CELL PROLIFERATION IN THE INTESTINAL EPITHELium

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE by

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<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMARY</td>
</tr>
<tr>
<td>DECLARATION</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
</tr>
</tbody>
</table>

### CHAPTER I

#### Section I
Introduction

#### Section II
Literature Review

### CHAPTER II
Rationale of the experiments

### CHAPTER III
General Methods and Materials

(a) Type of rats used and general conditions

(b) Methods of anaesthesia for all rats

(c) General methods used for opening the abdominal cavity (laparotomy)

(d) General methods used for opening the cranial cavity (craniotomy)

(e) Collection staining and sectioning of specimens of the small intestine

(f) Stereotactic methods used in these experiments

(g) Perfusion method for brain fixation

(h) Cerebral tissue sectioning and staining

(i) Method of staining neural tissue after vagotomy

(j) The stathmokinetic technique

(k) Statistical methods used in these experiments

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
</tr>
<tr>
<td>1.20</td>
</tr>
<tr>
<td>2.1</td>
</tr>
<tr>
<td>3.1</td>
</tr>
<tr>
<td>3.2</td>
</tr>
<tr>
<td>3.2</td>
</tr>
<tr>
<td>3.2</td>
</tr>
<tr>
<td>3.3</td>
</tr>
<tr>
<td>3.4</td>
</tr>
<tr>
<td>3.5</td>
</tr>
<tr>
<td>3.6</td>
</tr>
<tr>
<td>3.9</td>
</tr>
<tr>
<td>3.11</td>
</tr>
<tr>
<td>3.13</td>
</tr>
<tr>
<td>3.14</td>
</tr>
<tr>
<td>3.25</td>
</tr>
</tbody>
</table>
CHAPTER IV  
Experimental Investigations

Section 1
The effects of denervation and/or electrical stimulation of the nerve supply of small intestine.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction to section 1 experiments</td>
</tr>
<tr>
<td>2</td>
<td>Experiments,</td>
</tr>
<tr>
<td>(a)</td>
<td>The effects on crypt cell proliferation of division of the trunks of the abdominal vagus nerves.</td>
</tr>
<tr>
<td>(1) Methods,</td>
<td></td>
</tr>
<tr>
<td>(11) Results and Discussion,</td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>The effects on crypt cell proliferation of stimulation or sham stimulation of the abdominal vagus nerves,</td>
</tr>
<tr>
<td>(1) Methods,</td>
<td></td>
</tr>
<tr>
<td>(11) Results and Discussion,</td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>The effects of denervation of the small intestine on crypt cell proliferation,</td>
</tr>
<tr>
<td>(1) Methods,</td>
<td></td>
</tr>
<tr>
<td>(11) Results and Discussion,</td>
<td></td>
</tr>
<tr>
<td>(d)</td>
<td>The effects on crypt cell proliferation of local denervation of an implanted loop of small intestine,</td>
</tr>
<tr>
<td>(1) Methods,</td>
<td></td>
</tr>
<tr>
<td>(11) Results and Discussion,</td>
<td></td>
</tr>
<tr>
<td>(3) Discussion on section 1</td>
<td></td>
</tr>
</tbody>
</table>

Section 11
The effects of pinealectomy on crypt cell proliferation in the rat small intestine.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction to section 11 experiments,</td>
</tr>
<tr>
<td>2</td>
<td>Experiments,</td>
</tr>
</tbody>
</table>
(a) Effects, one, two, and three weeks after pinealectomy,
(1) Methods,
(11) Results and Discussion,
(b) Effects of pinealectomy combined with--
(A) Local denervation of the small bowel,
(1) Methods,
(11) Results and Discussion,
(B) Truncal abdominal vagotomy,
(1) Methods,
(11) Results and Discussion,
(c) Effects of combined diversion of bile into the midpoint of the small intestine and pinealectomy
(1) Methods,
(11) Results and Discussion,
(d) Effects of combined ligation and division of the bile duct and pinealectomy,
(1) Methods,
(11) Results and Discussion,
(e) Effects of combined pinealectomy and diversion of a jejunal loop into the colon, with restoration of continuity of the jejunum,
(1) Methods
(11) Results and Discussion,
(3) Discussion on section 11
Section 111
The effects on crypt cell proliferation of bilateral limbic system lesions, (with or without associated pinealectomy).
(1) Introduction to section 111 experiments,
(2) Experiments,
(a) The effects of bilateral hippocampal lesions, with or without associated pinealectomy
   (1) Methods
   (11) Results and Discussion,

(b) The effects of combined bilateral hippocampal lesions and local small bowel denervation,
   (1) Methods,
   (11) Results and Discussion,

(c) The effects of combined bilateral hippocampal lesions and bilateral truncal abdominal vagotomy,
   (1) Methods,
   (11) Results and Discussion,

(d) The effects of bilateral amygdaloid lesions, with or without associated pinealectomy
   (1) Methods
   (11) Results and Discussion

(e) The effects of bilateral septal lesions, with or without associated pinealectomy
   (1) Methods,
   (11) Results and Discussion,

(f) The effects of bilateral fornix lesions, with or without associated pinealectomy
   (1) Methods,
   (11) Results and Discussion,

(3) Discussion on section III.

Section IV
The amounts of food eaten during the various experiments.

(1) Introduction to section IV,
(2) Experiments,
(a) The amount of food eaten by pinealectomized rats compared with the amount eaten by sham-pinealectomized rats,

(1) Results and Discussion,

(b) Comparison of the food intake associated with pinealectomy and that associated with a feeding schedule,

(1) Results and Discussion,

(c) The amounts of food eaten during the various experiments,

(1) Results and Discussion,

(3) Discussion on section IV.

CHAPTER V
General discussion of the experimental findings,

CHAPTER VI
Appendix.

BIBLIOGRAPHY
Previous investigators have suggested that the control of proliferative activity in the crypts of Lieberkuhn of the small intestine is probably multifactorial and may include local mechanisms, direct and indirect effects of food in the lumen of the bowel, and systemic factors including humoral blood-borne factors, and effects mediated via the nervous system.

The main aim of the present study, using male Sprague-Dawley rats as experimental animals, was to use principally surgical experimental techniques to assess the influence of lesions of selected portions of the central nervous system (limbic system, pineal), and pertinent portions of the peripheral nervous system (vagus nerves, sympathetic nervous system), and combinations of these two, as well as certain other procedures judged to be appropriate to determine the mechanisms which control proliferative activity in the crypt cells, especially in relation to the nervous system and its particular role.

A total of 262 rats in 16 experimental procedures were used and the Colchicine stathmokinetic technique was used in all cases.

Bilateral cingulate or other neocortical lesions were not associated with any significant changes in crypt cell birth rate. On the other hand bilateral lesions of portions of the limbic system, viz., septum, fornix, hippocampus or amygdala, were associated with a rise in crypt cell birth rate of a considerable magnitude comparable with that following pinealectomy and unaffected by combinations of these lesions with pinealectomy. These results derived from the present study are consistent with the hypothesis that the effects of the
pineal on the gut are mediated via the limbic system, rather than the limbic effects being mediated via the pineal. The effects of limbic lesions on crypt cell proliferation rate have not been previously investigated, as far as can be determined from the available literature. The effects of pinealectomy are in accord with those of other workers using rats.

The effects of hippocampal lesions on the crypts appeared to be mediated in part by the autonomic nervous system, since they were diminished markedly by both vagotomy or local small intestinal denervation. Since the hippocampus is closely connected with the other parts of the limbic system, there is good reason to suspect that the effects of the lesions of other areas of the limbic system may also be mediated in a similar fashion. There does not appear to be evidence of such an investigation in the available literature.

Following pinealectomy there was a dramatic rise in the crypt cell birth rate. However, a finding of special interest is that crypt cell proliferation was found to be diminished below the expected level when pinealectomy was combined with, (i) truncal abdominal vagotomy, (ii) local small bowel denervation, but not by diversion of (iii) bile or (iv) luminal contents away from the intestinal epithelium. The findings regarding the effects of pinealectomy confirm those of other workers, but the effects of combining pinealectomy with the above mentioned changes have not been previously investigated, as far as can be determined from the available literature.

With regard to the functional implications of the above mentioned findings, the present investigator suggests that the pineal gland and the limbic system clearly appear to play some role in the general systemic modulation of crypt cell proliferation and exert such influence principally (although
probably not exclusively) via the autonomic nervous system. Further, it is still unclear whether the pineal gland and the limbic system are involved in day-to-day normal control mechanisms or act only under unusual conditions (e.g. stress).

Bilateral truncal abdominal vagotomy was found to be associated with a rise in crypt cell proliferation rate, in accordance with some workers but not others, since there is considerable difference of opinion in the literature on the effects of vagotomy on the crypts. However, stimulation of the abdominal vagus nerves was not associated with any significant change in the crypt cell proliferation rate, and this effect does not appear to have been previously investigated. With regard to the effects of vagotomy in association with pinealectomy or limbic lesions, it is notable that the effect of vagotomy on the crypts is to decrease the mitotic rate, suggesting that the vagus nerves have a different role when associated with such lesions.

Local denervation of loops of small intestine (mainly involving the sympathetic nerve supply) was associated with a significant fall in crypt cell proliferation rate which persisted for several weeks. Such effect was shown to be principally a direct neural one on the crypts, rather than being due to the local bowel ischaemia which may accompany this procedure. These findings are in accord with those of previous investigators, although the attempt to dissociate the neural from the local vascular effects does not appear to have been made by previous investigators.

Hyperphagia induced by dietary training was associated with a much smaller rise in crypt cell birth rate than that associated with limbic or pineal lesions, and neither pineal-
-ectomy nor limbic lesions were found to be associated with increased ingestion of food. In fact, there was not generally found to be any significant correlation between the amounts of food eaten and the rise or fall in crypt cell birth rate in the various experiments. The significance of this is discussed. There does not appear to have been an attempt to measure the amounts of food eaten in relation to the crypt cell mitotic rate made, on consulting the available literature.

This study has been made in an attempt to extend the amount of knowledge available about the topic of crypt cell proliferation control, especially in relation to the role of the C.N.S. in any such control mechanism, but obviously many questions remain unanswered as the mechanism of control of crypt cell proliferation is obviously a very complex one.
DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, it contains no material previously published or written by any other person, except where due reference is made in the text. The author consents to the thesis being made available for photocopying and loan if accepted for the award of the degree.

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### CHAPTER 1

<table>
<thead>
<tr>
<th>SECTION</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
</tr>
<tr>
<td>11</td>
<td>LITERATURE REVIEW</td>
</tr>
</tbody>
</table>
SECTION 1, INTRODUCTION

(A) THE RENEWAL UNIT

The renewal unit in the small and large intestinal epithelium is the crypt of Lieberkühn (see Fig. 1.1). The most common type of cell in the crypts is a columnar epithelial cell, the enterocyte which is capable of proliferation. At the base of the crypts of the small intestine large granulated Paneth cells of indeterminate function (see P-S, Fig. 1.1), are found, and higher in the crypts and on the villi are found mucous secreting goblet cells. There is good evidence (Aherne et al., 1977) that mature Paneth cells and goblet cells do not proliferate, so that in the present series of experiments, proliferation of the enterocytes only has been dealt with.

In both the small and large intestine, the crypt may be considered to form part of a two compartment system viz., (1) the proliferative compartment (P in Fig. 1.1), which supplies cells to the (2) functional compartment, which in the case of the small intestine is principally in the villi (F in Fig. 1.1) but in the case of the colon is the surface epithelium where there are no villi.

In the small intestine, the proliferative compartment occurs in the lower 2/3 of the crypts, whereas in the colon, (proximal colon), proliferating cells are concentrated in the middle 1/3 of the crypt, whilst
In the distal colon, they are virtually confined to the lower 1/3 of the crypt (Sunter et al. (1980); Tutton and Barkla (1976).

From the source of cell production in the crypts, the cells migrate upwards from several crypts to each villus in the small intestine (Bjerknes and Cheng, 1981, a-d), or to the surface epithelium, in the colon, where they are lost into the lumen. Crypt cells are generally thought to move upwards because of the proliferative activity of the cells below them. However, Loehry and Creamer (1969) established that there are many more crypts than villi, that very few of the crypts open on to the villi on all sides, and that in most cases much of each crypt opening is adjacent, not to villi but to other crypts. Thus they concluded that the migration of epithelial cells from crypts to villi cannot be a simple direct progression, as might be expected from two dimensional sections. Loehry et al. (1969) found that epithelial cells emerging from the crypts were probably channelled on to the villi rather than being desquamated at the crypt mouth. Wilson et al. (1984, 1985) observed that (1) villus cells appear to move upwards in a continuous sheet (2) there is little mixing of cell types on the villi, (3) there is no evidence of spiral movement and, (4) the cells move in straight lines and upwards. Cells can be lost from the sides of the villus as well as the villus tips.
A single crypt can feed cells to more than one villus.

Goodlad and Wright (1983) found a proximodistal gradient of villus cell number in the mouse, and also a similar gradient in the ratio of crypts to villi. They found that cell influx into the villi is approximately proportional to the villus population size and the villus population is almost directly equivalent to the crypt population.

Kapadia and Baker (1976) found that changes in villus shape are probably not determined by differences in the rate of crypt cell production. Sunter et al. (1980) found that although the mean crypt length varies quite considerably from one site to another along the length of the rat colon, the total crypt cell population varies only slightly, due to differences in the circumferences of the crypts at different levels.

Further up the crypt is the maturation compartment (Min Fig. 1.1) in which cells are thought to continue their differentiation but do not divide.

Aherne et al. (1977) concluded that the normal cell population in the crypt-villus complex is in a steady dynamic state.
Diagrammatic representation of the crypt-villus complex of the small intestinal mucosa.

V = Villus
C = Crypt of Lieberkühn
F = Functional compartment
M = Maturation compartment
P = Proliferation compartment.
P-S = Paneth cell and Stem cell compartment.

(after Appleton, Sunter, and Watson, 1980).
Besides these differentiated cells, there are other cells from which the crypt cells are derived i.e. stem cells.

These have been described by Cheng and Leblond, (1974 b), Leblond and Cheng (1976) as cells which have the ability to undergo divisions throughout the life of the organism and to give rise to progeny, (1) which ultimately undergo differentiation and (2) to others which are similar to themselves i.e. "functional stem cells", responsible for the population of the normal crypt.

Others have reserved the term "stem cells" for cells capable of repopulating the crypt after death of proliferative cells, following some toxic insult, i.e. "potential stem cells".

These latter cells are often referred to as "clonogenic" but the functional stem cells may also be "clonogenic" (Wright, 1978) and there is uncertainty as to how separate and well defined these two groups are i.e. as to whether they constitute the same, different or overlapping populations.

Cheng and Leblond (1974 a and b), Leblond and Cheng (1976) noted that the crypt base columnar cells of the small bowel shared certain morphological characteristics with the stem cells of other renewal systems and thus concluded that the basal crypt columnar cells were functional stem cells for all small bowel epithelial cells.

Cairnie et al. (1965), Al-Dewachi et al. (1974),
suggested that the basal cells, which are few in number, have longer cell cycle times than the cells in the proliferative compartment, which are derived from them, in both the small intestine and the colon. Boarder and Blackett (1976) concluded that a considerable portion of the potential stem cells were rapidly proliferating and that the slowly cycling cells did not make up a large proportion of the potential stem cells. It has been suggested that both slowly cycling basal crypt cells and rapidly proliferating basal cells have clonogenic properties and it is thought possible that the crypt repopulating mechanism may vary according to the degree of cell death.

Slowly cycling basal cells are thought to occur in human small bowel (Wright et al., 1973b). It has been suggested by Wright (1978), Tutton and Barkla (1976) that the slowly cycling functional stem cells, being exposed relatively longer to carcinogens than the other cells of the renewal system, may be more susceptible to the actions of carcinogens.
If the mucosa of the small intestine is destroyed at one spot, epithelialization occurs first by migration of cells from adjacent areas, followed by an increased rate of cell division in the crypts at the margin of the injured area. Later, new crypts form on its floor and the villi are regenerated. If the injury penetrates the muscularis mucosae that layer is not re-formed during repair (Davenport, 1971).

(C) THE MECHANISMS OF ADAPTIVE GROWTH OF THE INTESTINAL MUCOSA

Al-Dewachi et al. (1975a), Appleton et al. (1980) concluded that, under experimental but not normal conditions, there was evidence of non-cycling cells in the proliferative compartment of both the small bowel and colon.

Wright and Alison (1984) concluded that there were three potential mechanisms of achieving net growth or atrophy in the epithelium, viz.,

(1) change in the cell production rate,
(2) change in the life span of the cells of the functional compartment,
(3) changes in the absolute number of proliferative units or in the proportion of proliferative to functional units (in the small bowel, the crypt-villus ratio) and that the most important of these was changes in the cell production rate.
Wright and Alison (1984) considered the three parameters most important in modulating crypt cell production rate to be:

1. Absolute increase in the number of proliferating cells,
2. Increase in growth fraction, i.e., the ratio of proliferating to non-proliferating cells,
3. The cell cycle time, and these authors said that of these three factors, the most important in hyperplastic responses is the absolute size of the proliferative compartment. In hypoplastic responses (e.g., starvation) the absolute size of the proliferative compartment can be reduced and there is also evidence for an increase in cell cycle time. Experimentally, they concluded it appears that generally speaking, compensation is achieved either by a change in a single parameter or all parameters appear to change at the same time.

These parameters responsible for the proliferative events need to be related to changes in growth that might occur if the proliferative response is maintained e.g., to ensure an increase in the crypt population by increasing crypt cell production would necessitate a mechanism to ensure that crypt cells remained in the crypt, rather than migrated at a faster rate.

Since it is not known how the normal size of the crypt population is maintained (Wright and Alison, 1984), it is not known how the crypt increases in size, but it has been suggested that changes in
the plane of cell division are involved.

In hyperplastic responses, e.g. after partial intestinal resection, since usually the life span of the villus cells is unchanged, suggesting a constant transit time, the villus population and size are probably expanded primarily by an increase in crypt cell production. This was supported by the experimental findings of Clarke (1975).

Proliferative compartment size changes may be achieved, (potentially), by two mechanisms -

(1) an increase in stem cell efflux, with a constant number of transit divisions,
or

(2) a constant stem cell efflux and an increase in the number of transit divisions.

In some instances one mechanism is effective, and in others, the other is probably effective. Increased influx into the proliferative compartment alone probably does not explain the three dimensional expansion in crypt size seen in coeliac disease and after partial resection of the gut.

Studies on the human intestine (Wright and Alison, 1984), show that increased numbers of proliferative cells necessarily means an expansion of the maturation compartment. This presumably means that cells need to maintain a maturation sequence of a minimum length before emerging on to the surface.
In the present series of experiments the emphasis has been on measurements of crypt cell birth rate and comparison of this between various rats has been made. It is obvious from the present discussion that there are other factors involved in mucosal growth besides the crypt cell birth rate and that there are many variables involved. In these experiments comparison was made of the same parameter of cell production in similar tissues and similar rats, minimizing variables where possible to give some insight into some of the factors which influence the control of crypt cell production.
(D) CIRCADIAN RHYTHMS IN CRYPT CELL MITOTIC ACTIVITY IN THE INTESTINE.

Chang (1971), Chang and Nadler (1975), Hamilton (1979), Reeve (1975), Sigdestadt et al. (1969), Sigdestadt and Lesher (1970), Scheving et al. (1972, 1978) and Al-Dewachi et al. (1976) have all reported a circadian rhythm in the mitotic index and/or synthesis phase of the cell cycle (i.e. in proliferative activity) of the mouse or rat duodenum, small intestine or colon or rectum. The peak of DNA synthesis appeared to occur around the time of transition from dark to light and the lowest point was at the time of transition from light to dark. Throughout the mouse intestine the phasing appeared to be the same for stomach as for rectum but the lowest amplitude of the rhythm was found in the duodenum.

Sinha (1960), Clark and Baker (1963) reported a similar periodicity for the gastric epithelium, which persisted in hypophysectomized rats.

Al-Dewachi et al. (1976), suggested that one principal factor contributing to this variation appeared to be circadian fluctuations of the transit times of the G1 and G2 phases of the cell cycle.

Leblond and Stevens (1948) Bertalanffy (1960) Hunt (1952), Muhlemann et al. (1956), and Pilgrim et al. (1963) did not find cell proliferation.
rhythmnicity in the parts of the gut epithelium which they investigated.

However, the work of Scheving et al. (1972) involved sampling at 2 hourly intervals, and probably represented a more extensive study than some of these, especially that of Bertalanffy (1960). The weight of experimental opinion appears to be in favour of the existence of circadian rhythms of mitotic activity in the crypts.

(a) Regulatory aspects of circadian rhythm

Circadian rhythms in crypt cell mitotic activity may be influenced by:

1. Endogenous factors e.g.: Urogastrone, Epidermal Growth factor, Glucagon (Yeh et al., 1981), (Scheving et al., 1979, 1980), and possibly chalones (Thompson, 1974; Brugal and Pelmont, 1975; Sassier and Bergeron, 1977, 1980; Iversen, 1981) and glucorticoids, which have a photoreversible circadian rhythm (Dixon, Booth and Butler, 1967), and can inhibit cell proliferation (Bullough, 1968) may be involved.

2. Exogenous factors, e.g. the light-dark cycle (Scheving, Tsai, and Scheving, 1983; Sigdestadt and Lesher, 1971; Scheving et al., 1974) and feeding habits (Sakata and Tamata, 1978).

Edmunds (1984) thus considered that the circadian variations in intestinal cell proliferation were
probably a combined result of changes induced by exogenous influences, including feeding habits, and internal regulators.

(b) Possible mechanisms of the circadian rhythm in cell proliferation (see Scheving et al., 1983),

(1) The nutritional and abrasive effects of meals may play a direct role in the proliferative rhythm of the digestive tract.

(2) The effect of certain components of a meal may play a direct role in evoking the secretion of substances which increase mitosis.

(3) The light-dark cycle and meal presentation, acting neurally or hormonally on certain of the hypothalamic nuclei e.g. the suprachiasmatic and ventral medial nuclei, could induce local or systemic discharge of positive growth controlling signals, such as epidermal growth factor or insulin, possibly through the action of the sympathetic nervous system.

(4) Mesenchymal factors could affect the epithelial response to growth-promoting factors.

(5) These environmental stimuli might either directly or indirectly affect the rhythms in the biochemical properties of the cell surface receptors for positive growth factors.

(c) Neural control of cell proliferation rhythms.

Scheving et al. (1983) concluded that the suprachiasmatic nuclei of the hypothalamus (which is an important area for the timing of physiological processes) and which have exclusive input from the
retina are not "a biological clock" mechanism, which could generate and control circadian rhythms but may instead act as a circadian "pacemaker", a phase resetter and amplitude modulator.

He also concluded that the suprachiasmatic nuclei represent only one part of an overall neuroendocrine network of control.

von Euler (1956) found variation of adrenaline secretion in both humans and rats related to exercise and sleep. Bullough (1964) found that adrenaline acted in conjunction with a chalone to effect a mitotic depression in epidermis which was maximal during the stress and activity of the day. Tutton (1973a) showed that the crypt cell mitotic rate was not affected by physical exercise.

It was suggested that the higher crypt cell turnover at night reflected a greater need for absorptive cells in the nocturnal period of feeding, so it was thought that diurnal variations in crypt cell mitotic rate could reflect diurnal variations in feeding, which is itself secondary to the normal cycles of sleeping and waking (Alov, 1963).

Tutton (1975) found that chemical sympathectomy did not change circadian rhythm in food consumption but abolished the circadian rhythm of crypt cell mitotic activity.
Thus, normal secretion of noradrenaline by the neurones of the mucosal plexus may be a necessary cofactor for some other mechanism e.g. a chalone, which controls the circadian rhythm in crypt cell proliferation.

Anton-Tay (1971) suggested a role for melatonin secretion, which also shows light dependent circadian variations in the maintenance of crypt cell circadian rhythms.

(d) Endocrine influences on circadian rhythm.

Scheving (1983) found that (1) epidermal growth factor, (2) insulin, (3) glucagon, (4) A.C.T.H.-1-17 (an analogue) have an influence on cell proliferation rhythms, especially in relation to rhythms in the digestive tract.

However, he concluded that a general systemic release of epidermal growth factor is insufficient by itself to account for rhythmic variations in the tissues. The response to epidermal growth factor was tissue specific and related to the time of day at which it was administered.

Scheving et al. (1982) demonstrated that whilst glucagon and insulin affected the DNA synthesis in various organs, the effect varied from organ to organ in its extent, and the effects differed from those of epidermal growth factor. Thus, it is possible that all 3 of these factors may play a
role in the control of crypt cell proliferation at different circadian stages.

The effect of ACTH-1-17 (an ACTH analogue) on crypt cell proliferation rate in the intestinal tract varied with the time of day.

Thus, Scheving (1983) believes that no single peptide or hormone is responsible for the control of crypt cell proliferation but one modulates the other, and they are in turn modulated by neural influences such as the suprachiasmatic nuclei.

Klein (1980) has suggested that light and feeding schedules may act through a common intermediate, the adrenal hormones, which then directly affect cell division in different tissues.

Friedenwald and Buscke (1944), Klein and Torres (1978) found that adrenomedullary hormones, such as noradrenaline, may influence circadian rhythms in mitotic activity.

Tutton (1973b) showed that inhibition of small intestinal crypt cell proliferation was only partially reversed by glucocorticoid treatment, suggesting that adrenomedullary hormones may be important in the control of crypt cell division.

Goodrum et al. (1974), Mamontov (1977) suggested that adrenocortical, and Klein (1979a) suggested that adrenomedullary hormones are involved in the
maintenance of circadian periodicity in the adult and the initiation of mitotic rhythms in the neonate. It was suggested that perhaps noradrenaline levels and the sympathetic nervous system may be involved as intermediates in situations where adrenal hormones (Mamontov, 1977), and glucocorticoids have been shown to alter the circadian rhythmicity of mitotic activity (Goodrum et al., 1975).

Wright and Alison (1984) concluded that the circadian crypt proliferative index changes appear to be accompanied by a genuine fluctuation in the cell production rate and flux into the functional compartment, and, therefore, in the size of the functional compartment. They suggest that the circadian variation in mitotic activity is due to synchronization of cohorts of cells in the upper part of the proliferative compartment. This causes changes in the proliferative compartment size and, therefore, in the cell production rate, which is passed on to the villus or colonic surface as increased influx, giving variation in the villus population also. Circadian variation in cell demand, induced possibly by villus cell attrition or local factors, may be the main cause and they suggest that it may not be necessary to incriminate exogenous hormones in the process.
It is apparent from this discussion that circadian rhythms in crypt cell mitotic rate are very important, and it is also important when comparing crypt cell mitotic rates in various tissues, to ensure that the specimens of tissue are obtained at a similar time of day e.g. as close to midday as possible.
Since this series of experiments is primarily concerned with the factors involved in the control of crypt cell proliferation, the following literature review is mainly concerned with the known facts concerning the control of crypt cell proliferation.

The exact mechanism of control of crypt cell proliferation is not known but certain hypotheses have been advanced.

Wright and Alison (1984) have classified the hypotheses of control of crypt cell proliferation into two basic categories:

1. Hypotheses suggesting mainly inhibitory mechanisms,

2. Hypotheses suggesting mainly stimulatory mechanisms.

From a critical evaluation of the literature they suggest that neither of these mechanisms wholly explains the mechanisms of control but that each contributes to the total mechanism of control.

The mechanisms of control of crypt cell proliferation may be further subdivided into:

1. Local mechanisms of control

2. General mechanisms of control, but there is often some overlap between these mechanisms.
Wright and Alison (1984) further consider that loss of tissue mass e.g. villus destruction is more likely to involve feedback mechanisms, possibly with the mediation of chalones, whereas control by gastrointestinal hormones, luminal nutrition or workload are more likely to involve some form of stimulation of cell proliferation, whilst partial resection may involve decreasing the concentration of an inhibitor or stimulating the production from the remaining bowel of some factor which stimulates cell production.

Many investigators support the belief of Clarke (1973, 1975) that there is a close relationship between the digestive and absorptive capacity of the intestinal epithelium and the rate of turnover of crypt and villus cells. Although Dowling and Gleeson (1973), Dowling (1982) have shown that villus hyperplasia results in a relatively immature villus population, whilst a reduced villus population results in the presence of hypermature villus cells (because of changes in migration rate), the net effect is a variation in the total absorptive capacity which appears to correlate with the size of the villus population (Dowling and Booth, 1967) or the crypt cell production rate (Wright and Alison, 1984).

Senior et al. (1982) found that during regeneration of colonic mucosa, during prolonged organ culture,
the regenerating epithelium reproduced crypts of similar morphological appearance to those of normal mucosa, suggesting that the direction of cell migration and production of new crypts was determined by intrinsic regulatory factors within the tissue. They suggested that the various general factors affecting crypt cell proliferation rate e.g. luminal contents, etc, may simply be modulators of a local control mechanism.

Experimental evidence for a feedback mechanism in the gut.

Wright and Al-Nafussi (1982) using cytosine arabinoside, which led to a severe reduction of the total crypt cell population, found that this was followed by rapid recovery and subsequent fluctuations of crypt population. Comparison between these fluctuations and those of the villus population showed that the peak of cell production occurred at 4 days, when the villus population was minimal and the crypt population was increasing. Analysing the same experimental situation with a scheme devised by Cairnie (1976), they concluded that crypt cell proliferation did not increase until villus cell population depletion had occurred. This implied some form of feedback on the proliferating crypt compartment, which with other evidence, strongly supported the operation of a feedback system.
The concept of a feedback mechanism was supported also by the experimental work of Sato (1972) and Britton et al. (1982). The latter authors also proposed that the feedback system operated from both the maturation compartment and the functional compartment but acted exclusively on the stem cell compartment.

Thus the size of the villus population is linked with the size of the crypt population in this concept, although not necessarily in a linear relationship.

This hypothesis predicts (Wright and Alison, 1984) that (1) the onset of crypt cell proliferation after perturbation of the mucosa is directly related to the time taken for villus depopulation to reach some unknown critical level (suggested by Rijke et al. (1974) to be below a villus length of 60-70%),

(2) after perturbation, some form of inverse relationship should exist between the villus population size and the crypt cell production rate.

These predictions were supported by Galjaard et al. (1972), and Rijke et al. (1974).

Whilst Rijke et al. (1974) suggested that reduction below a critical villus length triggered off crypt cell proliferation, it was presumed that there was some level of maximal crypt cell production above which it was impossible to go, and Wright et al. (1973 a) suggested that this maximal level was reached before total villus depopulation and that the
feedback signal was saturated at extreme demand, as proposed by Sato et al. (1972).

Wright and Al-Nafussi (1982) concluded that although there appeared to be some form of feedback mechanism operating, there was probably not a linear relationship between crypt cell production rate and the villus population.

Hagemann (1980) using mouse jejunum and studying proliferative responses after damage, considered that crypt cellularity rather than villus cellularity was the important modulator of cell production.

However, as indicated by Wright and Alison (1984), the difficulty in drawing firm conclusions about whether villus or crypt cell depletion is the principal or sole factor is that where the crypt cell population is depleted experimentally, there are associated and subsequent changes in villus population which cause difficulties in interpretation.

Rijke et al. (1976) found that temporary ischaemia of the rat small intestine, resulting in rapid, selective, villus damage but no crypt population changes i.e. a pure villus cell depletion, was followed by the rapid onset of increased crypt cell proliferation and an increase in the size of the proliferative compartment, supporting the hypothesis that the feedback control emanated from villus cells.
Wright and Alison (1984), in a review of this topic, report another instance of pure villus depletion, viz., exposing a patient with coeliac disease on a strict gluten-free diet to gluten, where there was no reduction in crypt cell population in response to the gluten, was associated with the rapid onset of crypt hyperplasia.

These results suggest that villus compartment depletion is relatively more important in the feedback mechanism. This mechanism may possibly act via a relative increase in proliferative compartment size or, possibly, by an action on the stem cells. However, this mechanism is not the only one involved in cell proliferation control because there are many examples where a reduction in villus population is not seen. For instance, after partial resection both villus population and crypt cell production rate change in the same direction (not opposite as predicted by the feedback mechanism), (Clarke, 1975).

In starvation, where there is a reduction in the villus population, there is also a reduction in the crypt cell production rate.

In lactation, where villus population is expanded, so is the crypt cell production rate.

Despite these limitations, there is some support, at
least in some circumstances, for the "villus longistat",
or local mechanism, whereby villus length tends to
be maintained at a constant value despite circum-
stances which alter the life expectancy of villus
cells (Tutton, 1979).

Thus, although villus cells live longer in the intest-
ine of gnotobiotic animals and in by-passed segments
of intestine, villi do not become abnormally long
but instead the rate of cell production is reduced.
Conversely, as described previously, when villus loss
is increased by ischaemia, villus shortening is
avoided.

When crypt cell proliferation is temporarily suppressed
by cytotoxic drugs or irradiation, villi become
shorter, but when the crypt cells have recovered
from the inhibiting agent, production proceeds at a
rapid rate until, and only until, the villi have
been restored to their normal length.

These responses by which crypt cell production is
adjusted to match villus cell loss are highly
localized and can be demonstrated in one segment of
gut whilst cell proliferation occurs at the usual
rate in an adjacent segment (Tutton, 1979).

To explain the proliferative crypt response after
partial enterectomy, Booth et al. (1956) advanced the
"tissue mass" hypothesis which postulated that the
intestinal cells secrete a growth inhibitor, the
serum concentration of which falls after partial
enterectomy, thereby permitting the remnant to grow
until sufficient mass is regenerated to restore the concentration of the inhibitor to normal levels.

However, Tilson et al. (1972) found that there was a larger increment in villus height in the jejunum after bypass than in the remnant after excision, suggesting that other factors such as functional demand or luminal nutrition are more important than reduction of tissue mass after resection.

Possible mediators in the feedback mechanism

**CHALONES**

The action of chalones may be summarized in the following manner:

- **NORMAL CHALONE CONCENTRATION IN THE TISSUE** = GROWTH INHIBITION IN THE TISSUE,
- TISSUE IS DAMAGED,
- TISSUE DAMAGE RESULTS IN A DECREASED CHALONE CONCENTRATION,
- CELL DIVISION RESULTS DUE TO DECREASED CHALONE CONCENTRATION,
- TISSUE IS RESTORED.
Besides this local action of Chalones, possibly operative in local epithelial damage, it has also been considered possible that other more general factors e.g. the action of pancreatobiliary secretions or the effect of luminal contents, may act on the epithelium by changes in the level of these chalones.

Chalones are therefore derived from the epithelial tissue itself and many attempts have been made to extract such substances, which should have an inhibitory action on crypt cell mitotic activity.

Anderson (1973) for instance, failed to find any inhibitory action of an aqueous extract of whole rat small intestinal homogenate on the onset of jejunal epithelial cells in vivo whereas Tutton (1973, c:) found that the 80% ethanolic precipitate of an aqueous extract of crypt cells inhibited the onset of mitosis in rat jejunal mucosa in vivo and in vitro, whereas villus cell extracts had no such effect. The extract was found to be heat-labile and acted on colonic crypts, oesophagus or epidermis in a similar manner, and was not potentiated by β-adrenergic agents.

Brugal and Pelmont (1975), Brugal (1976). found that aqueous extracts of newt intestine had a similar inhibitory action on that intestine, but that this action may have been irreversible, which would not support the action of a chalone.
Sassier and Bergeron (1977) found that the inhibitory action of an aqueous extract of rabbit small intestine on crypt cell mitotic activity in mouse small intestine and colon in vivo was reversible but not tissue specific, since it inhibited mitotic activity in liver and kidney in vitro also.

Sassier and Bergeron (1978) further raised doubts about the specificity of the effects of these extracts by finding that, whilst rabbit colon mucosal extracts inhibited mitotic activity in mouse small intestine, this was also achieved using renal and testicular extracts.

However, the same authors (1980), using more purified preparations in the same experiment, did demonstrate their tissue specificity, and postulated the existence of a factor which plays a role in the regulation of crypt cell proliferation in both small and large intestine. Some support was lent to this concept by the findings of Buckholtz et al. (1976), and Obertrop et al. (1977) that partial resection of one organ led to apparent proliferative responses in the other but the concept of a chalone common to both small and large intestine was not supported by other investigators e.g. May et al. (1981). They found that whereas an aqueous extract (non-cytotoxic) of villus cells produced a profound, apparently tissue specific, and reversible reduction in cell pro-
liferation in a rat intestinal cell line in vitro, extracts from colonic mucosa were apparently without effect on this intestinal cell line.

Wright and Alison (1984), in a review of the topic, comment that besides the fact that Sassier and Bergeron (1978) used whole intestinal extracts and thus made the distinction between villus or crypt effects impossible, in many of such similar studies there was no attempt to show reversibility of action or lack of cytotoxic effect, and that small bowel extracts are known to contain cytotoxic factors (Chan et al., 1975).

They further suggested that, since the large molecular weights of the active extracts suggested aggregation with other substances e.g. proteins, the presence of cell proliferation inhibitors present in the mucosa e.g. Secretin (Johnson and Guthrie, 1974), or somatostatin (Wright and Alison, 1984) in the extracts may partially at least explain their action.

The possible role of chalones in the control of crypt cell proliferation thus appears to be unresolved.
Possible control of crypt cell proliferation by stimulation of cell proliferation in the crypts.

Just as there appears to be some evidence for a feedback mechanism in the control of crypt cell proliferation, it is also possible that in some circumstances control by stimulation of proliferation in the crypts is operative, and it is possible that each mechanism plays some part in the overall control. Stimulation of proliferation may, for instance, play a part in the responses of the crypts to partial resection of the bowel, to bypass of the small intestine, or lactation, where there is an increase in crypt cell proliferation.

Considering control by stimulation, Wright and Alison (1984), in a review of this topic, consider that there are three principal factors which produce such a stimulatory effect on the crypts, viz.,

1. the presence of food in the intestinal lumen,
2. the presence of pancreaticobiliary secretions in the intestinal lumen,
3. endogenous chemical stimulators, either hormonal or derived from the nervous system.

The presence of food in the intestinal lumen does not imply,
that all of this food is being absorbed, and the presence of food may be viewed in different ways. Gleeson et al. (1972) saw it as important in maintaining the morphology, and therefore functional efficiency, by local absorption of nutrients into the mucosa. Clarke (1975, 1977) viewed the presence of food in the lumen as inducing a functional demand on the intestine, producing a compensatory response to improve its absorptive capacity. However, the presence of food in the lumen is not a straightforward situation, as indicated by Johnson (1979) viz. -

Figure 1.2

FOOD IN THE G-I TRACT

DIRECT EFFECTS

INCREASED DESQUAMATION

LOCAL NUTRITION

PARACRINE EFFECTS

GROWTH OF MUCOSA

INDIRECT EFFECTS

HORMONE MOTILITY

RELEASE, SECRETION

NERVE STIMULATION

NON-G-I HORMONES

(after Johnson (1979), originally from Gastroenterology, 72, 788, (1977).

(N.B. paracrine effects are those exerted locally on cells in close proximity to the hormone containing cells).
Evidence supporting the importance of food in the lumen.

(1) Evidence from the effects of small bowel resection

(a) The effects of small bowel resection.
Williamson et al. (1978 a, b) found increased villus and crypt size in the remaining small intestine following 70% resection of the small bowel in the rat, commencing between two and four days after resection and occurring both proximal and distal to the anastomosis.

McDermott and Roudnew (1976 a), Hanson et al. (1977 b) found that maximum crypt population was reached at approximately 12 days after resection.

Hanson et al. (1977 a) found that the increase in cellularity was proportional to the length of bowel excised and Hanson (1982) found that the crypt: villus ratio remained constant.

Al-Mukhtar et al. (1982 a) found that the increase in crypt cell production rate was almost entirely due to an absolute increase in proliferative compartment size.

Booth et al. (1959), Dowling and Booth (1967), found that the increase in villus size was greater after proximal than distal small bowel resection, and was maximal near the anastomosis, tapering off
Nygaard (1967) considered that the number of villi increased after resection but Forrester (1972) considered this unlikely.

(b) The effects of small bowel resection in relation to the presence of food in the lumen.

Weser and Hernandez (1971), Nygaard (1967), Dowling and Booth (1967), Tilson and Wright (1970), Levine et al. (1976), Weser and Tawil (1976) all found that, provided the animal has been fed orally, the remaining small intestine after resection undergoes marked mucosal hyperplasia, as expected, especially in the remaining ileum after jejunal resections.

Dowling and Booth (1967) further found that rats fed with a bulk diet, with little nutrient value, did not produce the same degree of mucosal hyperplasia as animals fed a normal diet. It should be borne in mind when considering these results that the magnitude of the mucosal hyperplasia is dependent on the amount of gut resected also (Young, 1985), whilst crypt changes are independent of their position in the intestine.

The relatively more variable adaptive changes found in jejunal mucosa after ileal resection than the previous -ly mentioned resection (Young and Weser, 1974)
were interpreted as giving additional support to the concept of adaptation to the nutrient value of the luminal contents since following jejunal resection, the ileum receives a relatively richer supply of nutrients to stimulate the mucosa. However, whilst this is consistent with the concept of adaptation to the nutrient value of the luminal contents, the evidence is indirect and other factors which could have influenced these results include a possible fall in luminal pH or the effects of salivary, pancreato-biliary or duodenal secretions (which in turn may be affected by the amount and quality of the ingested nutrients).

Furthermore, as pointed out by Levine et al. (1976) (see also Fig. 1.2), although oral intake appears to be an important initial stimulus, feeding may lead to many changes in the hormonal and neurovascular environment of the gut and the presence of food affecting the nutrition of the mucosa locally may be one of many factors operative.

Young (1985) has pointed out that, whilst the majority of carbohydrate and fat absorption as well as vitamins and minerals occurs in the jejunum, the ileum is capable of becoming more efficient at these functions when the jejunum is resected because of the ileums' substantial capacity to undergo compensatory adaptation. Thus, he states the resection of proximal jejunum is followed by relatively little nutritional disturbance. On the other hand
the ileum is the major absorptive site for the
products of protein digestion and for specific absorption
of vitamin B12 and bile salts. The jejunum cannot
take over these latter two functions and is less
capable of compensatory adaptation. This contrasts
with the earlier view of the explanation for the
differing results of jejunal resection from ileal
resection.
Feldman et al. (1976) found that the villus hyperpla-
sia observed in orally fed dogs after jejunectomy was
not present if the dogs were fed parenterally and
the slight but significant fall in villus height was
comparable with that seen in parenterally fed dogs
without any operative procedure (Hughes et al.
(1978); Hughes and Dowling (1980)). These findings,
together with those of Al-Mukhtar et al. (1982 b)
supported the contention that luminal nutrition
or the presence of nutrients within the lumen was
essential for the ileal adaptive response to jejun-
ectomy.
(2)
Evidence from the effects of ileo-jejunal transposition

Gronqvist et al. (1975), Altmann and Leblond (1970),
Philipson (1975), Dowling and Booth (1967), Al-Mukhtar
et al. (1982 b), Rijke et al. (1977 a), and
McDermott (1979) showed that transposing an ileal
segment into the jejunal region resulted in hyper-
plastic mucosal changes with taller villi, and/or
a rise in crypt cell production rate in the
transposed ileal segment, with or without hypoplastic
mucosal changes in the transposed jejunal segment. This was interpreted as indicating that the ileal loop responds to the relatively greater luminal nutrition in its proximal (jejunal) site by becoming hyperplastic, whilst the now more distally located jejunum becomes hypoplastic as the result of its relatively lower luminal nutrition. Dowling (1982) however, felt that these conclusions could not be made with certainty, because the degree of proximal ileal absorption after transposition is unknown.

Furthermore, McDermott (1979) found after ileo-jejunal transposition in the rat that the size of both the crypt cell population and the proliferative zones of both transposed segments were increased. A pattern of descending villus size was re-established in a duodeno-ileal direction.

(3) Evidence from experiments on the effects of jejunal bypass.

Gleeson et al. (1972) found that if a loop of jejunum was isolated from luminal nutrients and pancreato-biliary secretions it became hypoplastic, with a narrower calibre, shorter villi, shallower crypts, slower cell migration and reduced segmental absorption, whilst mucosal hyperplasia was stimulated in the remaining ileum in continuity. Using similar bypassed (Thiry-Vella Loops) segments, Al-Mukhtar et al. (1982b) found a decreased crypt cell
production rate in the bypassed loop and the findings were also supported by experimental work carried out by Menge et al. (1970), using self-emptying blind loops. The studies of Dudrick et al. (1977), Fenyö et al. (1976), Grenier et al. (1974), Iversen et al. (1976) and most other investigators, have confirmed similar findings in man e.g. in patients undergoing jejuno-ileal bypass for obesity, but the findings of Tompkins et al. (1977), and Solhaug (1976) do not confirm this, however.

Williamson et al. (1978a), whilst confirming the above findings in self-emptying jejunal blind loops after exclusion, did not find mucosal hyperplasia in defunctioned upper ileum in rats with a Thiry-Vella fistula (Williamson and Bauer, 1978). They suggested that the ileal mucosa, being seldom exposed to high concentrations of intraluminal nutrients, was less sensitive to deprivation of food. These findings were supported by the experiments of Hanson, Rijke et al. (1977b), Imondi et al. (1968), Altmann (1972) and all were considered to support the luminal nutrition hypothesis.

However, it is difficult to separate luminal nutrition from other effects which may be due to the presence of food and since the bypassed segments still have an intact nerve and blood supply the action of hormones or neurotransmitters in this effect is possible, as well as the direct effects of the nutrients locally on the mucosa.
Evidence from experiments on the effects of starvation and semi-starvation.

It should be noted that in starvation, in addition to the direct absence of intraluminal nutrients, there is an accompanying reduction in endogenous gut secretion, since oral intake of food stimulates the flow of bile and pancreatic juice.

Ross and Mayhew (1983) found that whilst fasting did not result in a diminution of villus height, the villus mucosal surface was diminished throughout the rat small bowel, thus suggesting also the inadequacy of villus height as a measure of the overall mucosal surface.

Altmann (1972), Goodlad and Wright (1984), Hopper et al. (1968), Al-Dewachi et al. (1975a), Hagemann and Stragand (1977) found a progressive reduction in villus population size and reduction in crypt population during starvation in the rat and mouse. The absolute number of crypts and villi remained largely unchanged in starvation, and there was only a minor reduction in the total crypt number in the rat at 5 days (Clarke, 1972) while the crypt:villus ratio in the mouse remained constant during 48 hours starvation (Goodlad and Wright, 1984). Crypt cell production rate is reduced after starvation in the mouse (Weibbecke et al., 1969; Hagemann and Stragand, 1977) and rat small intestine.
(Al-Dewachi et al., 1975 a) and these changes are reversed by refeeding.

Thus it appears that the hypoplasia of starvation is reversed by refeeding.

However, as indicated previously, these experiments do not exclude the effect of the secondary effects of starvation on crypt cell proliferation.

In an attempt to overcome the effects of malnutrition experiments using total parenteral nutrition were carried out i.e. where there was no chance of a deficiency of calories or essential foodstuffs obscuring the effects of diminished topical nutrition to the intestinal mucosa.

(5) Evidence from experiments involving total parenteral nutrition.

Hughes et al. (1978), Hughes and Dowling (1980), Levine et al. (1976), Al-Mukhtar et al. (1982, b) found that exclusion of oral intake and replacement of this by total parenteral feeding was associated with hypoplasia and/or hypofunction of the intestinal mucosa. The disappearance of the proximo-distal gradient of mucosal mass in these circumstances was explained by suggesting that the jejunum, being exposed to relatively greater nutrients than the ileum, is more affected by the absence of luminal nutrients.

However, it should be noted that total parenteral nutrition also decreased crypt cell turnover in the
colon (Morin et al., 1980), where there is relatively little nutrient value in the luminal contents. This suggests that the bulk of the luminal contents may perhaps be more significant than the nutrient value.

The importance of the nutrient value of the contents as opposed to their bulk was supported by the findings of Spector et al. (1977), Levine et al. (1974), and Eastwood (1977), who showed that infusion of either 30% dextrose or 5% mixed amino acids into the rat stomach during total parenteral nutrition, maintained the mucosal mass of the jejunum as well as the proximo-distal gradient and, if these substances were infused into the ileum, ileal and more proximal gut hyperplasia were stimulated. Furthermore, similar effects on the intestinal mucosa could be obtained by gastric or ileal infusion of specific single amino acids (Spector et al. 1977 a)

Further support for the importance of the nutrient value of the contents of the bowel as opposed to bulk was provided by Jacobs et al. (1975 b) who found that an isotonic solution of the elemental diet "Vivonex" not only prevented the hypoplasia which otherwise occurs in Thiry-Vella loops where there is a lack of intestinal content, but it also stimulated an increase in villus length. Morin et al. (1982) found that oral administration of long chain triglyceride fat to jejunectomized rats on total parenteral nutrition (only 20% of the total energy
requirements) maintained intestinal structure and
function at the same level as in animals maintained
on a solid diet.
Menge et al. (1975 a and b), and Clarke (1977)
gave further support by showing that glucose,
galactose and methionine partially prevented the hypoplasia and hypofunction of self-emptying blind loops.
Dowling (1982) however, commented that this experiment
al model had its limitations, in that the iso-
peristaltic loops may not have been self-emptying and
thus it was likely that some luminal nutrients may
have refluxed into the most distal parts of the
bypassed loops with the result that there may have
been varying degrees of hypoplasia throughout the
"excluded" loop.
Clarke (1977) in turn, suggested that whilst his
findings supported the "luminal nutrition" theory
as far as villus height was concerned, they shed no
light on the mechanism of epithelial replacement,
since he considered that villus height was not an
adequate morphometric index.

Several other investigators added to the controversy
as to whether luminal bulk or nutrient value was
relatively more important. Storme and Williams (1981),
Janne et al. (1977) have shown a depression in
epithelial cell proliferation in rat colon submitted
to oral elemental diets, where faecal bulk is reduced
but not necessarily the nutrient value of the
colonic content.

Ryan et al. (1979) found that addition of inert bulk to an oral liquid diet restored normal DNA synthetic activity in the colon (without a concomitant increase in serum gastrin levels.).

From the foregoing analysis and discussion it can be seen that much of the evidence is indirect and not absolutely conclusive, but suggests that the role of nutrients in control of crypt cell proliferation cannot be ignored.

(6) Evidence from the effects of experimentally induced hyperphagia.

(a) Hyperphagia induced by exposure to low temperatures

Heroux and Gridgeman (1958) found that the hyperphagia and increase in luminal nutrition associated with hypothermia (6°C). for one month produced mucosal hyperplasia (i.e. increased villus height) and segmental hyperfunction. (Jacobs et al., 1975 a), and that these changes could be prevented by matching intake to that of normothermic, normophagic controls. These findings were supported by the work of Al-Mukhtar et al. (1982 b), who found increased cell production in the crypts.

(b) Williamson (1978 a) has reported villus hypertrophy occurring after several different models of experimental hyperphagia, including -
(1) intermittent starvation,
(2) tube feeding,
(3) high lactose diets,
(4) insulin injections,
He also notes that hyperphagia is a feature of certain conditions associated with increased small bowel growth, such as:
(1) lactation,
(2) hyperthyroidism,
(3) diabetes mellitus,
but pair feeding and other experiments suggest that hormonal factors contribute to adaptation in these cases, as well as hyperphagia,

(c) Hyperphagia associated with hypothalamic lesions or dietary training.

Brobeck, Tepperman and Long (1943) using female rats made bilateral hypothalamic lesions involving the ventrolateral portion of the central grey substance, including the adjacent portion of the ventromedial nucleus (note that this nucleus was not bilaterally destroyed). They found that many of the rats became very obese, with an increased amount of fat in every depot of the body, including beneath the skin, in the omentum, the mesenteries and retroperitoneal tissues.
Their gastrointestinal tracts were found to be both dilated and "hypertrophied". However, it was not stated whether this was mucosal or muscular
hypertrophy, or both and what parameters were used to measure it. It was suggested that this was due to their hyperphagia as Tepperman et al. (1943) reported the same changes in normal rats trained to eat relatively large amounts of food at each session.

However, it should be pointed out in this context that in man adaptation to subtotal jejunoileal bypass develops in spite of reduced oral intake (in the treatment for obesity), (Barry et al., 1977), and the early response of rat small bowel to partial resection is independent of hyperphagia (Menge et al., 1975). Dowling (1982) has commented that once again the evidence from the hyperphagia experiments is indirect.

(7) Proposed mechanisms involved in the effect of luminal nutrition.

Young (1985) has pointed out that energy sources and oxygen required by crypt and villus cells are not delivered equally to villus and crypt cells because of the countercurrent effect of blood flow in the mucosa, such that whilst the crypt cells receive substrates and oxygen in concentrations similar to that in the blood, the villus cells receive decreasing concentrations of these towards the villus tips. When isotopically labelled amino acids are injected intravenously they preferentially label crypt cells.
and when given directly into the lumen, they preferentially label villus cells. Thus, villus cells are particularly sensitive to changes in dietary intake and starvation is known to lead to a greater decrease in small intestinal protein mass than in any other tissue in the body.

It is not clear (Wright and Alison, 1984) how these changes in luminal nutrition are translated into changes in crypt cell production rate.

Dowling (1982) has suggested that the luminal nutrients may act by changes in-

1. the levels of trophic gut peptides of the APUD system, (see later in literature review),
2. the levels of biochemical and cell biological regulators of tissue growth, e.g., Ornithine Decarboxylase/diamine oxidase system (Luk and Baylin, 1982),
3. the neurovascular status of the mucosa,
4. the levels of other enterotrophic substances, e.g., the pancreatic-biliary secretions.

He suggested that any or all of these mechanisms may be operative and should not be considered mutually exclusive.

Although the difference between the crypt cell mitotic rates in orally as opposed to totally parenterally fed rats has been explained as due to the greater villus cell loss in the presence of food producing stimulation of the crypts to proliferate, Clarke (1975) investigating the effects of starvation and refeeding on the mucosa of the rat, concluded that increased villus tip shedding did not necessarily
produce increased crypt cell production.

Evidence not supporting the importance of food in the lumen in control of crypt cell proliferation.

(1) Jejunal adaptation following ileal resection.

Since ileal resection does not produce a relative increase in nutrient load in the jejunum, it might be expected that the adaptive hyperplasia after ileal resection might be accompanied by an increase in food intake, but most investigators have not found any such increase.

Several experimental effects appear to suggest that mechanisms other than luminal nutrition are operative, although this does not exclude the simultaneous action of luminal factors.

(2) Adaptation following colonic resection.

Wright et al. (1969 a, b) found in man and in experimental animals that total colonic resection was accompanied by modest adaptive changes in the ileum and the reduction in ileostomy effluent correlated with the increased villus height (see also Hulten et al., 1971).

Bucholtz et al. (1976), Masesa and Forrester (1977), Wright et al. (1969 a) found that subtotal colectomy in rats was associated with compensatory ileal hyperplasia, although this was relatively slow to develop.
Dowling (1982) commented that as far as is known there is no change in the amount of nutrition reaching the distal small bowel after colonic resection and that there was no change in food intake, although Masesa (1976) claimed a 40% increase in the food intake of colectomized rats.

Furthermore, adaptive changes occur in the shortened colon itself, although less pronounced than the ileal adaptive responses e.g. Williamson et al. (1982) observed modest hyperplasia in the right colon eight months after caecal resection or left hemicolecction in rats, and Masesa and Forrester (1977) found marked hyperplasia of the left colon three months after right hemicolecction.

St.Clair et al. (1984) found on transposing a 3 cms. segment of transverse colon into the ileum that there were no changes in proliferative activity in this segment of colon, which was of course being exposed to a very different nutrient environment.

Touloukian and Wright (1973) found that there was villus hypertrophy in the proximal and distal intestinal segments of newborn infants with jejunoileal atresia which was most marked in the distal segment of the bowel. Obviously nutrient factors appear not to be of great significance in this case.

Delvaux et al. (1984) found that on refeeding rats with a transverse colostomy after fasting, crypt cell
proliferation was increased, not only in the proximal colon, but also in the excluded colon.

Dowling (1982) concluded that the available evidence neither negated nor destroyed the luminal nutrition hypothesis, but rather suggested that in some cases another mechanism may be operative. It seems that luminal nutrition may play a role in crypt cell proliferation control but it would probably be an oversimplification of a complex system of control to assume that this is the only, or even the major factor, in a probable multifactorial control.

For instance, Touloukian et al. (1972a) have shown that there are changes in the adrenergic innervation of the gut after resection, and Tutton and Helme (1974) have demonstrated that adrenergic stimuli effect changes in crypt cell activity. Thus, adrenergic or neurovascular factors may participate in mediating the response to intestinal resection. Young et al. (1977c) found that oral feeding was associated with increased sympathetic activity. Riber et al. (1971) found that intraluminal mechanical stimulation of the cat small intestine was associated with increased mucosal blood flow, so the differences in crypt cell proliferation in the presence or absence of luminal food may be at least in part due to local changes in mucosal circulation.
This raises the question of the relative importance of the physical properties of the luminal contents as opposed to their nutrient value.

Skagen (1977) found that infusion of hypertonic saline (450 m Osm./Kg.) into a catheter implanted into the upper jejunum of unrestrained rats after three days of starvation resulted in a sharp rise in the rate of incorporation of isotopically labelled thymidine into DNA in the crypts. The response was similar to that associated with peroral feeding. No changes occurred if isotonic sodium chloride or an isotonic amino acid/carbohydrate solution was used. He suggested that osmoregulatory mechanisms may be involved in evoking the proliferative response to refeeding in the small intestine.

Clarke (1976) found that starving a rat with an isolated small intestinal sac for 48 hours further diminished the crypt cell renewal rate in this loop, already diminished by its isolation. However, infusion of distilled water into the isolated small intestinal sac for 72 hours was associated with a similar degree of stimulation of cell production in the sac as in fed as in starved rats. It was suggested that the fall in crypt cell production rate in the isolated loop in a starving rat is not due to non-availability of the substances required for cell synthesis.
Furthermore, Clarke (1977) found that infusion of sodium chloride into a surgically prepared rat upper small intestinal sac was associated with a small increase in crypt cell production locally (measured by the stathmokinetic technique) but a considerable increase in crypt cell production in the uninfused gut in continuity.

These findings raise the question of whether the mucosa responds to the increased "work load" i.e. the amount of work necessary to deal with the particular stress applied to it or to the amount of work necessary, for instance, to absorb the nutrients in the lumen. This question will be dealt with again at another point in the literature review.

Dooley (1984) has demonstrated small intestinal osmoreceptors but their function is not certain.

If osmolarity of the intestinal contents is important in control of crypt cell proliferation and osmoreceptors are present in the small intestine, it is necessary to postulate that changes in osmolarity of the intestinal contents do in fact occur.

In this respect, Ganong (1979), amongst others, has stated that in normal man the post-prandial luminal contents entering the upper jejunum are iso-osmolar to plasma, irrespective of the osmolarity of the ingested meal. However, Ladas et al. (1983) found that despite mixing with upper gastrointestinal secretions
and transepithelial movement of fluid and electrolytes, osmotic and electrolytic equilibration of intestinal contents with plasma is not produced at this level for up to two hours after a meal. Thus, it is conceivable that the osmolarity of the intestinal contents could play a role in crypt cell proliferation control.

If this were possible, a possible link with the sympathetic system is conceivable, since Sjovall (1984) found that sympathetic splanchnic nerve stimulation led to an increase in net fluid absorption in the gut, provided that glucose was present in the jejunal lumen. However, this is all very conjectural.

The presence of luminal food and/or pancreato-biliary secretions may have other significance, however, in determining the growth and production of the villi. Rowinski and Kaminski (1973), and Rowinski et al. (1977) found that in implanted fragments of mouse small intestine located under the kidney capsule of syngenic mice there were no intestinal villi but crypts persisted and epithelial cells could proliferate up to 30 days after transplantation. These epithelial cysts were devoid of direct contact with the usual luminal contents. There was not the usual innervation and the blood circulation within the transplant was probably different from that in its usual site. It is perhaps significant that the duration of the
cell cycle in these transplants of adult intestine was found to be similar to that found by Zinzar et al. (1973) for three month old transplants of foetal mouse small intestine. Quite possibly, the luminal contents play a part in the development of the villi but this does not necessarily mean that they continue to be active in its regulation. As noted elsewhere in this review, in Coeliac disease it is possible to have small villi with active proliferation in the crypts.

(3) Parabiotic and cross circulation studies.

Loran and Carbone (1968) in parabiotic animals and Laplace (1974), Williamson et al., (1978 b.), in cross circulated animals found that small bowel resection in one of the animals (the "donor" animal) was associated with an adaptive proliferative response in the other animal to which it was connected (the recipient) in which there was an exchange of circulation but no bowel connections. The food intake of both animals apparently remained within normal limits. These studies suggested that a humoral agent passed from one animal to the other and was responsible for the proliferative response. Timeshevcich et al., (1984 a, b, c) found that lymphocytes from rats in which half of the small intestine had been resected acquired the ability to stimulate mitotic activity in the crypts of the small intestine, large
intestine, and oesophagus of syngenic recipients. This raises the possibility that lymphocytes rather than or together with humoral agents are cross circulated. However, further investigation is probably required to substantiate these claims. Although the experiments appear to have adequate controls, the numbers of rats are not known, nor the details of methods of determination of the mitotic index. Whilst the findings tend to give support to factors other than luminal nutrition, they do not exclude the action of luminal nutrition in crypt cell proliferation control.

(4) The effects of resection on excluded intestine.

Tilson and Wright (1970), Williamson and Malt (1982) (using rats), and Feldman et al. (1978) (using dogs) found that resection of the part of the small bowel containing nutrients (approximately ⅓) could diminish or prevent the hypoplasia in a bypassed small bowel loop, which was not in contact with nutrients or pancreato-biliary secretions. McDermott and Roudnew (1978) found that after 40% resection of small intestine villus size was increased in the loop isolated from nutrients, supporting the operation of a humoral factor rather than the effects of nutrients, but found only limited evidence for the influence of a systemic factor on crypt cell proliferation.
These findings support the operation of humoral agents as part of the general mechanism of crypt cell proliferation control but of themselves do not exclude the simultaneous operation of luminal factors.

(5) Onset of post-resectional hyperplasia before luminal nutrition has been restored post-operatively.

Hanson (1977 b ) found that adaptive hyperplasia in the residual intestine commenced before the animals had fully recovered their appetite, after resection of the small intestine. This is suggestive evidence but not very conclusive.

In considering the amounts of food eaten in the various experiments, it should be borne in mind that food in the intestinal lumen may have 2 general effects, (1) it may nourish the mucosa and in this way affect its efficiency or (2) it may pose an increased "work load" to the mucosa i.e. the mucosa has to expend energy to provide secretions and absorption of the nutrients and this presumably increases with the amount of food presented to it.

In an attempt to answer this question, Menge et al. (1975. a, b ), Clarke (1975, 1977) measured the crypt cell production rate or assessed the mucosal architecture in isolated loops of small intestine perfused from an abdominal skin surface access.
They found that, whilst the mucosal architecture was little affected by isotonic solutions of galactose or glucose, galactose, which is absorbed but not metabolized, produced a similar rise in crypt cell proliferation rate to glucose, which is absorbed and metabolized. They considered that these findings supported a "work load" hypothesis, rather than a "luminal nutrition" hypothesis as the basis for the stimulus to cell production provided by the presence of food in the intestinal lumen.

Dowling (1982) in a review of the topic, doubted the reliability of the result, suggesting that there could have been some distal reflux of luminal content and that the self-emptying loops were not completely so.

Evidence supporting the importance of pancreato-biliary secretions in the mechanisms of control of crypt cell proliferation.

The presence of food in the intestinal lumen is associated with the release of pancreatic and biliary secretions and it is therefore difficult to determine to what extent the effects of food in the lumen are due to (1) the local nourishing effect of the food, (2) the "work load" imposed on the mucosa or (3) the effects of the associated pancreato-biliary secretions.

Gelinas et al. (1977) suggested, in a study on small bowel resection, that there was an association between
proximal resection with transient pancreatic hypersecretion.

Stock-Damgé et al. (1982) concluded that the pancreatic hyperplasia induced by extensive small bowel resection is independent of the level of endogenous gastrin in the plasma (see later in literature review) suggesting that endogenous gastrin is not a trophic factor for the exocrine pancreas.

Altmann (1971), Altmann and Leblond (1970) found that whilst diverting the bile duct into a self-emptying ileal loop increased villus height, diversion of the pancreatic duct induced an even larger increment in villus height.

Altmann (1974) also found that infusion of pancreatic extracts into the ileum also increased villus height.

These authors thus suggested that the pancreatic secretions are trophic to the small intestinal mucosa and that the proximo-distal gradient in villus size and small bowel mucosal mass may be due to the proximal position of the pancreatic secretions.

Derubertis et al. (1984) found that bile salts stimulate colonic epithelial proliferation.

The potentiating effect of pancreato-biliary secretions on intestinal adaptation.

Jacobs and Dowling (1975) found that diverting the pancreato-biliary secretions to the mid-point of the small intestine augmented the previously mentioned
ileal adaptive response to cold induced hyperphagia 
(Jacobs et al., 1975 a).

Effects of pancreatobiliary secretions on adaptation 
after small bowel resection.

Weser et al. (1977), Williamson et al. (1978 a) found 
that if the pancreato-biliary secretions were diverted 
to the mid-point of the small bowel remnant after 
resection of the jejunum, the degree of ileal hyper-
plasia was increased above the level expected after 
resection.

Shellito et al. (1978) found that their results sugges-
ted that some adaptive hyperplasia to resection of 
jejunum can occur after pancreato-biliary exclusion 
but the hyperplastic response is potentiated by the 
presence of pancreato-biliary secretions.

Martinez et al. (1984) found that, whilst bile salt 
absorption from the remaining gut was diminished 
seven days after a massive small bowel resection, 
this was restored to normal by 30 days post operativ-
ely. Perry (1975) similarly showed that after re-
section of the distal small bowel in the rat, a 20% 
compensatory increase in bile absorption (bile acids) 
by the jejunum occurred which helped to maintain the 
bile salt pool. It is possible then that there is 
a relatively larger amount of bile acids within the 
intestinal lumen after resection of the small bowel,
assuming the rate of secretion of the bile acids has not been diminished by the resection, so that these substances could possibly influence the result of bowel resection, especially during the early stages after resection, but probably not at a later stage of adaptation.

The effects of increasing pancreateo-biliary secretions during total parenteral feeding.

Hughes et al. (1980 a) found that during total parenteral feeding the pancreas also becomes hypoplastic. To attempt to distinguish between the effects of luminal nutrition and pancreateo-biliary secretions on the intestinal crypts Hughes et al. (1978, 1982) produced daily stimulation of pancreatic secretion by administration of cholecystokinin and secretin and in rats subjected to total parenteral nutrition they noted that the villus hypoplasia which usually results was abolished. However, in later experiments they showed that the source of the hormone preparation was important.

Hughes et al. (1979) examined the effect of infusion of non-boiled and boiled (to exclude heat-labile trophic factors) pre-harvested pancreatic juice into the small intestine, and found that both boiled, and especially non-boiled, pancreatic juice increased mucosal mass in self-emptying jejunal loops.
However, as pointed out by Wright and Alison (1984) the effects of this infusion are difficult to distinguish from those of the resultant, perhaps unusually large, amino acid load presented to the mucosa under these experimental conditions, due to the action of the pancreatic juice on the small intestinal content, and certainly Altmann (1974) conceded that infusion of an amino acid mixture into the bowel reproduced part of the villus height increase evoked by pancreatic extracts. It is also known that infusion of various amino acids can affect the villus height or small intestinal DNA content (Menge et al., 1975b; Weser et al. (1982); Morin et al. (1982)).

The effects of bile on crypt cell proliferation

Fry and Staffeldt (1964), Roy et al. (1975) found that the presence of unconjugated bile acids in the lumen of the small intestine (without pancreatic secretion) stimulated crypt cell proliferation, the effect occurring primarily in the ileum. Conversely, removal of bile from the lumen (by bile duct fistulation) decreased crypt cell renewal in the ileum, without any effect on the proximal jejunum (Roy et al., 1975), and it was found that decreased crypt cell depth and cell migration were restored by an infusion into the lumen of sodium taurocholate. Urban and Weser (1980) therefore suggested that jejunal and ileal mucosal growth may be regulated by somewhat different mechanisms.
Williamson et al. (1978a) found that high luminal concentrations of bile caused transient (48 hours to one week) cell proliferation in the ileal mucosa but the additional presence of pancreatic juice prolonged this adaptive response. Whereas the previously mentioned experiment was designed to expose the ileal mucosa to a more physiological concentration of bile than the infusion of sodium taurochololate for instance, it should be remembered that the ileal mucosa is not usually exposed to this level of concentration of bile so it is difficult to determine from these experiments how important the role of bile in the control of crypt cell proliferation is.

Ecknauer and Bohmer (1977) allowing for the effects of semistarvation (Al-Dewachi et al. (1975b) and Altmann (1972)), and restraint (Tutton, 1975a), which affect crypt cell proliferation rate, found that whilst external fistulation of the bile duct in rats caused a decrease in cell renewal in the crypts but no change in the morphology of jejunum and ileum, bile duct ligation resulted in smaller villi but no change in cell renewal as compared with two control groups. They suggested that the difference might be explained by the increased bile acid concentration in the blood associated with bile duct ligation.

On the other hand, Deschner and Raicht (1979) found that on bile duct fistulation colonic crypt
cell proliferation was reduced but alterations in crypt cell proliferation became less marked in specimens obtained more proximally, approaching the small bowel, suggesting that perhaps bile is relatively more important in the control of colonic crypt cell proliferation.

Fry et al. (1968) found no change in crypt cell proliferation rate in the small intestine of rats with external fistulation of bile (and probably pancreatic secretions) for 4 days.

In contrast, Raich et al. (1975) found increased crypt cell turnover two days after biliary fistulation.

However, bile duct ligation or fistulation may not be completely separate from the effects of associated changes in pancreatic secretion. For instance, Geratz and Lamb (1974) found that ligation of the proximal common bile duct in rats caused a diminution in secretion of pancreatic amylase, which was corrected by placing a drain proximal to the ligature, whereas there was a rise in amylase output when secretions were collected from a distal bile fistula.

Wright and Alison (1984) in a review of the topic, commented that the experiments of Altmann et al. (see above) did not include measurements of crypt cell proliferation but rather stressed changes in villus size. Clarke (1970a) noted that crypt cell production rate was constant throughout the small intestine, despite local variations in pancreato-biliary secretions, which did not support
the concept that pancreato-biliary secretions act directly in the control of crypt cell proliferation. Williamson et al. (1978a) commented that since infusion of fresh hog bile into isolated ileal loops in conscious rats did not increase villus size, perhaps the presence of food is needed as well as bile for the pancreato-biliary secretions to have their effect. Thus, it becomes difficult to separate the two effects e.g. in the effects of starvation, so it seems likely that these factors act together in some way.

Therefore, the case for a role for pancreatic and biliary secretions in the control of crypt cell proliferation is far from being clear cut and whilst some role in the general control seems likely, their effects cannot be completely separated from those of other factors and control does not appear to depend greatly on these secretions alone.

Evidence not supporting the importance of pancreato-biliary secretions in the control of crypt cell proliferation.

Jacobs and Dowling (1975) found that in orally fed rats, maintained at low temperatures, or normal temperatures (22°C, Miazza et al., 1982) or in totally parenterally fed rats, maintained at room temperature (Miazza et al., 1982), diversion of pancreato-biliary secretions away from the jejunum accentuated rather than abolished the proximal to distal gradient in villus heights, mucosal mass and
absorptive surface, and produced hyperplasia rather than hypoplasia of the jejunum, suggesting that the diversion procedure removed a tentative anti-trophic influence of pancreato-biliary secretions from the jejunum with a rebound increase in jejunal mucosal growth.

In the group of animals totally parenterally fed the intestinal adaptive response still occurred, despite the lack of food or pancreato-biliary secretions in the intestinal lumen, suggesting the operation of humoral factors.

Weser et al. (1977) also found that the absence of bile and pancreatic secretions from the jejunum resulted in an increase in jejunal mucosal mass in male rats.

These effects may in part be due to the damaging effect of the pancreato-biliary secretions on the villus cells under normal circumstances.

Fenyö (1977) also found that small bowel bypass stimulated the growth of functional jejunileal remnants even in the absence of duodenal, pancreatic and biliary secretions.

Similarly, Tilson et al. (1975) found that ileal hyperplasia occurs after oesophagoileostomy with diversion of gastroduodenal and pancreatobiliary secretions into the caecum.

Whilst these experiments suggest that these secretions are not essential to the proliferative response in
some cases, it does not completely exclude their
effect in other situations or their contribution to
total control mechanisms but it suggests that their
role is possibly not a major one, but probably
related to the effects of luminal nutrition.

Evidence supporting the operation of hormonal factors
in the control of crypt cell proliferation.

Hormonal factors have been implicated in the possible
role of luminal nutrition and, as Wright and Alison
(1984) comment, whilst there is considerable evidence
for their role in adaptive growth processes e.g.
after intestinal resection, this does not clarify
their role in the everyday control of crypt cell
proliferation where they may form part of a multi-
factorial mechanism.

Young (1985) observed that it must be borne in
mind that many hormones have multiple forms. However,
not all forms have been included in the experiments
which will be noted in this review. Also, whilst
the levels of hormones may be measured, the ability
to establish direct cause and effect relationships can
be difficult, and the action of the hormones may
depend on the characteristics of the hormone receptors
on the target cells.
Hormones may act systemically, or locally in a paracrine manner, on the intestinal cells.

Experiments supporting the systemic operation of
hormones in control of crypt cell proliferation.
Loran and Carbone (1968) in parabiotic animals, and Laplace (1974) and Williamson et al., (1978 b) in cross circulated animals i.e. animals connected by their circulation only and having no communication between their gastrointestinal tracts, found that a partial small bowel resection in one partner was matched by a proliferative response in the small intestine of the other partner, suggesting the cross-circulation of a blood borne factor.

Wright and Alison (1984) in a review of the topic, have commented that the measurement of cell proliferation in the crypts in these experiments, is in their opinion suboptimal, and this seems a justifiable criticism in at least several of these experiments on examining the methods of assessment.

Whereas Williamson and Bauer (1978) in the rat and Feldman et al., (1976), in the dog, respectively, found that partial resection of the small bowel in continuity reduced the mucosal atrophy in a segment of small intestine excluded from food and pancreatico-biliary secretions (confirmed by Al-Mukhtar et al., 1982. b.). Clarke (1975a) found that starvation of such animals increased the mucosal atrophy in the excluded segment of small bowel, although the loop was shown capable of responding by proliferative response to a suitable stimulus.

Williamson et al., (1978 d) showed that distal adaptation to jejunal bypass is slower to develop than the response to an equivalent small bowel
resection. They postulated an inhibitory hormone elaborated by the intestine in contact with the nutrients, the circulating levels of which would fall sharply after resection but more gradually after jejunal bypass.

Hanson et al. (1977 c) noted a marked proliferative response in isolated small intestinal loops after partial resection of the small intestine in continuity. They suggested that when a large small bowel resection was done, blood flow alterations would not only occur in the residual functional portion but also in the isolated intestinal loop, possibly causing the resulting changes in crypt cell turnover. They further suggested that this mechanism could allow gross adjustments to various changes in the luminal environment allowing the feedback mechanism of the villus to be a fine tuning and local control mechanism. Thus, they suggested a neurovascular explanation rather than a hormonal explanation of the findings.

However, Lundgren (1974) stated that neither the lowering of perfusion pressure to the small intestine nor the activation of the sympathetic vasoconstrictor fibres induce any significant decrease in villus blood flow i.e. villus blood flow remains almost constant when reducing perfusion pressure. Villus tip blood flow is slowed, however.

Thus, a neurovascular explanation for the findings does not appear to be adequate and the results are consistent with the action of hormonal agents.
Giving further support to the hormonal theory is the finding of Tilson and Livstone (1975) who observed a prominent proliferative response in ileal fragments beneath the kidney capsule after partial resection of the small intestine in continuity.

Al-Mukhtar et al. (1982), Wright et al. (1969 a), Woo and Nygaard (1978), Bucholtz et al. (1976) all found either an increase in crypt cell production rate or increased villus height, increased crypt depth or increased rate of cell migration in the ileum following subtotal colectomy.

Conversely, Sharp et al. (1983) found a significant proliferative response in the crypts of the descending colon after a greater than 50% resection of combined ileum and jejunum. These findings were supported by those of Obertop et al. (1977), Nundy et al. (1977), Tilson et al. (1976), Williamson et al. (1978, c).

It is also possible that the changes seen in this case might be related to changes in luminal nutrition as well as or instead of hormonal effects. However, the findings of Masesa and Forrester (1977) that the compensatory responses in the small and large intestine after resections of the colon cannot be closely linked to changes in food intake, and those of Dowling (1968, 1974) that increased food intake is not seen in rats after partial resection of the small intestine
do not give much support to the operation of luminal factors in these cases.
It was noted also that if the colon was bypassed to the same site as in subtotal colectomy instead of removing it, there was the same associated small intestinal changes and changes in food intake, suggesting that if the result was due to hormonal changes removal of the bowel was not required for the effect.

Winborn et al. (1974), Seelig et al. (1977) found hyperplasia of the gastric glands some months after resection of proximal or distal small intestine.

Wickbom et al. (1975) found that following massive small bowel resection in dogs there was an excellent correlation between increases in serum gastrin concentrations and heidenhain pouch gastric acid outputs. This was taken as suggesting that the hormone gastrin plays a role in the hypersecretion of gastric acid which follows massive small bowel resection.

As previously noted, jejunal hyperplasia occurs in animals following diversion of pancreato-biliary secretions, provided the animals are maintained on total parenteral nutrition (Dowling, 1982).

Bjerknes and Cheng (1981d) found a transient hyperplasia of endocrine cells of the gut after
Experiments supporting the local operation of hormones in the control of crypt cell proliferation.

Rijke et al. (1976) found that the proliferative mucosal response following local ischaemia of the mucosa was confined to the ischaemic areas.

Rijke et al. (1979 a) found that the proliferative activity in the transverse colon did not change after temporary ischaemia of the bypassed descending colon, suggesting that the response after ischaemia of the bypassed colon is a local one.

Clarke (1976) found an adaptive response in a "self-emptying" sac of small intestine emptying on to the bowel but not in a similar sac emptying on to the skin, after small bowel resection.

Wright and Alison (1984) noted that the compensatory hyperplasia following mucosal damage with gluten in coeliac disease was also a local phenomenon, being mainly confined to the jejunum. They also suggest that the local hypoplasia of isolated intestinal loops points to the likelihood of any hormonal mechanism which might be involved being a local one.

Experimental evidence suggesting that hormonal factors may not be of great importance in the control of
McDermott and Roudnew (1976b) found that in rats 40% jejunal resection did not influence the cell population kinetics of the crypts of the descending colon. An effect might have been expected in view of the previously mentioned experimental findings.

Kirschner and Osborne (1978) found no proliferative response in the non-resected partner of parabiotic rats after resection of one member of the pair. However, Wright and Alison (1984) have commented that the capillary cross circulation used in this experiment allowed insufficient transfer of blood, compared with the arterio-venous anastomoses used by some other workers (previously mentioned).

Sharp and Osborne (1981) found no proliferative response in the unirradiated partner of a rat subjected to temporary local irradiation of exposed jejunum and ileum but a proliferative response in the unirradiated jejunum and colon of the irradiated parabiotic partner, suggesting perhaps that the parabiotic union may have not been adequate to transmit a humoral factor which appears to be present in the irradiated partners' blood.

The findings of Clarke (1975, 1976) mentioned above have also been interpreted as evidence of the importance of local luminal rather than blood-borne factors.
In interpreting Clarkes' findings it should be borne in mind that both loops had an intact nerve and blood supply allowing for the possibility of neurovascular effects.

Considering the available evidence, there appears to be a likelihood that there is a humoral agent operative in the control of crypt cell proliferation but the extent of its action and the relative contribution which it makes to the overall mechanism of control are difficult to assess on the presently available data. It appears that there may be locally acting and systemically acting hormones or possibly the same hormone may act locally in one situation and systemically in another situation.

A further difficulty in postulating a hormonal mechanism is the occurrence of hypoplasia in bypassed loops of small intestine with the same blood supply as the remaining hyperplastic small intestine but as indicated above hormonal factors may form only part of the multifactorial control of crypt cell proliferation.

Possible mediators of hormonal action

(1) Experimental evidence for the action of gastrin in the control of crypt cell proliferation.

Mak and Chang (1976), and Crean et al. (1969) have shown that daily administration of synthetic
pentagastrin for 21 days produced parietal cell (gastric) hyperplasia in rats.

Johnson et al. (1969a, b), Chandler and Johnson (1972) found that pentagastrin stimulated protein and RNA synthesis in the stomach and upper duodenum.

Johnson and Chandler (1973), Johnson et al. (1975) found that antrectomy (removal of the gastric antrum), which is known to significantly reduce serum gastrin levels (Bloom and Polak, 1982) was followed by a reduction in the total DNA and RNA in the stomach and duodenum, which was reversed by the administration of pentagastrin.

Pansu et al. (1974), Bosshard et al. (1978), found a stimulating effect of pentagastrin on rat jejunal crypt cell renewal and an inhibiting effect of secretin on jejunal cell renewal.

Johnson et al. (1978) found that gastrin, when applied topically to the intestine, could increase the growth of the midgut, ileum and colon, suggesting that the effect was not mediated by absorption and subsequent recirculation of the hormone.

Johnson et al. (1975), Lichtenburger et al. (1976) showed that pentagastrin reversed the loss of mucosal mass which occurs after starvation.
Willems et al. (1972) have shown that gastrin administration increases the labelling index of the gastric fundic mucosal cells in the dog, and Willems et al. (1977) also claimed a significant correlation between the same labelling index and the serum gastrin level.

Johnson and Guthrie (1974) claimed that large doses of pentagastrin (synthetic gastrin) increased cell proliferation in the gastric, duodenal and ileal mucosae and stimulated proliferation in the stomach and duodenum of fasted rats. Whilst supporting the proliferative effect of gastrin on the gastric fundus and duodenum, Casteleyn et al. (1977) found that gastrin depressed proliferation in the antrum.

Kerr et al. (1968), and Carpentier et al. (1978) have suggested that the acid hypersecretion observed after bowel resection or exclusion was related to gastrin release and a lack of inhibition of gastric acid secretion. This of course is very indirect evidence for the involvement of gastrin in the compensatory reaction of the bowel to resection.

However, if gastrin is involved, its action may possibly be independent of the secretion of gastric acid. This view is supported by the findings of Deveney et al. (1983) who showed that after creating chronic endogenous hypergastrinaemia by implantation of the gastric antrum into the colon, the chronic endogenous hypergastrinaemia was associated with
pancreatic and colonic hypertrophy, independent of the level of gastric acid secretion. However, since the relatively crude method of assessing mucosal weight was used, this result is open to criticism.

Zelenkova and Gregor (1971) observed that at about the time of weaning when the intestinal mucosa becomes more like the mature adult type, there is a rise in antral gastrin concentration. Lichtenburger and Johnson (1974) found that pentagastrin administration to non-weaned rats resulted in a similar maturation of the intestinal mucosa. During weaning, solid food of various types enters the stomach, so it is possible that this may be the stimulus to increased production of gastrin and gastrin may play a role in the maturation of the intestinal mucosa. In fact, it is possible that this is the primary role of gastrin and it may not be as actively involved as other factors in crypt cell control in the adult.

(2) Experimental evidence not supporting the action of gastrin in the control of crypt cell proliferation.

Mayston et al. (1975) found that daily administration of subcutaneous pentagastrin for 15 days failed to produce any significant structural or functional changes in the rat small intestine.
Coutsoftides (1976), Coyle et al. (1976) found in humans that the serum gastrin levels did not change in jejuno-ileal bypass, although there was associated hyperplasia of the remaining bowel in continuity.

Moosa et al. (1976), Caussignac and Dupre (1978) found that after extensive small bowel resection there was no corresponding increase in gastrin secretion. This suggests that gastrin may not be involved in the hypertrophy of the residual small intestine seen after this procedure.

Dworkin et al. (1976) found that the serum gastrin levels did not differ in animals fed orally or intravenously, with isolated small bowel loops, although the mucosal DNA content was greater in the isolated loops of animals fed orally i.e. there was a greater cell production rate in these.

Oscarson et al. (1977) showed that following various gastric procedures designed to produce widely differing endogenous gastrin levels, there was no correlation between serum gastrin levels and the degree of proliferative response to subsequent partial intestinal resection. There was no prevention of starvation induced mucosal DNA loss in animals with high circulating gastrin levels. Although these investigators suggested that gastrin did not have a major role in
the control of crypt cell proliferation, they did suggest that some of the previous effects attributed to gastrin might have been due to increased permeability of the mucosa to metabolic precursors and that this may be the role of gastrin in the mucosa.

Morin and Ling (1978) found that administration of pentagastrin resulted in stimulation of DNA content of the gastric mucosa and that of the proximal small intestine only. The same authors found that intravenous infusion of pentagastrin for 8 days in rats maintained by total parenteral nutrition after small bowel resection did not result in hyperplasia of the remaining bowel distal to the site of the resection. If rats were given the same nutrient solution intragastrically, hyperplasia of the small bowel was striking. Neither finding is in support of a central role for gastrin in control of crypt cell proliferation.

In the Zollinger–Ellison syndrome, the short bowel syndrome or Addisonian pernicious anaemia where there are elevated levels of gastrin, although gastric mucosal hyperplasia has been recorded (Straus et al. (1974); Isenberg et al. (1973)) small bowel hyperplasia has not been noted (Dowling (1982); Walsh and Grossman (1975)).

Thus, it appears that there is some evidence that gastrin is trophic for the gastric mucosa but does not appear to be trophic for the small bowel mucosa.
Fatemí et al. (1984) suggested that gastrin and pentagastrin may act as secretagogues on the small intestine and the effects on cell proliferation noted previously may be secondary to that action. They further concluded that gastrin at physiological levels and pentagastrin at pharmacological levels may stimulate crypt cell proliferation in the colon. In support of the secondary action of gastrin Mayston and Barrowman (1973) have shown that in the atrophic pancreas of hypophysectomized rats, pentagastrin causes cellular hypertrophy and hyperplasia.

Conversely, hypergastrinaemia, with increased gastric acid production, as seen after massive small bowel resection, has been found in dogs to be associated with poor adaptive hyperplasia in the small bowel. This effect was improved by the administration of histamine 2-receptor antagonists, which suppress gastric acid secretion (not via the vagi). This suggests that one of the secondary effects of gastrin secretion at least is not conducive to adaptation in the small bowel (see Young, 1985).

Other possible mediators of hormonal action,

(3) Experimental evidence for the action of cholecystokinin in the control of crypt cell proliferation.

This has been considered as a possible enterotrophic hormone because of its peptide structure being
closely related structurally to gastrin. Its effects range from stimulation of the gall bladder to contract and augmentation of the action of secretin on pancreatic bicarbonate secretion to inhibition of gastric emptying. So it appears that some at least of its effects may be secondary to other effects.

Secretin on the other hand, which is linked with it, stimulates pancreatic and biliary bicarbonate secretion and augments cholecystokinin stimulated pancreatic enzyme secretion.

Hughes et al. (1978) found that, whereas bioextracted cholecystokinin and secretin appeared to prevent the intestinal mucosal hypoplasia of total parenteral nutrition, the synthetic cholecystokinin octapeptide had no effect. Hughes et al. (1980b) also suggested that cholecystokinin increased tritiated thymidine incorporation into intestinal DNA i.e. had a stimulatory effect on the intestinal epithelium crypts.

Weser and Tawil (1978) found that intravenous infusion of cholecystokinin in jejunally resected rats produced a greater than expected small bowel mucosal growth but not as great as in those fed the same amount of nutrient intragastrically for the same period of time, although greater than in those maintained on total parenteral nutrition.

Dowling (1982) whilst noting marked effects on the pancreas, found that infusions of both low and high
dose cholecystokinin and pancreozymin had no effects on intestinal structure and function. He concluded that there was no evidence for a significant role for cholecystokinin in the small intestine as a growth regulator but commented that in many of the studies on the effect of this substance it was impossible to distinguish between the direct effect of cholecystokinin and/or secretin on the gut from the indirect effects of these substances through the action of the pancreato-biliary secretions, which in turn have been noted to effect crypt cell proliferation (see above). For instance, Barrowman and Mayston (1973), Rothman et al. (1967), Snook (1969) have all shown that administration of cholecystokinin in rats can produce pancreatic enlargement, but it is not known whether this substance acts in this manner under physiological circumstances. Melmed and Bouchier (1969) have suggested that cholecystokinin may be involved in the physiological regulation of the structure and function of the exocrine pancreas so that it may act indirectly on the small intestinal epithelium but in any case the effect is probably not a major one in the control of crypt cell proliferation.

(4) Experimental evidence for the action of enteroglucagon in the control of crypt cell proliferation.

This is a peptide hormone produced mainly by the endocrine cells of the mucosa of the lower ileum.
Gleeson et al. (1971) described marked villus hyperplasia and intestinal dilatation in the small bowel of a patient with an enteroglucagon secreting tumour of the right kidney. These effects disappeared after removal of the tumour as the high circulating levels of enteroglucagon returned to normal. Bloom (1972) demonstrated that this tumour contained gut derived (enteroglucagon or gut glucagon-like immunoreactivity) rather than pancreatic-derived glucagon.

Jacobs et al. (1976), Bloom et al. (1978), Gregor et al. (1980), Al-Mukhtar et al. (1982a), Besterman et al. (1982) all found that the plasma levels of enteroglucagon rose after small bowel resection. Gornacz et al. (1984) also noted that the rise in enteroglucagon levels was sufficiently rapid and prolonged to be involved throughout the adaptive process, thus making it more likely to be involved than some of the other hormones considered. The same authors also noted that if resection was combined with the exclusion of loops of small bowel from nutrients, the circulating enteroglucagon and cholecystokinin levels closely matched enterocyte production, even when luminal influences were excluded.

However, Besterman et al. (1982) found that during colonic resection enteroglucagon levels were below the control values, raising the question of the significance of this hormone in crypt cell proliferation control in the colon.
These findings were supported by those of Kennedy et al. (1982), and Bloom and Polak (1982) speculated that the reason for this discrepancy may have been that after resection a major source of enteroglucagon may have been removed, and certainly the results of Kennedys' experiments were after total resection of the colon.

Frame (1977) reported high levels of enteroglucagon in patients after jejuno-ileal bypass. Since the initial weight loss noted in patients in which this operation is used as a therapeutic measure in the treatment of obesity is thought to be due to decreased intake of food, Bloom and Polak (1982) have suggested that the raised enteroglucagon levels may be responsible for the mucosal hypertrophy. This in turn would increase absorption of nutrients and tend to eventually negate the effects of the operation on weight loss.

These authors and Sarson et al. (1981) have found similar levels of enteroglucagon in patients in whom the upper small intestine containing the biliary and pancreatic juice is separated from the nutrient stream and anastomosed to the terminal ileum, the stomach being rejoined to the upper ileum leaving only a small segment of the bowel for digestion. Whilst the hormone levels tend to fall with time, the tendency to regain weight is much less.

Dowling (1982) also noted that the jejunal hyperplasia which follows pancreatobiliary diversion was also
accompanied by an increase in enteroglucagon levels in the blood (although this was noted to be temporary).

Besterman et al. (1978) found that where there was crypt cell hypertrophy but villus cell atrophy, as in coeliac disease, there was a high level of enteroglucagon also.

Elias and Dowling (1976) thought it probable that the intestinal hyperplasia associated with lactation, which was apparently not due to changes in food intake, was due to enteroglucagon and Jacobs et al. (1976,1982) found elevated levels of this hormone during lactation. They also found it elevated in cold-induced hyperplasia in rats (which was possibly related to increased food intake). Thus, it is not clear which of these factors is operative.

Bloom and Polak (1982), and Al-Mukhtar et al. (1982b) concluded from various animal experiments that there was a good correlation between elevation of enteroglucagon levels in the blood and the presence of mucosal hypertrophy in the gut (i.e. immunoreactive circulating enteroglucagon levels).

Bloom (1979) proposed that enteroglucagon might be trophic to the mucosa of the gut, and the findings of Uttenthal et al. (1982) that purified rodent enteroglucagon levels.
glucagon was one of the few substances which could stimulate nucleotide incorporation into DNA in cultured enterocytes lent some support to Blooms' suggestion.

Whilst this is one of the few direct connections between the levels of enteroglucagon and crypt cell proliferation, most of the evidence is indirect although very suggestive of the importance of this hormone. In fact, of the hormones so far considered it is probably the one most likely to be involved in crypt cell proliferation control.

Another piece of indirect evidence was provided by Goodlad et al. (1983) who found that during starvation plasma levels of gastrin and enteroglucagon fell, but whereas plasma gastrin rose slowly after refeeding, plasma enteroglucagon levels rose rapidly to levels significantly above controls and these levels could be correlated with crypt cell production at several sites along the bowel.

Possible mechanism of action of enteroglucagon.

Assuming that this hormone is important in the control of crypt cell proliferation, the mechanism by which it acts on the crypts has not yet been ascertained. It has been suggested that since luminal nutrients, especially fat, are known to release enteroglucagon into the circulation and luminal nutrients themselves appear to play a role in its control, this hormone
may provide the link between the effect of luminal nutrients and the crypts. For instance, after small bowel resection, there is a relatively increased amount of nutrient presented to the intestinal mucosa. This could result in an increase in the secretion of enteroglucagon with subsequent hyperplasia of the mucosa, although of course there may be other intermediary mechanisms involved e.g. neurovascular or neural mechanisms, in the chain of events leading to this.

However, the situation is apparently not as simple as this explanation would suggest. For instance, Dowling (1982) found that, whilst plasma entero-glucon levels were high soon after pancreateo-biliary diversion, they fell to normal subsequently although mucosal hyperplasia persisted. He suggested a "priming" role for enteroglucagon in intestinal adaptation, with the possibility that ornithine decarboxylase levels may act as the "trigger mechanism" to initiate adaptation.

This suggestion was supported by the findings of Luk et al. (1982) who have shown fluctuations in the activity of this enzyme which coincide with the onset and maintenance of the mucosal hyperplasia of lactation. Further support for the role of this enzyme in the adaptation following lactation was given by the findings of Yang et al. (1984). According to Luk and Baylin (1984), increased activity of ornithine decarboxylase was noted during the time after small bowel resection when there is initiation of
mucosal cell hyperplasia. They further claimed that by giving the specific irreversible inhibitor for this enzyme (α-difluoromethyl ornithine) orally prior to resection there was a suppression of this substance (as well as some other polyamines) and complete abolition of the intestinal adaptation, including crypt cell proliferation. They suggested that the increase in ornithine decarboxylase and other polyamine synthesis and activity are critical for the process of post-resectional crypt cell proliferation, at least in vivo, and that the critical step mediated by the polyamines in the adaptive process is the onset of new DNA synthesis.

Thus, it appears that whilst there seems to be a strong case for the operation of enteroglucagon in the control mechanism, its role is not clearly defined. Once again, such critical analysis reinforces the possible concept of the control of crypt cell proliferation being a multifactorial one.

From the clinical point of view, Bloom et al. (1979a), Bloom (1980) found that not only were enteroglucagon levels raised in small bowel resection and bypass but levels were raised in the "dumping syndrome" (Bloom et al., 1972) where rapid small bowel transit delivers a relative increase in luminal stimuli to the ileum, as well as levels of neurotensin (Blackburn et al., 1980). This syndrome is believed to result from osmotic changes in the
lumen of the gut with passive loss of fluid from the circulation into the intestinal lumen. In this case the hormonal changes do not appear to be concerned with crypt cell proliferation but may act together with the autonomic nervous system to produce the characteristic syndrome. In fact, the symptoms and the hormonal changes can be abolished by the administration of a viscous fibre (e.g. pectin). This raises the question of whether the stimulus to the secretion of these hormones is the nutrient content of the luminal contents or their physical characteristics. This point was raised when dealing with the effects of luminal nutrients and it is possible that the physical characteristics of the luminal contents may act as the stimulus to entero-glucagon production, which in turn "primes" the mucosa for the action of ornithine decarboxylase.

The possible role of pancreatic glucagon in the control of crypt cell proliferation.

Fatemì et al. (1980) found that injection of this substance increased mucosal growth throughout the gastrointestinal tract, whereas Rudo et al. (1976), Bell and Weser (1979) suggested that glucagon decreased mucosal mass and reduced villus cell migration rates. Rudo and Rosenberg (1973) suggested that this hormone might play a role in the intestinal adaptive changes of semi-starvation and experimental diabetes and Dowling (1982) suggested that these
Studies were probably unrelated to studies with entero-
glucagon.

(5) Experimental evidence for the action of somatostatin
in the control of crypt cell proliferation.

Somatostatin is found mainly in the D cells of the
gastric antrum and the pancreas, but also in the
duodenum and the jejunum. In the small intestine
somatostatin immunoreactive nerves are present close to
the epithelium (Keast et al., 1984) immediately
below the crypts and villi. It is also present
not only in the hypothalamus, but other areas of the
brain including the limbic system (Hökfelt et al.,
1978). Somatostatin has many actions including
inhibition of—,
(a) growth hormone,
(b) thyroid stimulating hormone,
(c) insulin,
(d) glucagon,
(e) pancreatic polypeptide,
(f) gastrin,
(g) secretin,
(h) gastric acid,
(i) gastric emptying,
(j) pancreatic bicarbonate and enzymes,
(k) gall bladder contraction,
(l) coeliac blood flow.
Because of its widespread distribution throughout the
body and its wide and powerful range of actions,
Polak (1978) has suggested that it probably acts mainly as a paracrine (local effect) hormone. It is also obvious that because of its widespread effects it may act via many indirect means on the crypts, and some of these factors have already been dealt with in this review and will not be repeated here. Arimura and Fishtack (1981) also supported the view that its actions are probably local in the gut because of its rapid inactivation and extremely low levels in the peripheral blood, as well as the factors mentioned above.

Lehy et al. (1979, 1980), Bosshard et al. (1980), Senegas-Balas et al. (1985) all found that somatostatin administration was associated with diminished mitotic activity in the gastric, jejunal or ileal mucosae.

It is of interest, because of the effects of diminished blood flow to the mucosa on crypt cell proliferation, that Pawlik et al. (1976), and Tyden et al. (1979) found that parenterally administered somatostatin, in dogs and humans, was associated with reduced mesenteric blood flow. This would be consistent with the reduced crypt cell mitotic rate, except that as previously demonstrated in this review, changes in mesenteric blood flow are not necessarily reflected in local changes in blood flow in the villi.
Since the question of the stimulus to crypt cell proliferation being the physical characteristics of the luminal contents has been raised in this review, it is of interest to note that Rouiller et al. (1979) found that installation of a nutrient containing hyperosmolar volume load into the lower ileum of dogs resulted in significantly higher levels of somatostatin—like immunoreactivity in the mesenteric vein. It is possible that the osmolarity of the intestinal contents may act via the local secretion of somatostatin and as will be seen in subsequent discussion, this may lead to activation of entero-glucagon. On the other hand it may be primarily concerned with the control of water and electrolytes absorption from the mucosa. Davis et al. (1980), Dharmsathaphorn et al. (1980 b), and Mitchenere et al. (1981) have proposed this substance as one of many possibly involved in transepithelial regulation of water and electrolyte transport.

Lehy et al. (1979) have suggested that somatostatin could suppress the growth hormone levels required for cell proliferation in the crypts. Whilst in rats it has been reported that growth hormone secretion can be abolished or reduced by longstanding somato-statin administration (Martin et al., 1974), in humans somatostatin appears to affect high growth hormone levels rather than basal normal physiological levels (Leblanc et al., 1975).
Wright and Alison (1984) recorded that somatostatin administration inhibited the rise in crypt cell production rate and a rise in circulating enteroglucagon following small bowel resection. This indicates that its action was linked with that of enteroglucagon.

Sagor et al. (1985) gave somatostatin to suppress enteroglucagon secretion and bombesin to stimulate enteroglucagon secretion in rats undergoing small bowel resection or transection. It was found that the changes in enteroglucagon levels corresponded closely with changes in crypt cell proliferation rate, suggesting once again that enteroglucagon is quite possibly the most favoured candidate for humorally mediated trophic influences on the small intestine. It seems possible, as remarked above, that somatostatin acts via the effects of enteroglucagon, forming part of a chain of effects in the control of crypt cell proliferation.

(6) Experimental evidence for the action of gastric inhibitory polypeptide in the control of crypt cell proliferation.

Pederson et al. (1982), Buchan et al. (1983) found after jejuno-ileal bypass in rats that there were high levels of circulating gastric inhibitory polypeptide, which they concluded were due to this substance being secreted by the blind loop. They also found a reduction in sensitivity of the β cells of
the pancreatic islets to this substance. However, they found that massive small bowel resection had little effect on the response of the $\beta$ cells to this substance. They suggested that the high basal levels of gastric inhibitory polypeptide were causally related to the decreased insulin response in the jejuno-ileal bypass rats. This experimental evidence of itself does not clearly indicate a role for this peptide in crypt cell proliferation control, and it seems more likely on this evidence that this substance might form another possible link in a chain of polypeptide action involved in this control.

(7) Experimental evidence for the action of epidermal growth factor in the control of crypt cell proliferation.

This substance has been isolated from the salivary and duodenal glands.

Johnson and Guthrie (1980), Dembrinski et al. (1982), and Al-Nafussi and Wright (1982) found that parenteral administration of this substance increased crypt cell production rates in several parts of the rat and mouse gastrointestinal tract.

Goodlad et al. (1984) found that intravenous but not intragastric urogastrone—epidermal growth factor was trophic to the intestine of parenterally fed rats.
Ford et al. (1982) found that early breast milk from rats who had given birth approximately 3 days earlier markedly improved the compensatory villus enlargement following 60% mid small bowel resection in neonatal rats, especially in the distal small bowel. This factor was thought not to be operative in adults as its effect became less with later breast milk. Young (1985) has commented that epidermal growth factor and epidermal growth factor-like factors can be found in the maternal milk and may be important in the maturation of the intestine. It is possible that the factor described by Ford et al. (1982) is in fact epidermal growth factor.

Hay and Brown (1985) demonstrated functional epidermal growth factor receptors in cells of a rat epithelial cell line in culture, suggesting a role for epidermal growth factor in the regulation of intestinal epithelial cell physiology. It seems possible, therefore, that this factor is operative in the control of crypt cell proliferation during neonatal life and it seems reasonable to imagine that the same factors may not be involved in this process throughout the life of an individual.

Clarke (1976) has suggested that adrenaline or noradrenaline may act as systemic factors in crypt cell control and certainly there is evidence that the central nervous system plays some role in control of crypt cell proliferation (see below).
It seems unlikely that any of the hormones so far mentioned could, alone, account for the compensatory changes in the epithelium after small bowel resection.

The importance of local factors cannot be disregarded and in fact probably form part of a composite mechanism involving local factors, hormones and possibly neural or neurovascular factors, which are interdependent.

With regard to another local factor, the basement membrane of the mucosal epithelium, Sharp et al. (1980) have commented that since the response to small bowel resection involves the entire small bowel and its magnitude is proportional to the amount of tissue removed, thus compensating for the absorptive defect, they could speculate that the basement membrane underlying the epithelium may be important. However, there is no experimental evidence to support this contention.

As discussed above, the action of somatostatin may involve the effects of growth hormone and it was noted that substances such as gastric inhibitory polypeptide influenced the secretion of the islets of the pancreas as well as the intestinal epithelium. It is therefore possible that the above mentioned hormones may act partly at least via the pituitary and its hormones.

(8) Experimental evidence concerning the possible action
of the pituitary and its hormones in the control of crypt cell proliferation.

Taylor et al. (1979) in a study on the effects of bowel resection in rats with associated hypophysectomy, using the changes in net weight of the mucosa as a measure of hyperplasia (not a very reliable method), found that in hypophysectomized rats subjected to jejunal resection the distal ileum retained the ability to hypertrophy, but not to the extent seen in pair-fed controls. Similar results were obtained using the ileum. These findings were interpreted as suggesting that the pituitary gland plays an indirect role in the mucosal response to gut resection. Hypophysectomy was followed by reduced food intake, which was followed by small intestinal mucosal hypoplasia. However, the hypoplasia was not totally explicable on the basis of reduced food intake because the adaptation to gut resection occurring in hypophysectomized rats was less than in the pair-fed controls.

Riecken et al. (1974) demonstrated a reduction in crypt depth in hypophysectomized rats compared with pair-fed control animals; the difference was abolished when the hypophysectomized rats were given thyroid and growth hormone replacement therapy.

Sharp et al. (1980) found that hypophysectomy reduced mitotic activity in the rat intestinal crypts. The length and weight of the intestine
as well as the number of crypts was also significantly reduced. They concluded that an intact pituitary was required for the process of crypt replication which leads to intestinal growth.

Bastie et al. (1982) also found mucosal atrophy after hypophysectomy, and like Taylor et al. (1979), and Al-Dewachi et al. (1975 b) concluded that these changes were not entirely due to changes in food intake.

Young (1985) in a review of the topic, claimed that normal pituitary function is critical for the maturation of the intestine at the time of weaning of the suckling animals and that this maturation can be brought on precociously by the injection of thyroxine or corticosteroids.

If the hypophysis is important in crypt cell proliferation, we may speculate that it may act via the pituitary hormones or other hormones over which it has influence.

In this respect, Tiscornia and Dreiling (1966) found that hypophysectomy caused exocrine pancreatic atrophy in the dog and Altmann (1971) found that exocrine pancreatic secretions were trophic to the intestinal epithelium, although as discussed above this is by no means established. Similarly, Crean (1963), Schapiro et al. (1970) found atrophic changes in the stomach, pancreas and submaxillary glands as
well as the small intestine, so it is possible that the associated effects of hypophysectomy may influence crypt cell proliferation, but considering the often not clear cut effects of these associated defects, as indicated in the previous discussion, the reason for the actions of the pituitary should probably be sought in the effects of its hormones.

In relation to this problem, Sharp et al. (1980) found that none of the hormones studied, viz.-

(a) gastrin,
(b) pentagastrin,
(c) isoprenaline,
(d) steroid hormones,

appeared to play a major role in the compensatory responses of the intestine to X-irradiation and resection in adult rats.

Considering the hormones produced by the pituitary gland and their known individual effects on crypt cell proliferation.

Experiments regarding the action of prolactin in the control of crypt cell proliferation.

Campbell and Fell (1964) attributed the considerable hyperplasia of the intestinal epithelium seen in lactating rats to the increased amount of food, or some component thereof, passing through the gut as daily food consumption increases by up to three-fold in lactating mice.
They attributed the observed increase to the brief hyperphagic period when the rats were fed once daily since the effect was abolished by feeding the same amount of food in three portions during the course of the day.

However, Harding and Cairnie (1975) using lactating mice found that their experimental results did not support the primacy of food in the intestinal response to lactation, as a very large response was obtained in mice on a restricted diet. They concluded that food consumption, the hormonal status of the mother, and the metabolic demands of lactation all play a role in the complex control of cell proliferation in the small intestine of the lactating mouse.

In support of these findings, Elias and Dowling (1976) found that the hyperplasia which accompanies lactation occurred even in isolated bowel loops. Prolactin was thus proposed as the mediator of these effects. Sharp et al. (1980) found, on administering prolactin to three month old female rats, that there was increased DNA-specific activity per crypt in the small intestine, especially in the ileum, although the effect was not a great one.

However, Muller and Dowling (1981) found that induced hyperprolactinaemia, produced by perphenazine administration and pituitary transplantation beneath the
kidney capsule did not affect intestinal morphology.

Prolactin secretion may therefore not be the only reason for the hyperplasia of lactation but it may play some part, if only as an intermediary hormone. For instance, Jacobs et al. (1976) found that enteroglucagon levels are raised in lactation and it may act in conjunction with this or other hormones whose outputs are increased during lactation.

The pituitary gland may affect the levels of thyroid hormones, which are important in metabolism, via the action of the thyroid stimulating hormone, so this is another possible pathway of action from the pituitary gland to the intestinal mucosa.

Carriere (1966) found a decrease in the weight and mucosal thickness of the jejunum after thyroidectomy. Leblond and Carriere (1955) found administration of thyroxine stimulated intestinal epithelial proliferation.

Sharp et al. (1980) found that thyroidectomy in suckling rats resulted in decreased incorporation of tritiated thymidine by the gastrointestinal tract i.e. decreased mitotic activity in the mucosa.

This evidence for the action of thyroid hormones in crypt cell proliferation is indirect and rather vague so that the role of this hormone is not clearly defined.
The levels of testosterone and oestrogen may be changed indirectly by the action of the pituitary, and since for instance the mitotic activity in the intestinal epithelium has been shown by Bullough (1946) to vary with various phases of the oestrous cycle, the effects of these hormones on crypt cell proliferation were examined.

Wright et al. (1972), Tutton (1982) have shown that jejunal crypt cell proliferation was promoted by androgenic hormones. These results were supported by those of Carriere (1965), Tuohimaa and Niemi (1968). It has been suggested that these effects may be due to general metabolic or nutritional effects of the anabolic hormones rather than a direct effect on the crypt cells. However, Tutton (1982) noted that neither castration nor androgen administration influenced colonic crypt cell proliferation.

It is known that the intestinal epithelium is not a target tissue for oestrogens (Jensen and De Sombre (1973); Pietras and Szego (1977)). Despite this, Bullough (1946) showed that epithelial cell mitotic activity in the small and large intestine varied with various phases of the oestrous cycle, primarily due to the stimulatory effect of oestrogens on mitosis. Chang and Hoff (1980) showed that oestrogens exerted an inhibitory effect on mouse colon epithelial proliferation. Agrez and Spencer (1982) were of the opinion that the little available evidence was not conclusive.
The role of the sex hormones would thus appear to be a relatively minor one and the effects are difficult to separate from the secondary effects of these hormones.

The pituitary gland may act on the adrenal cortex by means of the adrenocortico-trophic hormone (ACTH), increasing the amount of circulating adrenal cortical hormones so that it may act on the crypts indirectly by means of these hormones.

In support of this contention, Wall and Peters (1971) found that prednisolone-21-phosphate, a synthetic hormone with both glucocorticoid and mineralocorticoid activity, could produce small intestinal crypt hypoplasia.

Tutton (1973b) found that a single injection of prednisolone tertiary butyl acetate stimulated crypt cell proliferation on the 7th day after administration.

Wright et al. (1978) showed that a single injection of prednisolone tertiary butyl acetate depressed the crypt cell proliferation rate in the rat small intestine. Whilst recovery from the single injection occurred over 7 days, if multiple daily injections were given the crypt cell proliferation remained depressed.

The evidence presented is indirect and insufficient to definitely implicate adrenal cortical steroids in the
day to day regulation of crypt cell proliferation, although they may be involved. However, it is known that in stressed animals there is increased output of adrenal cortical steroids, so that it is likely that in those situations there is an effect on the crypts from these steroids.

In the various experiments concerning intestinal resection or bypass, little mention is made by the investigators of the effect of the changing bacterial flora of the bowel lumen on crypt cell proliferation itself. Obviously, the luminal bacterial flora is changed by excluding nutrients or pancreato-biliary secretions from the lumen, which may also have the effect of changing the pH of the luminal contents. Furthermore, there are differences between the bacterial flora of the small intestine and the colon.

Experimental evidence concerning the effect of the intestinal bacterial flora on crypt cell proliferation.

Matsuzawa and Wilson (1965), Guenet et al. (1970), Lesher et al. (1964), Abrams et al. (1963) studying germ-free mice and rats, found that the transit time of the small intestinal epithelial cells from the bottom of the crypts to the top of the villi was delayed (especially in rats). There was decreased mitotic activity in the crypts with a slower intestinal mucosal renewal rate in animals raised in a germ-free environment.
It is possible that the luminal bacterial flora produce some damage to the surface enterocytes of the villi even under normal conditions, and the resulting increased villus cell loss stimulates the crypts to increase production of cells at the rate seen in conventional animals. If the stimulating effect of the bacterial flora is lacking, as in germ-free animals, the crypt cell mitotic rate would be lower.

In support of this concept of the role of the luminal bacterial flora, Symons (1965) found elevated crypt cell proliferative rates accompanying infective enteritis (although there may be other factors operative in this case besides the change in bacterial flora).

Williamson (1978a, b) suggested that changing luminal flora could account for the ileal hyperplasia which follows subtotal colectomy, although Bucholtz et al. (1976) found that ileocaecostomy, which changes the flora of the ileum to a more active colonic type, does not promote ileal crypt hyperplasia.

The role of the bacterial luminal flora is not a straightforward one. E.g. Khoury et al. (1969) found that administration of antibiotics, such as neomycin or penicillin to mice produced mucosal changes which were the opposite.
to those observed in germ-free animals (not similar, as expected). He concluded that the effect was probably the result of direct action of the antibiotics on the mucosa.

Altmann (1974) found that instillation of bacterial suspensions into isolated intestinal loops was without obvious effect on the mucosa.

Ranken et al. (1971) suggested that changes in the level of free cholic acid in the intestinal lumen might be responsible for the observed changes but this was not supported by the findings of Meslin et al. (1974).

Interestingly, Sacquet et al. (1971) found a large increase in bacterial flora related to the creation of a blind small intestinal loop. This was supported by the findings of Nygaard (1967). Sacquet et al. (1971) also noted an acceleration of cell migration rate at all levels of the intestine in conventional rats fitted with blind loops but that this phenomenon was not observed in germ-free rats fitted with blind loops.

Thus, it is possible that the bacterial flora do play some part in the general mucosal reaction to small bowel bypass, although nutritional factors may also be involved.
Nygaard et al. (1967) also noted modification of the small bowel luminal flora after small bowel resection, suggesting that this might have a role in the response to small bowel resection.

Saquet et al. (1971, 1972) noted that the response to changes in the luminal bacterial flora induced by caecectomy in germ-free rats was not uniform at various levels of the small intestine. It had been noted that modification of the luminal flora produced mucosal effects which were not identical in different animal species.

The importance of luminal bacterial flora in the control of crypt cell proliferation is difficult to ascertain because of the number of variables concerned, especially the individual variation in flora and the ever changing characteristics of the flora in a particular animal.

It seems probable that the luminal bacterial flora does not play a major role in the mechanism of control of crypt cell proliferation.

The proliferative changes in the crypts associated with small bowel obstruction illustrate some aspects of crypt cell proliferation control.

Williams et al. (1968) found increased crypt cell proliferation after partial small bowel obstruction.
in the rat, whereas Kamei et al. (1973) found reduced crypt cell proliferation.

Ecknauer et al. (1977), using gnotobiotic rats, found a considerable increase in crypt cell renewal in the small intestine proximal to a complete mechanical obstruction after 72 hours, but no obvious changes in crypt cell renewal distal to the obstruction. They suggested that possibly:

1. A local chalone-like substance may be involved because of the lack of changes distal to the site of the obstruction.

2. Bacterial overgrowth may have occurred proximal to the obstruction due to stasis of intestinal contents, which is known to be associated with bacterial overgrowth of the luminal contents (Bloch (1975); Gracey et al. (1972); Tabaqchali and Booth (1970)).

3. This in turn is followed by extensive degradation of bile salts in the lumen and some of these degradation products produce cell damage (Gracey et al. (1973, 1975); Shiner (1969); Gianella et al. (1974)), or are able to influence crypt cell turnover in the small intestinal epithelium (Ranken et al., 1971).

Besides these factors, there are known to be vascular and fluid exchange disturbances proximal to a bowel obstruction and these changes may also influence crypt cell proliferation rate.

Burnham (1983) also found limitation of the hyper-proliferative response to the area immediately proximal
to an obstruction of the rat ascending colon, suggesting once again that the proliferative stimulus in colonic obstruction is a local one. It may be a local hormonal or a local neurovascular effect. It seems possible that all of the range of possible mechanisms of crypt cell proliferation control might not be used in any particular situation but rather a selection of mechanisms might be made for use in a particular situation.

So far, I have dealt with the proliferation of the crypt cells without considering the possible role of the underlying supporting mesenchymal tissue. Pascal et al. (1968), and Kaye et al. (1968) noted its intimate relationship to the epithelium. David (1972) noted that cultured or grafted rabbit gastric epithelium could not develop when deprived completely of the supporting mesenchyme, suggesting that the mesenchyme may be vitally important during organogenesis of foetal gut tissues.

Pascal et al. (1968), Parker et al. (1974), Marsh and Trier (1974 a,b ) found in adults that the normal crypts of both small and large intestinal mucosa are surrounded by a sheath of fibroblasts which undergo proliferation and migration to the upper parts of the crypts in synchrony with epithelial cell migration.

Pascal et al. (1968), Marsh and Trier (1974 a,b ), also found that in the small bowel the fibroblasts continue to migrate to the villus tips but it is
not known whether these fibroblasts are ultimately sloughed into the lumen or are "re-cycled" to the deeper layers of the lamina propria.

**Experimental evidence for the operation of neural factors in the control of crypt cell proliferation.**

Neural control of crypt cell proliferation presupposes the presence of nerve fibres located in such a way that they can act directly on the crypts, or related to blood vessels in close proximity to the crypts, so that they may act via changes in blood flow locally or via secretion into the blood vessels.

**Experimental evidence for suitably located nerve fibres**

Although early investigators such as Bizzozero (1893) disputed the presence of such suitably located nerve fibres, Dogiel (1895), and Cajal (1911) found a rich nerve supply to the mucous membrane of the intestine.

More recently, Dahlstrom et al. (1984) demonstrated cells with neuron-like properties immediately beneath the basal lamina of the rat ileal mucosa.

Palay and Karlin (1959) found many unmyelinated nerves associated with blood vessels in the lamina propria of the rat jejunum. Lane and Rhodin (1964) found that many bundles of axons arising in the submucosal plexuses passed into the villi.
Similar nerve fibres were noted to be related to arterioles and venules in the mouse colonic mucous membrane by Silva et al. (1968) the adrenergic innervation being sparse.

Similarly, both noradrenergic and cholinergic nerve fibres have been found immediately subjacent to the basement membrane of the mucosa throughout the intestine of —,

(1) cat and monkey, Jacobowitz (1965),
(2) guinea pig, Gabella and Costa (1968a),
(3) man, Baumgarten (1967),
(4) rabbit and rat, Costa and Gabella (1971).

The function of these plexuses cannot be directly related to the presence of neural control but their presence is necessary before neural factors can be considered a possibility.

The concept of neural control of cell proliferation has been supported experimentally in other tissues e.g. Byron (1975) found that stimulation of proliferation of haemopoietic stem cells of the bone marrow which receive an autonomic innervation can be produced by a cholinergic mechanism. Muir et al. (1975) showed that increased proliferation of the acinar cells of the rat parotid could be produced by stimulation of the sympathetic nerve supply of the gland.
The effects of denervation of the intestine on crypt cell proliferation.

(a) Sympathectomy.
Dupont et al. (1965) demonstrated that immunosympathectomy (i.e. generalized effect rather than localized effect) leads to increased crypt to villus migration time, whilst Musso et al. (1975) found that surgical preganglionic sympathectomy produced only a transient increase in crypt to villus transit time.

Iachat and Goncalves (1978) found that division of the abdominal splanchnic nerves (a more localized effect) only affected crypt cell dynamics in the early post operative period and they suggested that the reason why Musso et al. (1975) found very little effect after splanchnicectomy was that they used experimental times in excess of three days.

Tutton and Helme (1973, 1974), Klein and Torres (1978) in adult rats, and Klein and McKenzie (1980) in neonatal rats, found that chemical sympathectomy after treatment with either 6-hydroxydopamine or guanethidine sulphate was associated with a prolonged fall in the mitotic rate in the small intestine. These findings were supported by the findings of Klein (1979 b).
Tutton and Barkla (1977a) found that chemical sympathectomy (as above) also decreased the mitotic rate in the rat large intestine.
Division of two adjacent neurovascular pedicles to a loop of small intestine produces autonomic denervation (especially of sympathetic nerve fibres) of that segment (Schofield, 1960).

Tutton and Helme (1973, 1974) found that denervation of a loop of small intestine, as above, was associated with strongly inhibited crypt cell proliferation in that segment, without appreciably influencing cell proliferation in adjacent regions which have an intact nerve supply.

The circadian variations in crypt cell proliferation, in small and large intestine, previously mentioned in this review, are either obliterated (Tutton, 1975), or suppressed (Klein, 1980) following chemical sympathectomy.

Touloukian and Spencer (1971) found that hypertrophy of the ileal remnant following 50% midenterectomy in rats was accompanied by an increase in blood flow to the ileal remnant which was selective and paralleled the compensatory hypertrophy, suggesting a causal relationship.

Touloukian et al. (1972a) found that, following a similar procedure to above, in similar rats, endogenous cathecolamine activity in the hypertrophied ileal remnant was reduced by nearly 50% of normal and accompanied by a corresponding decrease in the density of adrenergic terminals within the myenteric plexus.
and arterial vasculature of the hypertrophied ileum. This suggested the possibility of the occurrence of adrenergic denervation of the hypertrophied gut remnant following major intestinal resection, with a possible increase in mucosal blood flow. It is known that decreased mucosal blood flow is associated with decreased crypt cell proliferation (Rijke et al., 1976) so possibly increased mucosal blood flow might lead to increased crypt cell proliferation.

Other evidence suggesting that the sympathetic nerves might act on the crypts via the blood supply was provided by the findings of Grim (1963), who showed that splanchnic nerve stimulation caused vasoconstriction of the splanchnic vasculature. Hultén et al. (1977) found that on stimulation of the regional splanchnic nerves in man and cats, the blood flow to the mucosa as well as to the other layers of the small and large intestine was diminished.

If compensatory post-resectional hypertrophy depends solely on mucosal blood flow changes, the fact that post-resectional hypertrophy is greater if the animal is fed orally rather than parenterally remains unexplained.

Dowling (1982) has suggested the possibility that the mechanism whereby luminal nutrition and hormonal factors stimulate adaptive hyperplasia is by increased mucosal
blood flow, in response to chemical, hormonal and neural stimuli.

Sympathectomy apparently can modify the effects of other procedures on crypt cell proliferation e.g. Levine et al. (1982) found that chemical sympathectomy with 6-hydroxydopamine exaggerated the intestinal mucosal hypoplasia in rats maintained on total parenteral nutrition. However, this effect could be prevented by intragastric infusion of luminal nutrients, suggesting that the effect of the sympathectomy was minor compared with that of the luminal nutrients.

(b) Vagotomy.

Silen et al. (1966) reported a fall in jejunal crypt cell proliferation rate two weeks after abdominal vagotomy in the dog. By six weeks postoperatively there was recovery and an increased rate of crypt cell proliferation.

Musso et al. (1975) found that the crypt cell mitotic index was diminished from the third to the tenth day after abdominal vagotomy but recovered thereafter.

Lachat and Goncalves (1978) also reported an initial decrease in the crypt cell mitotic rate for the first 75 hours after vagotony.

Tsibulevskii and Eletskii (1976) found that between seven and thirty days after bilateral subdiaphragmatic
vagotomy in dogs there were inflammatory and marked degenerative changes in the jejunal mucosa, followed by compensatory structural changes sixty to one hundred and twenty days postoperatively. These findings suggested a possible trophic action by the vagus nerves.

Ballinger et al. (1964) found an initial reduction in height of the ileal mucosa of the dog, reaching its maximum 2 weeks after bilateral truncal vagotomy, followed by compensatory hyperplasia for up to five months.

Ballinger et al. (1965a) suggested that the initial changes were ischaemic in origin, since vagotomy was shown to result in a profound decrease in mesenteric blood flow, and since dogs in which the vagal supply to the small bowel had been spared (selective vagotony) did not develop this atrophy of the intestinal mucosa.

Delaney (1967) found a significant increase in small bowel perfusion rates in dogs four to six weeks after abdominal vagotomy which would probably coincide with the recovery phase in the crypts.

Mackie and Turner (1971) found that a significant decrease occurred in blood flow in the dog jejunum and ileum within three weeks after vagotomy, returning subsequently to normal.

Conversely, Kewenter (1965) demonstrated that stimulation of the vagal nerve trunks had little if any
effect on mesenteric blood flow, although intestinal motility was altered.

However, changes in mesenteric blood flow are not necessarily reflected as changes in villus blood flow, as observed previously in this review.

Silen et al. (1966) suggested that the postvagotomy changes may have been due to changes in the luminal bacterial flora, which we have seen elsewhere in this review can affect the crypt cell mitotic rate. It is also possible of course that the vagi may act directly on the crypts, without intermediary mechanisms.

Llavag and Vaage (1972), Alpers and Kinzie (1973), and Mamontov et al. (1979) found an accelerated, not depressed, crypt cell proliferation rate after truncal abdominal vagotomy. Tsibulevskii and Orlova (1976) found an absolute increase in mitotic activity in the intestinal epithelium seven days after vagotomy.

Ellis and Pryse-Davies (1967) found no histological or histochanical alterations in the intestinal tract or in the growth rate of rats after vagotomy, and no degenerative changes were noted in the dog jejunal mucosa after vagotomy by Elliot et al. (1967). Bejar et al. (1968) found no changes in the intestinal mucosa in humans after vagotomy and pyloroplasty but possibly the differences from the results in
animals may be explained by species specificity or the effect of the accompanying pyloroplasty.

Thus, it appears that the experimental evidence concerning the effects of vagotomy on crypt cell proliferation is not conclusive, but conflicting. However, it appears that vagotomy has some effect and differences in such effect may in some instances be species specific. There is also considerable variation in the methods used to assess the crypt cell mitotic rate, and in some cases assessments of mucosal size are made rather than an accurate assessment of crypt cell mitotic activity.

In fact, the activity of the vagus in relation to crypt cell mitotic activity may be related to its effects on other factors e.g. pancreato-biliary secretions. Laplace (1982) found that cutting the afferent or sensory fibres of the vagus abolished the adaptive mucosal changes in the residual intestine of the pig twenty-eight days after partial enterectomy. As we have seen above, hormonal factors such as enteroglucagon may be involved in the adaptive process so there may be a link between secretion of these substances and the activity of the vagi.

As indicated previously, pancreato-biliary secretions have been implicated in crypt cell control. Hayama et al. (1963) found a marked reduction of secretion
from pancreatic fistulae in dogs following abdominal vagotomy, which they suggested was a direct effect of the vagus.

Pfeffer et al. (1952) found that the powerful effects of insulin and secretin on increasing the flow of exocrine secretions of the pancreas were eliminated by bilateral abdominal vagotomy, suggesting also the importance of the vagal or cephalic phase of pancreatic secretion. The effect on insulin produced secretion was not found in humans however (Kraft et al., 1962).

Rudick and Hutchinson (1964) showed that abdominal vagotomy interfered with normal emptying of the gall bladder in humans.

Experimental effects of sympathetic neurotransmitter agonists and antagonists on crypt cell proliferation.

Tutton and Helme (1974) found that administration of noradrenaline promoted, whilst adrenaline slowed down, crypt cell proliferation in the rat.

Tutton and Barkla (1977) found that the noradrenergic agonist metaraminol promoted crypt cell proliferation in the rat colon, whilst in both rat jejunum and colon, the α adrenergic antagonist phentolamine was found to slow crypt cell proliferation. However, the β adrenergic antagonists, propanolol, practolol and solatol lacked this effect.

It was thus suggested that noradrenaline stimulates
crypt cell production in rat intestine by acting on an adrenoceptor.
Kennedy et al. (1983) obtained similar results concerning the effects of \( \alpha \) adrenergic agonists and antagonists on crypt cell proliferation in both the small and large intestine of the mouse.
The influence of \( \alpha \) adrenoceptors may suggest a role for the adrenergic nerve fibres known to exist in relation to the crypts throughout the intestine (Gabella and Costa, 1968a).
It is known that changes in tissue levels of adrenaline and noradrenaline liberated from the intramural plexus may modify the motility and the blood supply of the small intestine (Furness and Costa, 1974), so that changes in these may be involved in control of crypt cell proliferation.
Autonomic denervation itself, as previously described, alters the liberation of adrenaline and noradrenaline locally and these substances could be involved in the changes seen after this procedure.

Experimental evidence of the effect of parasympathetic neurotransmitter agonists and antagonists on crypt cell proliferation.

Tutton (1975 b) found that stimulation of cholinceptors by either the cholinceptor agonist, carbachol, or by elevated levels of endogenous acetylcholine after treatment with the acetylcholinesterase inhibitor, neostigmine, promoted cell proliferation in the crypts of the rat
Jejunum. These effects were blocked by the nicotinic cholinoreceptor antagonist tubocurarine but were not blocked by the muscarinic cholinoreceptor antagonist, atropine sulphate.

Furthermore, he found that whereas tubocurarine alone, or hexamethonium alone, decreased the crypt cell mitotic rate, atropine alone increased the mitotic rate, and was in turn blocked by phentolamine, suggesting an adrenergic mechanism. He suggested that there may be functional interactions between cholinergic and adrenergic post-ganglionic neurons in the gut i.e. that the sympathetic and parasympathetic components of the gut innervation may not be completely independent of one another. This type of interaction has apparently been suggested by the work of Kosterlitz and Lees (1972) and Löffelholtz and Muscholl (1969). Tutton (1977) found that crypt cell proliferation was stimulated by cholinergic drugs.

The effect of stimulation of the neurovascular pedicles in the mesentery of the small intestine on crypt cell proliferation.

Tutton (1975a) found that application of electric stimuli to the neurovascular pedicle promotes crypt cell proliferation in the region of the small intestine supplied by that particular pedicle, when compared with sham controls. He considered this increase in mitotic rate to be not solely dependent upon the integrity of the sympathetic nerves, since it was
also demonstrated in chemically sympathectomized rats. The finding that nerves other than adrenergic influenced crypt cell proliferation was considered consistent with the previous findings (Tutton and Helme, 1974) that surgical denervation of a loop produced a more profound effect on crypt cell proliferation than chemical sympathectomy. They suggested that serotonergic nerves (found in the intestinal wall by Gershon et al., 1965), or histaminergic nerves (found in the intestine by Coujard, 1950) may be operative in crypt cell proliferation control. As to whether these neurally induced changes in the crypts were mediated by changes in local vasomotor activity or motility, the former appeared unlikely since in the intestine the vasculature receives only a sympathetic innervation (Davenport, 1966). Motility changes were also considered unlikely to be responsible because the effect of combined parasympathetic and sympathetic stimulation is bowel wall relaxation, whereas the effect of parasympathetic stimulation alone is contraction of the bowel wall (Davenport, 1966), and in contrast both forms of stimulation caused accelerated crypt cell proliferation.

With regard to the mode of action of the neurotransmitter substances, Tutton and Helme (1974) found that noradrenaline and adrenaline have opposing actions on crypt cell proliferation but Ahlquist (1948) had earlier noted that they were both potent mesenteric vasoconstrictors.
Conversely, adrenaline and isoprenaline both inhibit jejunal crypt cell proliferation but have opposing effects on mesenteric blood flow. Adrenaline causes constriction and isoprenaline (isoproterenol) dilatation of the vessels (Tutton and Helme, 1974). The same authors found that the inhibitory effect of isoprenaline on crypt cell proliferation was not due to its effects on local blood flow, since this substance inhibited crypt cell proliferation also "in vitro".

The possibility of these agents acting by means of changes in the motility of the bowel was considered by the same author, since noradrenaline decreases and carbachol increases motility (Weiner, 1980) but both agents stimulate jejunal crypt cell proliferation. Also, it was noted that nicotinic type cholinooceptors were involved in changes in intestinal proliferation, whereas muscarinic type cholinooceptors were involved in changes in motility.

The possible role of cyclic nucleotides as second messengers within intestinal crypt cells.

Goldberg et al. (1975) suggested that the intracellular molar ratio of $3',5'$-cyclic guanosine monophosphate (cGMP) to $3',5'$-cyclic adenosine monophosphate (cAMP) was involved, amongst other things, in the regulation of cellular proliferation and it is known that extracellular noradrenaline, acetylcholine, histamine
and serotonin affect the levels of these substances. There appears to be some experimental evidence linking substances such as noradrenaline with the activity of cGMP and cAMP, via \( \alpha \)-adrenoceptors and intracellular calcium ion levels.

Tutton and Barkla (1980a), studying the effects of various systemically effective derivatives of cAMP and cGMP, found that \( \text{N}^6-\text{O}^2' \)-dibutyryl cGMP stimulated crypt cell proliferation in the small and large intestine, whilst \( \text{N}^6-\text{O}^2' \)-dibutyryl cAMP inhibited crypt cell proliferation in the jejunum (Tutton and Barkla, 1982a), and colon (Tutton and Barkla, 1980a). Thus, these substances may provide a link between the actions of the sympathetic nervous system and the crypt cells.

It appears from the available evidence that neural factors, perhaps linked to the effects of neuropeptide secretions, and possibly cyclic nucleotides, could probably provide suitable mechanisms for the function of the more localized "villus longistat", as well as the more generalized control of crypt cell proliferation, which is needed to adjust to changing nutritional demands on the organism.

Experimental evidence concerning the role of serotonin in the control of crypt cell proliferation.

Ersparner (1954) estimated that approximately 60% of
the serotonin present in rats is within the digestive system, and Hakanson (1970) found much of it within the enterochromaffin cells.

Tutton (1974) found that small doses as well as medium doses of serotonin accelerated crypt cell proliferation in the small intestine, whereas very large doses (300 mg./Kg.) prolonged both cell cycle and mitotic times. Small doses of serotonin were also noted to accelerate crypt cell proliferation in adrenalectomized animals. Partial serotonin depletion following administration of 6-fluorotryptophan was associated with retarded crypt cell proliferation.

It is possible that serotonin may form part of the pathway of action of the autonomic nervous system in the control of crypt cell proliferation. For instance, the effects of noradrenaline or of sympathectomy may be mediated through alterations in the rate of release of serotonin from enterochromaffin cells.

Thompson and Campbell (1966), furthermore demonstrated that the serotonin content of the alimentary tract is significantly increased following immunosympathectomy in mice, and this was supported by the findings of Weber (1970) in rats.

Tansy et al. (1971) showed that noradrenaline infusion produced degranulation of guinea pig duodenal enterochromaffin cells (with presumed release of serotonin into the circulation), which may explain the increased crypt cell proliferation. It is difficult to be
very conclusive however in this case, since the amount of serotonin released is not known and the effect of serotonin is very variable, depending on the dosage of administration. The same authors showed that vagal stimulation caused enterochromaffin cell degranulation in normal but not in chemically sympathectomized guinea pigs.

Larsson (1981) in the cat, and Ohsumi et al. (1974) in the rat, found evidence of adrenergic type fibres in the vagus nerves, originating apparently in the superior cervical ganglion and going to the small intestine, which Larsson considered may have been involved in the demonstrated release of serotonin into the gut lumen following vagal stimulation. He also concluded that the splanchnic nerves participate in the regulation of serotonin release from the entero-chromaffin cells into the portal circulation by an adrenergic mechanism.

Tutton (1974) suggested that the increased crypt cell proliferation seen in coeliac disease (Trier and Browning, 1970) may possibly be related to altered levels of serotonin, since increased urinary levels of serotonin metabolites are found in patients with this disease (Haverback, 1958). However, this evidence is very indirect and the increased levels may be the result of other factors.
Experimental evidence for the effect of prostoglandins on crypt cell proliferation.

Tutton and Barkla (1980b) found that the prostoglandin analogue, $F_{2\alpha}$, was an accelerator of cell proliferation in rat jejunal crypt cells. Although this analogue may act directly on the crypts or via the various means so far mentioned, it has been shown, in some cases at least, to act by raising intracellular levels of cyclic guanosine monophosphate (Kuehl et al., 1973), although this may not be the mechanism in this particular case.

In support of the action of this analogue via changes in cGMP are the findings that other agents that have been shown to stimulate the formation of cGMP, e.g. noradrenaline (Schultz et al., 1975), acetylcholine (Goldberg et al., 1974), and serotonin (Goldberg et al., 1974), as well as dibutyryl cGMP itself, have been shown to accelerate jejunal crypt cell proliferation (Tutton and Helme (1974); Tutton (1977a); Tutton and Barkla (1980c)), respectively.
Experimental evidence regarding the factors concerned in control of crypt cell proliferation in neonates.

Klein (1980a) found that sympathectomy reduced crypt cell proliferation between 15 and 23 days after birth, and although there was still increased activity in the post-closure ileal epithelium crypt depth and villus height were also reduced. Sympathectomy delayed but did not permanently prevent the post-closure acceleration of proliferative activity in the ileum, since adult rats (neonatally sympathectomized) had normal compartment size (Dupont et al., 1965), but retained a slower rate of crypt cell proliferation (Klein, 1979b; Dupont et al., 1965; Klein, 1980; Klein and Torres, 1978).

Sympathectomy also delays the removal of vacuolated pre-closure cells from the epithelium of the villus, i.e., it delays the redifferentiation of the intestinal epithelium.

A similar reduction in crypt cell proliferation in both pre- and post-closure ileum can be induced by the administration of the \( \beta \) adrenergic stimulant isoprenaline (Klein, 1977), suggesting that adrenoceptors and catecholamines may function in a similar manner in the neonatal and adult rat intestinal epithelium.

Whilst the delaying effect of sympathectomy on the redifferentiation of the intestinal epithelium from the suckling to the more mature type is temporary,
hypophysectomy at 6 days results in a more permanent similar effect (Yeh and Moog (1975); Yeh (1977); Moog and Yeh (1979)). The latter effect may be overcome by treatment with thyroxine or cortisone (Yeh and Moog, 1975).

The glucorticoids and thyroxine also play an important role in intestinal development (Yeh and Moog, 1977), as suggested by the finding of depressed crypt cell mitotic activity associated with adrenalectomy or thyroidectomy, which may be restored to control levels by replacement therapy with cortisone and thyroxine, respectively.

The closure of the ileum at 18 to 19 days after birth in the rat and the concomitant acceleration of crypt cell proliferation take place during the rise of plasma corticosterone levels from 12 to 25 days after birth (Ader (1969); Allen and Kendall (1967); Haltmeyer et al. (1966); Redman and Sreebny (1976)).
Experimental evidence of the effects of stress on crypt cell proliferation in the small intestine.

Escobar (1963) found that in acutely stressed mice there was a reduction in the mitotic index in the stomach but an increase in the mitotic index in the intestinal epithelial crypts. This latter finding was challenged by Tutton and Helme (1973) on the grounds that the rate at which the cells entered mitosis had not been determined.

Tutton and Helme (1973) found that stress, applied in the form of frequent electrical shocks to the animal, inhibited crypt cell proliferation in the small bowel of intact rats, in both adrenalectomized rats and in rats subjected to $\beta$-adrenergic blockade. However, chemical (general), or surgical (local), sympathetomy appeared to prevent the inhibition of crypt cell proliferation due to the stress. They therefore suggested that the effects of stress are mediated via the sympathetic nerves to the small intestine. The foregoing is contrary to the findings of Selye (1955) who suggested that stress is mainly mediated via the pituitary-adrenal axis.

It is interesting to note, in view of the proposed importance of the sympathetic nervous system in the control of the reaction to stress, that rats subjected to repeated restraint stress had a reduction in the density of $\beta$-adrenergic receptors in the hypothalamus, cerebral cortex and brain stem.
which persisted for the duration of the chronic stress. Adaptation to stress was found to correlate positively with the loss of $\beta$ receptors, supporting the hypothesis that a reduction in the number of brain $\beta$-adrenergic receptors is one of the biochemical factors underlying adaptation to stress (Haeustler (1971); Stone and Platt (1982)).

Experimental evidence for a possible role for the hypothalamus in the control of crypt cell proliferation.

Jutisz et al. (1974) extracted and concentrated from the sheep hypothalamus a principle capable of inhibiting the in vitro multiplication of some cell strains stabilized in a continuous line. It was concluded that the substance was not a peptide. The sheep cerebral cortex and liver were also found to contain a similar factor inhibitory to mitosis, but in a lower concentration.

Hindoni et al. (1973) made radio-frequency lesions in the hypothalamus of male Wistar rats, which were killed three weeks later. The mean number of mitoses of epithelial cells per longitudinally sectioned tubule of small intestine removed from a site 10 cms. from the pyloric sphincter was obtained. Forty rats were used and the mitoses were counted in a total of 20,000 glandular tubules. In rats with lesions confined to the tubero-infundibular region of the hypothalamus, and in a few cases in which the lesions extended to
planes immediately posterior to it, the average number of mitoses per tubule was 25% to 50% higher than in controls. The differences were found to be highly significant, statistically ($P < 0.001$).

It was also noted in the animals with tubero-infundibular lesions that there was, as a rule, a correlation between volume of tissue destroyed and the increase in the average number of mitoses. Control radio-frequency lesions made in the hypothalamus, but outside the tubero-infundibular region, excluded the possibility of non-specific effects. The authors considered that these changes were probably not attributable to functional changes in the endocrine system, since whilst some of the animals were found to have regressive cellular changes in the pituitary, the rats with extensive tubero-infundibular lesions were also those with regressive changes in the pituitary and showed the greatest increase in the average number of mitoses per glandular tubule.

The pituitary changes could have resulted from the local effects of the lesion itself. The finding of pituitary regression in the face of crypt cell proliferation contrasts with the fall in crypt cell proliferation noted after hypophysectomy observed above in this review.

The method of assessing crypt cell activity may lend itself to some criticism, but a large number of mitoses were counted, so that it is likely that some change had occurred as a result of these lesions, although care should be taken in quantitating the effect.
Experimental evidence of the effect of removal of the pineal gland on crypt cell proliferation in the small intestine.

Bindoni (1971) summarized the results of various experiments on the small intestine, spleen, liver, and adenohypophysis in the following publications, viz.-

Bindoni and Cambria (1968),
Bindoni and Raffaele (1971),
Bindoni and Raffaele (1968),
Guiffrida et al. (1969).

They found that, using an estimate of the mitotic index (the number of mitoses per 100 nuclei), and a biochemical method based on the incorporation of $p^{32}$ into DNA, the cells of the spleen, intestinal crypts, liver and adenohypophysis from pinealectomized rats had a higher mitotic index and a higher $p^{32}$ incorporation into their DNA than the corresponding cells of sham-operated animals.

Pinealectomy did not induce changes in the mitotic rate of the regenerating liver, possibly because the process of liver regeneration may be controlled by the pituitary (Bindoni and Raffaele (1971); Wrba and Rabes (1967)).

Testicular atrophy was found to follow removal of the pituitary but this atrophy was found to be less pronounced in animals in which both the pineal and pituitary had been removed. An analogous effect was demonstrated in follicular spleen cells. On the basis of these results Bindoni concluded that the
stimulation of the mitotic activity by pinealectomy was probably independent of pituitary function. It was suggested that the effects of pinealectomy were due to direct effects of the pineal on the tissues.

Furthermore, Bindoni et al. (1976) prepared and partially purified an antimitotic substance from the pineal gland of the sheep which inhibited the in vitro multiplication of three cell strains. The substance was not destroyed by proteolytic enzymes, nor by boiling with 6M hydrochloric acid, and was established as being different from the known antimitotic agents, or melatonin, serotonin or noradrenaline which did not show any antimitotic activity under the same conditions.

It should be noted, however, that the method for assessing cell proliferation in the small intestine used by Bindoni in the experiments on pinealectomy lends itself to some criticism, in that only one dose of colchicine was given 3 hours before the animal was killed. With this in mind, these experiments have been repeated in the present series of experiments because it was felt that there had been an effect of pinealectomy demonstrated in this case.

An interesting effect was noted by Pearl et al. (1966), although not in relation to intestinal epithelium. He observed that stimulation of the anterior hypo-
thalamus produced marked hyperplasia of the mucous neck parietal and chief cells of the stomach, which did not occur if the vagi were sectioned before stimulation.

Miscellaneous effects on crypt cell proliferation, not necessarily related to the mechanism of control of crypt cell proliferation.

(1) Changes in the epithelium with age.

Hermos et al. (1971), Eastwood and Trier (1974), noted that rapid differentiation occurred in the mucosa of the small and large intestine of the rat during the last 5 days of gestation, from an undifferentiated stratified epithelium to a more adult form. The same authors noted very little change in the pattern of epithelial renewal during the early neonatal period until weaning, when there was a marked lengthening of the villi in both mice and rats (Koldovsky et al. (1966); Trasher (1967); Cheng and Bjerknes (1985)), which persisted throughout life from 4 weeks.

Lesher et al. (1961 a, b), Fry et al. (1961), and Cameron (1972) found that in rodents with advancing age crypt cell proliferation and migration became slower.

In the proximal intestine of old rats the proliferative zone was expanded and the crypt epithelial cell
numbers were greater than in young rats (Holt et al., 1984), suggesting to these authors that there was decreased survival of newly formed crypt cells in the aged.

Clarke (1977a) found that crypt-villus ratio rose with age along the length of the small intestine and the crypt depth increased.

Penzes and Skala (1977) noted that, whilst the mucosal area per unit serosal area diminished along the length of the small intestine in the young rat, in adults there was a relative constancy of these parameters along the length of the intestine.

Since there are variations in the kinetic status of the bowel mucosa with age, it is important in cell kinetic experiments on the bowel to consider groups of rats of roughly similar age, and in particular not to consider groups of rats composed of very immature and older rats.

(2)

Changes in the intestinal epithelium associated with vitamin deficiency in the rat.

Zile et al. (1977) suggested that, since vitamin A deficiency was associated with fairly marked changes in the cell kinetics of the mucosa, it may play a role in the regulation of cell division in the small intestine of the rat. However, there seems to be
little other evidence to support this contention, although vitamin A may play some part in the effects of the luminal contents as a whole.

Similarly Burge and Alpers (1973) found that oral administration of vitamin D\textsubscript{3} to vitamin D deficient rats was associated with stimulation of mucosal cell proliferation. They suggested that it may be a trophic factor, perhaps related to the stress of absorption of calcium from the lumen.

**Effect of vitamin B\textsubscript{12} deficiency.**

Arvanitakis (1978), Eichorn and Bryden (1955), Nebel (1975), Foroozan and Trier (1967), found shortening of the villi or decreased crypt cell mitotic activity in the small bowel and associated malabsorption in patients with pernicious anaemia. All of these changes were reversed after treatment with vitamin B\textsubscript{12}. Since vitamin B\textsubscript{12} is absorbed largely in the ileum, it might be supposed that partial resection of the ileum would result in vitamin B\textsubscript{12} malabsorption but no significant changes in absorption of this vitamin were found after resection by Stokkers et al. (1977).

(3) Changes in the small intestinal mucosa in diseased states, generalized or local.

Creamer (1964 a) and Salem et al. (1964) reported hypoplastic changes in the small intestinal mucosa in ulcerative colitis, and Creamer (1964 c) found hypoplastic mucosal changes in patients with primary
malignant disease in any part of the body, including the gut, which may or may not be a reflection of severe illness i.e. not necessarily specific to malignancy. These changes may contribute partially to the cachexia of late malignancy.

Creamer (1964 b) noted jejunal mucosal abnormalities where there was a definite change in the luminal environment e.g. near a gastrectomy stoma, and Creamer (1965) also noted changes in mucosal structure in chronic pancreatitis, chronic intestinal obstruction, and the Zollinger-Ellison syndrome.

Similar degenerative changes have been found in the ordinary population of southern India (Baker et al., 1962'), Uganda (Banwell et al., 1964'), and Thailand (Sprinz et al., 1962'), and in these cases dietary abnormalities and intestinal infestation appear to have been likely causes of abnormal intra-luminal environment.

In support of this, protein malnutrition was found primarily to affect the crypt, reducing the rate of cell renewal and rendering the crypts and villi atrophic (Takano (1964); Deo et al. (1965) in rodents), (Deo and Ramalingaswamy (1965) in monkeys). Furthermore, abnormal mucosae have been reported in Giardia Lamblia infections (Cameron et al., 1962), and Hookworm infestation (Sheehy et al. (1962); Salem et al. (1964')).
Anderson, Finlayson and Deschner (1974) noted that in coeliac sprue villi were absent, crypts were enlarged, and in the untreated disease there was a marked increase in the proliferative activity in the crypts. Cell loss from the mucosa was much higher than normal (Croft, Loehry and Creamer, 1968), confirming the rapid epithelial cell turnover in these patients.

(4) Changes in the mucosa in Diabetes Mellitus.

Schedl and Wilson (1971), Jervis and Levin (1966), Miller et al. (1977) found that in rats with Diabetes Mellitus induced by Alloxan or Streptozotocin there was increased mucosal growth and in particular greatly increased DNA synthesis and crypt cell proliferation in the ileum and jejunum which was independent of the dietary regimen. Stenling et al. (1984) found that these changes were more pronounced in the jejunum than the duodenum and that there was associated hyperphagia.

However, the findings of Arsenault and Menard (1984) that the mitotic index of mouse jejunal explants in vitro was increased by the addition of insulin to the medium suggested that the effect did not depend on other factors e.g. luminal nutrition. Sodoyez-Goffaux et al. (1985) found the presence of insulin receptors in the gastrointestinal epithelium of the foetal rat, suggesting that the gut is potentially at least an important insulin target.
Thus, it is possible that insulin may be one of the hormonal factors involved in the control of crypt cell proliferation, either acting independently, or more probably in concert with other hormones.

(5)
Changes in the intestinal mucosa in Uraemia.

Castrup et al. (1970), McDermott et al. (1974 a, b) found that intestinal epithelial cell proliferation was apparently inhibited in uraemia by a direct effect on the renewal cycle. This is thought to contribute to the development of uraemic lesions in the gastrointestinal tract.

In chronic renal failure where elevated serum gastrin levels are found (Korman et al. (1972); Maxwell et al. (1971)), since this hormone is considered by some investigators to be trophic to the intestinal mucosa, an increase in crypt cell proliferation might be expected but this was not found.

(6)
Changes in the intestinal mucosa following partial gastrectomy.

Zufarov et al. (1973, 1974) found that there was hyperplasia of gastric, small intestinal and colonic mucosa in dogs following gastric resection, albeit delayed for some months post operatively. It seems unlikely that changes in the luminal pH could explain such widespread changes in the gastrointestinal tract.
Lees and Grandjean (1958) found no consistent histological changes suggesting either atrophy or hypertrophy of the jejunal mucosa after partial gastrectomy. Polak and Bloom (1979) found that removal of the pyloric antrum resulted in a decrease in weight, RNA, DNA and protein content as well as DNA synthesis in the oxyntic glands, duodenal, ileal and colonic mucosa. Similar changes were observed in the pancreas. All such changes were partially or totally prevented by the administration of gastrin. Since the antral mucosa is a source of gastrin, removal of this area would be consistent with these changes but, as indicated elsewhere in this review, the importance of gastrin in the control of crypt cell proliferation is by no means established.

(?)
Changes in the intestinal mucosa in zinc deficiency.

Ament and Broviac (1973), Kelly et al. (1976) found in a rare zinc deficiency syndrome associated with zinc malabsorption, Acrodermatitis Enteropathia, that the intestinal mucosa was flat with a decrease in crypt numbers and some crypt necrosis, flattening of villi and inflammatory infiltration of the lamina propria. Zinc therapy resulted in reversal of these changes.
Elmes and Jones (1980) found that acute zinc deficiency in young rats resulted in degenerative changes in the enterocytes.

Southon et al. (1985) also noted a substantial decrease in the net influx of cells into the villi of zinc-deficient rats compared with controls. However, in the available literature there appeared to be no substantial evidence regarding effects on the crypts of the mucosa, only villus size.

Elmes (1977) concluded that the changes in zinc-deficient rats were relatively slight compared with the marked changes in the mucosa in fasted rats.

Thus, the place of zinc, which plays a role in DNA synthesis (Chesters, 1974), in the normal control of crypt cell proliferation is probably not a very significant one, but it may contribute to the local mechanisms of control as a dietary factor in the luminal nutrition of the enterocytes.

Finally, Kruse Jarres et al. (1975) found that in rabbits absorption of zinc was not disturbed remarkably by jejunectomy.

(8)

The effects of chronic alcohol ingestion on the mucosa.

Baraona et al. (1974) found in rats which received ethanol daily over several weeks into a totally liquid nutritionally adequate diet, the villi became shorter, and in the ileum there was stimulation of epithelial renewal perhaps in response to initial injury to the mucosa.
The effects of ionizing radiation on the intestinal mucosa.

Trier and Browning (1966), Rijke et al. (1975) found that after an initial depression of crypt cell mitosis and reduction of villus height in the small intestine there was a phase of increased crypt cell proliferation resulting in replenishment of the villus population of cells.

The effects of administration of Methotrexate, systemically, on the intestinal mucosa.

Trier (1962) found that a single dose resulted in profound depression of crypt cell mitotic activity which returned rapidly to normal after cessation of therapy.
Conclusions

Although many factors have been identified as affecting the rate of crypt cell proliferation, it is obvious that all of these factors are probably not involved in the day to day regulation of crypt cell proliferation under basal conditions, although some may be involved in the control under abnormal conditions.

Demonstration of elevated levels of a particular hormone in association with increased crypt cell proliferation does not necessarily implicate that substance as a causal factor. In some cases, the altered levels are coincidental or subsequent to the changes in crypt cell proliferation, so caution must be exercised in interpreting these changes.

There appears to be considerable evidence for the existence of a localized feedback system of control which is not operative under all conditions but plays an important part in it. It seems likely that this local mechanism is mediated by a humoral mechanism but local neurovascular factors may also be involved.

Besides the local mechanism of control, there is a more generalized mechanism which may be mediated by humoral agents such as enteroglucagon, somatostatin and others, or alternatively may be mediated by the action of the autonomic nervous system.
This more generalized system would appear to respond to changes in the luminal environment e.g. the presence or absence of food in the lumen and the composition of the luminal contents. Hence the luminal contents themselves are important in the control of crypt cell proliferation.

The luminal contents may act by providing nourishment to the lining epithelium i.e. the more luminal nourishment the more proliferation of the cells, but it seems more likely that the luminal contents act by presenting a "work load" to the mucosa in the form of metabolic demand for secretion of enzymes and absorption of nutrients. This is analogous with the effects of presenting an increased "work load" to a skeletal muscle, with resultant hypertrophy. Just as an actively exercising muscle requires a greater blood flow, so there is a demand for greater blood flow in the presence of greater luminal "work load". Thus, the changes in blood supply to the mucosa cannot be separated from the other mechanisms of control nor can neurovascular factors mediated by the sympathetic nervous system.

There seems to be some evidence from the literature reviewed that the physical characteristics of the luminal contents are possibly more, if not of equal importance in signaling the amount of "work load" which the crypts must respond to, as the nutrient value.

The sympathetic nervous system may be a means of
coordinating the various mechanisms and perhaps of monitoring the proliferative response.

The local bacterial luminal flora do not appear to play a major role in the control of crypt cell proliferation but disorders of this are reflected as changes in crypt cell mitotic rate.

Similarly the effect of pancreato-biliary secretions of themselves does not appear to be a major one, but it should be remembered that these secretions are continually modifying the physical and chemical nature of the luminal contents and so, in conjunction with the luminal contents and complementary to them, they may assume a greater importance.

Conclusions drawn from experiments involving resection or bypass of the small bowel should be drawn with caution, since both are very artificial situations and the mechanisms illustrated by them may be used only under certain circumstances. Also, considerable stress effects are placed on the animals and animals vary considerably in their individual reaction to such stresses.

It is difficult to find a place for the observations of Hindoni with regard to the effect of pinealectomy. Much is still to be learnt about the functions of the pineal gland, and it may be that it is only part of another control mechanism.
A localized feedback mechanism may be important in "defending" the mucosa against harmful influences, whereas as suggested previously the stimulatory effect of the luminal factors may be involved in the response to increased functional demand.

Prolactin and the pituitary gland appear to be involved in the development of the intestinal mucosa during and after lactation, but may have a more secondary role in adult life.

It is evident from the literature that stress affects the rate of crypt cell proliferation, probably via the sympathetic system. It is difficult to determine whether the type and degree of stress used in these experiments is similar to the usual day to day stresses in these animals i.e. are we looking at a grossly abnormal situation with a response which would not normally be evoked in these animals?

It appears that control is probably multifactorial and the extent to which each factor acts is as yet not determined.

The role of the stem cells in the response of the crypts is not clear, but they may represent the target on which some or all of the controlling factors act.
It is suggested by the literature that the hypothalamus may possibly exert some control over crypt cell proliferation but again it is not indicated whether this action is part of the day to day control mechanism or a mechanism for dealing with abnormal situations. It is conceivable that the hypothalamus could act via the autonomic system or via the secretions of the pituitary gland.

The effects of vagotony appear to less well defined in their effect on the small intestine than the effects of local sympathectomy or sympathetic nerve stimulation, suggesting perhaps a more significant role for the sympathetic part of the autonomic nervous system. The actions of adrenaline and noradrenaline and the effects of adrenergic agonists and antagonists also suggest the importance of the sympathetic nervous system. It also seems possible that the sympathetic nervous system may control the secretion of biogenic amines such as serotonin, which may in turn be concerned in the control of crypt cell proliferation.

Cyclic AMP and Cyclic GMP may also play some part in control of crypt cell proliferation as yet not defined, but perhaps as one of the final links in the chain of factors affecting the crypt cells.
Williamson (1978a) in a review of the topic noted that there are some features of the experimental evidence in the literature which appear to be inconsistent, viz.,

(1) whilst intraluminal nutrients and alimentary secretions appear to be important in the maintenance of mucosal integrity, the initial degree of ileal adaptation to jejunectomy is much greater than the effects of jejunal bypass or pancreato-biliary diversion, suggesting the operation of additional systemic influences,

(2) he also observed that altered chyme or enteric secretion could scarcely explain jejunal hyperplasia after distal enterectomy, ileal adaptation after subtotal colectomy or villus enlargement distal to atretic segments of intestine in neonates,

(3) the hypertrophic response of the small bowel to intermittent starvation in rats is inconsistent with the theory of luminal nutrition, for despite intense hyperphagia when allowed access to food, these animals consumed a lower nutrient load. In rats fed an elemental diet which gives full nutrient effect with minimal gastrointestinal stimulation, morphologic compensation for subtotal enteric bypass develops similar to that in animals given normal chow. Also, the early response to resection is independent of hyperphagia,

(4) furthermore, bypass stimulates growth of functional jejunoileal remnants, even in the absence of duodenal, pancreatic and biliary secretions (Fenyö, 1977)
Similarly, ileal hyperplasia occurs after oesophago-ileocolostomy with diversion of gastroduodenal and pancreatic-oblitary secretions into the caecum (Tilson et al., 1975).

Some clinical aspects of studies in intestinal adaptation.

After defunctioning the distal bowel by a proximal colostomy in rats the mucosal changes in the distal colon mimic those observed in bypassed loops of small bowel (Terpstra et al. (1981); Delvaux, et al. (1983); Rijke et al. (1979)). Terpstra et al. (1981) suggested that this hypoplasia reflected the loss of mechanical stimulus that follows faecal diversion, because closure of the transverse colostomy was followed by a burst of proliferative activity in the distal colonic crypts. The temporary diarrhoea which may ensue after colostomy in man has been attributed to the loss of absorptive surface area in the defunctioned bowel, which would be consistent with these findings in rats.

The gastric hypersecretion which may follow massive intestinal resection may be corrected by -

(1) Histamine-2 receptor blockade, which is thought to speed up the adaptive process.

or

(2) Vagotomy and pyloroplasty has been suggested, but whilst beneficial in dogs (Frederick and Craig,
1964), there is little evidence of improvement in patients following this procedure (Leonard et al., 1967), and the creation of an incontinent stomach is likely to be deleterious (Mc Kelvey, 1970).

Based on the experiments with rats it has been suggested that oral feeding should be commenced as soon as possible after bowel resection, to speed up the adaptive process.

Barros D'Sa et al. (1978) in a review of 29 published cases of reversed small intestinal segments after extensive small bowel resection found satisfactory to excellent results in 23 cases. Various other manoeuvres to delay the passage of intestinal content, and presumably favour absorption, have been suggested e.g. colonic interposition, recirculating loops, valves, sphincters, baffles and strictures. These don't really rely specifically on any effect of increased cellular proliferation in the mucosa.

Bilateral splanchnicectomy has been suggested as another measure which might improve the result of intestinal resections in humans, by increasing the blood flow to the bowel, and presumably by this means the crypt cell proliferation rate.

However, Mackby et al. (1965) found that whilst bilateral splanchnicectomy increased the blood flow in dogs with 85% resection of the small bowel
by 20%, these dogs fared less well than control animals after resection.

A critical evaluation and analysis of the literature on this topic leads the present author to suggest that control of gut cell proliferation is probably multifactorial and the extent to which each factor acts in this control is as yet not determined. Despite many years of research into this topic, it is notable that no definite mechanism of control has been determined. However, it is likely, in my own opinion, that the most rewarding areas for research in this field in the future will be regarding the influence of the neuropeptides, especially enteroglucagon, and the role of the C.N.S., which may overlap with that of the neuropeptides.
CHAPTER II

RATIONALE OF THE EXPERIMENTS

(a) General aims of the experiments.

(b) Factors determining the selection of the small intestine of the male Sprague-Dawley rat as the subject of the experimental studies.
GENERAL AIMS OF THE EXPERIMENTS

(1) To determine the effects of denervation of the small intestine on crypt cell proliferation in the small intestine. It was necessary to clarify, if possible, the effects of vagotomy, about which there is so much difference of opinion in the literature, as well as confirming the effects of local denervation of the small bowel.

(2) To determine whether the effects of vagal nerve stimulation on the crypts are consistent with the effects previously noted after vagal nerve section.

(3) To determine if possible local interference with the blood supply of the affected small bowel loop following local denervation could be an important factor in determining the effects on the crypts of this procedure or whether any effect obtained is principally due to the local denervation itself.

(4) To determine if the previously reported hyperproliferation of the crypts following removal of the pineal gland could be recorded again, and for a longer period of time, using a stathmokinetic technique.

(5) To determine if the effects of pinealectomy on crypt cell proliferation are primarily neurally mediated, by combining pinealectomy with local autonomic denervation of the bowel as in the previous experiments, it being then assumed that the effects of such denervation were primarily neural rather than neurovascular.

(6) To determine if the removal or modification of the luminal contents of the small bowel e.g. by exclusion of bile from the lumen, could affect the proliferation rate, and to further determine if these luminal changes could affect the effect of pinealectomy on the crypts, when combined with this procedure.
(7) To determine if lesions of the "limbic system" can affect the rate of crypt cell proliferation in the small bowel.

(8) To determine if bilateral lesions of parts of the "neocortex", including the cingulate gyrus, can affect the rate of crypt cell proliferation, because of their association with the "limbic system", and because lesions of these areas of the brain must be made in the present series of experiments so that access might be gained to the "limbic system".

(9) To examine the effects of combined lesions of the "limbic system" and pinealectomy on crypt cell proliferation rate in an attempt to determine if these regions act via the same or related neural pathways on the crypts.

(10) To examine the effects of combined "limbic" lesions (Hippocampal lesions used as an example) and autonomic denervation of the small bowel to determine if the "limbic system" affects the crypts of the small intestine via the autonomic nervous system.

(11) To estimate the amounts of food eaten under various experimental circumstances, associated with differing rates of crypt cell turnover, to determine if there is a consistent relationship between the amount of food eaten and the crypt cell turnover.

(12) It was hoped that any further knowledge acquired from these experiments regarding the normal mechanisms of control of crypt cell proliferation might act as a basis for further experimental investigation regarding abnormal mechanisms of crypt cell proliferation control e.g. in gastrointestinal epithelial malignancy.

(1) This species of animal is readily available and relatively easy to maintain.

(2) The anatomy of the rat gastrointestinal tract bears considerable similarity, functionally and structurally, to the human gastrointestinal tract so that through a study of the mechanisms of control in rats some insight may be gained (within certain limits) into the mechanisms of control of crypt cell proliferation in the human.

(3) A considerable volume of literature exists regarding the mechanisms of crypt cell proliferation in this species or in the related Wistar species of rats.

(4) Male rats were used in these experiments to obviate changes in the crypt cell proliferation rate known to be related to periodic changes in hormonal status in female rats (see Chapt. 1, Section 11).

(5) The small intestine was used as a model for studying the mechanisms involved in crypt cell proliferation because a considerable volume of the known literature on this topic concerns the small bowel rather than the colon. Moreover, it appears from the
literature that there is probably some similarity in the mechanism of control between the colon and small bowel.

(6) To minimize variations in crypt cell proliferation rate due to differences in age and environmental conditions an attempt was made, as far as possible, to select rats of roughly similar ages and to maintain them under as similar conditions of temperature and diet as possible.
CHAPTER III

GENERAL METHODS AND MATERIALS
(a) TYPE OF RATS USED AND GENERAL CONDITIONS
Male albino Sprague-Dawley rats were used and maintained at a temperature of approx. 22°C and in darkness from 0.22 hours until 0.08 hours. They were maintained on tap water and M and V mouse cubes, (CHARLICK and SONS). Specimens of small intestine were collected as close to midday as possible when using the stathmokinetic technique to minimize diurnal variation in crypt cell proliferation rate.

(b) METHOD OF ANAESTHESIA USED FOR ALL RATS
A combination of general and local anaesthesia was used in all cases. The rat was rendered unconscious by an intraperitoneal injection of dilute Nembutal. This was prepared by diluting a 60 mg./ml. solution of Pentobarbitone Sodium 10 times with Normal Saline (Abbot Labs.). The site of the incision was infiltrated with 2% Xylocaine solution (without Adrenaline). Rats were killed subsequently by an overdose of Ether.

(c) GENERAL METHODS USED FOR OPENING THE ABDOMINAL CAVITY (LAPAROTOMY)
The rat was lightly anaesthetized (to avoid respiratory depression) with intraperitoneal Nembutal, placed supine on a dissection board and restrained with adhesive tape. The abdominal wall was shaven, cleaned with alcohol, and infiltrated with 2% Xylocaine along the line of the proposed incision. The abdominal wall was then divided in the midline in layers.
When closing the abdomen, the margins were approximated in layers. The combined muscle and peritoneal layer was closed with a continuous 5-0 black silk suture and the fur was approximated with Michel Clips. Intramuscular Penicillin was given in all cases postoperatively in a dosage appropriate to the size of the rat.

(d) GENERAL METHODS USED IN OPENING THE CRANIAL CAVITY (CRANIOTOMY).

The rat was anaesthetized in the usual manner with intraperitoneal Nembutal and a small amount of 2% Xylocaine was injected into the base of the external ear (see A, Fig. 3.1).

This enabled the head to be stabilized between the ear bars of a head holder, painlessly. The remainder of the scalp was then shaved and infiltrated (see B, Fig. 3.1) with 2% Xylocaine solution.

A sagittal incision was made (see C Fig. 3.2), through the full thickness of the scalp to expose the periosteum, the margins of the wound being held apart by a self-retaining retractor.

The periosteum was then incised (see D Fig. 3.3) and scraped off the bone with a dry swab to expose bare bone in the area to be trephined.

A circular piece of bone (0.4 cms. in diameter) was removed over the calvarium of the skull (see D Fig. 3.3), using a dental trephine (see Appendix) and the area was continuously irrigated with normal saline to prevent overheating of the bone.

The bone was removed carefully, leaving the dura
mater intact. This was later divided with a knife blade.

Aspiration of cerebral tissue was performed by a small bore malleable sucker connected to low pressure suction (see Appendix). The sucker was cleansed and sterilized with Dettol solution. Haemostasis in brain tissue was achieved either by (1) aspirating with the sucker through a small gauze swab soaked in warmed normal saline (body temperature), or (2) by applying Gelfoam (Appendix). Bleeding from the cut bone margins was checked by the application of sterile bone wax (see Appendix).

The scalp wound was closed with interrupted black silk sutures (5/0) and Michel Clips. Penicillin was administered intramuscularly in a dosage appropriate to the size of the rat.

(e) COLLECTION, STAINING, AND SECTIONING OF SPECIMENS OF THE SMALL INTESTINE.

Unless otherwise indicated, all specimens of small intestine were approximately 2 cms. long and were collected from a point 5 cms. distal to the duodeno-jejunal flexure (designated proximal jejunum), or from a point 5 cms. proximal to the ileocaecal junction (designated distal ileum).

To obtain a "flat" specimen of bowel (after Tutton), during fixation the bowel was opened longitudinally, dipped into Bouin's solution for about 20 seconds and then flattened out on a piece of filter paper soaked in Bouin's solution. The specimen was then left adherent to the paper for approximately one minute,
Diagrammatic views of the dorsum of the rat head, showing the site of scalp incision.
after which time it remained "flat" and this facilitated longitudinal sections showing as much of the length of each crypt as possible.

Specimens of small intestine were fixed in 10% Bouins solution, dehydrated through alcohols, embedded in paraffin and then sectioned at 4\(\mu\)m. Sections of intestine were stained with Haemotoxylin and Eosin (see Appendix).

(f) STEREOTACTIC METHODS USED IN THESE EXPERIMENTS

The rat was anaesthetized and the scalp infiltrated with local anaesthetic, as described above. The scalp was clipped and the rat head fixed in a Stereotactic Instrument (see Appendix) by first placing the ear bars of the frame into the ear canals (which had been anaesthetized), so that they impinged on a ledge of bone immediately anterior to the ear drum. The ear bars were thus fitted snugly against the skull. Using the Vernier scale, the head was "centred" in the middle of the Stereotactic frame and the ear bars tightened so that the head pivoted freely about the axis of the ear canals.

The incisor bar was positioned immediately behind the upper incisor teeth and the nose clamp positioned over the nose to hold the head firmly.

The scalp was divided and the bone of the calvarium of the skull in the region of the proposed lesion cleared of periosteum (as previously described). Having found bregma and lambda (see Fig. 3.4), it was ascertained that these two points were on
the same horizontal plane before the skull holes were made. This was done by lowering the electrode to the surface of the skull at the level of lambda and bregma, respectively, and taking readings from the Vernier scale. Adjustments were then made to the incisor bar so that the dorsal surface of the skull was horizontal.

Usually, this meant that the incisor bar was 3-5 mms. below the level of the ear bars.

Because the skull suture lines are usually irregular, the approximate midpoint of the sagittal and coronal sutures was used to determine the position of the bregma and lambda.

Trephine holes were made over the region of the proposed entry of the electrodes, the dura mater was incised with a knife blade; and the electrodes were lowered to the level of the surface of the brain.

A reading was taken from the vertical Vernier scale, and using the stereotactic atlas the distance for the electrode to be lowered into the brain was added to this reading.

The other coordinates were similarly calculated using the bregma, lambda, and the sagittal suture as landmarks. The electrodes were lowered slowly and withdrawn slowly from the brain. They were cleaned after each lesion was made and the integrity of the insulated coating tested before each usage (see Appendix).

Haemostasis was achieved with Gelfoam, where necessary and the scalp closed (as previously described).
Dorsal view of the rat skull

A = Coronal suture
B = Sagittal suture
C = Bregma
D = Lambda
E = Occipital bone
F = Zygomatic arch
G = Nasal bone
The rat was placed in a jar containing a high concentration of Ether until respiration had just ceased, following loss of consciousness. A transverse incision was made from one side of the rat to the other, immediately inferior to the costal margin (see Fig. 3.5, A).

The diaphragm was incised to expose the heart whilst it was still beating. Two incisions were made along the lateral sides of the thorax (B, Fig. 3.5), and the flap (C, Fig. 3.5) was elevated towards the head. An incision was made into the left ventricle (D, Fig. 3.5), and an infusion cannula inserted (E, Fig. 3.5). This was held in position by plain forceps and advanced into the aorta.

Through this cannula, 40 mls. of 10% phosphate buffered Formalin was instilled under gentle pressure. An incision was made into the right atrium to allow outflow of perfusate and blood.

When the rat appeared to be fixed, using large scissors and bone nibblers, the head was excised, the occipital bone being partially removed to expose part of the cerebellum (to allow better infiltration of the the buffered Formalin.

The head was then stored in 10% buffered Formalin for at least several weeks before the brain was carefully removed from the skull.
Figure 3.5

Diagrammatic representation of the ventral surface of the rat,
(see text for description).
(h) CEREBRAL TISSUE SECTIONING AND STAINING

To examine histologically the part of the brain containing the lesions a block of brain tissue was removed such that the anterior and posterior surfaces of the block were parallel to the electrode tracks in the stereotactic plane.

The brain was rested so that the dorsal surface of the cerebrum and cerebellum was in contact with a flat horizontal surface (see Fig. 3.6). The vertical stereotactic plane was then at a 22° angle posterior to the perpendicular of the horizontal resting surface (D, Fig. 3.6).

Using a cutting guide constructed from perspex, and a razor blade, a cut was made approximately 2-3 mms. anterior to the area of the lesion at an angle of 22° posterior to the perpendicular of the horizontal resting surface and a similar cut was made posterior to the area of the lesion at the same angle.

Care was taken, as far as possible, to make the block of brain so removed as bilaterally symmetrical as possible.

The brain was then sectioned in 50 μm sections by means of a Lancer Vibratome, Series 1000 (see Appendix). Slides were coated (see Gel Gum, Appendix), the sections mounted and stained with Neutral Red (see Appendix).

Specimens of the pineal gland were fixed in 10% phosphate buffered Formalin, embedded in paraffin wax, sectioned at 5 μm, and stained with 1% Toluidine Blue (see Appendix).
Figure 3.6

Diagrammatic representation of the method of cutting blocks of fixed brain tissue for subsequent sectioning.

A = Rostral part of brain
B = Caudal part of brain
C = Perspex holder
D = The direction of cutting the brain
E = The direction of advancement of the brain within the perspex holder.

(Ref. "Neuroscience" by J.E. Skinner)
(1) METHOD OF STAINING NEURAL TISSUE AFTER VAGOTOMY

(After Rintoul, 1957)

A staining fluid consisting of 2% osmium tetroxide (25 mls.) and 3% Sodium Iodide solution (75 mls.), was made up immediately before use.

A small piece of tissue from the site of vagotomy was immersed in this solution for 24 hours in darkness.

The tissue was then washed in demineralized water and teased out on a slide in this water before mounting to remove excess osmic acid. The prepared tissue was floated on to a slide, rapidly dehydrated and mounted in "Depex".
(j) THE STATHMOKINETIC TECHNIQUE

This method of studying the crypt cell turnover rate relies on the ability of an agent, in this case Colchicine (Sigma Chemical Co., St. Louis, Mo., U.S.A.) to inhibit mitosis.

Rats were given intraperitoneal injections of Colchicine using the dose 0.1 mg./100 G. of body weight recommended by Tannock (1967), and killed at intervals between 1/2 hour and 4 hours after administration by an overdose of Ether. No rats were killed before 1/2 hour after administration of Colchicine because of the uncertainty that there would be full absorption of Colchicine from the peritoneal cavity before that time. Four hours was chosen as the maximum duration of Colchicine treatment because previous studies (Tutton, 1973a) had shown that, whilst some mitoses appeared pyknotic (darker than usual), the rate at which cells were accumulated in metaphase arrest during the fourth hour after injection of Colchicine did not differ significantly from that at which they were accumulated over the initial three hours of Colchicine treatment.

Counts of metaphase figures were made on each tissue specimen at high magnification, using light microscopy, in crypts sectioned longitudinally so that their glandular lumen could be identified throughout the lower one-fourth of their length. Only metaphase figures in the lowest ten cells on each side of the base of the crypt were counted. This procedure was adopted because in the rat all of these cells are proliferating, whereas some of the
more superficial crypt cells are differentiating and not dividing (Cairnie, Lamerton and Steel, 1965), (see Fig. 3.7).

Only mitoses (metaphase figures) in the focal plane of the crypt were counted to minimize errors due to section thickness and a correction factor for section thickness was thus not used (Tutton, 1973a).

Metaphase figures were identified according to the criteria of Stevens, Hooper (1961). All tissues were surveyed carefully for the presence of anaphase and telophase mitoses. If such mitoses were observed, the tissues of that particular rat were not included in the estimation of mitotic index, and that particular part of the experiment was repeated.

Paneth cells were excluded from all counting procedures. Since the mitotic rates derived from the counting of metaphase figures were only used for comparison of cell proliferation in rats of one experimental group with that in animals of another experimental group, no corrections were made for either the shape of the crypt (Tannock, 1967), or the relative size of the interphase nuclei and mitotic figures (Clarke, 1970).

All experiments were timed so that the rats were killed as close to midday as possible, to minimize errors due to diurnal variation in mitotic rate (Tutton, 1973a). Forty crypts were counted for each specimen of gut. The mean number of metaphases per 20 cells, per crypt was calculated (see Statistical Methods (k)), and from this figure the mitotic index (metaphase
index) was calculated, i.e. the proportion of cells in mitosis at the time of examination of the tissue. 

Graphs of mitotic (metaphase) index versus duration of colchicine treatment were constructed for each experimental group of tissues having mitoses collected for up to 4 hours, the mitotic (metaphase) index from each rat representing a point on the regression line. Using the least squares method (see Statistical Methods (k)), the slope (expressed as mitoses/cell/hour), and the standard error of the slope of the regression line were calculated.

The slope of the regression line describes the rate of accumulation of arrested metaphases following Colchicine. The measurement of the slope of this line gives an estimate of the "birth rate" or the rate of entry of cells into mitosis.

The statistical significance of apparent differences between the slopes of different regression lines was estimated by means of the "t" test (see Statistical methods (k)).
Figure 3.7

Diagrammatic representation of a typical small intestinal crypt. Estimates of metaphase index are made on the lowest 10 cells, (excluding the Paneth cells), which have exponential growth characteristics. The more superficial cells (B) include differentiating, non-dividing cells.
GENERAL COMMENTS ON STATHMOKINESIS

Stathmokinetic Agents

The most popular of these agents are -
Colchicine (and its derivative, Colcemid),
Vinblastine,
Vincristine.

These drugs act by interfering with the formation of the metaphase spindle, and thus cause cells to accumulate in the metaphase stage of mitosis.

An ideal stathmokinetic agent should have the following properties (according to Tannock, 1967) -
(a) At the dose level used and in the particular tissue under study, it should arrest all metaphases during the observation period, i.e. no telophases should been seen in the sections. As noted above, this principle was used in these experiments.

(b) The arrested metaphases should not degenerate into an unrecognizable state before the tissue is fixed and examined. In these experiments, by not exceeding a 4 hour period after Colchicine and in fact trying to keep below this time limit, the number of degenerating metaphases was minimized as was the error likely to occur when attempting to distinguish these degenerating figures from degenerating interphase nuclei.

(c) The metaphase arresting ability of the agent should not have adverse effects on interphase cells.

As far as is known this is not the case with the dosage of Colchicine used in these experiments.
Having satisfied these criteria as far as possible, an optimal dose was chosen (Tannock, 1967), so that a well-defined linear rate of accumulation of arrested metaphases over a reasonable period of time (4 hours) was obtained.

The value of the mitotic index is only a rough guide to the proliferative activity of a tissue because it depends on the duration of mitosis, as well as on the number (or proportion) of cells which are proliferating (Aherne et al., 1977).

An assumption is made in this case that the cells of the small intestinal crypts are in a steady kinetic state, not in an exponentially expanding state e.g. as in a tumour. There seems to be ample evidence from the literature that this is in fact the case.

Furthermore, since rats of the same species and sex maintained under the same conditions were used, it seems likely that the duration of mitosis would be similar in the rats used so that the mitotic index between different rats could be compared.

POSSIBLE ERRORS IN THE STATHMOKINETIC TECHNIQUE

(1) A sampling error at any particular site and a variation in proliferative activity from site to site in most tissues (Aherne et al., 1977).

(2) Error related to variation in the kinetic activity of a particular tissue between different rats.

These potential sources of error have been borne in mind in the discussion of the results derived from the present experiments and allowances made for them in deciding whether there was a real or apparent change in proliferative activity in a particular tissue.
Correctly applied, the stathmokinetic technique provides a useful kinetic parameter, the rate of entry of cells into mitosis or the cell "birth rate" (called in these experiments the mitotic rate). The method also has the advantage of giving results over a relatively short period of time compared with autoradiographic techniques and involves the study of paraffin sections only (Wright et al., 1980).

FURTHER LIMITATIONS OF THE METAPHASE ARREST TECHNIQUES

(1) THE DOSAGE OF THE METAPHASE ARRESTING AGENT

Tannock (1967) and Nome (1975) have shown that optimal dosages for the various agents may differ in the same tissue and that different tissues may have different optimal dosages for the same agent. Tannock found that an increase in the dose caused the metaphase index to increase. Thus, it was considered important in these experiments to give an adequate and constant dose of Colchicine.

Further increase in the dosage led to degenerating (pyknotic) metaphases becoming predominant and hence there was not only a loss of arrested metaphases but an actual decrease in the arrested metaphases as a proportion of the total number of cells.

High doses of Colchicine appeared to be toxic and to lead to rapid metaphase degeneration.

Clarke (1971) showed a decreasing number of arrested metaphases as the dosage of Colcemid increased from 1 to 5 mg./Kg.

(2) POSSIBLE EFFECTS ON THE RATE OF ENTRY OF CELLS INTO MITOSIS
An important requirement of the optimal dose is that it should not affect the flux of cells through other phases of the cell cycle.

Clarke (1971) concluded from a comparison of tritiated thymidine and stathmokinetic agents that metaphase blocking agents, at suitable doses, do not affect the rate of entry of cells into mitosis in the small intestine of the rat.

(3) ASSOCIATED STRESSFUL EFFECTS OF THE STATHMOKINETIC TECHNIQUE

This procedure requires a potentially stressful injection which could lead to the release of catecholamines and adrenocortical steroids, both of which may diminish proliferative activity (see Lit. Rev.). It is not known for certain how much variation between individual rats would have been in their reaction to this stress in these experiments but since comparisons are being made between groups of similar rats, in age and size, and subject to similar stresses, it seems probable that this factor would not affect valid comparisons of results in different rats to any extent.

(4) VARIATIONS IN METAPHASE FIGURE SIZE AFFECTING THE COUNT OF METAPEASES

Simnett (1968) considered metaphases arrested by Colcemid in certain circumstances were larger than interphase nuclei, whereas Clarke (1968, 1970) concluded that in other circumstances Colcemid arrested metaphases were smaller than interphase nuclei. Both suggested correction factors.
Tannock (1967) however, cast doubt on whether these correction factors were constant with time. Furthermore, in the crypts the cells in metaphase are nearer to the axis of the crypt than are interphase cells. Longitudinal sections (used in these experiments), may therefore include mitoses from columns of the crypt other than those whose interphase cells are being counted, and an overestimate of the mitotic index will result. These effects were minimized in the present series of experiments by using thin sections (4 μm.) and by counting only those metaphase figures (B, Fig. 3.8) which are in sharp focus and which are in focus when the luminal margin of the cells of the crypt is in sharp focus (A, Fig. 3.8).

(4) OBTAINING LINEARITY OF METAPHASE COLLECTION AND AN OPTIMAL PERIOD OF COLLECTION.

If e.g. only two readings of mitotic index were made at 0 and at 4 hours after Colchicine administration an assumption has been made that colchicine acts immediately after administration (which is known not to be so), and that metaphase collection was linear in form (which also cannot be assumed). Also, if Colchicine is administered by intraperitoneal injection then the rate of absorption is also much slower than after intravenous injection. Taylor (1965) obtained linearity with Colchicine up to eleven hours but there was considerable metaphase degeneration. To overcome these difficulties as far as possible collection of metaphases in these experiments was commenced at 1/2 hour (at the earliest)
Figure 3.8
Diagrammatic representation of a longitudinal section of a small intestinal crypt showing the luminal margin (A), and the position of a typical metaphase figure (B).
and continued no longer than 4 hours with 5 to 8 readings of mitotic index. The cell birth rate was thus measured over the linear portion of the collection line, as suggested by Tannock (1967).
(k) STATISTICAL METHODS USED IN THESE EXPERIMENTS

(1) CALCULATION OF THE MITOTIC INDEX, (METAPHASE INDEX).

In these experiments this was derived from the proportion of the crypt cells arrested in metaphase compared with the total number of cells examined in each crypt.

The results were grouped with regard to the frequency of occurrence of counted metaphases in relation to the possible numbers of metaphases which could possibly be counted per specimen.

(a) The mean metaphase count per specimen of gut at any particular time after administration of Colchicine was calculated. This was the arithmetic mean of grouped data (Connolly and Sluckin, 1962, p 22).

(b) Since 10 crypt cells were examined on either side of the lowest part of the crypt lumen, the total number of crypt cells examined per crypt = 20.

Hence, Mitotic Index = \[ \frac{\text{The mean metaphase count}}{\text{The number of cells examined per crypt}} \]

The standard deviation of the mitotic index for each time interval after the administration of Colchicine was determined by calculating the standard deviation for grouped data (Connolly and Sluckin, 1962, p 44), and dividing this figure by the total number of cells examined per crypt.

(c) Having determined the mitotic index, and knowing the time intervals after Colchicine administration, the relationship between these two parameters was
examined by drawing a graph of the mitotic index versus times after Colchicine administration and the correlation between these was assessed by the degree of "scatter" around the "line of best fit" (see below).

If the degree of inclination of the regression line was "steep" this was considered to denote a more rapid "birth rate" of the crypt cells than if the degree of inclination of the regression line was "more horizontal". The mitotic rate, calculated from the slope of the regression line (see below), was expressed as mitoses/cell/hour.

Providing there was fairly good correlation i.e. relatively little "scatter" of values on the line (see below) the "slopes" of regression lines from different rats could be compared (within certain limits) and thus the "birth rate" in the crypts of the small intestine of one rat could be compared with the "birth rate" in the crypts of the small intestine of another rat.

**CALCULATION OF THE "SLOPE" OF THE REGRESSION LINE**

This was calculated using the method of least squares to find the "line of best fit" (see Mould, 1976, p 56).

The slope of the line = a, and the intercept of the line = b, were determined by the following formulae (see Fig. 3.9).
Figure 3.9
Typical graphical representation of the relationship between mitotic index, (y), and time, (x). The slope of the line = a and the intercept = b.
(assuming a linear relationship)
Intercept \( b \) = \[ \frac{\sum(X).\sum(XY) - \sum(X^2) \cdot \sum(Y)}{[\sum(X)]^2 - N \cdot [\sum(X^2)]} \]

Where \( N \) = the number of points on the "scatter" diagram.

\( \sum = \) the sum of

\( Y \) = mitotic index,

\( X \) = time after administration of Colchicine.

The slope \( a \) is expressed by the formula,

\[ (a) = \frac{\sum(X).\sum(Y) - N \cdot [\sum(XY)]}{[\sum(X)]^2 - N \cdot [\sum(X)]} \]

To calculate the Standard Error of this slope, the following formula was used --

\[ \sigma_a = \sqrt{\frac{N \cdot [\sum(\Delta Y)^2]}{(N-2) \cdot \left[ \frac{\sum(\Delta X)^2}{N} - \frac{\sum(X)^2}{[\sum(X)]^2} \right]}} \]

Where \( \sum(\Delta Y)^2 \) is the sum of the squares of the deviation of each point from the line.
The Y value of the point on the graph by observation - (ax + b), which is the Y value of the point on the line calculated using the straight line formula.

Having determined the slope (i.e. the rate of birth of cells in mitoses/cell/hour), and the standard error of the slope, it was then essential to compare the slopes from different tissues and the "t" test was used for this comparison.

To determine whether the two linear trends were statistically significantly different from one another, the standard error of the difference between the two slopes was calculated from the formula (as suggested by Dr. P. Tutton of Monash University).

\[
SE_{b_1 - b_2} = \sqrt{WSE_{b_1}^2 + WSE_{b_2}^2}
\]

where the \( SE_{b_1} \) and \( SE_{b_2} \) on the right are standard errors of the slopes, (see p 3.28).

The weights, \( W_1 \) and \( W_2 \) are given by -

\[
W_1 = \frac{(n_1-2)}{(n_1+n_2-4)} \left\{ \frac{\sum (\Delta y_1)^2 + \sum (\Delta y_2)^2}{\sum (\Delta y_i)^2} \right\}
\]

and

\[
W_2 = \frac{(n_2-2)}{(n_1+n_2-4)} \left\{ \frac{\sum (\Delta y_1)^2 + \sum (\Delta y_2)^2}{\sum (\Delta y_i)^2} \right\}
\]
Comparing the difference between the two slopes with zero -

\[ t = \frac{(b_1 - b_2) - 0}{SE_{b_1 - b_2}} \]

where \( b_1 \) and \( b_2 \) are the respective values for the slopes in mitoses / cell/ hour. Using this formula, the value for "t" was calculated for a certain number of degrees of freedom.

The number of degrees of freedom ( \( df \) ) was calculated from the formula-

\[ (n_1 - 2) + (n_2 - 2) = n_1 + n_2 - 4, \]

\( n_1 \) and \( n_2 \) being the sizes of the two data sets in question.

From the tables for expected values of "t" for the two-tailed probability levels of \( P \) the values of "t" for the number of degrees of freedom used in the comparison was read off ( see Bliss, 1967, p 510-511). If the calculated value for "t" exceeded the table value for "t" the two tailed probability for this value to exceed the table value for "t" could be determined.

On the advice of Mr. P. Leppard of the Department of Statistics, University of Adelaide, only statistical differences significant at least at the 5 % level were considered to be valid in drawing conclusions regarding differences in mitotic rates between two data sets. On his advice also, the 95 % confidence intervals were not used for this comparison, as there appeared to be no additional advantage to be gained from this additional comparison.

To determine how closely the relationship between mitotic index and time of administration of Colchicine approached a straight line, the regression coefficient was calculated, as in Mould (1976, p 59). The regression coefficient (correlation coefficient) was determined by the following formula-
A perfect correlation i.e. all points on the line and no "scatter" of results would be denoted by \( r = 1 \). No correlation or widespread "scatter" of the points contributing to the line would be denoted by \( r = 0 \). A value for \( r = 0.92 \) would be considered satisfactory correlation for the points. This was calculated in all cases but has only been recorded in this thesis where there is no accompanying graphical representation of the relationship between mitotic index and time.
In calculating the mean and standard deviation of ungrouped data e.g. the values for pH determinations or measurements of the amounts of food eaten by rats, the following formulae were used.

\[
M = \frac{\sum X}{N}
\]

where \( M \) = the arithmetic mean
\( \sum X \) = the total sum of the values
\( X \) = an individual value
\( N \) = the number of measurements made

\[
\sigma = \sqrt{\frac{\sum X^2}{N} - \left( \frac{\sum X}{N} \right)^2}
\]

where \( \sigma \) = the Standard Deviation
\( X \) = the deviation of a value from the mean (as calculated).
\( N \) = the total number of measurements made.
(see Connolly and Sluckin, 1962).

The mean and standard deviation of these values having been calculated, they were compared statistically for significant difference by a modification of the "t" test (used where there are relatively few values and the means are relatively close).

The standard deviation of the difference of the means

\[
= \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}
\]
Where \( S_1 \) is the standard deviation for one mean, and \( S_2 \) is the standard deviation for the other mean. 
\( n_1 \) = the total number of values for one mean 
\( n_2 \) = the total number of values for the other mean.

\[
t = \frac{\text{The difference between the means}}{\text{The standard deviation of the difference of the means}}
\]

the "t" value may be read off the table for critical points of the "t" distribution (as before), knowing the degrees of freedom (as previously calculated). However, as a rough approximation, where the number of values was relatively few, for \( P < 0.01 \), the difference between means was considered to be probably significant if "t" > 2 and to be definitely significant if "t" was > 3.
CHAPTER IV

EXPERIMENTAL INVESTIGATIONS
SECTION I

THE EFFECTS OF DENERVATION AND/OR ELECTRICAL STIMULATION OF THE NERVE SUPPLY OF THE SMALL INTESTINE

(1) Introduction to section 1 experiments,

(2) Experiments

(a) The effects on crypt cell proliferation of division of the trunks of the abdominal vagus nerves,

(b) The effects on crypt cell proliferation of stimulation or sham stimulation of the abdominal vagus nerves,

(c) The effects of local denervation of the small intestine or crypt cell proliferation,

(d) The effects on crypt cell proliferation of local denervation of an implanted loop of small intestine.

(3) Discussion on section 1 experiments.
INTRODUCTION TO SECTION I EXPERIMENTS

As indicated in the literature review, there is considerable variance of opinion concerning the effects of truncal vagotomy on crypt cell mitotic rate in the small intestine with no uniform effect being reported.

As previously reported by this author (Callaghan, 1979) the effects of vagotomy were found to be a relatively small increase in crypt cell mitotic rate after several weeks. It was decided therefore to repeat the effects of truncal vagotomy, using a different method of statistical comparison of the results so that these results could act as a baseline for comparison of combined vagotomy and removal of the pineal gland (see Chapt. IV, Section II).

In this section the effects of vagal stimulation were determined so that they could be compared with those of vagotomy, on one hand, and with the effects of stimulation of the sympathetic nerves of the small intestine on the other (see Chapt. I, Section II).

In this way the relative importance of the parasympathetic as opposed to the sympathetic components of the autonomic nervous system in the control of crypt cell proliferation in the small intestine could be assessed.

Finally, the operation of local denervation (including sympathetic nerves), of a loop of small bowel entails division also of the local blood supply, although the collateral circulation is left intact as far as possible. Hence, to minimize the effect of local ischaemia on crypt cell proliferation (see Rijke, 1976) during local denervation a loop of small intestine was implanted in the anterior abdominal wall for five months so that it developed an accessory blood supply derived from the anterior abdominal wall which was not associated with
its usual sympathetic nerve supply via the branches of the superior mesenteric artery. It was intended that when the local denervation was later performed, the involved loop of bowel would remain adequately vascularized but not innervated by visceral sympathetics, thus separating the effects of these two factors on crypt cell proliferation. Even if the blood supply of the loop was not at the same level as previously, it would certainly be better than if the loop had not been implanted, and thus the effect of local denervation should be different if vascular factors were of prime importance in the effect of local denervation on crypt cell proliferation.

THE EFFECT OF BILATERAL TRUNCAL ABDOMINAL VAGOTOMY ON THE CRYPT CELL PROLIFERATION RATE IN THE SMALL INTESTINE.

METHODS

Twenty-three male Sprague-Dawley rats, weighing between 420-550 Gms. were randomly divided into 4 groups, anaesthetized (as in Chapt. 111(b)), and laparotomy was performed in all groups (as in Chapt. 111(c)).

In two of these groups, each of 5 rats, the trunks of the anterior and posterior vagus nerves were divided below the diaphragm (using as a guide the description of the vagus nerve in the rat by Legros and Griffith (1969). Any filaments of the vagus nerve lying on the oesophagus were removed using low magnification of the dissecting microscope. The stomach and small intestine were thus deprived of their vagal innervation.

The remaining 2 groups of rats were subjected to laparotomy only.

Penicillin was administered to all rats post-operatively and the rats were maintained on food and water ad libitum.

One of the groups subjected to vagotomy was killed after
one week, and the other after two weeks.

Similarly, one group subjected to laparotomy only (n=5), was killed after one week, and the other group (n=8), after two weeks.

All rats were killed by an overdose of Ether at intervals, between 1 hour and 4 hours after intraperitoneal colchicine. Specimens of small intestine were collected, sectioned and stained as described in Chapt. 111 (e). The mitotic rate was determined, as in Chapt. 111 (j) and (k).

Besides the use of silver staining at the site of vagal section to examine the nerve endings (as in Chapt. 111(i)), measurement of the gastric content pH was made post-mortem by suspending the gastric contents in normal saline and using a digital pH meter.

**TABLE 4.1**

**RESULTS**

Mitotic rate in the small intestine following-

<table>
<thead>
<tr>
<th>(1) ONE WEEK AFTER VAGOTOMY</th>
<th>(2) ONE WEEK AFTER LAPAROTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.0768 ± 0.0021</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0584 ± 0.0053</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.0705 ± 0.0629</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0023 ± 0.0023</td>
</tr>
</tbody>
</table>

Results in mitoses/cell/hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2
P<0.02, t = 3.345, df = 6, i.e. significant at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P<0.05, t= 2.451, df= 6, i.e. significant at the 5% level.
TABLE 4.2

Mitotic rate in the small intestine following-

<table>
<thead>
<tr>
<th>TWO WEEKS AFTER</th>
<th>TWO WEEKS AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAGOTOMY</td>
<td>LAPAROTOMY ONLY</td>
</tr>
<tr>
<td>PROXIMAL</td>
<td>0.0866 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0086 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.0889 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0023 ±</td>
</tr>
<tr>
<td></td>
<td>0.0605 ±</td>
</tr>
<tr>
<td></td>
<td>0.0031 ±</td>
</tr>
<tr>
<td></td>
<td>0.0651 ±</td>
</tr>
<tr>
<td></td>
<td>0.0018 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/cell/hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2,
P<0.01, t= 3.262, df = 9, i.e. significant at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P<0.00005,
t = 8.814, df = 9 , i.e. significant at the 5% level.

Marked gastric dilatation was observed post-mortem in all vagotomized rats when compared with similar controls. The mean pH of the gastric contents, one week and two weeks after vagotomy (4.40 ± 0.01, 5.23 ± 0.32, respectively) was found, using a modified "t" test (see Chapt. 111(k)), to be statistically significantly elevated over the mean pH of the gastric contents of ten rats of similar type and weight not subjected to vagotomy (3.51 ± 0.13), (P<0.01) i.e. significant at the 5% level. As previously mentioned, the completeness of vagotomy was also supported by staining for degenerating nerve fibres at the sites of transection of these nerves.

DISCUSSION

The results indicate completeness of vagotomy in all rats. Thus, whilst the increase in crypt cell proliferation rate after vagotomy was marginally significant statistically in the case of the distal ileum one week after operation, the significance was more definite in the case of the proximal jejunum. Furthermore, two weeks after vagotomy the mitotic rate was more definitely raised in both proximal jejunum and distal
ileum than after one week, when compared with controls. These findings will be more completely discussed later.

\[0.36 \pm 0.12\]

\[0.28 \pm 0.16\]

\[0.08 \pm 0.12\]

\[0.04 \pm 0.04\]

Graph of mitotic index versus time.

\[X = \text{Proximal jejunum, one week after bilateral truncal vagotomy.}\]

\[\bullet = \text{Proximal jejunum, one week after laparotomy.}\]
Graph of mitotic index versus time

X = Distal ileum, one week after bilateral truncal vagotomy.

● = Distal ileum, one week after laparotomy
Figure 4.3

Graph of mitotic index versus time

\( X = \) Proximal jejunum, two weeks after truncal vagotomy.

\( \bullet = \) Proximal jejunum, two weeks after laparotomy.
Graph of mitotic index versus time

X = Distal ileum, two weeks after truncal vagotomy.

○ = Distal ileum, two weeks after laparotomy, only.
4.10

(b) **THE EFFECTS ON CRYPT CELL PROLIFERATION OF STIMULATION OR SHAM STIMULATION OF THE ABDOMINAL VAGUS NERVES.**

**METHODS**

A group of ten male Sprague-Dawley rats, weighing 300-500 Gms. was divided into two groups, each of five rats.

All rats were anaesthetized (as in Chapt. 111(b)), and laparotomy performed (as in Chapt. 111(c)).

**GROUP (1) STIMULATION OF BOTH TRUNKS OF THE VAGUS NERVE, (5 Rats).**

Through the abdominal incision, the lower end of the oesophagus was exposed. A bipolar electrode composed of twisted well insulated copper wire was placed in close contact with the lower end of the oesophagus near the oesophago-gastric junction. The electrode was then covered throughout its length by a thick layer of insulating bone wax (see Fig. 4.5). The wound was closed around this electrode to help maintain the usual temperature around the stimulated area. Colchicine was given into the peritoneal cavity. Electrical stimuli were generated using a Grass model SD9 stimulator. A submaximal pulse rate of 10 per second and pulse duration of 10 m. sec., at 20 volts was used. This was noted to produce a contraction of the abdominal wall and a sustained contraction of the stomach wall. Volleys of impulses, of 10 seconds duration, were given every 15 minutes from the time that colchicine was given until the time the rats were killed, at intervals between 1 hour and 3 3/4 hours later.

**GROUP (2) SHAM VAGAL STIMULATION (5 Rats)**

The same procedure was followed, except that no electrical impulse was passed through the electrodes. The rats were
killed at intervals between 1/2 hour and 4 hours after intraperitoneal colchicine.

RESULTS

TABLE 4.3

Mitotic rate in the small intestine following-

<table>
<thead>
<tr>
<th>(1) VAGAL STIMULATION</th>
<th>(2) SHAM VAGAL STIMULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.0694 ± 0.0063</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0594 ± 0.0044</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

Note that there is no statistical difference on comparison of standard errors.
Figure 4.5

Diagrammatic representation of the positioning of the electrodes in stimulation of the vagus nerves.

Note the positive electrode is placed more proximally on the oesophagus.

A = The anterior vagus nerve.
B = The posterior vagus nerve.
DISCUSSION

It appears that electrical stimulation of the trunks of the abdominal vagus nerves as they lie on the oesophagus, of sufficient strength and duration to produce contraction of the stomach wall, is not associated with a statistically significant change in crypt cell mitotic rate in the proximal jejunum or distal ileum, over the duration of the stimulation period. In the placement of electrodes the description of the vagus nerves of the rat by Legros and Griffith (1969) was used as a guide.

During vagal stimulation, care was taken to ensure—

(1) adequate contact with the vagi and the lower oesophagus,
(2) adequate insulation of the electrodes from the anterior abdominal wall and the other viscera, to prevent dissipation of the electrical stimuli.
(3) that the voltage was sufficient to produce stimulation, by testing whether the voltage used could produce contraction of the anterior abdominal wall and the stomach wall.

Furthermore, the voltage used, pulse rate and duration of the experiment were deliberately chosen to be similar to those used in stimulation of the mesenteric nerves by Tutton (1975a), in which there was an obvious effect obtained on electrical stimulation of the mesenteric nerves over a similar duration, in similar rats. Thus, a comparison could be made of the relative degree of change in crypt cell proliferation rate in either case.

This experiment has not of course excluded a different effect of vagal stimulation on the intestinal crypts if the stimulation was of longer duration or a different voltage.
(c) THE EFFECTS OF LOCAL DENERVATION OF THE SMALL INTESTINE ON CRYPT CELL PROLIFERATION, (AFTER 5 DAYS).

METHODS

Five male Sprague-Dawley rats weighing 450-500Gms. were anaesthetized (as in Chapt. 111(b) ) and laparotomy was performed (as in Chapt. 111(c)). Two loops of jejunum (B in Fig. 4.6) and ileum (C, Fig. 4.6), respectively, were subjected to local denervation, (as described by Tutton and Helme (1974) and based on the experimental work of Schofield (1960)).

Two successive neurovascular bundles were ligated and divided (F, Fig. 4.7), in such a way that a collateral circulation could reach the affected segment (D, Fig. 4.7). The abdomen was closed, Penicillin administered and food and fluids given ad libitum. Five days later, the rats were killed by an overdose of Ether at intervals between 1 and 4 hours after intraperitoneal Colchicine. Specimens were obtained from the midpoint of the affected segments of proximal jejunum and distal ileum (D, Fig. 4.7), sectioned and stained (as in Chapt. 111(e)).

The mitotic rate was determined (as in Chapt. 111(j) and (k)).

RESULTS

| TABLE 4.4 |

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th>(1) ONE WEEK AFTER LOCAL DENERVATION</th>
<th>(2) ONE WEEK AFTER LAPAROTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL 0.0368 ±</td>
<td>0.0584 ±</td>
</tr>
<tr>
<td>JEJUNUM 0.0030 ±</td>
<td>0.0053 ±</td>
</tr>
<tr>
<td>DISTAL 0.0357 ±</td>
<td>0.0629 ±</td>
</tr>
<tr>
<td>ILEUM 0.0010 ±</td>
<td>0.0023 ±</td>
</tr>
</tbody>
</table>

Results in mitoses / cell / hour.
Diagrammatic representation of local sympathectomy of the small intestine.

A = Transverse colon.
B = Proximal jejunal loop.
C = Distal ileal loop.
D = The midpoint of the sympathectomized loop.
E = Anastomotic vascular channels.

F = Point of ligation and division of the neurovascular bundle.
G = Arterial arcade.
PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 1,
P<0.01, t = 3.724, df = 6, i.e. significant at the 5% level.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P<0.00005,
t = 11.826, df = 6, i.e. significant at the 5% level.

DISCUSSION

Thus, local denervation of either a loop of proximal jejunum or distal ileum is associated with a statistically significant decrease in crypt cell mitotic rate when compared with the effect of laparotomy alone. Local sympathectomy of the small intestine, as described by Tutton and Helme (1974) is in fact the same procedure as local denervation of a segment of bowel, without division of the intramural plexuses, as described in this experiment. Tutton and Helme (1973, 1974) have examined the process of subsequent intramural plexus degeneration histochemically, confirming the efficacy of this method.

Schofield (1960) found that, in histological studies, no more than 3 consecutive neurovascular bundles could be ligated and divided in rats without producing apparent stasis of blood in the operated segment. Tutton and Helme (1974) did not observe any histological evidence of local bowel ischaemia after local denervation for 5 days. In the present instance only 2 neurovascular bundles were ligated, and macroscopically there were no signs of ischaemia, as indicated by colour changes in the affected bowel at operation and subsequent histological examination. A similar length of small intestine was used in the present experiment as used by Schofield (3-7 cms.). He found an appreciable reduction in the number of nerve fibres at the middle of the operated segment within 24 hours and a more
pronounced reduction within 3 days after division of the mesenteric nerves. The reduction involved the plexuses in each layer of the intestine. There was progressively greater reduction in the number of nerve fibres in the operated segments over the next 72 hours. He noted no further reduction in the number of nerve fibres in rats surviving longer than 3 days post-operatively. Thus, at the period of 5 days post-operatively selected for the present experiments, maximal degeneration of the nerve fibres could be expected.

Tutton and Helme (1974) noted that both surgical (i.e. local denervation as in the present experiments), and chemical sympathectomy (intravenous 6-hydroxydopamine), resulted in similar histological changes, i.e. complete absence of varicose fluorescent material in nerve terminals usually seen around the jejunal arterioles in the myenteric ganglionic plexus, and in scattered nerve terminals in the submucosal and muscular layers of the jejunum (Gabella and Costa, 1968). However, they noted that enterochromaffin and mast cells, both of which contain biogenic amines, had a normal formaldehyde induced fluorescence appearance in denervated jejunal segments.

In both local denervation or chemical sympathectomy, there was a similar fall in crypt cell proliferation, (not a rise as might have been expected if vagal nerve fibres had been excised), suggesting that principally sympathetic nerve fibres were involved in the local denervation. However, Tutton (1975) suggested that whilst chemical sympathectomy slowed down crypt cell proliferation, total surgical denervation of a loop of small intestine caused a much greater slowing down of crypt cell mitotic rate. He suggested that innervation other than adrenergic probably also influenced crypt cell proliferation. This suggestion was supported by the finding that stimulation
of the neurovascular bundles to the small intestine produced a rise in crypt cell mitotic rate which was not solely depend-
ext upon the integrity of the sympathetic nerves because it was demonstrable in chemically sympathectomized rats. Thus, local denervation of a segment of small bowel has at least the same qualitative effect on crypt cell mitotic rate as chemical sympathectomy, suggesting that the effect of local denervation is due mainly to division of sympathetic nerve fibres, but not exclusively so. This is consistent with the findings of Malmfors et al. (1981), who found that in pigs following vagotony the innervation pattern of the jejunum appeared comp-
letely unaffected. However, following complete denervation by autotransplantation of a jejunal segment, the adrenergic fibres disappeared, whilst the peptide containing and acetylcholinester-
erase positive nerve fibres remained, apparently unaltered.

The fall in crypt cell birth rate noted in the present experiment, following local denervation, is thus consistent with the findings of Tutton and Helme (1973, 1974). In den-
ervating a segment of small bowel locally, it is necessary to also divide two adjacent mesenteric vessels. Although these were divided as far proximally as possible, to minimize vas-
ular disturbance in the affected loop, there remained the possibility that the fall in crypt cell mitotic rate might be due to local ischaemia (Rijke et al., 1976). An attempt to exclude this factor has been made in the experiment which follows.
Figure 4.8.

Graph of mitotic index versus time.

$X$ = Proximal jejunum, five days after local denervation

$\bullet$ = Proximal jejunum, seven days after laparotomy.
Graph of mitotic index versus time

X = Distal ileum, five days after local denervation.

○ = Distal ileum, seven days after laparotomy.
THE EFFECT ON CRYPT CELL PROLIFERATION OF LOCAL DENERVATION IN AN IMPLANTED LOOP OF SMALL BOWEL.

METHODS

Nine male Sprague-Dawley rats weighing between 250-524 Gms. were anaesthetized (as in Chapt. 111, b) and laparotomy was performed through a long midline incision (see Chapt. 111, c). The left margin of the incision was everted so that the parietal peritoneum was exposed and a longitudinal incision was made through the parietal peritoneum into the underlying muscle of the anterior abdominal wall (B, Figs. 4.10), to create a "trench" deep enough to cover a loop of small intestine.

A loop of jejunum, approximately 5 cms. in length, from a site 5 cms. distal to the duodenojejunal flexure was placed into this "trench", i.e. a loop of sufficient length to be supplied by two consecutive neurovascular bundles. The musculo-peritoneal defect was then closed around the bowel loop, as far as possible, so that only the mesenteric border of the loop was free within the peritoneal cavity (C, D, Figs. 4.11). The abdomen was then closed, Penicillin administered, and food and fluids given ad libitum. The purpose of this procedure was to allow the loop to develop an alternative blood supply from the abdominal wall (which was not accompanied by its principally sympathetic nerve supply following the mesenteric vessels).

Five months later, the abdomen was reopened and it was found that the implanted loop was part of the anterior abdominal wall or attached to it by a short mesentery with the newly developed vessels visible in this new "mesentery". The two neurovascular bundles supplying the affected loop were ligated and divided with 5X0 black silk sutures, taking care not to interfere with the loop in any other way. The abdomen was closed, Penicillin administered, and food and fluids
supplied ad libitum. Five days later, the rats were killed by an overdose of ether, at intervals between 1 and 4 hours after intraperitoneal Colchicine. The affected loop was dissected free of the anterior abdominal wall, fixed in Bouin's solution and both longitudinal and transverse sections were made, including the part of the abdominal wall immediately adjacent to the affected loop (F, Figs. 4.11). These specimens were sectioned and stained (as in Chapt. 111, e). The transverse section was used for detecting the presence, microscopically, of blood vessels invading the loop from the anterior abdominal wall. The sections were also examined histologically for the presence of signs of ischaemia. The mitotic rate was determined (as in Chapt. 111, j and k).

**RESULTS**

<table>
<thead>
<tr>
<th></th>
<th>LOCAL DENERVATION</th>
<th>CONTROL LOCAL DENERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL LOOP</td>
<td>0.0339</td>
<td>0.0368</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0012</td>
<td>0.0030</td>
</tr>
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Results in mitoses / cell / hour.

Note that there is no statistically significant difference on comparison of the standard errors.
Diagrammatic representation of the implantation of a jejunal loop (C) into the substance of the anterior abdominal wall (D), the parietal peritoneum (A) being cut at (B). A block for transverse section (F) was removed from the excised, previously implanted loop (E).
Figure 4.12 - Photomicrograph of a transverse section of the loop of jejunum implanted into the anterior abdominal wall, showing (A) numerous small blood vessels invading the sero-muscular layer of the bowel from the surrounding anterior abdominal wall, (Haemotoxylin and Eosin stain, X 150).
Figure 4.13 - Low magnification photomicrograph of a transverse section of the loop of jejunum implanted into the anterior abdominal wall. Note that villus height is not obviously diminished. (Haemotoxylin and Eosin stain).
Figure 4.14

Graph of mitotic index versus time.

X = Proximal jejunum, delayed local denervation.

o = Proximal jejunum, non-delayed local denervation.
DISCUSSION

It is evident from examination of the histological specimens that revascularization of the implanted loop of small bowel from the anterior abdominal wall has occurred very successfully over the 5 month period of implantation (see A, Fig. 4.12, fig. 4.13). This secondary blood supply does not carry with it the usual predominantly sympathetic innervation via the superior mesenteric artery plexus, so that when the vascular pedicles were ligated in the process of local denervation, the loop of small bowel involved had a blood supply from the anterior abdominal wall which did not carry the usual predominantly sympathetic innervation to the bowel. Subsequent local denervation by ligation of the neurovascular pedicles, whilst removing this innervation, allowed at least most if not all of the blood supply of the affected loop to remain intact. If this secondary blood supply to the loop was deficient, there would be expected to be ischaemic effects on the crypts, indicated by a diminished crypt cell mitotic rate (Rijke et al., 1976). Furthermore, there were no histological signs of ischaemia.

Since the crypt cell mitotic rate in the implanted loop was not found to differ statistically from that in the loop which had been denervated for 5 days only, it appears that the effect of the denervation on the crypt cell mitotic rate is primarily a neural rather than a vascular one.

GENERAL DISCUSSION ON THE EXPERIMENTAL FINDINGS IN SECTION 1

Since truncal abdominal vagotomy is associated with a rise in crypt cell proliferation rate in the small intestine, the vagus nerves are apparently not trophic to the crypts, as suggested by some authors (see below). In another study (Callaghan, 1984) this was supported by the finding that
there was a lack of obvious dystrophic changes in the small intestinal villi on scanning electron microscopy, between 2 and 9 weeks after truncal abdominal vagotony. It should be noted, however, that in this latter study, only gross changes in the surface of the villus structure would have been noted and no information gained concerning the crypts or the density of the villi. The present findings are in accord with research reports by Mamontov (1979) and Alpers and Kinzie (1973). They, too, found that vagotomy had a stimulatory effect on crypt cell proliferation in the small intestine.

However, in contrast to the present findings, Silen et al. (1966), Musso et al. (1975), Ballinger et al. (1964), Lachat and Goncalves (1978), Liavag and Vaage (1972) found an initial decrease in small bowel crypt cell proliferation rate after truncal vagotony, suggesting a trophic role for the vagus nerves. Additionally, Silen et al. (1966), Musso et al. (1975) and Liavag and Vaage (1972) reported a later hyper-proliferative response. Ellis and Pryse-Davies (1967) found no histological or histochemical changes in the intestine after vagotomy. Of course, this does not exclude cell kinetic changes of a minor nature.

There thus seems to be little uniformity of opinion on the effects of vagotomy on the crypts in the literature. Some of these differences are probably explainable by species differences, or differences in the duration of the response to vagotony between various strains of the same species. It is not known, for instance, if there was a minor fall in crypt cell production in the rats studied in the present experiments during the first week after vagotony. We can say that as far as the strain of rats studied in the present experiments is concerned, a sustained rise in crypt cell mitotic rate can be expected after one week, and this information will be
applied in section II and section III of this thesis when the effects of vagotomy are combined with those of pineal or limbic lesions. A survey of the relevant literature shows that there is considerable variation in the methods used to assess the crypt cell mitotic rate. For example, in some instances assessments of mucosal size are made rather than an accurate stathmokinetic technique, as in the present experiments.

It is interesting to note that Tsibulevskii and Eletskii (1976) found early combined inflammatory and vasomotor degenerative changes in the jejunum after vagotomy. It is possible that, in view of the other experimental findings, there could have been coincident vascular interference in this case. This raises the question whether the effect of vagotomy on the intestinal mucosa could be a vascular one, as suggested by Ballinger (1965 a) who showed that vagotomy resulted in a profound decrease in mesenteric blood flow. However, Rijke (1976) showed that ischaemia of the mucosa should be associated with a decreased crypt cell production rate, so that the present experimental findings do not appear to support an explanation on the basis of post vagotomy ischaemia.

Dowling (1982) has suggested that abolition of Cholecystokinin release after vagotomy may play a role in the response after vagotomy. The present experimental findings do not throw any light on this possibility. Another possible factor in the effects of vagotomy on the crypts is via the action of gastrin, which is not of course definitely supported by many investigators as a factor in crypt cell proliferation control (see Chapt. I Section IIII). However, Uvnäs- Wallensten et al. (1977), Stadil (1974), Malmstrom et al. (1977) have all related gastrin secretion to vagal function.
Silen et al. (1966) have suggested that the post-vagotomy changes may be due to changes in the luminal bacterial flora, which can affect the crypt cell mitotic rate (see Chapt. I, Section 11). It is also possible that the vagus nerves may act directly on the crypt cells without intermediary mechanisms. Whilst the crypt cell proliferation rate one week after vagotomy was significantly greater than in controls (albeit marginally in the case of the distal ileum), the effect was more significant after two weeks. It is interesting to note that three weeks after vagotomy (see Chapt. IV, Section 111) the rise in crypt cell proliferation rate is of a similar order of magnitude. It appears therefore that there is a gradual rise in proliferation rate up to two weeks and this rate is maintained at more or less the same level up to three weeks after vagotomy.

Tutton (1975a), using a similar voltage, pulse rate and duration of electrical stimulation in a similar strain of rats found that electrical stimulation of the mesenteric nerves to the small intestine was associated with an obvious increase in crypt cell mitotic rate. In the present experiment, however, vagal stimulation was not associated with any significant change in crypt cell proliferation rate. This may mean that the effect of vagal stimulation on the crypts is much slower after vagal stimulation, and that had vagal stimulation continued for a longer duration, there might have been an effect or it may mean that vagal efferent influence over the crypts is less than the efferent influence of other mesenteric nerves e.g. sympathetic nerves. In this context it may be significant that Andrews (1986), in a review of the topic, noted that many studies have indicated that the abdominal vagus is composed of 80-90% afferent fibres in non-ruminants, a finding supported
by Agostini et al. (1957), and Evans and Murray (1954). Thus the vagus nerves may perhaps be more important in the control of crypt cell proliferation as mediators of afferent information from the crypts to a hypothetical central neural centre which is concerned with crypt cell proliferation control.

It should be noted that when the effects of vagotomy in the present experiments are contrasted with the effects of local denervation, there is not a great difference in the relative quantitative effects of the two procedures on the crypts. However, the qualitative effects of vagal and mesenteric stimulation are very different.

It is possible that the vagus nerves may relay information regarding the physical or nutrient value of the luminal contents, which appears to be important in the mechanism of crypt cell proliferation control. In support of this contention Fox et al. (1976) have hypothesized that the vagus nerve relays glucose absorption and/or glucose storage information from the periphery (i.e. the viscera) to the brain (perhaps the lateral hypothalamus), and hence vagotomy produces carbohydrate intake decrements specifically. The vagi may also possibly be sensitive to other factors e.g. a rise or fall in the luminal bacterial levels or long term changes in diet.

The effect of local denervation in decreasing the rate of crypt cell proliferation in loops of jejunum and ileum, significant after five days, is maintained for at least three weeks after this procedure (see Chapt. IV, Section 111). The experimental findings are consistent with those of Klein (1980) who found that generalized sympathectomy reduced crypt cell proliferation between 15 and 23 days after birth.
(neonates), and also delayed but did not prevent the post-closure acceleration of proliferative activity in the ileum. Klein (1980 a) suggested that the mechanism for autonomic nervous system regulation of cell division may involve either direct or indirect pathways, including alterations of mucosal blood flow or gut motility (Dupont et al., 1965), or more direct regulation through the intestinal plexuses of nerves and their "diffuse neurohumoral synapse" (Dupont et al. (1965); Hillarp (1959)). Johnson et al. (1975 a) found that the noradrenaline levels in the intestine are lowered by 80% after Guanethidine treatment (generalized sympathectomy) and suggested that the depletion of noradrenaline may be directly responsible for the reduction of mitotic activity which occurs in sympathectomized rats. It has previously been suggested that noradrenaline plays a role in the normal regulation of circadian rhythms and the stimulation of mitotic activity during the nocturnal hours (Klein (1979 b, 1980); Klein and Torres (1978); Tutton (1973) and Tutton and Helme (1974)). Thus, it is possible that in the case of local denervation, local changes in noradrenaline levels in the intestinal wall may form part of the mechanism of production of decreased crypt cell proliferation.

Various studies have shown that crypt cell proliferation in rats is considerably retarded following immunosympathectomy, chemical or surgical sympathectomy. Dupont et al. (1965) demonstrated that immunosympathectomy (i.e. generalized effect rather than localized effect) leads to increased crypt to villus migration time, whilst Musso et al. (1975) found that surgical preganglionic sympathectomy produced only a transient increase in crypt to villus transit time.
Lachat and Goncalves (1978) found that division of the abdominal splanchnic nerves (a more localized effect) only affected crypt cell dynamics in the early post operative period.

They suggested that the reason why Musso et al. (1975) found very little effect after splanchnicectomy was that they used experimental times in excess of three days. Tutton and Helme (1973, 1974), Klein and Torres (1978) in adult rats, and Klein and McKenzie (1980 a) in neonatal rats, found that chemical sympathectomy, after treatment with either 6-hydroxydopamine or guanethidine sulphate, was associated with a prolonged fall in the mitotic rate in the small intestine, and this was supported by the findings of Klein (1979b). Tutton and Barkla (1977 a) found that chemical sympathectomy also decreased the mitotic rate in the rat large intestine. Tutton and Helme (1973, 1974) found that denervation of a loop of small intestine was associated with strongly inhibited crypt cell proliferation in that segment, without appreciably influencing cell proliferation in adjacent regions, which had an intact blood and nerve supply.

The circadian variations in crypt cell proliferation in small and large intestine, previously mentioned in Chapt. 1, Section 1, are either obliterated (Tutton, 1975) or suppressed (Klein, 1980) following chemical sympathectomy. The influence of both noradrenaline and of sympathectomy on crypt cell proliferation could be explained in terms of a direct stimulating effect of noradrenaline on the crypt cells, and there is ample evidence for an abundant supply of nerve fibres to the crypts (Gabella and Costa, 1968 a, see Chapt. 1 Section 11).
However, the influence of both exogenous noradrenaline and of sympathectomy on crypt cell proliferation may be mediated through an alteration in the release of serotonin from enterochromaffin cells in the intestinal wall. Considerable experimental evidence suggests the existence of an adrenergic innervation of the enterochromaffin cells. Thompson and Campbell (1966) demonstrated that in mice the serotonin content of the alimentary tract was significantly increased following immunosympathectomy. Similar elevation of gastrointestinal serotonin levels in rats was observed by Weber (1970) following chemical sympathectomy.

On the other hand, noradrenaline infusion was shown by Tansy et al. (1971) to cause degranulation of enterochromaffin cells in the duodenum of guinea pigs and they also showed that vagal stimulation caused enterochromaffin cell degranulation in normal but not in chemically sympathectomized guinea pigs. Tutton (1974) has shown that serotonin can affect the rate of crypt cell proliferation. Thus serotonin released from these degranulated enterochromaffin cells may be responsible for the acceleration of crypt cell proliferation which can be observed with the administration of noradrenaline (see Chapt. 1, Section 11). Furthermore, it is considered possible that the elevated urinary levels of serotonin metabolites observed in Coeliac Disease may be related to the increased level of crypt cell proliferation found in that disease (Tutton, 1974; Haverback and Davison, 1958; Kowlessar et al., 1958; Trier and Browning, 1970).

As mentioned previously, these present experimental findings are consistent with those of Tutton and Helme (1973, 1974). However, Lachat and Goncalves (1978) found that,
in variance with the present findings, the effect of sympathectomy on the intestinal crypts was more short lived but as sympathectomy in this case was achieved by division of the abdominal splanchnics, it is possible that some recovery of innervation may have occurred, a feature less likely in the case of division of the local neurovascular pedicles. It should be noted that, whilst the sympathetic nerves are principally involved in the procedure of local sympathectomy, other types of nerve fibres are contained in the mesenteric nerves and as demonstrated by Tutton (1975) these nerves may also affect the rate of crypt cell proliferation, probably to a lesser extent. It appears that the effects of mesenteric nerve stimulation on the crypts is principally via the sympathetic component of these nerves (Tutton, 1975).

Regarding the possible mechanism of action of the sympathetic nerves on the crypts, Lachat and Goncalves (1978) found that local denervation caused a delay in cell migration from the crypts in ileal epithelium. Dupont et al. (1965) suggested that lower intestinal motility after local denervation could explain the delay in migration of the crypt cells. Another possibility is the presence of an intrinsic and direct control of crypt cell migration through a local nerve plexus (Dupont et al. (1965); Sprintz (1971)). It has also been suggested that the disturbance in the local blood supply after autonomic denervation may be responsible for changes in the kinetic activity of the epithelium (Ballinger et al. (1965 a); Koch, (1959); Padula et al. (1968); Ross et al. (1963)). Another factor to be considered is that changes in tissue levels of adrenaline or noradrenaline, liberated from the intramural plexuses, may modify the motility and the blood supply.
of the small intestine (Furness and Costa, 1974). These substances are known to affect the crypt cell proliferation rate in the small bowel directly (Tutton and Helme, 1974). Autonomic denervation alters the liberation of these substances so that they could be involved in the effects on the crypts.

It is necessary to consider the relative importance of direct neural control of crypt cell proliferation by the sympathetic nerves and the very important luminal factors mentioned in the literature. In other words, where does neural control fit into the general mechanism of control? This is difficult to answer, given the amount of experimental evidence at present available. It is perhaps of interest to note that Levine et al. (1982) found that, whilst chemical sympathectomy with 6-hydroxydopamine exaggerated the intestinal mucosal hypoplasia in rats maintained on total parenteral nutrition, this mucosal atrophy could be prevented by intragastric infusion of luminal contents, suggesting that the effect of sympathectomy was less significant than that of the luminal nutrients.

Regarding the possibility that local vascular changes may be the means by which both vagal and sympathetic nerve fibres act on the crypts, it is interesting to note that Ballinger (1965a) suggested that the initial changes of hypoplasia of the ileal mucosa for up to two weeks after vagotomy found by him were ischaemic in origin since vagotomy was shown to result in a profound decrease in mesenteric blood flow. Moreover, since dogs in which the vagal supply to the small bowel had been spared (selective vagotomy) did not develop atrophy of the intestinal mucosa, this gave additional support to this concept. It is possible, however, that some variance in the findings may be explained on species differences
If however, there was mucosal ischaemia associated with vagotomy up to two weeks after vagotomy in rats and this was the principal factor involved in the vagal effects on the crypts, since it is known that mucosal ischaemia is associated with crypt cell hypoplasia (Rijke, 1976) in rats, a fall rather than a rise in crypt cell mitotic rate should have occurred. Delaney (1967) found a significant increase in small bowel perfusion rates in dogs 4 to 6 weeks after abdominal vagotomy, which would coincide with the period of increased crypt cell activity found by some researchers but not in the present experiments.

In contrast, Mackie and Turner (1971) found that there was a significant fall in blood flow in the dog jejunum and ileum within three weeks after vagotomy and that this later returned to normal. Kewenter (1965) demonstrated that stimulation of the vagal nerve trunks (the cell kinetic effect of this does not appear to have been studied) had little if any effect on mesenteric blood flow, although intestinal motility was altered. Furthermore, it is known that changes in mesenteric blood flow are not necessarily reflected as changes in villous blood flow (see Chapt. 1, Section 11). Davenport (1966) furthermore, showed that the intestinal vasculature receives only a sympathetic innervation. It seems unlikely, therefore, that the changes seen after vagotomy in the crypts are due to local changes in the villous blood supply, although no definite conclusions could be drawn from the present study. However, in the case of local denervation of the gut there is a possibility that this local denervation may directly result in some change in the blood supply to the crypts, so that the sympathetic nerves might act to some extent on the crypts by means of such changes.
Touloukian and Spencer (1971) found that hypertrophy of the ileal remnant following 50% midenterectomy in rats was accompanied by an increase in blood flow to the ileal remnant which was selective and paralleled the compensatory hypertrophy, suggesting a causal relationship. Touloukian et al. (1972a) found that, following a similar procedure to above, in similar rats, endogenous catecholamine activity in the hypertrophied ileal remnant was reduced by nearly 50% of normal and accompanied by a corresponding decrease in the density of adrenergic terminals within the myenteric plexus and arterial vasculature of the hypertrophied ileum. This suggested the possibility of the occurrence of adrenergic denervation of the hypertrophied gut remnant following major intestinal resection, with a possible increase in mucosal blood flow. It is known that decreased mucosal blood flow is associated with decreased crypt cell proliferation (Rijke et al., 1976) so possibly increased mucosal blood flow might lead to increased crypt cell proliferation.

Other evidence suggesting that the sympathetic nerves might act on the crypts via the blood supply was provided by the findings of Grim (1963) who showed that splanchnic nerve stimulation caused vasoconstriction of the splanchnic vasculature. Hultén et al. (1977) found that on stimulation of the regional splanchnic nerves in man and cats the blood flow to the mucosa as well as the other layers of the small and large intestine was diminished. However, since Rijke et al. (1976) noted that ischaemia of the small intestine was associated with decreased crypt cell mitotic rate, the findings of Hultén et al. (1977) would not be consistent with the hyper-proliferative effect on the crypts noted by Tutton (1975) on stimulation of the neurovascular pedicles. This suggests
that the sympathetic nerves to the small intestine do not produce their effect on the crypts primarily via local mucosal vascular changes.

However, although in the methods used in the present denervation experiments the effect on the local gut circulation was minimized as far as possible, this factor remains to be considered. As mentioned previously, the effect of implanting a loop of small intestine in the present experiments was to allow the establishment of a good collateral circulation over a period of five months, and this appeared to have been established. Removal of the mesenteric blood supply, with its associated sympathetic nerve supply derived from the splanchnic nerves allowed the loop to retain at least part if not all of its local blood supply. The fact that the crypt cell proliferation rate after local denervation was not altered after reimplantation for five months suggests strongly that the effect of local denervation on the crypts is primarily a neural one.

The other possibility to consider is that a temporary episode of local ischaemia, at the time of division of the neurovascular pedicles may have resulted in sufficient mucosal damage to produce a fall in crypt cell proliferation rate five days later. However, this seems unlikely since Wagner et al. (1979) found that at 5 to 7 days after a temporary episode of mesenteric ischaemia there is increased crypt cell proliferation as part of a regeneration process, which is completed by 8 days. Furthermore, Lundgren (1974) found that activation of the sympathetic vasoconstrictor fibres to the gut does not induce any significant decrease in villous blood flow in the small intestine (see Hulten et al., 1977) suggesting that the effects of sympathetic nerve lesions on the crypts are not mediated by changes in mucosal blood flow.
It should be noted that, as pointed out by Tutton and Helme (1974), adrenaline and noradrenaline which are both potent vasoconstrictors have opposite effects on crypt cell proliferation. Furthermore, adrenaline and isoprenaline, which are both known to decrease crypt cell proliferation (see Chapt. 1, Section 11) have opposite effects on the circulation in the small intestine. This raises some doubts concerning the importance of the intestinal circulation in the control of crypt cell proliferation. Thus, the available evidence suggests that the effect of the sympathetic (and other mesenteric fibres) and the vagal nerves on the proliferative activities of the intestinal crypts is probably principally, if not wholly, a direct neural one. It appears, therefore, that sympathetic fibres have a stimulatory effect on crypt cell mitotic rate in the small intestine, and since sympathectomy (local or general) of the gut results in a fall in crypt cell mitotic rate, this effect may be a tonic stimulatory one, possibly from a higher neural centre. In the case of the vagus nerves, they appear to have a depressant effect on crypt cell proliferation, which is removed by vagotomy. However, it is suggested that they play an afferent role rather than an efferent one, conveying information regarding the luminal contents, once again, possibly to a higher neural centre. As Andrews (1986) in a review of the topic remarks, there seems little doubt that areas of the hypothalamus do receive information from the gut, via the vagal afferents, tractus solitarius and its nucleus, medial forebrain bundle to the hypothalamus, concerning the chemical nature of its contents and the degree of distention and absorbed glucose levels. This information presumably is used for such functions as the
regulation of food intake and the control of autonomic out-
-flow to the gut. It is possible that information relayed from
the intestinal mucosa regarding the luminal contents, via the
vagus nerves, to higher centres in the central nervous system
is processed in these centres. The resulting discharge of
activity is mediated via the sympathetic component of the
autonomic nervous system (the more efferent component) to
modulate crypt cell proliferation rate.

This proposed mechanism may be operative in day to day
control of crypt cell proliferation or may possibly only
become operative under certain circumstances (see general
discussion). Since the presence of a central nervous system
centre for the regulation of crypt cell proliferation was
proposed, further experiments (Chapt. 11, Section 11)
were performed to investigate this.
SECTION 11

THE EFFECTS OF PINEALECTOMY ON CRYPT CELL PROLIFERATION IN THE RAT SMALL INTESTINE.

(1) Introduction to section 11 experiments,

(2) Experiments,

(a) Effects one, two, and three weeks after pinealectomy

(b) Effects of pinealectomy combined with -

(A) Local denervation of the small bowel,

(B) Truncal abdominal vagotomy,

(c) Effects of combined diversion of bile into the midpoint of the small intestine and pinealectomy,

(d) Effects of combined ligation and division of the bile duct and pinealectomy,

(e) Effects of combined pinealectomy and diversion of a jejunal loop into the colon, with restoration of continuity of the jejunum,

(3) Discussion on section 11 experiments.
INTRODUCTION TO SECTION 11 EXPERIMENTS

In Section 1, the role of the vagus and sympathetic nerves in crypt cell proliferation was investigated. In this section the possible influence of the pineal gland on crypt cell proliferation was examined. There is experimental evidence that the hypothalamus acts as a coordinating area for the autonomic nervous system, and that the hypothalamus in turn has many connections with other areas of the brain, more specifically with the limbic system. As has already been mentioned in the literature review (see Chapt. 1, Section 11, Bindoni et al., 1973) lesions of the hypothalamus may influence the level of crypt cell proliferation in the small intestine. However, in the present series of experiments, it was decided to investigate the effects of lesions of the pineal gland rather than the hypothalamus (which has many complicated and varied functions besides that of coordinating the autonomic nervous system.), bearing in mind that the pineal gland is connected with and influences the hypothalamus (Korf and Wagner (1980). Lesions of this gland have been shown previously to influence crypt cell proliferation (Callaghan and Firth, 1978).

The purpose of the following experiments therefore was to attempt to define the possible significance of the pineal gland in a hypothetical central nervous system mechanism of control of crypt cell proliferation, and to obtain more information regarding the interaction between neural control mechanisms and the luminal control mechanisms.

As noted above, Bindoni and his associates found an increase in the mitotic rate in the small intestinal crypts and other viscera after removal of the pineal gland. However,
because the experimental techniques used in those experiments did not satisfy the standards set in this investigation, it was felt that the effects of pinealectomy should be reassessed using a more precise stathmokinetic technique. Further information on the duration of action of this effect was obtained by examining the effects of pinealectomy for up to three weeks. Further, to determine if the mechanism of action of the pineal on the crypts is via the autonomic nervous system, the effect of pinealectomy on vagotomized or locally denervated small intestine after one week was examined. The effects of these denervations has been examined in section 11.

Previously, (Callaghan, 1978) it has been shown that diversion of a small intestinal loop away from the luminal nutrient content of the small bowel and exposing it to colonic contents was associated with significantly decreased crypt cell proliferation rate in the excluded loop. As indicated in the literature review, it appears that luminal factors play an important role in the control of crypt cell proliferation in the small intestine. To determine if a change in the luminal environment, such as the previous diversion of a small bowel loop, could alter the effect of pinealectomy, this diversion procedure was repeated with an associated pinealectomy.

As also indicated in the literature review, there is considerable evidence for (and some against) the importance of pancreatic and/or biliary secretions in the control mechanism of crypt cell proliferation - either acting directly or perhaps indirectly via their effects on the luminal contents. To determine if the absence of biliary secretions from the intestinal lumen could affect the degree of hyperproliferation resulting from pinealectomy, pinealectomy was combined with ligation and division of the bile duct, which results in the
absence of all bile products from the lumen (including bile salts), or diversion of the bile to the distal small bowel, which results in normal reabsorption of the bile salts in the distal small bowel but deprives the jejunum alone of biliary products. The effect of removal of pancreatic secretions from the lumen on the response to pinealectomy could not be examined with any precision, because total pancreatectomy would introduce other factors which could possibly affect the pinealectomy effects, e.g. diabetes, and partial pancreatectomy of even a major portion of the pancreas would not allow an accurate assessment of the decrease in pancreatic exocrine secretion produced.

Whilst the effect of pinealectomy after two weeks has previously been determined (Callaghan and Firth, 1978), this was repeated in this series of experiments because a different method of statistical comparison had been used previously. The effect of pinealectomy after three weeks was investigated to determine how sustained the effect on the crypts was and to act as a baseline for comparison with the effects of limbic lesions (see Chapt. IV, section 111).

EFFECTS ON CRYPT CELL PROLIFERATION, ONE, TWO, AND THREE WEEKS AFTER PINEALECTOMY.

METHODS

Thirty five male Sprague-Dawley rats weighing 350-500Gms. and maintained under standard conditions (see Chapt. 111 a) were randomly selected into 6 groups, each of 5 to 7 animals, and anaesthetized (see Chapt. 111 b).

GROUP (1)- PINEALECTOMY FOR ONE WEEK.

(7 RATS)
Craniotomy was performed (see Chapt. IIId). A disc of bone was removed over the Lambda (see Fig. 4.33) to expose the superior sagittal sinus and the pineal gland. The method was similar to that of Kusczak and Rodin (1976), (see A and E in Figs. 4.15, 4.16, and 4.17). The superior sagittal sinus was ligated (G, Fig. 4.16), and the caudal part of the sinus with surrounding dura mater (H, Figs. 4.16, and 4.17) was lifted to expose the pineal gland (E, Figs. 4.15, 4.16, and 4.17). The straight sinus (F, Fig. 4.15) was usually damaged during this procedure and bleeding was controlled with Gelfoam (see Appendix). The pineal gland was removed, placed in 10% Formalin to fix and stained with Toluidine Blue (see Appendix) to confirm pinealectomy. After fixation, 10μm thickness sections were made and examined histologically. The scalp was sutured with black silk and metal clips applied to the fur (see Appendix). Postoperatively, the rats were given food and fluids ad libitum and Penicillin was administered.

GROUP (2) SHAM PINEALECTOMY FOR ONE WEEK, (7 RATS)

The same procedure as pinealectomy was followed, except that the pineal gland was not removed; i.e. the superior sagittal sinus was also ligated. The result of the mitotic rate in these rats was later compared with that in those subjected to pinealectomy, as well as with that in those not subjected to any neurological procedure. On the seventh day postoperatively the rats of groups (1) and (2) were killed by an overdose of Ether, at intervals between 45 minutes and 4 hours after intraperitoneal Colchicine, and as close to midday as possible to minimize the diurnal variation in crypt cell mitotic rate. Brain perfusion was performed with 10%
Formalin (as in Chapt. 111, g) and the perfused head was excised and placed in 10% Buffered Formalin. If the histological sections of the excised pineal glands were not conclusive (unusual) regarding completeness of removal, a part of the brain around the site of the pineal was removed from the fixed brain and sections 10 μm in thickness were cut from this area and stained with neutral red (see Appendix).

Specimens of small intestine were collected, stained and sectioned (as in Chapt. 111 e). The mitotic rate was determined (as in Chapt. 111 j and k).

GROUP (3) PINEALECTOMY FOR TWO WEEKS.

(5 RATS)

Craniotomy was performed (Chapt. 111 d). Pinealectomy (as previously).

GROUP (4) SHAM PINEALECTOMY FOR TWO WEEKS.

(5 RATS)

The rats were subjected to the same procedure as above except that the pineal gland was not removed.

GROUP (5) PINEALECTOMY FOR THREE WEEKS.

(6 RATS)

These rats were subjected to pinealectomy, as above. The rats of groups 3, 4, and 5 were killed at 2 or 3 weeks, at intervals between 1 and 4 hours after intraperitoneal Colchicine. The brains were perfused, as previously. The pineal glands were examined, as previously described, to confirm pinealectomy. Specimens of small intestine were collected, sectioned and stained (as in Chapt. 111 e). The mitotic rate was determined (as in Chapt. 111 j and k).

GROUP (6) NO OPERATIVE PROCEDURE, (5 RATS). Rats were killed as above between 1 and 4 hours after Colchicine. Specimens were collected and processed, as above.
Diagrammatic representation of the dorsum of the rat brain (after Greene, 1935).

- **H**: See text
- **D**: Cerebellum
- **C**: Transverse hemispherium
- **B**: The cerebral sinus
- **A**: Superior sagittal sinus
- **E**: Point
- **F**: Sinus
- **G**: Sinus
- **P**: Sinus
## RESULTS

### TABLE 4.6

Mitotic rate in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) ONE WEEK</th>
<th>(2) ONE WEEK</th>
<th>(3) NO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFTER</td>
<td>AFTER SHAM</td>
<td>OPERATIVE</td>
</tr>
<tr>
<td>PINEALECTOMY</td>
<td>PROXIMAL</td>
<td>DISTAL ILEUM</td>
<td>PROCEDURE</td>
</tr>
<tr>
<td>PROXIMAL</td>
<td>0.1143 ±</td>
<td>0.0833 ±</td>
<td>0.0722 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0024 ±</td>
<td>0.0036 ±</td>
<td>0.0033 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1174 ±</td>
<td>0.0851 ±</td>
<td>0.0701 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0024 ±</td>
<td>0.0025 ±</td>
<td>0.0023 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.05, t = 2.54, df = 11, i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.02, t = 2.78, df = 11, i.e. significant at the 5% level.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 3, P < 0.20, t = 1.734, df = 9, i.e. not significant at the 5% level.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 3, P < 0.10, t = 1.833, df = 9, i.e. not significant at the 5% level.

### TABLE 4.7

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) TWO WEEKS AFTER</th>
<th>(2) TWO WEEKS AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PINEALECTOMY</td>
<td>SHAM PINEALECTOMY</td>
</tr>
<tr>
<td>PROXIMAL</td>
<td>0.1225 ±</td>
<td>0.0792 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0046 ±</td>
<td>0.0010 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1281 ±</td>
<td>0.0772 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0065 ±</td>
<td>0.0016 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.0001, t = 10.06, df = 6, i.e. significantly different at the 5% level.
DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.0002, t = 8.483, df = 6, i.e. significantly different at the 5% level.

TABLE 4.8

Correlation Coefficients -

<table>
<thead>
<tr>
<th></th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.9965</td>
<td>0.9997</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9955</td>
<td>0.9991</td>
</tr>
</tbody>
</table>

i.e. there was satisfactory correlation for each regression line.

TABLE 4.9

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) ONE WEEK</th>
<th>(2) TWO WEEKS</th>
<th>(3) THREE WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFTER PINEAL-ECTOMY</td>
<td>AFTER PINEAL-ECTOMY</td>
<td>AFTER PINEAL-ECTOMY</td>
<td></td>
</tr>
<tr>
<td>PROXIMAL</td>
<td>0.1143 ±</td>
<td>0.1225 ±</td>
<td>0.1283 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0072 ±</td>
<td>0.0046 ±</td>
<td>0.0017 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1174 ±</td>
<td>0.1281 ±</td>
<td>0.1289 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0017 ±</td>
<td>0.0065 ±</td>
<td>0.0017 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, DISTAL ILEUM, GROUPS 2 AND 3 - NOT STATISTICALLY DIFFERENT, ON COMPARISON OF STANDARD ERRORS.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 1, P < 0.20, t = 1.822, df = 8, i.e. no significant difference at the 5% level.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P < 0.20, t = 1.813, df = 8, i.e. no significant difference at the 5% level.
Figure 4.12

Graph of mitotic index versus time

X = Proximal jejunum, one week after sham pinealectomy.

● = Proximal jejunum, one week after pinealectomy.
Graph of mitotic index versus time.

$X$ = Distal ileum, one week after sham pinealectomy

$\bullet$ = Distal ileum, one week after pinealectomy.
DISCUSSION

The findings of the present study show that there was a statistically significantly increase in the mitotic rate in the crypts of the proximal jejunum and distal ileum one week after removal of the pineal gland, when compared with controls. The mitotic rate in the crypts was not statistically significantly different in rats subjected to craniotomy and ligation of the superior sagittal sinus when compared with non-operated controls, indicating that these procedures, used to expose the pineal gland, did not themselves significantly change the crypt cell proliferation rate. It should be noted that these results are for rats killed as near as possible to midday, to minimize the effects of diurnal fluctuations in pineal and/or crypt cell mitotic activity.

Two weeks after pinealectomy there was a statistically significant rise in crypt cell mitotic rate in the small intestinal crypts, when compared with controls. There was no statistically significant difference between the mitotic rates in the crypts, one, two, or three weeks after pinealectomy, i.e. the effect on the crypts was sustained.

Since it was found that the crypt cell mitotic rate in rats subjected to ligation of the superior sagittal sinus alone did not differ significantly from that in rats not subjected to any neurosurgical procedure, it is suggested that the pineal effect was a specific one and not due to neurosurgical interference alone. Ligation of the superior sagittal sinus, which could interfere with the venous drainage of the pineal gland into the great cerebral vein, is apparently without effect on crypt cell proliferation.
Thus, the dramatic rise in the crypt cell proliferation rate in the small intestinal crypts following pinealectomy demonstrated by Bindoni and Cambria (1968), Bindoni and Raffaele (1968), Giuffrida et al. (1969) was confirmed by repeating the effects of pinealectomy. It was felt, using the criteria suggested by Wright and Appleton (1980 a) that an insufficient number of metaphase counts had been made after administration of Colchicine in these experiments. Accordingly, the experiment was repeated, using a similar stathmokinetic technique, but with more metaphase count values, and a similar result was obtained.

Boeckmann (1980) in adult Sprague-Dawley and Wistar rats distinguished—,

(1) pineal tissue in the intercommissural region as the deep pineal, (2) a superficial pineal which represents the major part of the pineal complex, (3) nearly always a parenchymal stalk of variable length. Heidbüchel and Vollrath (1983) concluded that, despite the fact that the commonly used techniques of pinealectomy remove the superficial pineal only, it was found that in male Sprague-Dawley rats, three to six weeks after superficial pinealectomy, the deep pineal exhibited a clear decrease in volume due to an atrophy of the intrinsic pinealocytes, an atrophy which was still noticeable after six or twelve months. They also concluded that after superficial pinealectomy the deep pineal was not an important factor with respect to melatonin formation, as there are extrapineal sites of melatonin formation. In the present experiments, the superficial part of the pineal was removed but it appears that the effect on the pineal gland is the same as if all the gland had been removed.

It should also be remembered that the very nature of the
stathmokinetic technique using Colchicine, because of the increasing amount of degeneration of metaphases four hours after administration of that substance, means that after the four hour period any further rises in mitotic rate may be masked by this degeneration. That is, if the technique was prolonged after four hours, the values for the mitotic rate may tend to reach a "ceiling". This means that, e.g. three weeks after pinealectomy, the recorded rise in mitotic rate may be a little less than the actual rise in rate, but certainly would not be greater. The four hour period of observation has been chosen as an arbitrary one. Furthermore, the observations on the greatly increased mitotic rate after pinealectomy have been made as close to midday as possible, but since the mitotic rate in the small intestinal crypts is known to fluctuate in a diurnal fashion anyway, it is possible that the mitotic rate in the crypts is also greatly changed at midnight. That is, the rise may be part of a wider fluctuation in mitotic rate resulting from the effects of pinealectomy. To elucidate this problem further it would be necessary to determine the mitotic rate as close to midnight as possible, and further observations will be made on this in the future.

EFFECTS OF PINEALECTOMY COMBINED WITH LOCAL DENERVATION OF THE SMALL INTESTINE OR TRUNCAL ABDOMINAL VAGOTOMY

METHODS

(A) COMBINED WITH LOCAL DENERVATION OF THE SMALL INTESTINE.

Six male Sprague-Dawley rats, randomly selected from a group weighing 400-500 Gms. were anaesthetized (as in Chapt. 111, b) and laparotomy (as in Chapt. 111, c) performed. Local denervation of a loop of proximal jejunum, 5 cms. distal to the duodeno-jejunal flexure, and of a loop of distal ileum,
5 cms. proximal to the ileo-caecal junction, was performed (as in Chapt. IV, Section 1), (see Figs. 4.6 and 4.7). The abdomen was closed, Penicillin administered and food and fluids supplied ad libitum. Five days later the rats were again anaesthetized and pinealectomy performed (as in Chapt. IV, Section 11), (see Figs. 4.15, 4.16 and 4.17). Penicillin was again administered and food and fluids supplied ad libitum. Specimens of pineal gland were sectioned and stained, as previously, to confirm pinealectomy. One week postoperatively, the rats were killed by an overdose of Ether at intervals, between 35 minutes and 4 hours after intraperitoneal Colchicine. Specimens of the denervated segments of small intestine were collected, sectioned and stained (as in Chapt. 111, e). The mitotic rate was determined (as in Chapt. 111, j and k), for both groups. The brain was perfused (see Chapt. 111, g).

(B) COMBINED WITH BILATERNAL TRUNCAL ABDOMINAL VAGOTOMY

Six male Sprague-Dawley rats, randomly selected from a group weighing 350-550 Gms. were anaesthetized (as in Chapt. 111, b) and pinealectomy performed (as in Chapt. IV, Section 11), (see Figs. 4.15, 4.16, and 4.17). The specimens of pineal were examined histologically to confirm pinealectomy. Under the same anaesthetic, the abdomen was opened (as in Chapt. 111, c) and bilateral truncal abdominal vagotomy was performed (as in Chapt. IV, Section 1). Penicillin was administered postoperatively, and food and fluids supplied ad libitum. One week postoperatively, the rats were killed at intervals, between 3/4 hours and 4 hours after intraperitoneal Colchicine. The brain was perfused (as in Chapt. 111, g). Specimens of small intestine were collected, sectioned, and stained (as in Chapt. 111, e). The gastric contents were removed at death and suspended in normal saline. The pH of
these contents was determined, using a digital pH meter.

**RESULTS**

**TABLE 4.10**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) ONE WEEK WITHIN ONE WEEK AFTER PINEALECTOMY</th>
<th>(2) ONE WEEK WITHIN ONE WEEK AFTER PINEALECTOMY AND LOCAL DENERVATION</th>
<th>(3) ONE WEEK WITHIN ONE WEEK AFTER PINEALECTOMY AND VAGOTOMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal Jejunum</td>
<td>0.1143 ± 0.0024</td>
<td>0.0846 ± 0.0047</td>
<td>0.0856 ± 0.0047</td>
</tr>
<tr>
<td>Distal Ileum</td>
<td>0.1174 ± 0.0024</td>
<td>0.0831 ± 0.0040</td>
<td>0.0874 ± 0.0041</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

Proximal Jejunum, Group 1 > Proximal Jejunum, Group 2, P < 0.0001, t = 5.603, df = 9, i.e. significant at the 5% level.

Distal Ileum, Group 1 > Distal Ileum, Group 2, P < 0.0001, t = 7.145, df = 9, i.e. significant at the 5% level.

Proximal Jejunum, Group 1 > Proximal Jejunum, Group 3, P < 0.0001, t = 5.85, df = 9, i.e. significant at the 5% level.

Distal Ileum, Group 1 > Distal Ileum, Group 3, P < 0.0001, t = 6.666, df = 9, i.e. significant at the 5% level.

Proximal Jejunum, Group 3 > Proximal Jejunum, One Week After Truncal Vagotomy Alone, P < 0.20, t = 1.491, df = 7. i.e. not significant at the 5% level.

Distal Ileum, Group 3 > Distal Ileum, One Week After Vagotomy Alone, P < 0.0002, t = 7.347, df = 7, i.e. significant at the 5% level.

Proximal Jejunum, Group 2 > Proximal Jejunum, 5 Days After Local Denervation, P < 0.0001, t = 7.966, df = 7, i.e. significant at the 5% level.
DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, 5 DAYS AFTER LOCAL
denervation, \( P < 0.00005, t = 12.86, df = 7, \) i.e. significant at the 5 % level.

<table>
<thead>
<tr>
<th>TABLE 4.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>PINEALECTOMY AND VAGOTOMY</td>
</tr>
<tr>
<td>pH OF THE GASTRIC CONTENTS</td>
</tr>
</tbody>
</table>

Using a modified "t" test, (see Chapt. 111, k), the pH of the gastric contents of the combined vagotomy and pinealectomy group was found to exceed the pH of the gastric contents of the vagotomy alone group, (\( P < 0.01, t = 17, df = 8, \)) i.e. significant at the 5 % level.

**DISCUSSION**

Comparison of the pH of the gastric contents after pinealectomy combined with vagotomy and that after vagotomy alone (see Chapt. IV, Section 1), together with the previously mentioned criteria, supports the contention that vagotomy was complete in the present experiments. Thus, it appears that local denervation (especially sympathectomy) of the small intestine, allowing five days for degeneration of nerve fibres (see Tutton and Helme, 1974) when combined with pinealectomy resulted in the expected rise in crypt cell mitotic rate being statistically significantly smaller than after pinealectomy alone.

Similarly, concomitant bilateral truncal vagotomy was associated with a statistically significantly smaller than expected rise in crypt cell mitotic rate after pinealectomy. Comparison of the mitotic rates after local denervation or vagotomy associated with pinealectomy revealed no statistical
difference (on comparison of standard errors), although the mitotic rate in those subjected to combined local denervation and pinealectomy exceeded that in those subjected to local denervation alone (see Chapt. IV, Section 1). The difference in mitotic rates between those subjected to combined vagotomy and pinealectomy on one hand and those subjected to vagotomy alone on the other was not as clear cut, there being no statistically significant difference with regard to the proximal jejunum but a statistically significant difference with regard to the distal ileum. The mitotic rates in the proximal jejunum and distal ileum of those subjected to pinealectomy combined with truncal vagotorny or those combined with local denervation were not statistically significantly different from those subjected to sham pinealectomy only (on comparison of standard errors). It is perhaps not surprising that the effect on the crypts of combined pinealectomy and truncal vagotomy after one week approaches the baseline rate for craniotomy or no operative procedure, considering the relatively small effect of vagotomy on crypt cell mitotic rate after one week.

Thus, it appears that local denervation or vagotorny combined with pinealectomy will decrease the mitotic rate to a level statistically similar to that after sham pinealectomy, or alternatively, to a level statistically similar to that associated with no operative procedure, but not, in the case of associated local denervation particularly, to a level similar to the rate produced by local denervation alone, i.e. the effect of pinealectomy itself is alone cancelled out. It is not clear what the significance of this is but there are several alternative explanations. Since removal of the pineal gland appears to prevent the usual response of the crypts to
local denervation, it may be that the presence of the pineal gland is necessary for the full effects of local denervation particularly to be manifest. This may mean that the secretions of the pineal are necessary for the full effect of local denervation on the small intestine or that pinealectomy may have interrupted a neural pathway which allows the full effect of local denervation to be manifest. It is difficult to imagine this possibility being operative, although it must be considered. Alternatively, pinealectomy may have affected the sensitivity of the crypt cells to the principally sympathetic denervation, perhaps through a generalized effect on the autonomic nervous system. It is known, for instance, that the pineal has connections with the hypothalamus. (Korf and Wagner, 1980) and might act via this means to affect the autonomic nervous system.

Another possibility is that the hyperproliferative reaction following pinealectomy may be acting independently of and opposing that of the hypoproliferative effects of local denervation, and the resulting intermediate level of crypt cell mitotic rate may by chance be statistically similar to that of controls without operative procedure. In this respect, it is conceivable that the effect of pinealectomy was being mediated via the vagus nerves, when the sympathetics have been cut.

A further possibility which must be considered, and for which there appears to be some functional basis, is that the effects of pinealectomy may be mediated to some extent at least by other pathways besides the autonomic nervous system, although the autonomic nervous system would appear to be a major pathway for its action. These other possible pathways would include the action of humoral agents or the use of endocrine pathways.
It is known that the pineal gland may act on the crypts by means of a humoral agent. For example, Bindoni et al. (1976) prepared and partially purified an antimitotic substance from the pineal gland, which was established as being different from melatonin, serotonin and noradrenaline, all of which occur in the pineal gland. Melatonin, however, should be considered as a possible mediator of the pineal actions, since it occurs in both the pineal gland and the gastrointestinal tract of the rat throughout life (Bubenik, 1980). Raikhlin and Kvetny (1976) in fact found active biosynthesis of melatonin in the enterochromaffin cells of the gastrointestinal tract. Bubenik (1980) found that exogenously administered melatonin concentrated in all parts of the gastrointestinal tract, with most pronounced accumulation in the colon and rectum, suggesting that possibly melatonin produced elsewhere (e.g. in the pineal gland) might be accumulated in these tissues. However, since the removal of the pineal neither influences the levels of melatonin in rat hypothalamus (Koslow, 1974), nor in the digestive tract (Bubenik, 1980), and the removal of the pineal gland lowered melatonin levels in the plasma of ewes only temporarily (Kennaway, 1977), the pineal gland cannot be considered to be the only source of melatonin. Ozaki and Lynch (1976) further suggested that extrapineal sources of melatonin may contribute to the circulating pool in plasma, suggesting that after pinealectomy, other tissues may be able to take over the synthesis of melatonin.

If the pathway of action of the pineal gland on the crypts is via the autonomic nervous system to the gut, we may speculate on the means by which it achieves its actions. At present, no nerve fibres ending in the mucosa in close proximity to the
villi have been definitely implicated in the control of crypt cell proliferation, although it is thought likely that certain fibres are involved i.e. the cholinergic and adrenergic fibres, as well as other fibres (referred to as p-type (purinergic)) because they ultrastructurally resemble the peptide-containing neurosecretory neurons in the posterior pituitary (Baumgarten et al. (1970); Cook and Burnstock (1976)). These p-type fibres seem to contain peptides, such as substance P, vaso-active intestinal peptide, somatostatin, or encephalin, and others (Hakanson et al. (1981); Sundler et al. (1980); Keast et al. (1984)). All of these neuropeptides have effects on the gut and some e.g. somatostatin, are known to affect the rate of crypt cell proliferation in the small intestine (see Chapt. 1, Section 11). Furness and Costa (1979) concluded that somatostatin was possibly a neurotransmitter in the intestine that is released at neuro-humoral junctions and causes inhibition of intestinal movements (this does not exclude actions on the crypts). Jessen et al. (1980) suggested that the large numbers of peptide containing interneurons of the myenteric and sub-mucosal plexuses may act to modify the function of other neurons, so that outgoing signals from the plexuses are the result of filtering an incoming impulse flow through a closely knit neuronal web.

Malmfors et al. (1981) found in pigs that following vagotomy the innervation pattern of the jejunum appeared completely unaffected, but following complete denervation by autotransplantation of a jejunal segment the adrenergic fibres disappeared, whilst peptide-containing acetylcholinesterase positive nerve fibres remained apparently unaltered. The fact that the effect of pinealectomy was not completely
cancelled by local denervation of the gut may possibly indi-
cate that some of the effect of pinealectomy mediated by the
autonomic nervous system is transmitted via peptide-containing
neurons, and it is possible that the effects of pinealectomy
may be modulated by such nerves in the gut wall. Since somato-
statin secretion is linked with that of enteroglucagon ( see
Chapt. 1,Section 11) then this latter substance may also lie
in the pathway of action of the pineal gland on the crypts. In
support of this contention, Tutton (1975 a) observing the
effects of electrical stimulation of the mesenteric nerves of
the gut, considered that the resulting increase in crypt cell
mitotic rate was not solely dependent upon the integrity of
the sympathetic nerves to the gut, because it was also demon-
strated in chemically sympathectomized rats. So the pineal
may possibly act on the crypts via cholinergic, adrenergic or
peptidergic nerve endings. The neuropeptides previously implic-
ated in the control of crypt cell proliferation may thus
form part of the "chain of action" of the pineal on the small
intestinal crypts.

In fact, Lundberg et al. (1978,1979) confirmed that
additional peptide-containing nerve fibres may originate from
sources extrinsic to the gut, and reported that the vagal trunks
and splanchnic nerves contained such fibres. Somatostatin is
known to be present in the hypothalamus and in the submucosa
of the small bowel ( Polak, 1975). but in relation to the cont-
rol of crypt cell proliferation, there appears to be no obvious
significance to this observation.

The gut neuropeptides may thus have several different
roles in the control of crypt cell proliferation. Polak (1979)
has described several possible general roles for these subst-
ances -
(1) that of a classic circulating hormone, acting at some distance, (2) a local hormone acting on the neighbouring cells (a paracrine effect), (3) as a neurotransmitter (neuromodulator) being released from nerve terminals by axonal depolarization. Further experimentation needs to be done on the level of substances such as enteroglucagon, somatostatin, and others after pinealectomy, with or without denervation of the gut. The present investigations have studied the effects of pinealectomy over three weeks, but of course the long term effects of pinealectomy on the crypt cell proliferation rate are not known and further experimental work needs to be done on this aspect.

The pinealectomy effect could be a neurovascular one but although changes in local blood supply have been implicated in the effects of vagotomy on the crypts (see Chapt. 1, Section 11) it appears likely that local vascular effects are of not prime importance in the effects of local denervation on the crypts, so that there is nothing to suggest that the pinealectomy effects are due to local vascular changes.
Figure 4.20  TIME (HR)

Graph of mitotic index versus time

X = Proximal jejenum, Pinealectomy with local gut denervation

• = Proximal jejenum, one week after pinealectomy.
Figure 4.21

Graph of mitotic index versus time.

X = Distal ileum combined pinealectomy and local gut denervation

○ = Distal ileum, pinealectomy (one week)
Graph of mitotic index versus time.

$X = \text{Proximal jejunum, combined pinealectomy and truncal vagotomy.}$

$\bullet = \text{Proximal jejunum, one week after pinealectomy.}$
Graph of mitotic index versus time.

X = Distal ileum, combined pinealectomy and truncal vagotomy.

○ = Distal ileum, one week after pinealectomy.
THE EFFECT OF COMBINED DIVERSION OF BILE INTO THE MIDPOINT OF THE SMALL INTESTINE AND PINEALECTOMY.

METHODS

Twenty male Sprague-Dawley rats, weighing between 360-450 Gms. were randomly selected into three groups and anaesthetized (as in Chapt. 111, b).

GROUP (1), DIVERSION OF BILE INTO THE MIDPOINT OF THE SMALL INTESTINE.

(8 RATS),

An upper midline laparotomy was performed (as in Chapt. 111, c). The liver was retracted upwards and the green-coloured bile duct visualized above the duodenum by retracting the stomach upwards and to the left. The bile duct was mobilized and divided, the proximal end of the duct being cannulated with very fine polythene tubing. This was tied firmly in position with a 5 X 0 black silk suture. The distal end of the bile duct was ligated with black silk. The distal end of the polythene tube was inserted through a small stab wound into the midpoint of the small intestine, and fixed in position with a purse-string black silk suture (see Fig. 4.24). The abdomen was closed with black silk sutures, Penicillin was administered, and sucrose fluids only, supplied postoperatively for 24 hours, following which fluids and food were supplied ad libitum.

GROUP (2), PINEALECTOMY WITH BILIARY DIVERSION.

(7 RATS).

Laparotomy (see Chapt. 111, c) and diversion of bile into the midpoint of the small intestine was performed, as previously. Under the same anaesthetic, pinealectomy (see Chapt. IV, Section 11) was performed. Specimens of the pineal gland were stained and examined histologically, as previously,
to confirm pinealectomy. Postoperatively, Penicillin was administered. Sucrose fluids were supplied ad libitum for 24 hours following which food and fluids were supplied ad libitum. Postoperatively, all rats were killed after one week by an overdose of Ether, at intervals between 1/2 hour and 3 3/4 hours after intraperitoneal Colchicine. In group 2 the brain was perfused (as in Chapt. 111, g). Specimens of small intestine in both groups were collected, sectioned and stained (as in Chapt. 111, e). The mitotic rate was determined (as in Chapt. 111, j and k).

GROUP (3), LAPAROTOMY ALONE, (5 RATS).

Laparotomy was performed,(as in Chapt. 111, c). After one week the rats were killed by an overdose of Ether, at intervals between 1/2 hour and 4 hours after intraperitoneal Colchicine. Specimens of small intestine were collected, as previously, and the mitotic rate determined.

RESULTS

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th>(1) BILIARY DIVERSION</th>
<th>(2) PINEALECTOMY AND PINEALECTOMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1231 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0011 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1232 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0024 ±</td>
</tr>
<tr>
<td>PROXIMAL JEJUNUM, GROUP 1 &gt; PROXIMAL JEJUNUM, GROUP 2, P &lt; 0.005, t = 3.666, df = 10. i.e. significant difference at the 5% level.</td>
<td></td>
</tr>
<tr>
<td>DISTAL ILEUM GROUP 1 &gt; DISTAL ILEUM, GROUP 2, P &lt; 0.20, t = 1.757</td>
<td></td>
</tr>
</tbody>
</table>
df = 10, i.e. not significantly different at the 5% level.

**TABLE 4.13**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILIARY DIVERSION AND PINEAL-ECTOMY</th>
<th>(2) BILIARY DIVERSION ONLY</th>
<th>(3) LAPAROTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.1231 ± 0.0011</td>
<td>0.0646 ± 0.0020</td>
<td>0.0584 ± 0.0053</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1232 ± 0.0024</td>
<td>0.0665 ± 0.0020</td>
<td>0.0629 ± 0.0023</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.00005, 
\[ t = 29.25, \text{df} = 11, \text{i.e. significantly different at the 5\% level.} \]

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005, 
\[ t = 17.71, \text{df} = 11, \text{i.e. significantly different at the 5\% level.} \]

PROXIMAL JEJUNUM AND DISTAL ILEUM OF GROUP 2 DID NOT DIFFER ON COMPARISON OF STANDARD ERRORS FROM PROXIMAL JEJUNUM AND DISTAL ILEUM OF GROUP 3.
Figure 4.24
(Modified from Richards et al., 1964)

Drawing of a composite of the duct system of the rat pancreas. Stomach, spleen, and splenic segment of the pancreas have been reflected cephalically.

A = The proximal end of the bile duct, containing a cannula

B = The ligated distal end of the bile duct.

C = The stomach

D = The spleen

E = The transverse colon

F = The point of entry of the polythene cannula into the small intestine.

G = The duodenum

H = The head of the pancreas.
Figure 4.25

Graph of mitotic index versus time.

X = Proximal jejunum, combined biliary diversion and pinealectomy.

● = Proximal jejunum, one week after pinealectomy.
Figure 4.26  TIME (HR)

Graph of mitotic index versus time

X = Distal ileum, combined biliary diversion and pinealectomy.

● = Distal ileum, one week after pinealectomy.
DISCUSSION

The findings of the present study show that the mitotic rate in the proximal jejunum of rats subjected to combined biliary diversion and pinealectomy significantly exceeded that in the proximal jejunum of those subjected to pinealectomy only for one week, although this was not the case in the distal ileum. However, in this experiment the significant result from the point of view of elucidating the pathway for the effects of the pineal gland on the crypts was that the effects of combined biliary diversion and pinealectomy were associated with a clearly greater hyperproliferative effect on the crypts than biliary diversion alone. The hyperproliferative effects of pinealectomy were once again confirmed and were not diminished by the associated biliary diversion to the level associated with biliary diversion alone.

It is interesting to note that biliary diversion alone was not associated with any significant change in crypt cell mitotic rate over the effects of laparotomy alone. It should be noted that the patency of the tube used for the diversion of bile was checked after death of the rat to confirm that bile had reached the distal part of the small intestine. Since the tube was implanted at the midpoint of the small bowel, the reflux of bile to the proximal five cms. of the proximal jejunum (where specimens were collected) was considered unlikely. Bile salts, which pass into the small intestine in the bile, are reabsorbed in the ileum for the most part (the enterohepatic circulation) only about 2% of bile salts being lost in the faeces in one single circulation (Perry, 1975). In the experiments on ligation of the common bile duct it was hoped to study the effect of interruption of the
enterohepatic circulation of bile acids, as opposed to the experiments in which the bile was diverted into the midgut, in which the circulation of bile acids through the ileum remained intact. It appears from the results that luminal bile salts, in particular, are not a major factor in the control of crypt cell proliferation, although they form part of the luminal environment.

It is difficult in the rat, in particular, to separate the effects of diminished pancreatic and diminished biliary secretions on the crypts. In an attempt to do this, the bile duct was cannulated, or ligated, superior to the pancreas and the first part of the duodenum to minimize any incidental effects on the flow of pancreatic secretions. However, it is still difficult to dissociate the effects of biliary and pancreatic secretions, as Geratz and Lamb (1974) found that ligation of the proximal common bile duct in rats caused a marked decrease in spontaneous and trypsin-inhibitor induced secretion of pancreatic amylase, partially corrected by placing a drain proximal to the ligature (as in the biliary diversion experiments). There appears to be conflicting reports on the effects of biliary diversion or ligation in the literature. Roy et al. (1975) and Williamson et al. (1978a) concluded that removal of bile from the small bowel lumen (by fistulation) decreased crypt cell renewal in the ileal mucosa, which was corrected by luminal infusion of sodium taurocholate (a bile salt) without any effect on the proximal jejunum, but these findings were not supported by the present experiments. Ecknauer and Böhmer (1977) found that external fistulation of the bile duct caused a decrease in cell renewal in the small bowel crypts but bile duct ligation resulted in smaller villi
but no change in crypt cell renewal. They suggested that in bile duct ligation there was increased bile acid concentration in the blood (cholestasis) which they thought might explain the difference. In contrast to these findings, Raicht et al. (1975) found increased crypt cell turnover two days after biliary diversion. Williamson et al. (1978a) found that diversion of bile through a cannula from the common bile duct to the midpoint of the small intestine (as in the present experiments) resulted in a transient increase in crypt cell proliferation in the ileum of male Sprague-Dawley rats (for one week) and this effect was more prolonged (up to one month) if there was an associated diversion of pancreatic secretions. Weser et al. (1977a) also found that diversion of bile and pancreatic secretions into the ileal lumen stimulated the growth of ileal mucosa.

It is not clear why the results of biliary diversion in the present experiments are different from those obtained by Williamson et al. (1978a), especially since the methods used are similar and the rats are of the same species. Perhaps the difference lies in the different methods of assessing crypt cell proliferation in both cases. Also, the transient effect on cell proliferation for one week noted by Williamson et al. (1978a) may have subsided to normal by the time the experimental data were collected in the present experiments. Altmann (1974), on the other hand, noted that fresh hog bile does not increase villus size when infused into isolated ileal loops in conscious rats.

The evidence regarding the role of bile in control of crypt cell proliferation does not appear to be conclusive, and the present experiments have not given much support for its role in this. It may play a part in altering the physical or
nutrient qualities of the luminal contents, in much the same way that the pancreatic secretions do, and thus may be part of the effect of the luminal contents, without having a specific effect related to its content of bile salts or pigments. It should again be pointed out that the patency of the plastic tube used in biliary diversion was tested for the presence of bile in the ileum in the experiments on biliary diversion. Thus, there is little chance that the rats subjected to biliary diversion had in fact biliary obstruction. It appears that the presence or absence of bile in the intestinal lumen does not significantly diminish the effects of pinealectomy and this finding is consistent with the effects of pinealectomy being mediated via the autonomic nervous system or by other means and suggests that the effect is independent of the changes in the intestinal luminal contents which accompany biliary diversion. Since the luminal contents have been implicated so clearly in the probable day to day mechanism of control of crypt cell proliferation, the results raise the possibility that the pineal may be involved in some mechanism of control other than the day to day control. It is possible that it acts on the crypts only under certain special conditions. This will be further discussed.

THE EFFECT OF COMBINED LIGATION AND DIVISION OF THE COMMON BILE DUCT AND PINEALECTOMY.

METHODS

Fifteen male Sprague-Dawley rats were randomly selected from a group weighing 370-490 Gms. into two groups and anaesthetized (as in Chapt. 111, b).

GROUP (1), LIGATION AND DIVISION OF THE BILE DUCT. (8 RATS).

Upper midline laparotomy was performed (as in Chapt.
The liver was retracted upwards (see Fig. 4.27) and the green coloured bile duct visualized above the duodenum by reflecting the stomach upwards and to the left. The bile duct (A in Fig. 4.27) was ligated (B in Fig. 4.27) a little distance above the superior aspect of the duodenum, so that the ligature was proximal to the small number of para-biliary ducts entering above the superior surface of the duodenum. The abdomen was closed, Penicillin was administered and sucrose fluids, only, were given for 24 hours, followed by food and fluids ad libitum.

GROUP (2), COMBINED BILIARY LIGATION AND DIVISION WITH PINEALECTOMY.

(7 RATS).

Laparotomy (as in Chapt. 111, c) was followed by ligation and division of the common bile duct, as above. The abdomen was closed and, under the same anaesthetic, pinealectomy was performed (Chapt. IV, Section 11). The specimens of pineal gland were stained, as described previously, and examined histologically to confirm pinealectomy. The scalp was closed, Penicillin administered, and sucrose fluids supplied, alone, ad libitum, for 24 hours postoperatively. Following one week with food and fluids ad libitum, both groups of rats were killed at intervals, between 3/4 hour and 3 1/2 hours after intraperitoneal Colchicine. In group 2 the brain was perfused (see Chapt. 111, g) and specimens of small intestine were collected from both groups. These were sectioned and stained as in Chapt. 111, (e). The mitotic rate was determined in all rats (as in Chapt. , j and k). Statistical comparison was made with the group subjected to laparotomy only (see previous experiment).
Drawing of a composite of the duct system of the rat pancreas

Stomach, spleen, and splenic segment of the pancreas have been reflected cephalically,

A = The bile duct, above the site of ligation,
B = The bile duct, at the site of ligation,
C = The duodenum,
D = The stomach,
E = The spleen,
F = The head of the pancreas,
G = The transverse colon.

( dotted line above the duodenum and below B represents the site of the parabiliary ducts ).
### RESULTS

#### TABLE 4.14

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILIARY LIGATION</th>
<th>(2) PINEALECTOMY AND PINEALECTOMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEEJUNUM</td>
<td>0.1292 ± 0.0018</td>
<td>0.1143 ± 0.0024</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1104 ± 0.0036</td>
<td>0.1174 ± 0.0024</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.001, t = 4.966, df = 10, i.e. a significant difference at the 5% level.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P < 0.20, t = 1.666, df = 10, i.e. not significantly different at the 5% level.

#### TABLE 4.15

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILIARY LIGATION</th>
<th>(2) BILIARY LIGATION</th>
<th>(3) LAPAROTOMY PINEALECTOMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEEJUNUM</td>
<td>0.1292 ± 0.0018</td>
<td>0.0694 ± 0.0042</td>
<td>0.0584 ± 0.0053</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1104 ± 0.0036</td>
<td>0.0682 ± 0.0038</td>
<td>0.0629 ± 0.0023</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.00005, t = 13.28, df = 11 i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005, t = 8.115, df = 11, i.e. significantly different at the 5% level.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 3, P < 0.20, t = 1.746, df = 9, i.e. not significantly different at the 5% level.

DISTAL ILEUM, GROUPS 2 and 3 - NO STATISTICAL DIFF.
Figure 4.28

Graph of mitotic index versus time

X = Proximal jejunum, biliary ligation and pinealectomy.

● = Proximal jejunum, one week after pinealectomy.
Figure 4.29  TIME (HR)
Graph of mitotic index versus time
X = Distal ileum, biliary ligation and pinealectomy.
• = Distal ileum, one week after pinealectomy
DISCUSSION

These results show that the mitotic rate in the proximal jejunum of those subjected to combined ligation and division of the bile duct with pinealectomy was significantly greater than the rate in those subjected to pinealectomy alone, although at first sight these values appear to be of a similar order. Indeed, in the case of the distal ileum of these two groups, the difference between them does not reach statistical significance. However, what is important from the point of view of elucidating the possible pathway of action of the pineal on the small intestinal crypt cells is that the mitotic rate in the crypts of those subjected to combined biliary ligation and pinealectomy clearly exceeds that in the crypts throughout the small intestine of those subjected to ligation of the bile duct alone, once again confirming the hyperproliferative effect of pinealectomy. Curiously, the effect of biliary ligation alone does not produce any significant change in mitotic rate over that in those subjected to laparotomy alone. The complete ligation of the bile duct of course means complete interruption of the enterohepatic circulation of bile salts, so that considering the previously determined effects of biliary diversion with retention of this enterohepatic circulation (see above) it appears that the presence or absence of bile salts in the intestinal lumen does not significantly affect the crypt cell mitotic rate. This is despite the apparent importance of the presence of the unconjugated bile acids, especially sodium taurocholate, in the lumen in stimulating crypt cell proliferation, primarily in the ileum, reported by Fry and Staffeldt (1964), and Roy et al. (1975). It was suggested (Urban and Weser, 1980) that because of
the different effects of bile salts on the ileal and jejunal mucosa growth may be regulated by different mechanisms in the two regions. The present results of biliary ligation and diversion do not support this view.

Williamson et al. (1978 a) found that high concentrations of luminal bile caused transient (48 hours to one week) increased cell proliferation in the ileal mucosa. However, removal of all bile from the lumen, as in the present experiment, was not associated with the expected fall in crypt cell mitotic rate. However, it is possible that the concentration of bile in that experiment may have been much higher than usual and this may have resulted in local mucosal damage. This may be the reason for the increased crypt cell mitotic rate in that case. Additionally, the presence of pancreatic juice was noted to prolong the effect (Williamson et al. (1978 a), and it is difficult to dissociate the effects of these two secretions on the crypts.

Despite the precautions taken in the technique of operation to prevent damage to the pancreatic ducts, bile duct ligation or fistulation may not be completely separate in its effects from those associated with changes in pancreatic secretion. For example, Geratz and Lamb (1974) found that ligation of the proximal common bile duct in rats caused a diminution in secretion of pancreatic amylase, which was corrected by placing a drain proximal to the ligature, whereas there was a rise in amylase output when secretions were collected from a distal bile fistula. Clarke (1970 a) noted that crypt cell production rate was constant throughout the small intestine, despite local variations in pancreato-biliary secretions.

This did not support the concept that pancreato-biliary
secretions act directly in the control of crypt cell proliferation. Williamson et al. (1978 a) commented that, since infusion of fresh hog bile into isolated ileal loops in conscious rats did not increase villus size, perhaps the presence of food is needed as well as bile for the pancreatobiliary secretions to have their effect. However, as indicated in the previous discussion, the effects of bile salts, bile pigments and pancreatic secretions cannot be precisely segregated because they are associated with changes in the luminal contents which of themselves affect the mitotic rate in the crypts.

It appears that the results of biliary ligation, as with those of biliary diversion, combined with pinealectomy are consistent with the effect of pinealectomy on the crypt cell mitotic rate being mediated by the autonomic nervous system or some other means rather than by changes in the luminal environment, of which the pinealectomy effect seems to be largely independent, i.e. the effects of pinealectomy are independent of the presence or absence of luminal bile.

THE EFFECT OF COMBINED PINEALECTOMY WITH DIVERSION OF A LOOP OF JEJUNUM INTO THE COLON

METHODS

Twenty three male Sprague-Dawley rats were randomly selected from a group weighing 300-460 Gms. into four groups. All were anaesthetized (see Chapt. 111, b).

GROUP (1), PINEALECTOMY AND JEJUNAL DIVERSION,
(5 RATS).

Laparotomy (see Chapt. 111, c) was followed by isolation of a proximal jejunal loop and attachment to the colon, with restoration of continuity of the jejunum. A 5 cms. long
jejunal loop, isolated except for its blood and nerve supply (see Fig. 4.30, B) was selected from a site 5 cms. distal to the duodeno-jejunal junction, closed at either end and attached to the transverse colon, with an opening of approximately 1 cm. in diameter (see Fig. 4.30). The continuity of the residual jejunum was restored by end to end anastomosis (see A in Fig. 4.30). The abdomen was closed, and under the same anaesthetic, pinealectomy was performed (as in Chapt. IV, Section 11). Pineal specimens were processed, as previously described, and examined histologically to confirm pinealectomy. Penicillin was administered and the rats maintained on food and water, ad libitum, following 24 hours with sucrose fluids only ad libitum.

**GROUP (2), SHAM PINEALECTOMY AND JEJUNAL DIVERSION.**

(5 RATS).

These rats were subjected to isolation of a proximal jejunal loop and attachment to the colon, as above, as well as craniotomy and ligation of the superior sagittal sinus but the pineal gland was not removed. Postoperatively, Penicillin was administered, and following 24 hours of sucrose fluids only, food and fluids were supplied ad libitum. Two weeks post-operatively, both groups were killed at intervals, between 1/2 hour and 4 hours after intraperitoneal Colchicine. In both groups the brain was perfused (see Chapt. 111, g) and sectioned. Specimens were collected from the midpoint of the loop attached to the colon, from the proximal jejunum 5 cms. distal to the duodeno-jejunal junction and from the distal ileum 5 cms. proximal to the ileo-caecal junction. These specimens were sectioned, and stained, as in Chapt. 111, (e).

**GROUP (3), DIVERSION OF A JEJUNAL LOOP ONLY.**

(5 RATS). *(WITHOUT PINEALECTOMY)*
Laparotomy was performed as above and a jejunal loop isolated as in group 1 and group 2 but no pinealectomy was performed and the rats were killed after 2 weeks, at intervals between 1/2 and 4 hours after intraperitoneal Colchicine. Specimens were treated as above.

GROUP (4), LAPAROTOMY ONLY. (8 RATS).

Laparotomy only was performed. Rats were killed after 2 weeks, at intervals up to 4 hours after Colchicine, and specimens of small bowel obtained as above. Mitotic rate was determined in all groups, as in Chapt. 111, (j) and (k).

RESULTS

Comparison of the mitotic rates in the small intestine following -

(1) Two weeks after pinealectomy and diversion of a jejunal loop.
(2) Two weeks after pinealectomy,
(3) Two weeks after sham pinealectomy and diversion of a jejunal loop,
(4) Two weeks after diversion of a jejunal loop,
(5) Two weeks after laparotomy only.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1244</td>
<td>0.1225</td>
<td>0.0876</td>
<td>0.0655</td>
<td>0.0605</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>± 0.0127</td>
<td>± 0.0046</td>
<td>± 0.0047</td>
<td>± 0.0068</td>
<td>± 0.0031</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1225</td>
<td>0.1281</td>
<td>0.0883</td>
<td>0.0567</td>
<td>0.0651</td>
</tr>
<tr>
<td>ILEUM</td>
<td>± 0.0067</td>
<td>± 0.0065</td>
<td>± 0.0078</td>
<td>± 0.0027</td>
<td>± 0.0018</td>
</tr>
<tr>
<td>LOOP OF</td>
<td>0.1279</td>
<td>± 0.0073</td>
<td>0.0437</td>
<td>± 0.0378</td>
<td>0.0039</td>
</tr>
<tr>
<td>DIVERTED JEJUNUM</td>
<td>± 0.0073</td>
<td>0.0033</td>
<td>± 0.0039</td>
<td>0.0039</td>
<td></td>
</tr>
</tbody>
</table>

Results in mitoses/cell/hour.
PROXIMAL JEJUNUM, GROUP 1 \( \gtrless \) PROXIMAL JEJUNUM, GROUP 3, 
\( P < 0.05, t = 3.016, df = 6, \) i.e. significantly different at the 5\% level.

DISTAL ILEUM, GROUP 1 \( \gtrless \) DISTAL ILEUM, GROUP 3, \( P < 0.02, t = 3.226 \)
\( df = 6, \) i.e. significantly different at the 5\% level.

ISOLATED JEJUNAL LOOP, GROUP 1 \( \gtrless \) ISOLATED JEJUNAL LOOP, GROUP 3
\( P < 0.00005, t = 11.37, df = 6, \) i.e. significantly different at the 5\% level.

PROXIMAL JEJUNUM AND DISTAL ILEUM, GROUP 1 NOT STATISTICALLY DIFFERENT FROM PROXIMAL JEJUNUM AND DISTAL ILEUM, GROUP 2, ON COMPARISON OF STANDARD ERRORS.

JEJUNUM, GROUP 5 \( \gtrless \) ISOLATED JEJUNUM, GROUP 4, \( P < 0.005, t = 4.283, df = 9, \) i.e. significantly greater at the 5\% level.

ISOLATED JEJUNAL LOOP, GROUP 3, NOT STATISTICALLY DIFFERENT FROM ISOLATED JEJUNAL LOOP, GROUP 4, ON COMPARISON OF STANDARD ERRORS.

| TABLE 4.17 |
|---|---|---|---|---|---|
|   | 1   | 2   | 3   | 4   | 5   |
| PROXIMAL JEJUNUM | 0.9822 | 0.9965 | 0.9561 | 0.9798 | 0.9882 |
| DISTAL ILEUM JEJUNAL LOOP | 0.9932 | 0.9955 | 0.9912 | 0.9943 | 0.9957 |
| ISOLATED JEJUNAL LOOP | 0.9928 | 0.9897 | 0.9747 |

i.e. there was satisfactory correlation for each regression line.
Diagrammatic representation of the site of attachment of the isolated jejunal loop, (B), to the transverse colon, with end-to-end restoration of continuity of the small bowel at (A).
Figure 4.31

Graph of mitotic index versus time.

\( X = \) Diverted jejunal loop, combined pineal-ectomy and jejunal loop diversion.

\( \bullet = \) Diverted jejunal loop, combined sham pinealec-ectomy and jejunal loop diversion.
Graph of mitotic index versus time.

X = Diverted jejunal loop, diversion of a jejunal loop.

● = Proximal jejunum, laparotomy, only.
DISCUSSION

Whilst there is a statistically significant difference between the mitotic rate in the proximal jejunum and distal ileum of those subjected to combined pinealectomy and those subjected to sham pinealectomy, both accompanied by diversion of a jejunal loop, this difference is of the order expected from the previous comparison of the effects of pinealectomy and those of sham pinealectomy. However, the interesting finding in this experiment is that the mitotic rate in the isolated jejunal loop of those subjected to combined pinealectomy and diversion of a jejunal loop greatly exceeded that in the isolated jejunal loop of those subjected to sham pinealectomy or those subjected to diversion of a jejunal loop without any cranial operation, once again confirming the hyperproliferative effect of pinealectomy. The mitotic rate in the isolated jejunal loop should, on comparison with that after jejunal diversion alone, be much lower. Thus, the hyperproliferative effect of pinealectomy has not been diminished by diversion of a jejunal loop, although it is obvious from the results that such a diversion usually results in a significant fall in the mitotic rate in such an isolated loop.

Since the effect of pinealectomy does not appear to be influenced by luminal factors, let us examine these factors and the possible reasons why the mitotic rate is lower in the isolated loop in an effort to understand the possible mechanism of pineal action on the crypts. Isolation of a jejunal loop by attaching it to the colon has the effect not only of removing the usual luminal nutrient content, with which it is normally associated, but also exposing it to the colonic contents, which are not only lower in nutrient value but have
a different bacterial flora (it was noted in all cases that the isolated loop contained faecal material). It is interesting to note that the hyperproliferative effect of pinealectomy was undiminished by such a change in luminal environment.

The presence of food in the lumen of an "absorptive" organ such as the small intestine appears to be an important factor in the mechanism of control of crypt cell proliferation (Weser and Hernandez (1971); Nygaard (1967); Dowling and Booth (1967); Tilson and Wright (1970); Weser and Tawil (1976); Young and Weser (1974)). Clarke (1974) showed that a significantly diminished crypt cell proliferation rate occurred in blind sacs of rat small intestine diverted on to the skin or into the small intestine, the most profound effects being observed in the former instance. The interpretation of these results was that the small intestine in contact with ingesta had a higher cell proliferation rate than that without such contact. However, this work also raised the possibility that the more profound depression of crypt cell proliferation rate in the small intestinal loops exposed to the skin rather than to the small intestinal contents may have reflected a difference in the bacterial flora between these two sites.

In the present experiment, involving the attachment of a loop of jejunum to the colon, there was a lack of the usual luminal nutrient content in the attached loop, and it was exposed to colonic contents which have a lower nutrient value and a higher bacterial content than the small intestine. Whilst these findings were consistent with those of Clarke (1974), the additional presence in the present experiment of a higher than usual luminal bacterial content might have been expected to oppose these nutritional effects, as suggested by the findings of Lesher et al. (1964), Abrams et al. (1963), on
the effects of the luminal bacterial content on crypt cell proliferation. These results possibly support the contention that the effect of lack of nutrients on the mucosa is greater relatively than the effect of the increased bacterial content of the lumen.

It might also have been expected that small intestine exposed to the unaccustomed colonic flora, as well as the more abrasive faecal material would have been more subject to local mucosal damage than usual, with increased surface desquamation and a proliferative crypt cell response to compensate for this. However, it is interesting to note that Clarke (1975) found that increased desquamation, as a result of injury, is not necessarily associated with any increase in crypt cell mitotic rate. The present experimental findings are consistent with those of Rijke et al. (1977 b) who found that in bypassed jejunum there was a lower crypt cell production rate.

The isolated loop of jejunum in the present experiment is also deprived of pancreato-biliary secretions, and the diminished crypt cell proliferation rate is also consistent with the lack of pancreato-biliary secretion, the importance of which has been supported by the findings of Altmann (1971), Altmann and Leblond (1970), Altmann (1974), Jacobs et al. (1975 b), Jacobs and Dowling (1975), Weser et al. (1977), Williamson et al. (1978 a). However, as noted by Altmann (1974), and Menge et al. (1975 b), the products of pancreatic action on the luminal contents may be as important as the pancreato-biliary secretions themselves, and it is difficult to dissociate the relative effects of these two factors. It should be noted that there was no significant reactive hyperplasia in the proximal jejunum and distal ileum of the small intestine not involved in the loop in this experiment.
This differs from the findings of Gleeson et al. (1972), Al-Mukhtar et al. (1982), Dudrick et al. (1977), Iversen et al. (1976), Fenyo et al. (1976), Grenier et al. (1974), Williamson and Bauer (1978), Tilson and Wright (1970) who found reactive hyperplasia in the remaining small bowel in continuity. Perhaps the very short segment (5 cms.) used in this present experiment was insufficient to produce this generalized effect, i.e. possibly there is a critical length of small bowel which must be excluded to produce this generalized effect. It would appear that the effect of a humoral agent is probably not operative in this case.

The findings in this experiment are consistent with the previous findings, suggesting that the pineal acts by means other than via luminal factors, in fact despite the expected effects of luminal factors. The findings regarding the crypt cell mitotic rate in the isolated jejunal loop without associated pinealectomy give some weight to the importance of luminal factors in crypt cell proliferation control. It is not clear from the present experimental findings to what degree, if any, pineal control over the crypt cells is operative under normal circumstances because it must be remembered that pinealectomy itself creates an artificial situation in the body which might not usually exist. The effect of the pineal on the crypts may be only occasional, under certain circumstances, or may be due to the release of another mechanism which controls crypt cell proliferation.

GENERAL DISCUSSION ON SECTION I

It would be reasonable to conclude that the hyperproliferative effect of pinealectomy on the crypts, confirmed by the present experiments, may either be a primary effect
from the presumably tonic mitotic supressant effect of the pineal gland itself or pinealectomy may have released a proliferative mechanism elsewhere which it usually suppresses. Whatever the mechanism of action of the pineal, the effect of associated vagotomy or local denervation of the intestine (mainly sympathetic but also other fibres) is to dramatically cancel out this effect, so that it appears that the effect is probably mediated via the autonomic nervous system, at least to some extent.

However, the fact that local denervation of the bowel associated with pinealectomy did not return the mitotic rate in the crypts of the denervated bowel to the level expected after local denervation (see Chapt. IV, Section 1) could mean that part of the pinealectomy effect could be by means of pathways other than the autonomic nervous system. The considerably more positive effect of vagotomy in cancelling out the effect of associated pinealectomy one week after pinealectomy compared with the less definite and opposite effect (see Chapt. IV, Section 1) one week after vagotomy alone suggests that the vagus nerves are probably directly involved in the transmission of the effect of pinealectomy to the crypt cells. The pineal may act via cholinergic, adrenergic or peptidergic nerve endings on the crypts, and the neuropeptides may also be involved but no information regarding these possibilities could be gained from the present experiments.

The fact that Bindoni and Raffaele (1971) demonstrated that the hyperproliferative effects of pinealectomy affected diverse organs in various parts of the body is consistent with a humoral effect as well as an autonomic nervous system mediated effect. Bindoni (1971) concluded (see Chapt. 1, Section 11) that the stimulation of mitotic activity by
pinealec-tomy was probably independent of pituitary function and in fact suggested that the effects of the pineal were due to a direct action on the tissues. In support of the humoral action of the pineal gland on the tissues, Bindoni et al. (1976) prepared and partially purified an antimitotic substance from the pineal glands of sheep which inhibited the in vitro multiplication of three cell strains. This substance was not destroyed by proteolytic enzymes nor by boiling with 6M hydro-chloric acid, and was established as being different from the known antimitotic agents or melatonin, serotonin or adren-aline, which did not show antimitotic activity under the same conditions. These findings of course may not be directly applicable to the present experimental situation because of species differences, and it would have been interesting to note the effect of such a pineal extract on the crypt cells in vivo.

As mentioned in Chapt. 1, Section 11, there is ample evidence for nerves suitably approximating the mucosal crypts and the basement membrane of the cells to allow neural trans-mission of an effect on the crypt cells, although the function of these plexuses cannot be directly related to the presence of neural control in our present state of knowledge.

If the autonomic nervous system is involved in the effect of pinealec-tomy, we must consider various pathways by which this could occur. The pineal gland is known to be supplied with sympathetic nerve fibres via the superior cervical ganglion. It is interesting to note that in a previous experiment (Callaghan, 1979 a) bilateral removal of the superior cervical ganglia was found not to be associated with any significant change in the crypt cell mitotic rate in the
small intestine, although it is known (Reiter and Hester, 1966) that the endocrine activities of the gland depend on an intact sympathetic innervation. The cell bodies of the post-ganglionic neurones are in the superior cervical ganglia and processes of these terminate within the pineal gland (the nervii conarii), (Thieblot et al. (1947); Kappers (1960)), having followed blood vessels, although the nerve terminals do not form morphological synapses with the pinealocytes. Serotonin is synthesized within and released from the pinealocytes (Axelrod, 1974) whereas the bulk of the noradrenaline within the gland (a suitable humoral agent) is confined to the sympathetic nerve endings (Matsushima et al., 1981).

It is thought that the superior cervical ganglion is involved in the effects of light and darkness on the secretory activity of the pineal gland. Romijn (1975) and Sheridan (1975) reported ultrastructural changes in the pinealocytes of rabbits and hamsters after superior cervical ganglionectomy and Reiter et al. (1975) showed that this procedure reduced the physiological activity of the gland. If superior cervical ganglionectomy is not associated with decreased crypt cell proliferation rate, despite the diminished function, it would suggest that the increased crypt cell proliferation rate after pinealectomy is probably not due to the lack of intrinsic function of the gland itself but rather due to the effects of pinealectomy on the functions of other portions of the nervous system partly under its influence. Moreover, Cardinali et al. (1981 a) point out that since the effects of pinealectomy do not mimic those of superior cervical ganglionectomy in other instances, superior cervical ganglionectomy should not be considered as merely "pineal denervation". 
Cardinali et al. (1981b) further point out that the superior cervical ganglion also has other connections besides the pineal gland e.g. they suggested a functionally relevant link between the superior cervical ganglion and the medial basal hypothalamus, and that the superior cervical ganglion appears to constitute a peripheral neuroendocrine centre. Pelegrino de Iraldi et al. (1963) found that superior cervical ganglionectomy in the rat produced a decrease of serotonin level by 73% in the pineal gland after 5 days, and Reiter (1981) found that superior cervical ganglionectomy reduced the metabolic activity of the pineal gland. Since these changes in the pineal gland function after superior cervical ganglionectomy are not associated with changes in crypt cell proliferation, it is likely that such changes are not operative in the effects on the crypts after pinealectomy.

In support of the possible humoral effects of the pineal gland on the crypts, it should be noted that, in comparison with other organs, the pineal gland has a greater blood supply per Gm. of tissue than any other endocrine gland and is surpassed only by the kidney (Reiter, 1981). The blood supply is greater at night than during the day (Quay, 1972). However once again, superior cervical ganglionectomy, which reduces the metabolic activity of the gland, also decreases blood flow through the organ to 2/3 of the usual level but is not associated with any significant change in crypt cell proliferation rate.

Ligation of the superior sagittal sinus, without removal of the pineal gland could reasonably be expected to disturb the venous drainage of that area, including the pineal, because of its connections with the great cerebral vein,
which drains the pineal, but as seen in these experiments, this is without effect on crypt cell proliferation rate despite the high rate of blood flow through the gland. Thus, it appears that whilst the effects of superior cervical ganglionectomy are many, including the maintenance of circadian rhythms, none of these effects can be linked with changes in the crypt cell proliferation rate in the small intestine.

The result of superior cervical ganglionectomy raises several other possible explanations for the action of the pineal. One possible explanation is that denervation of the pineal may remove its secretory activity but leave intact various fibre tracts which can produce the effects of the intact pineal gland. Another is that pinealectomy achieves its effect by damage to neighbouring structures, which are left intact by denervation alone. It has been found by Pazo (1981) that superior cervical ganglionectomy may spare one or more pineal functions, and he concluded that the bulk of the inputs into the pineal gland come through its stalk. In this regard, Reiter (1981), Rønnekliev and Møller (1979) described other nerves of obscure function passing directly from the diencephalon through the pineal stalk. It is perhaps of interest to note that Ahlman et al. (1978) described evidence of sympathetic nervous innervation of the small intestine of the cat, via the vagus nerves from the superior cervical ganglion. The significance of this connection, if any, is not clear but there are no known efferent neural connections from the pineal gland to the superior cervical ganglion.

Another possibility is that the pineal gland may act directly via neural pathways on the gastrointestinal crypts. In the present experiments with pinealectomized rats, bilateral truncal abdominal vagotomy and local denervation of
loops of jejunum and ileum both resulted in a considerably diminished hyperproliferative effect in the crypts of the affected bowel. However, whilst vagotomy decreased crypt cell proliferative activity to a level comparable with that obtained after truncal vagotomy alone, local denervation of the gut did not quite lower the crypt cell proliferative activity to the level seen after local denervation alone, suggesting that some humoral or neurohumoral factor is possibly involved in the mediation of the pineal effect, as well as the more obvious mediation of the effect via the autonomic nervous system. Since the innervation of the pineal via the superior cervical ganglion does not appear to provide a possible efferent pathway for the action of the pineal gland via the autonomic nervous system, it must be assumed that the central connections of the pineal gland are possible routes for mediating the effect of pinealectomy via the autonomic nervous system, possibly via both the vagi and the sympathetics.

Let us then examine the possible central connections of the pineal gland as a possible pathway for its action via the autonomic nervous system or possibly neuroendocrine system, which is regulated by regions within the limbic and hypothalamohypophyseal portions of the brain. Shapiro and Salas (1971) demonstrated that even after bilateral superior cervical ganglionectomy light impulses on the retina can influence rat pineal function. Dafny (1980) suggested that photic stimuli to the pineal were transmitted via two separate routes - (1) a fast one via a central neural pathway, probably via the habenular region, and (2) a slower one via the superior cervical ganglia and the peripheral sympathetic pineal innervation. Semm and Vollrath (1979 a) further showed that two functionally different, sympathetically influenced categories of pinealocyte are present.
in the guinea pig pineal which respond to sympathectomy. They suggested that the activity of the two different cell categories may depend on the peripheral and the central innervation of the pineal, respectively. These facts may form part of the basis for the lack of effect on crypt cell proliferation following superior cervical ganglionectomy, in that the particular cells affected by this procedure may not be the ones involved in the effects of pinealectomy. Semm et al. (1979 b) demonstrated fibres from the habenular nuclei reach the guinea pig pineal. Additionally, fibres within the striae medullaris also reach the habenulae somewhat more dorsally. They suggest that the habenular nuclei (probably the lateral ones) may modify pineal gland activity, while, vice versa, the pineal may influence single unit activity in the habenular nuclei. It is not clear how habenular units can be influenced by pineal stimulation. Hence they established the presence of a central pineal innervation. In addition to the habenulo-medullary striae-pineal nerve fibres of guinea pigs, pineal-petal vasopressin and oxytocin containing extrahypothalamic neurosecretory fibres have been demonstrated in the rat in the area of the subcommissural organ and the pineal gland (Buijs et al. (1978); Buijs and Pévet (1980)). Buijs and Pévet (1980) also showed that these fibres ran via the subcommissural organ or the habenular commisure into the pineal stalk, in order to terminate in the anterior part of the pineal. They speculated that these fibres might be involved in the regulation of water balance.

Korf and Wagner (1980) found probably identical fibres in the guinea pig pineal and the origin of these fibres in the region of the paraventricular magnocellular hypothalamic nucleus could possibly suggest their peptidergic nature.
From the paraventricular nucleus, the fibres course via the habenular or the posterior commissure and the pineal stalk to the distal portion of the pineal organ. Also, according to Smith and Ariens-Kappers (1975), in mammals the pineal is not only involved in the regulation of the function of the parvo-cellular but also in that of the magnocellular hypothalamic nuclei (Ariens-Kappers et al., 1974).

Since the vagi appear to be concerned in the actions of the pineal gland on the crypts, it is interesting to note that Swanson and Sawchenko (1980) showed that there was a connection between the paraventricular nucleus (see above) and the dorsal vagal complex. Furthermore, the paraventricular nucleus is connected with the median eminence and the pituitary. Jean-ningros and Mei (1977) found that electrical stimulation of the splanchnic and vagus nerves in cats resulted in responses being recorded bilaterally in an area corresponding to the ventromedian nucleus of the hypothalamus. Whilst this evidence for a descending pathway connecting the pineal with the gut via the vagi and the sympathetics is tenuous, there may well be such a path from the pineal via the hypothalamus and the sympathetic nervous system to the gut, but as remarked previously, this proposed pathway is probably not the only means of action of the pineal on the gut crypts. In fact, the pineal need not be part of such a neural connection.

Dafny (1977) also observed that photic, acoustic, olf-actory bulb, ventromedial hypothalamus and amygdaloid complex stimulation each elicit electrophysiological responses in the rat pineal, suggesting possible afferent input of information from these areas. We shall return to the amygdaloid complex later (see Chapt. IV, Section 111).
This is consistent with the proposed role of the pineal in assimilation of information and modulation of lower centres (Oksche and Pevet, 1981). As noted already, Bindoni concluded from his experiments that the pituitary gland was probably not involved in the actions of the pineal on the intestinal crypts. Clementi (1965) and Palkovits (1965) have suggested that the pineal gland could be involved in water and electrolyte metabolism, and certainly the magnocellular neurons of the paraventricular nuclei of the hypothalamus (which we have seen are connected to the pineal) and the supraoptic nuclei, synthesize and release vasopressin and oxytocin in response to dehydration. As noted above, vasopressin and oxytocin fibres have been associated with the pineal gland (Buijs and Pevet, 1980). It is not clear if there is any significance for this possible involvement of the pineal gland in fluid and electrolyte metabolism in regard to the control of crypt cell proliferation. Oxytocin and vasopressin have not been shown to have any independent effect on crypt cell mitotic rate. Dennhardt et al. (1979) found that in rats the permeability of the luminal membrane of the gut was enhanced by vasopressin and Kerr et al. (1977) found that in the monkey vasopressin was a potent vasoconstrictor of the inferior mesenteric arterial circulation. Erwald et al. (1976) found that synthetic vasopressin diminished hepatic and portal venous blood flow in man. Millet et al. (1975) demonstrated that an increase in intragastric pressure in the rabbit produced a significant rise in plasma vasopressin concentration.

Skagen (1977) and Clarke (1976) have suggested the importance of the physical characteristics of the luminal contents, and in particular the osmolarity of the contents, in the possible mechanism of control of crypt cell proliferation,
and it has been demonstrated by Summers (1978) that there are osmoreceptors in the rat small intestine. Whether or not the pineal could be involved in effects on the intestine via the action of vasopressin or oxytocin is highly conjectural. Any such mechanism seems unlikely to be involved in the control of crypt cell proliferation. For instance, if it operated by means of changes in mesenteric blood flow, these have a very imprecise effect on the submucosal blood flow (Wilson et al., 1975). Furthermore, Sunny-Long et al. (1983) found that pinealectomy influences the release of oxytocin but not vasopressin from the hypothalamo-neurohypophyseal system of the dehydrated rat exposed to a 12 hour light photoperiod.

Another possibility to consider is whether the pineal could act to some extent via the adrenal gland. Kinson et al. (1967), Dickson and Hasty (1972), Pivat et al. (1973), DeFronzo and Roth (1972) produced evidence that the pineal gland had an inhibitory effect on the adrenal cortex, probably mediated by an inhibitory effect on the secretion of A.C.T.H.

However, following pinealectomy the effects on the size and weight of the adrenal glands or the histochemistry and histology of the adrenal cortex were found not to be consistent (Yamada (1961); Wurtman et al. (1959); Hoffman and Reiter (1966); Vollrath (1981)). Kinson (1967) found that pinealectomy was without demonstrable effect on corticosterone production, whereas Jacobs (1974) claimed that it restricted it. Baráth and Csaba (1973) found that pinealectomy depressed adrenal medullary function. Milne et al. (1966, 1968) found that severe stress in rats resulted in regressive structural changes in the pineal followed by enhanced activity, and Lynch et al. (1977) attributed the effects of stress on the pineal to an action on the adrenal medulla.
No definite conclusions may therefore be made regarding the role of the adrenal gland in the effects of pinealectomy but it would appear not to serve a major role in the effects of pinealectomy on the crypts. Carriere (1966), and Sharp (1980) found regressive changes in the mucosa of the gastrointestinal tract after thyroidectomy. After pinealectomy, there is an increase in thyroid gland function (Csaba et al. (1968); Ishibashi et al. (1966)). It is therefore conceivable that the pineal gland could act on the crypts of the small intestine by means of an effect on the rate of thyroid hormone production. The fact that vagotomy and local sympathectomy appear to modulate the effects of pinealectomy suggests, however, that if operative the effect of these hormones is not a major one.

Pinealectomy was found to produce a biochemical syndrome characterized by diminished glucose tolerance, a decrease in hepatic and muscular glycogenesis, and an increase in pyruvate concentration (Milcu et al. (1971); Milcu and Milcu (1958); Milcou et al. (1963); Milcou et al. (1957)). Csaba and Barath (1971) noted that pinealectomy is followed by a decrease in blood sugar levels. On comparing the effects of diabetes on crypt cell proliferation (see Chapt. 1, Section 11) and those of pinealectomy, it seems unlikely that the effect of pinealectomy on the crypts is mediated by an effect on the islets of Langerhans.

Another means by which pinealectomy may have partially achieved its effect is by increasing the intake of food, thereby increasing the luminal nutrition of the mucosa (see Chapt. 1, Section 11). However, there was no significant change if food intake in pinealectomized rats in the present experiments (see Chapt. IV, Section IV).
The present author's findings are in accord with those of Ishibashi et al. (1966) and Takahashi et al. (1976). Baum (1970) also found that pinealectomy was without effect on changes in feeding rhythms induced by changes in environmental illumination.

Another possible mechanism of action of the pineal gland on the crypts is via the secretion of growth hormone. Hester (1966) found that the pineal gland exerts an inhibitory influence on growth. Sorrentino et al. (1971a) concluded that the pineal gland can regulate the growth of sensory deprived rats, either directly by modifying pituitary growth hormone stores or indirectly by way of the pituitary gonadotrophins, or by some other endocrine pathway. The inhibitory influence of the pineal gland on the hypothalamic-hypophyseal-adrenal axis has been suggested by the work of Reiter and Fraschini (1969), Farmer (1974), and Farrell (1959). An anti-stress effect by blocking the stress-induced release of A.C.T.H., mediated by pineal principles, was suggested by Miline (1968) and Motta et al. (1971). Relkin (1972) concluded that increased pineal function probably inhibits secretion of growth hormone releasing factor, which decreases pituitary synthesis and release of growth hormone. In rats, pinealectomy abolishes the dark-induced decrease in growth hormone level in both pituitary and plasma (Relkin, 1972) and prevents the decrease in growth hormone levels which occur after light deprivation alone.

Sorrentino et al. (1971a, b) and Smythe and Lazarus (1973) further proposed that the pineal gland may regulate growth by its melatonin secretion acting as a blocker to secretion of growth hormone at the level of the hypothalamus, but this effect was not confirmed in humans (Smythe and
Lazarus, 1974). Romijn (1978) suggested that, on the basis of various studies (including the above), the pineal gland had more of a modulatory than an inhibitory or stimulatory effect on growth. It is conceivable, therefore, that in the rat the pineal gland could exert some of its effect via the changes in the secretion of growth hormone. Certainly, Sharp et al. (1980) concluded that an intact pituitary was required for the process of crypt cell replication which leads to intestinal growth. Crean (1963), Schapiro et al. (1970), Levin (1969), Leblond and Carriere (1955), Bastie et al. (1982) all found small intestinal mucosal atrophy following hypophysectomy. However, growth hormone as such has only been indirectly implicated in crypt cell proliferation control. Growth hormone secretion cannot, therefore, be definitely implicated in the mechanism of the pineal gland affecting the small intestinal crypts. Furthermore, as previously noted, Bindoni et al. (1968) observed that the rise in cell renewal following pinealectomy also occurred if pinealectomy was accompanied by hypophysectomy, suggesting that its effect did not require an intact hypophysis.

It is also possible of course that the mechanism by which the pineal affects the crypts of the small intestine may be different from the usual mechanism of control of crypt cell proliferation. Since the pituitary gland is known to play an important role in the normal maturation of the small intestinal mucosa at the time of weaning, if the pineal gland exerts a modulatory effect on pituitary function, the pineal may well be important in the maturation of the intestinal mucosa at the time of weaning, but this is very conjectural.

Another possible factor to be considered in the action of the pineal gland on the crypts is the secretion of prolactin.
Sharp et al. (1980) found that prolactin administration increased crypt cell proliferative activity in the small intestine and that prolactin may be important in the hypertrophic response of the intestine in lactating animals, but it may not be entirely responsible for the observed changes. Pinealectomy is followed by an increase in pituitary prolactin levels (Relkin, 1972 a, b, 1973), and a decrease in blood prolactin concentration. However, the diurnal rhythm of this hypophyseal hormone is not greatly affected by pinealectomy (Niles et al., 1977). The effect appears to be mediated by changes in the levels of a hypothalamic inhibitory factor, which in turn regulates prolactin secretion, probably through the agency of melatonin (Reiter et al., 1976, 1977). It is conceivable that the pineal gland may affect the growth of the small bowel mucosa during lactation by modulation of the secretion of prolactin, but this is not necessarily relevant to the results obtained in the present experiments. Thus, the results of the present experiments appear to indicate that whilst the effect of pinealectomy on the crypt cell proliferation rate of the crypts of the small intestine is probably mediated mainly via the sympathetic and parasympathetic nervous systems, the fact that local denervation of the small intestine does not reduce the hyperproliferative effect on the small bowel crypts following pinealectomy as fully as might be expected from the effects of concomitant vagotomy suggests that perhaps the pineal may act via other pathways to some extent also e.g. via such humoral agents as the adrenal cortical hormones, thyroid hormones, growth hormone or prolactin. These humoral mechanisms would be consistent with the fairly widespread effects of pinealectomy described by Bindoni. Certainly, neural connections to the hypothalamus.
have been demonstrated (Korf and Wagner, 1980) so that the pineal gland could act via the hypothalamus and the sympathetic nervous system or via the neuroendocrine pathways, or via a combination of both.

It appears that the nature of the luminal contents, whether food or biliary secretions is not important in the action of the pineal gland on the small intestinal crypts and in fact the effects of pinealectomy on the crypts may override the effects of changes in the luminal environment which would produce only minor changes in crypt cell proliferation rate.

The question which must be answered is whether the pineal gland is involved in the day to day regulation of crypt cell proliferation. The available experimental evidence does not allow any definite conclusion as to its role in normal control of this process. Perhaps the fact that pineal gland excision effects may override the other effects, e.g. luminal nutrition, known to be probably significant in the day to day control of crypt cell proliferation in the small intestine, and the fact that the effects are relatively so great may suggest that the pineal is involved in crypt cell mitotic control in "unusual situations". It is difficult to suggest what these situations are. Perhaps the pineal gland is involved in the maturation of the small intestine and/or in reactions of the body to stress.

The apparent "damping down" effect on crypt cell proliferation raises interesting possibilities regarding the possible role of the pineal gland in the development or maintenance of neoplastic conditions in the bowel. Williamson (1979), Williamson and Malt (1980) have pointed out that the adaptive growth following proximal small bowel resection
enhances the development of chemically induced colonic carcinoma, although there are obviously many other unknown factors involved. It is conceivable that given the right conditions, e.g. the correct luminal environment, changes in the level of pineal secretion may influence the development of mucosal neoplasia. Relkin (1976) reviewing the available literature on the relationship between the pineal gland and malignancy, concluded from the many studies examined that there was a suggestion that—

(a) the pineal gland itself does not provide fertile environment for the growth of neoplastic tissue,

(b) the presence of the pineal seems to retard the growth and spread of tumours,

(c) the pineal gland in humans having neoplastic disease becomes larger and degenerates, as a result of the disease. Relkin considered that it was likely that this degeneration was the end result of a gland which had been chronically hyperactive, (thus its increased size), in an effort to secrete substances such as melatonin which are capable to some extent of controlling neoplasia. The proposed degeneration could explain the findings that these pineals are lighter than those obtained in patients dying of non-neoplastic diseases. However, Tapp (1980 a ) contended that the weights of pineal glands of those dying of malignant conditions were not statistically different from those dying of non-malignant conditions, although he did concede that in most age groups the glands tended to be smaller in patients dying of malignancy, and patients dying of carcinoma of the breast and from melanoma had larger glands than those dying of sarcoma. The same author (1980 b ) found increased metabolic activity in the pineal glands of tumour bearing animals, although the
pineal weights did not differ from controls.

Lapin (1976), in a review of the relationship between the pineal gland and malignancy, commented that experimental as well as clinical data have shown that the pineal gland and its compounds may be involved in the development and growth of neoplasia, possibly by means of both endocrine and neural activities of the gland. Some evidence was also available that the pineal substances could modify cell division and the mitotic process. Rodin (1963), Dasgupta and Terz (1967), Barone and Dasgupta (1970), Lapin (1974) found that pinealectomy enhanced the growth of transplantable tumours. Considering the possible role of melatonin in the pineal effect, Lapin and Frowein (1981) noted a negative correlation between melatonin content and the size of a Yoshida Sarcoma. Bartsch and Bartsch (1981) found that experimental tumours were stimulated by melatonin injections in the morning and inhibited by late afternoon injections. El Domieri and Dasgupta (1973) found a reversal by exogenous melatonin of the effects of pinealectomy on tumour growth in pinealectomized hamsters. However, Huxley and Tapp (1972) demonstrated that no anti-tumour effects could be detected following the five day administration of melatonin on the growth of Yoshida Sarcoma in intact rats. Thus, this remains a controversial subject and the present experimental findings do little to elucidate it.

It should be remembered that pinealectomy itself creates an artificial situation in the body, and removal of the pineal gland may produce excess activity in another part of the central nervous system, so that the possibility that the effects of pinealectomy are secondary to the release of
other effects should be borne in mind. This possibility will be further considered later in this discussion.

Considering the nature, magnitude, and distribution of the proliferative response in the gut after resection, and the demonstrated dependence on luminal factors, it seems unlikely that the response of the small bowel to resection or bypass involves the pineal gland in its mechanism. Similarly, the effects on the small bowel mucosa of starvation and refeeding do not appear to be mediated by the pineal gland.

The effects of pinealectomy would appear to fit into a systemic rather than a local effect and it is possible that the pineal forms part of a feedback mechanism in which excessive proliferation is suppressed by the secretions of neural connections of the gland. The fact that there is a dramatic effect on crypt cell proliferation rate after pinealectomy seems to imply either a tonic suppressive effect by the pineal gland on crypt cell proliferation in the small bowel or that the pineal is opposed by another mechanism which tends to increase crypt cell proliferation. There is no indication from the present experimental findings as to which of these two mechanisms is more likely, and no indication from the literature of a possible central nervous system mechanism tending to tonically increase crypt cell proliferation. The pineal gland may possibly form part of a mechanism to prevent excessive swings of hyperproliferation in the crypts due to other factors, and thus may possibly be active only when the normal mechanisms of control of crypt cell proliferation are exceeded. Pinealectomy does not appear to result in totally unrestricted crypt cell proliferation, so that other mechanisms must be operative to prevent the unrestrained proliferation seen in malignancy.
Oksche and Pevet (1981) considered it highly probable that the pineal gland had multiple functions. They said that by its neural and/or endocrine activity this gland centrally modulates endocrine and probably also non-endocrine regulatory systems. More specifically this may be in the nature of tuning their function in response to external and internal conditions for the benefit of the organism. They also considered that melatonin is probably not the pineal effector compound "par excellence". Reiter (1981) also suggests that as well as melatonin there may be other active principles produced by the pineal gland, as yet undiscovered, which may be responsible for its actions. He considered that the pineal keeps the organism in proper synchrony with the prevailing environmental conditions, especially in relation to the photoperiod, since it has been implicated amongst other things in the regulation of body temperature, influencing lipid metabolism or determining activity rhythms in the body.

Melatonin, however, should be considered as a possible mediator of pineal activity on the crypts, since it occurs in both the pineal gland and the gastrointestinal tract of the rat throughout life (Bubenik, 1980). Raikhlin and Kvetny (1976) in fact found active biosynthesis of melatonin in the enterochromaffin cells of the gastrointestinal tract. Bubenik (1980) found that exogenously administered melatonin concentrated in all parts of the gastrointestinal tract with most pronounced accumulation in the colon and rectum, suggesting that possibly endogenously produced melatonin might also accumulate in these tissues. However, since pinealectomy neither influences the levels of melatonin in rat hypothalamus (Koslow, 1974), nor in the digestive tract (Bubenik, 1980), and removal of the pineal gland lowered melatonin levels in
the plasma of ewes only temporarily (Kennaway, 1977), the pineal gland cannot be considered to be the only source of melatonin. Ozaki and Lynch (1976) further suggested that extra-pineal sources of melatonin may contribute to the circulating pool in plasma, implying that after pinealectomy other tissues may be able to take over the synthesis of melatonin. Since it appears that the effects of pinealectomy are not affected by luminal factors, it is difficult to reconcile the effects of vagotomy in removing the pineal effect and the presumed mostly afferent role ascribed to the vagus nerves by the results reported in Section 1, Chapt. IV. If the vagus nerves are involved in relaying luminal information to the central nervous system, it might possibly be expected that, since luminal factors have no influence on the effects of pinealectomy, the vagi might have less effect also on the pineal effect. The vagi in respect to the effect of the pineal on the crypts appear to have more of an efferent role. This raises the possibility that the effect of the pineal gland on the crypts is an "abnormal" mechanism, not usually operative in the day to day control of crypt cell proliferation. Since luminal information itself does not influence the pineal effect, the effect of lesions of the "visceral brain", the limbic system, will be considered in the next section of this thesis.

As noted above, the pineal gland has connections with the hypothalamus, and it may be that it exerts its effects via the hypothalamus, which in turn regulates the autonomic nervous system. The hypothalamus, as noted in Chapt. 1, Section 11, itself has been noted to have an effect on crypt cell proliferation (Jutisz et al. (1974); Bindoni et al. (1973)) so that the pineal may represent only part of a more extensive C.N.S. mechanism for controlling crypt cell proliferation.
SECTION 111

THE EFFECTS ON CRYPT CELL PROLIFERATION OF BILATERAL LIMBIC SYSTEM LESIONS, (WITH OR WITHOUT ASSOCIATED PINEATECTOMY).

(1) Introduction to section 111.

(2) Experiments,

(a) The effects of bilateral hippocampal lesions,
   (with or without associated pinealectomy),
(b) The effects of combined bilateral hippocampal lesions and local small bowel denervation,
(c) The effect of combined bilateral hippocampal lesions and bilateral truncal abdominal vagotomy,
(d) The effect of bilateral amygdaloid lesions,
   (with or without associated pinealectomy),
(e) The effect of bilateral septal lesions,
   (with or without associated pinealectomy),
(f) The effect of bilateral fornix lesions,
   (with or without associated pinealectomy).

(3) Discussion on section 111.
INTRODUCTION TO SECTION 111 EXPERIMENTS

It appears from the results in Section 11 of this thesis that the pineal gland acts, at least to some extent, via the autonomic nervous system in its effects on the crypt cell mitotic rate in the small intestine. Presumably, if we are to postulate that the pineal has the effect of decreasing the crypt cell proliferation rate if intact, then it is not inconceivable that another area in the central nervous system may have a role in opposing this effect. The area of the brain known as the "limbic system", which includes the amygdaloid nuclei, hippocampus, septal and fornix regions, is not only known to be involved in the expression of emotions but is referred to by some authors as the "visceral brain" i.e. a higher centre concerned with the reception and interpretation of afferent information from the viscera and concerned with visceral function.

The major portions of the limbic system are extensively interconnected with and influenced by the association cortex, more particularly the prefrontal cortex. In turn it has many two-way connections and a major role in influencing the hypothalamus in the regulation of functions of that structure. It is also evident that there is a lot of overlap in the functions of the different components of the limbic system with those of the hypothalamus. It deserves to be stressed that as to what extent and how the various components of the limbic system are involved in complex visceral functions remains to be elucidated.

Since the pineal gland is known to have connections with the paraventricular magnocellular hypothalamic nucleus (Korf and Wagner, 1980), and the limbic system is known to
have hypothalamic connections, it was considered possible that they might both act via the hypothalamus and possibly the autonomic nervous system in their effect on the crypts. Of course it will be recalled that the hypothalamus may exert its influence, if any, on the intestine in at least three ways. Firstly, via two-way polysynaptic connections with the various nuclei of the vagus nerve in the medulla and the sacral parasympathetic outflow. Secondly, via descending sympathetic influences which link the hypothalamus with autonomic nuclei and regions in the medulla, and more specifically with the lateral horn of the thoracolumbar spinal cord. Thirdly, via the neuroendocrine links involving the neuro-hypophysis and adeno-hypophysis.

Accordingly, lesions were made in the limbic area of the brain to determine if they affected the crypt cell mitotic rate in the small intestine. By associating these lesions also with pinealectomy, it was hoped to determine if these limbic areas were associated functionally with the pineal effect on crypt cell proliferation.

To determine if the autonomic nervous system could play a role in the mediation of effects from the limbic system to the crypts, and since all areas of the limbic system are known to be interconnected, it was decided to select one particular key area of the limbic system, the hippocampus, and associate lesions of this area with bilateral truncal abdominal vagotomy or local denervation of the small intestine.
THE EFFECTS OF HIPPOCAMPAL LESIONS, (WITH OR WITHOUT ASSOCIATED PINEAL LESIONS), ON CRYPT CELL PROLIFERATION IN THE SMALL INTESTINE.

METHODS

Twenty nine male Sprague-Dawley rats weighing between 268-495 Gms. were randomly divided into four groups and anaesthetized, as in Chapt. 111, (b) and (d).

GROUP (1), CRANIOTOMY ONLY PERFORMED.

(8 RATS).

Bilateral trephine holes were made in the skull (as in Chapt. 111, d) at the same site as for "neocortical" lesions (see Chapt. IV, Section 111, and A in Fig. 4.33). The discs of bone were removed and the dura mater incised, but damage to the underlying cerebral cortex avoided. The scalp was closed with black silk, Penicillin was administered and food and fluids supplied ad libitum postoperatively. Three weeks later, the rats were killed, at intervals between 1 1/4 hours and 3 1/4 hours after intraperitoneal Colchicine.

GROUP (2), BILATERAL "NEOCORTICAL" LESIONS.

(8 RATS).

Craniotomy was performed (see Chapt. 111, d). Bilateral trephine holes (A in Fig. 4.33, 4.34) were located approximately 2 mms. posterior to bregma, 2 mms. lateral to the sagittal suture and 2 mms. anterior to lambda (see Figs. 4.33 and 4.34 (E)). The dura mater was incised with a knife and removed from the trephine hole with scissors. The underlying cortex was aspirated by gentle suction, using a narrow bore malleable sucker and low pressure suction by a water pump (Kartell, No. 1395). The cerebral cortex was found to be approximately 1 mm. thick and removal of this exposed the
fibres of the corpus callosum. This was gently aspirated and the lateral ventricle of the brain entered. Bleeding was controlled by drawing air with the sucker through a cotton pellet soaked in warm normal saline. The hippocampus could be seen within the ventricle but care was taken not to damage this. Gelfoam was placed in the trephine hole and the scalp closed. Penicillin was given postoperatively and food and fluids supplied ad libitum. Three weeks later the rats were killed, at intervals between 1 1/4 hours and 4 hours after intraperitoneal Colchicine. The brains were perfused (see Chapt. 111, g) and later removed for examination. The methods used for these cerebral lesions were similar to those of Hart (1976).

GROUP (3), BILATERAL HIPPOCAMPAL LESIONS, (8 RATS).

Craniotomy was performed (Chapt. 111, d) and the "neocortex" was exposed, as previously. The "neocortex" was aspirated as was the corpus callosum, as previously, and the fluid filled lateral ventricle entered (see B in Fig. 4.35). The hippocampus was then clearly visible (see A in Fig. 4.35) in the floor of the ventricle as a shiny surface which was whiter than the overlying white matter of the cortex. The hippocampus was gently aspirated within the confines of the overlying trephine hole to a depth of approximately 1 mm. when the underlying reddish coloured thalamus was clearly visible. Care was taken not to damage the thalamus. Haemostasis was achieved with Gelfoam. The scalp was closed with black silk sutures and clips. Penicillin was administered postoperatively and food and fluids supplied ad libitum. Three weeks later, the rats were killed, at intervals between 1 hour and 4 hours after intraperitoneal Colchicine. All brains were perfused (see Chapt. 111, g) and later removed for
sectioning (50μm.) and stained with Neutral Red (see Chapt. 111, h) or preserved for dorsal surface photography. The method used was once again similar to that of Hart (1976).

GROUP (4), COMBINED PINEALECTOMY AND BILATERAL HIPPOCAMPAL LESIONS,
(5 RATS).

Bilateral hippocampal lesions were produced, as previously, and under the same anaesthetic, pinealectomy was performed (as in Chapt. IV, Section 11). The scalp was closed and specimens of the pineal gland were stained and sectioned, as previously, and examined histologically to confirm pinealectomy. Penicillin was administered postoperatively and food and fluids supplied ad libitum. Three weeks later the rats were killed, at intervals between 1 1/4 hours and 3 1/2 hours after intraperitoneal Colchicine. The brain was perfused (as in Chapt. 111, g) and either sectioned and stained with Neutral Red (as in Chapt. 111, h) to confirm the extent of the lesions or the dorsal surface was photographed after removal from the skull. In all groups specimens of small intestine were collected, sectioned and stained (as in Chapt. 111, e). The mitotic rate in the small intestine was determined (as in Chapt. 111, j and k).
**Figure 4.33**

Diagrammatic representation of the dorsum of the skull, showing the areas of skull removed in craniotomy, (A), (associated with "neocortical" lesions).

B = Bregma.
C = Lambda
D = Sagittal suture
E = Coronal suture.
Diagrammatic representation of the dorsal surface of the brain (rat), showing the site of the trephine holes (A).

B = The coronal suture (skull)
C = The sagittal suture (skull)
D = The lambdoid suture (skull)
E = Distances to trephine hole, see text.
F = The cerebellum
G = The Frontal cortex
H = Olfactory lobe
I = Pineal gland
Figure 4.35

Diagrammatic representation of the right and left trephine holes made during exposure of the hippocampus. (A), B = the lateral ventricle. C = the margin of the trephine holes.
Figure 4.36
Dorsal view of typical bilateral "neocortical" and hippocampal lesions (A) and pineal lesion (B) (X 4.5).
Coronal section of the rat brain at the level of the "neocortical" lesions (A) showing the extent of the dorsal hippocampal lesions (B). Note the underlying thalamus (C) has not been damaged.

D = the cerebral aqueduct.

E = the periaqueductal grey.

F = the remainder of the hippocampus.

Section stained with neutral red (X 9.6).
RESULTS

Mitotic rates in the small intestine following-

(1) CRANIOTOMY + "NEOCORTICAL" + CORPUS CALLOSUM LESIONS

<table>
<thead>
<tr>
<th></th>
<th>PROXIMAL</th>
<th>DISTAL</th>
<th>ILEUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEJUNUM</td>
<td>0.0693 ±</td>
<td>0.0678 ±</td>
<td>0.0037 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

The mitotic rates in the proximal jejunum and distal ileum of both groups were not statistically different, on comparison of standard errors.

Correlation Coefficients -

<table>
<thead>
<tr>
<th></th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.9922</td>
<td>0.9968</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9956</td>
<td>0.9920</td>
</tr>
</tbody>
</table>

i.e. there was satisfactory correlation for each regression line.

Mitotic rates in the small intestine following -

(1) BILATERAL "NEOCORTICAL" LESIONS ONLY

<table>
<thead>
<tr>
<th></th>
<th>PROXIMAL</th>
<th>DISTAL</th>
<th>ILEUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>JEJUNUM</td>
<td>0.1205 ±</td>
<td>0.0057 ±</td>
<td>0.0013 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.
PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.00005, t = 8.827, df = 12, i.e. statistically significant at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005, t = 10.520, df = 12 i.e. statistically significant at the 5% level.

**TABLE 4.21**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th>(1) HIPPOCAMPAL LESIONS AND PINEALECTOMY</th>
<th>(2) HIPPOCAMPAL LESIONS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.1219 ± 0.0029</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1213 ± 0.0020</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

There was no statistically significant difference between these groups on comparison of standard errors.

**TABLE 4.22**

Correlation Coefficients -

<table>
<thead>
<tr>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.9960</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9987</td>
</tr>
</tbody>
</table>

i.e. there was satisfactory correlation for each line.

**TABLE 4.23**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th>(1) HIPPOCAMPAL LESIONS AND PINEALECTOMY</th>
<th>(2) PINEALECTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.1219 ± 0.0029</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1213 ± 0.0020</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.
PROXIMAL JEJUNUM, GROUP 2 ≠ PROXIMAL JEJUNUM, GROUP 1, P < 0.10, t = 2.064, df = 7, i.e. not statistically different at the 5% level.

DISTAL ILEUM, GROUP 2 ≠ DISTAL ILEUM, GROUP 1, P < 0.02, t = 3.04, df = 7 i.e. significantly different at the 5% level.

Figure 4.38

Graph of mitotic index versus time.
X = Proximal jejunum, hippocampal lesions.
• = Proximal jejunum, "neocortical" lesions.
Graph of mitotic index versus time.

X = Distal ileum, hippocampal lesions.

○ = Distal ileum, "neocortical" lesions.
DISCUSSION

The results of this portion of the study show that the crypt cell mitotic rate in the small intestine after bilateral lesions involving the "neocortex" and corpus callosum was not statistically different from the mitotic rate in the small intestine following craniotomy and division of the dura mater only. The term "neocortex" is perhaps too inclusive as the area lesioned is only one of several areas of neocortex. However, the term "neocortex" is used here merely for convenience to designate a particular part of the neocortex overlying the part of the hippocampus to be lesioned.

The crypt cell mitotic rate in the small intestine of rats subjected to bilateral (1) craniotomy, (2) "neocortical" lesions, (3) lesions of the corpus callosum, (4) hippocampal lesions clearly exceeds that in the small intestine of rats subjected to bilateral (1) craniotomy, (2) "neocortical" lesions, (3) lesions of the corpus callosum, only. It is thus deduced that the sharp rise in crypt cell mitotic rate in hippocampal lesions is associated specifically with the hippocampal lesions themselves.

It should be noted that the same area of "neocortex" at the same site was removed in all cases and that only the dorsal hippocampus was lesioned in all cases, care being taken (and histological confirmation being obtained) that the underlying thalamus was not damaged. The period of three weeks after lesioning was arbitrarily chosen on the basis of other researchers reports, as the period likely to allow recovery from the initial procedure and subsidence of local oedema around the lesion, so that the effects of oedema and possible
associated loss of function of the neighbouring brain areas could be minimized. The hippocampus is the largest component of the hippocampal formation and is an infolding of the parahippocampal gyrus. It consists of three regions: the hippocampus, the dentate gyrus, and the subiculum. It is a C-shaped structure in coronal sections, bulging into the inferior horn of the lateral ventricle. It resembles, in shape, a ram's horn and is closely associated with the adjacent dentate gyrus and together they form an S-shaped structure. The hippocampus forms a prominent ridge in the floor of the lateral ventricle overlying the thalamus. The surface of the hippocampus is covered by a layer of white fibres (alveus) which is composed of hippocampal projection fibres and these are later referred to as fimbria.

The dorsal surface of the hippocampus was lesioned in this experiment at about its midpoint. If this surface of the structure was followed anteriorly, it would be found to become smaller in size and to merge into a large bundle of fibres (fornix) which interconnects the hippocampus with the septum and the hypothalamus. Moving even further anteriorly, the fornix fibres split into (1) a compact post-commissural division (usually called the "columns of the fornix") which descends just posterior to the anterior commissure, and (2) a more diffuse precommissural division that travels throughout the septal region. The anterior-most aspect of the hippocampus therefore, is composed largely of fibres of the fornix. More posteriorly, the hippocampus has a "rolled" appearance on cross-section and still more posteriorly, the hippocampus is seen in two locations - a dorsal component and a ventral component.

The ventral component is the tip of the "horn" which has curved downwards and anteriorly into the temporal
lobe region (the detailed internal architecture of the hippocampus is complicated and not relevant to the present discussion). The hippocampus receives axons from many cortical areas, particularly the sensory association regions, including the amygdaloid nucleus, via the parahippocampal gyrus, and forms a component of the "limbic system" which will be discussed throughout this thesis.

The traditional view of hippocampal efferent function was -

1. the hippocampus was thought to act as a single unit and common functions were ascribed to the portion of the hippocampus adjacent to the entorhinal cortex (anterior or ventral hippocampus) and to its more posterior and dorsal portion.
2. it was thought that the common functions of the hippocampus were subserved by common efferent projections, i.e. that virtually the entire influence of the hippocampus was conveyed via the fornix to the hypothalamus and the thalamus. It was thought that the hippocampus and the amygdala (the amygdaloid nuclei—also part of the "limbic system") function independently through their respective projection systems and have common functions only to the extent that their respective efferent systems project to the same diencephalic structures.
3. hippocampal influence was thought to be almost entirely inhibitory, primarily because behavioural studies showed an arrest of behaviour during hippocampal activation.
4. the hippocampal efferent system, the fornix, was thought to project almost exclusively to the septum and the mammillary bodies. From here its influence was believed to be relayed to the anterior thalamic nuclei, to the cingulate cortex, and finally back into the hippocampus—the limbic circuit proposed by Papez (1937). Poletti (1986) in a review of more recent work, including his own, has presented a reformulated view
of hippocampal efferent function. viz—,
(1) although hippocampal influence at the behavioural level
appears to be inhibitory; at the neuronal level in the basal
diencephalon, at least, its influence is predominantly excitat-
ory.
(2) hippocampal influence is not focused on the region of the
mammilary bodies but is distributed throughout the hypothalamus.
Furthermore, the influence of the hippocampus on the preoptic
areas and basal forebrain may be even greater than its influ-
ence on the hypothalamus. Similarly, the fornix system in the
primate projects only a relatively small portion of fibres to
the mammillary region, with other projections throughout the
hypothalamus and comparably prominent direct projections to
both the medial and lateral preoptic areas as well as thro-
ughout the basal forebrain.
(3) a major portion of hippocampal influence on the basal di-
encephalon is not mediated via the fornix system.
(4) the anterior and posterior portions of the hippocampus
have different anatomical and physiological functional pro-
jections: the posterior hippocampus influences the basal di-
cephalon exclusively via the fornix system with major in-
puts to the basal forebrain, the preoptic region, and with
less prominent inputs to the hypothalamus.

In contrast, the major influence of the anterior hippo-
campus on the hypothalamus and basal forebrain is exerted via
the amygdala, whereas the major influence of the anterior
hippocampus on the preoptic region appears to be mediated via
the fornix system. Accordingly, the hippocampus and the amygdala
now are shown not to act as separate structures within
the temporal lobe. The hippocampus has a direct prominent
effect on amygdala unit activity and the amygdala in turn
integrates and exerts a major portion of the anterior hippocampal influence on the hypothalamus and basal forebrain.

The functional influence of the anterior hippocampus may be merely relayed via the amygdala or one of the functions of the amygdala is to integrate and modulate the influence of the anterior hippocampus. This latter formulation is thought to be the more correct i.e. anterior and posterior hippocampus have opposing influences. Poletti also quotes evidence that there are opposing influences of different parts of the amygdala on the basal diencephalon. This formulation of course raises the possibility that lesions of the anterior hippocampus may affect the crypt cell proliferation rate differently from those of the posterior hippocampus. In the present experiment, it was not possible to define precisely as to which area, anterior or posterior, the lesions of the hippocampus belonged. When the results of the other experiments in the present series are considered and the position of the hippocampus within the limbic system evaluated, a view could be taken that the position of the lesions within the hippocampus probably was not very important in the resulting effect on crypt cell proliferation. It is not obvious, when considering lesions in such a complex structure with so many connections, how these lesions have resulted in the changes in crypt cell proliferation seen in this case or why this effect should be present. However, when the hippocampus is considered subsequen-

tly in more holistic terms as part of the limbic system
it will be seen that there is a possible explanation in terms of that system. Let us consider the direction of the principal influence of the hippocampus. Previously, it was considered that the principal influence of the hippocampus was directed into the Papez circuit. Papez (1937) said that hippocampal influence on the diencephalon was relayed primarily to the cingulate cortex and then back to the hippocampus to complete the Papez circuit.

However, Poletti (1986) in a series of experiments demonstrated (1) that the primary influence of the hippocampus was not projected on the cingulate cortex but in all likelihood on the basal diencephalon. The hippocampus was thus thought to have a major dual influence exerted throughout the basal diencephalon. This dual influence, with opposing effects on the ventromedial nucleus of the hypothalamus, is mediated directly via the fornix and through the amygdala.

(2) the influence of the hippocampus on the cingulate cortex and its inferred participation in the limbic circuit postulated by Papez appears to be comparatively minor.

(3) in contrast the temporal lobe limbic system has a major influence on the brain stem and the periaqueductal grey and also direct projections to the spinal cord. Based on extensive physiological and anatomical data, the author proposes that the limbic system does not function as a reverberating circuit rimming the telencephalon. Instead, that the temporal lobe limbic structures exert their functional role primarily by a direct influence on structures downstream in the neuraxis; the diencephalon, the midbrain and even the spinal cord.

This concept implies that the temporal lobe limbic system plays a significant direct role in most of the functions associated with the basal diencephalon and on some specific functions of
the brainstem and spinal cord.

The working hypothesis thus formulated is that the temporal lobe limbic system modulates virtually all of the functions of the brainstem, including behavioural responses to "fight and flight", pain suppression, sexual maturation and autonomic and endocrine activity. It is conceivable therefore that the limbic system may act on the crypts by modulation of the activity of the autonomic nervous system on the crypts, since this is known to influence crypt cell mitotic activity. With this in mind, the effects of combined hippocampal lesions with vagotomy or local denervation of a small bowel loop were considered subsequently.

Newman (1974) in a review of the mechanisms of cerebral control of the hypothalamus, suggested that another way of looking at the role of the hippocampus is from the point of view of a visceral control centre which has to deal with unpredictable emotional situations so that a given effect can never be guaranteed to follow a given stimulus. It was further suggested that there might be a feed-back to the hypothalamus itself and in this way appropriate adjustments of the hippocampal output could be made so that under conditions of intense excitement such as occurs in emotional stress, inhibition is overcome by the release of excitatory transmitter and the result is a powerful driving force on the centres controlling the viscero-endocrine output. Thus, the hippocampus may only have its effect on the crypts, for instance, under unusual conditions, e.g. of stress, and not under the usual conditions, when the luminal factors in the intestine may perhaps play a more prominent role.

With regard to the neocortex, Nauta (1986) states that traditionally in humans the neocortex is considered to
embody the highest levels of sensory analysis and integration as well as certain of the more differentiated components of motor function as the principal substrate of thought processes. He adds that its role in rodents of course may differ to some extent from this. The limbic system, by contrast, is viewed as the main cerebral representative of the internal milieu, expressing its functions in the form of states of affect and motivation. However, it is now known that nearly all of the neocortex is connected with both the limbic system and the corpus striatum, in the case of the limbic system by way of the variously complex sequences of cortico-cortical connections. Influences of these connections ultimately converge on ventral and medial regions of the temporal cortex, which in turn project to the amygdala as well as to the entorhinal area, the major cortical source of afferents to the hippocampus. The cortical conduction line to the amygdala is reciprocated, in part at least, by direct amygdofugal projections to the inferior temporal and prefrontal cortex. Thus, the "neocortex" lesioned in these experiments cannot be considered to be a separate functional unit from the hippocampus. In this context it is strange that lesions of the "neocortex" do not produce changes in crypt cell proliferation rate but lesions of the hippocampus do, for some reason which is not evident.

Since the limbic system is rather ill-defined, the definition of the components of the limbic system itself are variable and there appears to be no universal agreement on its exact composition (perhaps for the reasons mentioned above, and because of species differences). For the purposes of this thesis we have used the view of Hamilton (1976) who states that the structures most commonly included in the description of the rat limbic system are,
the hippocampus, the cingulate gyrus, the septum, the amygdala, the entorhinal cortex, and parts of the hypothalamus and midbrain. For the purposes of the other experiments in this section, this description will be used.

Lesions of the corpus callosum are noted also to be not associated with changes in crypt cell proliferation rate in this experiment. This structure consists of a bundle of nerve fibres, the vast majority of fibres coursing transversely, interconnecting the two hemispheres and continuous with the external capsule which marks the lateral border of the amyg-daloid complex. Many of the connections between cortical and subcortical regions pass through this thick band of fibres.

In considering the effects of combined hippocampal lesions and pinealectomy, it was found that the mitotic rate in those rats with combined lesions did not differ significantly from those with hippocampal lesions alone or pinealectomy alone. These results are more consistent with the effects of pinealectomy being mediated via the limbic system, rather than the limbic effects on the crypts being mediated via the pineal gland. There are no known direct neural connections between the pineal gland and the hippocampus. However, Ueck (1979) found evidence supporting the existence of direct connections between the amygdaloid nuclei and the pineal, and of course the amygdaloid nuclei are known to be connected with the other parts of the limbic system, including the hippocampus. It is possible that the limbic system and the pineal gland may form part of an integrated system affecting the crypt cell proliferation rate, since both the limbic system and pineal have connections with the hypothalamus and the hypothalamus plays an important part in the autonomic nervous system; it is referred to by Smith et al. (1980) as the "head ganglion"
of the autonomic nervous system. In this context it is interesting to note that the effects of pinealectomy are modified by autonomic nervous denervation of the gut. Thus, it is possible that the effects of lesions in both areas may be mediated via the autonomic nervous system. In the following experiment the effect is observed of autonomic nervous denervation of the intestine combined with hippocampal lesions to determine if the hippocampal lesion effect on the crypts is likely to be mediated via the autonomic nervous system.

THE EFFECT OF BILATERAL HIPPOCAMPAL LESIONS ON CRYPT CELL MITOTIC RATE IN DENERVATED SMALL INTESTINE.

METHODS

Eleven male Sprague-Dawley rats were randomly selected from a group weighing 345-540 Gms. into two groups and anaesthetized (as in Chapt. 111, b).

GROUP (1), LOCAL DENERVATION OF SMALL INTESTINE ONLY, (5 RATS).

Laparotomy was performed (as in Chapt. 111, c) and local denervation of a loop of proximal jejunum and distal ileum was performed (as in Chapt. IV, Section 1). The abdomen was closed, Penicillin administered and food and fluids administered ad libitum.

GROUP (2), BILATERAL HIPPOCAMPAL LESIONS AND LOCAL DENERVATION OF SMALL INTESTINE, (6 RATS).

Laparotomy was performed (as in Chapt. 111, c) and local denervation of proximal jejunum and distal ileum performed (as in Chapt. IV, Section 1). The abdomen was closed, and under the same anaesthetic, bilateral hippocampal lesions were made (as in Chapt. IV, Section 111). The scalp was closed, and Penicillin administered. Food and fluids were
supplied ad libitum. Three weeks later, the rats of both
groups were killed, at intervals between 1 hour and 3 1/4 hours
after intraperitoneal Colchicine. In group 2, the brain was
perfused (as in Chapt. 111, g) and sectioned to examine the
extent of the lesions (as in Chapt. 111, h). These lesions
were compared with previous hippocampal lesions. Specimens of
the denervated small intestine were collected, sectioned and
stained (as in Chapt. 111, e). The mitotic rate in the
crypts was determined (as in Chapt. 111, j and k).

RESULTS

TABLE 4.24

Mitotic rates in the small intestine following-

<table>
<thead>
<tr>
<th></th>
<th>(1) HIPPOCaMPAL LESIONS</th>
<th>(2) HIPPOCaMPAL LESIONS AND LOCAL DENERVATION</th>
<th>ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.0368 ±</td>
<td>0.1205 ±</td>
<td></td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0025 ±</td>
<td>0.0057 ±</td>
<td></td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.0373 ±</td>
<td>0.1199 ±</td>
<td></td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0044 ±</td>
<td>0.0054 ±</td>
<td></td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 1, P<0.00005,

\[ t = 12.492, \text{df} = 10, \text{i.e. significantly different at the 5}\% \text{level.} \]

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P<0.00005,

\[ t = 11.472, \text{df} = 10, \text{i.e. statistically different at the 5}\% \text{level.} \]

TABLE 4.25

Mitotic rates in the small intestine following-

<table>
<thead>
<tr>
<th></th>
<th>(1) HIPPOCaMPAL LESIONS</th>
<th>(2) LOCAL DENERVATION AND LOCAL DENERVATION</th>
<th>ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.0368 ±</td>
<td>0.0297 ±</td>
<td></td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0025 ±</td>
<td>0.0032 ±</td>
<td></td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.0373 ± 0.0314</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0044 ± 0.0045</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.20, t = 1.830, df = 7, i.e. not significantly different at the 5% level.

DISTAL ILEUM, GROUP 1, NOT DIFFERENT FROM DISTAL ILEUM, GROUP 2, ON COMPARISON OF STANDARD ERRORS.

**DISCUSSION**

The results of this portion of the study show that local denervation (principally sympathectomy) of a loop of jejunum or ileum in the presence of bilateral hippocampal lesions (as in the previous experiment), results in a crypt cell mitotic rate which is not only diminished to a level very much below the hyperproliferative level associated with hippocampal lesions but is not significantly different from the level expected after local denervation. As in the previous experiments, the corpus callosum and "neocortex" were removed to expose the hippocampus and care was taken not to damage the underlying thalamus. It could be argued that the procedure of local denervation of the small intestine used itself had resulted in local ischaemia of the crypts, which is known (Rijke et al., 1976) to be associated with decreased crypt cell mitotic rate. However, as was found in the experiments in Chapt. IV, Section 1, it is probable that the effect of local denervation on the crypts is not a vascular but primarily a neural one. It seems likely in this case then that the effects of hippocampal lesions on the small bowel crypts have been interrupted by loss of the principally sympathetic innervation. That is, the effects of the hippocampus on the crypts are probably mediated in part at least via the sympathetic nervous
Figure 4.40. TIME (HR)

Graph of mitotic index versus time.

X = Proximal jejunum, hippocampal lesions and local gut denervation

● = Proximal jejunum, hippocampal lesions.
Figure 4.41

Graph of mitotic index versus time.

X = Distal ileum, hippocampal lesions and local gut denervation

● = Distal ileum, hippocampal lesions.
system, and the effect on the crypts would appear to be a
direct neural one rather than a vascular one. When compared
with the results of pinealectomy on the crypts in similarly
denervated loops of small bowel, it should be noted that the
degree of fall of crypt cell proliferation rate was only to
the level of that associated with sham pinealectomy, but still
exceeded that associated with local sympathectomy, alone, in
contradistinction to the present experimental findings. This
may possibly suggest that the hippocampus acts directly and
principally on the crypts via the sympathetic nervous syst-
em, in contradistinction to the pineal which may act also by
other pathways as well as the sympathetic nerves.

There are no known direct pathways from the hippocampus
to the crypts via the sympathetic nervous system, but since
the hippocampus is known to be functionally linked with the
hypothalamus, it is possible that the hippocampus acts via the
hypothalamus which of course has connections with the sympat-
getic nervous system. Saper (1976) found evidence for differ-
ential input to the various thoracic cord segments from the
sympathetic nervous system, suggesting that there may be
"specific pathways" from higher centres, rather than the
older "mass action" concept of undifferentiated autonomic
output. In the previous discussion it has been shown that there
are suitably placed autonomic nerve fibres through which the
hippocampal effect could be mediated directly to the crypts.
The path may be more complex than this, and it is difficult to
determine if the mitotic suppressant effect of the hippocampus
on the small bowel crypts is a tonic effect of the hippocampus
or lesions of the hippocampus result in the release of a prob-
ably neural mitotic promoting effect by some other area.
THE EFFECT OF COMBINED HIPPOCAMPAL LESIONS AND BILATERAL TRUNCAL ABDOMINAL VAGOTOMY.

METHODS

Eleven male Sprague-Dawley rats, weighing 385-540 Gms. were randomly selected into two groups and anaesthetized (as in Chapt. 111, b).

GROUP (1), HIPPOCAMPAL LESIONS AND VAGOTOMY (6 RATS).

Laparotomy was performed (as in Chapt. 111, c), and bilateral truncal abdominal vagotomy carried out (as in Chapt. IV, Section 1). Under the same anaesthetic, bilateral hippocampal lesions were performed (as previously). The scalp was closed and Penicillin was administered. Food and fluids were supplied ad libitum, post-operatively.

GROUP (2), VAGOTOMY ONLY (5 RATS).

Laparotomy was performed (as previously), and bilateral truncal abdominal vagotomy performed (as in Chapt. IV, Section 1). Penicillin was administered post-operatively and food and fluids supplied ad libitum.

Three weeks later, all rats were killed, at intervals between 1 1/2 hours and 3 1/4 hours after Colchicine. In group 1, the brain was perfused (as in Chapt. 111, g) and following removal was sectioned and stained (as in Chapt. 111, h). The gastric contents of both groups were suspended in normal saline and the pH determined using a digital pH meter (see Appendix). Specimens of small intestine were collected, sectioned and stained (as in Chapt. 111, e) and the mitotic rate was determined (as in Chapt. 111, j and k).
RESULTS

TABLE 4.26

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) HIPPOCAMPAL LESIONS</th>
<th>(2) HIPPOCAMPAL LESIONS AND VAGOTOMY</th>
<th>ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.0919 ±</td>
<td>0.1205 ±</td>
<td></td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0055 ±</td>
<td>0.0057 ±</td>
<td></td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.0823 ±</td>
<td>0.1199 ±</td>
<td></td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0031 ±</td>
<td>0.0054 ±</td>
<td></td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 1, P<0.01, t = 3.530, df = 10, i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P<0.00005, t = 5.150, df = 10, i.e. significantly different at the 5% level.

TABLE 4.27

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) HIPPOCAMPAL LESIONS</th>
<th>(2) VAGOTOMY AND VAGOTOMY</th>
<th>ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.0919 ±</td>
<td>0.0858 ±</td>
<td></td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0055 ±</td>
<td>0.0026 ±</td>
<td></td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.0823 ±</td>
<td>0.0932 ±</td>
<td></td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0031 ±</td>
<td>0.0015 ±</td>
<td></td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 DOES NOT DIFFER FROM PROXIMAL JEJUNUM, GROUP 2, ON COMPARISON OF STANDARD ERRORS ALONE.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P<0.05, t=2.725, df = 7, i.e. significantly different at the 5% level.
TABLE 4.28

GASTRIC pH

(1) HIPPOCAMPAL LESIONS (2) CONTROLS
AND VAGOTOMY ( NO OP. )

<table>
<thead>
<tr>
<th></th>
<th>(1)IPP OCAMAL LESIONS</th>
<th>(2) CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH OF GASTRIC CONTENTS</td>
<td>7.91 ± 0.18</td>
<td>3.51 ± 0.13</td>
</tr>
</tbody>
</table>

Using a modified "t" test (see Chapt. 111, k), the gastric contents were found to be clearly alkaline and to exceed the pH of controls ( P<0.005, t = 55.90, df = 15 ) i.e. significant at the 5% level.

TABLE 4.29

GASTRIC pH

(1) THREE WEEKS AFTER VAGOTOMY (2) CONTROLS ( NO OP. )

<table>
<thead>
<tr>
<th></th>
<th>(1) THREE WEEKS AFTER VAGOTOMY</th>
<th>(2) CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH OF GASTRIC CONTENTS</td>
<td>7.55 ± 0.13</td>
<td>3.51 ± 0.13</td>
</tr>
</tbody>
</table>

Using a modified "t" test (see Chapt. 111, k), there was found to be a significant difference between these values ( P<0.01, t = 5.77, df = 12 ) i.e. significantly different at the 5% level.

DISCUSSION

The degree of alkalinity of the gastric contents and the presence of excessive gastric dilatation strongly suggest that there was completeness of bilateral truncal vagotomy in both groups. The crypt cell mitotic rate in the proximal jejunum of those subjected to combined hippocampal lesions and vagotomy was not statistically different from that in the proximal jejunum of those subjected to vagotomy alone. In the case of the distal ileum, there was a borderline significance,
Graph of mitotic index versus time.

X = Proximal jejunum, hippocampal lesions and vagotomy.

○ = Proximal jejunum, hippocampal lesions.
Figure 4.43  
Graph of mitotic index versus time.  
$X =$ Distal ileum, hippocampal lesions  
and vagotomy.  
$\bullet =$ Distal ileum, hippocampal lesions.
(table value of "t" = 2.365). Hence, the rates were of a roughly similar order of magnitude. However, the interesting finding from the aspect of elucidating the pathway of action of the hippocampus on the crypts was that the crypt cell mitotic rate in those subjected to combined vagotomy and hippocampal lesions was significantly less than in those subjected to bilateral hippocampal lesions only, and of a similar order to that associated with truncal vagotomy only. This suggests strongly that the vagus nerves are involved to some extent in the pathway of action of the hippocampus on the crypts, and that the hippocampus has a suppressant effect on crypt cell mitotic activity.

It is not clear whether this is a tonic effect by the hippocampus or that lesions of the hippocampus may have removed its suppressant effect on another centre which can increase crypt cell proliferation. With regard to the possible primary pathway of action of the hippocampus via the vagus nerves to the crypts, Hopkins and Holstege (1978) showed that the central nucleus of the amygdala (which has connections with the hippocampus) projects to the dorsal motor nucleus of the vagus. Beattie (1932), Brooks (1967,1977), Colin-Jones and Himsworth (1970), Grijalva (1980, a, b), Grossman (1967), Henke (1979), Misher and Brooks (1966), Porter et al.(1953) all support the view that anterior brain structures, particularly those within the hypothalamus and those associated with the limbic system, exert strong modulatory influence upon sub-diaphragmatic visceral activity via the efferent components of the vagus nerve.

In connection with vagal afferent fibres, Andrews (1986) states that there seems little doubt that areas of the hypothalamus do receive information from the gut. Such input includes the chemical nature of its contents, the degree of
distention, and the absorbed glucose levels (note the importance of luminal factors in the control of crypt cell proliferation). This information presumably is used for such functions as the regulation of food intake and the control of autonomic outflow to the gut. Andrews (1986) said it was open to conjecture whether vagal influences projected to "higher centres" rostral to the hypothalamus. In this regard Bachmann et al. (1977) found that stimulation of the cervical vagus nerves evoked activity in the cingulate cortex, but this may not be relevant to stimulation of the abdominal vagus nerves. Dunlop (1958) in a study of the marsupial phalanger, showed that electrical or chemical stimulation of the gastric mucosa evoked electrical activity in the hippocampus but not in the amygdala, and it was assumed that the pathway was via vagal afferents. Rogers et al. (1980) suggested that limbic and hypothalamic effects upon the dorsal motor nucleus of the vagus were probably exerted via connections with the medial magnocellular reticular formation, i.e. indirectly. It would be tempting to suggest that there is a "centre" for the control of crypt cell proliferation in the limbic system or in the hypothalamus, which is affected by the afferent input of information from the gut via the vagus nerves. Such a "centre", in turn, exerts its effect on the crypts via an efferent effect via the vagi or even the sympathetic nerves.

The situation is not as simple as this as there are many variables and more information is needed regarding the various pathways. For instance, vagotomy alone promotes crypt cell proliferation, but if vagotomy is combined with pinealectomy, or with limbic lesions, its effect is very different. Then it acts to decrease the amount of crypt cell proliferation.
below the expected rate. Thus, it seems that the vagi can act in a different manner in respect to the crypts depending on the circumstances, and this is in keeping with the rather complex nature of the vagus nerves themselves.

So, whilst there seems to be evidence of connections, directly or indirectly between the limbic system and the vagus nerves, it is not possible to propose a simple gut-to-brain-to-gut circuit via the vagus nerves. However, as Brooks (1983) has remarked, it is not correct to think of a vagus afferent pathway leading to a vagus centre that organizes vagal action. The vagus is not that simple an entity: its afferents and efferents serve a complex of functions, not a function. Perhaps a more holistic concept is more appropriate that all parts of the brain, from cortex to medulla, are involved in organizing vagal responses. In fact, Brooks (1983) suggests that all body sensors can affect vagus nerve function, but special sensory receptors relate to particular functions. The vagus nerve is an afferent-efferent cable. Its afferent fibres connect with a great diversity of sensors and carry signals to a large number of interconnected centres in the brain. The actions of the vagus nerve are therefore extremely complex. It is known, for instance, that whilst the vagus nerve may be classified in part as an efferent cholinergic parasympathetic nerve, it contains sympathetic efferents that liberate adrenergic transmitters and is a producer, transporter and liberator of compounds such as cholecystokinin and gastrin. An interesting link between the gastrointestinal tract and the limbic system was provided by Kim (1976) who reported that large bilateral hippocampal lesions produced an increase in gastric pathology (mucosal erosions) over controls in restrained rats and that this effect was blocked by peripheral vagotomy.
Further links between the limbic system and the gastrointestinal tract were found by Henke (1980) who showed that lesions of the amygdala apparently reduced restraint-induced stomach pathology, whereas electrical stimulation of this area induced some gastric pathology in rats and cats, this effect being eliminated by prior vagotomy. Henke's findings were corroborated by Innes and Tansy (1980), Sen and Anand (1957) and Henke and Savoie (1982). With reference to this, it has been suggested that afferent vagal fibres reached this region via the anterior hypothalamus and preoptic region. The foregoing observations of course cannot be directly related to the effects of limbic lesions on crypt cell proliferation in the small intestine, but it is interesting to note that limbic lesions do affect the integrity of the gastric mucosa, although how this is achieved is not evident.

THE EFFECTS OF BILATERAL AMYGDALOID NUCLEUS LESIONS ON CRYPT CELL PROLIFERATION, (WITH OR WITHOUT ASSOCIATED PINEALECTOMY)

METHODS

Twenty one male Sprague-Dawley rats, weighing 270-445 Gms. were randomly arranged into three groups, and anaesthetized (as in Chapt. 111, b).

GROUP (1), BILATERAL LESIONS OF THE AMYGDALOID NUCLEUS (7 RATS).

The scalp was divided, the periosteum cleared from the dorsum of the skull over the relevant area, and trephine holes made (see Chapt. 111, d) so that the centre of the diameter of the trephine hole approximated to the site of entry of the electrode into the brain (B in Figs. 4.44 and 4.45) viz. 3 mms. posterior to the Bregma, 4.5 mms. lateral to the sagittal suture (A in Fig. 4.45). The earth electrode was clipped to the margin of a cut made into the Temporalis muscle on
that side. The stainless steel electrode (see Appendix for method of preparation) was lowered through a small incision in the dura mater at the site of entry of the electrode (determined from the stereotactic atlas by Paxinos and Watson (1982). The electrode was passed down into the brain substance for 10mm. Using a Grass LM 4 lesion maker (see Appendix), a current of 30 MA. was applied to the electrode to produce an amygdaloid nucleus lesion (F in Fig. 4.45). The electrode was withdrawn, cleaned, the insulation checked (see Appendix) and the procedure was repeated on the other side of the brain. The scalp was closed over Gelfoam. Penicillin was administered postoperatively and food and fluids supplied ad libitum.

**GROUP (2), SHAM BILATERAL LESIONS OF THE AMYGDALOID NUCLEUS (9 RATS).**

Exactly the same procedure was followed as in the placement of the electrodes for bilateral amygdaloid lesions, except that no electric current was passed through the electrodes. Penicillin was administered postoperatively and food and fluids were supplied ad libitum.

**GROUP (3), BILATERAL AMYGDALOID LESIONS WITH ASSOCIATED PINEALECTOMY (5 RATS).**

Bilateral amygdaloid lesions were made as described above and pinealectomy (see Chapt. IV, Section 11) performed. Specimens of the pineal gland were sectioned and stained, as previously, to confirm pinealectomy. Three weeks later, all groups of rats were killed, at intervals (between 1 hour and 3 3/4 hours for group 1, between 1 hour and 3 3/4 hours for group 2, and between 1 1/4 hours and 3 1/2 hours for group 3) after intraperitoneal Colchicine. The brain was perfused,
Specimens of small intestine were collected, sectioned and stained (as in Chapt. 111, e). The mitotic rate for the small intestine was determined (as in Chapt. 111, j and k).

RESULTS

**TABLE 4.30**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>BILATERAL AMYGDALOID LESIONS</th>
<th>SHAM AMYGDALOID LESIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1251 ±</td>
<td>0.0690 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0045 ±</td>
<td>0.0015 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1260 ±</td>
<td>0.0667 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0032 ±</td>
<td>0.0022 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.00005, t = 13.046, df = 12, i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005, t = 16.027, df = 12, i.e. significantly different at the 5% level.

**TABLE 4.31**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>PINEALECTOMY AND AMYGDALOID LESIONS</th>
<th>AMYGDALOID LESIONS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1250 ±</td>
<td>0.1251 ±</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0021 ±</td>
<td>0.0045 ±</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1222 ±</td>
<td>0.1260 ±</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0021 ±</td>
<td>0.0032 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.
PROXIMAL JEJUNUM AND DORSAL ILEUM OF GROUPS 1 AND 2 DID NOT DIFFER ON COMPARISON OF STANDARD ERRORS.

**Table 4.32**

Correlation Coefficients-

<table>
<thead>
<tr>
<th></th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.9994</td>
<td>0.9960</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9993</td>
<td>0.9972</td>
</tr>
</tbody>
</table>

i.e. there was satisfactory correlation for each regression line.

**Table 4.33**

Mitotic rates in the small intestine following-

<table>
<thead>
<tr>
<th></th>
<th>(1) AMYGDALOID LESIONS AND PINEALECTOMY</th>
<th>(2) PINEALECTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.1250 ±</td>
<td>0.1283 ±</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1222 ±</td>
<td>0.1289 ±</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1, IS NOT DIFFERENT FROM PROXIMAL JEJUNUM, GROUP 2, ON COMPARISON OF STANDARD ERRORS.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P < 0.05, t = 2.392, df = 7, i.e. significantly different at the 5% level.
Figure 4.44
Diagram of the dorsum of the skull showing -

A = Bregma.
B = The site of the electrode entry.
C = The sagittal suture of the skull.
D = The coronal suture.

Figure 4.45
Diagram of the dorsum of the skull (detail of previous figure),

A = Bregma.
B = The site of electrode entry.
E = The electrode.
F = The amygdaloid lesion.
Figure 4.46

Coronal section of rats brain, showing typical bilateral amygdaloid lesions (A) (Neutral Red stain, X 10.2).

Note the amygdaloid lesions do not appear to encroach on the neighbouring lateral hypothalamus. However, the overlying cortex (Pyriform) is involved to some extent in the lesion (see general discussion).

On the right hand side of the section the overlying cortex has been removed during the processing of the section. The hippocampus is not involved in these lesions.

B = Caudate-Putamen.
C = Corpus callosum.
D = Stria Medullaris.
E = Thalamus.
F = Lateral Hypothalamus.
G = Optic tract.
H = Hippocampus.
Figure 4.47

Graph of mitotic index versus time.

$X =$ Proximal jejunum, sham amygdaloid lesions.

$\bullet =$ Proximal jejunum, amygdaloid lesions.
Figure 4.48  
TIME (HR )
Graph of mitotic index versus time.
$X = \text{Distal ileum, sham amygdaloid lesions}$.
$\bullet = \text{Distal ileum, amygdaloid lesions}$. 
DISCUSSION

The results of the present study show that the crypt cell mitotic rate is significantly increased three weeks after bilateral amygdaloid lesions, when compared with controls. However, the mitotic rate after amygdaloid lesions was not significantly different from that after combined pinealectomy and amygdaloid lesions. Whilst the mitotic rate in the proximal jejunum of those subjected to combined amygdaloid lesions and pinealectomy did not differ significantly from that in the proximal jejunum of those subjected to pinealectomy only, in the case of the distal ileum the rate in the group subjected to pinealectomy significantly exceeded that in the combined lesion group. It should be noted however, that this is a borderline significance (table value of "t" = 2.365; experimental value of "t" = 2.392) as the two values are in fact of a similar general order of magnitude. Thus, it appears that the amygdaloid nuclei can exert a depressing effect on crypt cell mitotic rate but whether there is a tonic depressing effect operative by the amygdaloid nuclei is not clear from the present experimental data.

Since pinealectomy has the same effect (in fact, of a similar order of magnitude) it is interesting to note that associated pinealectomy did not diminish the effect of the amygdaloid lesions. The result is more consistent with the pineal acting via the amygdaloid nuclei than the reverse. It appears that these structures may form part of a complex controlling crypt cell proliferation.

The effects of combined amygdaloid lesions with local denervation of the gut or vagotomy were not carried out in this case but since the amygdaloid nucleus has many connections with the hippocampus—both forming part of the limbic
system, it is conceivable that the amygdaloid nuclei act via the autonomic nervous system on the crypts also.

In the process of making the amygdaloid lesions, which were verified histologically, the area of cortex overlying it was involved in the lesions, in a very local fashion. This is the pyriform lobe, which has been described by Hamilton (1976) as part of the limbic system. Certainly, as will be seen in subsequent discussion, it is so closely linked with the amygdaloid nuclei that its effects could not be considered as separate but rather a lesion of this area is part of the amygdaloid lesions.

The amygdaloid nuclei form almond shaped structures (Hamilton, 1976), which are situated between the inferomedial aspect of the temporal cortex (uncus) and the lateral border of the hypothalamus. The anterior to posterior extent of this nuclear mass is approximately the same as that of the hypothalamus in the rat, and a convenient landmark for the anterior border of the amygdala is the appearance of the "slit-like" third ventricle and the posterior border communicates with the posterior extent of the mammillary complex. The poorly demarcated anterior amygdaloid area merges almost imperceptibly into the hypothalamus at its medial border and into the cortex at the level of the rhinal fissure. With regard to its afferent connections, although the amygdala receive direct fibres from the olfactory bulb, the principal functions of the amygdala are not concerned with smell. Other afferent pathways are from the thalamus, hypothalamus, prefrontal cortex and brain stem reticular formation as well as from the somatosensory, visual and auditory systems. There are two main efferent pathways from the amygdala, the stria terminalis and the ventral amygdofugal pathway. The stria terminalis
is the main outflow tract of the amygdala. From its sites of origin it follows a "C"-shaped course caudally, dorsally, anteriorly and then ventrally along the medial surface of the caudate nucleus to reach the region of the anterior commissure where it branches to supply-

(1) the septal nuclei, (2) anterior olfactory nucleus, (3) anterior nuclei of the hypothalamus, (4) ventromedial nucleus of the hypothalamus, (5) bed nucleus of the stria terminalis, (6) habenular nuclei via the stria medullaris thalami. The ventral amygdalofugal pathway, on the other hand, originates from both nuclear groups of the amygdala, as well as from the peri-amygdaloid cortex. It distributes fibres to-

(1) the pyriform cortex, (2) dorsomedial nucleus of the thalamus, (3) midbrain reticular formation, (4) claustrum, (5) anterior olfactory nucleus, (6) caudate nucleus, (7) putamen, and (8) hypothalamus. The two amygdaloid nuclei communicate with each other through the stria terminalis and the anterior commissure, and the nuclear groups within each complex communicate with each other. The functions of the amygdaloid nuclei are difficult to separate from those of other neighbouring structures because of their many connections. Ablation or stimulation experiments have implicated them in the following-

(1) increase and decrease of autonomic functions, (2) orienting responses to normal events in the visual environment,
(3) lesions of the dorsomedial region appear to be associated with aphagia, decreased emotional tone, fear, sadness and aggression, (4) lesions of the lateral region of the amygdala produce hyperphagia, happiness and pleasure reactions, (5) stimulation of the rostral and lateral regions of the amygdala are associated with fear and flight, (6) stimulation of the
caudal and medial regions produce a defence and aggression reaction, (7) stimulation of the dorsal regions of the amygdala produces an arousal response similar to that following stimulation of the reticular activating system of the brain stem. However, the amygdalar response is independent from the reticular activating system response, (8) stimulation of the ventral regions of the amygdala produce a decrease in arousal and sleep. Ablation of the amygdala as a whole produces a sluggish hypoactive animal. This was, incidentally, not a very obvious feature of behaviour in the present experiments, in which the lesions of the amygdala were extensive and almost certainly involved all of the nuclei of the amygdala, so that the question of the different actions of different parts of the amygdala does not enter into consideration in the effects on crypt cell proliferation but rather in these experiments the effect of a total lesion of the amygdala is being observed.

(9) Bilateral lesions of the amygdala produce hypersexuality (there was no occasion to observe this in these experiments).

(10) Stimulation of the amygdala produces complex rhythmic movements related to eating, e.g. chewing, licking, etc, but it is believed that since many if not all (Afifi and Bergman, 1980) of the above functions can be observed after stimulation or ablation of e.g. the hypothalamus or septal regions, the amygdala play an integrative role in all of the above functions.

Newman (1974) noted also that the amygdala are capable of producing widespread changes in the autonomic nervous system. He noted, however, that on the input side of the amygdala there is no relationship between central and peripheral impulse patterns and, on the output side entirely opposite effects may be elicited on the same function. He suggested
that the amygdaloid is not directly involved in the events of receptor mechanisms or in their primary discharge patterns and that, even if more specific functions are discovered in the future, it is now evident that the amygdaloid neurons show an enormous flexibility of response to the majority of incoming signals. With regard to output, it was found that stimulation of the amygdaloid could modify the activity of the hypothalamus in response to a peripheral stimulus. Newman concluded that flexibility of action appeared to be the keynote of the amygdaloid mechanism and this now appears to be confirmed by the variations and inconsistencies observed in the reactions to electrical stimulation of the amygdaloid or in the character of the behavioural disturbances resulting from lesion experiments. He suggested that the amygdaloid was only part of a more extensive functional system whose modulatory influences and flexibility are essential to the organization of conscious behaviour.

In the context of the present study, it appears unlikely that in the case of the influence of the amygdala on the crypts there will be found to be a well defined crypt-to-amygdala-to-crypt pathway but rather that the effect will be an indirect one. This concept is in accord with a recent suggestion. Nauta (1986) noted that the neocortex is connected with the limbic system by way of the variously complex sequences of cortico-cortical connections which ultimately converge on ventral and medial regions of the temporal cortex, which in turn project to the amygdala as well as to the entorhinal area, the major cortical source of afferents to the hippocampus (as noted previously). The cortical conduction line to the amygdala is reciprocated, in part at least, by direct amygdofugal projections (see above) to the inferior
temporal and prefrontal cortex. Thus, these areas (hippocampus, amygdaloid nuclei, and entorhinal areas) are interconnected so closely that it is likely that, since amygdaloid and hippocampal lesions are associated with increased crypt cell proliferation rate, the lesions of the entorhinal cortex which forms part of the complex would be expected to have a similar effect to amygdaloid lesions alone.

It was thought previously that the hippocampal and amygdala areas functioned independently through their respective projection systems and had common functions only to the extent that their respective efferent systems project to the same diencephalic structures. However, recently Poletti (1986) has concluded that the hippocampus and the amygdala do not act as separate structures within the temporal lobe. He suggests that the hippocampus has a prominent direct effect on amygdala unit activity and the amygdala, in turn, integrate and exert a major portion of the anterior hippocampal influence on the hypothalamus and basal forebrain. He also noted that there is evidence that there are opposing influences of different parts of the amygdala on the basal diencephalon. Further, he added that the temporal lobe limbic structures, including those being considered at the moment, do not act as a reverberating circuit but exert their functional role primarily by a direct influence on structures downstream in the neuraxis—the diencephalon, the midbrain and even the spinal cord. He has formulated the hypothesis that the limbic system modulates virtually all of the functions of the brainstem, including behavioural responses to "flight and fight", pain suppression, sexual maturation, and endocrine and autonomic activity.

Thus, it is possible for the amygdala to act to modulate
crypt cell mitotic activity possibly via the autonomic nervous system, either directly or indirectly via other areas of the limbic system. Therefore, it is not possible to determine to what extent the effect of the amygdaloid lesions is being mediated by effects on the hippocampus, for example. The results of the present study have shown that the amygdaloid nuclei have a suppressant effect on crypt cell proliferation in the small intestine, which is manifested by increased crypt cell proliferation rate after amygdaloid lesions. In any case it is not possible to say to what extent this effect is due only to amygdaloid activity and how much it is due to release of hippocampal activity as these two areas are so closely linked functionally. There appears to be a possibility that amygdaloid effects are mediated by the sympathetic nervous system, as are the hippocampal effects, but no clear-cut descending pathways can be defined. Rather, it appears likely that the amygdaloid nuclei, which have access to much afferent visceral information, along with the hippocampus exert their effect by a modulating influence on the sympathetic nervous system, and the effects on the crypts may be a manifestation of perhaps increased activity in that system.

The pineal effect on the crypts is similar to that of limbic lesions and it would appear likely that it exerts its effect via the limbic system with which it has connections. Since the limbic system is also involved in the mechanism of "flight and fight", it is possible that the effect of the amygdaloid lesions on the crypts may only be present in conditions of stress, especially conditions involving the emotions.
THE EFFECT OF BILATERAL SEPTAL LESIONS, ( WITH OR WITHOUT ASSOCIATED PINEALECTOMY), ON CRYPT CELL PROLIFERATION IN THE SMALL INTESTINE.

METHODS

Twenty six male Sprague-Dawley rats, weighing between 365-515 Gms. were randomly selected into four groups, and anaesthetized ( as in Chapt. 111, b ).

GROUP (1), CRANIOTONY WITH LIGATION AND DIVISION OF THE SUPERIOR SAGITTAL SINUS

( 5 RATS ).

A disc of bone approximately 0.4 cms. in diameter was removed from the midline of the dorsum of the skull, immediately posterior to the Bregma, to expose the dura mater ( see Chapt. 111, d ), ( see A in Fig. 4.51 ). Incisions were made on either side of the superior sagittal sinus and the sinus was ligated through these incisions in the dura mater with 6 X 0 black silk ( see C in Fig. 4.49 (b) ,B in Fig. 4.51 ). The scalp was closed with black silk sutures and lips. Penicillin was administered postoperatively and food and fluids supplied ad libitum. Three weeks later the rats were killed, at intervals between 1 hour and 3 1/2 hours after intraperitoneal Colchicine. The brain was perfused ( as in Chapt. 111, g ) and later removed and stored in 10 % Formalin. Subsequently, the brain was removed from the skull for examination.

GROUP (2), BILATERAL CINGULATE CORTICAL LESIONS ONLY

( 7 RATS ).

A trephine hole was made ( see Chapt. 111, d ) over the Bregma ( B in Fig. 4.33 ) and a circular piece of bone
was removed to expose the dura mater and the superior sagittal sinus. Incisions were made on either side of the superior sagittal sinus and the sinus was ligated (see B in Fig. 4.49 (b)). Part of the cingulate cortex was thus exposed (see D in Fig. 4.49 (c), Din Fig. 4.51) and subsequently aspirated within the limits of the trephine hole (B in Fig. 4.50).

The lesion extended from the dorsal surface of the cerebral hemisphere to the medial surface so that the corpus callosum could be seen, but damage to this structure was avoided (see E in Fig. 4.49 (d)). Haemostasis was achieved with Gelfoam and the scalp wound closed with black silk and clips. Postoperatively, Penicillin was administered and food and fluids supplied ad libitum.

GROUP (3), BILATERAL SEPTAL LESIONS ONLY

(6 RATS).

Two trephine holes were made (as in Chapt. 111, d) one over Bregma, and the other slightly overlapping it so that when the two discs of bone were removed, the two defects were confluent (A in Fig. 4.49 (a)) and the dura mater and the most anterior part of the superior sagittal sinus were exposed. Incisions were made through the dura mater on either side of the superior sagittal sinus and it was ligated (C in Fig. 4.49 (b)), the cut ends being retracted to expose the anterior part of the cingulate cortex on either hemisphere (D in Fig. 4.49 (c)). This was aspirated, after removal of a spur of bone anteriorly, to facilitate exposure (B in Fig. 4.49 (b)). This resulted in the anterior part of the corpus callosum being exposed between the anterior parts of the medial aspects of the cerebral hemispheres (E in Fig. 4.49 (d)). A transverse cut was made through the substance of the corpus callosum, with the sucker (F in Fig. 4.49 (e)).
The anterior cerebral artery was then aspirated above the corpus callosum and the corpus callosum aspirated carefully in an anterior direction to expose the greyish septum ( H in Fig. 4.49 (f) and J in Fig. 4.52 ) medially, and the anterior horns of the lateral ventricles laterally ( G in Fig. 4.49, (f) ) separated by the septum. Keeping anterior to the fornix ( K in Fig. 4.52 ) the septum was carefully aspirated so that the two anterior horns of the lateral ventricles were in continuity ( see J in Fig. 4.52 ) and the damage to the fornix was avoided as far as possible ( see in Fig. 4.49, (g) ). Haemostasis was achieved with Gelfoam and the scalp was closed with black silk and clips. Penicillin was administered and food and fluids supplied ad libitum.

GROUP ( 4 ), SEPTAL LESIONS AND PINEALECTOMY

( 8 RATS ).

The septum was aspirated, as above, and a further trephine hole was made over Lambda, through which the pineal gland was removed. ( see Chapt. IV, Section 11 ). Haemostasis was achieved with Gelfoam and the scalp was closed with black silk sutures and clips. Penicillin was administered and food and fluids supplied ad libitum. Specimens of the pineal gland were processed, as previously, and examined histologically to confirm pinealectomy. Three weeks later, all groups were killed, at intervals between 1/2 hour and 3 1/2 hours after Colchicine. The brains were perfused ( as in Chapt. 111, g ) and subsequently either sectioned and stained with Neutral Red ( as in Chapt. 111, h ) after removal from the skull or photographed. Specimens of small intestine were collected from all groups, sectioned and stained ( as in Chapt. 111, e ). The mitotic rate was determined, as in Chapt. 111, j and k.
Figure 4.49, Diagram of dorsal view of skull.

(a) (b) (c) (d) (e) (f) (g)

(see text)
Figure 4.50  Diagram of dorsal view of rat brain showing the area of cingulate cortex removed (E)
A = coronal suture
C = cingulate cortex
D = cerebral hemisphere
E = cerebellum.
F = lambdoid suture.

Diagram of coronal section of brain at the site of the lesion, showing, A, the excised bone, B = the ligation of the superior sagittal sinus, C = the dura mater, D = the cingulate gyrus.
Figure 4.52

Diagram of sagittal section of rat brain

M = Cerebellum
L = Corpus callosum
K = Fornix
J = Septum
N = Third ventricle
O = Anterior commissure
P = Olfactory lobe

The direction of the arrow indicates the direction of approach to the septum.
### RESULTS

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th>Group</th>
<th>(1) CRANIOTOMY</th>
<th>(2) CRANIOTOMY WITH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRANIOTOMY WITH</td>
<td>DIVISION OF SUPERIOR</td>
</tr>
<tr>
<td></td>
<td>ONLY</td>
<td>SAGITTAL SINUS</td>
</tr>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.0732 ±</td>
<td>0.0756 ±</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.0678 ±</td>
<td>0.0773 ±</td>
</tr>
<tr>
<td>Results in mitoses/ cell/ hour.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROXIMAL JEJUNUM, GROUP 1</td>
<td>DID NOT DIFFER</td>
<td>ON COMPARISON OF</td>
</tr>
<tr>
<td>STANDARD ERRORS FROM PROXIMAL</td>
<td></td>
<td>COJPARISON OF</td>
</tr>
<tr>
<td>JEJUNUM, GROUP 2.</td>
<td></td>
<td>STANDARD ERRORS</td>
</tr>
<tr>
<td>DISTAL ILEUM, GROUP 2</td>
<td>DID NOT DIFFER</td>
<td>FROM PROXIMAL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JEJUNUM, GROUP 2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.20, t = 1.461</td>
</tr>
<tr>
<td></td>
<td></td>
<td>df = 9. i.e. not</td>
</tr>
<tr>
<td></td>
<td></td>
<td>significantly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>different at the 5 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>level.</td>
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</tbody>
</table>

| TABLE 4.35 Correlation Coefficients-
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) PROXIMAL JEJUNUM</td>
<td>(2) DISTAL ILEUM</td>
</tr>
<tr>
<td></td>
<td>PROXIMAL JEJUNUM</td>
</tr>
<tr>
<td></td>
<td>DISTAL ILEUM</td>
</tr>
<tr>
<td>i.e. there was satisfactory</td>
<td></td>
</tr>
<tr>
<td>correction for each regression</td>
<td></td>
</tr>
<tr>
<td>line.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 4.36 Mitotic rates in the small intestine following -</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) BILATERAL, CINGULATE GYRUS LESIONS</td>
</tr>
<tr>
<td>PROXIMAL JEJUNUM</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
</tr>
<tr>
<td>ILEUM</td>
</tr>
</tbody>
</table>
Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 1, P < 0.01, t = 3.64, df = 8, i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, P < 0.05, t = 2.605, df = 8 i.e. significantly different at the 5% level.

**TABLE 4.37**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILATERAL SEPTAL LESIONS</th>
<th>(2) LIGATION OF SUPERIOR SAGITTAL SINUS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1223 ± 0.0018</td>
<td>0.0756 ± 0.0019</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0026</td>
<td></td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1210 ± 0.0026</td>
<td>0.0773 ± 0.0019</td>
</tr>
<tr>
<td>ILEUM</td>
<td></td>
<td>0.0010</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.00005, t = 19.45, df = 7, i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005, t = 11.205, df = 7, i.e. significantly different at the 5% level.

**TABLE 4.38**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILATERAL SEPTAL LESIONS</th>
<th>(2) CINGULATE CORTICAL LESIONS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1223 ± 0.0018</td>
<td>0.0665 ± 0.0017</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0026</td>
<td>0.0019</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1210 ± 0.0026</td>
<td>0.0674 ± 0.0019</td>
</tr>
<tr>
<td>ILEUM</td>
<td></td>
<td>0.0019</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.
PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P<0.00005, t = 23.25, df = 9, i.e. significantly different at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P<0.00005, t = 18.482, df = 9, i.e. significantly different at the 5% level.

<p>| TABLE 4.39 |
| Mitotic rates in the small intestine following - |</p>
<table>
<thead>
<tr>
<th>(1) SEPTAL LESIONS AND PINEALECTOMY</th>
<th>(2) SEPTAL LESIONS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.1272 ± 0.0017</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.1266 ± 0.0021</td>
</tr>
</tbody>
</table>

Results in mitoses/cell/hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P<0.10, t = 1.812, df = 10, i.e. not significant at the 5% level.

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P<0.20, t = 1.696, df = 10, i.e. not significant at the 5% level.

<p>| TABLE 4.40 |</p>
<table>
<thead>
<tr>
<th>Correlation Coefficients - (1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.9985</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9989</td>
</tr>
</tbody>
</table>

i.e. there was satisfactory correlation for each regression line.

<p>| TABLE 4.41 |
| Mitotic rates in the small intestine following - |</p>
<table>
<thead>
<tr>
<th>(1) SEPTAL LESIONS AND PINEALECTOMY</th>
<th>(2) PINEALECTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.1272 ± 0.0017</td>
</tr>
</tbody>
</table>
DISTAL
ILEUM

Results in mitoses/ cell/ hour. THESE TWO

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DISTAL</td>
<td>0.1266</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.0021</td>
</tr>
</tbody>
</table>

GROUPS DID NOT DIFFER STATISTICALLY

Figure 4.53
TIME (HR)
Graph of mitotic index versus time.
X = Proximal jejunum, septal lesions.
○ = Proximal jejunum, cingulate lesions.
Figure 4.54

Graph of mitotic index versus time.

X = Distal ileum, septal lesions.

○ = Distal ileum, cingulate lesions.
Coronal section of rat brain, showing typical septal lesion (A, A). Section is stained with Neutral Red (X 9.4).

A = Previous site of septum (note that immediately inferior to this site part of the fornix has also been removed).
B = Anterior commissure.
C = Corpus callosum.
D = Site of removal of the cingulate gyrus.
E = Third ventricle.
F = Optic tract.
G = Hypothalamus.
Figure 4.56
Dorsal view of cortical lesions associated with septal lesions (B) and pinealectomy (A) (X 4.5).
DISCUSSION

Since ligation of the superior sagittal sinus was part of the procedure to expose the cingulate cortex, and subsequently the septum, and since this may have resulted in local cortical damage itself, the effect of ligation of the superior sagittal sinus alone was compared with that of craniotomy alone. It was found that there was no statistically significant difference between the crypt cell mitotic rate associated with craniotomy alone, from that associated with ligation of the superior sagittal sinus, despite there being a very small area of local cingulate cortical damage associated with ligation and division of the superior sagittal sinus alone. Craniotomy and ligation of the superior sagittal sinus was found to be associated with a higher crypt cell mitotic rate than that associated with bilateral cingulate lesions. It is not clear why this should be so, since there was some cortical damage in both cases.

However, bilateral septal lesions were associated with a crypt cell mitotic rate which significantly exceeded that associated with either ligation of the superior sagittal sinus or cingulate lesions, indicating that the effect on the crypts was due to the septal lesions and not the ligation of the superior sagittal sinus or the cingulate lesions which are part of the procedure for producing septal lesions.

Combination of septal lesions with pinealectomy was associated with a rise in crypt cell mitotic rate which was statistically similar to that associated with pinealectomy alone, or septal lesions alone. These findings are more consistent with the pinealectomy effects on crypt cell mitotic rate being mediated via the limbic system than the limbic
effects being mediated via the pineal gland and the pineal and limbic system may in fact form part of the same mechanism which is affecting the crypts in this case.

The septal region forms part of the limbic system, and in view of the effects on crypt cell mitotic rate associated with lesions of other parts of the limbic system, e.g. the hippocampus, and the amygdala, and the close connections between various parts of that system, the effect in this experiment is not surprising. However, the reason why the cingulate cortical lesions are not also associated with an increase in crypt cell mitotic rate is not so obvious.

The cingulate cortex extends the entire medial aspect of each hemisphere from the dorsal surface down to the corpus callosum, i.e. the cingulate gyri form the walls of the longitudinal fissure. This area of cortex is sometimes described as being part of the limbic system, especially in the case of humans, and in fact is arbitrarily divided into an anterior and posterior part (the more anterior part of this structure being lesioned in these experiments). The cingulate cortex is known to be closely interconnected with the thalamic nuclei and to project to a variety of other subcortical areas including the basal ganglia. However, Hamilton (1976) found that in the rat there is no evidence for terminations in the traditional limbic system structures such as the septum, amygdala, hippocampus and mammillary bodies. According to Hamilton (1976) this lack of close interrelationship with the limbic system draws into question the somewhat routine inclusion of the cingulate cortex as an integral part of the limbic system. However, the cingulate gyrus has indirect access to traditional limbic system influences, and there is some convergence
of limbic system and cortical influences in the midbrain. 
This lack of direct connection with the amygdala, hippocampus, 
etc., may explain the lack of a rise in crypt cell mitotic 
rate following cingulate lesions in this species. In fact, 
there was a slight unexplained fall in mitotic rate. Damage to 
the corpus callosum occurred in these experiments but it has 
been shown in a previous experiment that a lesion in this area 
is not associated with changes in crypt cell mitotic rate. 
It is therefore suggested that the marked increase in crypt 
cell mitotic rate in this experiment is due to the bilateral 
septal lesions. Unfortunately, whilst the lesions are mainly 
septal, there appears to be some damage to the fornix also. 
It is probably arbitrary to attempt to separate these two 
lesions using the aspiration methods, especially as they 
form interrelated parts of the limbic system. For the purpose 
of these experiments, therefore, the term " septal" lesion 
denotes a lesion which is mainly septal and a " fornix " lesion 
denotes a lesion which is mainly fornix. 

On the other hand statistical comparison of the mitotic 
rates in the proximal jejunum and distal ileum of rats sub-
jected to bilateral septal lesions revealed no statistical 
difference from the mitotic rates in the proximal jejunum and 
distal ileum of those subjected to bilateral hippocampal les-
ions despite the fact that it is unlikely that there is any 
direct overlap between these lesions.

The septum itself ( Hamilton, 1976 ) is composed of a 
group of nuclei and fibres which are nested beneath the corpus 
callosum and situated between the lateral ventricles. The 
ventral border of the septum is somewhat less clearly defined 
but is approximately at the level of the anterior commissure, 
In the most anterior regions of the septum, two thin bands of
cells make up the medial and lateral septal nuclei. Near the posterior border of the septum, the fibres of the fornix columns descend through this structure and two additional septal nuclei become apparent. The ventral border of the septal region is continuous with the anterior part of the hypothalamus (preoptic nucleus). In the present experiments both the medial and lateral septal nuclei were lesioned. The septal area receives afferent fibres from the hippocampus, amygdala, hypothalamus, and midbrain (Afifi and Bergman, 1980). Fibres from the hippocampus reach the septal area via the fornix, in fact it appears that specific areas of hippocampus project upon specific regions of septum. Fibres from the amygdala reach the septal area via the stria terminalis and fibres from the hypothalamus reach the lateral septal area via the medial forebrain bundle. Fibres from the midbrain reach the septal area via the medial forebrain bundle, arising primarily from the periaqueductal gray region and the ventral tegmental area. The septal area has efferent fibres to the hippocampus, hypothalamus, habenular nucleus and midbrain. Fibres from the medial septal region reach the hippocampus via the fornix. Septal efferents reach the hypothalamus via the medial forebrain bundle and project primarily upon the lateral nuclear group of the hypothalamus. In addition, the medial septal area is connected with the supraoptic and paraventricular nuclei of the hypothalamus (which also have connections with the pineal, Korf and Wagner, 1980) and this connection is believed to be important in the regulation of oxytocin release. Fibres reach the habenular nucleus via the stria medullaris thalami and projections to the midbrain course through the medial forebrain bundle.

The functional importance of the septal region is
considered to lie in providing a site of interaction between limbic and diencephalic structures, e.g. the connection of the hippocampus with the hypothalamus via the septal region.

Stimulation and ablation experiments have implicated the septal region in various roles e.g. emotional behaviour, water consumption regulation, changes in activity or in learning, and stimulation of the septal region has an inhibitory effect on autonomic function. It is perhaps by this latter path that the septal region affects the crypts, since the autonomic nervous system has been shown to have an influence on crypt cell proliferation (see Chapt. IV, Section 1), and the effects of hippocampal lesions (a related area) on the crypts have been shown to be diminished by concomitant lesions affecting the autonomic nervous supply of the crypts.

Thus, whilst septal lesions have been associated in this case with a considerable increase in crypt cell mitotic rate, which is of a similar order of magnitude to that observed in association with other limbic area lesions, it is difficult to completely separate the effects of septal lesions from those of other limbic areas because of the intimate neuronal connections between these various areas and in fact the effects cannot be separated from those of the pineal ablation. It would then appear likely that the pineal and limbic system are part of the same mechanism which can affect crypt cell mitotic rate under certain circumstances. Interestingly, the cingulate cortex, whilst it has an indirect connection with the limbic areas examined, in that its connections converge on the same areas, does not appear to be involved in this mechanism, which appears to require direct connections between the areas involved. Furthermore, MacLean (1986) whilst classifying the cingulate cortex as part of the limbic system, suggested
on the basis of experimental evidence that the cingulate
cortex was involved in (a) nursing, in conjunction with mater-
ernal care, (b) audiovisual communication for maintaining
maternal offspring contact, and (c) playful behaviour.

THE EFFECT OF BILATERAL FORNIX LESIONS, WITH OR WITHOUT
ASSOCIATED PINEALECTOMY.

METHODS

Twelve male rats, weighing 385-530 Gms., were randomly
divided into two groups and anaesthetized ( as in Chapt. 111,
b ).

GROUP (1), BILATERAL FORNIX LESIONS
( 6 RATS ).

Two trephine holes were made ( see Chapt. 111, d ), one
over Bregma and the other overlapping it and posterior to it
( A in Fig. 4.57 ,(a) ), so that when the two discs of bone
were removed, the two defects were confluent and the dura mater
and superior sagittal sinus were exposed ( Fig. 4.57,(b) ).
Incisions were made through the dura mater on either side of
the superior sagittal sinus and it was ligated and divided
( C in Fig. 4.57,(b) ). The cut ends of the sinus were retract-
ed to expose the cingulate gyrus on either cerebral hemisph-
ere ( D in Fig. 4.57,(c) ). This was aspirated to expose
the corpus callosum, between the medial aspects of the cerebral
hemispheres ( E in Fig. 4.57,(d) ). A transverse cut was
made with the sucker ( E in Fig. 4.57,(d) ) through the subs-
tance of the corpus callosum (as in septal lesions ). The
corpus callosum ( F , Fig. 4.57,(e) , K in Fig. 4.58) was then
aspirated to expose the septum ( G in Fig. 4.57,(e) ) and
the lateral ventricles, as well as the part of the corpus
callosum immediately overlying the fornix ( H , Fig. 4.57,(e) ).
A hole was deepened through this part of the corpus callosum
immediately overlying the fornix (I in Fig. 4.57,(f)) to expose the cavity of the third ventricle (see also J in Fig. 4.58) thus creating a fornix lesion (J in Fig. 4.58) with as minimal as possible damage to the septal nuclei (O in Fig. 4.58, G in Fig. 4.57, (e)). Haemostasis was achieved with Gelfoam. The scalp was closed with black silk sutures and clips and Penicillin was administered. Food and fluids were supplied ad libitum.

**GROUP (2), BILATERAL FORNIX LESIONS AND PINEALECTOMY**

(6 RATS).

The fornix was lesioned as described above, and a trephine hole was made over the lambda, through which the pineal was removed (see Chapt. IV, Section 11). Haemostasis was achieved with Gelfoam and the scalp was closed with black silk sutures and clips. Penicillin was administered and food and fluids supplied ad libitum. Specimens of the pineal gland were processed, as previously, and examined histologically to confirm pinealectomy. Three weeks later both groups were killed at intervals (group 1, between 1 hour and 3 1/4 hours, group 2 between 1/2 hour and 3 1/2 hours) after Colchicine. The brains were perfused (see Chapt. 111, g) and either sectioned and stained (see Chapt. 111, h) or photographed after removal from the skull. Specimens of the small intestine were collected, sectioned and stained (as in Chapt. 111, e). The mitotic rate in the small intestine was determined (as in Chapt. 111, j and k).
Figure 4.57
Dorsal view of the skull, (see text).

(a)

(b)

(c)

(d)

(e)

(f)
Figure 4.58
Diagram of sagittal section of rat brain, showing:

L = The cerebellum
K = The corpus callosum
J = The direction of approach to the fornix.
P = The olfactory lobe.
O = The septum.
N = The anterior commissure.
M = The third ventricle.
RESULTS

Mitotic rates in the small intestine following -

(1) BILATERAL FORNIX (2) LIGATION OF THE

<table>
<thead>
<tr>
<th>LESIONS</th>
<th>SUPERIOR SAGITTAL SINUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1314 ± 0.0045</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0045 ± 0.0005</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1207 ± 0.0026</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.00026 ± 0.00026</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2, P < 0.00005,

\[ t = 12.400, df = 7, \text{ i.e. there was a statistically significant difference at the 5\% level.} \]

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005,

\[ t = 11.421, df = 7, \text{ i.e. there was a significant difference at the 5\% level.} \]

---

TABLE 4.43

Mitotic rates in the small intestine following -

(1) BILATERAL FORNIX (2) CINGULATE CORTICAL

<table>
<thead>
<tr>
<th>LESIONS</th>
<th>LESIONS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1314 ± 0.0045</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.0045 ± 0.0005</td>
</tr>
<tr>
<td>DISTAL</td>
<td>0.1207 ± 0.0026</td>
</tr>
<tr>
<td>ILEUM</td>
<td>0.00026 ± 0.00026</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 1 > PROXIMAL JEJUNUM, GROUP 2,

\[ P < 0.00005, t = 15.829, df = 9, \text{ i.e. significantly different at the 5\% level.} \]

DISTAL ILEUM, GROUP 1 > DISTAL ILEUM, GROUP 2, P < 0.00005,

\[ t = 17.193, df = 9, \text{ i.e. significantly different at the 5\% level.} \]
**TABLE 4.44**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILATERAL FORNIX LESIONS AND PINEALECTOMY</th>
<th>(2) BILATERAL FORNIX LESIONS ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1215 ± 0.0048</td>
<td>0.1314 ± 0.0045</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.1212 ± 0.0028</td>
<td>0.1207 ± 0.0026</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9956</td>
<td>0.9957</td>
</tr>
<tr>
<td>PROXIMAL JEJUNUM, GROUP 2 &gt; PROXIMAL JEJUNUM, GROUP 1, P &lt; 0.20, t = 1.455, df = 8, i.e. not significantly different at the 5% level.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DISTAL ILEUM, GROUP 2 NOT DIFFERENT FROM DISTAL ILEUM, GROUP 1. ON COMPARISON OF STANDARD ERRORS.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 4.45**

<table>
<thead>
<tr>
<th>Correlation Coefficients</th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL JEJUNUM</td>
<td>0.9956</td>
<td>0.9957</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9976</td>
<td>0.9962</td>
</tr>
</tbody>
</table>

i.e. there was satisfactory correlation for each regression line.

**TABLE 4.46**

Mitotic rates in the small intestine following -

<table>
<thead>
<tr>
<th></th>
<th>(1) BILATERAL FORNIX LESIONS AND PINEALECTOMY</th>
<th>(2) PINEALECTOMY ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROXIMAL</td>
<td>0.1215 ± 0.0048</td>
<td>0.1283 ± 0.0017</td>
</tr>
<tr>
<td>JEJUNUM</td>
<td>0.1212 ± 0.0028</td>
<td>0.1289 ± 0.0026</td>
</tr>
<tr>
<td>DISTAL ILEUM</td>
<td>0.9956</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

Results in mitoses/ cell/ hour.

PROXIMAL JEJUNUM, GROUP 2 > PROXIMAL JEJUNUM, GROUP 1, P < 0.20,
\[ t = 1.446, \text{df} = 8, \text{i.e. not significantly different at the 5\% level.} \]

DISTAL ILEUM, GROUP 2 > DISTAL ILEUM, GROUP 1, \( P < 0.05, t = 2.483, \text{df} = 8, \text{i.e. significant at the 5\% level.} \]

---

**Figure 4.59**

Graph of mitotic index versus time.

- \( X = \text{Proximal jejunum, fornix lesions} \)
- \( \bullet = \text{Proximal jejunum, cingulate lesions} \).
Figure 4.60

Graph of mitotic index versus time.

X = Distal ileum, fornix lesions.

● = Distal ileum, cingulate lesions.
Coronal section of rat brain showing the site of typical bilateral fornix lesions (A,A) (Neutral Red stain, X 9.3).

B = The previous site of the third ventricle.
C = The stria medullaris thalamos.
D = The caudate-putamen.
E = The globus pallidus.
F = The site of the corpus callosum lesion.
G = The site of the bilateral cingulate cortical lesions.

(compare with Plate 17, Paxinos and Watson, 1982.)
Figure 4.61
Figure 4.62

Dorsal view of typical fornix lesion (F) and pineal lesion (P), (X 4.5).
DISCUSSION

The crypt cell mitotic rate in the proximal jejunum and distal ileum of rats subjected to ligation of the superior sagittal sinus, removal of part of the corpus callosum, cingulate cortical and fornix lesions significantly exceeded that in the proximal jejunum and distal ileum of those subjected to the other procedures, excluding lesions of the fornix, either singly or in combination. Since the other procedures mentioned, which are necessary to expose the fornix region have each been shown singly (see above) to be not associated with a significant increase in crypt cell mitotic rate, these results allow the conclusion that the increase in crypt cell mitotic rate in this present experiment was associated specifically with the fornix lesions. As mentioned in the discussion on the previous experiment, there is some difficulty in completely separating fornix and septal lesions when using aspiration methods for lesioning, which require an approach from the surface of the brain, but the histological specimens of brain obtained (see Fig. 4.61) suggest that the lesions in this experiment were primarily in the fornix, although it is not possible to exclude some incidental damage to the septal area also.

The crypt cell mitotic rate associated with combined pinealectomy and bilateral fornix lesions was not statistically significantly different from that associated with bilateral fornix lesions alone, over a similar period of time. Whilst the mitotic rate associated with the combined lesions was not statistically significantly different from that in those rats subjected to pinealectomy only, with regard to the proximal jejunum, there was a significant difference using the "t" test (pinealectomy rate greater) with regard to the distal
ileum. However, in the latter case it should be noted that the table value for "t" = 2.306 for df = 8, whereas the experimental value was "t" = 2.483. Thus, the significance of the difference is "borderline" and it appears that the rates are of a similar general order of magnitude. Therefore, it appears that associated pinealectomy certainly has not diminished the effect of bilateral fornix lesions to any extent, and this is more consistent with the effects of pinealectomy being mediated via the fornix than the effects of the fornix lesions being mediated via the pineal gland. It is likely that both structures form part of a common mechanism which affects crypt cell mitotic rate. When the mitotic rate in the proximal jejunum and distal ileum of those subjected to bilateral fornix lesions was compared with that associated with bilateral hippocampal lesions, there was found to be no statistically significant difference between these rates (proximal jejunum, P < 0.20, "t" = 1.434, df = 10; distal ileum - no difference on comparison of standard errors). Similarly, there was no statistically significant difference between the mitotic rate associated with bilateral fornix lesions and that associated with bilateral septal lesions (proximal jejunum, P < 0.10, "t" = 2.068, df = 8; distal ileum, no difference on comparison of standard errors). Thus, it appears that the pineal gland, fornix, septum, hippocampus, and probably the amygdaloid nuclei, all have a similar hyperproliferative effect on the crypts of a similar order of magnitude when lesioned, the pineal probably acting via the other areas.

The fornix is situated at the inferior border of the septum, which separates the two lateral ventricles, and courses below the corpus callosum. It is C-shaped fibre bundle which
connects the hippocampus and the mammillary body (Afifi and Bergman, 1980). In fact the efferent fibres of the hippocampus travel via the fornix, which is a continuation of the alveus, which lies on the ventricular surface of the hippocampus and converges further to form the flattened band of white matter - the fimbria. The fornix itself becomes obvious at the level of the anterior poles of the hippocampus (Hamilton, 1976) and here it contains fibres of the fimbria and from this point the fibres arch downwards and split into two components. One component descends anterior to the level of the anterior commissure into the preoptic area. The other post-commissural component descends to the mammillary bodies.

The fornix is a bundle of fibres which forms partially reciprocating connections between the hippocampus, septum, hypothalamus, thalamus, and midbrain. As such, it represents one of the major fibre systems involved with the interconnection of the limbic system structures. It represents the major pathway for the interconnection of the septohippocampal complex with the hypothalamus and midbrain. The septum and hippocampus share internal circuitry that suggests a high degree of interaction. The bi-directional connections between these forebrain structures and the sensory, visceral, and motor aspects of the hypothalamus and midbrain may provide a clue to the possible function of this system (Hamilton, 1976).

The fornix thus distributes fibres to (1) the cingulate gyrus, (2) septal nuclei, (3) preoptic region of the hypothalamus, (4) the lateral region of the hypothalamus, (5) the mammillary body, (6) the anterior nucleus of the thalamus. Poletti (1986) also found physiological evidence for direct projections of fornix fibres to a number of other structures, not previously known to receive fornix afferents e.g. the medial preoptic
area and the perifornical nucleus of the hypothalamus. However, he found only a small portion of the fornix fibres going to the mammillary bodies and that most of the hippocampal influence on the hypothalamus in the intact animal came from the anterior hippocampus via non-fornix pathways e.g. via the amygdala. From these findings of Poletti it would seem that the relationships of the fornix to the other areas of the limbic system are probably not yet completely resolved and it is difficult to determine from the connections of the fornix the mechanism of its action on the crypts. In any case, it is probable that the fornix acts on the crypts via the sympathetic nervous system via its links with the hypothalamus. There are similarly no clear cut pathways between the pineal and the fornix, so probably both areas act through the medium of the hypothalamus and the sympathetic nervous system.

GENERAL DISCUSSION ON SECTION 111

In drawing any conclusions from the effects of C.N.S. lesions, certain limitations of the lesion technique for investigation should be considered.

(1) Small local lesions may have actions beyond the area where it is desired to make the lesion, e.g. disruption of capillaries, oedema, pressure reactions and subsequent degeneration of axons and cell bodies at a distance from an amygdaloid lesion, in the lateral hypothalamus, whilst not apparent histologically in the present experiments, may be present to some extent. Thus, the boundaries of a lesion are perhaps not as definite as they seem histologically.

(2) The changes produced consist of a combination of transient, reversible effects, and sustained irreversible
effects. An attempt to minimize the degree of transient effects on crypt cell proliferation was made in the present experiments, by allowing the animals to survive for at least three weeks so that local reactions could settle down.

(3) Because the destruction of an area is irreversible additional animals have to be used, all with the same lesion. The problem then arises of making the lesion exactly the same in all animals, and of allowing for individual variation of the animals in the series, in regard to the reaction to that lesion. An attempt has been made in this series to minimize this problem by determining histologically that the lesions were as identical as possible.

(4) The reparative processes may histologically obscure the limits of destruction as a function of the time between the lesion and when the animal is killed. An attempt to minimize this effect has been made by killing all rats with C.N.S. lesions at the same time interval after lesioning.

(5) Kuypers (1962) observed that vastly different effects are seen from C.N.S. lesions in immature animals when compared with mature ones. Rats of a similar maturity were used in these experiments where possible, minimizing this effect.

Bearing these precautions in mind, it has been found that bilateral hippocampal, amygdala, septal, or fornix lesions were associated with an increase in crypt cell mitotic rate of a similar order of magnitude to that associated with pinealectomy and combination of these lesions with pinealectomy did not diminish the effect on the crypts to any extent. This suggests that the pineal acts via the other areas lesioned and quite possibly forms part of a common mechanism affecting the crypts.
Whilst there are no known neural pathways between the septum and the pineal, Ueck (1979) noted that short latency responses in the pineal body of the rat were found following amygdaloid, ventromedial hypothalamic, olfactory and acoustico-stimulation, supporting the concept that there are direct connections between the amygdaloid nuclei and the pineal. The amygdaloid nuclei are known to be connected to the other parts of the limbic system, the septal, fornical, and hippocampal nuclei.

The hippocampus, amygdala, septum and fornix form part of the limbic system which is known to be concerned with self-preservation on the one hand, especially in regard to food intake, and on the other with emotional states pertaining to interpersonal relationships. However, whilst in the human limbic system the cingulate gyrus is also considered to be part of this system, in the rat (Hamilton, 1976) it is not. In this context it is interesting to note that cingulate lesions in the rat were not associated with a significant change in crypt cell mitotic rate. Thus, it appears that the limbic system, as a unit, is concerned with this effect on the crypts. There appears to be no well defined neural pathway between the limbic system and the crypts, just as there is no well defined neural pathway between the pineal and the crypts. The effect of the hippocampal lesions on the crypt cell mitotic rate was found to be cancelled completely (more completely than in the case of the pineal) by autonomic denervation of the small bowel which necessarily involves the terminal vagal fibres. It is of interest to note that the effect of vagotomy in this case being the opposite to the effect of vagotomy alone, this suggests that the vagus nerves play a different role in the case of the action of the limbic system and that
the limbic system acts largely via the autonomic nervous system as a whole. However, although the exact pathway could not be determined in these experiments, since the limbic system, pineal and hypothalamus are intimately, anatomically and functionally, related (in fact it is considered that the limbic system modulates the activity of the hypothalamus) it seems likely that the limbic system (and probably the pineal) acts via the hypothalamus which forms an important part of the the autonomic nervous system.

The fact that the limbic system is involved in reactions to "flight or fight" reactions and has been referred to by MacLean (1949) as the "visceral brain" emphasizing its role in connection with the viscera, together with the apparently different (more efferent) action of the vagus nerve in the effects of the limbic system on the crypts, may suggest that the limbic system depressant effect on crypt cell mitotic rate in the gut may be part of a specific action of the limbic system perhaps operative only under certain circumstances e.g. similar situations to "flight or fight" situations or conditions of stress.

Allowance was made in the experimental design for the stressful effect of the limbic and pineal lesions by the use of experimental controls.

It appears from the available literature that the role of the luminal nutrients in the control of crypt cell proliferation is of prime importance, and it is difficult to imagine that control of crypt cell proliferation via the limbic system or pineal could be the principal mode of day to day control, especially since changes in the luminal environment do not modify the hyperproliferative effects of pinealectomy. Since the limbic system is involved in the reception and
assimilation of a considerable amount of afferent data, including that from the viscera, it is possible that the vagi may play an afferent role in this process (see Andrews, 1986), and at the same time under other circumstances perhaps an efferent role in mediating the effects of the limbic system. As well as the sympathetic nerves, other nerves (see Chapt. IV, Section 1) e.g. serotonergic, histaminergic or cholinergic nerves could also be involved in the efferent effects of the limbic system or pineal on the crypts. Certainly the innervation of the crypts is such that (see Chapt. IV, Section 1) this would be possible, these nerves coming into close relationship with the crypt cells. As considered in Chapt. IV, Section 1, besides direct neural action on the crypts it is possible that the autonomic nervous system may act via other local changes involving the crypts, e.g. changes in motility of the bowel, changes in local blood supply of the small bowel, but the reasons why these mechanisms are unlikely to be operative have been dealt with in Chapt. IV, Section 1, so that it seems likely that the effects of the limbic system and pineal gland are probably direct neural effects on the crypts.

As described in Chapt. IV, Section 1, the effects of lesions of the pineal gland or the limbic system on the crypts which involve the action of the autonomic nervous system could also involve the local effects of noradrenaline or serotonin as intermediaries in the neural effect. Just as it has been postulated that the limbic system has a modulatory role on the lower centres in the brain, and possibly even the spinal cord, Poletti (1986), Oksche and Pevet (1981) also suggested a modulatory role for the pineal with regard to endocrine and probably non-endocrine regulatory systems, tuning their
function to external and internal conditions for the benefit of the organism concerned. Once again, this view would be consistent with the pineal and limbic system acting in concert on the crypts. Although the limbic system appears to act on the crypts mainly via the autonomic nervous system, its actions are complex, and it may act on the crypts also possibly by other pathways. For instance, hippocampal and septal lesions have been associated with changes in levels of growth hormone (Borer et al. 1977, 1979) and hippocampal lesions have been reported to alter certain parameters of anterior pituitary function e.g. the secretion of A.C.T.H. (Bohus 1975); Cassady and Taylor (1976); Johnson and Moberg (1980); Mangili et al. (1966), of growth hormone (Martin et al., 1973) and luteinizing hormone (Taleisnik and Beltramino, 1975). These are thought to be perhaps mediated by an action of the hippocampus on the medial hypothalamic neurons which also receive fibres from the amygdaloid region (Krettek and Price 1978; Swanson and Cowan 1976). Endröcz and Lissák (1962) suggested that the hippocampus serves as an integrative mechanism for the pituitary-adrenal cortical system. Kawakami et al. (1968) reported that lesions of the dorsal fornix reduce corticosterone biosynthesis. Mangili et al. (1966) found that there was evidence that the limbic structures may diminish or augment adrenal stress responses.

With reference to the results of the foregoing studies it is worth remembering that these mechanisms are probably involved in emotional reactions. More specifically it is known that hippocampal-pituitary-adrenal cortical system is aroused in conditions of distress, depression, and feelings of helplessness, whereas the amygdaloid-sympathetic-adrenal medullary system is aroused in the "fight or flight" reactions.
(Henry(1980); Henry and Stephens(1977)). Since, as noted above, the adrenal cortical and medullary secretions are known to affect the rate of crypt cell proliferation, it is possible that the limbic system may be involved to some extent in mechanisms which affect, amongst other things, the crypt cell proliferation rate in stressful situations. Brown et al. (1979), Seggie and Brown (1976) support the notion that there is a central extrahypothalamic neural mechanism controlling responses to stress that is independent of those mechanisms that are responsible for the homeostatic control of hormone levels. Normal resting hormone responses are not necessarily disturbed even when there is extensive damage to this neural centre. It has been suggested that the septum and the basolateral amygdala are an integral part of this network i.e. non-homeostatic needs are controlled by a specific neural network. This raises the possibility of a similar mechanism, involving the limbic system acting on the crypts and affecting proliferation, perhaps in reaction to stress. In examining the effects of various operative procedures on crypt cell proliferation are we disregarding the possibility that a different mechanism of crypt cell proliferation control may be operative under these unusual conditions than is operative under non-operative (and non-stressful) conditions?

Another possibility to examine is the neural connections, if any, between the limbic system and the vagus nerves such that vagotomy can modify one of the actions of the limbic system, as suggested in the case of the hippocampal lesions in these experiments. Hopkins and Holstege (1978) showed that the central nucleus of the amygdala projected to the dorsal motor nucleus of the vagus. Beattie (1932), Brooks (1967,1977) Colin-Jones and Himsworth (1970), Grijalva (1980,a b)
Grossman (1967), Henke (1979), Misher and Brooks (1966), Porter et al. (1953) support the view that anterior brain structures, particularly those within the hypothalamus and those associated with the limbic system, exert a strong modulatory influence upon the subdiaphragmatic visceral activity via the efferent vagus nerve. Saper (1976) found evidence of primary descending projections of the paraventricular nucleus of the hypothalamus terminating in the dorsal motor nucleus of the vagus in the medulla. Rogers et al. (1980) suggests that the paraventricular nucleus, which has connections with the pineal gland, acts as an integrator of visceral afferent information i.e. paraventricular nuclear neurons may receive significant visceral sensory input from the brainstem structures and this information may be relayed to higher order limbic and hypothalamic structures or integrated and referred back to autonomic preganglionic loci or to the neurohypophysis.

Rogers et al. (1980) suggest that limbic and hypothalamic effects upon the dorsal motor nucleus of the vagus are probably exerted via connections with the medial magnocellular reticular formation i.e. indirectly. Thus, there seems to be evidence of connections, directly or indirectly, between the limbic system and the vagus nerves. It is not possible to propose a simple gut-to-brain-to-gut circuit via the vagus nerves, however. As Brooks (1983) has remarked it is not correct to think of a vagus afferent pathway leading to a vagus centre that organizes vagus action. The vagus is not so simple an entity. Vagal afferents and efferents serve a complex of functions, not a function. It is a better concept that all parts of the brain, from cortex to medulla, are involved in organizing vagal responses. It is better to think that all
body sensors can affect vagus nerve function, but special sensory receptors relate to particular functions. The vagus is an afferent-efferent cable. Its afferent fibres connect with a great diversity of sensors and carry signals to a large number of interconnected centres in the brain. The actions of the vagus nerve itself are therefore complex. It is known, for instance, that whilst the vagus nerve may be classified as a cholinergic parasympathetic nerve (efferent) it contains sympathetic efferents that liberate adrenergic transmitters, and is a producer, transporter and liberator of compounds such as cholecystokinin and gastrin.

With regard to the action of the limbic system on the crypts via the sympathetic nervous system, it seems likely that this action is mediated via the hypothalamus. Smith et al. (1980) concluded that the hypothalamus is the "head ganglion" of the autonomic nervous system. Saper (1976) found evidence for differential input to the various thoracic cord segments, suggesting that there may be "specific pathways" from higher centres, rather than the older "mass action" concept of undifferentiated autonomic output.

Let us look now at the structures that compose the limbic system and the manner in which they act on other areas. Hamilton (1976) states that the structures most commonly included in the various descriptions of the rat limbic system are the hippocampus, the cingulate gyrus, the septum, the amygdala, the entorhinal cortex, and parts of the hypothalamus and midbrain. However, there appears to be no universal agreement on its composition and as indicated previously there is evidence that the cingulate cortex does not form part of this system in the rat. Components of the limbic
system have major connections with the olfactory system, as well as with other parts of the forebrain and with the midbrain (projection, commissural, association fibres). Most of these pathways are multisynaptic and reciprocally connected. Because of the multiple interconnections, it is difficult to assign specific functions to individual structures in the limbic system (i.e. limbic-olfactory system). Activities of the limbic system are essential for the self preservation of the organism (e.g. feeding, "fight or flight") and preservation of the species (e.g. mating, procreation, care of the young). The limbic system is influenced by all of the sensory systems (olfactory, optic, auditory, etc), general exteroceptive and interoceptive systems, and emotional status.

The main outlet of the limbic system is via the hypothalamus to the brainstem and the spinal cord, mainly via the autonomic nervous system, but also via the somatic motor system (e.g. in food acquisition and ingestion, behavioural patterns in attack and defence) and also to the pituitary gland and the endocrine system. It appears that, from data collected from ablation experiments, limbic structures inhibit the hypothalamus and that non-limbic cortex (neocortex) in turn modifies the inhibitory effects of the limbic system. The limbic system has been traditionally viewed as the main cerebral representative of the internal milieu, expressing its functions in the form of states of affect and motivation. However, as stated by Nauta (1986), at the current state of knowledge it is no longer easily conceivable that great categories of brain function can be localized so exclusively to one or other subdivision of the brain, because over recent decades these regions have become less sharply delineated from one another and an increasing number of cross connections
between them have been reported with convergence and blending of efferents in a variety of foci. Papez (1937) defined a limbic system that included all principal afferent and efferent connections of the limbic lobe, i.e. that the limbic system was a largely self-contained functional system whose principal efferent and afferent connections were relayed to and from other limbic structures. However, as mentioned previously, Poletti (1986) found that experimentally the hippocampus has most of its influence on the diencephalon, i.e. that this region is not primarily involved in a functional limbic circuit within the limbic lobe but exerts its role by influencing the functions associated with structures downstream, especially the basal diencephalon. Other limbic structures, e.g. the amygdala as well as the hippocampus have been shown to directly affect the midbrain and even the spinal cord. He thus hypothesized that the limbic system modulates virtually all of the functions of the brainstem, including behavioural responses to flight and fight, pain suppression, sexual maturation, and autonomic and endocrine activity. He postulated, as opposed to the view of Papez, that the limbic system serves behaviour less related merely to survival and allows some early cognitive functions and rudimentary primary emotions, in association with the neocortex. This view supports the concept of the limbic system possibly acting on the crypts via the autonomic nervous system.

Alternatively, the limbic system has been considered as the "visceral brain" concerned with visceral activity and emotional behaviour (Newman, 1974, MacLean, 1949). The "visceral brain" may be regarded as comprising, (1) a receptor region for visceral afferent impulses, (2) a distribution centre for directing influences within the network, and
(3) a motor region for controlling the patterns of hypothalamic discharges. However, some authors, e.g. Altenre and Curthoys (1978) claim that this collection of structures cannot rightly be called a "system" because it has insufficient anatomical or functional organization. However, for the purposes of this discussion, we will refer to it as a "system". Having defined the "limbic system" (see Fig. 4.63) is it possible to consider each of the lesions in the present series of experiments as separate, discrete lesions or merely lesions of the system as a whole? It is probably not possible to completely separate the effects of hippocampal, amygdaloid lesions, and in particular the septal and fornix lesions are so intimately related that, using the method of lesioning by direct aspiration, these lesions could be considered as "septo-fornical lesions, with perhaps more damage relatively in one area in one experiment, and more damage in another area in another experiment.

There appears therefore to be no evidence for a definite "centre" for the control of crypt cell proliferation in the limbic system but it is presumed that information from the viscera is made available to the limbic system as part of the general afferent input and the limbic system (and/or pineal) possibly exerts a constant modulatory (probably tonic suppressant) effect on crypt cell mitosis via the lower brain centres and the autonomic nervous system. It is also possible that the effect of the limbic system and the pineal is to oppose a stimulatory effect on crypt cell mitosis by some other as yet undetermined brain centre but it is difficult to conceive what part of the brain could serve that purpose, so that the former concept seems more likely. It is considered (Hamilton, 1976) that the limbic system is not
essential to any special sensory function, such as vision or olfaction, nor is it a necessary part of any homeostatic regulatory system; all of these functions can be retained following virtually any type of limbic system damage, and additionally, primitive organisms lacking a limbic system can exhibit all of these basic functions. There is general agreement that the major function of the limbic system is one of modulation of sensory, motor, and homeostatic systems (Issacson (1974); McCleary (1966); Nauta (1973)). In support of this conclusion, for instance, it has been found that surgical destruction of the septum can (1) change food intake by altering the responsiveness to taste factors, internal cues, or reinforcement contingencies (Beatty and Schwartzbaum (1968); Hamilton et al. (1974)), (2) change the responsiveness to environmental stimuli (Brown and Remley, 1971), or (3) change certain types of motor responses (McCleary, 1966). None of these effects represents a loss of the basic function but rather an impairment of some factor that controls the organisms' response to that class of stimulus.

Nauta (1973) has suggested that the entire limbic system (he included the prefrontal cortex) functions as a sort of "sounding board" having the ability to preview alternative behaviours and to evaluate the likely consequences.

Given a certain situation the limbic system may enable the organism to take into account the internal environment (e.g. the depletion of energy stores), and the external environment (e.g. the presence of food), along with the events that would be likely to follow certain types of behaviour (e.g. potential attack by a predator). According to this view, the limbic system is integral in allowing the organism to cope with a complex and constantly changing environment.

A = The cingulate cortex.
B = The thalamus.
C = The hippocampus.
D = The amygdaloid nuclei.
E = The hypothalamus.
F = The septum.
Thus, this type of system (modulatory) imposed upon the basic sensory and regulatory systems, has a great deal of survival value. Apparently, the system may be activated from within. Zeman and Innes (1963) have suggested that there are such centres which may inhibit or dampen down the activity of the limbic system, such as the septal nuclei, and the precommissurole hippocampus, and others which may activate the system, such as the amygdala. It is possible that the limbic system may act to modulate the day to day regulation of crypt cell proliferation, in the mechanism of which such factors as the luminal nutrition, neural activity or hormonal substances, amongst others are important.

The limbic system and the pineal gland, which may act together, may have a role in preventing excessive swings of hyperproliferation, which might otherwise occur in the day to day course of regulation of crypt cell proliferation. Certainly, the effect of these structures can "override" the other factors which appear to be important in crypt cell proliferation control, suggesting that the limbic system and pineal are probably not part of the ordinary control mechanism, but rather a mechanism which acts only in certain circumstances, perhaps for instance during reactions to stress.

The apparently similar maximum levels of crypt cell proliferation after limbic lesions or pinealectomy may be artificial to some extent, due to the limitations of the Colchicine stathmokinetic technique, with its metaphase figure degeneration setting perhaps a "false ceiling" on the level of crypt cell proliferation which can be registered.

However, it is evident that even without the postulated restraint on crypt cell proliferation exercised by the limbic system and pineal, uncontrolled crypt cell proliferation
does not supervene, i.e. of the nature of malignant proliferation. Thus, it appears that there are other mechanisms which prevent this occurrence. It would be interesting to speculate that if this limbic/pineal mechanism was faulty allowing repeated "wide swings" of crypt cell hyperproliferation, whatever mechanism which prevents uncontrolled crypt cell proliferation may be so stressed that it may become "exhausted" with resulting uncontrolled crypt cell proliferation. However, this is highly speculative and there are many variables which have not been considered here. Certainly, some experimental importance has been attached to the probable presence of excessive crypt cell proliferation prior to the development of gastrointestinal tumours (Williamson (1979); Williamson and Malt (1980)) but this relationship is not a simple straightforward one.

Papez (1937) proposed that the limbic system of humans was involved in the expression of emotion, principally by its connections with the hypothalamus and the sympathetic nervous system, with or without the aid of the pituitary gland. It appears that the interplay between psychological and bodily events occurs principally in the limbic system. Newman (1974) suggested that the hippocampus could be seen as a visceral control centre which has to deal with unpredictable emotional situations, so that a given effect can never be guaranteed to follow a given stimulus. Under conditions of intense excitement, such as occur under emotional stress, there is a resulting powerful driving force on the centres controlling the visceral-endocrine output via the hippocampus and the hypothalamus. It would therefore be interesting to study further the effects of emotional disturbance, which is a little difficult to distinguish from other forms of stress.
in rats, on crypt cell proliferation. It is known that the limbic system is the principal site for the interplay between psychological and bodily events. Emotion consists of three interrelated facets or components (1) a feeling, (2) autonomic manifestations and (3) physical expressions, so that it is often difficult to separate the three components. The various areas which have been lesioned in these experiments, and which are intimately neurally interrelated, are also associated with a wide range of emotional changes following stimulation or ablation. Thus, emotional changes themselves may be the initiating factor in the effect of the limbic system on the crypts. These emotional changes could of course act indirectly on the crypts in other ways e.g. by changes in the amount of food eaten or endocrine changes, as mentioned above. The former changes could alter the luminal milieu and thus affect crypt cell proliferation in this way (see Chapt. 1, Section 11). The possibility that such changes in food intake could be operative in limbic or pineal lesions will be investigated in Section IV of this thesis.

Certainly, whilst pineal lesions, presumably acting on the crypts via the autonomic nervous system, apparently have the same effects on the crypts irrespective of luminal contents, there are other instances of neural effect on the crypts being modified by the luminal contents. Levine et al. (1982) for instance, found that whilst chemical sympathectomy of the intestinal crypts exaggerated the intestinal mucosal hypoplasia in rats maintained on total parenteral nutrition this mucosal atrophy could be prevented by intragastric infusion of luminal contents.

It is perhaps significant to note that lesions of the hypothalamus, which it has been postulated may be on the
pathway from the limbic system to the crypts, have also been shown to be associated with an increase in crypt cell mitotic rate. As mentioned in Chapt. 1, Section 11, Bindoni et al. (1973) found that hypothalamic lesions of the tubero-infundibular region resulted in an increase in crypt cell mitotic rate in the small intestine three weeks later, but this did not occur if the lesions were sited elsewhere in the hypothalamus. Furthermore, these authors considered that these changes were probably not attributable to functional changes in the endocrine system, since whilst some of the rats were found to have regressive cellular changes in the pituitary gland (which is also linked with the hypothalamus). The rats with extensive tubero-infundibular lesions were also those with regressive changes in the pituitary gland and showed the greatest increase in crypt cell mitotic rate. The pituitary changes could have resulted from the local effect of the tubero-infundibular lesion itself. The findings of pituitary regression in the face of crypt cell proliferation contrast with the fall in crypt cell mitotic rate found after hypophysectomy observed by Sharp et al. (1980) (see Chapt. 1, Section 11). Thus, the changes observed by Bindoni et al. are at least consistent with a neurally mediated effect on the crypts (as mentioned in Chapt. 1, Section 11). Jutisz et al. (1974) extracted and concentrated from the sheep hypothalamus a principle capable of inhibiting the in vitro (cell culture) multiplication of some cell strains stabilized in a continuous line. This perhaps suggests that the hypothalamic effect may be mediated in part by a humoral or neuro-humoral means, but the results are not easy to interpret because of possible species differences and the use of an in vitro rather than an in vivo test for the effects of this substance.
It should be noted that there is no evidence in the available literature of previous investigation of the effects of limbic lesions on crypt cell mitotic rate in the small intestine. Noback (1967) states with regard to the hypothalamus, that it has a significant role in the regulation of autonomic activities, and essentially it acts as a modulator influencing the autonomic centres in the brain stem and spinal cord. He states, for example, that anxiety generates neocortical activity that may be projected to the hypothalamus and in turn the hypothalamic output to the cardiovascular integrative centre in the medulla has a modulatory effect which can affect the heart rate and blood pressure. The anterior hypothalamus has an excitatory parasympathetic ( inhibitory to sympathetic activity ) role and the activity in this region produces a parasympathetic tone. Lesions of this region often result in sympathetic effects. The posterior hypothalamus has an excitatory sympathetic role. Stimulation of this area often results in the production of a sympathetic tone. A lesion in this region may reduce both the sympathetic and parasympathetic effects of the hypothalamus upon other centres because, in addition to destroying the hypothalamic sympathetic centres, the lesion interrupts the caudally projecting pathways from the parasympathetic centres. Thus, the possible role of the hypothalamus in mediating the effects of limbic lesions to the sympathetic nervous system is probably not a simple straightforward one. In any case, it appears from the results that both the sympathetic and parasympathetic nervous systems are involved in the transmission of the limbic and pineal effects to the crypts.
SECTION IV

THE AMOUNTS OF FOOD EATEN DURING THE VARIOUS EXPERIMENTS.

(1) Introduction.

(2) Experiments,

(a) The amount of food eaten by pinealectomized rats compared with the amount of food eaten by sham-pinealectomized rats.

(b) Comparison of the food intake associated with pinealectomy and that associated with a feeding schedule.

(c) The amounts of food eaten during the various experiments.

(3) Discussion on section IV.
INTRODUCTION TO SECTION IV

The importance of the nutrient value of the intestinal content in the maintenance of the normal crypt cell proliferation rate in the rat small intestine was supported by the findings of Spector et al. (1977), Levine et al. (1974), Eastwood (1977), and Jacobs et al. (1975). Furthermore, Hughes et al. (1978), Hughes and Dowling (1980), Levine et al. (1976), Al-Mukhtar et al. (1982 b) found that exclusion of oral intake and replacement of this by total parenteral feeding was associated with hypoplasia and/or hypofunction of the intestinal mucosa. Heroux and Gridgeman (1958) found that hyperphagia for one month produced small bowel mucosal hyperplasia and these findings were supported by the work of Al-Mukhtar et al. (1982b) and Williamson (1978 a). Although the evidence is indirect, there appears to be a role for luminal nutrients in the control of crypt cell proliferation in the rat small intestine. Whilst there appears to be no evidence in the available literature regarding the effects of pinealectomy on oral intake of food, numerous studies have indicated that damage to limbic system structures, such as the septum and amygdala can produce changes in feeding behaviour (Hamilton, 1976).

However, the literature concerning the amounts of food eaten after specific lesions of the limbic system is confusing e.g. the literature on the effects of amygdaloid nuclear lesions on food intake generally shows conflicting results, viz - Anand and Brobeck (1952), Anand and Dua (1955), Allikmets and Ditrikh (1965), Fuller et al. (1957), Green et al. (1957), Lewinska (1967), Morgane and Kosman (1957), Singer and Montgomery (1969), Brutkowski et al. (1962), Fonberg (1966), Yamada and Greer (1960). Similar conflicting results were obtained regarding food intake after hippocampal lesions,
In view of these findings it was decided to investigate the intake of food associated with the pineal and limbic lesions as well as with the other experimental procedures performed in this series, and to determine if there is any correspondence between the mitotic rate in the crypts and the intake of food.

**THE AMOUNT OF FOOD EATEN BY PINEALECTOMIZED RATS COMPARED WITH THE AMOUNT EATEN BY SHAM-PINEALECTOMIZED RATS**

Results are summarized in Fig. 4.65.

Note that whilst there is a temporary lag in the mean weights of food eaten by pinealectomized rats, when compared with those eaten by sham-pinealectomized rats for the first seven days (presumably due to the effects of the operation) after seven days the mean amounts of food eaten were identical. Both amounts of food are within the normal range of intake for rats (Williams, 1976) during the duration of the experiment, despite the fact that the crypt cell mitotic rate in the pinealectomized group clearly exceeded that in the sham-pinealectomized group (see Chapt. IV, Section 11). The results support the contention that the food intake cannot be directly related to crypt cell mitotic rate in the rat small intestine.

**COMPARISON OF THE FOOD INTAKE ASSOCIATED WITH PINEALECTOMY AND THAT ASSOCIATED WITH A FEEDING SCHEDULE. (IN RELATION TO THE MITOTIC RATE IN THESE TWO GROUPS).**

**INTRODUCTION**

Since the importance of luminal nutrition in the control of crypt cell proliferation has been stressed in the literature (see Chapt. 1, Section 11), in a previous investigation (Callaghan, 1979 b) it was decided to study the effects of hyperphagia induced by a dietary training technique,
Figure 4.65: Graph of mean weights of food eaten versus time.
(Brobeck et al., 1943) on the rate of crypt cell proliferation in the rat small intestine.

A total of twenty five male albino rats were allowed food for three hours only every day for twenty four days, at the end of which time it was found that the mean daily weight of the rats fell rapidly within a few days and did not approach the normal level again throughout the duration of the trial. The food intake, however, rose to reach the upper level of normal intake (see Williams, 1976) from a lower level but the mitotic rate did not exceed the values in control rats. Thus, the rats were made hyperphagic within a few days by this dietary regimen, and the relative concentration of nutrients within the intestinal lumen at any time during the three hours per day allowed for feeding was also by inference much higher than normal. The small intestinal mucosa was thus stressed to provide a means of absorbing nutrients more effectively and more rapidly than usual.

This it could do by increasing its surface area (necessitating at least in part an increase in crypt cell mitotic rate) or increasing its absorptive capacity or both. In fact the capacity for compensation of the small intestinal mucosa was obviously only partial, since the original mean weight of the rats was not approximated during the duration of the experiment. The fact that the daily mean weight of the rats did not continue to fall (i.e. after the fifth day) seemed to indicate that there was some degree of compensatory increase in absorptive capacity also. The intestinal mucosa did not respond to this situation by a significant increase in crypt cell proliferation, although this may have occurred had the trial lasted beyond twenty four days. When compared with the mitotic rate in the crypts of rats subjected to pinealectomy,
in which there was no significant increase in food intake but a considerable increase in mitotic rate, there was seen to be a significant difference between the mitotic rates in these two groups, (proximal jejunum, $P < 0.00005$, $t = 19.34$, $df = 27$; distal ileum, $P < 0.00005$, $t = 14.62$, $df = 27$). Thus, there was a significant difference in both jejunum and ileum at the 5% level. These findings suggest that there is no significant correlation between mitotic rate in the crypts and the intake of food. These results show that the crypt cell mitotic rate in the proximal jejunum and distal ileum of rats three weeks after pinealectomy clearly exceeds that in the proximal jejunum and distal ileum of those subjected to a feeding trial designed to increase their daily intake of food, for twenty five days. This was despite the increase in food intake of the order of 60% during the feeding schedule. The crypt cell mitotic rate during the feeding trial was much lower than the level after pinealectomy, which was not associated with any significant increase in food intake.

This of course does not mean that luminal nutritional factors may be disregarded in the process of crypt cell mitotic control because the weight of evidence supports a significant role for these factors. However, it appears that significant increases in crypt cell mitotic rate can occur without concomitant significant increases in the amount of food eaten and vice versa. It should be pointed out that the rats involved in the feeding schedule were observed to be subject to considerable stress and emotional excitement during their periods of limited feeding, so that it is interesting that the effects of such daily emotional stress appear not to have resulted in a significant change in the crypt cell mitotic
rate overall, although there may have been temporary daily fluctuations in rate.

<table>
<thead>
<tr>
<th>Experimental Procedures</th>
<th>Mean Weight of Food Per Day (GMS.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Bilateral cingulate lesions</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>(2) Bilateral hippocampal lesions and pineal-</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>-ectomy</td>
<td></td>
</tr>
<tr>
<td>(3) Bilateral neocortical lesions</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>(4) Bilateral hippocampal lesions</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>(5) Bilateral amygdaloid lesions</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>(6) Bilateral amygdaloid lesions and pineal-</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>-ectomy</td>
<td></td>
</tr>
<tr>
<td>(7) Bilateral fornix lesions</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>(8) Bilateral septal lesions</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>(9) Local gut denervation for two weeks</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>(10) Diversion of bile from the gut lumen</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>(11) Ligation of the bile duct and pinealectomy</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>(12) Ligation of the bile duct</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>(13) Craniotomy</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>(14) Diversion of bile from the gut lumen and</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>pinealectomy</td>
<td></td>
</tr>
<tr>
<td>(15) Bilateral fornix lesions and pinealectomy</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>(16) Bilateral septal lesions and pinealectomy</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>(17) Bilateral hippocampal lesions and local</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>gut denervation</td>
<td></td>
</tr>
<tr>
<td>(18) Local gut denervation for three weeks</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>(19) Vagotomy for three weeks</td>
<td>11 ± 3</td>
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TABLE 4.47 (cont'd)

<table>
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<tr>
<th>EXPERIMENTAL PROCEDURES</th>
<th>MEAN WEIGHT OF FOOD PER DAY (GMS.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20) Bilateral hippocampal lesions and vagotomy</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>(21) Sham amygdaloid lesions</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>(22) Pinealectomy for three weeks</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>(23) Pinealectomy for one week</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>(24) Sham pinealectomy for one week</td>
<td>17 ± 2</td>
</tr>
</tbody>
</table>

Analysis of these results reveals the following-
(1) There appears to be a certain uniformity in the amounts of food eaten by very different experimental groups.
(2) As expected the amounts of food eaten were not significantly different where the mitotic rates were similar in compared groups.
(3) However, in some cases, where there was a considerable difference in mitotic rate between groups, the amounts of food eaten were similar e.g. local gut denervation after three weeks compared with pinealectomy after three weeks; combined hippocampal lesions and local gut denervation compared with hippocampal lesions alone; combined biliary ligation or biliary diversion with pinealectomy alone.
(4) The amount of food eaten could not be directly related to the level of crypt cell mitotic rate.

DISCUSSION ON SECTION IV

The results of this section of the study show that the crypt cell mitotic rate cannot be correlated with the amount of food eaten. It is surprising that in all of the experimental groups of animals there was considerable uniformity in food
intake which very probably reflects the efficiency of the feeding control mechanism in maintaining a fairly constant food intake. Thus, whilst luminal factors are clearly important in the mechanism of control of crypt cell mitotic rate, rises or falls in crypt cell mitotic rate are not necessarily accompanied by corresponding changes in food intake.

This is not entirely unexpected considering the complex mechanisms operative in the control of food intake, involving many levels of the brain— not just the "satiety centres" which presumably monitor the luminal nutrient content. The fact that pineal lesions are not associated with change in food intake suggests that the pineal gland is not involved in the mechanism of control of food intake, but the same cannot be said for the other centres considered e.g. the limbic centres which have been implicated in the control of food intake. Numerous studies have indicated that damage to limbic system structures such as the septum and amygdala can produce changes in feeding behaviour (Hamilton, 1976). The literature on the effects of amygdaloid nucleus ablation on food intake generally shows conflicting results. For example, it has been reported that amygdaloid lesions have no effect on food intake (Anand and Brobeck (1952); Anand and Dua (1955)), or may produce hyperphagia (Allikmets and Ditrikh (1965); Fuller et al. (1957); Green et al. (1957); Lewinska (1967); Morgane and Kosman (1957); Singer and Montgomery (1969)), or hypophagia (Brutkowski et al. (1962); Fonberg (1966); Yamada and Greer (1960)). Increased food intake was reported following hippocampal lesions (Fisher and Coury, 1962). However was not confirmed by other studies (Jarrard, 1965). In fact, to draw attention to the complexity of the control
of food intake it is indicated that Robinson (1964) demonstrated that feeding could be elicited by electrical stimulation of virtually any area in the brain, especially limbic system sites. The present experimental findings suggest that the lesions of the limbic system considered here i.e. hippocampus, amygdaloid nucleus, fornix and septum appear not to be involved in the control of the amount of food eaten, although it is known that lesions in some of these areas are associated with abnormal feeding patterns. The lack of correlation between the marked increase in crypt cell mitotic rate after pinealectomy and any change in food intake is consistent with the demonstrated lack of effect of deficiency of luminal factors, e.g. intestinal contents or bile, on the crypt cell mitotic effects of pinealectomy found in the present series of experiments (see Chapt. IV, Section 11).
CHAPTER V

GENERAL DISCUSSION OF THE EXPERIMENTAL FINDINGS.
GENERAL DISCUSSION OF THE EXPERIMENTAL FINDINGS

In this thesis an attempt has been made to review the available literature, which is considerable and largely inconclusive, on the mechanisms of control of crypt cell proliferation in the intestine, and to examine in particular certain aspects of this control, in particular the effects of certain neurological lesions on crypt cell mitotic rate. It is accepted, however, that neurological factors are but part of a very complex and as yet incompletely understood mechanism for controlling crypt cell proliferation.

Whilst the effects of vagotomy on crypt cell proliferation in the small bowel are disputed in the literature, it was found in the present series of experiments that vagotomy after one week, whilst producing a marginally significant increase in crypt cell proliferation rate in the distal ileum, produced a more significant increase in the proximal jejunum. The increase in crypt cell mitotic rate in both proximal jejunum and distal ileum was more definite two weeks after vagotomy, but small compared with the hyperproliferative effects noted after pinealectomy or limbic lesions. Some of the differences in the effect of vagotomy on the crypts noted in the literature are probably explainable by species differences, or differences in the duration of the response to vagotomy between various strains in the same species. Furthermore, it was not known if there had been a minor fall in the crypt cell mitotic rate during the first week after vagotomy, followed by a rise. In the literature there is also considerable variation in the methods used to assess the crypt cell mitotic rate, and in some instances, assessments of mucosal size rather than rate of cell turnover were made.

Whilst the effects of vagal stimulation on mucosal
blood flow had been previously investigated (Kewenter, 1965), the effect on the crypt cell kinetics had not. It was perhaps surprising that there was no significant demonstrable effect on the crypt cell mitotic rate following electrical stimulation of the vagus nerves, which was continued for a similar time interval, using similar voltages and taking similar experimental precautions, to the stimulation of the neurovascular bundles of the small intestine performed by Tutton (1975a). The latter, which apparently principally involved sympathetic nerve fibres, was associated with quite a significant rise in crypt cell mitotic rate. Local denervation of a segment of small bowel, described by Tutton and Helme (1973, 1974) as surgical sympathectomy, because of the predominantly anti-sympathetic effect, was found experimentally to produce the same mitotic depressant effect as that observed by Tutton and Helme after the same procedure and the effect on the crypt cell mitotic rate was similar to that observed after chemical sympathectomy or immunosympathectomy (i.e., generalized sympathectomy).

Local ischaemia of the intestine, which could possibly be associated with the procedure of local denervation because of the close relationship between the mesenteric nerves and vessels, and could result in a similar fall in mitotic rate, was not noted as a side effect at operation by Tutton and Helme (1973, 1974) or to be present in the present experiments, and was furthermore carefully excluded histologically in both cases. Furthermore, Schofield (1960) noted that the ischaemic effects of this procedure became evident only if three or more neurovascular bundles were ligated simultaneously whereas in the present experiments only two neurovascular bundles were ligated. However, to add further to the evidence that the effects of local denervation on the crypts were not primarily
due to local ischaemia, in the present experiments it was found that even if a loop of jejunum is allowed to develop its blood supply from an alternative source e.g. the anterior abdominal wall, unassociated with its usual autonomic nerve supply, local denervation of such a well vascularized loop is associated with the same degree of fall in crypt cell mitotic rate. It was thus concluded from this and the other evidence in the literature that the effect of local denervation on the crypts was primarily a neural one, principally involving the sympathetic nerves (Malmfors et al., 1981) but also probably other fibres e.g. histaminergic, cholinergic, serotonergic (Tutton and Helme, 1974). Further evidence supporting the likelihood that the effects of cutting the neurovascular bundles on crypt cell mitotic rate are primarily neural rather than vascular was provided by the findings of Grim (1963), and Hultén et al. (1977) who showed that splanchnic nerve stimulation resulted in vasoconstriction of the splanchnic vasculature and decreased mucosal blood flow, and those of Rijke et al. (1976) who showed that a decrease in mucosal blood flow is associated with a fall in crypt cell mitotic rate, not the rise found by Tutton (1975a) following neurovascular bundle stimulation.

Toyohara et al. (1986) noted that even after one hour of ischaemia degenerative changes in the sympathetic nerves occurred in the rat distal colon, so that it is possible that some of the effects of ischaemia on crypt cell mitotic rate noted by Rijke may be attributable, in part at least, to changes in the sympathetic innervation of the crypts. Furthermore, Tutton and Helme (1974) found that whilst adrenaline and noradrenaline were both potent vasoconstrictors of the mesenteric circulation, they had opposite effects on the crypt cell
mitotic rate, and adrenaline and isoprenaline, which are both associated with a decrease in crypt cell mitotic rate have opposite effects on small bowel circulation.

Diminished blood flow through the mesenteric blood vessels does not have a straightforward effect on the flow of blood to the villi. Lundgren (1974) for instance, found that neither the lowering of perfusion pressure to the small intestine nor the activation of the sympathetic vasoconstrictor fibres induces any significant decrease of villous blood flow. However, although the villous blood flow stays almost constant when reducing perfusion pressure, mean transit time through the villus vascular loops is greatly increased, leading to villus hypoxia.

As noted previously, Levine et al. (1982) suggested that the effect of chemical sympathectomy on the crypts was less significant than that of the luminal contents, suggesting that neural control of the crypts is probably complementary to control by luminal factors. With regard to the effect of truncal vagotomy on crypt cell mitotic rate, no definite conclusions regarding the mechanism of action of the vagi on the crypts could be formed from the present experimental findings e.g. whether they are due to local changes in motility of the bowel, blood flow changes, changes in the bacterial flora of the lumen or direct neural action on the crypts. A survey of the available literature does little to elucidate the mechanism of action of the vagi at the crypt cell level.

However, vagotomy has been shown to be associated with a profound decrease in mesenteric blood flow (and possibly mucosal ischaemia, Ballinger, 1965 a), and since Rijke et al. (1976) found that such ischaemia should be associated with a decrease in crypt cell mitotic rate, the fact that the
crypt cell mitotic rate is increased after vagotomy suggests that vascular factors (especially ischaemia) are not of prime importance in the crypt cell changes found after truncal vagotomy. Furthermore, Kewenter (1965) showed that stimulation of the vagal trunks had little or no effect on mesenteric blood flow, and Davenport (1966) showed that the intestinal vasculature receives only a sympathetic innervation.

The vagus nerves may possibly act directly on the crypts, there being a sufficient number of suitable nerve fibres in close proximity to the crypts or they may act via some other means e.g. by a change in luminal bacterial flora, and certainly the fact that the effects of vagotomy are still manifest after three weeks would at least be consistent with a change in the luminal flora occurring.

Whereas Tutton (1975a) noted that stimulation of the mesenteric neurovascular bundles (concluded to be mainly but not exclusively sympathetic fibres) was associated with an increase in crypt cell mitotic activity, it might have been expected that if vagotomy was associated with an increased crypt cell mitotic rate, stimulation of the vagus nerves might have been associated with a decrease in crypt cell mitotic rate. However, as noted previously, there was no significant change in crypt cell mitotic rate associated with stimulation of the vagus nerves for a similar period of time, at a similar voltage, and in a similar strain of rats to those used by Tutton (1975a) for mesenteric nerve stimulation. This is consistent with the vagus nerves having either a slower effect on mitotic rate when stimulated or having a less direct efferent and a more indirect afferent effect on crypt cell mitotic rate, since cutting the vagus nerves appears to have a more definite effect on the crypts.
Since the vagi are also known to be largely of afferent fibre composition, this would be consistent with the vagi having a more afferent role in the day to day control of crypt cell mitotic rate. Of course it is known that the vagus nerves can relay information regarding the composition of the luminal contents to the hypothalamus. As indicated in Chapt. I, Section 11, afferent information concerning the luminal milieu is thought to be important in the mechanism of control of crypt cell mitotic rate, so transmission of this information to the central nervous system may be the role of the vagus nerves in crypt cell proliferation control.

It has been suggested by various authors (see Chapt. I, Section 11) that noradrenaline and/or serotonin may form part of the proposed pathway of action of the sympathetic nerves on the crypts, so that the effects of the sympathetic nerves on the crypts may possibly be mediated by these substances. Furthermore, these substances are present in the wall of the intestine and have been shown to have an effect on crypt cell mitotic rate, independently of the sympathetic nervous system (see Chapt. I, Section 11). In fact, as mentioned in the discussion after Chapt. IV, Section 1, it is possible that the effects of local denervation on the crypts may be due to some extent to local changes in adrenaline or noradrenaline levels in the intestinal wall. In this connection there is experimental evidence to suggest the existence of adrenergic innervation of the serotonin producing entero-chromaffin cells (Thompson and Campbell (1966); Weber (1970); Tansy et al. (1971)). Furthermore, Ohsumi et al. (1974) in the rat found evidence of adrenergic-type fibres in the vagus nerves. Larsson (1981) considered adrenergic fibres in the vagus nerves of the cat may have been involved in the
demonstrated release of serotonin into the gut lumen following vagal stimulation, and he concluded that the splanchnic nerves participate in the regulation of serotonin release from the enterochromaffin cells into the portal circulation by an adrenergic mechanism.

However, the concept of innervation of the alimentary tract by an autonomic nervous system composed of independent sympathetic (adrenergic), and parasympathetic (cholinergic), components is probably an oversimplification of the real situation, as suggested by Tutton (1975 b). He found, on the basis of experiments on cholinoreceptor stimulation and blockade, that there were probably functional interactions between the cholinergic and adrenergic postganglionic neurons innervating the intestine and many examples of these have been described in a review by Kosterlitz and Lees (1972) in other areas in the body.

Thus, it is suggested that the autonomic nervous system appears to have some role in the control of crypt cell proliferation, possibly by collecting information about the luminal milieu and relaying this via the vagi to the C.N.S. where this information is acted on. In some way possibly the crypts are then acted on via mainly the sympathetic nerves as part of the mechanism of crypt cell proliferation control. This is not to say there is exclusively a C.N.S. mechanism acting to control all of the daily adjustments in crypt cell proliferation, but possibly in some instances a neural mechanism may be operative e.g. the control of diurnal variations in crypt cell mitotic rates.

With regard to the enteric plexus of nerves, most studies of this have been in regard to its absorptive or secretory functions. There has been no previous investigation of the
role of this plexus within the intestinal wall in crypt cell proliferation control, so that our knowledge of the functions of this plexus are in terms of its control of absorptive or secretory functions.

In a review of this topic Cooke (1986) described the enteric division of the autonomic nervous system as an independent integrative system that differs in structure and function from the parasympathetic and sympathetic divisions of the autonomic nervous system. It consists of the myenteric plexus within the muscular layers and the submucosal plexus in the submucosa, and both are ganglionated and interconnected into a single functional system. The integrative circuitry continuously processes information from intramural and mucosal sensory receptors, and programs via motor neurons appropriate behaviour of the effector systems.

Most of the neurons within the submucosal ganglia project to the mucosa, suggesting that the submucosal plexus functions to control and coordinate absorptive and secretory function, blood flow, and contractility of the muscularis mucosae. Although the plexuses are separate, neural connections between the two suggest that they comprise a single integrative system. Nerve fibres from the ganglionated submucosal plexus extend into the mucosa to form a nonganglionated mucosal plexus adjacent to the muscularis mucosae, surrounding the crypts and subjacent to the villus cells. It was previously assumed that the sole function of the enteric ganglia was to pattern smooth muscle activity that is appropriate for the motor function of the gut, but now (Cooke, 1986) it is held that myenteric and submucosal ganglia control and coordinate the activity of the entire set of gastrointestinal effector systems. Thus, it is difficult to completely separate effects on crypt cell
proliferation mediated via the sympathetic nervous system from the other effects mediated via the sympathetic nervous system, but perhaps in some instances the direct effect on the crypt cells may predominate over, for instance effects on the local circulation. Cooke has further claimed that the previous hypothesis that submucosal ganglia contained only sensory neurons has not withstood rigorous scientific testing. Several lines of evidence indicate that both interneurons and motor neurons function in these ganglia. Experimental evidence suggests that the interneural circuits of the submucosal ganglia receive sensory information from luminal sensory receptors, integrate and encode this information and transmit commands as action potentials via motor axons to release neurotransmitters at the mucosal effector systems. The function of the enteric nervous system is continuously modulated by input from the extrinsic parasympathetic and sympathetic nerves (Cooke, 1986). With regard to transport function of the mucosa, which has been extensively studied, sympathetic influences on mucosal transport function (i.e. including the absorption of nutrients) may include the release of noradrenaline/somatostatin at synapses with cholinergic and noncholinergic motor neurons within the submucosal ganglia, as well as at junctions with transporting cells.

In addition, noradrenaline may act on the vasculature to alter Starling Forces across the capillary walls and it may act on the smooth muscle in the gut wall to inhibit motility. Thus, the sympathetic nerve influences on the mucosa are quite complex and it is difficult to completely separate the direct effects on the crypt cells completely from those on other factors in the mucosa, although in the case of the pineal and limbic lesions we can suggest that the effect is primarily a
5.11

neural rather than a vascular one. It is difficult, similarly, to dissociate the effects of the emotional disturbances arising from the limbic lesions from the direct effect of the limbic system on the crypts, because the emotional state of the individual, and the extent and composition of the extracellular volume all may influence the degree of extrinsic neural input to the enteric ganglia, and subsequent activation of vascular, muscular or epithelial motor responses that ultimately lead to alterations in intestinal transport processes and body fluid homeostasis (Cooke, 1986) and as we have seen these luminal factors themselves may have a role in the control of crypt cell proliferation.

With regard to the apparent significance of luminal factors in the control of crypt cell proliferation, it is of interest to note that mechanoreceptors, chemoreceptors, and thermoreceptors, which can give information about the physical properties of the luminal contents, have all been identified in the small intestine (Cooke, 1986). Mucosal receptors that exhibit the properties of either or both mechanoreceptors and chemoreceptors are present within the mucosa and probably function to sense the texture, fluidity and chemical composition of the luminal contents. For example, stroking the mucosa or changing the luminal glucose concentration, osmolarity or acidity evokes spike discharges in the plexuses. Some receptors are responsive to distention or distortion of the gut wall and probably monitor the volume of the intestinal contents. These findings are consistent with the findings of Skagen (1977) and Clarke (1976) who found that the physical characteristics of the luminal contents were as important, if not more important, than the nutrient value of the contents. The autonomic nervous system could have a role
in evaluating the characteristics of the luminal contents, so that this information could be relayed to other centres. The transport function of the mucosa parallels its functional activity and it is evident from the literature (Cooke, 1986), that coded information from a variety of sensory receptor types is undoubtedly important for coordinating the absorptive and secretory functions of the intestine with motility and blood flow, both from the standpoint of providing sensory information to the brain and local processing in the enteric circuits. With regard to the effects of extrinsic denervation of loops of intestine on the crypts, the effects of local stimulation of intestinal contents may influence the function of the mucosa irrespective of its lack of extrinsic innervation, e.g. tactile stimulation or distention of the intestinal lumen stimulates intestinal fluid secretion in extrinsically denervated Thiry-Vella loops of canine intestine (Cooke, 1986) and mechanical stimulation of the intestinal mucosa in the cat evokes a reflex vasodilatation.

The "luminal nutrition" aspect of the "work load" concept of the role of the luminal contents does not consider that the effects of local nutrient absorption can rarely be separated from those of ion absorption, which requires active absorption or "work load". For instance, it is known (Cooke, 1986) that alterations in chemical composition of the intestinal lumen can evoke changes in intestinal transport. In the cat it has been proposed (Cooke, 1986) that luminal glucose not only stimulates sodium absorption by the villus cells, it activates neural pathways that evoke a sodium secretory flux from the crypts. The physiological significance would be to ensure a high luminal sodium concentration necessary for maximal glucose uptake. It is thus difficult to separate the
osmotic effects of the intraluminal glucose from the purely locally nutrient effects on the enterocytes or the effect of the absorbed glucose on the blood glucose levels. Once again, this highlights the probable role of the autonomic nervous system in the mechanisms of local absorption of nutrients, the luminal levels of which also have some significance in the control of crypt cell proliferation.

To complicate the picture, there is evidence that noradrenaline or adrenaline stimulate sodium, chloride and water absorption and decrease bicarbonate secretion in rabbit ileum or rat jejunum in vitro (see Cooke, 1986). In order for neurons to have a direct influence on the epithelium (as has been suggested by the experimental results) neurotransmitters must be released in close proximity to the enterocytes. The terminal portions of the nerve fibres in the mucosa are varicose and are found to be subjacent to the basement membrane of transporting epithelial cells and entero-endocrine cells (Cooke, 1986). Often membrane to membrane contact between axon varicosities or nonvaricose axons and enterocytes occurs, but only occasionally can these contacts be considered true synapses, although apparently a synapse is not always necessary for effective interaction between a nerve and gland or muscle. The neurons exert their influence on effector cells by release of neurotransmitters from vesiculated varicosities and subsequent diffusion of the messenger to receptors on mucosal effectors. Occasionally, adrenergic fibres run directly subjacent to the villous epithelium, and therefore the possibility exists that noradrenaline/somatostatin released during stimulation of sympathetic pathways could directly affect the epithelial cells and/or indirectly alter epithelial function by influencing neurons within the synaptic circuitry.
of the submucosal plexus.

As Cooke (1986) in a review of the subject comments, caution must be exercised in ascribing neurotransmitter function to a chemical messenger on the basis of its immunocytochemical localization in enteric neurons. It is clear that many peptides coexist with other putative neurotransmitters within submucosal neurons but the significance of this coexistence is unknown.

In order for neurotransmitters to influence mucosal function directly, receptors specific for each chemical messenger must be present on the enterocytes. Receptor studies on the intestinal mucosa indicate that receptors are present for acetylcholine, vasointestinal peptide, somatostatin and noradrenaline but unfortunately receptor binding studies done on the intestinal mucosa cannot distinguish between receptors on specific types. Receptors that bind catecholamines are present on small intestinal and colonic enterocytes, and a mixed population of $\alpha_1$ and $\alpha_2$ adrenoreceptors was found in the rat, with the suggestion that $\alpha$ and $\beta$ adrenoreceptors are present in rat colonocytes. Thus, it is clear that neurons containing noradrenaline, somatostatin, acetylcholine and other peptides innervate the mucosa. The presence of receptors for these putative neurotransmitters on enterocytes suggests that these neurons function as motor neurons to the epithelium. There is thus a multiplicity of putative neurotransmitter substances localized to submucosal neurons, as well as a large number of specific receptors within the neural circuitry and on enterocytes. The presence of a messenger or a receptor however does not necessarily imply its function.

There is at present no satisfactory scheme for a functional classification of types of submucosal neurons. However,
the extent of synaptic input to the submucosal ganglia suggests that most neurons are interneurons or motor neurons involved in the regulation of mucosal function rather than sensory neurons. Another mechanism possibly involved in the secretory and motor functions of the intestinal mucosa, and thus possibly involved in local effects on crypt cell proliferation is the release of endocrine messengers. Because of the proximity of nerve fibres and endocrine cells (see Cooke, 1986) it is possible that neural stimulation releases messengers from the cells within the mucosa either (a) into the blood (b) locally into the interstitial space or, (c) into the intestinal lumen. Glucagon / glicentin, 5-hydroxytryptamine, and somatostatin are important endocrine messengers in the intestine and are involved in the production of secretion.

It is interesting to note that in connection with the role of the peptides in control of crypt cell proliferation, whilst electrical stimulation of the vagus nerves in vivo was not associated with any significant change in crypt cell proliferation rate, it has been reported to be associated with release of 5-hydroxytryptamine, motilin, gastrin, somatostatin, and substance P into the blood or lumen of the stomach or intestine (Ahlman et al. (1978a); Uvnas-Wallensten (1978); Fox et al. (1983)). It cannot be assumed that these substances are not involved in crypt cell proliferation control, however, and in fact the influence of these substances released on neural stimulation, on mucosal function has yet to be clarified, and the neurotransmitters released at neuroenterocyte junctions in response to electrical field stimulation have not all been identified. Recent studies provide strong evidence that acetylcholine is released at junctions of nerves and enterocytes (see Cooke, 1986), but that there is also a noncholinergic
part of the neurally evoked secretory response, and it has been suggested that serotonin may stimulate cholinergic submucosal neurons (via the submucosal plexus) to influence mucosal function.

It is clearly evident from the foregoing discussion that the role of the enteric plexus in the mucosal functions which have already been studied, e.g. absorption, electrolyte balance and secretion, is a very complex one. It can be expected that when the role of the enteric nervous system in the control of crypt cell proliferation has been defined, it will be of a similar order of complexity, involving neural effects, with or without local effects of released hormones or neurotransmitters.

The central nervous system monitors sensory information from the intestine and sends commands via the parasympathetic and sympathetic nervous system to the enteric ganglia or possibly directly to mucosal cells to modulate activity of the effectors. Preganglionic parasympathetic fibres originate in the lower medulla oblongata (dorsal motor nucleus of the vagus) and are carried by vagal efferent fibres to the small intestine and proximal part of the large intestine. All of the preganglionic parasympathetic fibres terminate on intrinsic neurons but direct synaptic connection within the ganglia between preganglionic fibres and the enteric motor neurons is unlikely because the number of efferent fibres in the vagal (and pelvic) nerves is about five orders of magnitude less than the number of enteric ganglion cells (see Cooke, 1986). Preganglionic sympathetic fibres that supply the intestines arise from neurons in the intermediolateral spinal columns between the fifth thoracic and second or third lumbar segments and pass through the sympathetic chain without synaptic relay to
synapse in prevertebral sympathetic ganglia. Postganglionic fibres from the coeliac and superior mesenteric ganglia supply the small intestine. The sympathetic fibres to the gut termi-nate in the enteric ganglia and blood vessels and some fibres may terminate subjacent to epithelial cells (as previously mentioned). Little is known about the central nervous system control of parasympathetic outflow to the intestine.

Jeanningros and Mei (1977) found that electrical activity was recorded in the hypothalamus, especially in the median nucleus, following electrical stimulation of the splanchnic and vagus nerves. The C.N.S. appears to be implicated in any neural mechanism of control of crypt cell proliferation, with the possible site of the controlling element being in the hypothalamus, since this area is an essential part of the autonomic nervous system, in addition to its other functions (it is sometimes referred to as the "head ganglion" of the sympathetic nervous system). Furthermore, the hypothalamus is a centre where afferent visceral information is collected and it is known that the sympathetic efferent effects manifested in e.g. "flight or fight" reactions probably emanate from this area. Since the hypothalamus has many and varied functions besides its involvement in the autonomic nervous system, including neuroendocrine and hormonal effects, it was decided that rather than examine the effects of hypothalamic lesions on crypt cell proliferation, initially at least, the effects of lesions of the pineal gland on these cells would be studied.

The hyperproliferative effect on the crypts associated with pineal lesions noted previously by Bindoni (1971) was confirmed, and the effect was noted to persist as long as three weeks after pinealectomy. It was found that the effects of pinealectomy were independent of changes in the luminal
environment, e.g. changes in the amount of nutrients, bile salts, and even exposure to the effects of the colonic bacterial flora. Whilst the effects of changes in the levels of the luminal bile salts in the small intestine were found to have a minimal effect on the crypt cell mitotic rate in the present experiments, there was not general agreement in the available literature regarding this (see Chapt. 1, Section 11).

When the effects of combining local gut denervation or vagotomy with pinealectomy on crypt cell proliferation were examined it was found that the large hyperproliferative crypt effect was diminished to the level associated with local denervation alone, suggesting perhaps that the pineal gland acts on the crypts principally via the autonomic nervous system but it may of course act also via other pathways e.g. via various endocrine changes which are known to be associated with pinealectomy, but probably not via the action of melatonin (as indicated previously). Whilst melatonin secretion probably is not the main effector pathway for the action of the pineal on the crypts, this substance may be another important link between the actions of the pineal and the limbic system. Critchley (1982) noted that specifically high affinity binding of $^3$H melatonin had been observed in central nervous system tissue and brain membranes, the highest affinity being for the limbic areas, including hippocampus, and the hypothalamus.

These possible humoral pathways of action of the pineal may of course be operative principally in relation to the other actions of the pineal which are not concerned with crypt cell proliferation. Humoral pathways of action of the pineal gland on the crypts would also be consistent with the widespread effects of pinealectomy on other organs described by
Bindoni and Raffaele (1971), although he did conclude from experimental evidence that the pituitary gland was probably not involved in the effects on crypt cell proliferation. Although there seems to be evidence for the involvement of the autonomic nervous system in the effects of the pineal gland and the limbic system on the crypts, there are no clear cut pathways between these areas and the crypts. However, there are possible indirect pathways between these areas of the C.N.S. and the crypts, via the vagi and sympathetic nerves, as described in the previous discussion, although the superior cervical ganglion which innervates the pineal does not appear to be involved in its actions on the crypts. Presumably the pineal acts principally on the crypts via the hypothalamus, with which it has connections, and the autonomic nervous system, via cholinergic, adrenergic and possibly other fibres e.g. serotoninergic, peptidergic fibres. Since the effect of local denervation of the gut on the crypts does not appear to be primarily a vascular one, it is suggested that the effects of the intact pineal, presumably to decrease crypt cell mitotic rate, are not mediated via local vascular effects but probably via local neural mediation.

The effects of vagotony when combined with pinealectomy were noted to be opposite to the effects on the crypts of vagotony alone and to resemble qualitatively those associated with local denervation, with or without associated pinealectomy. It is thus suggested that both the vagus nerves and the sympathetic nerves are involved in the actions of the pineal on the crypts in a principally efferent manner, as may also be the other nerve fibres in the neurovascular bundles e.g. histaminergic, serotoninergic, etc. Furthermore, the fact that the pinealectomy effect on crypt cell mitotic rate appeared
to be independent of changes in the luminal environment of the small bowel, which has been shown to have a role in the normal day to day control of crypt cell proliferation, suggested that the pineal effect on the crypts may be part of a mechanism operative only under "unusual" conditions, e.g. in stressful situations and not in day to day crypt cell regulation. The pineal has been postulated to have a modulatory effect on the other functions of the C.N.S. and endocrine functions (Oksche and Pevet, 1981) and it is conceivable that because of its suppressive effect on crypt cell proliferation, it may be part of a mechanism for the "damping down" of excessive swings in the crypt cell mitotic rate. Thus, in some way it may be involved in a protective mechanism against malignant change, e.g. in the intestine, where excessive crypt cell proliferation has been linked with predisposition to malignancy (Williamson, 1979; Williamson and Malt, 1980). However, this is highly conjectural, since there are many other factors, as yet unknown, which could be operative in the production of bowel tumours, besides excessive fluctuations in crypt cell proliferation.

It was noted by Bindoni et al. (1973), and Jutisz et al. (1974) that tuberoinfundibular lesions of the hypothalamus were also associated with increased crypt cell mitotic rate. Thus, the pineal gland may form merely one part of a more widespread mechanism affecting crypt cell proliferation, and it is of interest to note that Noback (1967) considered the hypothalamus to be a modulator of autonomic nervous activity in addition to its other functions. Bindoni et al. (1986) found a significant increase of cell multiplication in inoculated ascitic and solid tumours in both DBA/2 and C57BL/6 mice as well as wistar rats after radiofrequency lesions in the
median hypophysis (ventromedial and dorsomedial nuclei; part of the arcuate nucleus). Although they had previously demonstrated a similar proliferative effect following lesions of this area of hypothalamus on stratum germinativum of the epidermis, Yoshida ascites tumour in the rat, and Erlich's tumour and L1210 ascites tumour in the mouse, they did not find any similar effect when the anterior or posterior hypothalamus or the cerebral hemispheres were lesioned. No mention has been made in this study of pineal or limbic lesions but it is quite possible that the hypothalamic areas lesioned could be on the pathway of action of the pineal or limbic system, and the effects on intestinal tumours of these lesions have not yet been investigated. Thus, it is difficult to extrapolate the results of this experiment to the functions of the hypothalamus in the production or maintenance of gastrointestinal tumours. However, it is clear that further experimental work on the effects of lesions of the hypothalamus and/or limbic system/pineal on tumours of the gastrointestinal tract should be done in the future.

It is of interest that they noted that in animals with hypothalamic lesions in the areas mentioned, there was a slight decrease in the secretory activity of the adenohypophysis, as might be expected since this part of the hypothalamus is involved in the secretion of several neurohormones governing the function of the hypothalamus. However, because a fall in hypophysis function is generally associated with a fall in cell multiplication rate in normal and neoplastic tissues, they suggested that the increase in cell proliferation after hypothalamic lesions was more likely due to suppression of an inhibitory mechanism located in the hypothalamus which is independent of the hypophysis. However, they concluded that
a humoral mechanism was probably involved to some extent in the
effect, since it was noted even in cells suspended in ascitic
fluid. Thus, the role of the limbic and pineal areas in the
production or maintenance of tumours is only conjectural at
present. As mentioned in Chapt. IV, Section 11, the relation-
ship between the pineal gland and malignancy has been studied
in a general way. Thus, Relkin (1976) suggested that whilst
the pineal gland does not provide fertile soil for the growth
of tumours, the presence of the pineal seems to retard the
growth and spread of tumours. Whilst there was some controversy
regarding the weights of the pineal glands in those dying of
malignant disease, there was experimental evidence for increased
tumour activity in the pineals of tumour bearing animals, and
Lapin (1976), on reviewing the experimental data available at
that time, claimed the pineal gland may be involved in the
growth and development of neoplasia. This finding was support-
ed by the findings of Rodin (1963), Dasgupta and Terz (1967),
Barone and Dasgupta (1970), and Lapin (1974) who found that
pinealectomy enhanced the growth of transplantable tumours.
The possible role of melatonin in this effect on tumours was
considered to be controversial. It is not possible to apply
the findings in the present experiments directly to elucidate
this problem, but further work needs to be done on this aspect
of the effects of the pineal. Since the intact pineal is pre-
sumed to have a depressant effect on crypt cell mitotic rate,
it was decided to examine the possibility that there were other
C.N.S. areas which tended to accelerate the crypt cell mitot-
ic rate and opposed the action of the pineal. There is the
possibility, of course, that the pineal forms part of a more
extensive system which has a tonic depressant action on crypt
cell mitotic activity, without any opposing system.
The limbic system, besides being involved in the expression of the emotions, is known by some authors as the "visceral brain", i.e. a higher centre concerned with the reception and interpretation of afferent visceral information and concerned with visceral efferent function. Since this area has many connections with the hypothalamus and the autonomic nervous system as well as the pineal gland, it was decided to observe the effects of lesions in the limbic area. By combining lesions in this area with pinealectomy it was found that the effect of the limbic lesions on crypt cell mitotic rate was not diminished by the associated pinealectomy, suggesting that the pinealectomy effects were probably mediated at least in part via the limbic system.

Lesions of the limbic system, with the exception of the cingulate gyrus (which is not considered as part of the rat limbic system by Hamilton (1976)) resulted in a hyperproliferative effect on the crypts of a similar magnitude to that associated with pinealectomy, and unaffected by the associated pinealectomy, suggesting that both areas belong to the same system affecting crypt cell rate.

Since both the limbic system and the pineal have hypothalamic connections, and thus autonomic nervous system connections, and both pineal and limbic lesions were greatly modified in their effects by associated autonomic nervous denervation of the gut, these findings are consistent with the pineal and the limbic system mediating their effect on the crypts via the hypothalamus. Whilst the effect of autonomic nervous denervation was only combined experimentally with hippocampal lesions, rather than all limbic lesions, the connections of the hippocampus with the remainder of the limbic system are so extensive that it can be logically assumed that the effects
of lesions of the other limbic areas would also be negated by autonomic nervous denervation of the gut. In fact the negation of the effects on the crypts by autonomic denervation of the crypts was if anything more marked in the case of the limbic system than in the case of pinealectomy.

Layton et al. (1981) suggested an additional neural pathway whereby the amygdala can influence neuroendocrine regulation i.e. through its actions on the excitability of medial preoptic tuberoinfundibular neurons (see Bindoni et al. (1973); Jutisz et al. (1974)). The amygdala may influence neuroendocrine secretion via synaptic actions on tuberoinfundibular neurons in both the mediobasal hypothalamus and the medial preoptic/ anterior hypothalamic areas.

As previously indicated, the limbic system does have effects on the level of growth hormone, A.C.T.H., etc, and it is possible that this system may affect the crypts additionally via means of changes in the levels of these hormones e.g. over a longer period of time, although it would appear that the effect is primarily a direct neural one in the short term at least.

Davson and Segal (1976) state that the hypothalamus contains neuronal organizations or centres that by receiving afferent input from the viscera and initiating efferent output to the appropriate effectors permit control over such functions as eating, drinking, defence, etc. Furthermore, they remark that the medial forebrain bundle is an important tract carrying impulses to the hypothalamus from higher centres such as the amygdala and septum, and in general, relating it to the other parts of the limbic system ("visceral brain"). Lesions in the hypothalamus that interrupt the tracts which connect it with the "visceral brain" will produce effects equivalent
to destruction of centres elsewhere. Thus, lesions in the lateral hypothalamus that cause aphagia and adipsia probably are the result of the medial forebrain bundle, which passes through the whole lateral hypothalamic area, being severed by these cuts. As indicated above, this bundle relates the hypothalamus to the septal area, which receives connections with the hippocampus and amygdala, whilst there is a more direct communication with the same regions.

So it appears that the pineal gland and the limbic system form part of a system suppressing the rate of crypt cell proliferation, but probably not concerned in the daily adjustments of crypt cell mitotic rate, which are probably influenced mainly by the effect of luminal factors and, from the available literature, may also be mediated via humoral factors. The limbic and pineal effect appears to be primarily neurally mediated, rather than due to local vascular changes, and to involve the vagus nerves, the sympathetic nerves, and possibly the other fibres of the intestinal plexuses in probably mainly an efferent fashion. However, caution must be used to not oversimplify the connections of the limbic system and the pineal with the gut crypts. The involvement of the vagus nerves in the effects on these areas is probably not a simple straightforward one, as the work of Brooks (1983) has suggested that the vagal afferents and efferents serve a complex of functions, not a single function, and that its afferent fibres connect with a great diversity of sensors and carry signals to a large number of interconnected centres in the brain. Again, whilst the vagus nerve is classified as a cholinergic parasympathetic nerve, it contains sympathetic efferents that liberate adrenergic transmitters and is a producer, transporter, and liberator of compounds such as cholecystokinin and gastrin.
Whereas the limbic system was considered as a reverberating circuit (the Papez circuit) modulating the effects of other C.N.S. areas for many years, the experimental work of Poletti (1986) suggests that the limbic system exerts its functional role primarily by a direct influence on structures downstream in the neuraxis, the diencephalon, midbrain, and even the spinal cord, and this is consistent with the limbic system acting more directly on the crypts neurally rather than the crypt effects being coincidental to a widespread autonomic discharge.

The present experimental findings also suggest that luminal factors are probably important in the day to day control of crypt cell mitotic rate, e.g. there is an obvious fall in the mitotic rate in a jejunal loop isolated from the remainder of the small bowel and thus deprived of its usual nutrient content. However, biliary secretions, from the present experimental findings, do not appear to be of great importance of themselves, although their effects are difficult to separate from those of pancreatic secretions, and the experimental findings recorded in the available literature are controversial. We may speculate that, generally speaking, in the day to day mechanism of crypt cell proliferation control, the vagus nerves have a more afferent role in any neural mechanism involved and it is possible that the C.N.S. may be involved in the luminal mechanism of control by assimilating information regarding the luminal milieu and the C.N.S. may thus play only a supplementary role in a complex mechanism of control involving many factors. However, these actions of the vagi and sympathetics suggested here may quite possibly be separate from their actions when concerned with the mediation of the effects of the limbic system or pineal on the crypts.
The limbic system is seen in the literature (Poletti, 1986) as a system which modulates virtually all of the functions of the brainstem, including behavioural responses to "flight or fight", pain suppression, sexual maturation, and autonomic and endocrine activity so that the limbic system is not simply concerned with the mechanism of control of crypt cell proliferation, and its effects noted in these experiments could not be directly linked with any of its other effects e.g. the effects of emotional disturbance of stress. It is possible, for instance, that the hypoproliferative effect on the crypts associated with stress noted by Tutton and Helme (1973) may have been mediated by the limbic system, the hypothalamus, and the autonomic nervous system. They concluded that, whilst the pituitary-adrenal axis had been postulated previously as the major system mediating the response to stress, experimental evidence did not support this mechanism in the effects of stress on the crypts. They suggested that the integrity of the sympathetic nervous system rather than the presence of the adrenal gland appeared to be of great importance in the response of crypt cell proliferation to stress. In support of this, they recorded that similar crypt cell cycle times were observed in the jejuna of stressed intact animals, in surgically (locally) denervated segments of jejunum in stressed animals and in similar denervated segments of jejunum in non-stressed animals. They thus found that sympathectomy inhibited crypt cell proliferation in these situations just as much as stress, and in sympathectomized animals stress caused no further inhibition of crypt cell proliferation. They postulated that stress inhibits crypt cell proliferation by reducing or abolishing the activity of the sympathetic nerves which usually promote cell division. This view would at first appear to be consistent
with the present experimental findings of the effects of limbic lesions on the crypts and the apparent dependence of these effects on the integrity of the sympathetic nervous system and would presuppose that, if involved in the effects of stress, the limbic system exerted the depressant effect on crypt cell proliferation during the period of stress. However, if the limbic system only acts in a depressant manner on the crypts during periods of stress, and not at other times, this does not explain why the crypt cell mitotic rate rises after limbic lesions without periods of stress, which suggests a constant limbic depressant effect on the crypts, not just during periods of stress, which is released by limbic lesions and cancelled by local sympathectomy. Thus, there is no simple explanation for the effects of stress on the crypts involving the limbic system directly.

With regard to the role of the pineal gland in the effects of stress on the crypts, this does not appear on the available evidence, to be major one. Parfitt and Klein (1976) have concluded that whilst it is known that the sympathetic nervous system usually stimulates sympathetically innervated tissues during stress, apparently this effect is relatively minor in the intact pineal gland, which does receive heavy sympathetic innervation. They suggest that in the pineal gland the sympathetic system plays more of a passive homeostatic role during stress. They suggested that these changes in the pineal would tend to protect against large stress-induced changes in pineal indole metabolism during daylight hours when this parameter is normally low.

It is therefore not possible to establish a C.N.S. "centre" for control of crypt cell proliferation at the present level of knowledge.
Since the limbic system is known to be involved in the expression of the emotional status of the individual, and since the pineal has been implicated in the causation of malignancy (see above), although its role is controversial, we should consider if there is any evidence for changes in emotional status which could involve the limbic system being associated with the causation of malignancy. From the available literature, there appear to be tenuous links made by various experimenters between malignancy and the emotional status of individuals. From the present experimental findings no conclusion can be made concerning this relationship, but perhaps further experimental work to define this relationship is warranted.

A possible link has been observed for a long time. For instance, Galen observed that cancer in women was more frequent in "melancholic" rather than "sanguine" women. Kissen et al. (1969) in carefully experimentally controlled studies showed that people with lung cancer showed poor and restricted outlets for emotional discharge even before the onset of the cancer.

Bahnson et al. (1969) and Bahnson (1976) found that their data supported the presence of extreme repression and denial in cancer patients. Various reviews (quoted by Brown and Seggie, 1979) do point out some degree of consistency in finding that certain personality factors tend to be characteristic of cancer patients, even prior to the onset of illness, with other possible precipitating psychological factors. Meerloo (1954) has suggested that these psychological manifestations may be coincidental, i.e. stemming from different causes or, possibly, a single underlying neural or endocrine factor may cause both emotional disturbance and cancer, and that stress may be a causative factor in the development of cancer through the intermediary of the stress hormones. Psychological disturbance
may disturb the body chemistry so much that a cancer "in situ" may become a malignant tumour, according to this line of argument. It is difficult to assign any precise role to the limbic system in these psychological reactions, however, because it forms only one part of a very complex system reacting to stress involving the actions of hormones as well. Peters and Mason (1979), in discussing the possible ways in which autonomic responses to stress might affect cancer growth or metastasis, have postulated on the basis of experimental evidence both neurological and endocrine pathways involving to some extent both the limbic system and the hypothalamus. They further concluded that an extensive network of C.N.S. neurotransmitter, endocrine and other biological processes may be involved in the mediation of the effect of psychological influences on immune function (which is probably significant in the development of tumours) and that the interaction of biopsychological phenomena with the immune system may, in turn, alter the susceptibility to and course of neoplastic disorders. Thus, there seems to be some suggestion that the limbic system may be involved in possible changes in the immune system predisposing to the malignant process as well as being associated with changes in the mitotic rate in the crypts. Further experimental work needs to be done to clarify the role of areas such as the limbic system in the production of malignancy. The hypothalamus and the limbic system have been implicated also in theories concerning the genesis of breast cancer. Stoll (1977) in a review of the subject states that the hypothalamus may exert control over the immune response mechanism, in addition to controlling the secretion and release of anterior pituitary hormones. He quotes several reports of an increased tendency to breast cancer being associated with specific
personality or behavior patterns or preceded by mental stress and suggests on the basis of his own investigations that there may be a link between psychological attitudes and the degree of tumor activity in breast cancer. He suggests that on a theoretical basis the growth and spread of breast cancer could be affected by emotional stress and affective disorders through the hypothalamo-pituitary pathway. He also notes that the hypothalamus has widespread connections with the forebrain and limbic system, so that stimuli originating in those regions could modify the release of pituitary secretions. For example, it is known that emotional stress can affect the circulating levels of prolactin, growth hormone, gonadotrophin and adrenocorticotrophin, some of which factors are known also to affect crypt cell proliferation. He concluded that through the hypothalamus, stress might be able to influence the immune response also, and therefore through both mechanisms the growth of breast cancer could be affected.

It is possible similarly that changes in crypt cell proliferation associated with the action of the limbic system or pineal may be associated with changes in the immune response, and more experimental work needs to be done on this topic. However, Stoll (1977) points out that even if the presence of persistent emotional stress and increased risk of breast cancer could be confirmed, this does not necessarily mean a cause and effect relationship because it is possible for both to arise from a common cause. The same principle would apply to links established between emotional stress and carcinoma of the colon, and any such links could be expected to be of a very complex nature. So any possible relationship between the present experimental findings and the previous discussion must remain a very tenuous one, pending further experimental investigation.
However, more definite links between the gastrointestinal tract mucosa and lesions of the limbic system have been provided by the findings of Kim (1976) who reported that large bilateral hippocampal lesions produced an increase in gastric pathology (mucosal erosions) over controls in restrained rats and that this effect was blocked by peripheral vagotomy. Both the preoptic-hypothalamic areas, as well as the amygdala, have been implicated in the development of experimental gastric ulcers and haemorrhage (Grijalva et al., 1980b). Henke (1980) found that lesions of the amygdala apparently reduced restraint-induced stomach pathology, whereas electrical stimulation of this area induced such gastric pathology in rats and cats, this effect being eliminated by prior vagotomy (Henke (1980); Innes and Tansy (1980); Sen and Anand (1957)). Henke and Savoie (1982) have suggested that afferent vagal fibres reach this region via the anterior hypothalamus and preoptic region. The same authors found that lesions in the posterior cingulate cortex of male Wistar rats increased the stomach pathology in restrained and unrestrained rats, whereas lesions in the anterior cingulate gyrus apparently attenuated the effects of immobilization stress on such pathology. These effects cannot of course be directly related to the effects of limbic lesions on crypt cell proliferation in the small intestine, but it is interesting to note that limbic lesions do affect the integrity of the gastric mucosa, although how this is achieved is not evident, and in particular by what neural pathway it is produced.

Since luminal factors are obviously important in day to day control of crypt cell proliferation, it was felt that some assessment of the amounts of food eaten should be made during the various hyperproliferative and hypoproliferative responses
of the crypts. It was hoped that it may be possible to demonstrate a link between the amount of food eaten and the proliferative status of the crypts, but no such correlation was found. It is of interest that the amount of food eaten was not increased following the limbic lesions, despite their hyperproliferative effect and the known involvement of the limbic system in the regulation of food intake. Also, the emotional effects of limbic lesions themselves might have been expected to affect the amount of food eaten. Thus, it appears that the factors which control normal food intake level maintain it at a fairly constant level despite changes in crypt cell mitotic rate. It appears likely, from the available literature, that the normal level of crypt cell mitotic rate requires a certain minimal level of luminal nutrients but excessive crypt cell proliferation does not require an increased intake of nutrients. Thus, conclusions regarding the mechanism of crypt cell proliferation control based on experiments involving starvation should be made with some caution. Experiments have also shown that placing the intestinal mucosa under stress to absorb nutrients more rapidly, resulted in an increase in food intake of the order of 60% but there was no significant increase in crypt cell mitotic rate (see Chapt. IV, Section IV).

During starvation, when little or no nourishment is being conveyed by the blood stream to the enterocytes in the mucosa, it is important for the survival of the animal that what little nutrients are available in the intestinal lumen should be available to nourish the enterocytes, thereby maintaining their function so that they may absorb more food. It is not clear whether luminal nutrition is only important under such conditions of starvation or whether it is equally important in the day to day control, since many of the
experimental situations studied in the literature, are artificial ones. It is possible that luminal nutritional factors may be more important where an animal cannot obtain a certain minimum amount of food intake.

It should be also be remembered that oral food has other effects besides supplying luminal nutrition to the intestinal mucosa—,

(1) it may promote mucosal nutrition,

(2) it produces mechanical stimuli—stretching of the bowel associated with changes in motility, and increased abrasion and removal of the villus tip cells,

(3) it promotes the production of pancreato-biliary secretions,

(4) the activity of the sympathetic nervous system is affected by eating. Young and Landsberg (1977 a, b ) suggested, from a series of experiments, that the central sympathetic outflow is influenced by the nutritional state of the animal i.e. there is nutritional modification of the central sympathetic outflow. These factors may in themselves affect crypt cell proliferation,

(5) ingestion of food causes activity in the "satiety centres" of the brain. In respect to these centres it is of interest to note that the limbic system has been implicated in this system. Robinson (1964) demonstrated that feeding could be elicited by electrical stimulation of virtually any area in the brain, especially limbic system sites. Fonberg (1969) found that bilateral stereotactic lesions limited to the dorsomedial part of the amygdaloid complex in dogs resulted in aphagia with subsequent long lasting hypophagia, adipsia, vomiting, apathy, lack of motivation and loss of interest in the surroundings. However, in the present experiments there was no significantly
decreased intake of food, and apart from some transient irritability following limbic lesions, behaviour was not that different from control rats. The aphagia observed in dogs of course may be a species difference. Additionally, it must be remembered that in the present study the limbic lesions involved all of the amygdaloid complex and not just part of it as in the case of these dogs. In fact, there was no significant change in the amount of food eaten following the various limbic lesions, suggesting that the limbic system alone does not play a significant part in the control of intake of food. This observation is consistent with the more recent view in which the notion of centres controlling certain behaviours, e.g. eating, has been replaced by the concept of circuits involving interactions among a number of different structures. As observed in the discussion at the end of Chapt. IV, Section IV, the literature on the effects of amygdaloid nucleus ablation on food intake generally shows conflicting results. The results of the present experiments are consistent with such observations.

The presence of excessive or diminished proliferation of the crypts in itself, of course, could not be expected to cause an increase or decrease in the demand for food since this is determined by many other factors. The net effect of all appetite-regulating mechanisms in normal adult animals and humans is adjustment of food intake to the point where caloric intake balances energy expenditure with the result that body weight is maintained (Ganong, 1977). Amongst the factors which are concerned in the regulation of food intake are the level of glucose utilization in the "satiety centres" of the brain, and this is a function of the arterio-venous blood glucose difference, the size of body fat depots, the environmental temperature, and the amount of distention of the gastrointestinal
tract. Some of these factors may also be involved in the regulation of crypt cell proliferation but the relationship between the control of food intake and crypt cell proliferation does not appear to be a close one.

It has been seen in the literature review that if the small intestine is implanted beneath the kidney capsule, where it is not subjected to any stress to absorb nutrients, the villi disappear leaving a flat mucosa (Rowinski et al., 1977). This raises the question whether the villi are only present when there is a stress to absorb nutrients, and when absorption is not occurring, they are removed. In other words, they may be present because there is a constant "stress" on the mucosa during life, and when there is a greater demand for absorption e.g. in the presence of increased luminal nutrients the villi respond by enlarging. Alternatively, when the luminal nutrient value falls, there is less stress on the mucosa with a decrease in villus size. The stimulus to development and the maintenance of villus architecture may thus possibly be the amount of nutrients absorbed. In coeliac disease, in which there is decreased absorption from the lumen, for various reasons, the villi are very underdeveloped whilst the crypts are often hyperactive. This concept would presuppose that the amount of absorption of nutrients was registered firstly, and the villus growth was subsequently monitored according to the amount of absorption which was occurring. This notion is difficult to reconcile with the feedback control theory of crypt cell mitotic activity which appears (Rijke, 1976) to presuppose that the villus height is an important factor in the control of crypt cell turnover. As stated previously, it seems likely that control of crypt cell proliferation is multifactorial, and different factors may be operative under different circumstances.
As observed previously, the presence of food in the lumen of the gut does not imply necessarily that all of this food is being absorbed, and the presence of food in the lumen may be viewed in different ways. Gleeson et al. (1972) saw it as important in maintaining the morphology, and therefore the functional efficiency of the mucosa, by local absorption of nutrients into the mucosa ("luminal nutrition"). Clarke (1975, 1977) viewed the presence of food in the lumen as inducing a functional demand on the intestine to produce a compensatory response to improve its absorptive capacity ("epithelial work load"). However, the presence of food in the lumen is not a straightforward situation, as indicated by Johnson (1979) (see Fig. 1.2 Chapt. 1, Section 11).

One of the most important stimuli to the growth of most tissues appears to be functional demand. If one kidney is removed, the other hypertrophies; if a muscle is exercised, it enlarges, so that it is often assumed that mucosal growth is mainly controlled by functional demand, although that is a little more difficult to define in comparison to other organs. Whilst this is an attractive principle around which the mechanisms of control of crypt cell proliferation could be shaped, it is not without its inconsistencies. Falkner and Tanner (1978) have noted some of these—,

(1) the organs of an embryo grow considerably before they become functional, yet their rates of growth are carefully controlled,

(2) in the adult organism, most organs are represented by several times more tissue than is required for their subsistence. This provides a margin for safety, in cases of emergency,

(3) when the physiological activities of an organ are
bypassed, the resultant disuse atrophy seldom leads to the complete disappearance of the tissue concerned. A minimal mass is preserved from which regrowth can occur if necessary. In this case structure has been preserved although there is no function. Hence, functional demand is not the complete answer to the problem of control of crypt cell proliferation, although it certainly appears to be important in it. Possibly the limbic system and the pineal have a role in containing excessive proliferative activity in response to increased functional demands.

Furthermore, it is difficult to define what the functional demand on the mucosa is. Is it the demand to absorb nutrients or in fact the ability to deal with changes in the osmolality of the luminal contents? Alternatively, is the stimulus to increased proliferation associated with the luminal contents the result of the mechanical stimulation of these contents? This could explain the differences between the crypt cell mitotic rate in orally and parenterally fed rats. For instance, Biber et al. (1971) found that intraluminal mechanical stimulation of the cat small intestine was associated with increased mucosal blood flow, which it has already been noted can affect crypt cell mitotic rate (Rijke, 1976). Thus, the differences in crypt cell mitotic rate associated with the presence or absence of luminal contents may be at least in part due to local changes in mucosal circulation due to mechanical stimulation. The local stimulatory effects themselves could be mediated via the vagus nerves, for instance.

Besides this effect, when there is an increased "work load" on the mucosa there is an increased demand for secretion of enzymes and absorption of nutrients. As noted previously, this is analogous with presenting an increased "work
load" to a skeletal muscle, with resultant hypertrophy. Just as the actively exercising muscle requires a greater blood flow, so there is a demand for a greater blood flow in the presence of greater luminal "work load". Thus, the changes in the blood supply to the mucosa cannot be separated from the other factors possibly operative in the control mechanism with any precision, and neurovascular changes may be operative in the control mechanism.

With regard to the effects of luminal osmolarity on the crypts, Skagen (1977) found that infusion of hypertonic saline (450mOsm./Kg.) into a catheter implanted into the upper jejunum of unrestrained rats after three days of starvation resulted in a sharp rise in the rate of incorporation of isotopically labelled Thymidine into D.N.A. in the crypts. The response was similar to that associated with peroral feeding. No changes occurred if isotonic sodium chloride or an isotonic amino acid/carbohydrate solution was used. He suggested that osmoregulatory mechanisms may be involved in evoking the proliferative response to refeeding in the small intestine. It should be borne in mind, of course, that the effect was noted after a period of starvation which may have induced changes in the mucosa itself, e.g. the effects of changes in osmolarity of the luminal contents may be relatively greater after a period of starvation than in the course of normal feeding, i.e. the mucosa may be more sensitive to such changes at this time. However, further support for the importance of the physical properties of the luminal contents as opposed to the nutrient value was given by the findings of Clarke (1976). He found that starving a rat with an isolated small intestinal sac for forty eight hours further diminished the crypt cell renewal rate in this loop, already diminished by its isolation.
However, infusion of distilled water into the isolated small intestinal sac for seventy two hours was associated with a similar degree of stimulation of cell production in the sac in fed as in starved animals. He suggested that the fall in crypt cell production rate in the isolated loop in a starving rat is not due to non-availability of the substances required for cell synthesis. Furthermore, Clarke (1977) found that infusion of sodium chloride into a surgically prepared rat upper small intestinal sac was associated with a small increase in crypt cell production locally (measured by the stathmokinetic technique) but a considerable increase in crypt cell production in the unfused gut in continuity. Since this effect was similar qualitatively to that obtained by infusion of isotonic glucose or galactose, he concluded that "luminal nutrition" was less important than "work load". These findings raise the question of whether the mucosa responds to the increased "work load" i.e. the amount of work necessary to deal with the particular stress applied to it e.g. osmotic, or to the amount of work necessary to absorb the nutrients in the lumen. In this connection, Dooley (1984) has demonstrated small intestinal osmoreceptors but their function is not certain. If osmolarity of the intestinal contents is important in control of crypt cell proliferation and osmoreceptors are present in the small intestine, it is necessary to postulate that changes in osmolarity of the intestinal contents do in fact occur from time to time, especially associated with eating. In this respect, Ganong (1979), amongst others, has stated that in normal man the post-prandial luminal contents entering the upper jejunum are iso-osmolar to plasma irrespective of the osmolarity of the ingested meal. However, Ladas et al. (1983) found that despite mixing with upper gastrointestinal secretions and
transepithelial movement of fluid and electrolytes, osmotic and electrolytic equilibration of intestinal contents with plasma is not produced at this level for up to two hours after a meal. Thus, it is conceivable that the osmolarity of the intestinal contents could play some role in crypt cell proliferation control. If this were possible, a possible link with the sympathetic nervous system is conceivable, since Sjovall (1984) found that sympathetic splanchnic nerve stimulation led to an increase in net fluid absorption in the gut, provided that glucose was present in the jejunal lumen. Studies using stimulation or vagotomy on the influence of the vagus nerves on fluid and electrolyte transport in the intestine have given inconsistent results (Cooke, 1986). The effects resulting from splanchnic nerve stimulation are believed to be due to stimulation of mechanoreceptors (Cooke, 1986) but very little is known about the central nervous system processing and integration of afferent input from these receptors.

The effect of changes in luminal osmolarity affecting crypt cell proliferation may be seen in another way. Goodlad et al. (1987) found that in rats water absorption capacity of the small intestine, as measured by the segmented flow single pass perfusion method, could be correlated significantly with changes in crypt cell proliferation rate. Taking the amount of water absorption as a reliable indicator of functional capacity, they concluded that in "steady state" models of hypoplasia and hyperplasia, cell production is closely linked to functional capacity and both of these are also related to the food intake. In the present experiments, whilst information was obtained concerning food intake, none was obtained regarding fluid intake, so that this might be an important additional measurement to be made in future experiments on crypt cell
mitotic rate. However, it seems likely that if fluids were supplied ad libitum, as food intake increased so also would fluid intake, although the relationship between the two may not be always a constant one.

Another possibility to be considered in the local effects of the luminal contents is the varying amount of surface cell shedding of the luminal enterocytes, which presumably would depend upon the physical properties of the luminal contents to some extent. According to Galjaard et al. (1972) the rate of cell shedding would itself affect the rate of crypt cell proliferation. Increased surface luminal shedding would presumably be associated with oral intake of food as opposed to total parenteral feeding, and would be expected to be associated with a greater rate of crypt cell proliferation. However, whilst this appears to be a reasonable explanation for this difference in crypt cell mitotic rate between the two routes of feeding, Clarke (1975) found that his experimental findings did not support the hypothesis that increased cell shedding from the tips of the villi stimulated increased cell production in the small bowel crypts.

The presence or absence of luminal food and/or pancreatic-biliary secretions may have some significance, however, in determining the growth and production of the villi (as suggested above). Rowinski and Kaminski (1973) and Rowinski et al. (1977) found that in implanted fragments of mouse small intestine located under the kidney capsule of syngenic mice, there were no intestinal villi but crypts persisted and epithelial cells could proliferate up to thirty days after transplantation. These epithelial cysts were devoid of direct contact with the usual luminal contents. Additionally, there was not the usual innervation and the blood circulation
within the transplant was probably different from that in its usual site. It is perhaps significant that the duration of the cell cycle in these transplants of adult intestine was found to be similar to that found by Zinzar et al. (1973) for three month old transplants of foetal mouse small intestine. Quite possibly, the luminal contents play a part in the development of the villi initially but this does not necessarily mean that they continue to be active in its regulation. As noted in Chapt. 1, Section 11, in coeliac disease it is possible to have small villi with quite active proliferation in the crypts.

The findings of Rowinski and Kaminski (1973), mentioned above, in which the isolated intestine was deprived of its nerve supply but remained vascularized are consistent with the operation, at least to some extent, of humoral factors in the control of crypt cell proliferation, an observation corroborated by the findings of other researchers (see Chapt. 1, Section 11).

Clarke (1976) suggested, on review of the possible roles of growth hormone, cortisol, and glucagon as blood-borne effectors in the control mechanism, that in view of the demonstrated effects of the autonomic nervous system on crypt cell mitotic rate (Silen et al. (1966); Dupont, Biggers and Sprinz (1965); Tutton and Helme (1973, 1974)) circulating adrenaline was a possible systemic effector in the control mechanism. Thus, it is possible that the autonomic nervous system may play some part in the control of crypt cell proliferation via humoral as well as direct neural pathways. There is morphological evidence that intestinal crypts receive an abundant supply of adrenergic nerve fibres (Gabella and Costa, 1968 a). Whitfield (1980) states that catecholamines bind to specific receptors in the cell membrane from which they directly or indirectly stimulate the synthesis and/or release of intracellular
mediators such as calcium ions and cyclic nucleotides (cyclic adenosine 3'-5' monophosphate, cyclic guanosine 3'-5' monophosphate, see Chapt. 1, Section 11) which are known to affect cell proliferation, amongst other effects. Whilst it follows from this that the catecholamines should be physiologically important modulators of cell proliferation, this has not been proven (Whitfield, 1980).

With regard to the effects of prostaglandin analogues on the crypts (see Chapt. 1, Section 11) 16,16- dimethyl PGF$_{2\alpha}$ has been shown to act, in some cases at least, by raising intracellular levels of cyclic guanosine monophosphate (cGMP) (Kuehl et al., 1973). Three other agents that have been shown to stimulate the formation of cGMP i.e. noradrenaline (Schultz et al., 1975), acetylcholine (Goldberg et al., 1973), and serotonin (Goldberg et al., 1974), as well as dibutyryl cGMP itself, have been shown to accelerate jejunal crypt cell proliferation (Tutton and Helme (1974); Tutton (1974, 1977); Tutton and Barkla (1980 a)). Thus, the complexity of possible mechanisms of control is highlighted. Clarke (1974), however, in a review of the topic strongly argued against blood-borne tissue specific stimulants of crypt cell production, although other researchers have supported the existence of such agents. Whilst there appears to be some difference of opinion regarding the role of blood-borne stimulants of crypt cell mitotic activity, there seems to be some evidence that some hormones e.g. epidermal growth factor may play a role in the development of the suckling rat colon via an enteral route. Whilst the presence of hormones and other substances in the breast milk of many species is well documented (Koldovsky, 1980) and it has also been demonstrated that a number of these compounds are absorbed via the gastrointestinal tract both in intact form or
as altered metabolites. Pollack et al. (1987) concluded experimentally that breast milk epidermal growth factor (see Chapt. 1, Section II) has a physiologic role in the development of the rat suckling colon.

Thus, it appears that whilst luminal factors are probably the most important in the control of crypt cell mitotic rate, some role must be given to the autonomic nervous system, either acting as a means of interpreting the status, nutrient or otherwise, of the luminal contents and/or acting in an efferent manner on the crypts, either via direct neural connections or via humoral mechanisms, e.g. adrenaline, but obviously the autonomic nervous system is not the only mechanism involved in this control.

It has already been noted that the neuropeptides may affect the rate of crypt cell proliferation and changes in the levels of these substances have been associated with increased crypt cell proliferation due to other causes (see Chapt. 1, Section II). It is possible that these neuropeptides are functionally part of the central nervous system effects on the crypt cell proliferation rate in some as yet undetermined manner. A sizeable proportion of autonomic fibres are now considered to be peptidergic, rather than adrenergic or cholinergic, as previously noted by Gershon and Erde (1981), Polak and Bloom (1979). A number of these peptides have been found to be present in both brain and peripheral tissues (Grossman, 1979). In the periphery peptides are found in both nerve fibres and in endocrine cells. The diversity of their actions suggests that they may act differently depending on their location. It has been suggested that many of these peptides function as both gastrointestinal hormones and as neurotransmitters in the C.N.S. and peripheral nervous system (Loonen and Soudign, 1979).
Many of these peptides were originally discovered in the brain and subsequently found in the gastrointestinal tract of several mammalian species e.g. somatostatin (Arimura et al., 1975, Costa et al., 1975). Conversely, certain peptides were first isolated in the gastrointestinal tract and were later shown to be present in the brain e.g. cholecystokinin CCK-8 (Dockray, 1976), gastrin (Rehfeld, 1977, Uvnas-Wallensten, 1977).

The importance of the autonomic nervous system in the control of crypt cell proliferation has been suggested strongly by the present series of experiments. It may well be that the autonomic nervous system plays an important role in the hyper-proliferative effects on the crypts associated with small bowel resection, which has been studied so extensively in an attempt to elucidate the mechanism of crypt cell control. For instance, as noted in Chapt. 1, Section 11 Touloukian et al. (1972a) have shown that there are changes in the adrenergic innervation of the gut after resection, and Tutton and Helme (1974) have demonstrated that adrenergic stimuli effect changes in crypt cell activity. Thus, adrenergic or neurovascular factors may possibly participate in mediating the response of the crypts to intestinal resection. Furthermore, as noted previously, Young et al. (1977c) found that oral feeding was associated with increased sympathetic activity (note the difference in mitotic rate between orally and parenterally fed rats).

Thus, neural control of crypt cell proliferation appears to be well established as being involved in the regulation of crypt cell proliferation. This is clearly established in the light of previous published results and the findings of the present study. It seems likely that the autonomic nervous system serves to assimilate information regarding the
characteristics, physical or otherwise, of the luminal contents, and to integrate this information, perhaps with other sensory information, at various levels of the central nervous system. It is suggested that the effects on the crypts themselves, even when they are the result of changing luminal environment, may be mediated at least in part by the effector part of the autonomic nervous system, although as noted in literature review, the action of blood-borne (probably not tissue specific) agents cannot be ignored, amongst which may be adrenaline. It is suggested by the experimental results that this well integrated mechanism of control may be over-ridden on some occasions by a suppressant effect on the crypt cell mitotic rate and that this influence is transmitted by the vagus nerves and sympathetics to the gut and originates in the higher centres of the central nervous system, in particular the pineal gland and the limbic system, which appear to act as a functional unit. The full significance of this latter mechanism has not as yet been adequately elucidated.
CHAPTER VI

APPENDIX
Details of materials used in the experiments.

(1) Fur was shaven with an electric razor and cleansed with absolute alcohol.

(2) A 2% solution of Xylocaine (without adrenaline, Astra Pharmaceuticals) was used to produce local anaesthesia.

(3) For induction of general anaesthesia in the rats a solution of Pentobarbitone Sodium ("Nembutal"), 60 mgm. / ml. (T. M. Abbott Laboratories, distributed by CEVA Chemicals, Australia, Pty., Ltd., N. S. W.) was diluted 10 times with 0.9% Sodium Chloride Injection, B.P. ("Normal Saline", Soluvac, by Travenol Laboratories Pty, Ltd, Australia, D 4765).

(4) For killing the rats Anaesthetic Ether, B.P. by Hoechst, Australia, Ltd, Melbourne, Vic, 475 ml. bottle was used.

(5) For injections, Terumo hypodermic needles were used, usually 19 G x 1 1/2", or 25 G x 5/8".

(6) For most wounds, Black Silk sutures were used, 5 x 0 Metric 1, 16 mm conventional cutting - eyeless sutures - 75 cms. no. W - 500 H, and for ligating the Superior Sagittal Sinus, 6 x 0, Metric 0.7, 13 mm, cutting C-2, 30 cms., eyeless needle sutures made by Ethnor, Pty, Ltd, Sydney, Australia.

(7) For incisions Swann-Morton carbon steel sterile surgical blades sizes 12 and 11 were generally used.

(8) For closure of wounds, besides the black silk sutures, in the case of abdominal wounds, Justrite,
wound clips, no. 3. 2335 / A , (7654), 14 mm. by Clay Adams , Parsippany, N.J. U.S.A. were used, and for the closure of scalp wounds Totco Autoclips , 9 mm., no. 7631, by the same manufacturer.

(9) Bleeding from the skull margins was controlled by Ethicon bone wax (sterile) by Ethicon, Ltd.

(10) Bleeding from cerebral tissue was controlled by Gelfoam - 7 mm. absorbable gelatin sponge, U.S.P., The Upjohn Co. Kalamazoo, Michigan, 49001, U.S.A.

(11) Post-operatively, Aquacaine L/A Suspension, Benzathine Penicillin G with Procaine Penicillin G injection was used (each ml. contains 225 mg, 300,000 Units) of Benzathine Penicillin G and 150 Mg. (150,000 Units) of Procaine Penicillin G with Sodium Citrate, Carboxymethylcellulose and Lecithin as buffering and suspending agents. The suspension contains 0.002 % w/v Phenyl-mercuric nitrate as an antiseptic. The dosage appropriate to the size of the rat was used.

(12) Colchicine powder was used and this was dissolved in Normal Saline, as above, taking care to preserve sterility, to make a solution of 1 mgm. / ml. This solution was refrigerated whilst not in use and discarded after 7 days.

(13) Surgical instruments were sterilized in a solution of Solyptol (Faulding).

(14) Specimens of gastric contents for examination of pH were suspended in deionized water, with which the pH meter had been calibrated, and stirred vigorously using an electrical stirrer made by
Townson Scientific Equipment Manufacturers, Pty., Ltd.,
Cat. no. 211, 240 V, 50 Hz., distributed by
Townson and Mercer, Pty., Ltd.

(15) A T.P.S. Digital pH Meter was used for pH
measurements and double deionized water was used
as a standard to calibrate this.

(16) For stereotactic procedures a stereotactic
apparatus made by David Kopf Instruments, California, U.S.A. was used.

(17) For aspiration lesions of the brain, a water
pump, manufactured by Kartell (no. 1395) was
used. This provided low pressure suction when
connected to a tap in the laboratory.

(18) Electrodes for the amygdaloid lesions were made
from 25 gauge stainless steel needles. It was found
in a pilot study that a very satisfactory durable
coating for the electrode providing good insulation
could be provided by repeatedly coating the electrode,
after thorough cleansing with alcohol, with "Avon"
Super Strengthener for Nails. This provided a simple
and very effective cover which was not only insulat-
ing but water resistant. The insulating cover was
cleared for approximately 0.5 mm at the tip for
lesion making.

(19) The efficiency of this insulating cover was
tested after each lesion by the method described by
Hart (1976). A stainless steel needle was connected
with the positive terminal of a 9 Volt battery and
inserted into a beaker containing normal saline. The
electrode to be tested was connected to the negative
terminal of the same battery and inserted into the same solution. If the insulating cover of the electrode was intact, gas bubbles appeared only at the tip of the electrode, otherwise bubbles appeared at the site where insulation of the electrode was deficient along its length.

(20) For making the lesions, a Grass L.M. 4 Lesion Maker was used, Manufactured by Grass Medical Instruments, Quincy, Mass. U.S.A.

(21) The amount of food eaten by the rats was measured with Soehnle scales.

(22) Sections of the brain for photomicroscopy were cut using a Lancer Vibratome, Series 1000, which allowed sectioning to be done in a fluid bath by a vibrating blade, without freezing or embedding (manufactured by Lancer, U.S.A.).
Histological Stains used in the preparation of tissues

Haemotoxylin and Eosin Method
(1) Blocks were fixed in Bouins solution and embedded in Paraffin wax.
(2) Paraffin sections were then cut, mounted on glass slides and dried.
(3) The slides were dewaxed in xylene and hydrated in descending strengths of alcohol to distilled water.
(4) They were stained in haemotoxylin for two minutes, rinsed in running tap water and "blued" in ½ % ammonia for 30 seconds, and rinsed again.
(5) The slides were then stained in 1 % eosin Y for 30 seconds.
(6) They were dehydrated in absolute alcohol, rinsed in xylene and mounted.

Neutral Red Method
(1) 50 μm. sections were immersed in -
(a) 70 % alcohol overnight,
(b) 96 % alcohol for 2 mins,
(c) absolute alcohol for 10 mins,
(d) 96 % alcohol for 2 mins,
(e) 70 % alcohol for 2 mins,
(f) distilled water for 2 mins,
(2) the neutral red stain, 1 Gm. of neutral red in 500 ml. of water with 2 ml. of glacial acetic acid, was applied for 20-30 mins,
(3) sections were washed in distilled water,
(4) sections were immersed in -
(a) 96% alcohol, length of time varied from 1-2 minutes depending on how darkly the sections were stained,

(b) absolute alcohol for 2 mins. only

(c) a 50/50 mixture of absolute alcohol and xylol until the fibres in the section appeared white.

(4) sections were then mounted on slides coated with gel gum.

Method for Toluidine Blue stain used for pineals.

(1) Sections embedded in paraffin were dewaxed and brought to distilled water,

(2) The sections were stained in 1% Toluidine Blue for approximately 5 minutes,

(3) They were differentiated in 95% alcohol,

(4) The sections were then dehydrated, cleared and mounted in P. I. X.


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