PLASMA PROTEIN BINDING

OF

VITAMIN B_{12}

THESIS

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CONTENTS

INTRODUCTION

The introduction of radioactive isotopes into investigative medicine less than two decades ago has aided greatly in the understanding of the processes of absorption, utilization, turnover and excretion of many biological substances; the advances in the knowledge of the mechanisms of normal and abnormal haemopoiesis have been at least as substantial as those in other disciplines. Several isotopes are available for labelling erythrocytes, leukocytes and platelets, and three important haemopoietic building blocks, namely, iron, cyanocobalamin and folate, are available in one or more radioactive forms.

The radioactive isotopes used in the investigation of vitamin B_{12} metabolism fulfil several of the important criteria laid down for biological acceptability; the radioactive cobalt atom is an integral part of the molecule, and not an extraneous "tag", and labelling is therefore specific. However, for a number of reasons, investigations of vitamin B_{12} metabolism have not proceeded as far as comparable studies of iron metabolism. These reasons include:

i) the vitamin is physiologically active in relatively low concentrations;

ii) the radioactive isotopes generally used have specific activity so low as to make it difficult, if not impossible, to carry out investigations

with truly physiological quantities;

iii) cyanocobalamin has a number of biologically active analogues, and it is still not certain in which form or forms the vitamin exists in man;

iv) whereas iron is transported in plasma by a specific binding protein, transferrin, there are at least two specific in vivo binding proteins for vitamin B_{12}, and the interrelation between these two specific binders, which is still not clarified, may be altered in disease;

v) whereas iron is bound in vitro by transferrin only, vitamin B_{12} is bound by a number of electrophoretically separable protein moieties.

Purpose of the Present Study

In general terms, a study of the plasma protein binding of vitamin B_{12} has relevance in at least three areas; first, plasma protein binding of vitamin B_{12}, especially in vitro, is a facet of the metabolism of the vitamin which is not fully understood; second, there are important aspects of protein chemistry involved; and third, an understanding of the abnormality of protein binding of vitamin B_{12} seen in myeloid leukaemia may well throw some light on the understanding of this disorder.

The several specific, and interrelated, aims of the present study are:-

i) to evaluate critically the dialysis method of measurement of vitamin B_{12} binding capacity in vitro, and to evolve a standardized method which takes into account those factors which produce variation in binding;

ii) to investigate, by different methods of protein separation, the in vitro binding of vitamin B_{12} in normals, and in those situations where vitamin B_{12} binding is abnormal, such as chronic myeloid leukaemia;

iii) to assess the effect of leukocytes on vitamin B_{12} binding by plasma proteins;

iv) to investigate the vitamin B_{12} binding properties of isolated protein fractions.

Significance of Results.

It is considered that the following conclusions may be drawn from the results of the material presented and discussed in this thesis, and that these conclusions are original observations:-

1. That a method of measurement of in vitro vitamin B_{12} binding capacity of plasma proteins has been described which takes into consideration a number of variable factors not previously assessed in other methods;

2. That in vitro binding of vitamin B_{12} to an abnormal protein in myeloid leukaemia occurs preferentially, and not as an overflow phenomenon following upon saturation of normal binding proteins;

3. That separated α_1 acid glycoprotein will bind significantly more vitamin B_{12} added in vitro than all other protein fractions;

4. That increased in vitro binding can occur in patients with myeloid leukaemia at a time when the leukocyte count is normal, as a result of therapy. It has been considered previously that the increased capacity for binding vitamin B_{12} was seen characteristically in patients with untreated myeloid leukaemia, and that this increased binding capacity fell towards normal when treatment was instituted to lower the total leukocyte count and eliminate primitive forms from the peripheral circulation.

In addition, evidence is presented which adds to, and extends, previous knowledge in this field. It has been shown that:-

- Increased binding of vitamin B_{12} to plasma proteins takes place in patients with myeloid leukaemia;

- The abnormal vitamin B_{12} binding protein in myeloid leukaemia has many of the characteristics of α_1 acid glycoprotein, or a component of this protein complex;
- Normal granulocytes, or the breakdown products of normal granulocytes, may influence plasma protein binding of vitamin B_{12} added in vitro.

Format of the Thesis.

The format of this thesis is a conventional one; three features only require comment.

i) In the light of recent statements concerning thesis references (Witts, 1967), the references in this thesis are given in full.

ii) Two case histories are appended in detail (Appendix A) because of the importance of findings in these two patients to certain of the material contained in the thesis.

iii) In addition to the two papers attached, several other papers based on this material have been presented at meetings of the Australian Society for Medical Research, the Haematology Society of Australia, and at the XIth Congress of the International Society of Haematology, Sydney, 1966; a list of these papers is attached at Appendix C. A further list of publications of material contained in this thesis

is attached at Appendix B, including a number of abstracts of papers referred to in Appendix C.

Conventions

i) The references for this thesis are styled in the manner laid down in the World Medical Association's publication, "World Medical Periodicals", 3rd edition, 1961.

ii) The following conventions of terminology have been observed throughout the thesis:

(a) except where otherwise specifically indicated, the term "Vitamin B_{12}" is understood to refer to cyanocobalamin. In general, the terminology used by authors whose work is referred to in the literature review has been maintained in this respect.

(b) the term "myeloid leukaemia" has been used throughout the work, and this term is used to cover other synonymous (or nearly synonymous) terms used by various authors, including granulocytic leukaemia and myelocytic leukaemia.

(c) the units of measurement of vitamin B_{12} used are in general, those used by the authors

referred to in the literature review; thus, in some parts, the terms nanogram (ng.) and picogram (pg.) are used, and in other parts millimicrograms (mμg.) and micromicrograms (μμg.) are used, according to the style of the authors.

(d) the only abbreviations used in this thesis are those referring to bacteria such as Lactobacillus leichmanii (abbreviated as L. leichmanii) and Euglena gracilis (abbreviated as E. gracilis) and those referring to certain chemicals in which the commonly used name is a shortened form of the correct chemical name, e.g. DEAE cellulose is used for diethylaminoethyl-cellulose, CM cellulose is used for carboxymethyl-cellulose, and the term "tris" is used for tris (hydroxymethyl) aminomethane.