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STUDIES ON SOME CARDIOVASCULAR DRUGS IN MAN.

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by

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DECLARATION AND ACKNOWLEDGEMENTS

I declare that this thesis is of my own composition and records original work conducted during the years 1960 - 1964 mainly in the Department of Human Physiology and Pharmacology, University of Adelaide. The work in Part 1 of the thesis was undertaken while holding the position of Medical Registrar in the Cardiac Clinic, Royal Adelaide Hospital.

I would like to record my gratitude to Dr. C. J. Cooper, Dr. J. D. Fewings, Dr. G. C. Scroop and Dr. S. L. Skinner with whom I have had the privilege of working during various stages of these investigations, and especially to Professor R. F. Whelan and Dr. I. S. de la Lande for guidance, invaluable discussion and active participation in many of the experiments.

I would also like to thank Miss C. Applebee, Mrs. M. Contos, Mr. C. Jennings and Mr. A. McNeil for expert technical assistance, and those colleagues, students and patients who volunteered for the investigations.

Finally, I am grateful to the National Heart Foundation of Australia for the Research Fellowship granted to me from January, 1963 - March, 1964.

PREFACE

PREFACE

The present series of studies arose as the result of a clinical trial of a drug for the treatment of hypertension. During this trial it became obvious that tolerance to the drug developed in the majority of patients. Although explanations for the phenomenon of tolerance had been suggested on the basis of animal work, very few investigations on humans had been performed.

The original hypotensive drug and several others were therefore studied on humans in the laboratory not only from the point of view of tolerance, but also to determine their effects on blood vessels of the upper limb. In addition to these acute studies the vascular responses of a number of patients on chronic oral hypotensive drug therapy have been followed, as have those of a patient with phaeochromocytoma before and after operation. An investigation of the effects of noradrenaline on the human forearm is included in view of the importance of this subject in the interpretation of blood flow measurements in that segment, since the response of forearm vessels to noradrenaline is frequently used as a measure of vascular sensitivity.

All the studies reported in this thesis have been made on humans. Experiments with human subjects pose a number of special problems, including ethical considerations, technical difficulties with conscious subjects, relatively small numbers, and the small drug dosages that can be employed. In spite of these difficulties and limitations, it is very important that, where possible, a step of what

may be called human pharmacology should be interposed between pharmacological studies on animals and the administration of drugs to patients in clinical trials.

PART 1.

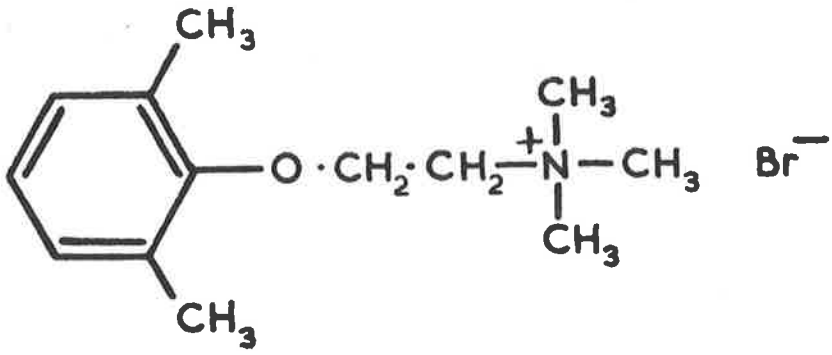
THE PROBLEM OF DRUG TOLERANCE IN HYPOTENSIVE THERAPY.

A clinical study of bretylium tosylate in hypertensive patients.

INTRODUCTION

The development of the post-ganglionic adrenergic-blocking agent bretylium tosylate was a major advance in the hypotensive drug field since it allowed a more specific attack on the raised peripheral resistance which is one important feature of hypertension. This increase in specificity obviated the serious side-effects resulting from parasympathetic blockade seen with the ganglion-blocking drugs previously used.

An earlier drug, choline 2,6-xylyl ether bromide (TM10) (Fig. 1), synthesized by Hey in Leeds in 1951, was the first substance whose major action was to prevent the release of noradrenaline from sympathetic nerve endings resulting from post-ganglionic adrenergic nerve stimulation (Bain, 1960). TM10 was synthesized as one of a group of "onium" compounds in an investigation of nicotine-like stimulant activity (Hey, 1952), and later Hey & Willey (1954) examined some of its pharmacological properties in animals, special attention being devoted to its local anaesthetic action. The sympathetic-blocking action was investigated more directly by Exley (1956, 1957). He found that in cats the effects of adrenergic nerve stimulation were abolished by TM10 without interfering with the responses of effector organs to adrenaline. The output of catecholamines from the stimulated adrenals was unimpaired. After examination of other closely related ethers in which local anaesthetic action was strong, but the sympathetic-blocking action was absent, he concluded that these two properties were quite separate. The adrenergic blockade was considered to be due to 'interference in some

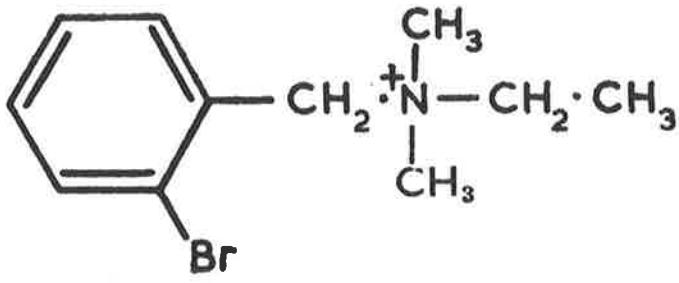


TM 10

Fig. 1.

other way with the release or synthesis, or both, of the adrenergic mediators'. Bain & Fielden (1957) on the basis of incubation studies on human chromaffin-cell tumor postulated that the adrenergic-blocking action of TM10 was due to an interference with the biosynthesis of noradrenaline from dopamine.

The parasympathomimetic side-effect of TM10 precluded its use in clinical practice, but its unique sympathetic-blocking action stimulated the search for other drugs with this property. Boura, Copp & Green (1959b) found that a series of benzyl quaternary ammonium compounds had such an action, and that, with one exception, the members were free of serious parasympathomimetic action. One of these was bretylium tosylate (Fig. 2). Although also a quaternary ammonium compound, TM10 is not otherwise closely related chemically to bretylium. The pharmacological properties of bretylium in animals have been reported by Boura, Green, McCoubrey, Laurence, Moulton & Rosenheim (1959a); Boura et al. (1959b); Boura & Green (1959); Duncombe & McCoubrey (1960); Boura, Copp, Duncombe, Green & McCoubrey (1960), Vernikos-Danellis & Zaimis (1960), and Boura & Green (1962). Probably the first human pharmacological investigations of bretylium were those carried out by Dornhorst (cited by Boura et al., 1959a), in which three subjects received intra-arterial infusions of the drug, and others received intravenous infusions. The predominant action of the drug, confirmed by animal and human pharmacological studies, is to cause post-ganglionic adrenergic neurone blockade. Initially it may have a sympathomimetic effect and may cause a transitory depression of ganglionic transmission. Catecholamine stores, including the adrenal



BRETYLIUM

Fig. 2.

medulla are little affected.

The first clinical report on bretylium was that by Boura et al.(1959a) who gave the drug orally to 36 patients for periods of 1 to 26 weeks. They found that tolerance was not a serious problem. The drug satisfactorily lowered the blood pressure of almost all of their patients, and side effects were few. The experience of Dollery, Emslie-Smith & McMichael (1960) with 50 patients over 6 months was less impressive, owing partly perhaps to a higher proportion of more severe hypertensive patients. Although some tolerance was observed, most patients required only moderate increases in dosage to maintain blood pressure control. Hayden & Boake (1960) and Mackie (1960) reported small clinical trials and observed the need for progressive dose increase in some patients. These workers did not regard drug tolerance as a serious threat to the efficacy of long-term therapy.

Our own experience in a 6 months' trial on a small group of mild to moderate hypertensive patients was far less reassuring, in that we found tolerance a serious problem. Six out of 9 patients finally required from 3 to 10 times the originally effective dose of bretylium tosylate to maintain comparable, or even less effective, blood pressure control. It is to illustrate this feature particularly that the following results are presented.

METHODS

Nine patients were studied, 6 of whom had received hypotensive therapy previously; 4 were males and 5 females, with ages

ranging from 41 to 60 years. The period of follow-up investigation for these patients varied from 16 to 25 weeks. The relevant clinical and laboratory findings are summarised in Table 1.

The minimum acceptable criteria for the trial were a blood pressure exceeding 100 mm Hg diastolic, or 190 mm Hg systolic, associated with Grade II retinal changes on the Keith-Wagener scale or electrocardiographic evidence of left ventricular hypertrophy or "strain" (McPhie, 1958), or radiologically demonstrable left ventricular enlargement. The bretylium tosylate used was in the form of 200 mg compressed scored tablets for oral administration.

On a patient's admission to the trial, treatment with chlorothiazide (0.5 g/day) was commenced, and this dosage was continued throughout the trial. All other therapy except digitalis was stopped. After approximately two weeks the patient was brought into hospital for the recording of his resting blood pressure. Readings were taken at least four times daily in lying and standing positions. The "resting B.P." (Table I) represents the lowest pressure recorded during three days of bed rest which were allowed to elapse before bretylium tosylate therapy was initiated. One patient was not admitted for stabilization, but a long series of consistently raised blood pressure readings over several years was available.

Bretylium tosylate was administered initially in doses of 100 mg three times a day approximately half-an-hour before food. Daily dosage increments of 100 to 300 mg were added as required. Patients were ambulant at this stage.

TABLE I.

Clinical and Laboratory Data of the Nine Patients Studied in the Trial.

Patient.	Age. (Years.)	Sex.	Previous Treatment and Complications.	Presenting Symptoms.	Blood Pressure. (Mm. of Mercury.)		Changes in Fundi.	Apex Beat.	Congestive Cardiac Failure.	Urine.	Intra-venous Pyelogram.	Blood Urea Nitrogen Content. (Mg. per 100 ml.)	Electrocardiogram.
					Casual.	Resting.							
R.	42	M.	"Mevasine", constipation, impotence.	Increasing dyspnœa, orthopnœa.	200/160	160/120	Grade I.	Anterior axillary line.	Œdema; jugular venous pressure 3". Liver enlarged to 4 fingers' breadth; Crepitations at lung bases.	Trace albumin. Hyaline casts.	Normal.	23	Ischæmia. Infarction.
H.	60	M.	No previous treatment.	Severe dyspnœa.	240/150	180/120	Grade II.	Mid-clavicular line.	Mild œdema. Liver enlarged to 3 fingers' breadth. Crepitations at both lung bases.	Casts.	Normal.	17	Left ventricular hypertrophy and ischæmia.
J.	54	M.	"Serpasil", "Mevasine"; constipation.	1955: Thrombosis of spinal artery: Brown - Séquard syndrome. No symptoms at present.	230/135	170/115	Grade I.	Anterior axillary line.	Mild œdema only.	Normal.	Normal.	20	Mild ischæmic damage.
W.	60	F.	"Mevasine", "Chlotride"; severe constipation.	"Blackout" lasting 2 to 3 minutes.	250/120	250/110	Grade II.	Mid-clavicular line.	Nil.	Normal.	Normal.	—	Mild ischæmia.
M.	55	F.	"Mevasine"; depression, constipation.	Headaches, palpitations, mild dyspnœa.	200/140	180/120	Grade II.	Mid-clavicular line.	Nil.	Normal.	Normal.	14	Normal.
G.	48	F.	"Mevasine", "Chlotride".	Headaches, dyspnœa, visual blurring.	230/130	210/120	Grade II.	Mid-clavicular line.	Nil.	Normal.	Normal.	12	Mild ischæmia.
E.	46	F.	"Mevasine", "Serpasil" constipation.	Dyspnœa, orthopnœa, congestive cardiac failure in 1957.	220/150	220/130	Grade II.	Mid-clavicular line.	Mild œdema; jugular venous pressure 1". Liver enlarged to 1 finger's breadth. Crepitations bases of both lungs.	Casts. Leucocytes. Sterile.	Normal.	15	Left ventricular hypertrophy.
F.	55	M.	No previous treatment.	Hypertension found on routine examination.	180/120	Not admitted to hospital.	Grade II.	Mid-clavicular line.	Nil.	Trace of albumin.	Normal.	—	Left ventricular hypertrophy.
V.	41	F.	No previous treatment.	Mild dyspnœa, nocturia.	195/110	160/100	Grade I.	Mid-clavicular line.	Nil.	Leucocytes. Sterile.	Normal.	12	Left ventricular hypertrophy.

The blood pressure was recorded two hours after each dose of bretylium tosylate during the in-patient period. When the blood pressure was satisfactorily controlled the patient was discharged, and subsequently followed up as an out-patient at the Cardiac Clinic. Patients attended at least one morning and one afternoon each week, when the blood pressure was taken hourly over three hours. In Figs. 3 to 6, the expression mean standing blood pressure = (systolic + 2 x diastolic) / 3 is used.

RESULTS

During the early phase of bretylium tosylate administration, a material fall in mean standing blood pressure was obtained with all but one patient. This fall, occurring over several successive days, reached between 15 and 20 mm in two patients, between 20 and 25 mm in two others and exceeded 30 mm in the remaining four. It was in the longer-term maintenance and in stabilization of dosage that difficulties became increasingly apparent.

Fig. 3 illustrates the one patient (R) in whom, after an initial limited hypotensive response and progressive dosage increments, no satisfactory control of blood pressure was achieved. The case was regarded as a failure of treatment, and bretylium tosylate was abandoned. Six patients fell into a second group, represented in Fig. 4 by patient H., in which a progressive increase in dosage was necessary to maintain blood pressure control. Fig. 5 shows the individual details of the gradual threefold to tenfold increases required over 5 to 6 months in

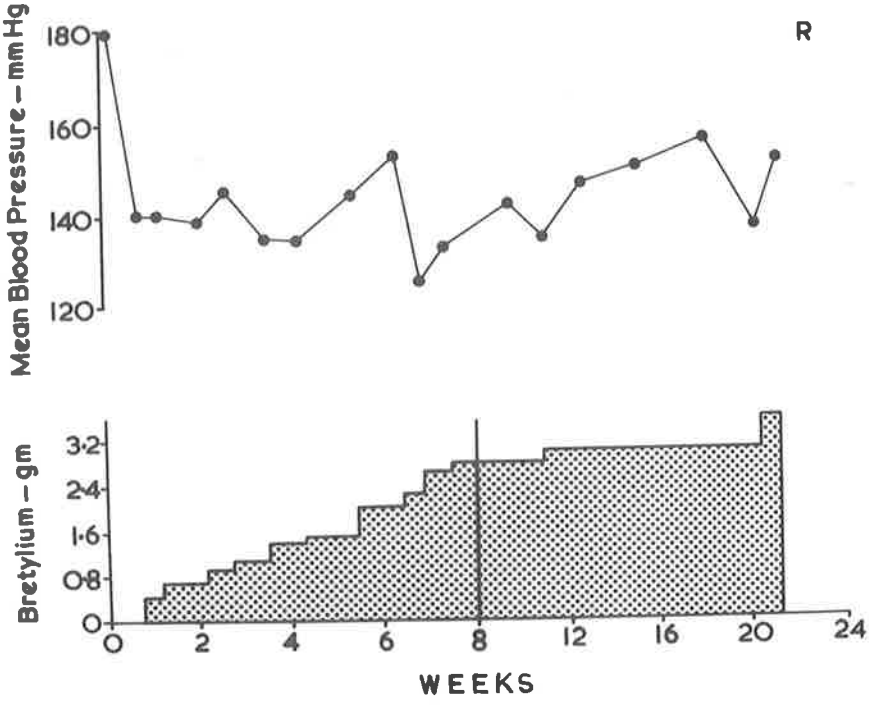


Fig. 3.

Drug dosage and mean blood pressure of the patient R.; the failure of treatment.

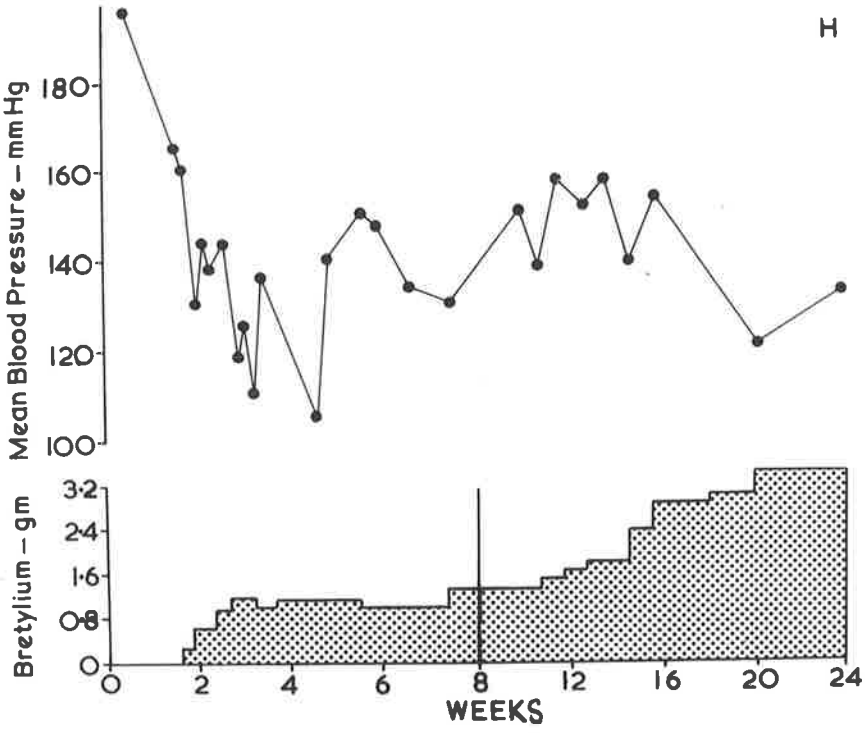


Fig. 4.

Drug dosage and mean blood pressure in one of the six patients developing tolerance (patient H).

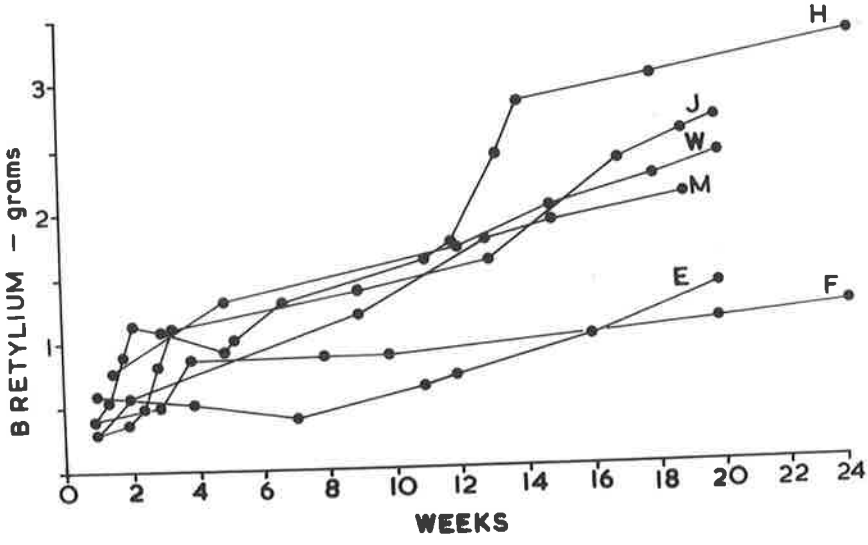


Fig. 5.

The rise of drug dosage in the six patients who developed tolerance.

this group of six patients. Satisfactory control was maintained with a fairly constant dose in two patients. One of these (V) is illustrated in Fig. 6. Table II summarizes the results and the drug side-effects which were seen. As in other reports, serious side effects were relatively few. One patient (R), while taking a dose of 3.6 g/day (the highest in this series), suffered no side effects; some nasal congestion present at the commencement of therapy being only temporary. Two patients complained of parotid pain, so severe in one (W) that therapy had to be stopped. Other complaints were minor, and not ascribable with certainty to the drug.

DISCUSSION

Experience with bretylium tosylate was limited at the time these patients were studied (Hodge & McPhie, 1960), but all reported trials had demonstrated considerable individual variation in sensitivity to the drug and in its absorption. Although mention had been made of tolerance in some patients, it was not considered a serious objection to the drug.

With the exception of one (R), all the patients in the present study showed a satisfactory initial response, and consequently first impressions were very favourable. However the subsequent development of tolerance in six patients became progressively more disturbing. The conclusion was that it constituted a serious practical objection to long-term therapy, particularly in view of the high cost of the large doses found necessary, and the frequent medical attendance required over a prolonged period. Furthermore, it was not

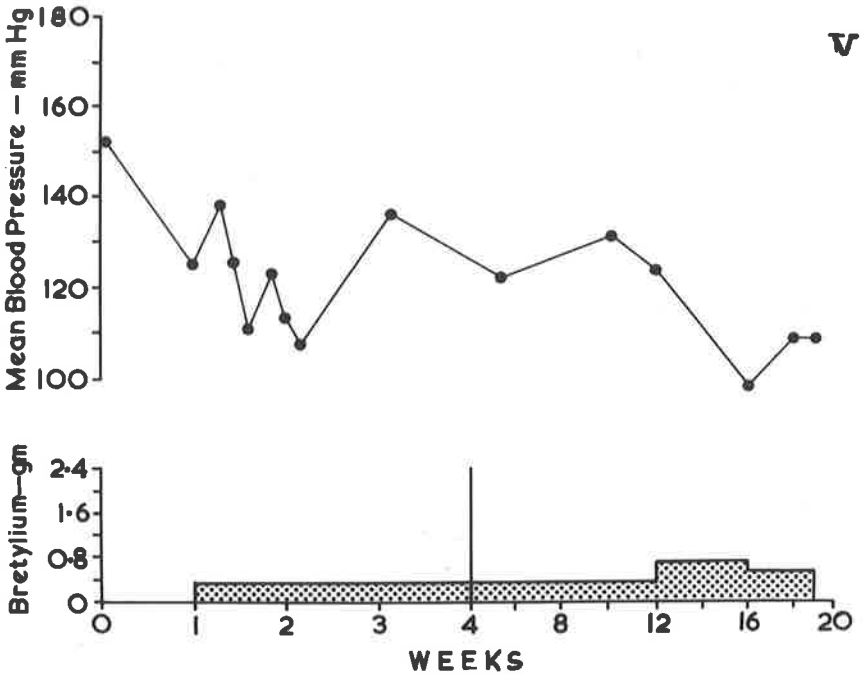


Fig. 6.

Drug dosage and mean blood pressure in patient V., one of the two satisfactorily controlled by the drug.

Results of Treatment, Period of Follow-up Investigation, and Side Effects of the Drug.

Patient.	Present Dosage. (Grammes per Day.)	Period of Follow-up. (Weeks.)	Comment.	Side Effects of Drug or Symptoms During Follow-up.
R.	3.6	21	Bretylium discontinued. Rapidly rising dose with unsatisfactory control of blood pressure. Failure of treatment.	Stuffy nose. Regained potency. Otherwise very well.
H.	3.4	24	Tolerance increasing.	Nil.
J.	2.7	20	Tolerance increasing.	Nil.
W.	2.4	20	Tolerance was increasing. Discontinued at 20 weeks because of parotid pain.	Fatigue. Parotid pain, becoming severe.
M.	2.1	19	Tolerance increasing.	Fatigue. Heaviness in chest. Very unstable personality.
G.	0.9	16	Satisfactory control.	Nil. Short follow-up.
E.	1.0	24	Tolerance increasing.	Muscle and parotid pain. Some deterioration of vision.
F.	1.2	25	Tolerance increasing slowly.	Fatigue.
V.	0.5	19	Satisfactory control. Stable.	Nil.

possible to predict, even in retrospect, whether an individual patient would acquire tolerance, or whether a stable dose would finally be attained.

This study was one of the first in which tolerance of such a severe degree was reported. Subsequent workers have confirmed this finding (Lowther & Turner, 1960; Evanson & Sears, 1960; Somers, 1962). Currently bretylium, although of great interest pharmacologically, is seldom used in the treatment of hypertension because of the phenomenon of drug tolerance (Wolf, Mendlowitz, Naftchi & Gitlow, 1963).

SUMMARY

1. Nine mildly to moderately hypertensive patients have been treated with bretylium tosylate and a fixed dose of chlorothiazide.

2. In one case blood pressure control was not achieved despite an ultimate daily dose of 3.6 g.

3. In six cases adequate control was attained in the face of steadily increasing drug tolerance. Ine one of these treatment was terminated after 20 weeks because of severe parotid pain.

4. Two patients have been satisfactorily treated with a steady dose.

PART 2.

THE ROLE OF VASCULAR HYPERSENSITIVITY IN TOLERANCE.

Sections A; B (i), (ii) & (iii); C; D.

PART 2.

Section A.

The effects of noradrenaline on skin and muscle vessels in the forearm, and the assessment of sensitivity changes.

INTRODUCTION

The response of the forearm blood flow to noradrenaline has often been used in the assessment of changes in vascular sensitivity in man. There are, however, conflicting reports on the effects of this agent on the forearm vessels. During intravenous infusions, Duncanson, Stewart & Edholm (1949) described a decrease in forearm flow as did Barnett, Blacket, Depoorter, Sanderson & Wilson (1950) while Barcroft & Konzett (1949) noted an increase which they interpreted as a passive response to the rise in blood pressure. Barcroft, Gaskell, Shepherd & Whelan (1954) also described an increase in forearm flow which they attributed to a reflex vasodilatation in muscle.

In the case of adrenaline the response of the total forearm flow is the resultant of the differential action of this drug on skin and muscle vessels (Skinner & Whelan, 1962) and the level of skin flow has a marked influence on the pattern of the total response (Whelan & de la Lande, 1963). Little direct information is available on the differential actions of noradrenaline on the vascular beds which comprise the forearm circulation. The present study was designed to analyse the effects of this drug on the two major vascular beds in the forearm, namely those of skin and muscle, and particularly to assess the role of the skin circulation in modifying total forearm blood flow responses to this amine.

METHODS

The subjects, who were ourselves and volunteer medical students, lay supine on a couch in a temperature-controlled laboratory for at least

30 min before observations began.

In order to study the contribution of the skin circulation to toal forearm blood flow changes some of the subjects were heated or cooled since it has been shown that the changes in total forearm blood flow during such procedures are confined to skin (Roddie, Shepherd & Whelan, 1956). Cooling was achieved by having the subject lie semi-clothed at rest in a room at 18°C with fans directed on the exposed trunk. Heating was carried out by raising the room temperature to 30°C and covering the subject with blankets. In all other experiments the room temperature was maintained at 24 - 25°C.

Muscle venous samples were obtained from a nylon catheter ("Intracath", Bardic) inserted via an 18-gauge needle into a deep muscle branch of an antecubital vein. Skin venous samples were similarly obtained from one of the superficial veins near the cubital fossa. The catheters were kept patent by occasional injections of a small amount of heparinized saline.

Intra-arterial infusions of noradrenaline were administered through a 23-gauge short-bevel needle inserted into the brachial artery of the catheterized forearm and connected by a length of polythene tubing to a constant infusion apparatus delivering 4 ml./min. During the control periods normal saline (0.9% w/v) was administered and was used as a vehicle for the drugs. In other subjects intravenous infusions were given into an antecubital vein of the opposite forearm.

Blood flow through one or both forearms was measured using water-filled, temperature-controlled plethysmographs (see Appendix 1). During each recording period the hand circulation was arrested by a

pneumatic wrist cuff inflated to 200 mm Hg.

In the experiments using intra-arterial infusions, the venous oxygen saturation was not measured simultaneously with the blood flow since the collecting-cuff pressure during flow measurements may divert skin venous blood into the muscle bed (Roddie et al., 1956). Two consecutive 5 or 10 min infusions were given between which at least 30 min were allowed to elapse, one response being measured during each infusion. During intravenous infusions blood flow and venous oxygen saturations were measured simultaneously in opposite forearms.

Blood flow measurements were made 3 to 5 times per min and blood samples taken at frequent intervals before, during and after the infusion period. Venous oxygen saturations were determined spectrophotometrically (Roddie, Shepherd & Whelan, 1957a).

To check that the venous samples obtained were from an area of forearm perfused by noradrenaline, Evans Blue dye (T 1824; 0.5 mg/min) was infused during a control period and blood samples from both catheters were centrifuged and inspected for dye content.

RESULTS

Intra-arterial infusions

Figure 7 illustrates the changes in total forearm blood flow and in skin and muscle venous oxygen saturation in one subject during intra-arterial infusion of noradrenaline (0.1 μg/min). The flow was reduced throughout the infusion and this was accompanied by a reduction in the saturation of muscle and skin blood, indicating a constriction in

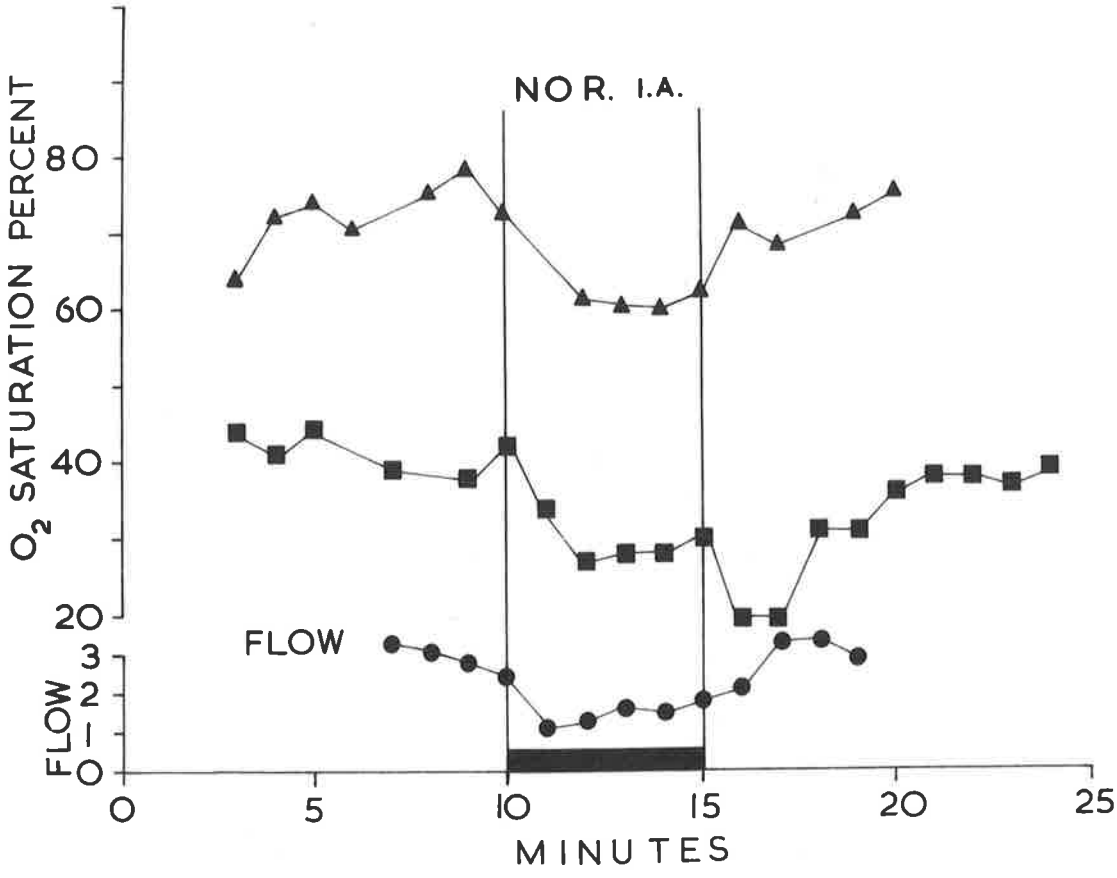


Fig. 7

The effect of noradrenaline infusion into the brachial artery on forearm skin (triangle) and muscle (square) venous oxygen saturation and on forearm blood flow (ml./100 ml./min). The period of infusion of noradrenaline (0.1 µg/min for 5 min) is indicated by the black rectangle.

both these vascular compartments. The magnitude of the constriction of muscle and skin vessels, and therefore of the fall in total flow, increased with increasing doses of noradrenaline. A similar result was obtained in five other experiments on four subjects. When the skin blood flow was elevated by indirect heating the fall in total forearm flow was correspondingly greater with the same dose (Fig. 8).

No "after-dilatation" was seen with the doses and infusion periods used. No changes were seen in the control side that could be ascribed to systemic effects of the drug.

Intravenous infusions

Total forearm blood flow.

Seventeen infusions of noradrenaline of 5, 10 or 20 μg/min for 5 or 10 min were given in eight subjects, and forearm flow measured. The changes in total forearm flow to a given dose showed considerable variation between subjects; a rise, a fall, or no change in flow being observed. Typical responses in three subjects to 20 μg/min are illustrated in Fig. 9 . In three subjects to whom all three doses were given, a constrictor response was obtained which was most marked with the lowest dose and became less with increasing doses; in two others an increase in forearm flow was seen with 20 μg/min.

Muscle and skin circulations

In addition to measurements of forearm flow, the venous oxygen saturation of blood from skin or from muscle or both was determined in twenty experiments on eight subjects and the responses in three of these are illustrated in Fig. 9 . The effect of noradrenaline on skin vessels was usually constrictor, the oxygen saturation of the skin venous blood

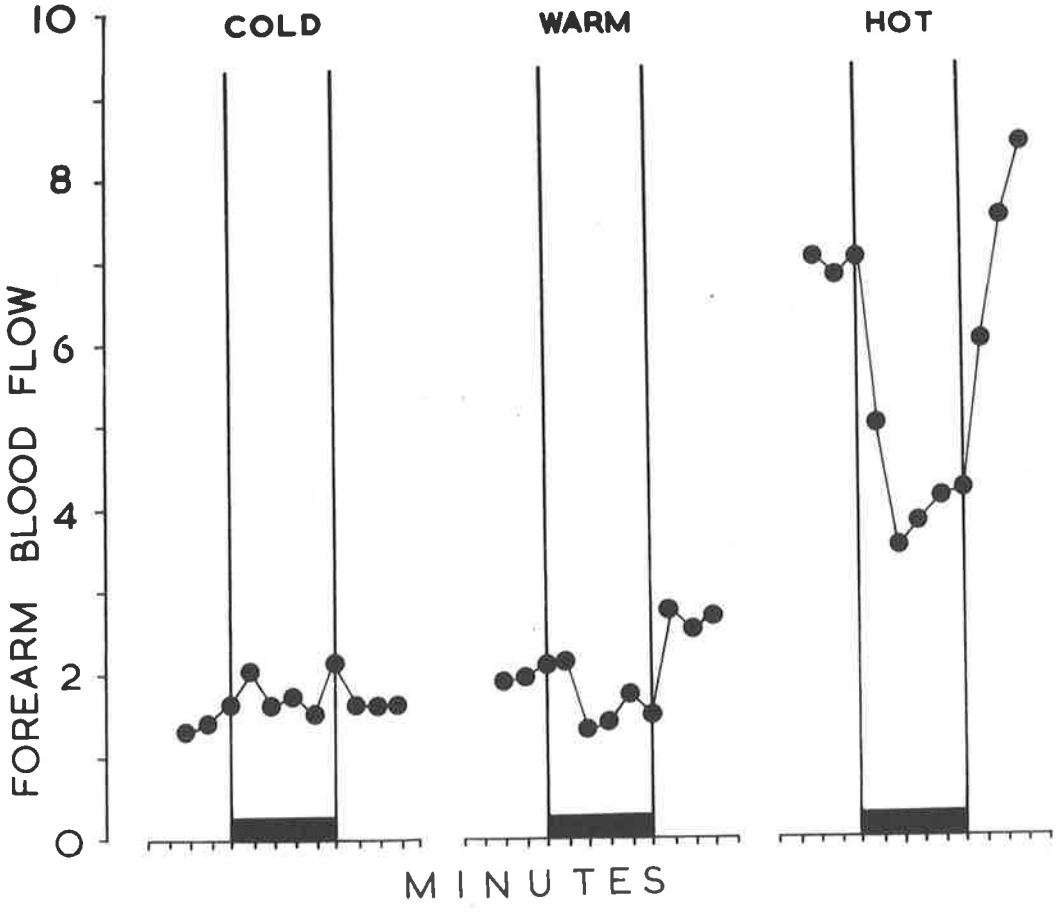


Fig. 8

The effect of intra-arterial infusions of noradrenaline (0.1 µg/min for 5 min) on forearm blood flow (ml./100 ml./min) in the same subject when cold, warm and hot. The periods of noradrenaline infusion are indicated by the black rectangles.

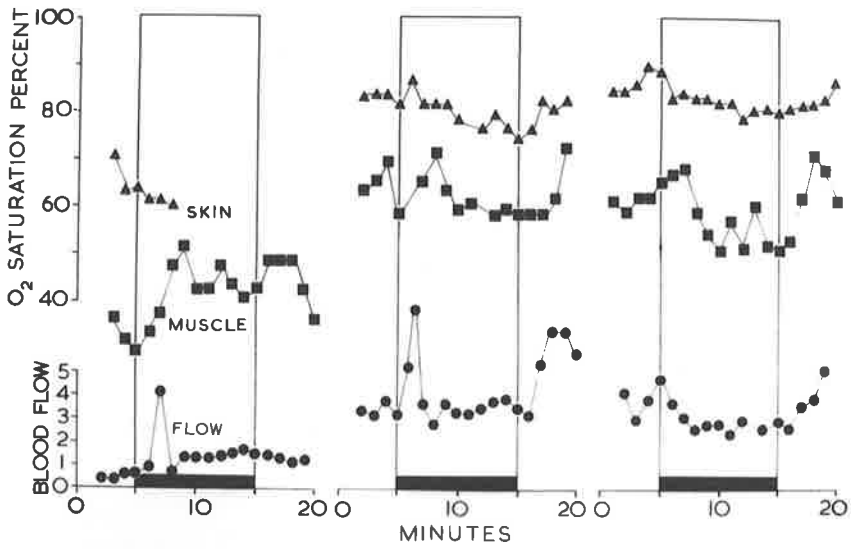


Fig. 9.

The responses of the venous oxygen saturation of forearm skin and muscle and of forearm blood flow to intravenous infusion of noradrenaline in three different subjects. The period of infusion of noradrenaline (20 μg/min for 10 min) is indicated by the black rectangle in each case.

falling in ten of fourteen infusions in which it was measured, remaining unchanged in three and showing a slight increase in one. The response of the muscle vessels in twenty infusions was more variable, showing a dilatation in eight infusions, no change in four and a constriction in eight. As in the case of flow measurements, the larger the dose the more common was a muscle dilator effect, and in two subjects there was a reversal of response, 5 or 10 μg/min causing a constriction of muscle vessels and 20 μg/min a dilatation (Fig. 10).

In those subjects in whom the muscle vessels dilated on intravenous infusion the direction of change in total forearm flow depended on the resting level of skin flow. If this was high, then the constriction masked the dilatation in muscle and resulted in an overall fall in total forearm flow. However, if it was low then the fall in skin flow was insignificant in relation to the muscle dilatation so that the total forearm flow rose. Fig. 11 illustrates such a subject in whom a variable directional change in total forearm blood flow was obtained with three infusions of 10 μg/min of noradrenaline at three different levels of resting skin flow achieved by body heating and cooling. This dose of noradrenaline had previously been shown in this subject to cause dilatation of muscle vessels (Fig. 9, left hand column).

In those subjects in whom the muscle vessels constricted the total forearm flow always fell, the magnitude of the fall with any particular dose being dependent upon the level of skin flow.

An "after-dilatation" followed the cessation of the intra-

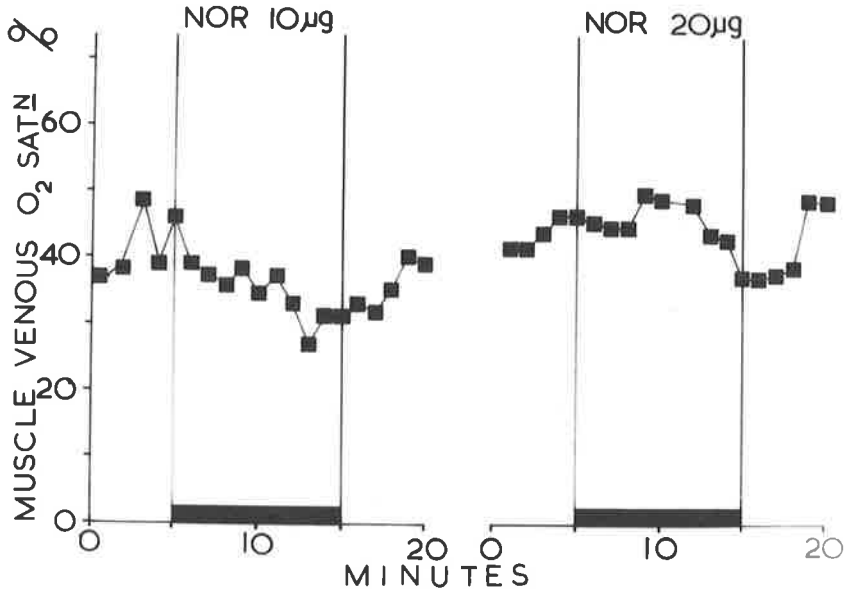


Fig. 10.

The effect on muscle venous blood oxygen saturation in one subject of intravenous infusion of noradrenaline at 10 µg/min (left) and 20 µg/min (right). The periods of infusion are indicated by the black rectangles.

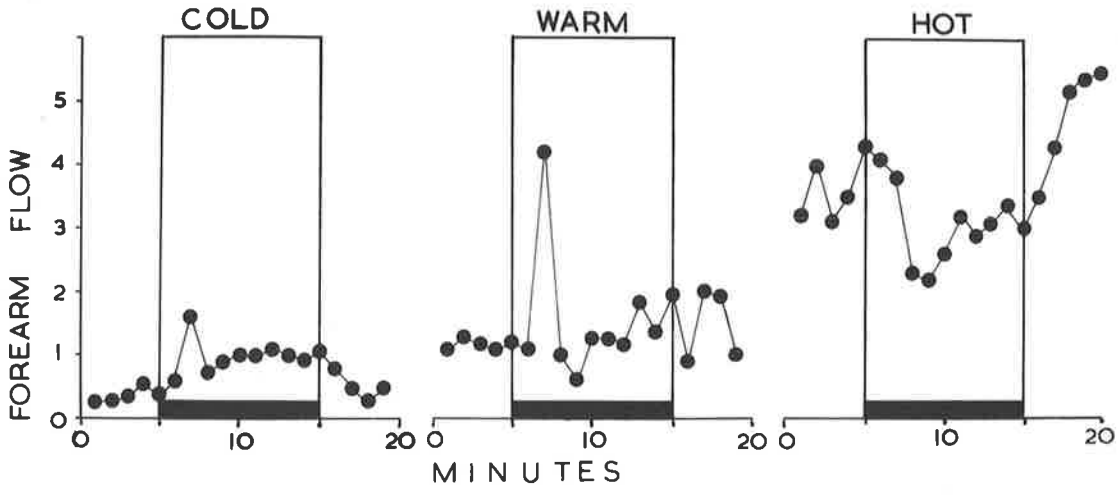


Fig. 11

The effect of intravenous infusions of noradrenaline (10 µg/min - black rectangles) on forearm blood flow (ml./100 ml./min) in one subject at different levels of skin flow.

venous infusion in fourteen of twenty infusions. This was confined to the muscle vessels and its magnitude increased with increasing doses. It usually reached its maximum 3 to 5 min after the end of the infusion, at a time when the arterial blood pressure had returned to its normal level.

The mechanism of the muscle vasodilatation

In order to determine the role of sympathetic nervous reflexes in the mechanism of the vasodilatation in muscle seen in some of the subjects, the responses of the muscle vessels to intravenous noradrenaline were measured before and after the introduction of bretylium tosylate into one arm (Fig. 12). Thirty minutes after bretylium, at a time when sympathetic reflexes have been shown to be abolished, (Blair et al., 1960), noradrenaline infusion resulted in a fall in muscle venous oxygen saturation in contrast to the marked increase seen with the same dose prior to administration of the blocking agent. Blood pressure was recorded from a needle in the brachial artery using an electromanometer and an ultraviolet recorder. The blood pressure response was similar on the two occasions.

In a different experiment in another subject, total forearm blood flow was measured on each side. Bretylium was infused into the artery of one side in a dose of 20 mg, and after a period of 30 min noradrenaline was infused intravenously at a rate of 10 μg/min for 5 min. The forearm flow on the side treated with bretylium fell, but that on the opposite side rose. The results of this experiment are illustrated in Fig. 13.

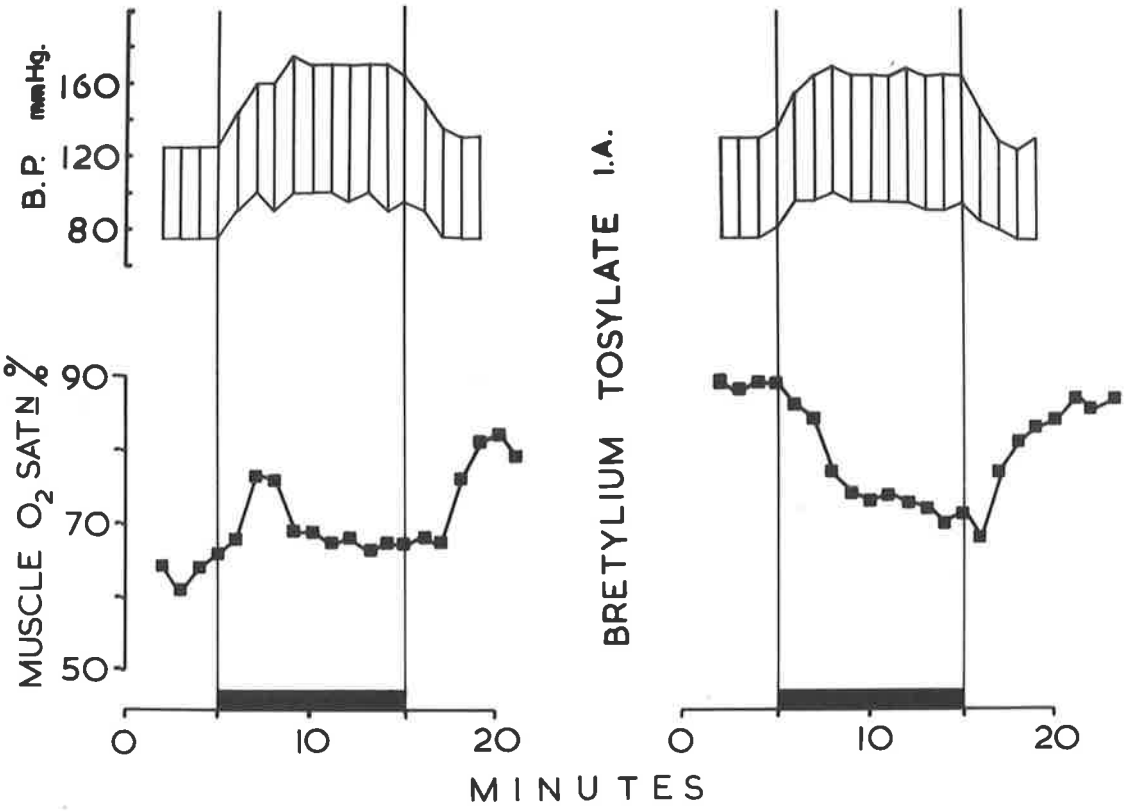


Fig. 12

The response of the muscle venous oxygen saturation and of the brachial arterial blood pressure to intravenous noradrenaline (10 µg/min - black rectangles) before (left) and after (right) intra-arterial infusion of bretylium tosylate (4 mg/min for 5 min)

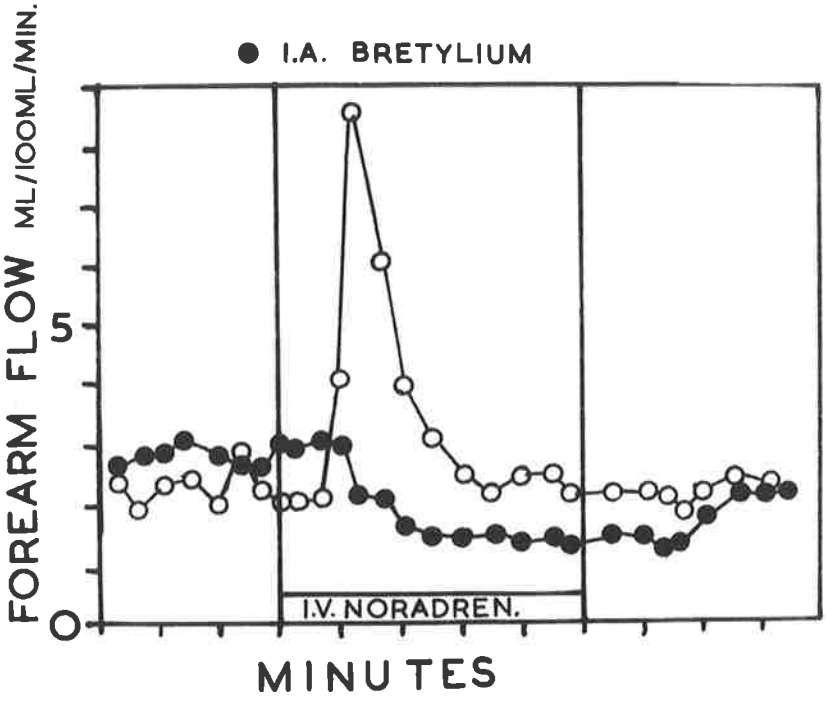


Fig. 13.

The effect of intravenous noradrenaline (10 µg/min for 5 min) on forearm blood flow of both sides. One side had previously received intra-arterial bretylium 4 mg/min for 5 min.

DISCUSSION

The results of the experiments described above demonstrate that the effect of noradrenaline on the forearm blood flow represents the resultant of the responses of the two principal vascular beds involved. The direct local action of the drug, seen when it is given intra-arterially, is to constrict both skin and muscle vessels so that the total forearm flow response is the additive effect on these vessels, and the magnitude of the response increases with increasing dose.

In the case of intravenous infusions, however, a vasodilatation of muscle vessels was often seen. This effect opposed the direct constrictor action of the drug, and was found to be more common with higher doses. In other experiments, where the muscle vessels showed no change or a constriction, the direct constrictor action presumably balanced or outweighed the dilatation. Thus the resultant action on the muscle vessels depends upon the summation of these two opposing effects.

The total forearm blood flow response, however, depends not only on the response of the muscle vessels, but is also profoundly influenced by the magnitude of the constriction in skin vessels which is greater if the skin flow is high prior to the infusion. It is thus possible to obtain a fall in total forearm flow if the skin flow is high, even when the muscle vascular bed shows a dilatation. These variables in skin and muscle explain why it is sometimes impossible to obtain a constant total forearm blood flow response from time to time even in

the same subject with the same intravenous dose of noradrenaline.

An increase in forearm blood flow during intravenous infusion of noradrenaline was observed by Barcroft & Konzett (1949) who attributed the effect to a passive dilatation, probably of muscle vessels, due to the rise in the arterial blood pressure. Whelan & Young (1953) also found an increased forearm flow, and noted that a large transient increase occurred at the beginning of the infusion. Barcroft et al. (1954) further investigated the dilator response of forearm vessels by examining the effect of sympathetic nerve block and of surgical sympathectomy and concluded that the increased flow was not due to a rise in perfusion pressure but must be due to a reflex vasodilatation of the forearm vessels, presumably those of muscle. The results of the present experiments establish that all phases of the forearm dilatation during the intravenous infusion of noradrenaline, and the after-dilatation, are confined to the muscle vessels.

The demonstration in two experiments (Figs. 12 & 13) that the increase of muscle flow in one and of forearm flow in the other is abolished by blockade of the sympathetic nerves in the arm by bretylium tosylate, after which a vasoconstrictor response alone ensues, points to the sympathetic reflex nature of the vasodilatation and demonstrates that increased perfusion pressure is not an important factor. As Barcroft et al. (1954) pointed out, the stimulus for the reflex could be a central effect of noradrenaline on the vasomotor centre or might be a consequence of the rise in blood pressure acting on the barereceptors. The fact that Roddie, Shepherd & Whelan (1957b) have demonstrated reflex alteration in calibre of forearm muscle vessels

in response to changes in central venous pressure and that alterations in the arterial pressure in the carotid sinuses have failed to induce reflex vasomotor responses in limb vessels (Ernsting & Parry, 1957; Roddie & Shepherd, 1958) suggests that venous pressure changes may be responsible; and noradrenaline has been shown to cause a marked elevation of central venous pressure in man (Fowler, Westcott, Scott & McGuire, 1951). The "after-dilatation" in muscle is presumably of the same nature as the dilatation seen during intravenous infusions of noradrenaline since it was also abolished by sympathetic blockade and the finding that it is most marked at a time when the arterial blood pressure has returned to normal provides support for the suggestion that the systemic arterial pressure is not the stimulus for the reflex dilator effect. Further experiments will, however, be necessary before the suggested reflex nature of the dilator response of muscle vessels to intravenous noradrenaline and the "after-dilatation" can be regarded with more certainty.

Alteration in the sensitivity of the peripheral vessels to circulating amines has been postulated to occur following sympathectomy (Duff, 1955), in hypertension (Doyle, Fraser & Marshall, 1959) and following the administration of sympathetic blocking agents (Green, 1961; Zaimis, 1961). Attempts to follow such changes by measurement of forearm blood flow during administration of intravenous noradrenaline will lead to difficulties in interpretation, in view of the differential effects on the different vascular beds involved, and because of the unpredictability of the reflex muscle dilator component. The magnitude of this component of the response will depend, presumably, upon the

degree of central reflex effect, which might be a response to elevation of systemic arterial or central venous pressure. Either of these could, in turn, be dependent at least in part on the degree of the constriction of the peripheral vessels produced by the direct action of the noradrenaline.

The use of the intra-arterial route of administration will eliminate the complication of the reflex responses, and could prove a suitable means of following forearm vessel sensitivity to noradrenaline since both muscle and skin vessels are constricted when the drug is given by this route, and since both vascular beds appear to show increased responses to increasing doses. However, the profound influence of the level of skin flow on the total forearm flow responses to noradrenaline, whether given intravenously or intra-arterially, necessitates careful control of environmental temperature.

For these reasons, responses of hand rather than forearm vessels have been used when investigating changes of sensitivity to noradrenaline after antihypertensive drugs as described in the following sections. Although the responses of forearm vessels to antihypertensive drugs are of interest and have been examined, little weight can be placed on the evidence they provide regarding the presence or absence of hypersensitivity.

SUMMARY

1. The skin vessels are constricted by both intra-arterial and intravenous infusions of noradrenaline. However, the magnitude of the skin constriction with any given dose, and therefore its contribution

to the total forearm flow response, is greater when the resting level of skin flow is high.

2. The direct action of noradrenaline on the muscle vessels is constrictor but in the case of intravenous infusions a vasodilator component opposes the direct constrictor action and the resultant effect on the muscle vessels is a balance of these two forces.

3. With intra-arterial infusions the constrictor effects on skin and muscle vessels are additive and a fall in forearm flow is seen with all doses.

4. With intravenous infusions the change in total forearm flow is variable and will depend upon the resultant of its direct constrictor actions on the skin and muscle vessels, and the opposing reflex dilator action on the muscle vessels.

5. The conversion by bretylium of a dilator response of muscle vessels to intravenous noradrenaline to a constrictor response, and the abolition of an "after-dilatation" suggest that both dilatations are mediated reflexly by the adrenergic nerves, and that increased perfusion pressure is not a major factor.

PART 2.

Section B.

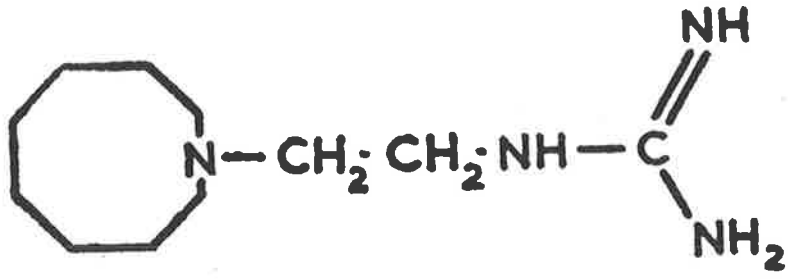
THE ACUTE EFFECTS OF ADMINISTRATION OF SOME ANTIHYPERTENSIVE DRUGS ON PERIPHERAL VESSELS AND ON THEIR SENSITIVITY TO NORADRENALINE.

(i) Bretylium tosylate and guanethidine.

INTRODUCTION.

Following shortly after bretylium, another substance capable of blocking the post-ganglionic adrenergic neurone was introduced, guanethidine. This chemically distinct substance is 2-(octahydro-1-azocinyl)-ethyl -guanidine sulphate (Fig.14), the detailed pharmacology of which has been described by Maxwell, Mull & Plummer (1959) and Maxwell, Plummer, Schneider, Povalski & Daniel (1960a). In addition to the adrenergic neurone blockade which is of long duration, it depletes most tissues of their catecholamines, with the important exceptions of the brain and the adrenal medulla (Sheppard & Zimmermann, 1959; Cass, Kuntzman & Brodie, 1960). Extensive clinical trials of guanethidine have established it as one of the most important drugs in the treatment of hypertension with relatively few side-effects (Page & Dustan, 1959; Leishman, Matthews & Smith, 1959, 1961; Dollery, Emslie-Smith & Milne, 1960; Barnett, Kincaid-Smith & Simpson, 1960).

Bretylium and guanethidine have a number of pharmacological properties in common, but one striking difference in their clinical characteristics is that the tolerance so frequently seen with bretylium (see Part 1) is not encountered to any significant degree with guanethidine. The development of tolerance was attributed by Boura et al. (1959a), by Green (1961) and by Zaimis (1961) to an increasing sensitivity of peripheral blood vessels to noradrenaline which was shown to occur in animal experiments. If this is so in humans, the noradrenaline might be normally circulating from the adrenal medulla, released by



GUANETHIDINE

Fig. 14.

sympathetic nervous activity (since in an ambulant patient on treatment it is impracticable to block completely all the sympathetic nerves), or released from a peripheral store by the action of the hypotensive agent.

The difficulty in accepting the phenomenon of hypersensitivity as the sole explanation of tolerance is that pharmacological studies show that guanethidine also causes hypersensitivity (Maxwell et al., 1960a; Maxwell, Plummer, Povalski & Schneider, 1960b), yet tolerance is not a feature of its use in patients. Further difficulties are that much of the evidence is derived from animals or animal tissues, and that drug dosages used are frequently much higher than those that can be used in humans. Again, inferences on sensitivity changes in blood vessels have frequently been made as the result of blood pressure changes following intravenous injection of drugs, but as will be discussed, such assumption are not justified.

The purpose of the present investigation was to determine whether an acute increase in sensitivity to noradrenaline accompanied the sympathetic-blocking action of bretylium and guanethidine on the blood vessels of the human hand and forearm. Observations were also made on the direct actions of these drugs on the blood vessels and on sympathetic transmission in these areas.

METHODS.

The subjects were healthy adults aged 20 to 35 years who lay supine on a couch for 30 to 60 min before observations began. The blood flow through the forearms or hands was measured three to four

times each minute by venous occlusion plethysmography using water-filled, temperature-controlled plethysmographs (see Appendix 1). Drugs were infused into one or other brachial artery near the ante-cubital fossa through a 3.5 cm long, 23 gauge, short-bevel needle connected by a length of polyethylene tubing to a mechanically-driven syringe which delivered 4 ml. of solution per min. Normal saline (0.9% NaCl w/v) containing ascorbic acid (1:20,000) was used for infusion during control periods and as a vehicle for the drugs.

To follow the time course of the sympathetic block resulting from intra-arterial infusion of bretylium or guanethidine ice was applied to the neck of the subject for 30 sec at intervals before and after infusion, and the resulting vasoconstriction in both hands was recorded. This procedure proved a potent sympathetic stimulus and both hands responded with an equally intense vasoconstriction if the sympathetic nerves were intact. The relationship of the response of the treated side to that of the control side was expressed as a ratio between the percentage falls in flow induced by ice in the injected and control hands respectively, multiplied by 100. Thus, if the flow on both sides fell equally the result would be expressed as 100% response; if after injection of the sympathetic blocking drug the control side showed an 80% fall and the injected side a 40% fall, then the result would be expressed as a 50% response.

To test for sensitivity to noradrenaline, two or three different doses of noradrenaline bitartrate (Levophed, Winthrop) were infused before and after the introduction of bretylium or guanethidine. In this way the dose response relationships in the two periods could be

compared. In other experiments the same dose of noradrenaline was given at intervals before and after the introduction of the sympathetic blocking agent. The mean of the flow values in the 2 min preceding the infusion was compared with that in the last 2 min of the 3 min infusion period. Where appropriate, account was taken of general spontaneous changes in limb blood flow by reference to the control side (see Appendix 2).

Bretylium tosylate (Darenthin, Burroughs Wellcome) was given in a dose of 10 or 20 mg over a period of 5 min and guanethidine hemisulphate (Ismelin, Ciba) in a dose of 5 mg for the same period. In a number of experiments the effect of phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline and French, 1.2 or 2 mg in 5 min) on the response of the limb vessels to the above drugs was observed. Doses of the drugs are expressed as weights of the salts.

RESULTS.

Effects on limb vessels.

The effects of intra-arterial infusions of bretylium (10 or 20 mg) and guanethidine (5 mg) on forearm and hand flow were qualitatively similar; vasoconstriction occurred after the administration of the drug, followed by a prolonged vasodilatation (Fig. 15). The vasoconstriction lasted longer after guanethidine than after bretylium, and with each drug was more prolonged in the forearm than in the hand.

In the forearm bretylium caused a transient three- to four-fold increase in flow during its infusion. The hand vessels responded some-

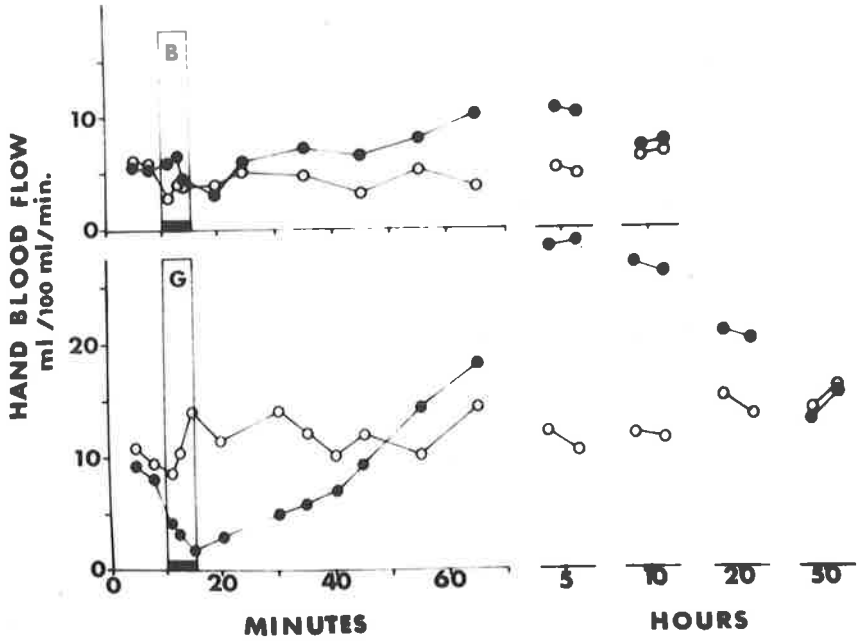


Fig. 15.

The response of the blood flow through the hand to infusions of bretylium (20 mg, B - upper section) and guanethidine (5 mg, G - lower section) into the brachial artery. ●, injected side; O, control side.

what differently, as only a moderate vasodilatation preceded the vasoconstriction in six of eight subjects, being more pronounced when the resting flow was low. No such effect was seen with guanethidine, which produced an immediate fall in hand and forearm flow during its infusion (Figs. 15 & 16).

The constrictor effect of bretylium on forearm and hand vessels was abolished by the prior administration of the adrenaline antagonist phenoxybenzamine. The constrictor effect of guanethidine was not completely abolished, a slight residual fall in flow remaining despite the abolition of the response to noradrenaline (Fig. 16). No change in flow was seen during infusion of the vehicle, and the residual constriction thus appeared to be due to a direct action of the drug on the vessels.

Sensitivity to noradrenaline

In Fig. 17 is illustrated an experiment in which the sensitivity of the vessels of one hand to a dose of 0.05 μg/min of noradrenaline was determined at intervals before and after intra-arterial infusion of guanethidine. The reflex constrictor responses of the vessels of both hands to ice applied to the neck are also shown. The results from this and from seven other such experiments are summarized in Fig. 18. In every experiment within 5 to 10 min of infusion of guanethidine the percentage reduction in flow caused by noradrenaline was greater than before the infusion. At this time the blocking action of the drug was incomplete as judged by the proportion of the sympathetic response (elicited by application of ice) which survived the block. At 30 min,

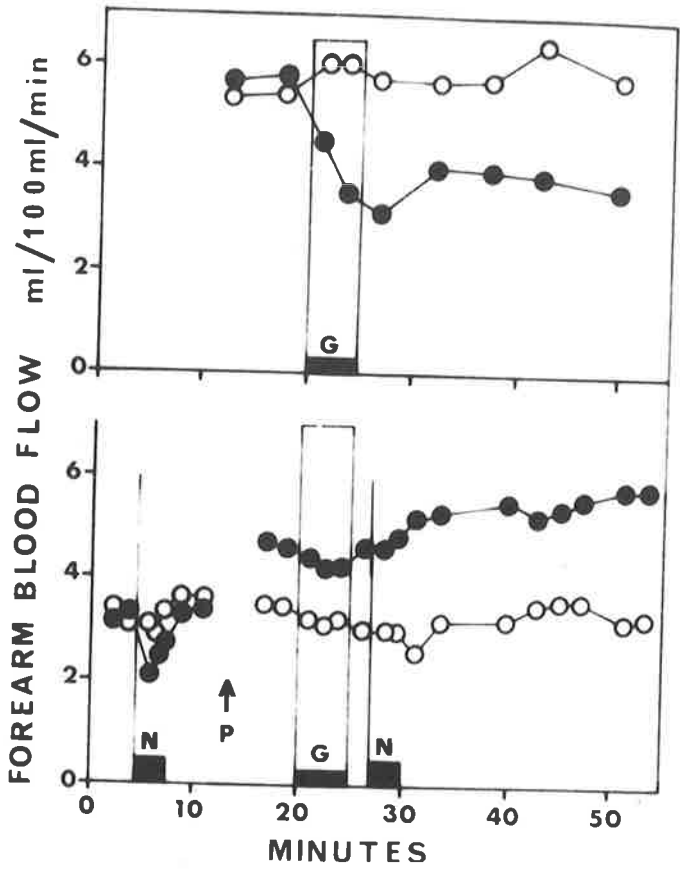


Fig. 16.

Lower section: the effect of phenoxybenzamine infused (at arrow) into the brachial artery on the response of the forearm vessels to guanethidine (G). ●, injected side; O, control side. At N, noradrenaline (0.025 μg/min for 3 min) was infused intra-arterially to check the efficacy of the block by phenoxybenzamine.
Upper section: the response of the forearm blood flow to guanethidine (G) alone in the same subject on a previous occasion.

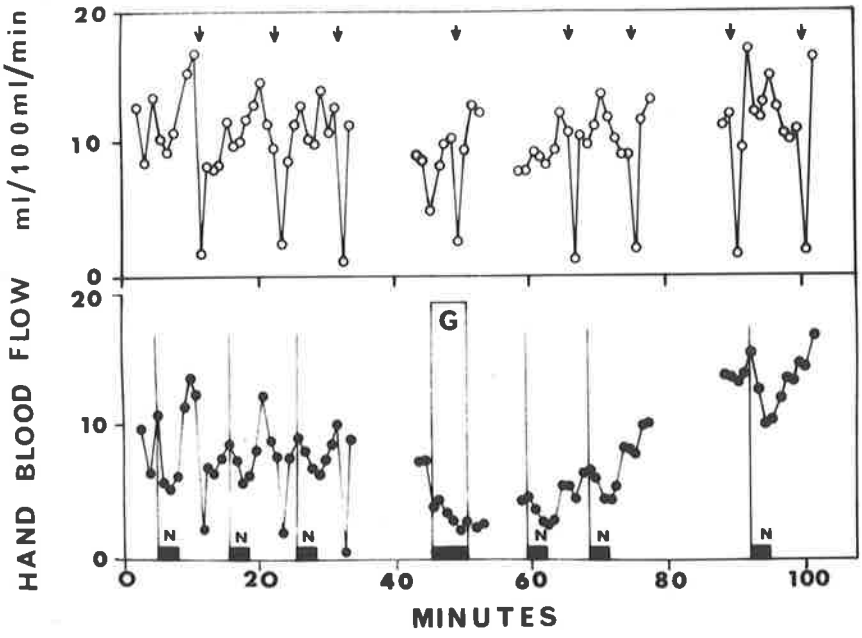


Fig. 17.

The response of the hand blood flow to application of ice to the neck (at arrows) and to intra-arterial infusions of noradrenaline (N) at intervals before and after intra-arterial infusion of guanethidine (G). ●, infused side; O control side.

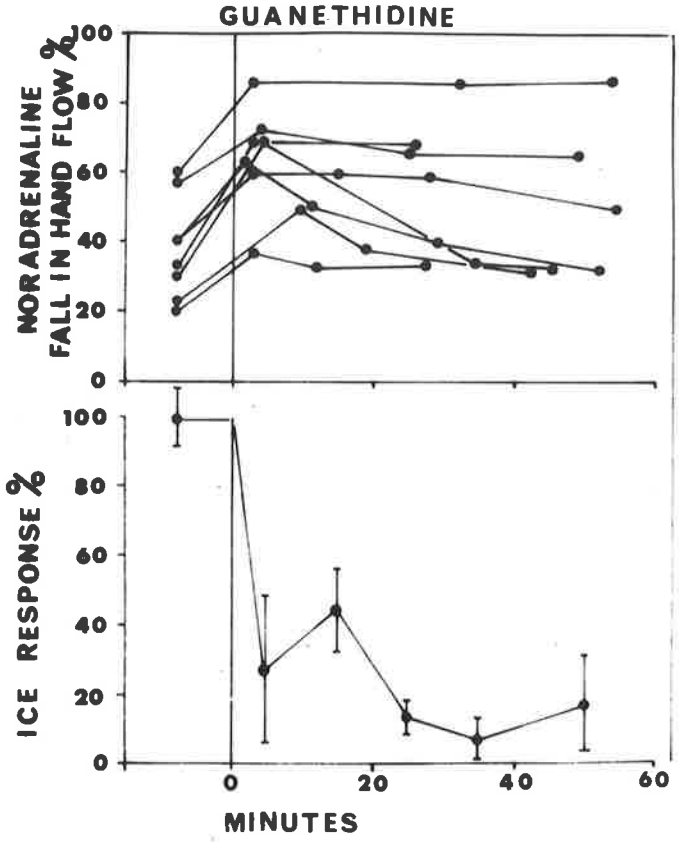


Fig. 18.

Upper section: the percentage fall in hand flow in eight subjects in response to intra-arterial infusions of noradrenaline (0.05 μg/min) before and at intervals after intra-arterial administration of guanethidine at time zero. Lower section: pooled data of the responses of the hand blood flow to ice applied to the neck in the above subjects. The response (ordinate) is expressed as the ratio of the falls in blood flow on the infused and controlled side respectively, times 100. Each point represents the mean of all observations in the appropriate 10 min period, and the vertical line through each point represents the standard deviation.

when block was complete and blood flow levels had returned to or had risen above control values, the responses to noradrenaline were still increased in six of the eight subjects.

The results of six similar experiments with bretylium are illustrated in Fig. 19 . In four subjects there was an increase in the percentage fall in flow with noradrenaline within 5 min of the end of the infusion of bretylium. At this time the sympathetic block was minimal and the vasoconstrictor action of the drug was most marked. By the time sympathetic block was complete (30 to 35 min after bretylium) the blood flow level was restored and the responses to noradrenaline had returned to pre-infusion levels in all but one subject in whom a 10% increase persisted.

With the forearms the time course of the blocking action of the drugs was not defined since the response of the forearm vessels to the application of ice was inconsistent. The dose-response curves of the forearm blood vessels to noradrenaline obtained before and after administration of bretylium and guanethidine did not demonstrate any increase in vascular sensitivity (Fig. 20). The dilatation of the forearm vessels in response to mental arithmetic, which is mainly due to cholinergic nerve activity (Blair, Glover, Greenfield & Roddie, 1959), was not affected by either bretylium or guanethidine.

DISCUSSION

The responses of the vessels to bretylium and guanethidine

The effects of bretylium on the blood flow in the forearm and hand in the present study are similar to those described by

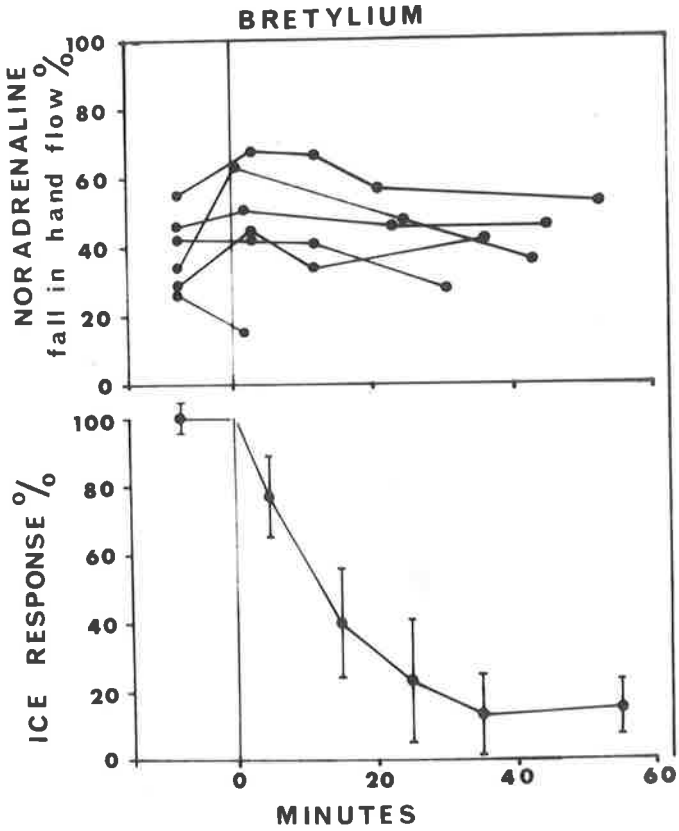


Fig. 19.

Upper section: the percentage fall in hand flow in six subjects in response to intra-arterial infusions of noradrenaline (0.05 μg/min) before and at intervals after intra-arterial administration of bretylium at time zero.
Lower section: pooled data of the responses of the hand flow to ice applied to the neck in the above subjects, calculated as for Fig. 18.

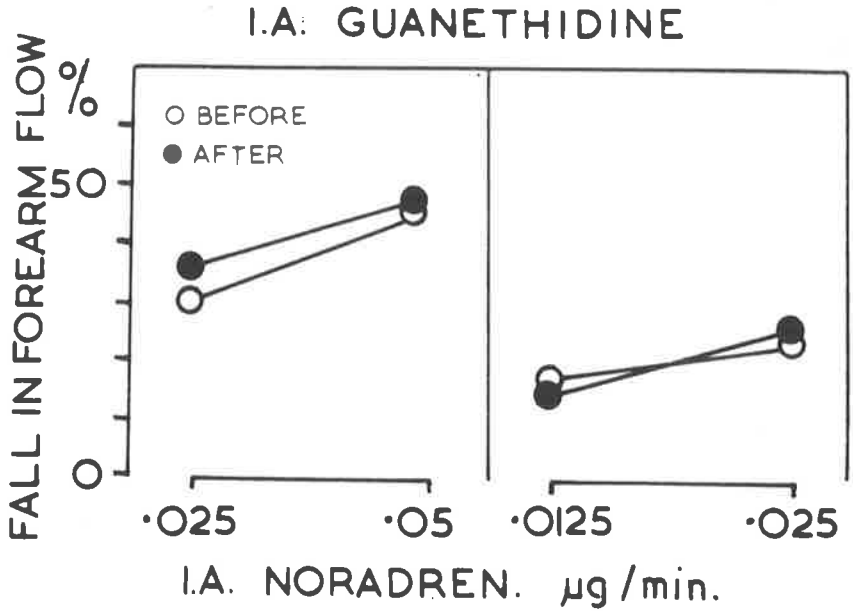


Fig. 20.

The responses of the forearm blood vessels to two doses of noradrenaline given into the brachial artery before (O) and after (●) intra-arterial infusion of guanethidine in two subjects.

Dornhorst (1959), by Blair, Glover, Kidd & Roddie (1960), and by French & Matthews (1961). The initial transient vasodilatation in the forearm was attributed by Blair et al. (1960) to a direct action of the drug on the vessels since it occurs in the nerve-blocked limb. The constriction of hand and forearm vessels is probably due to local release of catecholamines from the tissue since we found it was abolished by phenoxybenzamine. Animal experiments have also demonstrated that this drug releases catecholamines (Gaffney, 1961; Gillis & Nash, 1961; Gilmore & Siegel, 1962).

The pattern of response of the blood vessels of the hand and forearm to guanethidine (5 mg) differed from that to bretylium (10 to 20 mg) in that no initial vasodilatation was observed. The constrictor phase became evident during the period of infusion and both it and the subsequent dilatation persisted for longer periods than after bretylium. Most of the constrictor effect can be attributed to release of catecholamines, but a small residual fall in flow occurred after treatment with phenoxybenzamine which was presumably due to a direct action of the drug on the vessels. These actions of guanethidine are consistent with those observed in animals and isolated tissues which demonstrate that it causes a much greater release of catecholamines than does bretylium (Gillis & Nash, 1961), and accord with clinical observations that its hypotensive action is more prolonged (Page & Dustan, 1959).

Tolerance and hypersensitivity

Hypersensitivity of the cat nictitating membrane to noradrenaline has been reported both after bretylium and guanethidine (Green,

1962), although tolerance in clinical use, which is usually ascribed to vascular hypersensitivity, is prominent only with bretylium. Much of the evidence for vascular hypersensitivity comes from studies on intact animals or humans, and is more difficult to interpret than results from isolated tissues. Thus in acute experiments, Abboud, Eckstein & Wendling (1962) found an increased pressor response to noradrenaline infusions in dogs and man 30 min after intravenous administration of guanethidine and demonstrated an increased constriction of the forearm vessels in man, which was attributed to hypersensitivity of the vessels. However, inhibition of the baroreceptor reflex are by a drug, irrespective of whether this occurs centrally or at the level of the baroreceptors themselves or by interruption of the efferent pathways, will result in an exaggerated response of the limb vessels to noradrenaline; but this is not necessarily evidence of true hypersensitivity of the vascular smooth muscle. For example, hexamethonium, which blocks the baroreceptor reflex pathway, produces no increase in vascular sensitivity to intra-arterial injection at a time when the responses to intravenous administration are definitely increased (see Section B (iii)).

In the present study the intra-arterial route of administration was used so that a high concentration of bretylium and guanethidine could be applied to the vessels, while the amount entering the systemic circulation would be insufficient to have any general effect. Noradrenaline was also given intra-arterially to avoid the complex reflex responses which result from blood pressure changes. A small increase in the percentage fall in hand blood flow with noradrenaline

was found in four of six subjects given bretylium, but in only one did this persist for more than 30 min. The more definite and prolonged increase in sensitivity to noradrenaline after injection of guanethidine occurred before sympathetic block was complete and thus appeared to be unrelated to the blocking action.

The immediate hypersensitivity to noradrenaline after bretylium and guanethidine may be due in some way to their property of releasing noradrenaline from the tissues. A similar phenomenon has been reported in animal tissues immediately after treatment with reserpine by Innes (1960) and by Nakamura & Shimamoto (1960). A possible explanation for this phenomenon is that the exogenous noradrenaline and that released from peripheral stores have an additive effect on the receptors (Trendelenburg, 1963). If correct, this would explain why the acute noradrenaline hypersensitivity was much more prominent after guanethidine than after bretylium, since the former releases much more noradrenaline than the latter.

With the forearm vessels no increase in sensitivity to noradrenaline was observed after bretylium or after guanethidine. This absence of an increase in total forearm blood flow response to noradrenaline, however, does not altogether exclude the possibility of a change in sensitivity of one or other of the vascular beds which together make up the forearm circulation, but which cannot be differentiated by the plethysmographic method of flow measurement. It can be concluded that if any such change did occur it was not sufficiently great to alter the change in overall forearm vascular resistance produced by noradrenaline.

The slight transient increase in sensitivity of the hand

vessels to noradrenaline after bretylium (to which tolerance is marked), the more sustained increase in sensitivity with guanethidine (to which tolerance is negligible) and the absence of any increase in overall forearm vascular sensitivity all suggest that it is unlikely that this acute hypersensitivity is the cause of tolerance.

It is possible that an increase in vascular sensitivity to noradrenaline of slower onset may occur in patients on chronic therapy with these hypotensive drugs, and that this may be connected with tolerance. This possibility is examined in Section 2C.

SUMMARY

1. Infusions of bretylium and guanethidine into the brachial artery of normal subjects caused a fall in forearm and hand blood flow followed by a sustained increase in flow.

2. The constrictor phase was attributed to catechol amine release since it was blocked by phenoxybenzamine.

3. A slight and transient increase in response of the hand vessels to noradrenaline was seen after bretylium in the early constrictor phase before sympathetic block was complete. Guanethidine produced a more definite and sustained increase in the response of the hand vessels to noradrenaline.

4. It is concluded that the phenomenon of tolerance which is conspicuous with bretylium and negligible with guanethidine is unlikely to be due to the hypersensitivity of the peripheral vessels observed in these experiments.

PART 2.

Section B.

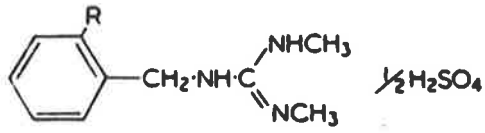
(ii) Berbamidine.

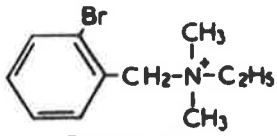
INTRODUCTION

In the treatment of hypertension, two drugs are available which block the transmission of impulses at sympathetic nerve terminals without interfering with the action of catecholamines. These are bretylium and guanethidine. Each, however, has certain disadvantages; tolerance is prominent with the first (see Part A) and the onset and offset of action slow with the second, while undesirable side-effects may occur with both. The search for an ideal hypotensive drug continues and a series of compounds related to bretylium and guanethidine was described by Boura, Copp, Green, Hodson, Ruffell, Sim, Walton & Grivsky (1961), the most potent of these being bethanidine (B.W. 467C60; N-benzyl-N'N"-dimethylguanidine sulphate) (Fig. 21).

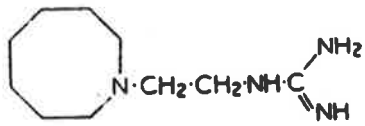
Pharmacological studies on animals and isolated preparations have shown this drug to be a potent hypotensive agent resembling guanethidine and bretylium in its actions, causing blockade of postganglionic sympathetic nerves, inducing hypersensitivity to catecholamines and possessing a transient sympathomimetic action (Boura & Green, 1963). Other pharmacological studies are those of Boura, Duncombe, Robson & McCoubrey (1962), and Costa, Kuntzman, Gessa & Brodie (1962). Clinical trials of the drug in hypertensive patients have shown that it may be a useful alternative to guanethidine and its effects have been ascribed to adrenergic blockade (Montuschi & Pickens, 1962; Smirk, 1963).

No data are available on the direct action of the drug on the blood vessels in man, and the present investigation was designed to





Bretylium



Guanethidine

Fig. 21.

The formula of bethanidine (above) with those of bretylium and guanethidine for comparison.

determine the nature and the mechanism of its effects on the limb blood vessels in normal human subjects. Intra-arterial and intra-venous infusions were given and measurements made of forearm and hand blood flow, systemic arterial pressure, the degree and time-course of sympathetic blockade and the development of sensitivity to noradrenaline.

METHODS

The subjects were normal adults between the ages of 20 and 35 years who lay supine on a couch in a laboratory maintained at 23-25°C for half-an-hour to one hour before observations began. The blood flow through the forearms or hands was measured 3 - 4 times each minute by the technique of venous occlusion plethysmography using water-filled, temperature-controlled plethysmographs (see Appendix 1). Plethysmograph temperatures for forearm and hand were 34°C and 32°C respectively. Drugs were infused into one or other brachial artery near the cubital fossa through a 3.5 cm, 23 gauge, short-bevel needle connected by way of a length of polythene tubing to a mechanically-driven syringe which delivered 4 ml of solution per minute. Saline (0.9% NaCl w/v) containing ascorbic acid 1:20,000 was used for infusion during control periods and as a vehicle for the drugs. Doses of drugs are expressed as weights of the salts.

Bethanidine was given intra-arterially in a dose of 1 mg/min for 5 min and its effects on the circulation through the hand or forearm recorded. The responses of the vessels to intra-arterial noradrenaline

and to a potent sympathetic stimulus (application of ice to the neck) were observed before and after bethanidine administration. The blood flow through the opposite hand or forearm was simultaneously measured as a control.

In a number of subjects bethanidine was given intravenously (8 mg/min for 5 min) and its effects on blood pressure, heart rate and forearm blood flow determined both with the subject horizontal and tilted 45° feet down.

RESULTS

Effects on limb vessels.

Fig. 22 (upper frame) illustrates a typical response of the vessels of the hand to an infusion of 5 mg bethanidine into the brachial artery of one side. An initial transient fall in flow was followed by a sustained vasodilatation which persisted for more than 5 hours. When re-examined 20 hours after the infusion the flow had returned to about the resting value. This pattern of response of the hand vessels to bethanidine was observed in 7 other experiments in 6 subjects.

The effect of intra-arterial bethanidine on the vessels of the forearm was qualitatively similar to that on the hand vessels, differing in that the initial constrictor effect was much more prolonged, lasting up to 2 hours. The subsequent dilatation was less marked than in the case of the hand, and had subsided at some time between 10 and 20 hours after the infusion. Fig. 22 (lower frame) illustrates one of four such experiments on the forearm. The influence of phenoxybenzamine

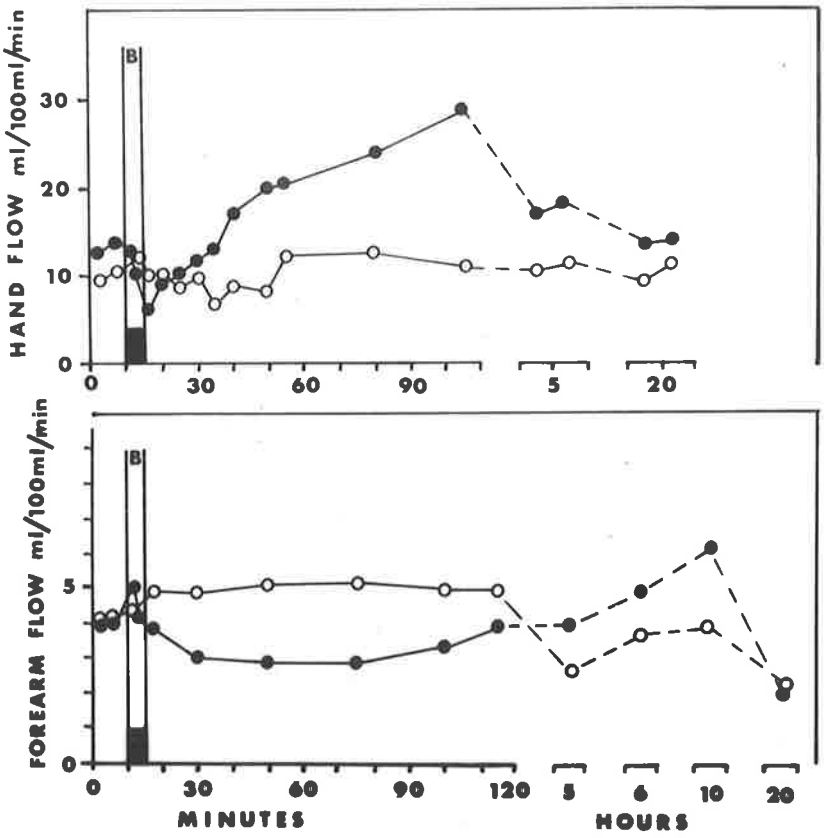


Fig. 22.

Response of the blood vessels of the hand (upper frame) and of the forearm (lower frame) to infusion of bethanidine (1 mg/min for 5 min) into the brachial artery of one side.

● infused side

O control side

B. - period of infusion of bethanidine.

Note the change of time scale.

given into the brachial artery in a dose of 3 mg in 5 min on the subsequent response of the forearm vessels to bethanidine is shown in Fig. 23. An infusion of noradrenaline (0.05 µg/min for 3 min) was given before and after the phenoxybenzamine to demonstrate that drugs given by this route were reaching the vascular bed under study and as a control for the adrenergic blocking action of the phenoxybenzamine. Infusion of phenoxybenzamine resulted in a rise in forearm blood flow presumably as a consequence of blockade of sympathetic constrictor tone. When the new level of flow had become stabilised, bethanidine was infused. Little or no fall in flow was now seen, in contrast to the responses illustrated in Fig. 22 (lower frame). The constrictor effect of bethanidine on hand blood vessels was also abolished by the prior administration of phenoxybenzamine.

Sympathetic blockade

Fig. 24 shows the pooled data from 8 experiments on 6 subjects in whom the reflex vasoconstrictor response of the hand vessels to ice applied to the neck for 15 sec was recorded before and at intervals after infusion of bethanidine into the brachial artery of one side. The application of ice is a potent sympathetic stimulus to which both hands respond with an equally intense vasoconstriction if the sympathetic nerves are intact. The relationship of the response of the treated side to that of the control side was expressed as a percentage by applying the formula I/C x 100 where I and C were the percent falls in flow induced by ice in the injected and control hands respectively. Blockade was complete in less than an hour, and in three subjects who were followed

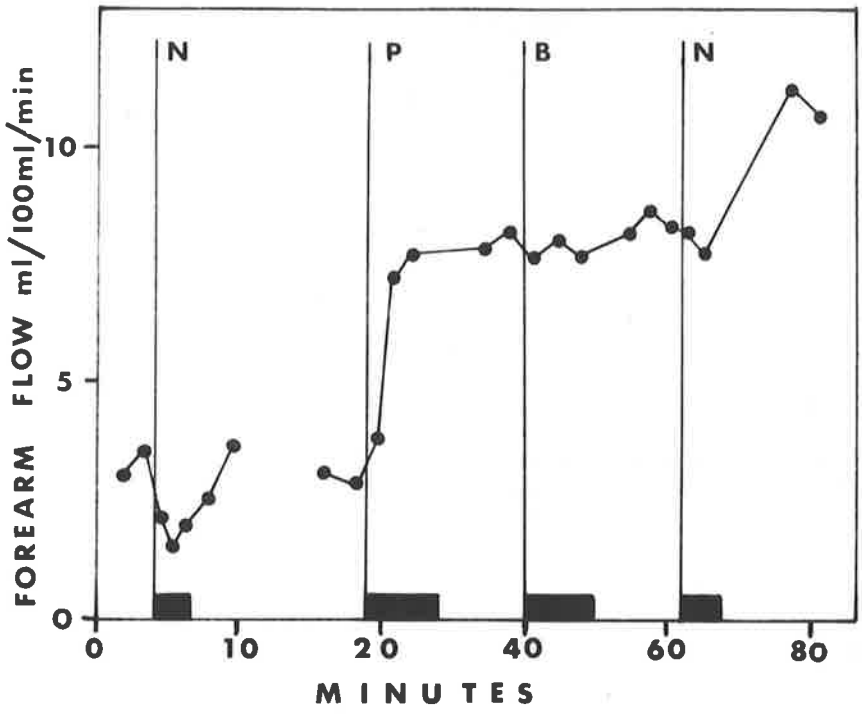


Fig. 23.

The effect of phenoxybenzamine on the response of the forearm vessels to bethanidine. N - infusion of noradrenaline (0.05 μg/min for 3 min) into the brachial artery. P - infusion of phenoxybenzamine (0.6 mg/min for 5 min). B - infusion of bethanidine (1 mg/min for 5 min).

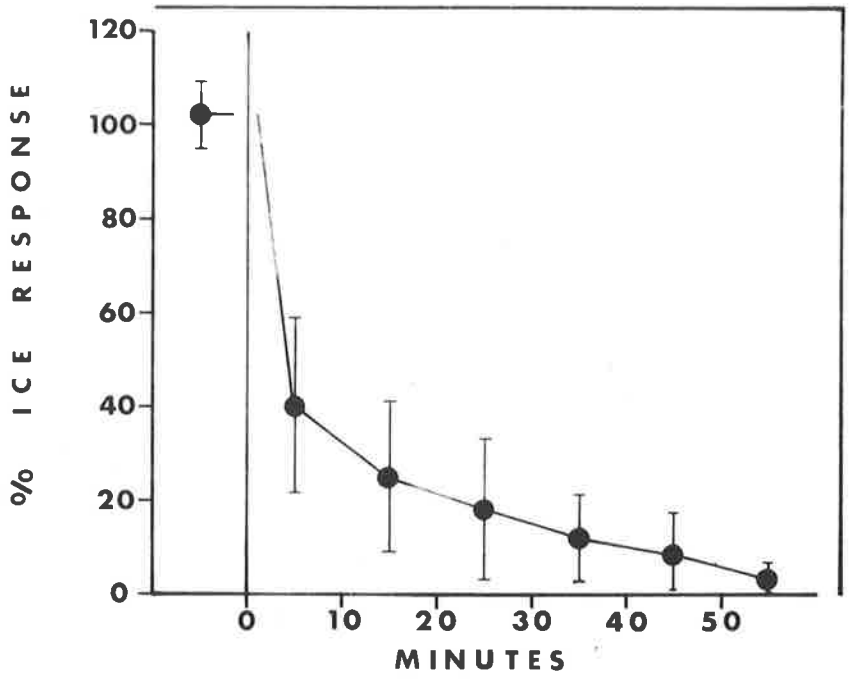


Fig. 24.

Sympathetic blockade of the hand blood vessels following bethanidine (1 mg/min for 5 min) given at time zero. The constrictor response of the hand vessels to ice applied to the neck is expressed as a percent change from the resting level (see text). Each point represents the mean of observations on four subjects for the appropriate 10 min period and the vertical line through each point represents the standard deviation of the individual values from the mean.

for a sufficient time recovery to 75-80% of the control side had occurred by 5 hours.

Sensitivity to noradrenaline

In 4 subjects illustrated in Fig. 25, hand blood flows were measured while noradrenaline (0.05, 0.1 or 0.2 μg/min) was infused for 3 min into the brachial artery of one side before and at intervals after infusion of bethanidine. The average of the flows recorded in the last 2 min of each noradrenaline infusion was compared with that of the flows in the 2 min immediately preceding the onset of the infusion. Where appropriate, account was taken of general spontaneous changes in limb blood flow by reference to the control side and a correction made (see Appendix 2). An increase in sensitivity occurred within 10 to 15 min of the end of the infusion of bethanidine in all cases and in 3 of the subjects this persisted until the experiments were terminated 35, 45 and 55 min after infusion. In the fourth subject the noradrenaline response had returned by the end of 30 min to below the control value.

Intravenous infusion

Blockade of the baroreceptor reflex responses to tilting was seen within 20 min of intravenous infusion of 40 mg of bethanidine over 5 min in 2 subjects. In neither subject was a fall in supine blood pressure below the pre-infusion level seen at any stage of the experiment. On the contrary, a rise of mean pressure of 9 mm and 11 mm occurred towards the end of the intravenous infusion of bethanidine.

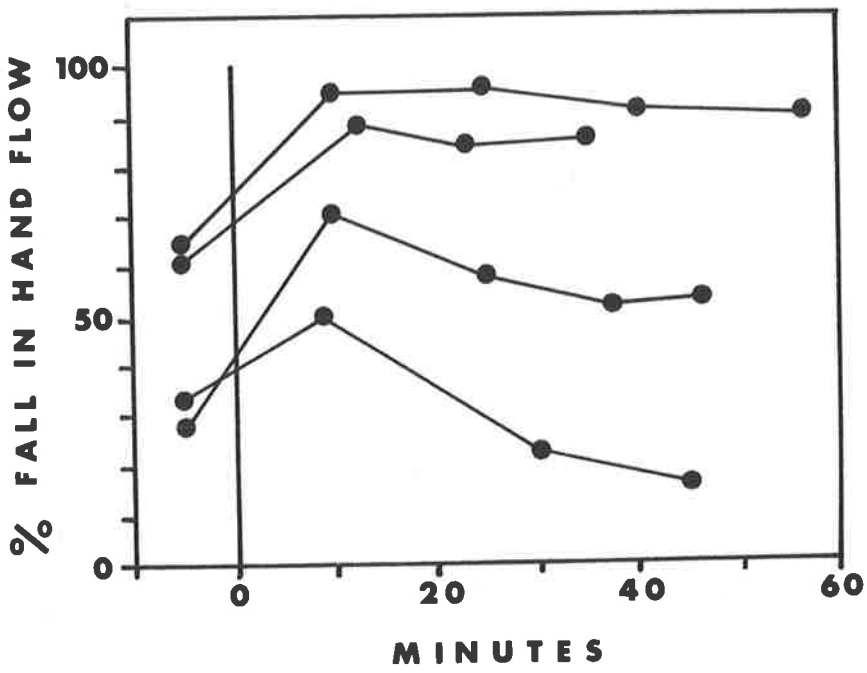


Fig. 25.

The response of the hand blood vessels to repeated doses of noradrenaline before and at intervals after bethanidine (1 mg/min for 5 min at time zero) in four subjects. The constrictor effect of noradrenaline is expressed as a percent fall in flow from the resting level. Drugs were given by the intra-arterial route.

On tilting 45° feet down after bethanidine, a profound fall in blood pressure rapidly developed and with one subject the supine position had to be restored after 2.75 min because of impending syncope, whereas before bethanidine a tilt of 5 min was tolerated without a fall in blood pressure (Fig. 26).

The responses to tilting of blood pressure, heart rate, forearm vascular resistance and blood flow before and after bethanidine in the subject illustrated in Fig. 26 are shown in Fig. 27. The normal compensatory increase in pulse rate and in forearm vascular resistance seen during tilting before bethanidine was modified or abolished after the drug. Thus, an initial small increase in pulse rate occurred which was not maintained, and by the end of the tilt, when fainting was imminent, the rate had fallen below the resting level. No increase in forearm vascular resistance occurred during the tilt after the drug. The fall in forearm blood flow at this time must therefore have been due solely to the reduced perfusion pressure.

DISCUSSION

As was discussed in Part 2B (i), the intra-arterial route of drug administration was used for most of the experiments reported here because an effective local concentration of drug can be achieved in the limb by this means, but dilution to sub-threshold levels when it reaches the general circulation avoids drug-induced changes in blood pressure or general vasomotor tone. Correction of spontaneous changes in blood pressure or vasomotor tone can be made by reference to the control side (see Appendix 2).

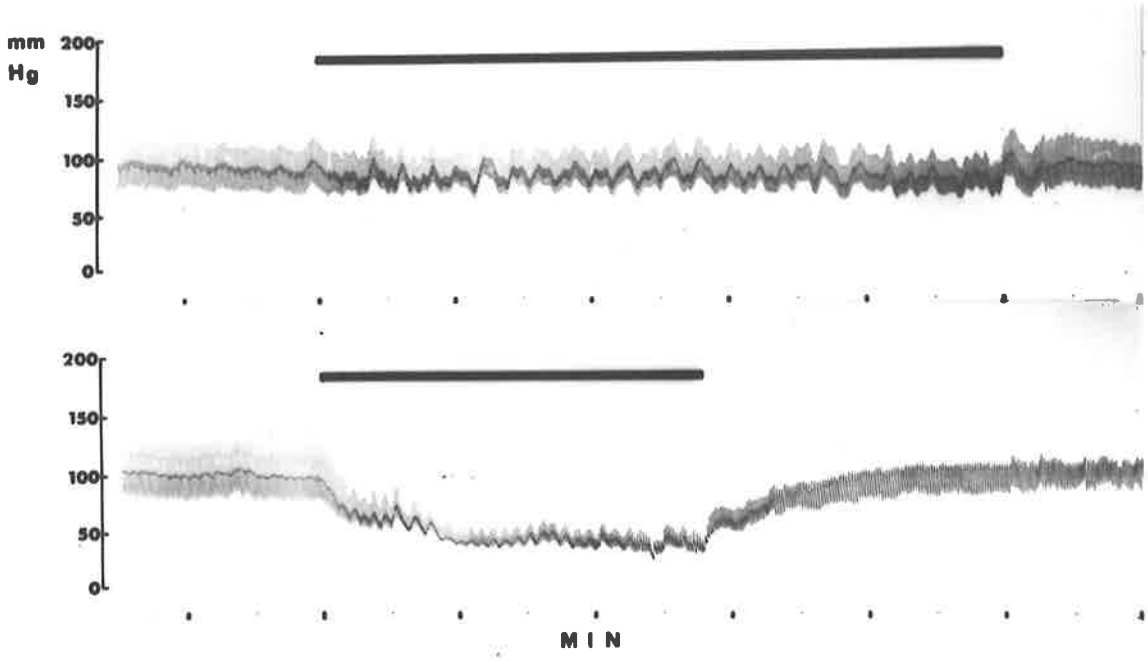


Fig. 26

The effect of tilting 45° feet down from the supine position on the brachial arterial blood pressure in one subject before (upper record) and 20 min after (lower record) intravenous infusion of bethanidine (8 mg/min for 5 min). The horizontal bar represents the duration of the tilt in each case. The heavier time signals are at minute intervals.

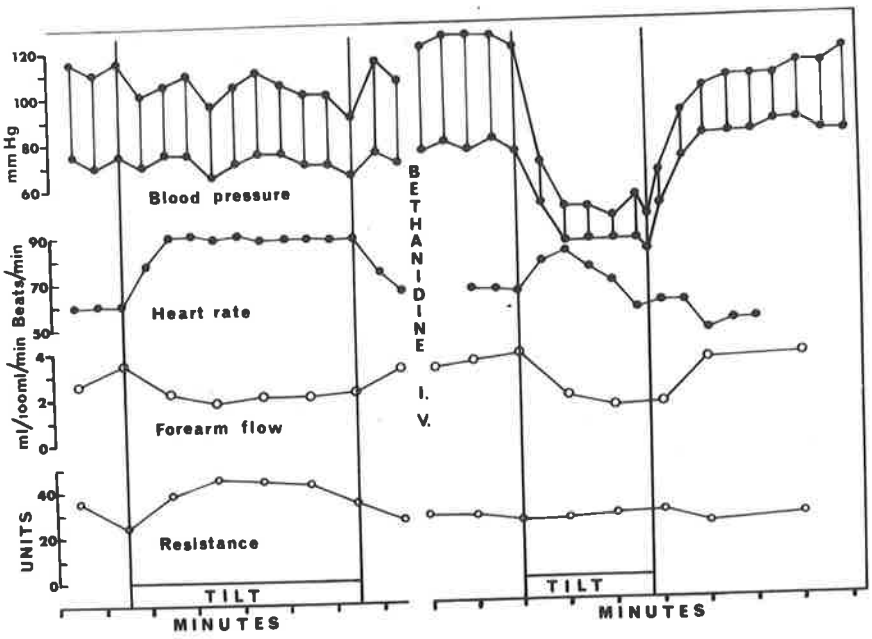


Fig. 27

The effects of tilting on the arterial blood pressure, heart rate, forearm blood flow and calculated forearm vascular resistance before (left hand frame) and 20 min after (right hand frame) intravenous infusion of bethanidine (8 mg/min for 5 min). Same subject as figure 26.

The effects of bethanidine on the forearm and hand vessels when given intra-arterially are intermediate between those previously observed with bretylium and guanethidine given by the same route (Blair et al., 1960; French and Matthews, 1961; Cooper, Fewings, Hodge and Whelan, 1963). The initial vasoconstriction, of shorter duration than after guanethidine, was abolished by phenoxybenzamine and is probably due to catecholamine release. The subsequent dilatation is associated with the increasing sympathetic blockade. If used in appropriate dosage, bethanidine, like bretylium and guanethidine, can produce virtually complete sympathetic blockade of the hand vessels.

The increase in sensitivity of the hand vessels to noradrenaline after bethanidine infusion into the arm commenced within 20 min, before sympathetic blockade was complete, and persisted in 3 of the 4 subjects for the remainder of the experiment. A similar effect has been demonstrated with bretylium and guanethidine, but was very transient in the case of the former, having disappeared by the time sympathetic blockade was complete (see Part 2B (i)). Bethanidine resembles guanethidine in the persistence of the increased sensitivity. If, as suggested in the discussion in Part 2B (i), this acute hypersensitivity is due to local release of noradrenaline, the duration of the increased sensitivity with bethanidine would be consistent with the findings of Boura et al. (1961) that this drug resembles guanethidine more closely than it does bretylium with regard to noradrenaline release.

The intravenous dose of bethanidine necessary to block the

baroreceptor reflex mechanism to a degree sufficient to cause severe hypotension on tilting is larger than might be expected from published data on oral administration in hypertensive patients. This may have been due to the fact that the present study was carried out on normotensive subjects.

The slight increase in blood pressure seen during and shortly after infusion of bethanidine is probably due to the vasoconstrictor action of catecholamines released from tissue, and suggests that used alone, bethanidine would be unsuitable for administration intravenously in hypertensive emergencies. Although from the clinical pharmacological point of view this drug is an effective sympathetic blocking agent, its place in therapy will depend on clinical trials of the drug in patients, and especially on whether tolerance or undesirable side-effects develop.

SUMMARY

1. Bethanidine (N-benzyl-N'N"-dimethylguanidine) has been administered intra-arterially and intravenously in normotensive subjects and its effects on the limb blood vessels, arterial blood pressure, vascular sensitivity to noradrenaline and the degree and time course of sympathetic nerve blockade studied.

2. The drug causes an initial constriction of hand and forearm vessels which is attributed to release of catecholamines since it is abolished by the prior administration of phenoxybenzamine. The constriction is followed by a sustained vasodilatation lasting between 5 and 20 hours.

3. Sympathetic blockade of hand vessels commences immediately on infusion and is complete within one hour of administration. Recovery occurs in about 5 hours.

4. Sensitivity to intra-arterial noradrenaline was increased within 10 - 15 min of infusion and before sympathetic blockade was complete.

5. Intravenous administration caused a slight rise in supine blood pressure. Baroreceptor reflex responses of heart and of limb vessels elicited by tilting feet down were abolished by bethanidine and postural hypotension occurred.

6. Bethanidine is a potent sympathetic blocking agent in man with properties intermediate between those of bretylium and guanethidine.

PART 2.

Section B.

(iii) Hexamethonium.

INTRODUCTION

The development of tolerance is a feature of the use of ganglion-blocking drugs as well as of the sympathetic-blocking drug bretylium tosylate (see Part 1). The mechanism of tolerance to ganglion-blocking drugs such as hexamethonium is still a matter of debate. An increase in the pressor response to injected adrenaline and noradrenaline has been demonstrated following ganglion-blocking agents in animals and in man (Page & Taylor, 1947; Corcoran & Page, 1947; Paton, 1951; Gefen & Ross, 1956). While this increased responsiveness has been attributed by some workers solely to blockade of compensatory baroreceptor reflexes (Moe, 1948; Page & Taylor, 1950; Bartorelli, Carpi & Cavalca, 1954), the findings of others suggest that an increase in the sensitivity of the peripheral vessels to circulating amines also plays a part (St. Clair & Stone, 1951; Mantegazza, Tyler & Zaimis, 1958; Maengwyn-Davis, Walz & Koppanyi, 1958). The latter proposition has not, however, been supported by the findings on all preparations tested nor for all types of hypotensive agents used (Prado & Carlini, 1959; Lum & Rashleigh, 1961; Blackman & Laverty, 1961), nor have there been any direct observations made on the development of vascular sensitivity to these drugs in man.

The present investigation was designed to determine whether increased sensitivity to noradrenaline developed in the peripheral vessels of the upper limb following hexamethonium administration in normal human subjects.

METHODS

The subjects were normal adult males between the ages of 18 and 35 years who lay supine on a couch in a room at a temperature of

21 - 23°C for one-half to one hour before observations began. The blood flow through the hands or forearms was measured by the technique of venous occlusion plethysmography using water-filled, temperature-controlled plethysmographs (see Appendix 1). Hexamethonium bromide (Vegolysen, May and Baker) in a dose of 10 mg over 5 or 10 min was infused into the forearm or hand through a 3.5 cm (23 s.w.g.) short-bevel needle introduced into the brachial artery at the elbow of one side and connected by way of a length of polythene tubing to a mechanically driven syringe which delivered 4 ml. of solution per min. Saline (0.9% sodium chloride w/v) was infused at a rate of 4 ml./min during control periods. Noradrenaline (Levophed, Bayer) was given intra-arterially in doses ranging from 0.0125 to 0.1 µg/min or intravenously in a dose of 10 or 20 µg/min. The drug was made up in saline containing ascorbic acid 1:50,000, so that the dose per min was contained in 4 ml. Flow records were taken 3 or 4 times/min.

Infusions of noradrenaline were given for 3 or 5 min and the level of flow during the last 2 min of infusion was expressed as a % fall from the previous resting level, determined as the average of all the flow measurements during the 2 min period immediately preceding the infusion. Account was taken of any progressive general drift in blood flow during the experiment by reference to the flow in the opposite control side when this was appropriate, or to the pre- and post-infusion flow in those cases of intravenous administration where both sides were receiving the drug (see Appendix 2).

RESULTS

Fig. 28 shows the responses of the forearm blood vessels to intra-arterial infusion of noradrenaline in 5 subjects before and immediately after infusion of hexamethonium into the same artery. An interval of 4 or 5 min was allowed to elapse between the end of one infusion and the beginning of the next, which was sufficient for the blood flow to return to its previous resting level. The first dose of noradrenaline to be given after the end of the hexamethonium infusion was commenced immediately without any interval. This dose was usually repeated at the end of the series of infusions. The last infusion of noradrenaline was commenced within 20 - 30 min of the end of the infusion of hexamethonium. While there were variations between the responses before and after hexamethonium, these differences were small and not constant. There was no evidence of an increased sensitivity to noradrenaline after hexamethonium. Similar experiments were carried out on hands with the same result.

A similar conclusion was reached from the experiment illustrated in Fig. 29, where a different procedure was adopted. While measuring forearm blood flow, a noradrenaline infusion into the brachial artery was commenced in a dose of 0.025 μg/min. After a period of 4 min, by which time the blood flow had reached its new level, the noradrenaline solution was replaced by one containing noradrenaline 0.025 μg/min and hexamethonium 2 mg/min. This was maintained for 5 min, after which the noradrenaline alone was continued for a further 5 min. The addition of hexamethonium to the noradrenaline infusion did not result in any further reduction in blood flow. That the vessels were capable of further

Fig. 28

The constrictor responses of the vessels of the forearms to infusions of 2 doses of noradrenaline into the brachial artery in five subjects before (0-0) and after (●-●) infusion of hexamethonium (C6) 10 mg into the same artery.

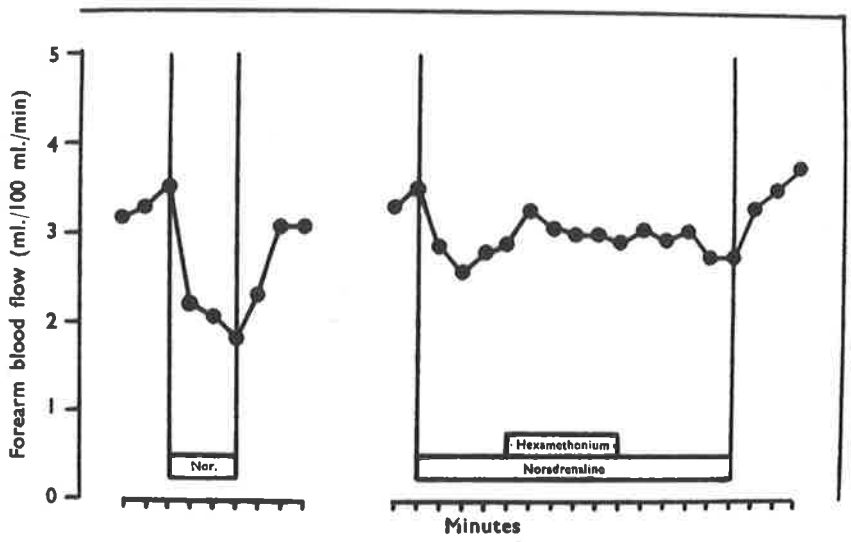


Fig. 29

The response of the blood flow through the forearm to infusions of noradrenaline (0.05 and 0.025 µg/min) into the brachial artery. Hexamethonium 2 mg/min was given for 5 min during the second infusion of noradrenaline. Each point represents the average of the four flow records taken in each min.

constriction is demonstrated by the greater response to a dose of 0.05 μg/min of noradrenaline given previously.

A similar experiment with hand vessels gave the same result.

In 3 subjects hexamethonium was given into the brachial artery of one side (1 mg/min for 10 min). Test doses of noradrenaline (2.5 μg/min for 5 min) were given intravenously, before, during and after the blocking agent, and hand blood flow measured on both sides. There was an increased constrictor response during and after the hexamethonium infusion in 2 of the subjects, but this occurred in the control hand as well as in the hand treated with hexamethonium, and to an equal degree (Fig. 30).

DISCUSSION

The intra-arterial route of administration of noradrenaline and hexamethonium was chosen for the reasons given in Section B (ii). With a resting level of flow in the forearm of 5 ml./100 ml./min, a forearm volume of 500 - 1,000 ml., infusion of hexamethonium 20 mg/min results in a local concentration of the order of 0.4 to 0.8 g/l. during the 10 min period of infusion. This is much greater than the blood concentration likely to be attained during oral or intravenous administration of the drug in hypertensive therapy where the highest recommended initial intravenous dose is 50 mg or 10 mg/l. of circulating blood volume. Hexamethonium itself given intra-arterially had no direct effect on the limb vessels.

No evidence of an increase in the constrictor response to noradrenaline was obtained either in the vessels of the hand or of the

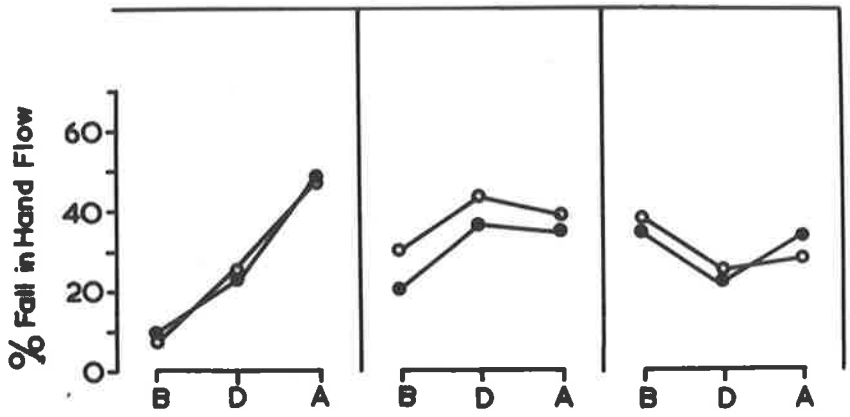


Fig. 30

The response of the blood flow through the right (0) and left (●) hands to intravenous infusions of noradrenaline (2.5 µg/min) in three subjects before (B), during (D) and after (A) infusion of hexamethonium (10 mg) into the brachial artery of the left side.

forearm whether the noradrenaline was given after hexamethonium or the hexamethonium was administered during infusion of noradrenaline when its constrictor effect had been established.

The evidence in the literature for changes in sensitivity to pressor amines after ganglion blocking agents is conflicting. Evaluation is made difficult by the different preparations, techniques and ganglion blocking agents used. One important feature that emerges from studies using blood-pressure responses of cats and dogs is that apparent sensitization by ganglion blocking drugs can occur as long as any cardio-vascular baroreceptor reflexes remain intact, and that to abolish these completely, animals must be pithed and vagotomised. Page & Taylor (1950) found total abolition of augmented responses in vagotomised, lumbodorsally-sympathectomised animals only after cord destruction below C6 and inactivation of the carotid sinus mechanism. Bartorelli et al. (1954) found potentiation to noradrenaline after hexamethonium in vago-tomised animals with spinal section, but this was abolished by pithing. Moe (1948) used pithed cats and found that the pressor responses to noradrenaline were not altered in the presence of a ganglion blocking agent. Lum & Rashleigh (1961) found no potentiation of vasoactive subtance by hexamethonium in vagotomised pithed cats or in cat carotid arterial strips.

Mantegazza et al. (1958) reported an increased blood pressure and fall in venous outflow from the hind-limb of the cat in intra-arterial and intravenous infusions of noradrenaline following hexamethonium, and inferred an increase in vascular sensitivity. However, the possible influence of a reduced perfusion pressure and increased vascular tone on the responses to intra-arterial noradrenaline has not been completely

excluded. Blockade of baroreceptor reflexes could have been responsible for the increased effect of intravenous noradrenaline.

In the present experiments the apparent increases in sensitivity of the hand vessels seen on intravenous infusion of noradrenaline in two subjects may be accounted for by the ganglion blocking action of the hexamethonium on reaching the general circulation, even though administered intra-arterially. If the response of the hand vessels to intravenous noradrenaline represents a balance between the direct constrictor effect of the drug on vessels and an indirect reflex dilatation as a consequence of the rise in blood pressure or some other central action, then blocking of this reflex effect by hexamethonium would now result in the direct action of noradrenaline being unopposed and therefore more marked. The fact that both hands behaved similarly though one received a very much higher concentration of hexamethonium than the other further supports this interpretation.

It is concluded that hexamethonium has no immediate local sensitising effect on the vessels of the upper limb in man, and, if sensitisation to circulating amines plays a part in the occurrence of tolerance to the drug, this must be a slowly developing effect. The immediate increase in responsiveness of the limb vessels to circulating noradrenaline can be accounted for by blockade of baroreceptor reflexes.

SUMMARY

1. Intra-arterial infusion of hexamethonium into the brachial artery had no potentiating effect on the constrictor response of the vessels of the forearm or hand to noradrenaline given by the same route.

2. The response of the hand vessels to intravenous infusion of noradrenaline was enhanced after intra-arterial hexamethonium, but this was attributed to entry of the blocking agent into the general circulation resulting in blockade of baroreceptor reflexes since the potentiation was seen to an equal degree on both sides.

3. It is concluded that if increased sensitivity to noradrenaline plays a part in the phenomenon of tolerance to hexamethonium this must be a slowly developing effect.

PART 2.

Section C.

The effect of long-term therapy with bretylium and guanethidine on vascular sensitivity to noradrenaline.

INTRODUCTION

In the previous section (Part 2B (i)) the conclusion was reached that the increase in noradrenaline sensitivity of hand blood vessels occurring immediately after bretylium and guanethidine was not related to the phenomenon of tolerance, but no evidence was obtained to indicate whether or not hypersensitivity occurred in the course of long-term oral therapy, or whether such sensitivity changes, if they occurred, could explain tolerance. As there is no information on these points in man the vascular sensitivity of a number of patients to noradrenaline has been studied before and during oral therapy with bretylium and guanethidine.

METHODS

Studies were commenced on five patients but completed only on two. Of the three incomplete studies one patient was so sensitive to the smallest available dose of guanethidine (10 mg) that treatment was discontinued and later changed, the second left the metropolitan area and the third was an unreliable patient living in the country who was not consistent in taking his tablets. The two patients on whom the study was completed, P.B. and D.A., were followed for ten and five months respectively. Their clinical management was undertaken by the author at a medical outpatient department in the Royal Adelaide Hospital. Neither had received treatment for hypertension previously and each was treated only with the hypotensive drug under investigation. The clinical details and results of investigations are given in Table III.

TABLE III.

Clinical and laboratory data of the two patients studied.

Patient.	P.B. Male. Age 52 yr.	D.A. Female. Age 43 yr.
Presenting complaint.	Headaches, loss of memory, disturbed vision.	Headache.
Abnormal physical signs.	B.P. 240/140. Fundi Grade IV. Cardiac apex beat beyond mid-clavicular line. Mild ankle oedema. Mild albuminuria.	B.P. 230/140. Fundi Grade II. Cardiac apex beat at mid-clavicular line. Moderate albuminuria.
Electro-cardiogram.	Indicates considerable L. ventricular hypertrophy and ischaemia.	Indicates ischaemia (inverted T-waves in leads V5 and V6).
Chest X-ray.	Gross cardiac enlargement.	Mild enlargement of heart.
Microurine.	An occasional red cell.	Epithelial cells only.
Intravenous pyelogram.	Normal.	Normal.
Urine content of 3-methoxy-4-hydroxy-mandelic acid.	Normal.	Normal.
Blood urea nitrogen	Upper limit of normal (20 mg/100 ml.)	Normal.
Serum electrolytes.	Normal.	Normal.
Haemoglobin & white cell count.	Normal.	Normal.
Erythrocyte sedimentation rate.	-	Normal.

Each patient was studied before treatment commenced, and on three occasions (patient P.B.) and two occasions (patient D.A.) while under treatment. Patient P.B. was studied 22 and 26 weeks after commencing treatment with bretylium tosylate, and 16 weeks after guanethidine was substituted. His blood pressure was never satisfactorily controlled with bretylium, a tolerance developing to doses raised progressively from 200 mg/day to 1.8 g/day. He was subsequently controlled by guanethidine 50 mg/day which replaced bretylium. Patient D.A. was studied 11 and 21 weeks after commencing treatment with guanethidine. Control of blood pressure, as evidenced by mild postural hypotension and the appearance of side-effects, was achieved 4 weeks after commencing treatment, the dose then being 40 mg/day. No further increase in dose has been necessary.

When in the laboratory the patients' hand blood flow and blood pressure were measured as described in previous sections. Noradrenaline was infused either intra-arterially into the brachial artery of one side or intravenously, and blood flow in both hands measured; with intravenous infusion, blood pressure was recorded continuously. During the final study, each patient was tilted 45° feet down to verify that the drug used was causing postural hypotension (Fig. 31). On each occasion three different doses of noradrenaline were infused intra-arterially for 4 min, and the average of the hand blood flows on the same side for the last 2 min of the infusion was compared with that for the 2 min preceding the infusion and a percentage change calculated. Hand blood flows of the opposite, uninjected side were averaged for the same periods and used in making corrections for general fluctuations in vasomotor tone or blood pressure (see Appendix). Two different doses of noradrenaline

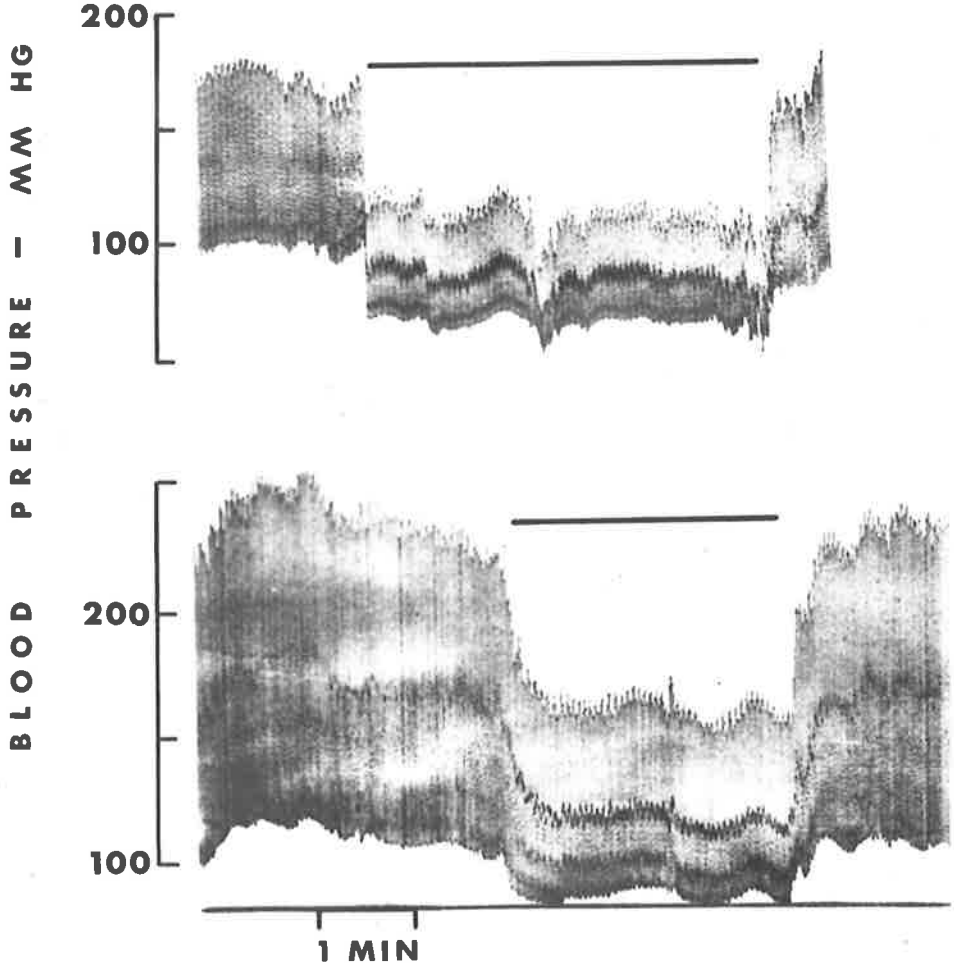


Fig. 31

The effect of tilting 45° feet down on the arterial blood pressure of patients D.A. (upper frame) and P.B. (lower frame). The period of the tilt is indicated by the black bar above each blood pressure record.

were infused intravenously for 5 min, and blood pressure was recorded continuously. Mean blood pressure ($\frac{\text{systolic} + 2 \times \text{diastolic}}{3}$) was determined from the record for the 2 min preceding the noradrenaline infusion and the last 2 min of the infusion and the percentage change calculated.

RESULTS

Fig. 32 shows the results of the four studies made on patient P.B. The response of mean blood pressure to intravenous noradrenaline and of hand blood vessels to intra-arterial noradrenaline was augmented after hypotensive therapy was commenced. These responses on the last occasion while the patient was taking guanethidine were slightly greater than when he was taking bretylium.

Fig. 33 shows the results of the three studies made on patient D.A. The responses to intra-arterial and intravenous noradrenaline were augmented after guanethidine was commenced.

An incidental observation that deserves further investigation was made during the final study on patient D.A. During the application of ice to the neck to test for sympathetic transmission the hand blood flow rose. This rise had been observed in many previous experiments when sympathetic blockade was profound, and was presumed to be due to increased perfusion pressure from a rise in blood pressure. On this occasion, however, a continuous recording of blood pressure during application of ice to the neck was made. The dilator response of hand vessels to ice was accompanied by a fall in blood pressure (Fig. 34). This implies that the increased flow must have been due to active vasodilatation in either the skin or the muscle of the hand.

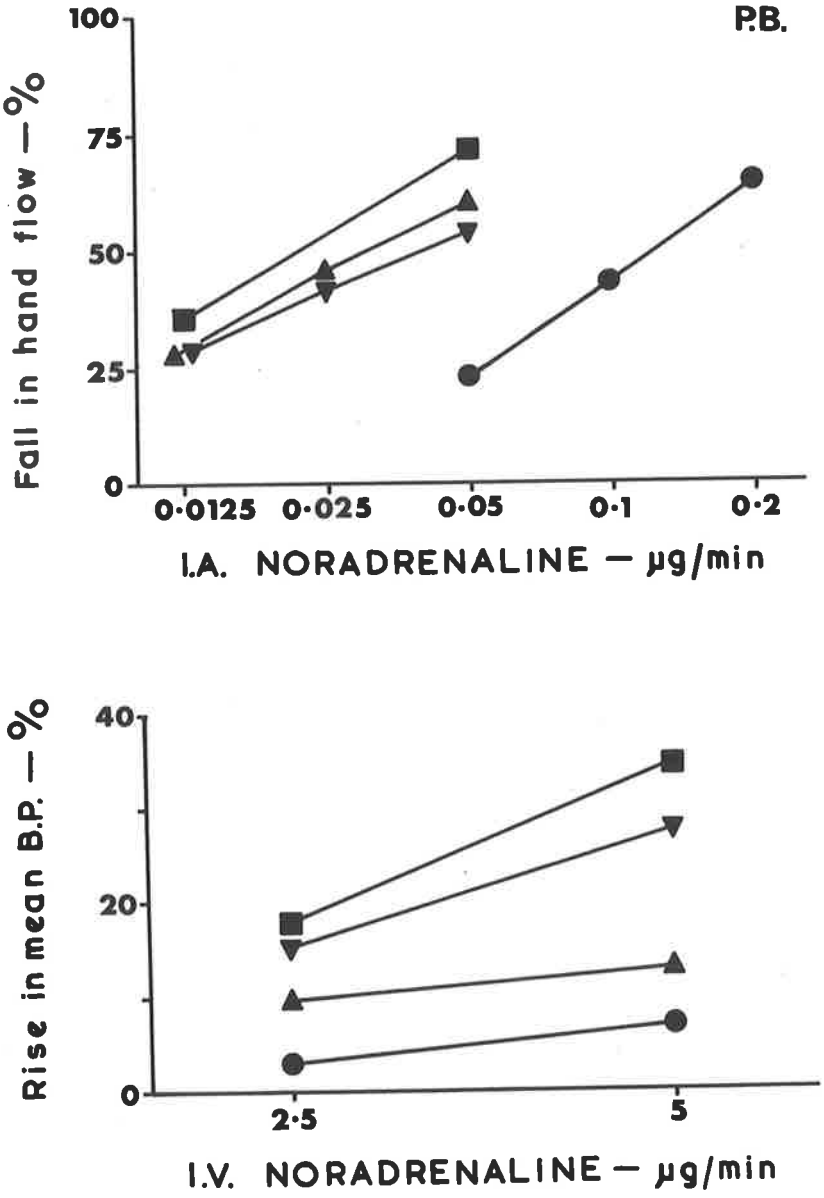


Fig. 32

Upper frame: the responses of hand blood vessels of patient P.B. to three doses of intra-arterial noradrenaline before therapy (●) and 22 weeks (▲) and 26 weeks (▼) after bretylium was commenced, and 16 weeks (■) after guanethidine was commenced.

Lower frame: the rise in mean blood pressure to two doses of intravenous noradrenaline on the same occasions as above.

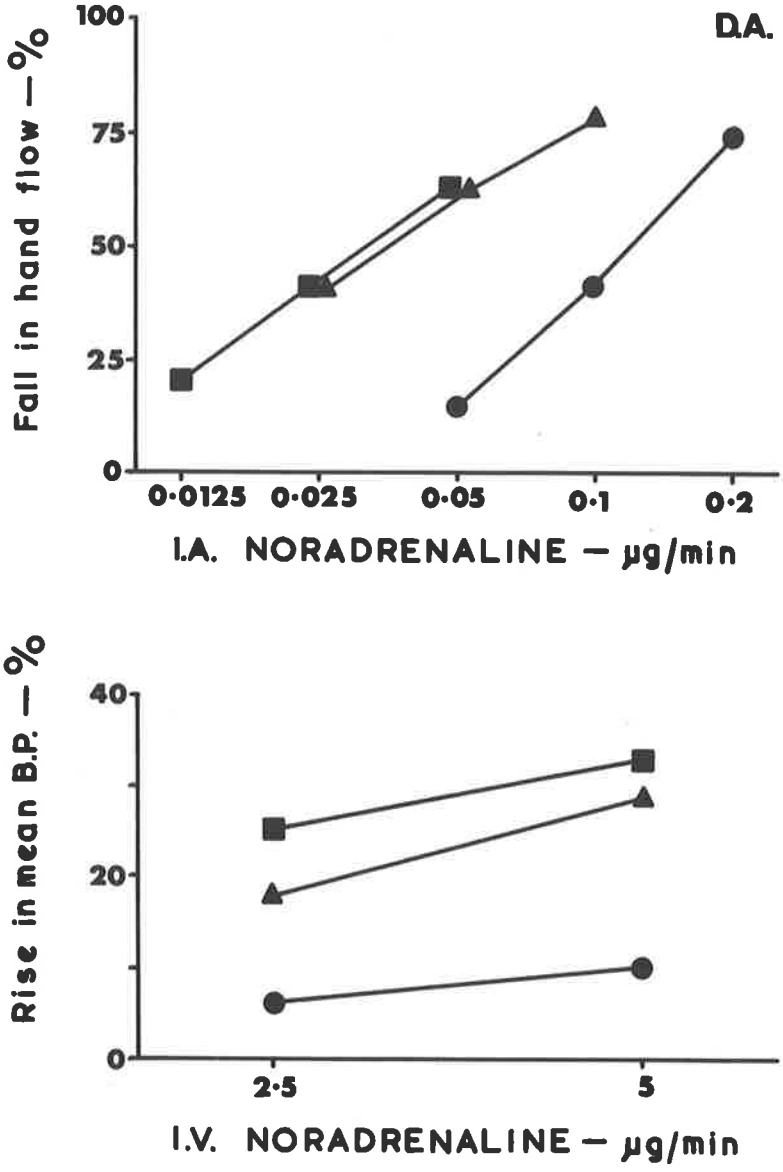


Figure 33.

Upper frame: the responses of hand blood vessels of patient D.A. to three doses of intra-arterial noradrenaline before therapy (●) and 11 weeks (▲) and 21 weeks (■) after guanethidine was commenced.

Lower frame: the rise in mean blood pressure to two doses of intravenous noradrenaline on the same occasions as above.

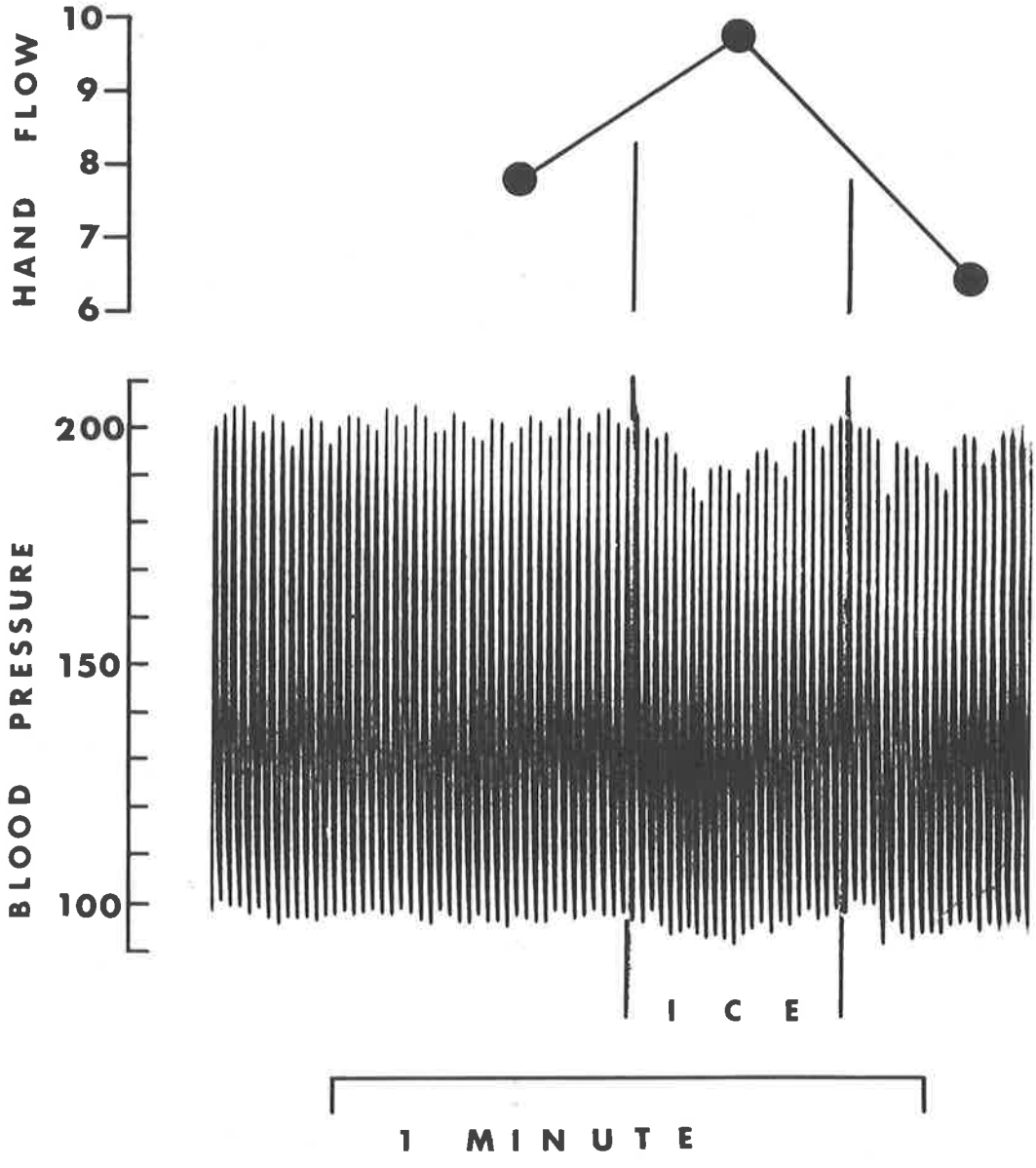


Fig. 34

The response of hand blood flow & of blood pressure of patient D.A. to ice applied to the neck (between the two vertical lines).

DISCUSSION

The findings on the two patients studied indicate that blood pressure response and sensitivity of hand blood vessels to noradrenaline is increased after the administration of the adrenergic neurone blocking drugs bretylium and guanethidine. As discussed in Part 2B (i) augmented pressor responses to noradrenaline after a drug which blocks the baro-receptor reflex arc do not necessarily indicate that true vascular hyper-sensitivity has occurred. In the present studies, however, the hand vessels have become more sensitive to local (intra-arterial) noradrenaline.

The results of the intra-arterial noradrenaline infusions in patient P.B. are particularly interesting since they show that the vascular sensitivity while he was taking guanethidine was at least as great as when he was taking bretylium. This makes it unlikely that vascular hypersensitivity is the sole cause of tolerance (see Introduction to Part 2B (i)) since patients develop this phenomenon with bretylium but not with guanethidine. In the face of the development of hypersensitivity to both drugs another explanation must be sought for tolerance.

The findings of Boura and Green (1962) in animal experiments may be relevant to tolerance in humans. They reported that after prolonged administration of bretylium, but not after guanethidine, the passage of low rates of stimuli occurred along adrenergic neurones. In some cases responses of the cat nictitating membrane to low rates of stimuli exceeded those in untreated controls. In the light of this it is possible that in humans on chronic bretylium therapy low-frequency tonic impulses may pass down sympathetic vasoconstrictor neurones and effect a rise in blood pressure, while in those on guanethidine such impulses would be blocked.

A further possibility is that the level of catecholamines in the blood of patients taking guanethidine may be lower than in those taking bretylium. At the present time there is no evidence on this point.

The problem of tolerance to hypotensive drugs is not unique, for many classes of drugs exhibit this phenomenon. In a comprehensive review of this subject in drugs affecting the nervous system, Seevers & Deneau (1963) broadly classify the possible mechanisms responsible for tolerance as (1) factors modifying access of the drug to the effector cell, including altered absorption, distribution, excretion and biotransformation; and (2), factors related to altered cellular response, including changes in cell membranes, receptors and enzymes.

Seevers & Deneau (1963), in giving an account of the large amount of work that has been done on the subject of tolerance to ethyl alcohol and the barbiturates conclude that no single factor can explain the phenomen, and that several of the mechanisms mentioned above play a part. Thus the conclusion from the present study that tolerance to bretylium is unlikely to have a single explanation (vascular supersensitivity to noradrenaline) is not surprising. No information, however, is available on other mechanisms that might play a part in the development of tolerance in the patients on bretylium, such as alterations of absorption, distribution, metabolism or cellular responses to the drug.

The present study provides the only evidence on changes in vascular sensitivity to noradrenaline in humans on long-term therapy with bretylium and guanethidine.

SUMMARY

1. Studies of vascular sensitivity to noradrenaline were carried out on two patients with essential hypertension before and during oral therapy with adrenergic neurone blocking drugs. One patient was treated with guanethidine, the other was treated initially with bretylium and later changed to guanethidine.

2. The responses of hand blood vessels and of blood pressure to noradrenaline were augmented during hypotensive therapy, and in the second patient were at least as great during guanethidine as during bretylium therapy.

3. As severe tolerance occurs to bretylium but not to guanethidine during oral therapy, vascular hypersensitivity to noradrenaline cannot be invoked as the sole cause of this phenomenon.

PART 2.

Section D.

Antagonism of adrenergic neurone blocking drugs by amphetamines.

INTRODUCTION

In studying the first adrenergic blocking drug TM10, Bain & Fielden (1956) noted that the effectiveness of nerve stimulation on a rabbit gut preparation previously treated with the drug was immediately restored by dopamine, but the block usually returned after washing out the bath. Further work did not disclose the mechanism of the reversal of the block. Nasmyth & Andrews (1959) found that TM10 failed to block adrenergic nerves in preparations from animals that had previously received cocaine. Further investigation showed that cocaine administered after TM10 would restore sympathetic transmission.

Boura & Green (1959) found that cocaine antagonised the adrenergic blocking effects of bretylium on rabbit gut and cat nictitating membrane. Day (1961) carried the investigations further, finding that dopamine antagonised the blocking action of bretylium and guanethidine, and in later studies (Day, 1962) a wider group of drugs was examined for antagonism of TM10, bretylium and guanethidine. He found that the sympathomimetic amines dexamphetamine, mephentermine, hydroxyamphetamine, ephedrine and phenethylamine were all effective in preventing or reversing adrenergic neurone blockade in isolated rabbit ileum or cat nictitating membrane. Tyramine and dopamine were effective only in the former preparation. Monamine oxidase inhibitors, if possessing sympathomimetic activity (phenelzine, pheniprazine and tranycypromine), were also antagonists. Extending the observations of Nasmyth & Andrews (1959) with TM10 and of Boura & Green (1959) with bretylium, Day (1962) found that cocaine also antagonised the blocking action of guanethidine. Day & Rand (1962a) confirmed these findings with other preparations, and after

subsequent work (Day & Rand, 1963) postulated that the antagonism of guanethidine by dexamphetamine was competitive. Bentley (1962) and Bentley & Sabine (1963) added noradrenaline, histamine, 5-hydroxytryptamine and carbachol to the list of antagonists to bretylium and guanethidine in guinea-pig hypogastric vas deferens preparations.

A small number of clinical reports of antagonism of adrenergic neurone blocking drugs have appeared. Wilson & Long (1960) found that four hypertensive patients taking amphetamine compounds for weight reduction failed to respond to bretylium, but that satisfactory control was established after withdrawal of the amphetamine. In a number of their hypertensive patients with blood pressure satisfactorily controlled by bretylium, the administration of amphetamine caused a temporary rise in blood pressure. Laurence & Rosenheim (1960) reported that 20 - 30 mg methylamphetamine given to two patients on guanethidine suffering from postural hypotension antagonised the postural fall in mean blood pressure.

With the widespread use of the post-ganglionic adrenergic neurone blocking drugs in the treatment of hypertension, and particularly in view of the long period of action of guanethidine, the most commonly used of this class of drug, it is most important that an effective antagonist should be available. Animal experiments suggest that the amphetamine group of drugs will reverse the blockade induced by guanethidine or similar drugs. The evidence, however, rests on the use of high concentrations of amphetamines often for a relatively long period, conditions which may not be reproducable in a patient. The evidence of Wilson & Long (1960) and Laurence & Rosenheim (1960), shows that blood pressure can be restored by the amphetamines after bretylium and guanethidine. Such evidence in

the human, however, does not necessarily prove that sympathetic transmission has been restored by the amphetamines.

In giving the reasons for this statement, it should be remembered that the amphetamines is a group of indirectly-acting sympathomimetic drugs requiring noradrenaline stores for release before they can have their action (Carlsson, Rosengren, Bertler & Nilsson, 1957; Burn & Rand, 1958). Further, neither bretylium nor guanethidine depletes the adrenal medulla of catecholamines (see Parts 1 and 2B (i)) and the amphetamines are capable of releasing catecholamines from the gland (Schümann & Phillipu, 1962). There is no evidence to indicate the degree of peripheral depletion of catecholamines nor the proportion of adrenergic nerves still capable of transmission in an ambulant patient under treatment with bretylium or guanethidine, but it is unlikely that all adrenergic nerves are completely blocked and that all peripheral stores of catecholamines are depleted.

Thus it can be seen that the amphetamines could raise blood pressure in a patient by releasing adrenal and possibly peripheral stores of catecholamines without unblocking a single adrenergic neurone. The effect of the catecholamines in raising blood pressure would be intensified if they were released into a hypersensitive or hyper-reactive vascular system.

In view of the lack of direct evidence for reversal of adrenergic neurone blockade in the human it was decided to investigate this problem in normal volunteers with bretylium, bethanidine and guanethidine, and in hypertensive patients being treated with guanethidine.

METHODS

The subjects were normal volunteers who lay supine on a couch capable of being tilted 45° feet down. Hand blood flow was measured as described in Appendix 1. Blood pressure, from a 22-gauge needle inserted into a brachial artery at the cubital fossa via a differential capacitance electromanometer and a carrier amplifier, was recorded on ultraviolet light-sensitive paper. In some experiments the subject was tilted 45° feet down to determine the effect on hand blood flow and blood pressure. As the hands and precordia of the subjects were in the axis of the tilt, little change in vertical height of those parts occurred with tilting. The sympathetic stimulus was ice applied to the neck as described in Part 2B (i).

Sympathetic blockade of one hand was achieved in some subjects by intra-arterial infusion of 20 mg bretylium, 5 mg guanethidine or 5 mg bethanidine, infused over a period of 5 min; in others, both hands were blocked by infusing the same dose of drug into each brachial artery simultaneously. In the latter group, before the adrenergic blocking drug was given, noradrenaline was infused simultaneously into each side in the same dose and the resulting fall in hand blood flow measured to ensure that an approximately equal perfusion of each hand was occurring. A fall in hand blood flow on each side differing by not more than 10% was regarded as acceptable. Generalised adrenergic neurone blockade was achieved in one subject by the intravenous infusion of 50 mg bethanidine over 5 min.

When local or general sympathetic blockade had been achieved, methylamphetamine hydrochloride, 20-50 μg/min for 1 - 2 min intra-

arterially or 1 mg/min for 2 min intravenously was infused to determine its effect on the blockade.

RESULTS

Unilateral sympathetic blockade of hand vessels

In two subjects in whom hand blood flow was being measured bethanidine 5 mg over 5 min was infused into the brachial artery of one side and its effect on vasoconstrictor responses to ice observed to determine the duration of the sympathetic nerve blockade caused by the drug. On a second occasion bethanidine was again infused into the same arm of each subject and when sympathetic blockade was established methylamphetamine (20 μg/min for 2 min) was infused at a time when in the previous experiment sympathetic responses were still abolished. The results of the four experiments on these two subjects are shown in Fig. 35. As only one side has been treated, the responses to ice of that side have been related to those of the control side by applying the formula I/C.100, where I and C are the percentage falls in flow on the injected side and control side respectively resulting from the application of ice. Subject G.L. showed a considerable restoration of sympathetic transmission after methylamphetamine, but subject G.S. showed a much smaller degree of restoration.

Bilateral sympathetic blockade of hand vessels

In order to obtain more controlled conditions than in the two subjects just described, sympathetic blockade was induced in both hands of 6 subjects by simultaneous bilateral brachial arterial infusion of one of the adrenergic neurone blocking drugs. Three subjects received

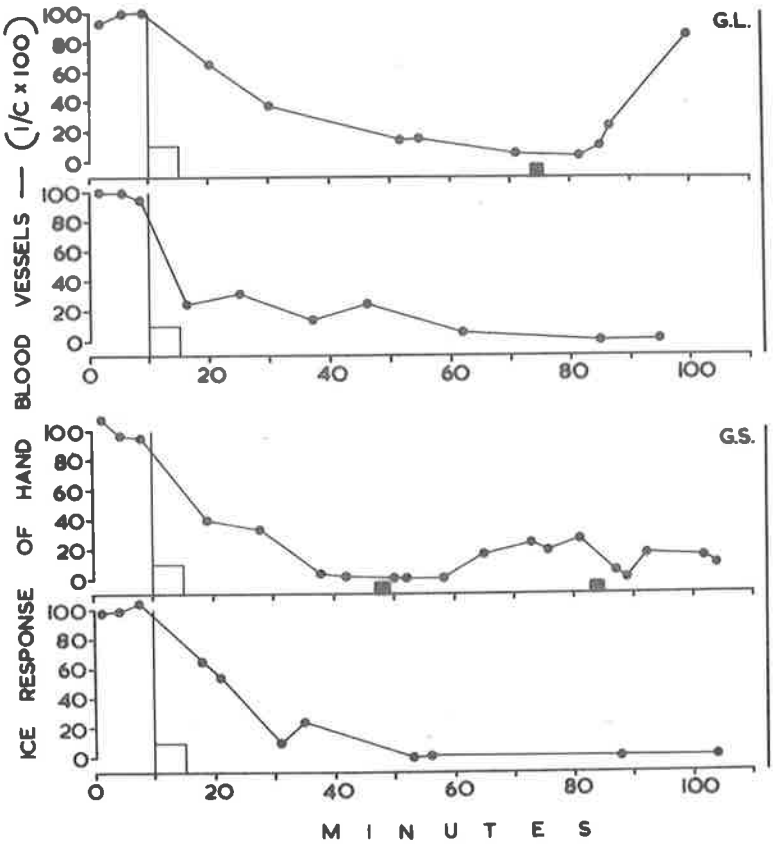


Fig. 36

The responses of the hand blood vessels of two subjects to ice applied to the neck before and after the intra-arterial infusion of bethanidine 1 mg/min for 5 min (hollow rectangle).

During one of the two experiments on each subject intra-arterial methylamphetamine (20 ug/min for 3 min) was infused on one or two occasions (black rectangle). The response to ice is calculated as the ratio of the fall in blood flow of the injected hand (I) to that of the control hand (C) times 100.

bethanidine, two received bretylium and one received guanethidine. When sympathetic blockade was complete on both sides, as judged by the absence of constrictor responses of hand vessels or the appearance of dilator responses to ice applied to the neck, methylamphetamine was infused into the brachial artery of one side until the blood flow on that side fell by 50 - 70%. This took 1 - 3 min with the doses used (20 - 50 μg/min). After the methylamphetamine infusion the ice stimuli were continued and the responses measured in each hand. If the blood flow on the side that had received methylamphetamine rose towards control values the drug was infused again. The details of one of these experiments are shown in Fig. 36, and the results of all six experiments are illustrated in Fig. 37.

It can be seen that the effect of methylamphetamine in restoring sympathetic transmission is not consistent but that in all cases there has been some return in response when compared with the opposite side.

Intravenous administration

One subject was given 50 mg bethanidine intravenously over a period of 5 min. The brachial arterial blood pressure and hand blood flow were measured and the responses to ice applied to the neck and to tilting 45° feet down were observed before and after the bethanidine infusion.

Sixty-four min after the drug, at a time when constrictor responses of hand vessels to ice were abolished, tilting caused the systolic blood pressure to fall to 75 mm Hg and the subject to feel faint. The horizontal position had to be restored 8 min after tilting, whereas before bethanidine the subject was tilted for 10 min with no

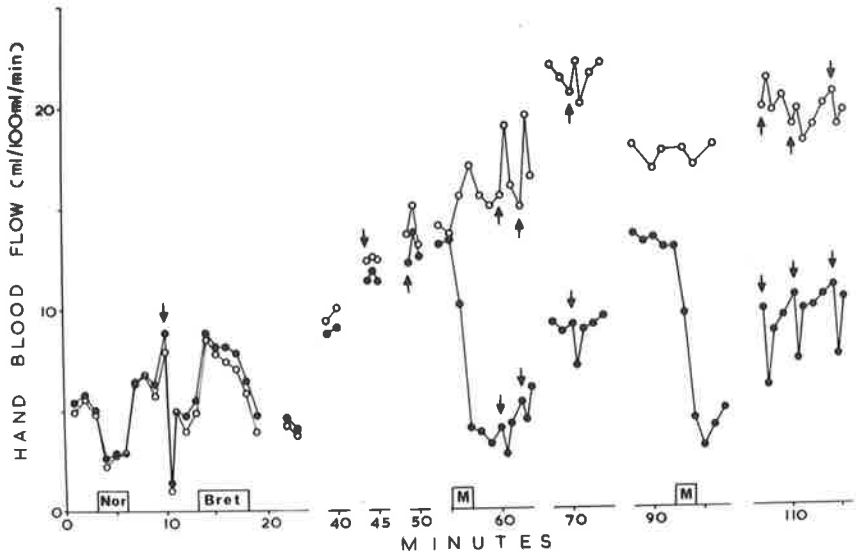


Fig. 38

The details of an experiment in which an attempt was made to reverse the sympathetic blockade of hand blood vessels induced by bretylium. Nor and Bret, bilateral intra-arterial infusion of noradrenaline and bretylium 0.05 µg/min for 3 min and 4 mg/min for 5 min respectively; M, intra-arterial methylamphetamine 80 µg/min for 2 min into one side (●); upward or downward pointing arrows, application of ice to the neck.

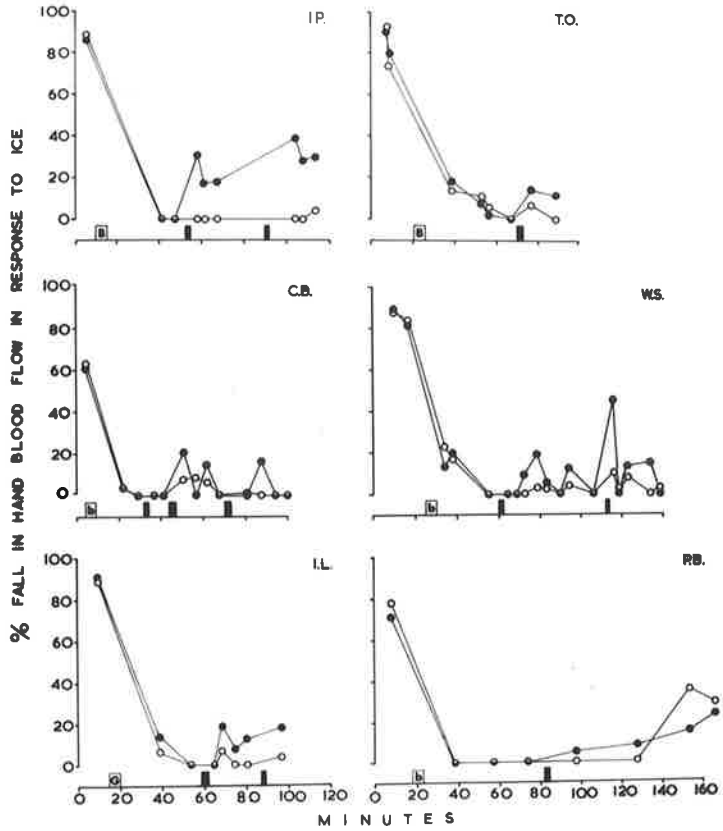


Fig. 37

The results in six subjects of the type of experiment illustrated in Fig. 36. The open and closed circles represent the percentage falls in the blood flow in each hand in response to ice applied to the neck. B, bilateral intra-arterial (I.A.) bretylium; b, bilateral IA. bethanidine; G, bilateral I.A. guanethidine; black rectangles, unilateral methylamphetamine (20-50 µg/min for 1-3 min) into the side represented by the closed circles.

feeling of faintness and little or no fall in systolic pressure. Twenty min later the subject was again tilted and when he reported that he felt "funny", methylamphetamine 1 mg/min was infused intravenously for 2 min and the 45° tilt maintained. The blood pressure was rapidly restored and rose above the pre-tilt level, reaching 190 mm Hg systolic 5 min later. In spite of this rise of blood pressure the hand vessels continued to show no constrictor response to ice. Fig. 38 shows the blood pressure recordings of this experiment.

DISCUSSION

The results of the experiments in which methylamphetamine was infused intra-arterially indicate that it partially reverses the sympathetic blockade induced by the adrenergic neurone blocking drugs, but the degree of this reversal is variable and usually modest. As Day & Rand (1963) postulate that the antagonism of guanethidine by dexamphetamine is competitive, and as Bentley (1963, personal communication) says that a guanethidine-blocked hypogastric-vas deferens preparation may require treatment with a high concentration of amphetamine for up to 40 min, it may be argued that the hand vessels in the present experiments required a higher concentration or a more prolonged administration of an amphetamine for a dramatic reversal of sympathetic blockade to occur. However, in some earlier experiments intra-arterial methylamphetamine in a barely threshold dose (sufficient to cause 10 - 20% fall in hand blood flow) for 10 - 15 min caused no detectable return of the ice response, and with a greater concentration of methylamphetamine than that used in the experiments illustrated in Fig. 37, the hand vessels constricted to such a degree that no further constriction could be seen or expected when ice was

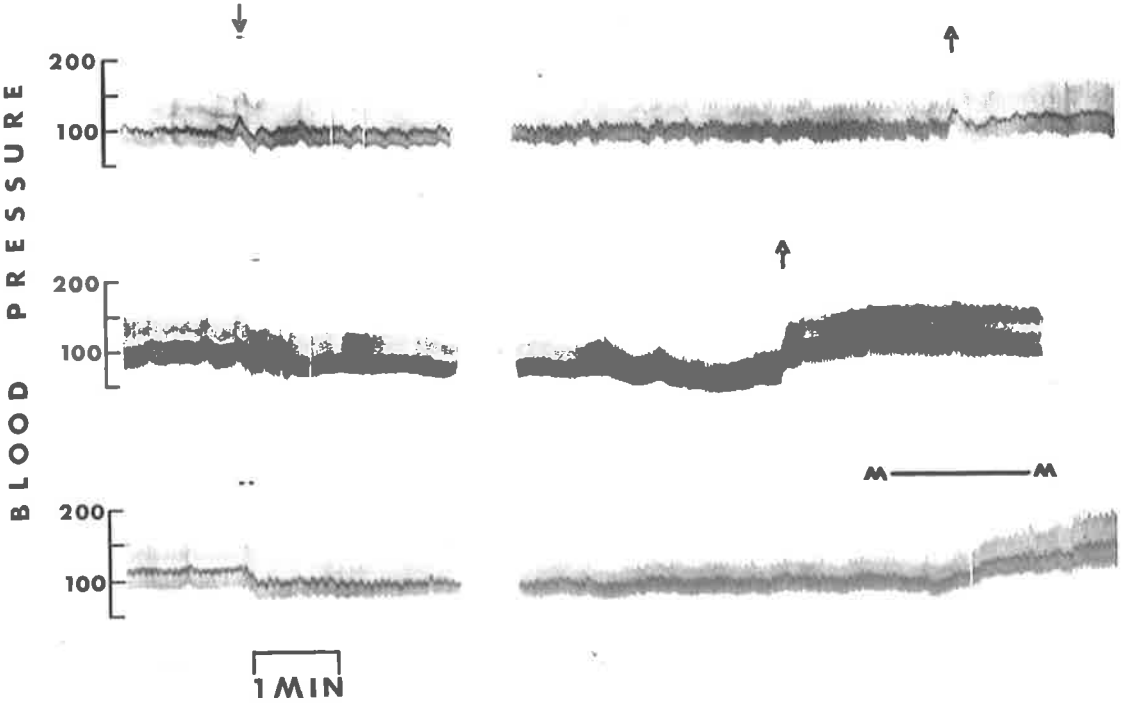


Fig. 38.

The responses of the arterial blood pressure (mm Hg) of a subject to tilting 45° feet down before (upper record) and after (middle and lower records) intravenous bethanidine 10 mg/min for 5 min. M–M, the intravenous infusion of methylamphetamine 1 mg/min for 2 min while the tilted position was maintained; ↓ , the time in each record at which the subject was tilted 45° feet down; ↑ , subject restored to horizontal position (upper and middle records). The break in each record was for 2.5 min.

applied to the subject's neck. It seems, therefore, that the limitations of the experimental technique used here will prevent the observation of complete reversal of sympathetic blockade by an amphetamine. However, it has been shown that partial reversal can occur in hand blood vessels following methylamphetamine infusion.

The subject given intravenous bethanidine and methylamphetamine illustrates the point made in the introduction that postural hypotension may be reversed by an amphetamine without it being necessary to postulate that unblocking of sympathetic nerves has occurred, for this subject developed postural hypotension paralleling the onset of blockade of hand vessels which was reversed by methylamphetamine without any return in the response to ice of hand vessels. Although it cannot be denied that sympathetic transmission may have been restored in other vascular beds, it is unlikely that the hand vessels would not have shared some of the restoration of sympathetic nerve function.

SUMMARY

1. Sympathetic blockade of the hand vessels of 8 normal subjects following intra-arterial infusion of an adrenergic neurone blocking drug was partly reversed by methylamphetamine by the same route and in a dose which reduced the blood flow by 50 - 70%.

2. Intravenous methylamphetamine reversed the postural hypotension occurring in a normal subject after the intravenous administration of bethanidine, but did not restore the constrictor responses of the hand blood vessels to ice.

PART 3.

GUANETHIDINE IN PERIPHERAL VASCULAR DISEASE.

Iontophoresis as a possible mode of therapy.

INTRODUCTION

The drug treatment of peripheral vascular disease presents many problems. If an effective dose of a vasodilator is taken orally the resultant hypotension may further lower the blood supply to an ischaemic area, especially if the vessels supplying the area are the seat of organic changes impairing their ability to dilate. Intra-arterial administration is one possible solution as the high local drug concentration that can be achieved is greatly reduced on reaching the general circulation, thereby minimizing undesirable systemic effects. However, technical difficulties and the possibility of a traumatic aneurysm at the site of repeated arterial punctures are disadvantages; also the action of most drugs given in this way is evanescent. The most satisfactory is probably reserpine (de la Lande, Parks, Sandison, Skinner & Whelan, 1960), but even with this drug the dilatation only lasts for about 24 hrs.

Another possible mode of local drug administration is iontophoresis (moving ions into the body by a galvanic current). This simple technique has been used since 1908 to introduce many drugs (Leduc, 1908; Abramowitsch & Necoussikine, 1946) and has the advantage that the area of administration and the dose of drug can be controlled. The experience gained with guanethidine (see Part 2B (1)), its long duration of action and wide use in the treatment of hypertension suggested that it may be of use in certain types of peripheral vascular disease if it retained its pharmacological properties after introduction by iontophoresis. The following studies were undertaken to investigate this possibility.

METHODS

Healthy male volunteers were studied. Experiments were carried out in a temperature-controlled laboratory at 23 ± 2°C. The drug was introduced by iontophoresis into the distal portion of a finger as follows. 10 mg guanethidine were dissolved in 10 ml. distilled water in a glass beaker; the terminal portion of the finger to be treated and the anode (copper or platinum) were then placed in this solution. Copper was found to be a more effective anode than platinum but had the disadvantage of causing blue staining of the subject's finger. This problem was overcome by using an agar-saline bridge from a beaker containing the copper electrode in saline to a second beaker containing the drug and the subject's finger. This system proved to be more effective than either copper or platinum used alone. The cathode consisted of a 15 cm x 10 cm piece of metal gauze covered with a cloth pad soaked with normal saline (0.9% NaCl w/v) and firmly strapped to a leg. Two radio "B" dry-cell batteries connected in series provided an electromotive force of 90 volts across a potentiometer of 1000 ohms resistance. An ammeter reading from 0 - 5 milli-amps, and an on-off switch were also placed in the circuit (see Fig. 39). A current of 4 milli-amps was used as more than this produced pain. The period of iontophoresis varied from 10 - 30 min. Make or break shocks were avoided by increasing or decreasing the current slowly. Nitrogen was bubbled through the drug solution to maintain a uniform concentration.

To determine the effects of guanethidine on blood flow, the heat elimination from corresponding fingers of each hand of 10 subjects was measured by calorimetry (Greenfield & Shepherd, 1950; Whelan, 1951).

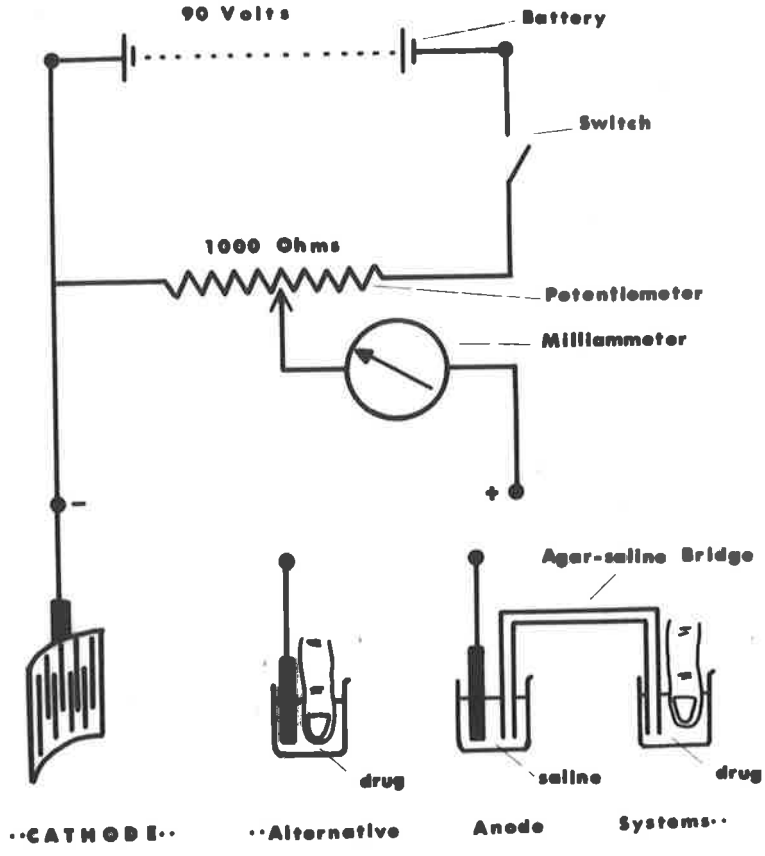


Fig. 39

The circuit diagram and arrangement of apparatus for iontophoresis.

Guanethidine was iontophoresed into one of the fingers while the other was untreated and acted as a control. The heat elimination from each finger was measured for 10 min before iontophoresis and for the same period 20 min after iontophoresis. Twenty min was allowed to elapse before the second period of measurement because earlier experiments had shown that iontophoresis of saline could cause a small rise in heat elimination lasting up to 15 min. The heat elimination of a finger is expressed in calories per 100 ml. of finger tissue per min, and in presenting the results the mean of the readings in the 10 min recording periods have been used.

The degree of sympathetic block due to iontophoresis of guanethidine was assessed in five subjects by immersing a foot in ice-cold water for 10 min and comparing the reduction in heat output of the treated finger with that of the control finger.

The bromcresol-green dye-conjugation method of Dollery, Emslie-Smith & Milne (1960) was used for assaying the guanethidine remaining in the beaker after iontophoresis to indicate the amount that had entered the finger. (All assays for guanethidine were performed by Messrs P. L. Reilly & D. W. Thomas.)

RESULTS

Fig. 40 shows data from experiments on 10 subjects. Guanethidine was iontopheresed into one finger for 10 min in 2 subjects, and for 30 min in 8 subjects, 5 of whom returned 24 hrs later for further readings. As the heat output from the two fingers during the control period was rarely identical, the relationship of the two has been expressed as a percentage according to the formula $\frac{T}{C} \times 100$,

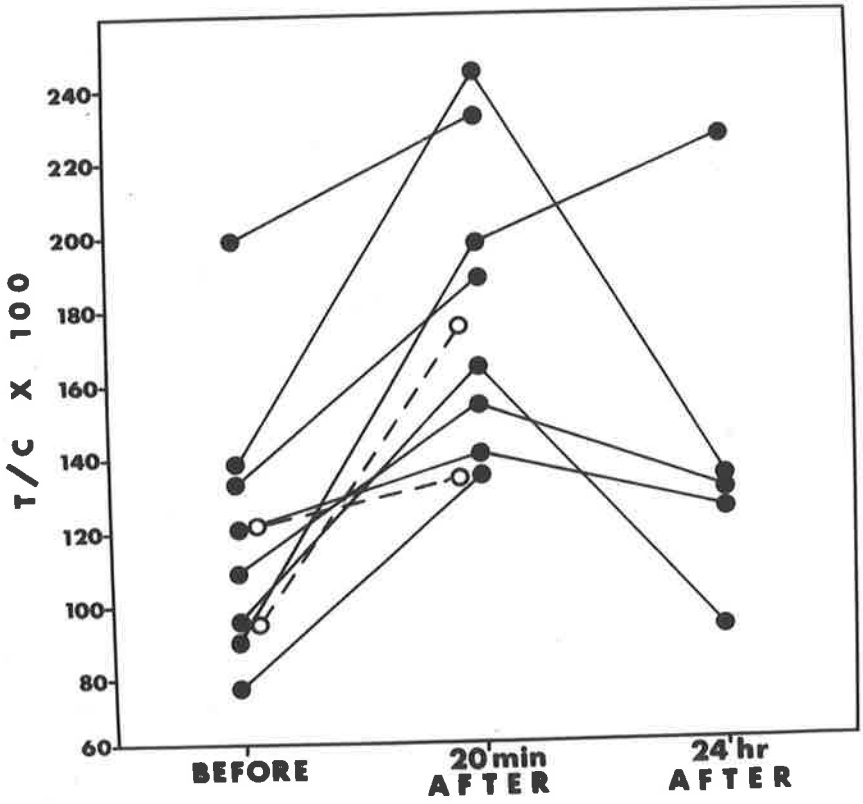


Fig. 40.

Changes in heat output of the fingers of 10 subjects after iontophoresis of guanethidine. Points represent heat elimination (cal/100 ml./min) of a guanethidine-treated finger (T) divided by that of a control finger (C) multiplied by 100. The lines join results of individual subjects before and after iontophoresis of guanethidine for 30 min (closed circles) or 10 min (open circles).

where "T" and "C" are the heat eliminations from the treated and control fingers respectively. When the heat output of the iontophoresed finger is higher than that of the control side the value is above 100%, and when lower, is below 100%. As can be seen in the figure the heat elimination from the iontophoresed finger was higher in all cases when measured 20 min after treatment, but had returned to control levels in 4 of the 5 subjects who were examined 24 hr later. The responses in the two subjects treated for 10 min did not differ from those treated for 30 min. The mean increase in heat output at 20 min was 53% (range 8% - 115%). In contrast, in an experiment in which 5 mg guanethidine was introduced into the brachial artery the heat elimination increased by 192% and remained elevated for more than 24 hr.

In 5 experiments immersion of the foot in ice-cold water for 15 min gave variable results with calorimetry. Two of the subjects showed no difference between iontophoresed and control fingers, while the other three showed responses on the iontophoresed side which were 50%, 60% and 71% of those on the control side.

Only a few subjects noticed a sensation of slight warmth in the finger after iontophoresis. Mild reddening of the skin occurred in the finger of most subjects immediately after iontophoresis, but was no more prominent after guanethidine than after saline.

The results of all calorimeter experiments were examined for evidence of vasoconstriction immediately after the iontophoresis of guanethidine, since an initial vasoconstriction occurs after intra-arterial administration (Cooper, Fewings, Hodge & Whelan, 1963), but no

such evidence was found. It is possible however, that the dilatation due to iontophoresis itself could have masked a short-lived constriction due to guanethidine.

For a 30 min iontophoresis at 4 milli-amps, the amount of drug entering the finger varied widely with each of the electrode systems used, and ranged from 0.7 to 4.6 mg. Irrespective of the electrode, there was no correlation between the dose of drug the subject received and its effect in increasing the heat elimination from the finger.

The passage of an electrical current through a guanethidine solution does not affect its concentration. This was determined in control experiments by electrolysis of a solution containing 10 mg of guanethidine for 30 min at 4 milli-amps.

In 6 of the subjects, blisters appeared on the treated fingers from 1 - 6 days after iontophoresis. The blisters later broke down to leave ulcers that healed slowly with scar formation. These subjects had had a wide range of doses of guanethidine introduced over 30 min.

In one subject saline alone was iontophoresed for 1 hr at 4 milli-amps without subsequent harm to the skin of the finger.

DISCUSSION

The increase in heat elimination and the degree of sympathetic nerve blockade seen after iontophoresis of guanethidine are very modest when compared with the results of intra-arterial administration of the drug. In the experiments reported an effect was obtained after iontophoresis of a range of doses from 0.7 - 4.6 mg. As the finger volume

used for iontophoresis was about 6 ml., these doses represent drug concentrations of approximately 0.1 - 1 mg/ml. of tissue. With intra-arterial injection, 5 mg guanethidine was introduced into 1200 ml. of forearm and hand tissue, representing a concentration of about 0.004 mg/ml. of tissue. Obviously most of the guanethidine during iontophoresis is not reaching the blood vessels or sympathetic nerve terminals. We cannot say yet whether its passage through the tissues during iontophoresis renders the guanethidine therapeutically inactive, or whether there are physical barriers to its passage in effective amounts.

The occurrence of blisters and subsequent skin necrosis due to guanethidine has not been reported in man, but Bein (1960) has stated that the drug in concentrations of 0.5 and 1% caused "strong local irritation" of guinea pig skin after intracutaneous injection, and Boura & Green (1962) noted that moderately severe necrosis often occurred at the site of guanethidine injections in cats.

It is concluded that iontophoresis of guanethidine is not useful clinically, as introduction of large doses of the drug causes only a moderate increase in blood flow and incomplete sympathetic blockade, and may also cause tissue necrosis. These findings do not necessarily apply to other sympathetic blocking drugs in existence or which may be developed in the future. The possibility of introducing them by this potentially useful method should be tested. Much work on the behaviour of drugs during iontophoresis into human skin remains to be done.

SUMMARY

In a search for more efficient methods of treatment of peripheral vascular disease, the technique of iontophoresis of guanethidine into fingers of healthy male volunteers has been investigated. The effects of this drug on heat output in 10 subjects and on sympathetic vasoconstrictor reflexes in 5 subjects was measured by calorimetry. Although an increase in heat output and partial sympathetic blockade occurred, the degree of these changes was much less than after intra-arterial guanethidine, and the doses of drug required per unit volume of tissue were greater. Furthermore, the occurrence of ulcers after iontophoresis precludes this form of administration from clinical practice.

PART 4.

STUDY OF VASCULAR SENSITIVITY AND THE USE OF ANGIOTENSIN IN A CASE OF PHAEOCHROMOCYTOMA.

INTRODUCTION

Phaeochromocytoma was first described by Fränkel in 1886 in a young girl of 18 years who suffered paroxysms of palpitation, pallor, dizziness, headache and nausea. She died during an attack and at autopsy bilateral adrenal tumours were found. The next description of the association of paroxysmal hypertension with phaechromocytoma was that of Labbé, Tinel & Doumer in 1922; the first (unsuccessful) attempt at removal was made by Masson & Martin in 1923; Roux in 1926 excised a tumour successfully in an undiagnosed patient (all cited by Millar, 1957). Mayo (1927) removed a tumour above the left kidney from a young patient suffering from paroxysmal hypertension with complete cure following operation. The first correct preoperative diagnosis with successful surgery was made by Pincoffs in 1929 (cited by Hume, 1960).

In recent years large series of this condition have been reported (Graham, 1951; Hume, 1960; Gifford, Kvale, Mahler, Roth & Priestley, 1961; Price & Secher, 1961; Glenn & Mannix, 1963 and Crandell & Myers, 1964), including extensive descriptions of clinical features, diagnostic methods and surgical and anaesthetic management. Most authors agree on the essential role of the adrenaline antagonists to control hypertensive episodes during operation, and on the use of directly - or indirectly - acting sympathomimetic drugs for the hypotension commonly occurring after removal of the tumour or the clamping of its pedicle. The use of these hypertensive agents, however, still poses a number of problems that remain to be solved.

This paper reports investigations carried out on a patient with phaeochromocytoma who was studied before operation to determine the relative merits of noradrenaline and of angiotensin as pressor agents in the presence of an adrenaline antagonist, phentolamine, and describes his management during operation when angiotensin was successfully used to elevate blood pressure. This is thought to be the first report of the use of angiotensin during surgery for this condition. The results of studies of vascular sensitivity to noradrenaline before and after removal of the phaeochromocytoma are also presented.

CASE REPORT

The patient, a 46-year-old male was admitted to the Royal Adelaide Hospital under the care of Prof. H. N. Robson with a 6-year history of attacks of pallor and paraesthesiae of the hands and feet. For the last 2 years the attacks had increased in frequency and during the attacks the patient suffered the additional symptoms of palpitation, headache and vomiting. Before admission, attacks were occuring at least once a day. Known precipitating factors were emotion, cold, vigorous exercise and large meals.

On examination the B.P. was 150/90 mm Hg. There were no other abnormal physical findings, and the urine was normal. Laboratory investigations gave the following results:- haemoglobin 17.5 g/100 ml., packed cell volume 52%. Normal values were recorded for blood platelets, total and differential white cell count, erythrocyte sedimentation rate, blood urea nitrogen, serum sodium, potassium, calcium, phosphate, CO_2

combining power, alkaline phosphatase, oxo and oxogenic steroids, protein-bound iodine and iodine^{131} uptake. Three 24-hour urine specimens contained 9.0, 9.3 and 10.5 mg 3-methoxy-4-hydroxymandelic acid (M.H.M.A.). (Normal for the laboratory should not exceed 7 mg/24-hour specimen.) E.C.G. showed inversion of T-waves in leads II, III, aVF and the lateral chest leads, suggesting myocardial ischaemia. Intravenous pyelography showed the possibility of a mass 5-6 cm in diameter overlying the upper pole of the left kidney. Total blood volume, red cell mass and plasma volume were normal (chromium^{51} method).

Catecholamines were assayed fluorimetrically in preoperative urine and peripheral arterial blood, in left renal vein blood taken during operation and in the tumour. They were isolated on "Dowex 50" resin and estimated by a trihydroxyindole method (after Vendsalu, 1960). The results of these estimations, with normal values in parentheses, are as follows:- Arterial blood; adrenaline 0.20 ng/ml. (0.23), noradrenaline 1.33 ng/ml. (0.31). Urine; adrenaline 175 μg/day (11.5), noradrenaline 28 μg/day (29). Renal vein blood during operation; before squeezing of tumour, adrenaline 92 ng/ml., noradrenaline 42.1 ng/ml.; after squeezing of tumour, adrenaline 1.28 μg/ml., noradrenaline 0.56 μg/ml. (normals for peripheral venous blood are adrenaline 0.07 ng/ml. and noradrenaline 0.40 ng/ml. - no figures are available for normal renal vein blood). Tumour (3 separate areas assayed); adrenaline 9.81, 12.55 and 15.4 mg/g (1.17), noradrenaline 1.72, 2.11 and 6.4 mg/g (0.87).

The fluorimetric assays of catecholamines were carried out by Dr. C. J. Cooper; other laboratory investigations were performed by the Institute of Medical and Veterinary Science.

The intravenous injection of 0.02 mg histamine-acid-phosphate caused a rapid rise in B.P. to over 300 mm Hg. systolic with pallor, sweating, restlessness, headache and palpitations. The attack was terminated by the intravenous injection of 10 mg phentolamine ("Regitine").

The operation was performed by Professor R. P. Jepson, using an abdominal approach through transverse subcostal incisions. A solitary tumour, 8 cm in diameter was found above the L. kidney and was removed without difficulty. Histological examination of the tumour confirmed the diagnosis of phaeochromocytoma.

The patient was treated with oral phentolamine 20 mg 4-hourly for 3 days before operation. Premedication was intramuscular morphine 10 mg, atropine 0.6 mg and phentolamine 5 mg given half-an-hour before induction of anaesthesia. Anaesthesia was induced with thiopentone 500 mg and succinyl choline 75 mg, and maintained with nitrous oxide, oxygen, d-tubocurarine and pethidine. Controlled ventilation was used throughout the operation.

Blood pressure from a cannula in the L. Brachial Artery via an electromanometer and amplifier, and pulse rate were monitored during the operation. Fig.41 illustrates the B.P. during the operation, the relevant surgical events and the times of phentolamine and angiotensin administration.

The post-operative course was uneventful, and the B.P. remained at 120 mm Hg, without hypertensive therapy. Seven days after operation the M.H.M.A. content of a 24-hour urine specimen was 5.9 mg, and the E.C.G. showed a normal tracing. He has had no more

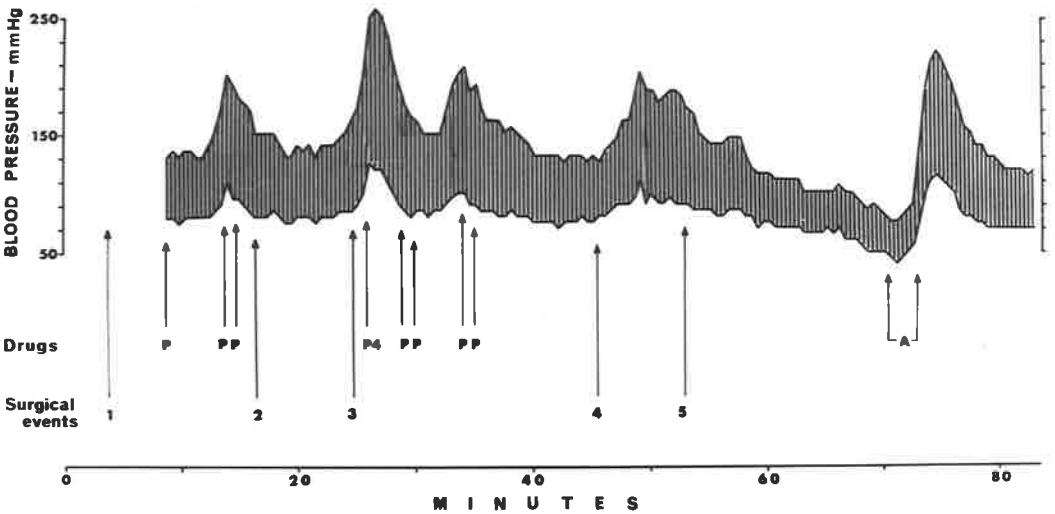


Fig. 41

The arterial blood pressure, drugs used and surgical events during the operation on the patient for removal of the phaeochromocytoma. P, phentolamine 2 mg and P_4, 4 mg intravenously; A, angiotensin 5 ug/min for 2.5 min intravenously; 1, skin incision; 2, peritoneum opened; 3, manipulation in region of left kidney; 4, tumour massaged; 5, pedicle clamped.

of the attacks which brought him to hospital.

CLINICAL PHARMACOLOGICAL TESTS

One series of investigations carried out before operation was designed to compare the effect of the adrenaline antagonist phentolamine on the responses of hand blood vessels to noradrenaline and angiotensin. The patient's blood flow through both hands was measured simultaneously by venous occlusion plethysmography (Greenfield, Whitney & Mowbray, 1963). Drugs were infused by a constant-infusion pump into one side through a needle inserted into the brachial artery near the cubital fossa. The blood flow in the opposite uninjected hand acted as a control. The doses of drugs given by the intra-arterial route were such that no systemic effects occurred, as judged by changes in arterial blood pressure and hand blood flow on the control side. The constrictor action of noradrenaline (0.2 μg/min for 3 min) was reduced to one fifth by the prior administration of phentolamine (0.05 mg/min for 3 min), whereas that of angiotensin was quite unimpaired. Fig.42 shows the results of this experiment.

Sensitivity tests were carried out four days before and nineteen days after operation to determine whether the removal of the tumour and the consequent reduction of circulating catecholamine levels had any effect on vascular sensitivity to noradrenaline. In these experiments, the responses of hand blood vessels to three different doses of intra-arterial noradrenaline was measured on the two occasions. Fig.43 gives the doses of noradrenaline and the responses of hand blood vessels on each occasion. Before operation the sensitivity was less

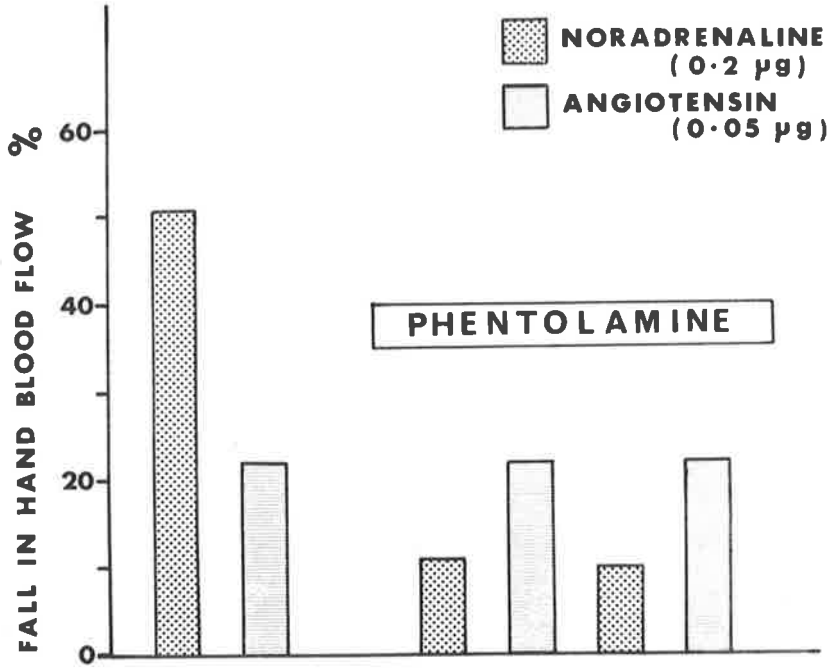


Fig. 42

The effect of phentolamine on the responses of the hand blood flow of the patient to noradrenaline 0.2 µg/min for 3 min and to angiotensin 0.05 µg/min for 3 min. All drugs were given intra-arterially.

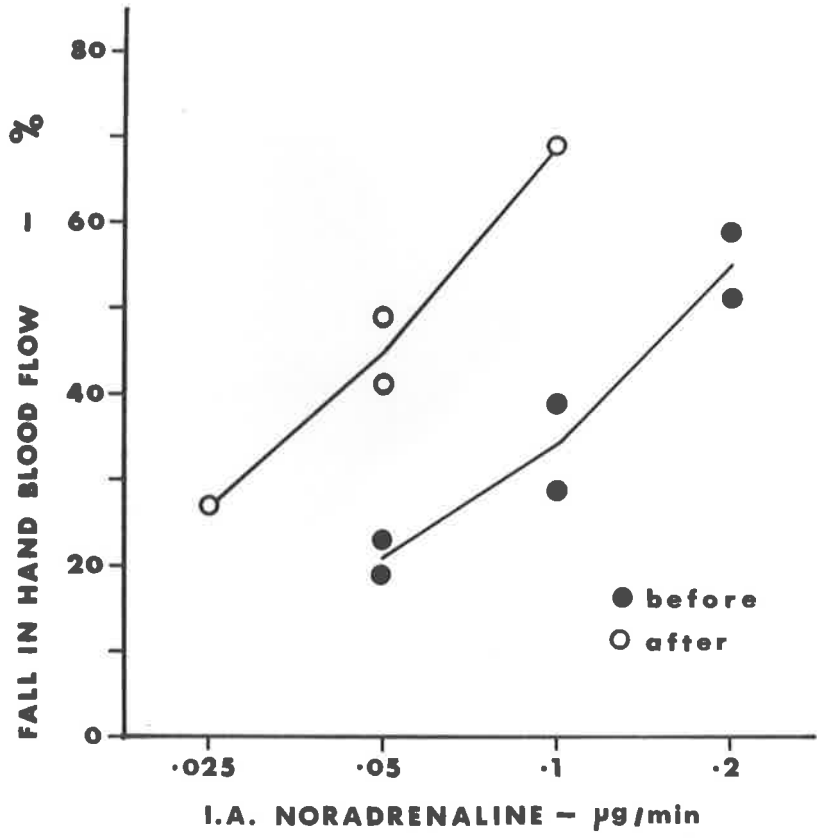


Fig. 43

The responses of the hand blood vessels of the patient to three doses of intra-arterial noradrenaline four days before and to three different doses nineteen days after operation.

than half that seen after removal of the phaeochromocytoma.

Intravenous noradrenaline was also infused on each occasion in doses of 5 and 2.5 µg/min for 5 min. The response has been calculated in terms of arbitrary peripheral resistance (P.R.) units for hand vessels by dividing mean blood pressure (mm Hg.) by hand blood flow (ml./100 ml. of hand tissue/min). The average P.R. for the 2 min prior to the noradrenaline infusion is compared with that for the last 2 min of the infusion, and a percentage change calculated. Fig. 44 shows the results obtained with the two doses of noradrenaline before and after operation. The response to 5 µg/min increased from 19% to 111% and that to 2.5 µg/min from 7% to 95%.

DISCUSSION

The use of adrenaline antagonists was an important advance in the management of phaeochromocytoma. Phentolamine is the most commonly used member of this class of drug because of its relatively short duration of action. Grimson, Longino, Kernodle & O'Rear (1949) first used the drug during and immediately preceding operation, and in 1951 Iseri, Henderson & Derr gave it orally for three days before operation as well as using it systemically during operation. In addition to their well-known action in blocking alpha receptors (Ahlquist, 1948; Nickerson, 1959), the antagonists protect the heart against arrhythmias induced by high catecholamine levels without affecting the positive chronotropic or inotropic actions (Nickerson, 1959).

The use of oral phentolamine before operation was at first simply for the purpose of protecting against persistent or paroxysmal hypertension (Iseri et al., 1951). Later, however, a further reason for its preoperative use was provided by the work of Brunjes, Johns &

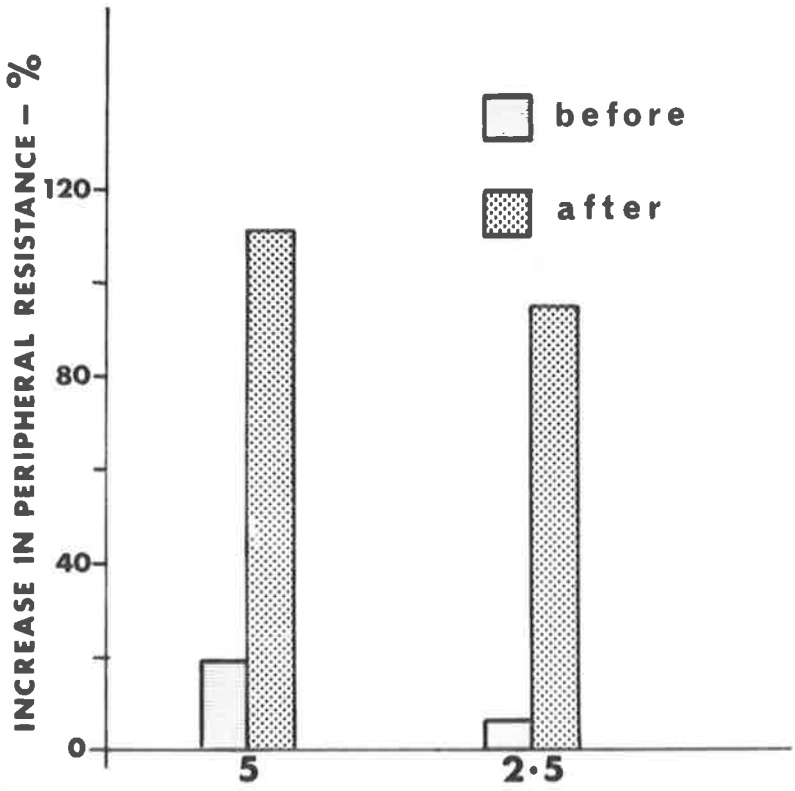


Fig. 44.

The increase in peripheral resistance of the patient's hand vessels with two doses of noradrenaline given intravenously four days before and nineteen days after operation.

Crane (1960) who showed that in phaeochromocytoma the whole-blood volume was reduced and suggested this could be one factor in the postoperative shock in these patients. In later studies (Johns & Brunjes, 1962) the blood volume abnormality was again demonstrated in a case, and was corrected before operation by the oral administration of phentolamine. Earlier investigators had shown in normal subjects that infusions of adrenaline decreased plasma volume (Kaltreider, Meneeely & Allen, 1942; Finnerty, Buchholz & Guillaudeu, 1958), supporting the above findings with phaeochromocytoma.

The results of investigations carried out before operation on this patient demonstrate that the constrictor effect of angiotensin is unimpaired by the presence of phentolamine in a dose sufficient to depress responses to noradrenaline. The failure of adrenaline antagonists to affect the response to angiotensin has also been described in animals (Bock, 1962). This property provides a strong reason for the use of angiotensin rather than noradrenaline in treating hypotension during surgery in cases of phaeochromocytoma, since an antagonist (usually phentolamine) will have been used to control the preceding hypertension. Furthermore, it is frequently impossible to curtail the administration of phentolamine in time to ensure that its effects will have gone by the time noradrenaline is required. These considerations have led some authors to question the desirability of phentolamine, except when severe rises of blood pressure make its use unavoidable (Price & Secher, 1961). The use of angiotensin will abolish this conflict and allow full doses of phentolamine to be given whenever blood-pressure elevations occur.

The evidence from the present patient that sensitivity to

noradrenaline increases (presumably to the normal level) after removal of the tumour provides another argument against the use of noradrenaline since infusion of this drug would prevent or delay the increase in vascular sensitivity necessary for a normal response to the new lower levels of endogenous catecholamines. The oral administration of an adrenaline antagonist before operation may also help to increase sensitivity to noradrenaline, but there is no evidence to substantiate this possibility.

There have been a number of reports of the cardiovascular actions of angiotensin in man (Corcoran, Kohlstaedt & Page, 1941; Bradley & Parker, 1941; Finnerty, Massaro, Chupkovich & Tuckman, 1961 and de Bono, Lee, Mottram, Pickering, Brown, Keen, Peart & Sanderson, 1963) and of its clinical use in various types of hypotension and shock (del Greco & Johnson, 1961; Derrick, 1962; Nassif, Nolan & Corcoran, 1963 and Wedeen & Zucker, 1963). However, none has reported or recommended its use in the surgery of phaeochromocytoma. In addition to the specific advantages of its use in this condition mentioned earlier, the clinical reports above provide further evidence supporting the use of angiotensin, for, in contrast to noradrenaline, it did not cause tissue sloughs, venoconstriction, oliguria or cardiac arrhythmias, and tachyphylaxis did not occur.

SUMMARY

1. The case report of a patient with phaeochromocytoma is presented.

2. The constrictor effect of angiotensin on the patient's hand blood vessels was unimpaired by phentolamine in a dose which greatly

reduced their responses to noradrenaline.

3. After the removal of the tumour the responses of the hand blood vessels to intra-arterial noradrenaline increased more than two-fold, and intravenous noradrenaline caused a much greater rise in the resistance of hand vessels than occurred before operation.

4. Angiotensin was successfully used to elevate the patient's blood pressure during operation after the tumour was removed.

ADDENDUM

Since the report of this case was written, a second case of phaeochromocytoma has been diagnosed and the tumour successfully removed at operation. Vascular sensitivity studies were done before operation, but have not been repeated after operation as yet.

Angiotensin was infused intravenously with a constant infusion pump during operation after removal of the tumour in a dose of 2 μg/min for 2 min with a consequent rise in blood pressure. This dose was smaller than that used with the first patient (5 μg/min for 2.5 min) and produced a less severe and therefore more satisfactory rise in pressure. Fig. 45 shows the blood pressure, the drugs used and the main surgical events during the operation.

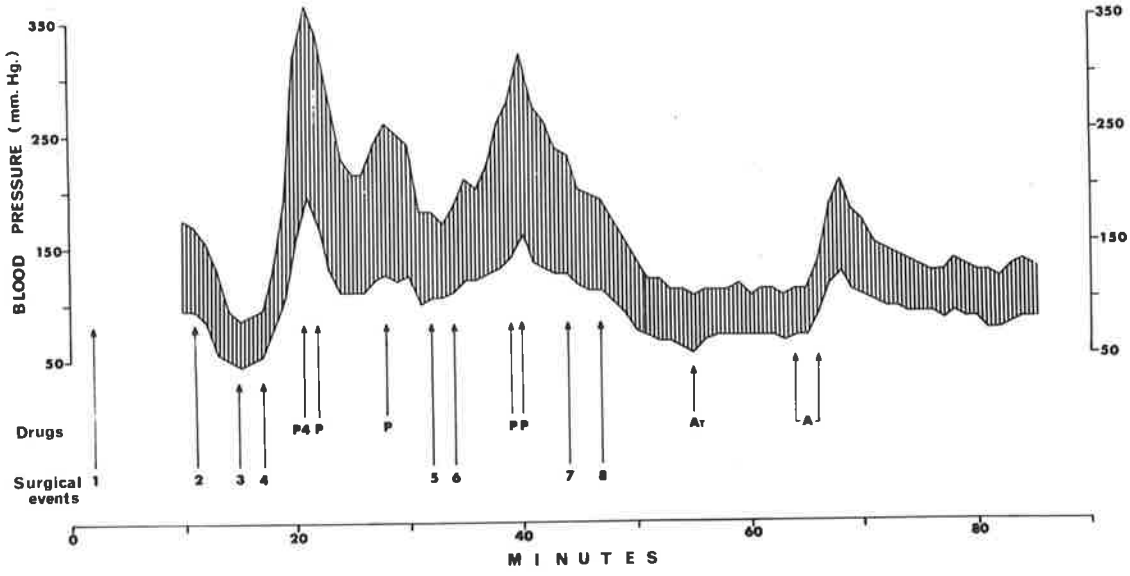


Fig. 45

The arterial blood pressure, drugs used and surgical events during the operation on a second patient for removal of phaeochromocytoma. P, phentolamine 2 mg and P4, 4 mg intravenously (I.V.); At, atropine 0.3 mg I.V.; A, angiotensin 2 µg/min for 2 min I.V.; 1, skin incision; 2, peritoneum opened; 3, right adrenal massaged; 4, left adrenal massaged; 5, tying main adrenal vein; 6-7, dissection of and removal of tumor; 8, searching rest of abdominal cavity.

APPENDIX 1.

PLETHYSMOGRAPHY

The history and principles of venous occlusion plethysmography have been extensively and adequately reviewed (Barcroft & Swan, 1953; Whelan, 1951; Skinner, 1961; Greenfield, 1960 and Greenfield, Whitney & Mowbray, 1963). The type of plethysmograph used to measure forearm or hand blood flow in most of the experiments in this thesis was that described by Greenfield (1954). Latterly, the metal rings holding the cuff diaphragms and the method of water drainage have been modified to conform with the type described by Greenfield et al. (1963). The airways connecting the plethysmographs with the float recorders and the interposed three-way taps and syringes are identical to those described by Barcroft & Swan (1953).

The method for inflating and deflating the wrist or arm cuffs, although similar in principle to that described by Barcroft & Swan (1953), has been extensively modified in detail and made automatic. The reservoir air tanks are fed by cylinders of compressed air through suitable valves. The inflow and outflow of air from the cuffs is controlled by valves (domestic washing machine type) operated electrically by an automatic sequence timer. This timer allows the periods of cuff inflation and deflation to be controlled independently for periods between 1 and 60 sec. There is also a three-position switch to allow manual control of the cuff inflation and deflation if this is required (e.g. to catch short-lived spikes in flow). The diagram of this apparatus is shown in Fig. 46 and the actual arrangement is seen in the photograph in Fig. 47 . The circuit diagram of the sequence timer is shown in Fig. 48.

The experimental procedure outlined by Barcroft & Swan (1953)

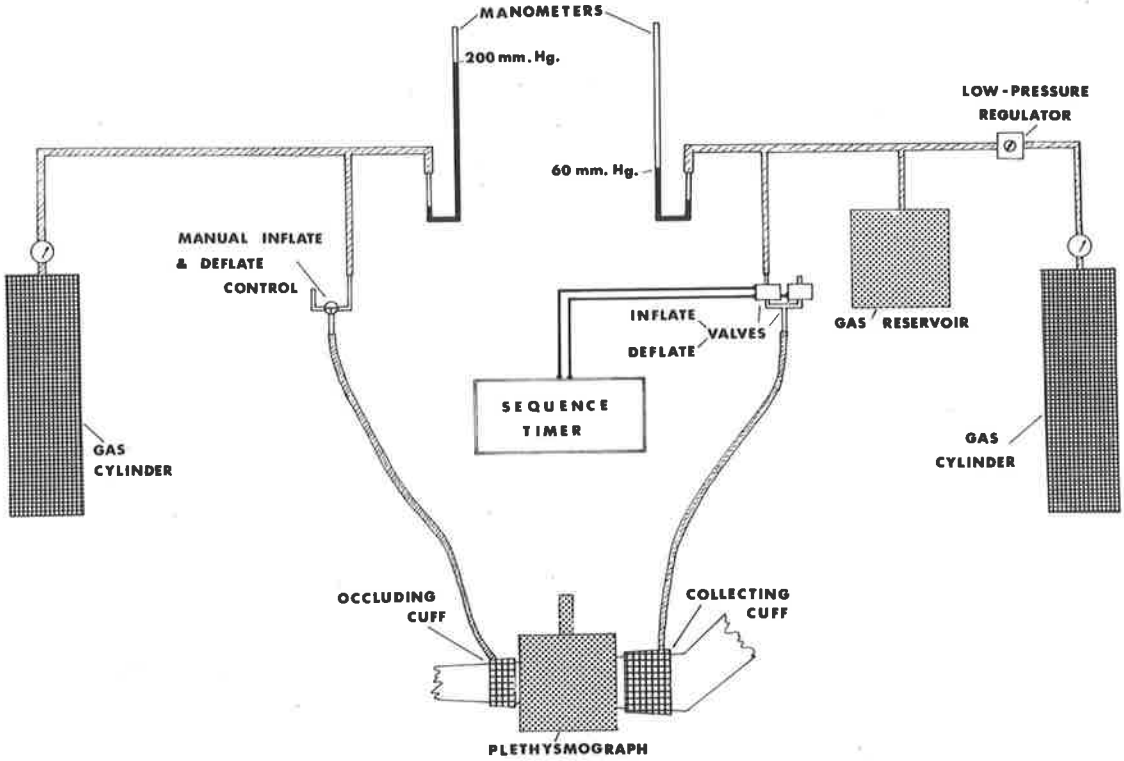


Fig. 46

Diagram of apparatus used for automatic inflation & deflation of cuffs.

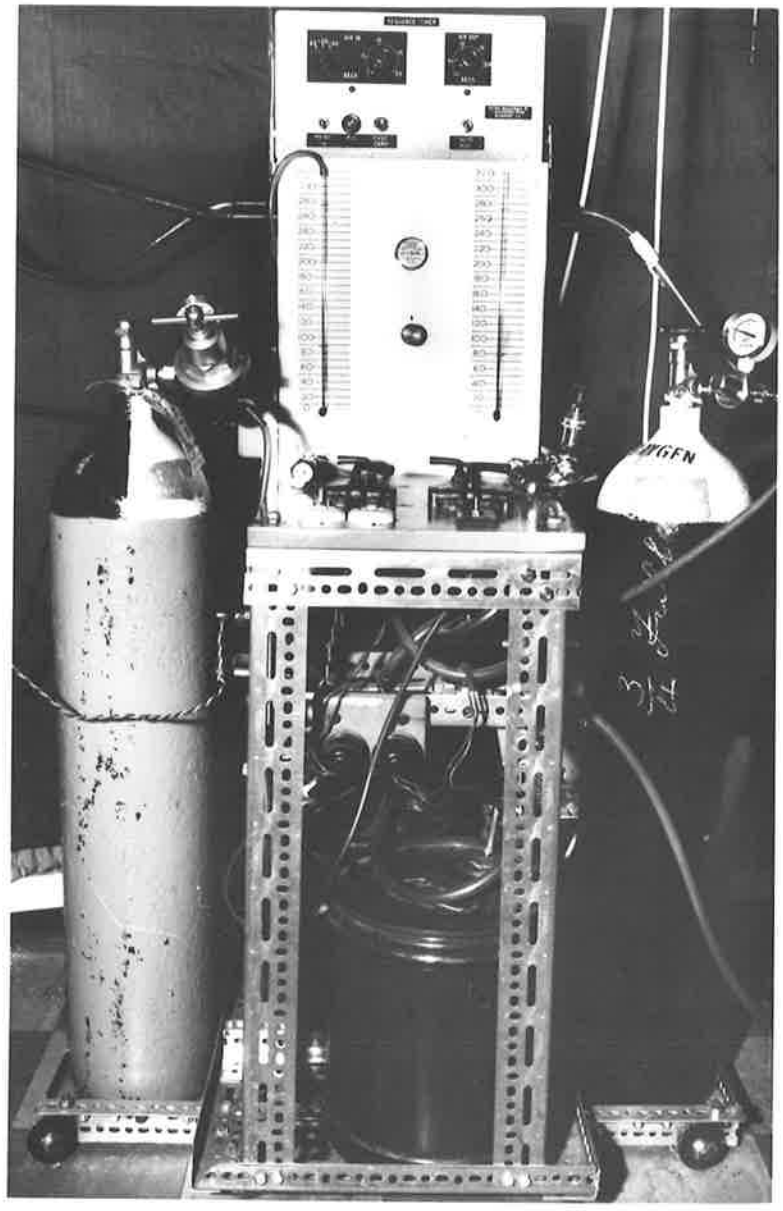


Fig. 47.

Photograph of the automatic apparatus used for inflating and deflating the cuffs.

Relays R1 & R4 RPL Type 11R.

Relays R2 & R3 RPL Type 2R mechanically interlocked.

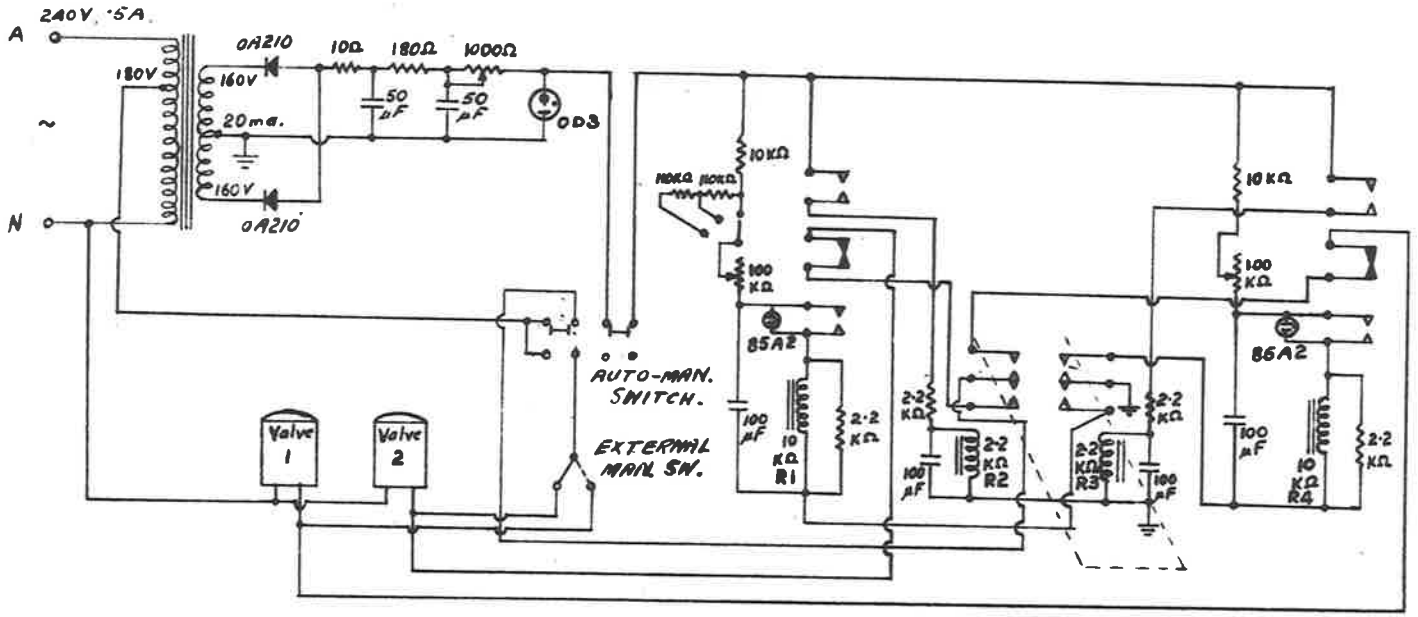


Fig. 48.

Circuit diagram of the sequence timer.

and Greenfield (1960) has been closely followed. Plethysmograph temperatures were maintained at 32 - 33°C and at 34 - 35°C during measurements of hand and forearm blood flow respectively. Room temperature was maintained at 23 ± 1°C except for special experiments. Full explanation of the experimental procedure to the subject, an adequate period of rest on the couch before recording is commenced, the positioning of laboratory personnel out of sight of the subject and as little noise as possible are all important points if steady blood flows, especially of hands, are to be obtained.

APPENDIX 2

The interpretation of blood-flow responses to intra-arterial drug infusions.

The level of blood flow in the forearms and hands is subject to continual alterations, even when the local environment of the segments under study is kept constant. These fluctuations in blood flow are much more pronounced in hands than in forearms and are due, at least in part, to variations in sympathetic vasoconstrictor tone and in blood pressure. Cooper, Cross, Greenfield, Hamilton & Scarborough (1949) noted that the changes in hand blood flow are approximately equal on both sides, and Greenfield & Patterson (1954) showed that fluctuations in the forearm flow of one side follow those in the other side reasonably closely. Both observations confirm the fact that the influences responsible for the fluctuations in forearm or hand blood flow affect both sides to an equal degree.

Utilizing this principle, Duff (1952) evolved an important formula for use in assessing the effects of intra-arterial adrenaline on the hand blood flow of one side, and later Greenfield & Patterson (1954) treated their own results in the same way in assessing the effects of increased transmural pressure on forearm blood flow. This method can be used in any circumstance in which blood flow in both hands or forearms is measured and in which a form of treatment is applied to one side only, provided (i) that the treatment does not prevent the control (untreated) side from responding normally to generalised fluctuations in vasomotor tone and in blood pressure, and (ii) that the local effects of the treatment have worn off before another treatment is applied (i.e. both sides must be capable of behaving similarly in the pre-treatment period). The method allows the effects of the local treatment on blood flow to be distinguished from those of general changes acting on both sides.

In using the formula given by Duff (1952) the assumption is made that the ratio of the averages of the blood flow measurements of the treated and control sides stays constant, so that a theoretical value can be obtained for the treated side during the treatment period which represents the level of flow which would have been expected had no treatment been applied. From this expected value and that actually observed as a result of treatment, an absolute and a percentage change can be calculated.

The formula is $\frac{Tb}{Cb} = \frac{Te}{Cd}$, and hence $Te = \frac{Tb.Cd}{Cb}$, where Cb and Cd are the average blood flow levels of the control side before and during the treatment period, Tb is the level of flow of the treated side before treatment and Te is the expected level of flow of the treated side without treatment. If To is the observed level of flow during treatment, then Te - To gives the change due to treatment, and $\frac{Te - To}{Te}.100$ gives the percentage change.

In this thesis Duff's formula has been used in assessing the constrictor effects of intra-arterial noradrenaline except after the infusion of the adrenergic neurone blocking drugs bretylium, guanethidine and bethanidine, since these drugs themselves change the level of blood flow on the treated side and also prevent it from responding to those changes in general vasomotor tone mediated by the adrenergic nerves. The blood flows on the teated and control sides during the two-minute period preceeding the noradrenaline infusion and those during the last two minutes of the infusion are averaged to give the values used in the formula.

As this method of assessment cannot be used after adrenergic blocking drugs, the drift in flow of the treated side itself has to be used to indicate where the blood flow level would have been had noradrenaline not been infused. Thus, the average flow for the pre-infusion two-minute period is joined to that for the two-minute period after the effects of noradrenaline have worn off. The fall in flow due to noradrenaline is then measured as the amount by which the true flow fell below this line. An example of this method is illustrated in Fig. 49, showing the details of part of an experiment in which intra-arterial guanethidine had been infused, and hand blood flow was being measured. The different behaviour of the control and treated sides is clearly seen.

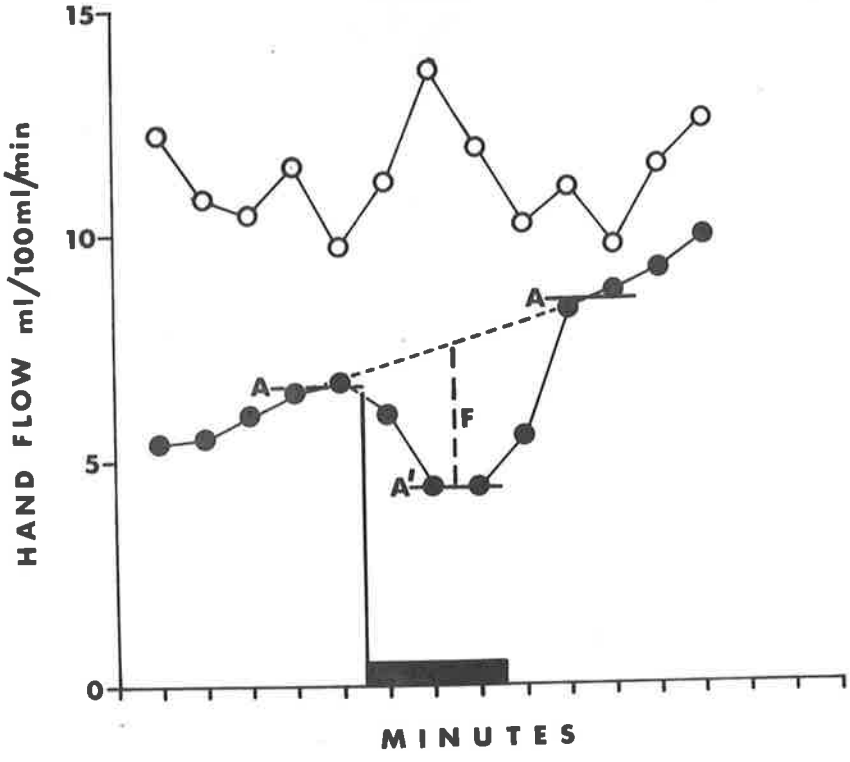


Fig. 49.

Method of measuring the response to intra-arterial noradrenaline of the hand blood flow of a side previously treated with guanethidine 1 mg/min for 5 min. ●, treated side; O, control side; A, average hand blood flow for 2 min before noradrenaline and for 2 min after its effect has worn off; A---A, line joining the two average flows; A', average blood flow for last 2 min of noradrenaline infusion; F, the fall in flow due to noradrenaline; black rectangle, noradrenaline 0.05 μg/min for 3 min.

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