THE PRINCIPLES AND PRACTICE OF VITAMIN B_{12} THERAPY

With reference to the treatment of patients with pernicious anaemia and other vitamin B_{12} deficiency states.

A Thesis submitted for the Degree of Doctor of Medicine. University of Glasgow.

by

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"Would you tell me, please, which way I ought to go from here?"

"That depends a good deal on where you want to get to," said the Cat.

"I don't much care where - so long as I get somewhere," Alice added as an explanation.

"Oh, you're sure to do that," said the Cat, "if you only walk long enough."

Alice's Adventures in Wonderland,
Chapter 6.

CONTENTS

PREFACE

The investigations reported in this thesis had their basis in some apparently simple problems posed by the introduction of vitamin B_{12} in the treatment of patients suffering from Addisonian pernicious anaemia and other vitamin B_{12} deficiency states. It might be thought that with the experience gained from the wide use of vitamin B_{12} in the last decade few, if any, problems remained about the treatment of such patients. That there are problems however, is clearly apparent if only from the widely varying recommendations about vitamin B_{12} therapy to be found in textbooks, monographs and articles.

The onus of solving these problems lies with the clinician who must bear the ultimate responsibility for the treatment of patients and this thesis is a record of studies carried out by a clinician with the object of clarifying some aspects of the principles and practice of vitamin B_{12} therapy in patients with pernicious anaemia and other vitamin B_{12} deficiency states.

INTRODUCTION

It seemed likely that one outstanding clinical problem arising in the treatment of patients suffering from pernicious anaemia with vitamin B_{12}, namely that of defining adequate maintenance therapy, could be solved by meeting two requirements and applying them to the study of patients with this disease. The first requirement was a method of determining the loss of injected vitamin B_{12} accurately: with such a method the site and extent of the loss could be located and measured and an estimate made of the amount remaining in the body. The second requirement was a method of establishing the time for a known amount of vitamin B_{12} in the body to be utilised. During investigations into these requirements various other problems relevant to the use of vitamin B_{12} in therapeutic practice, were encountered and studied and some of these investigations are also recorded in this thesis.

It has been found convenient to present the material in such a manner that each chapter appears to be a report of an isolated investigation.

/It will be obvious however, that each apparently limited inquiry forms an integral part of one study.

Plan of the thesis

The thesis is in two volumes. Volume 1 contains the text with relevant figures and tables in eight chapters and, a note of acknowledgments. Volume 2 is an appendix containing tables, details of technical methods, details of some of the patients studied and a bibliography. The pages of each volume are numbered consecutively. Title pages are coloured for easy reference.

Tables and figures in the text are each numbered consecutively and are placed as near to relevant points in the text as possible. Detailed results, which are presented in summary or figures in the text are given in full in the tables in the Appendix; these tables are numbered consecutively with the prefix A; thus Table 21 is in the text and Table A.21 in the Appendix. Technical methods are mentioned briefly in the text and full details are given in appropriate sections in the Appendix.

References cited in the text or elsewhere are given in full in conventional sequence and form

/in a bibliography in the Appendix. Included therein are titles of several communications presented to the Second European Symposium on Vitamin B_{12} and Intrinsic Factor held at Hamburg in August 1961. Publication of these communications originally scheduled for 1961, has been delayed and rather than await publication, and thus delay submitting this thesis for at least six months, it has been decided to refer to relevant communications by the title of the abstract or of the manuscript. The proceedings should be published by the time this thesis is submitted.

Terminology

Certain conventions have been followed and a few disregarded. The term vitamin B_{12}, hallowed by clinical usage is preferred to the more scientific term cyanocobalamin. The term Co vitamin B_{12} is used to indicate vitamin B_{12} with radioactive cobalt incorporated in the molecule: where a particular radioactive isotope is incorporated the appropriate prefix is employed e.g. ^{58}Co vitamin B_{12}. The term serum vitamin B_{12} level is used, as by most other

/authors, in preference to serum vitamin B_{12} concentration although, the term concentration is used in preference to level in other contexts. The terms dose and injection are often used, as in pharmacological practice, in preference to the term mass. The phenomenon of equilibration is occasionally referred to as mixing. The mass, dose or concentration of vitamin B_{12} or Co vitamin B_{12} is expressed as microgrammes (ug) or micromicrogrammes (uug) and the terms milli-microgrammes or milligrammes have been avoided. The term control has not necessarily been used to indicate a normal subject: the meaning will be clear from the context in which it is used. Both case initials and case numbers are used. The former, e.g. Case J.F.A.,for patients, particularly those with vitamin B_{12} deficiency states who were often the subject of more than one investigation: details of these patients, and some others of particular interest are given in a section in the Appendix. Case numbers are used for patients, most believed not to have any upset of vitamin B_{12} metabolism, who were

/usually the subject of one investigation only. Details of these patients are given in Tables in the text and it will be obvious from these that Case 6, say, of an investigation reported in Chapter 1 is not the same person as Case 6 of an investigation reported in Chapter 7.

CHAPTER I

THE URINARY EXCRETION OF ASSAYABLE VITAMIN B_{12} AND OF RADIOACTIVITY AFTER PARENTERAL ^{58}Co VITAMIN B_{12} IN MAN.

The Concept, Development, Proof and Implications of a Technical Method.

The measurement of the vitamin B_{12} excreted in urine, bile and faeces after parenteral administration presents difficulties. Colorimetric and spectroscopic methods are obviously valueless. Chemical methods are uncertainly specific, complicated and clumsy. Microbiological assay methods are not specific, subject to a considerable variation and are more suitable for measuring low concentrations of vitamin B_{12} than the high concentrations which may be found in urine after parenteral vitamin B_{12}. Isotope dilution and reversed isotope dilution techniques are theoretically ideal but are time consuming and technically difficult demanding rigorous purification techniques. In addition, the measurement of injected vitamin B_{12} excreted in faeces is complicated by the fact that faeces normally contain large amounts of vitamin B_{12}, and the measurement of vitamin B_{12} in bile is complicated by the fact that bile inhibits the growth of at least one microbiological assay organism.

Because of these difficulties, consideration was given to the feasibility of using Co vitamin B_{12} to measure the loss in urine directly after parenteral administration.

THEORETICAL CONSIDERATIONS

It is generally believed that vitamin B_{12}, whether absorbed from the intestine or given by injection, functions in the body as an active substance and does not undergo chemical change. It follows therefore, that if a patient who had no vitamin B_{12} in his body and who was not excreting any radioactive substance in his urine were given an injection of ^{58}Co vitamin B_{12}, it would be a reasonable assumption that the amount of radioactivity excreted in the urine would be a true indication of the amount of ^{58}Co vitamin B_{12} excreted. For example, if this hypothetical patient were given 1000 ug ^{58}Co vitamin B_{12} parenterally and 20% of the radioactivity were excreted then that 20% would represent 200 ug ^{58}Co vitamin B_{12}. Patients with vitamin B_{12} deficiency presenting in haematological relapse are the

/clinical equivalents of this hypothetical patient and it seemed likely that this assumption would also apply to them. Proof that this were so would be agreement between the values for radioactivity excreted and assayable vitamin B_{12} excreted in the urine after an injection of ^{58}Co vitamin B_{12}.

It was obviously essential that the normal subject should be studied also. Because of the large body stores of vitamin B_{12} in normal subjects however, it could not be assumed that the excretion of radioactivity after parenteral ^{58}Co vitamin B_{12} would be a true indication of the amount of vitamin B_{12} excreted. The possibility had to be considered that injected Co vitamin B_{12} might equilibrate, or mix, with the body stores. Under these circumstances the amount of radioactivity appearing in the urine after an injection of ^{58}Co vitamin B_{12} would not be a true indication of the mass of vitamin B_{12} excreted but would represent only a fraction of it. For example, if a patient with 3000 ug

/non radioactive vitamin B_{12} in his body were given 1000 ug ^{58}Co vitamin B_{12} parenterally and 20% of the dose of radioactivity were excreted in the urine, then that 20% would represent 20% of (1000 + a proportion of 3000) ug vitamin B_{12} depending on the extent of equilibration.

Preliminary investigations showed that the normal subject excreted about 60% of the dose of radioactivity after a parenteral injection of 1000 ug ^{58}Co vitamin B_{12} and it was obvious that complete equilibration could not have taken place otherwise this would have represented 2400 ug if the body stores were 3000 ug - an absurd situation. It was clearly important to determine whether any significant degree of equilibration did, in fact, occur and again the method of giving parenteral ^{58}Co vitamin B_{12} and comparing the urinary excretion of radioactivity and of assayable vitamin B_{12} seemed to be suitable. If there was good agreement between the results obtained by the two methods this would indicate that equilibration had not taken place whereas if a significant degree

/of equilibration did occur the amount of assayable vitamin B_{12} in the urine would be in excess of the amount indicated by radioactivity present. For example, if a subject were given 1000 ug ^{58}Co vitamin B_{12} parenterally and 50% of the radioactivity were excreted and a total of 500 ug of vitamin B_{12} were found by assay then this would be evidence against equilibration of significant degree: if however, 50% of the radioactivity were excreted and 750 ug of vitamin B_{12} were found by assay this would suggest that the 1000 ug ^{58}Co vitamin B_{12} had equilibrated with 500 ug of the body stores of non radioactive vitamin B_{12}.

Comparison of the radioactivity excreted and the assayable vitamin B_{12} excreted after parenteral ^{58}Co vitamin B_{12} in normal subjects to determine if there was any significant degree of equilibration appeared to be an acceptable procedure provided that the assay results were accurate. In the example already given where 50% of the radioactivity were excreted and the assayable vitamin B_{12} was

/750 ug it is obvious that this could happen not only as a result of equilibration but also as a result of erroneously high assay readings when there was no equilibration. The problem raised by the possibility of such a constant error in the assay causing misleading results could obviously be solved by duplicating the experiment in patients with little or no vitamin B_{12} in the body. For example, if a normal subject were given 1000 ug ^{58}Co vitamin B_{12} and 50% of the radioactivity were excreted in the urine and 600 ug of vitamin B_{12} were found by assay this would suggest either that there had been some equilibration or that the assay result was too high. If the same result was obtained in a patient with little or no body stores of vitamin B_{12} then it would be clear evidence that the assay reading were high.

Repeated injections of ^{58}Co vitamin B_{12} could be given to vitamin B_{12} deficient subjects without prejudicing the value of the results as, even if equilibration did occur, the injected ^{58}Co vitamin B_{12} would equilibrate with ^{58}Co vitamin B_{12} remaining in the body from the

/previous injection. For example, if a control patient were given 1000 ug ^{58}Co vitamin B_{12} and 50% were excreted he would retain in his body 500 ug ^{58}Co vitamin B_{12} with 50% of the dose of radioactivity given. If a second injection of ^{58}Co vitamin B_{12} 1000 ug radioactivity 100% were given and 60% of the dose of radioactivity were then found in the urine this would still represent 600 ug since the total of the body stores plus the second injection would equal 1500 ug radioactivity 150% for practical purposes.

The use of vitamin B_{12} deficient subjects as controls would not only serve to reveal a constant error in the assay, if such were present, but would also give some indication of the degree of equilibration, if any, which took place when normal subjects were given parenteral ^{58}Co vitamin B_{12}.

With these theoretical conditions in mind an experiment was planned to throw light on the possibility of using ^{58}Co vitamin B_{12} to estimate urinary losses after injection in

/vitamin B_{12} deficient and normal subjects and to investigate the extent, if any, of equilibration of injected ^{58}Co vitamin B_{12} with the body stores of vitamin B_{12} in normal subjects.

PLAN OF EXPERIMENT

The object of the experiment was to compare the values of radioactivity and of assayable vitamin B_{12} excreted in the urine after parenteral injections of ^{58}Co vitamin B_{12} in normal subjects, control subjects who were suffering from vitamin B_{12} deficiency and subjects who had been treated for vitamin B_{12} deficiency and whose body stores of vitamin B_{12} would probably lie between those of the normal and control group.

PATIENTS, MATERIALS AND METHODS

The control patients were ward inpatients suffering from vitamin B_{12} deficiency as shown by a macrocytic anaemia, megaloblastic erythropoiesis seen on bone marrow biopsy smears, a low serum vitamin B_{12} level and reticulocyte

/response and rise in peripheral blood values on treatment with parenteral ^{58}Co vitamin B_{12}. Details of these patients are given in the Case Reports in the Appendix.

These patients were given injections of ^{58}Co vitamin B_{12} daily, or on alternate days, until a suitable number of results was obtained. On general principles any other drug therapy was avoided during the investigation but in some cases drugs were given deliberately as part of studies reported elsewhere in this thesis. In every case it was established that the presence of drugs or their end products in urine did not affect the assay result and it was also established that they did not affect the amounts of radioactivity excreted.

The 'normal' patients were ward inpatients with a variety of diseases in which depletion of body vitamin B_{12} stores or upset of vitamin B_{12} metabolism is not a known feature. All had a normal serum vitamin B_{12} level. These patients were given a single intramuscular injection of ^{58}Co vitamin B_{12}. Some of these

/patients were receiving treatment at the time of the experiment and it was established that this did not affect the assay result. Details of these patients are given in Tables A.5, A.12 & 13, A.16, A.20 & A.24 in the Appendix.

The 'borderline' patients were regular attenders at an outpatient clinic and all had been diagnosed as having a vitamin B_{12} deficiency state when first seen. At the time of the injection of ^{58}Co vitamin B_{12} they were known to have serum vitamin B_{12} levels at the lower limit of normal suggesting that their body stores of vitamin B_{12} lay between these of the control and normal groups. Details of these patients are given in the Case Reports in the Appendix.

The nature and objects of the experiments were explained to the patients, all of whom cooperated willingly.

The doses of ^{58}Co vitamin B_{12} used were 50 ug 0.3 uc, 54 ug 0.3 uc, 100 ug 0.2 uc, 1000 ug 0.2 uc and 1140 ug 0.2 uc. In experiments using doses of 54, 540 and 1140 ug

/the same batch of ^{58}Co vitamin B_{12} was used for all patients in each dose group ('matched' series) and in experiments with doses of 100 and 1000 ug which were conducted over a longer period of time, different batches of ^{58}Co vitamin B_{12} were used ('miscellaneous' series).

Urine collections were made for 24 hours prior to, and subsequent to, the injection of ^{58}Co vitamin B_{12} in the normal subjects. In the case of the control patients a 24 hour collection was made prior to the first injection of ^{58}Co vitamin B_{12} and 24 hour collections were made thereafter until the experiment was terminated. None of the borderline patients had been given any radioactive isotope and urine was collected from these patients for the 24 hours after injection only. There was no reason to believe that the collections were other than complete.

The methods of preparing the ^{58}Co vitamin B_{12} solutions for injections and the methods of collecting urine are described in the Appendix. The amount of microbiologically active material

/in each urine collection was measured by the Euglena gracilis assay in at least two different assay batches and the mean results expressed as ug of total assayable vitamin B_{12}. The amount of radioactivity was measured and the value expressed as radioactivity excreted as a percentage of the amount in a dose. Details of the assay and isotope techniques used are given in the Appendix.

The regression equations relating the isotope values (y) to the assay values (x) for each group of patients in each dose group were calculated and the coefficient of correlation was also calculated. The regression coefficients in each dose group were compared to each other. Details of the statistical methods used are given in the Appendix.

RESULTS

The urine collections obtained prior to injections of ^{58}Co vitamin B_{12} were not found to contain detectable amounts of radioactivity and the amounts of assayable vitamin B_{12} in these collections were trivial.

/ Deterioration of the ^{58}Co vitamin B_{12} solutions in vitro did not occur as judged by serial microbiological assays during the time each batch was in use.

The disposition of patients into experimental groups is shown in Table A.1 and full details of individual results are given in Tables A.2-24 in the Appendix.

The results are summarised in Figs.1-5 and the essential statistical results shown in Tables 1 & 2. In each group of patients in each dose group there was a linear correlation between the amounts of radioactivity excreted and the amounts of assayable vitamin B_{12} excreted, the coefficients of correlation differing significantly from zero. In each dose group the regression coefficients were not found to differ significantly from each other ($t = <2.0$, $P = >0.05$). Minor differences in the results presented in Table 1 and those submitted by Adams (1961a) are due to the fact that the calculations were done by different workers, those in Table 1 being done by the author;

/a difference in the regression equations in the 1140 ug series was due to accidental transposition of results; those given in Table 1 are correct.

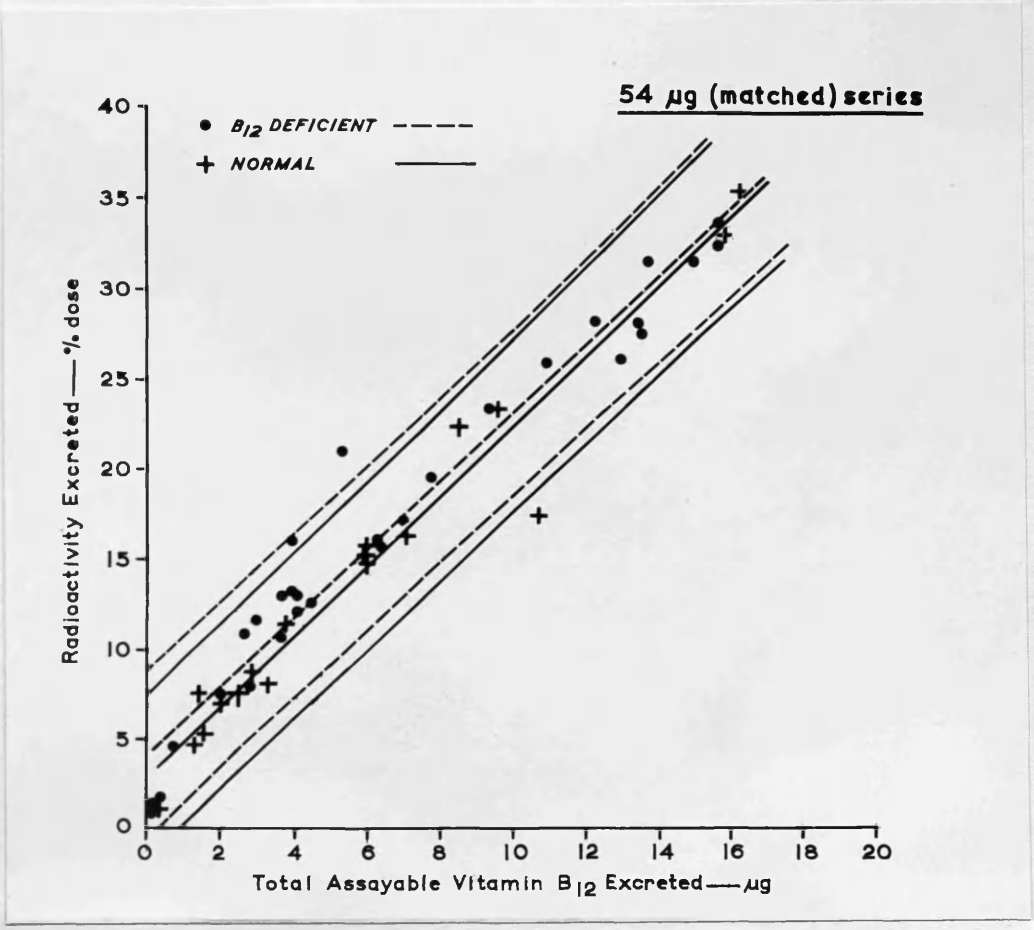


Fig. 1: Scattergram of results obtained in the 54 ug dose series. The regression lines and 95% confidence limits for both groups of patients are also shown.

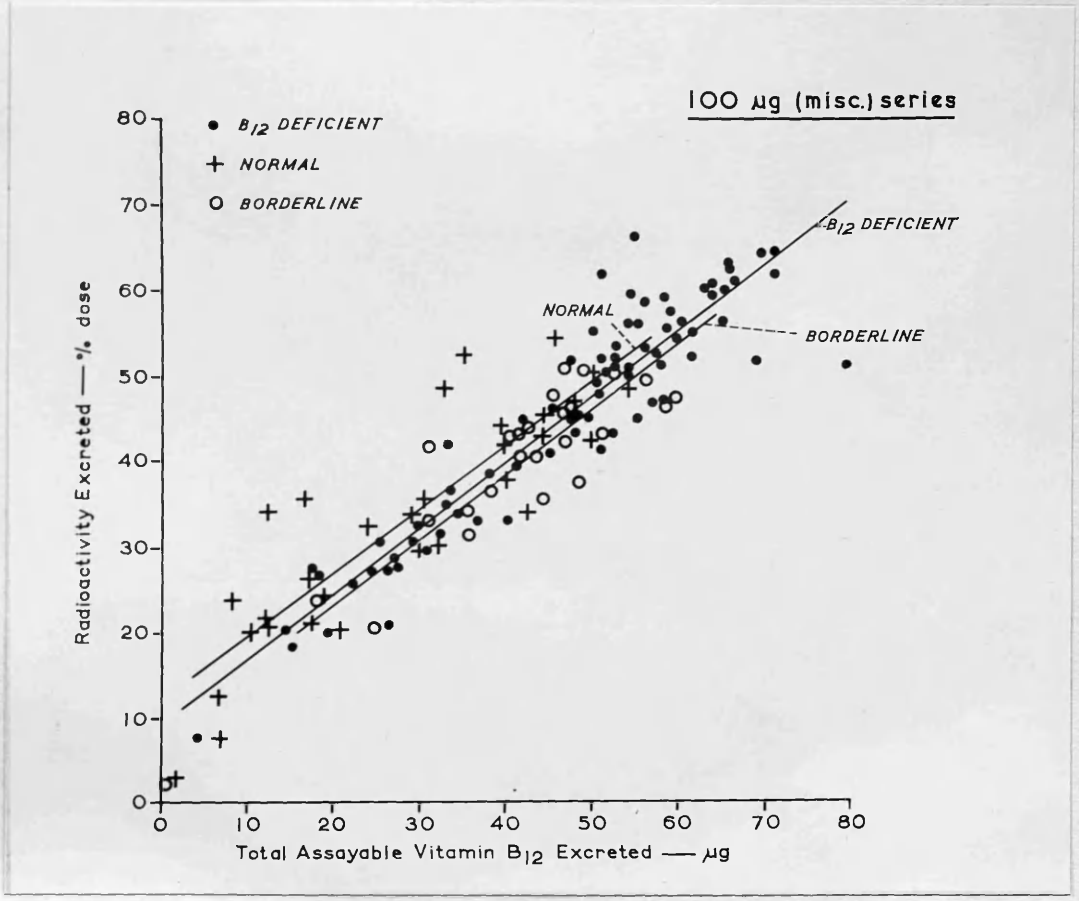


Fig. 2: Scattergram of results obtained in the 100 ug series. The regression lines for each group of patients are also shown.

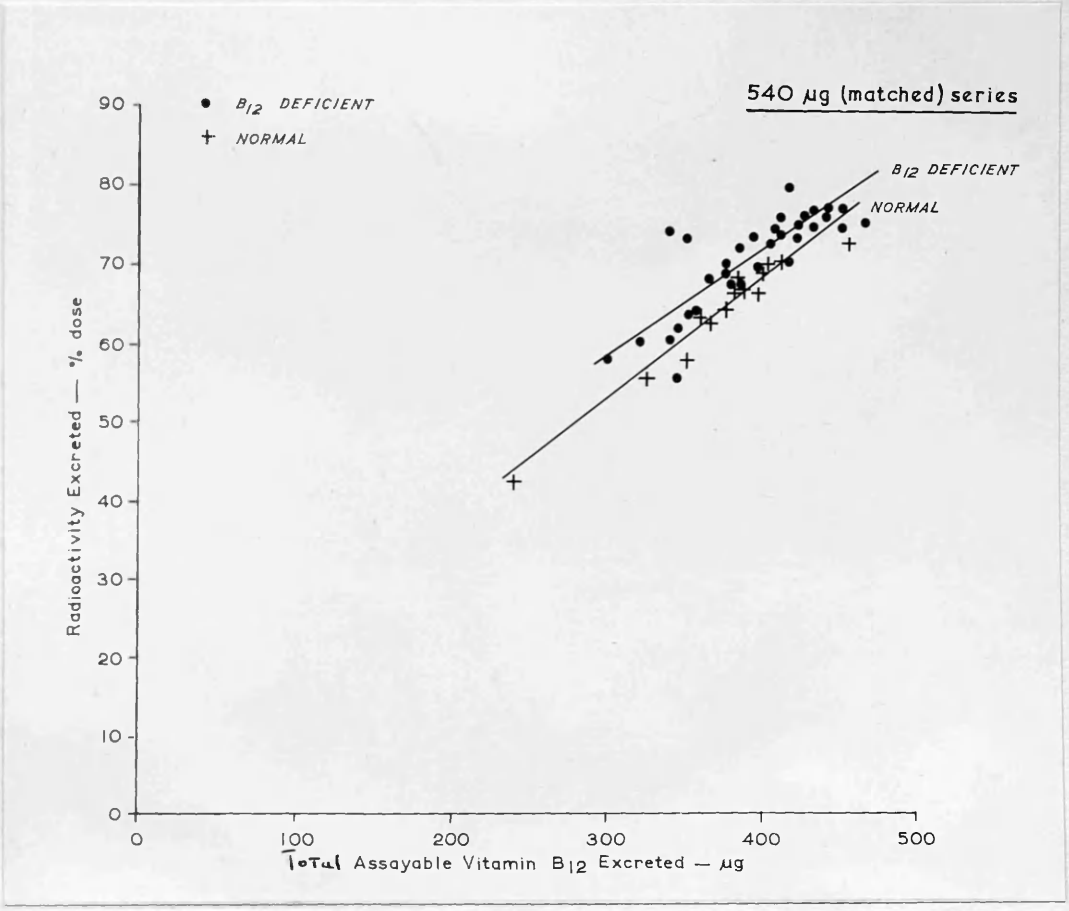


Fig. 3: Scattergram of the results obtained in the 540 ug series. The regression lines for both groups of patients are also shown.

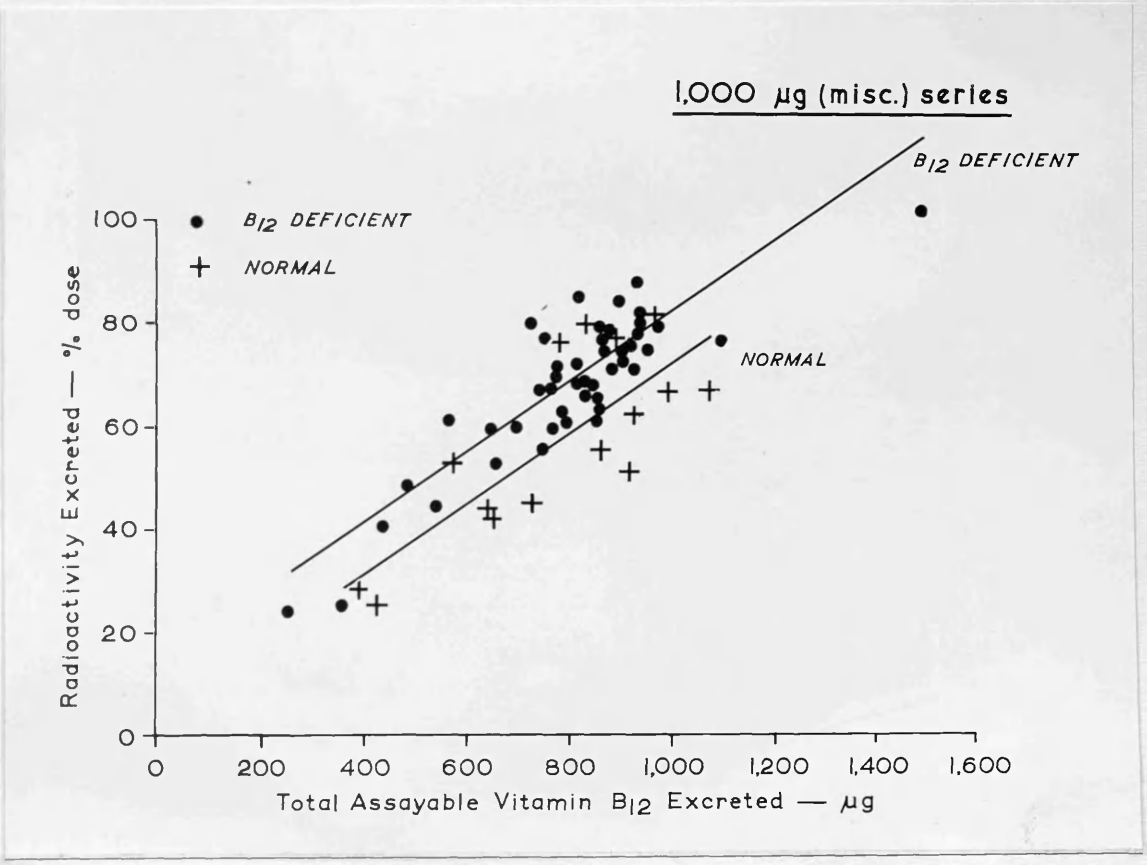


Fig. 4: Scattergram of the results obtained in the 1000 ug series. The regression lines for both groups of patients are also shown.

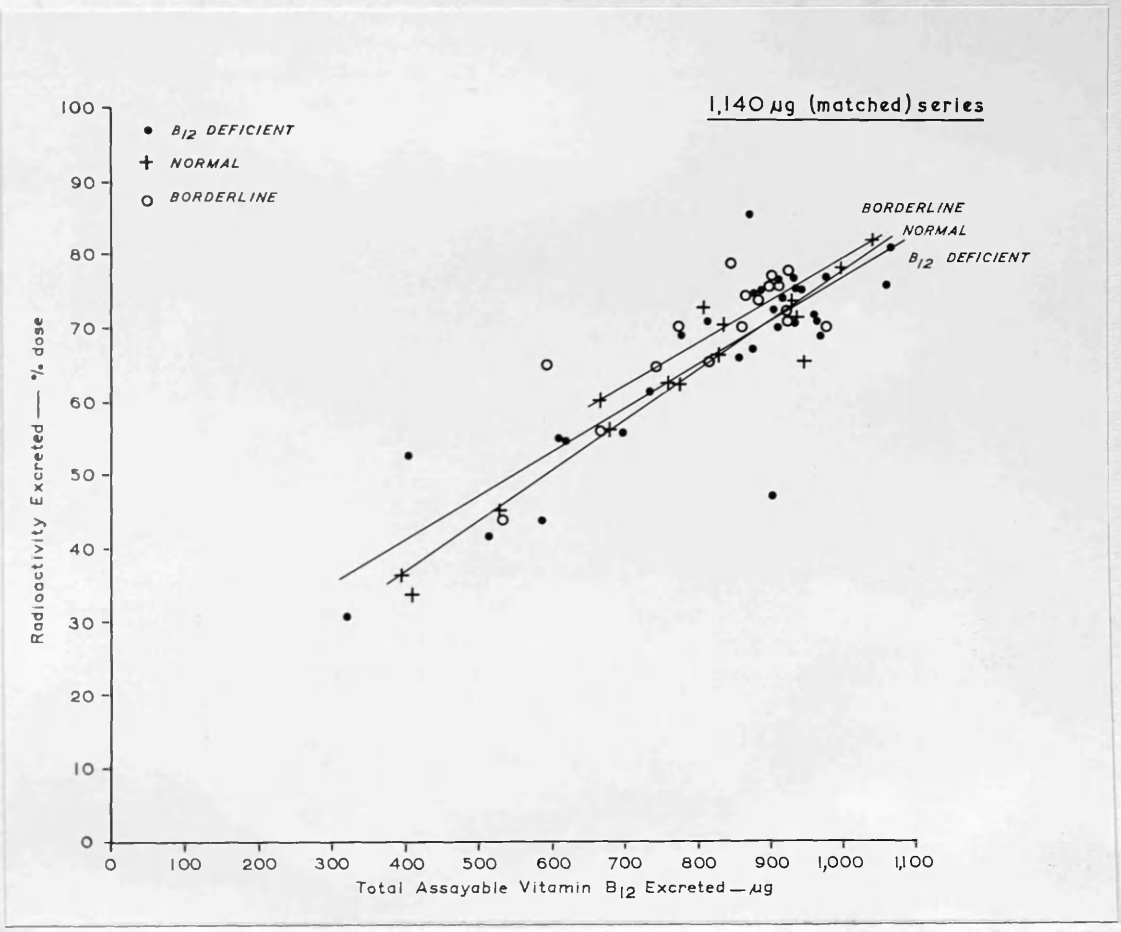


Fig. 5: Scattergram of the results obtained in the 1140 ug series. The regression lines for each group of patients are also shown.

SERIES	GROUPS	REGRESSION OF ISOTOPE VALUE (y) ON ASSAY VALUE (x)	COEFFICIENT OF CORRELATION 'r'	P
54ug (matched)	control	Y = 1.8797x + 4.2155	0.9752	< 0.001
	normal	Y = 1.9473x + 2.8223	0.9811	< 0.001
100ug (misc.)	control	Y = 0.7798x + 8.7053	0.9296	< 0.001
	borderline	Y = 0.7646x + 7.8021	0.9057	< 0.001
	normal	Y = 0.7598x + 11.5273	0.8721	< 0.001
540ug (matched)	control	Y = 0.1384x + 16.9635	0.8567	< 0.001
	normal	Y = 0.1567x + 6.2723	0.9736	< 0.001
1000ug (misc.)	control	Y = 0.07156x + 10.4905	0.8990	< 0.001
	normal	Y = 0.06903x + 3.4174	0.7914	< 0.001
1140ug (matched)	control	Y = 0.0594x + 17.5713	0.8518	< 0.001
	borderline	Y = 0.0567x + 23.0572	0.8135	< 0.001
	normal	Y = 0.0689x + 9.8847	0.9627	< 0.001

Table 1: showing essential statistical data. The isotope value (y) is the percentage of the dose of radioactivity excreted and the assay value (x) is the total assayable vitamin B_{12} excreted.

SERIES	GROUP	NUMBER OF OBSERVATIONS n.	MEAN VALUES EXCRETED	
			RADIOACTIVITY % dose excreted	TOTAL ASSAYABLE VITAMIN B_{12} ug.
54 ug (matched)	control	30	17.0600	6.8333
	normal	18	14.1166	5.8000
100 ug. (misc.)	control	77	45.5948	47.3064
	borderline	26	39.4653	41.4115
	normal	31	32.9903	28.2483
540 ug (matched)	control	33	71.2666	392.3636
	normal	14	64.7214	373.0000
1000 ug (misc.)	control	46	68.0869	804.8695
	normal	15	56.9800	775.9333
1140 ug (matched)	control	30	66.6333	825.9660
	borderline	17	69.9647	827.2941
	normal	15	62.8733	769.0660

Table 2: showing further statistical results.

DISCUSSION

Review of the literature

Apart from a report giving results from a smaller series of cases investigated by the same techniques (Adams 1961a) no comparable investigation appears to have been carried out in man.

The appearance of radioactivity in the urine following injections of Co vitamin B_{12} to rats and to man had been noted by Barbee & Johnson (1951) Horrigan & Heinle (1952) Mollin et al (1956) and others (vide infra) as an incidental observation prior to the present investigation. There was also evidence from extraction, chromatographic and isotope dilution studies that the urinary radioactivity was due to intact Co vitamin B_{12} molecules and not to free Co. (Chow et al 1951; Smith 1952, 1953; McLean & Bloch 1954). Acceptable quantitative evidence however, that the radioactivity excreted after parenteral Co vitamin B_{12} is a true measure of the assayable vitamin B_{12} excreted, such as is reported here, had not been

/produced.

After the present investigations had been completed it was found that the conclusion that the radioactivity excreted in urine following parenteral Co vitamin B_{12} was a measure of the vitamin B_{12} excreted had in fact been reached by several workers. The significance of these reports is difficult to assess as details of work justifying this conclusion were not given by Rosenblum et al (1952), Smith et al (1952), Smith (1953), Harte et al (1953) or Lang et al (1953), while the evidence presented by Yamamoto et al (1951) from a few experiments on rats certainly does not justify that conclusion. It appears that these conclusions were not acceptable as the isotope method of measuring urinary loss of injected Co vitamin B_{12} has not become a standard technique and the implication of the finding in relation to the question of equilibration of injected Co vitamin B_{12} with the body stores has never been raised.

Discussion of techniques

A possible criticism of the results is that

/the assay technique used did not give accurate results within the limitations of the method. When comparing results obtained from normal, borderline and control groups this is not important as the groups were studied in the same period of time and any error would be constant; it is however, relevant when considering whether the isotope method gives a true indication of the amount of ^{58}Co vitamin B_{12} excreted by vitamin B_{12} deficient subjects. A significant degree of inaccuracy is unlikely. Falsely high results, such as occur with the presence of pseudovitamin B_{12} (Baker et al 1956a) are virtually excluded by the low values obtained from the preinjection collections of urine and by the fact that the injected vitamin B_{12} was pure. Falsely low results, due to the presence of inhibitors to the growth of Euglena gracilis, such as drugs and their breakdown products, were excluded by the results of recovery experiments on the urines of patients taking drugs and a few patients not taking drugs. In view of these facts it can be taken

/that the assay did measure urinary vitamin B_{12} accurately and that the results are valid. The value for assayable vitamin B_{12} present in the 24 hour preinjection urine collection was not subtracted from the value for the post injection 24 hour urine collection; it might have seemed logical to do this on the assumption that the value for the preinjection collection represented a basal output of vitamin B_{12} in urine but it is obvious that such a refinement would not have made any difference to the results.

Ideally the results for the control group should have been obtained only from the first injection of ^{58}Co vitamin B_{12} given to a large number of untreated vitamin B_{12} deficient patients. The method used is apparently open to criticism on the grounds that after the first injection of ^{58}Co vitamin B_{12} no patient was then vitamin B_{12} deficient. Strictly speaking this is so but consideration of the facts presented in the introduction shows that, for the purpose of the experiment, it is perfectly permissible to include all the results obtained

/from these patients.

The investigation was deliberately limited to a period of 24 hours after an injection of Co vitamin B_{12}. The amounts of radioactivity and of assayable vitamin B_{12} excreted after this period are very small in the majority of cases (see Tables A.2-4, A.6-10, A.14 & 15, A.17-19, A.21 & 22) and there did not seem to be any good reason for extending the investigation to include this period.

As already noted the effect of some drugs and differing routes of administration was studied concurrently in some of the vitamin B_{12} deficient patients to find out if these affected the amounts of radioactivity excreted. It was established by recovery experiments that the presence of the drugs, or their end products, in urine did not affect the assay result and it was therefore legitimate to include the results in this investigation as the object was to compare the amounts of radioactivity excreted and assayable vitamin B_{12} excreted. In fact, as will be shown later none of these measures

/affected the amounts of radioactivity excreted. Similarly it is of no importance in this context that the cause of the vitamin B_{12} deficiency state in the control patients differed from case to case: the fact that they were vitamin B_{12} deficient is all that matters.

Significance of results

Two facts emerge clearly from the results. The first, which is of practical value, is that when man is given ^{58}Co vitamin B_{12} parenterally, in the dose range 54 - 1140 ug, the amount of radioactivity excreted in the urine in the subsequent 24 hours is a true indication of the amount of assayable vitamin B_{12} excreted: this is established whether the body stores of vitamin B_{12} are normal, grossly depleted or at a value between these two extremes. This is an important observation because it means that this direct isotope method of estimating urinary excretion of injected vitamin B_{12} can be used with complete confidence instead of measuring the amount excreted by microbiological assay. This method, which makes use of a very

/small dose of radioactivity, has considerable advantages over the assay method. As a technical method it is inherently more accurate than the assay method, the isotope counting technique used having an accuracy of 1 - 2% as opposed to the accepted error of ± 20% with the assay technique used (Ross et al 1957, Killander 1957a, 1958d, Shinton 1959, Girdwood 1960a). In addition any errors in the assay method may be magnified by the necessary use of urine dilutions of from 1.10 to 1.1000 depending on the original concentration although this potential source of error may possibly be offset by the greater recovery which occurs, at least with sera, when high dilutions are used (Girdwood 1960a). With the direct isotope method results may be obtained quickly and with but little expenditure of time and labour whereas the assay method requires a considerable amount of work and time before an acceptable result is obtained. A result may be obtained by the direct isotope method within a few minutes of the specimen being collected. Even when urine dilutions are

/assayed in only two different assay batches, and many samples were assayed in at least three different batches to ensure accuracy in this study, the final result is rarely available within two weeks of receipt of the sample. This interval could be reduced by using one of the more rapid assay methods (Girdwood 1954, Spray 1955, Tiffin & Williamson 1958) but even so the gain in time with the direct isotope method would still be considerable. The saving in labour with the direct isotope method can only be fully appreciated by those who have a working knowledge of microbiological assays. In brief a technical method of some precision has been shown to be superior on all counts to a laborious and relatively inaccurate method. It is also clear that the direct isotope method is more convenient, rapid and easy to use than chemical, isotope dilution or reversed isotope dilution methods and it is unlikely that it is less accurate than these methods.

The second fact which emerges from the results, and with equal clarity, is of more

/academic interest at present. It is that ^{58}Co vitamin B_{12}, given parenterally in the doses used, does not equilibrate with the body stores of vitamin B_{12} at least in the 24 hours after injection.

For the purposes of experimental design it was assumed that vitamin B_{12} deficient patients in haematological relapse had no vitamin B_{12} in their tissues, although it was appreciated that this was not the case. The amount of assayable vitamin B_{12} in the liver of a patient with pernicious anaemia in haematological relapse is small (see Chapter 7) but it cannot be assumed that it is negligible when considering the question of equilibration particularly with injections of ^{58}Co vitamin B_{12} of the order of 54 ug. The striking lack of difference between the results for the vitamin B_{12} deficient and normal subjects in the 54 ug series in particular implies that if equilibration did occur at all it did so to no greater extent when the body stores were normal than when they were grossly depleted. This seems very unlikely and

/it can be taken for practical purposes that the lack of equilibration is complete in the dose range of 54 to 1140 ug. Furthermore, in the 54 ug series it is permissible for the regression lines and standard deviation curves to be extended to meet the abscissa and ordinate. The regression line for the normal group meets the ordinate at the point abscissa 0, ordinate 2.8223 and the regression line for the control group meets the ordinate at the point abscissa 0, ordinate 4.2155. The lower standard deviation curve for the normal group meets the ordinate at the point abscissa 0, ordinate -7.29 and the lower standard deviation curve for the control group meets the ordinate at the point abscissa 0, ordinate -6.03. As it was not assumed that either regression line would pass through the point abscissa 0, ordinate 0, these findings are good evidence that the lack of equilibration occurs in the entire dosage range up to 1140 ug.

An obvious explanation for the findings is that the urinary excretion of injected ^{58}Co vitamin B_{12} is so rapid that there is

/insufficient time for equilibration, particularly if the body stores of vitamin B_{12} are in 'compartments' or 'subpools'. Observations on the excretion of injected vitamin B_{12} show that it is a rapid process (Chow et al 1950, Chesterman et al 1951, Krevans et al 1951, Sokoloff et al 1952) and observations on the excretion of parenteral ^{58}Co vitamin B_{12}, reported in Chapter 4, have confirmed and elaborated these findings. As a rule, about 50% of the total ^{58}Co vitamin B_{12} excreted in the urine after a parenteral injection is eliminated within three hours of injection. Equilibration however, is an extremely rapid process and to attribute the lack of equilibration solely to rapid excretion implies that the injected ^{58}Co vitamin B_{12} does not enter the 'subpools' in the body for an appreciable time. That this is not the case is apparent from the results reported by several workers from studies on the plasma clearance of injected Co vitamin B_{12}. Glass et al (1955), Estren et al (1958), Glass (1959) and Brody et al (1960) all report

/that the hepatic uptake of radioactivity is very rapid while Mollin et al (1956), Miller et al (1957), Estren et al (1958) and Hall (1960) all report very rapid clearance of injected ^{58}Co vitamin B_{12} from the blood stream. These results show that injected ^{58}Co vitamin B_{12} enters the tissues rapidly. Thus the argument that injected Co vitamin B_{12} does not equilibrate with the body stores because there is insufficient time for this to occur before it is excreted cannot be sustained.

Another explanation, which is also obvious, is that equilibration does not occur because vitamin B_{12} does not exist as such in the body. This, in turn, implies that injected vitamin B_{12} retained in the body is converted to an analogue or active form. This concept entails a radical departure from the current notions of vitamin B_{12} metabolism (Estren et al 1958) but it cannot be avoided. Furthermore, there is already evidence that this may be the case. A recently isolated enzyme, 5:6 dimethyl benzimidazole cobamide coenzyme, (Weissbach et al 1959, Barker et al 1960a) has been identified in

/rabbit liver in a concentration which, allowing for losses in isolation, corresponds to about 80% of the reported vitamin B_{12} content of beef liver (Weissbach et al 1959). This finding has led to the suggestion that this enzyme, or similar enzyme (Barker et al 1958, Barker et al 1960ab) may be the main form of vitamin B_{12} or B_{12}-like compounds which are catalytically active in the enzymatic reactions of living cells (Weissbach et al 1959), a suggestion recently strengthened by the isolation of 5:6 dimethyl benzimidazole cobamide coenzyme from human liver (Barker 1961). It has also been reported that 5:6 dimethyl benzimidazole cobamide coenzyme competes with vitamin B_{12} in a 1:1 ratio for attachment to intrinsic factor or liver homogenate receptors and that it exerts a haemopoietic effect equivalent to that of vitamin B_{12} when given intramuscularly in doses of 1 ug to patients with pernicious anaemia (Wasserman et al 1960). This enzyme, and others isolated by Barker et al (1958, 1960b) have distinctive absorption spectra but have not

/previously been detected in tissues reported to contain cobalamins. Weissbach et al (1959) suggest that the reason for this is that the enzymes are rapidly converted to vitamin B_{12} by procedures invariably used to extract cobalamins from tissues, such as exposure to light, heat or cyanide ions. It should be noted that the isolation of the enzymes is very difficult, all procedures being carried out in the dark or in a very dim light and nearly always at temperatures of 0 - 4°C. (Barker et al 1960a). In addition there is also evidence that hydroxocobalamin (vitamin B_{12a} or B_{12b}), which forms the bulk of the total cobalamins in some animal proteins (Tarr 1952), may be the metabolically active form of vitamin B_{12} (Smith 1961). This view has recently been strengthened by the finding that the bulk of the radioactivity in the livers of dogs given oral or parenteral Co vitamin B_{12} was present as Co hydroxocobalamin implying conversion of the retained Co vitamin B_{12} to Co hydroxocobalamin in the body (Rosenblum 1961).

/ The belief that, in man, vitamin B_{12} whether absorbed from the intestine or given by injection, functions in the body as an active substance is based essentially on five points:

(1) the clinical and haematological benefit obtained by the exhibition of pure vitamin B_{12} to patients with pernicious anaemia.

(2) the results of recovery experiments in which vitamin B_{12} is added to tissues and the effect measured by microbiological assay; an increase in microbiological activity, low in the tissues and body fluids of patients with pernicious anaemia, being obtained corresponding to the amount of vitamin B_{12} added (Ross et al 1957, Killander 1957a, Shinton 1959).

(3) on the results of microbiological assays of tissues with Ochromonas malhamensis which is the most specific assay organism for vitamin B_{12} (Ford 1953) as well as with Euglena gracilis (Ross & Mollin 1957).

(4) on the findings that the distribution of radioactivity in the body after an injection

/of Co vitamin B_{12} differs from that resulting from an injection of Co chloride (Meyer et al 1956).

(5) on the finding that the hepatic radioactivity, present post mortem in subjects who had received Co vitamin B_{12} before death, had the solubility and chromatographic characteristics of ^{60}Co vitamin B_{12} and that there was no evidence of any microbiologically active material containing ^{60}Co other than ^{60}Co vitamin B_{12} (Glass & Mersheimer 1958, Schloesser et al 1958).

It is now clear that none of these findings is evidence against the concept that vitamin B_{12} does not exist in the body or exists in the body to only an insignificant degree and that injected vitamin B_{12} is converted to an active form in the body. The fact that a chemically pure substance induces relief from signs and symptoms does not necessarily imply that the cause of the signs and symptoms was deficiency of that substance - at least five analogues of vitamin B_{12} appear to be as

/haemopoietically active as vitamin B_{12} itself (Blackburn et al 1957). The fact that the distribution of radioactivity in the body following an injection of ^{60}Co vitamin B_{12} differs from that following an injection of ^{60}Co chloride can only be taken as evidence that the metabolic pathways of these substances or their derivatives differs. The procedures involved in microbiological assay and chromatographic techniques may alter the chemical structure of the active principle: even with such a refined technique as the reverse isotope dilution method it is salutory to note that the radioactivity in the liver of dogs given Co vitamin B_{12} is largely in the form of Co vitamin B_{12} if nitrous acid is used in the preparation of tissues and largely in the form of Co hydroxocobalamin if the tissues are prepared with papain or trypsin (Rosenblum et al 1960, Rosenblum 1961). The extreme instability of the coenzymes particularly when exposed to visible light, cyanide ions or mild acid hydrolysis (Weissbach et al 1959) must be

/remembered in this context. It may be that the problem lies not in the techniques but in the preparation of tissues for analysis but this does not affect the issue. Nearly all the microbiologically active material - 'assayable vitamin B_{12}' - in the tissues is in the bound form. Binding implies at least a physical change since the assayable vitamin B_{12} in liver is microbiologically active but not dialysable (Pitney et al 1955, Ross & Mollin 1957). It is possible that the process of binding also involves chemical changes in the structure of injected vitamin B_{12}. This speculation, however tempting as an explanation for some of the facts must remain a speculation only at present.

For present purposes it is enough to know that the amount of radioactivity excreted in the urine in the 24 hours after an injection of ^{58}Co vitamin B_{12}, at least in the dose range 0 - 1140 ug, is a true measure of the ^{58}Co vitamin B_{12} excreted as judged by microbiological assay whether the body stores of vitamin B_{12}

/or its active principle are normal or depleted. Investigations using this direct isotope method of measuring the urinary loss after parenteral Co vitamin B_{12} will be reported in subsequent chapters. For convenience much current terminology will be retained although with the full appreciation that it is inaccurate: for example the term 'body stores of vitamin B_{12}' will be used rather than the more precise term 'body stores of the metabolically active form of vitamin B_{12}' and the term 'vitamin B_{12} deficiency state' will be retained in preference to the clumsy but more accurate term 'deficiency state relieved by vitamin B_{12}, the deficiency being of an as yet unidentified active principle probably an analogue of vitamin B_{12}.'

SUMMARY

The difficulties in measuring the amount of vitamin B_{12} excreted, especially in urine, after injections of vitamin B_{12} are described briefly.

Theoretical considerations affecting the use of ^{58}Co vitamin B_{12} in measuring the urinary loss directly are discussed, particular attention being paid to the possibility of equilibration of injected ^{58}Co vitamin B_{12} with the body stores.

An experiment, planned to examine the feasibility of using ^{58}Co vitamin B_{12} to measure urinary loss after injection in normal, vitamin B_{12} deficient and 'borderline' subjects is described.

The results show that there is linear correlation with a coefficient of correlation differing very significantly from zero between the vitamin B_{12} excreted as judged by microbiological assay using Euglena gracilis and the radioactivity excreted after injections of ^{58}Co vitamin B_{12} in doses of 54 - 1140 ug in all groups of patients. The

/regression coefficients for normal, vitamin B_{12} deficient and 'borderline' subjects do not differ significantly from each other in each dose group.

The literature on the subject is reviewed and the validity of the techniques discussed. The significance of the results is discussed and it is concluded that the amount of radioactivity present in the urine in the 24 hours after an injection of ^{58}Co vitamin B_{12} in the dose range 0 - 1140 ug is a true measure of the amount of assayable vitamin B_{12} excreted. The ease, simplicity and accuracy of this method - the direct isotope method - are stressed. It is also concluded that the results demonstrate lack of equilibration of injected ^{58}Co vitamin B_{12} with the body stores. The nature of this phenomenon is discussed and the likeliest explanation is considered to be that vitamin B_{12} does not exist as such in the body but in an active form. The nature of this active form is discussed in the light of recent work.

CHAPTER II

OBSERVATIONS ON THE URINARY EXCRETION OF INJECTED ^{58}CO VITAMIN B_{12} BY NORMAL AND VITAMIN B_{12} DEFICIENT SUBJECTS AND ON THE EXCRETION OF ^{58}CO VITAMIN B_{12} BY VITAMIN B_{12} DEFICIENT SUBJECTS GIVEN REPEATED INJECTIONS OF ^{58}CO VITAMIN B_{12}.

One might expect that a vitamin B_{12} deficient subject would retain in his or her tissues a greater proportion of an injection of vitamin B_{12} than a normal subject. One might also expect, particularly in the case of the vitamin B_{12} deficient subject, that with repeated injections of vitamin B_{12}, the amount excreted would increase, pari-passu with the increase in tissue stores, possibly reaching a stage at which the tissues were saturated when virtually all the injected vitamin B_{12} would be excreted.

Reference to the literature showed that there was general agreement that the vitamin B_{12} deficient subject excreted the same amount of vitamin B_{12} after an injection as did the normal subject; there was also general agreement that the amount excreted increased as the body stores were augmented by repeated injections. Critical appraisal of these reports however, left room for doubt about the conclusions and as the urinary excretion of parenteral vitamin B_{12} can be measured directly with ease and accuracy by using ^{58}Co vitamin B_{12} it was

/decided to reinvestigate these points.

PLAN OF EXPERIMENTS, PATIENTS & METHODS

Two separate experiments were undertaken.

The first experiment was designed to find out if vitamin B_{12} deficient subjects excreted significantly different amounts of injected ^{58}Co vitamin B_{12} in the urine than normal subjects. For this purpose the amounts excreted by nine previously untreated vitamin B_{12} deficient subjects after an intramuscular injection of 100 ug 0.2 uc ^{58}Co vitamin B_{12} were compared to the amounts excreted by thirty one normal subjects each given a single intramuscular injection of 100 ug 0.2 uc ^{58}Co vitamin B_{12} and to the amounts excreted by eight chronically anaemic patients who had normal serum vitamin B_{12} levels and were also given a single intramuscular injection of 100 ug 0.2 uc ^{58}Co vitamin B_{12}. Similarly the amounts excreted by twelve previously untreated vitamin B_{12} deficient subjects after an intramuscular injection of 1000 ug 0.2 uc ^{58}Co vitamin B_{12} were compared to the amounts

/excreted by fifteen normal subjects given a single intramuscular injection of 100 ug 0.2 uc ^{58}Co vitamin B_{12} and to the amounts excreted by nine chronically anaemic subjects who had normal serum vitamin B_{12} levels and were also given a single intramuscular injection of 1000 ug 0.2 uc ^{58}Co vitamin B_{12}.

All the vitamin B_{12} deficient subjects had a macrocytic anaemia, megaloblastic erythropoiesis seen on marrow biopsy smears and a low serum vitamin B_{12} level and all responded to parenteral ^{58}Co vitamin B_{12}. These patients are listed in Tables A.25 and A.27 and full details of each case are given in the Case Reports in the Appendix. The normal subjects were those studied in the investigation reported in Chapter 1 and details of these patients are given in Tables A.12, A.13 and A.20. Details of the chronically anaemic subjects who had normal serum vitamin B_{12} levels are given in Tables A.26 and A.28 in the Appendix.

Urine was collected from all subjects for 24 hours prior to, and subsequent to the

/injection of ^{58}Co vitamin B_{12}. Solutions of ^{58}Co vitamin B_{12} for injection were prepared as described in the Appendix. Several different batches of ^{58}Co vitamin B_{12} were used and each batch was assayed microbiologically each week it was in use to detect deterioration. The isotope counting method used is described in the Appendix. The values obtained from the three groups of subjects in each dose group were compared by an analysis of variance.

The second experiment was designed to find out if the proportion of injected ^{58}Co vitamin B_{12} which was excreted altered as the body stores of vitamin B_{12} or its active form, were replenished. This was done by measuring the radioactivity excreted by vitamin B_{12} deficient subjects given repeated injections of the same dose of ^{58}Co vitamin B_{12}. The doses used ranged from 54 ug 0.3 uc to 30,000 ug 0.1 uc and thirty subjects were studied. All presented with a macrocytic anaemia, megaloblastic erythropoiesis seen on marrow biopsy smears and a low serum vitamin B_{12}

/level and all responded to treatment with parenteral ^{58}Co vitamin B_{12}. In addition five other patients (Cases F.A., J.McF., I.R., A.S. & R.T.) who had presented with evidence of vitamin B_{12} deficiency and who had previously received parenteral vitamin B_{12} and were in full haematological remission were studied by the same method. The investigation in Cases F.A. and I.R. was interrupted and during this period they were given 1000 ug vitamin B_{12} intramuscularly each month. In some patients the effect of drugs or different routes of administration on the excretion of ^{58}Co vitamin B_{12} was studied concurrently. As will be shown later, in Chapter 3, these were without effect and it is therefore legitimate to include the results obtained from these patients in this investigation. Details of the patients are given in the Case Reports in the Appendix.

Urine was collected for 24 hours prior to the first injection of ^{58}Co vitamin B_{12} and for 24 hours after the injections of vitamin B_{12}.

/In some cases where injections were given frequently 24 hour collections were made uninterruptedly for the period of the investigation. Solutions of ^{58}Co vitamin B_{12} for injection were made up as described in the Appendix. Several batches of ^{58}Co vitamin B_{12} were used and each was assayed microbiologically each week it was in use to detect deterioration. The isotope counting method used is described in the Appendix.

In both experiments all patients were aware of the nature and objects of the experiment and all cooperated willingly.

RESULTS

No radioactivity was detected in any urine collected prior to the first injection of ^{58}Co vitamin B_{12}. Deterioration of the ^{58}Co vitamin B_{12} solution in vitro was not observed.

First experiment

The amounts of radioactivity excreted by the vitamin B_{12} deficient subjects are given in

/Tables A.25 & 27, by normal subjects in Tables A.12, A.13 & A.20 and by the chronically anaemic subjects with normal serum vitamin B_{12} levels in Tables A.26 & 28 in the Appendix. These results are summarised in Figs. 6 & 7 and the essential statistical results in Table 3. The mean values in each dose group do not differ significantly from each other: in the 100 ug series $F = 0.76$, $P = > 0.05$ and the standard error of a single measure is 12.01: in the 1000 ug series $F = 0.16$, $P = > 0.05$ and the standard error of a single measure is 15.44.

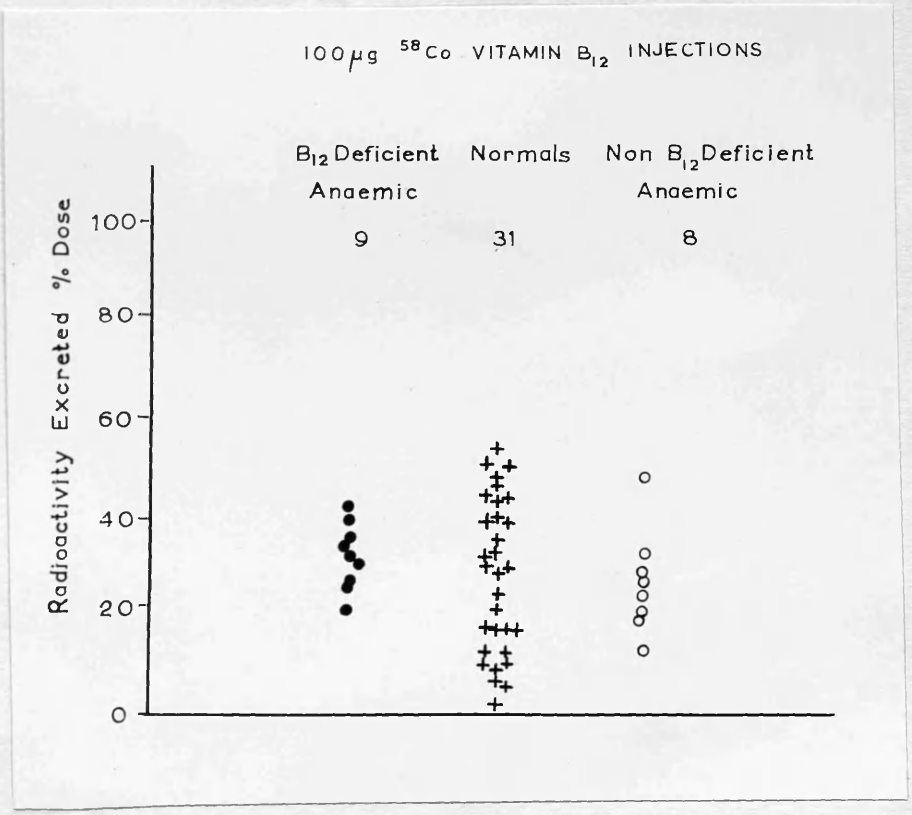


Fig. 6: Showing amounts of radioactivity excreted by anaemic vitamin B_{12} deficient patients, normal subjects and chronically anaemic patients with normal serum vitamin B_{12} levels given 100 ug ^{58}Co vitamin B_{12} intramuscularly.

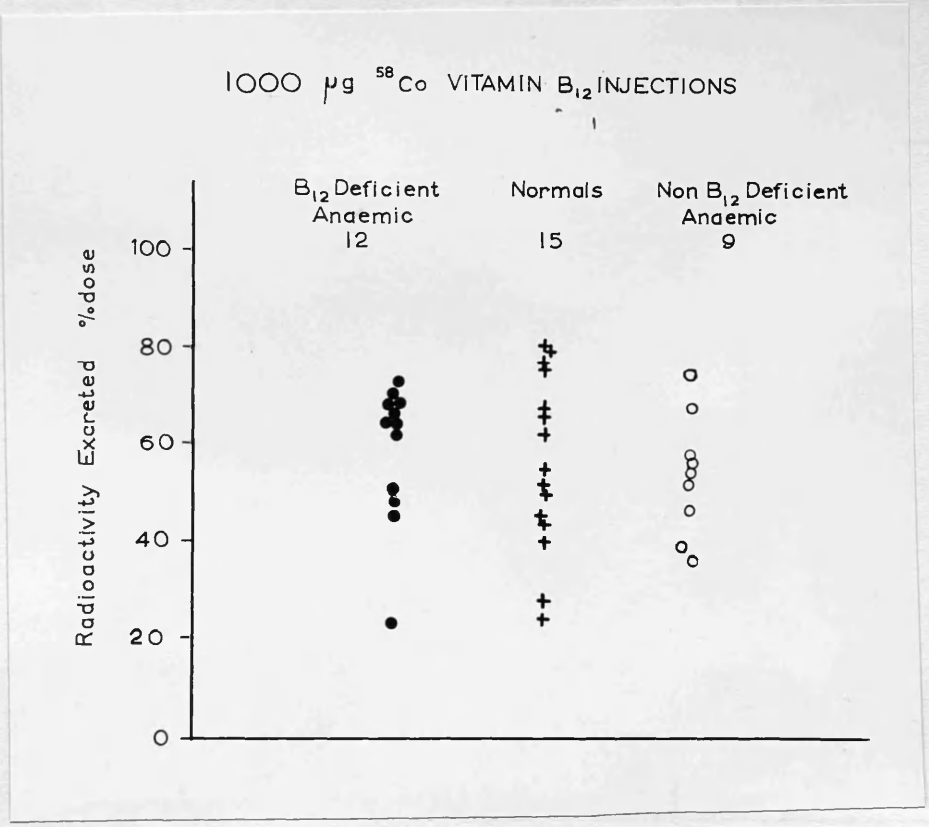


Fig. 7: Showing amounts of radioactivity excreted by anaemic vitamin B_{12} deficient patients, normal subjects and chronically anaemic patients with normal serum vitamin B_{12} levels given 1000 ug ^{58}Co vitamin B_{12} intramuscularly.

DOSE	PATIENTS	NUMBER OF RESULTS	MEAN VALUE EXCRETED % dose
100 ug	B_{12} deficient Anaemic	9	31.9222
	Normals	31	32.9903
	Anaemic Non B_{12} deficient	8	27.1000
1000 ug	B_{12} deficient Anaemic	12	58.7166
	Normals	15	54.7666
	Anaemic Non B_{12} deficient	9	56.9800

Table 3: showing mean values excreted by anaemic vitamin B_{12} deficient patients, normal subjects and patients with chronic anaemia and normal serum vitamin B_{12} levels, given injections of 100 ug and 1000 ug ^{58}Co vitamin B_{12}. The mean values in each dose group do not differ significantly from each other.

RESULTS (cont.)

Second experiment

The amounts of radioactivity excreted by patients given repeated injections of ^{58}Co vitamin B_{12} in the dose range 54 - 30,000 ug are shown in Figs. 8 - 42. The amount of ^{58}Co vitamin B_{12} retained by each patient, obtained by subtracting the amount excreted from the amount injected, is shown in Table 4. Full details of individual results are given in Tables A.2 - 4, A.6 - 10, A.14 & 15, A.17 - 19, A.21 & 22, A.29 - 48. As there was obviously no constant trend to an increase in the amounts of ^{58}Co vitamin B_{12} excreted during the observation periods, the results were not subjected to detailed statistical analysis.

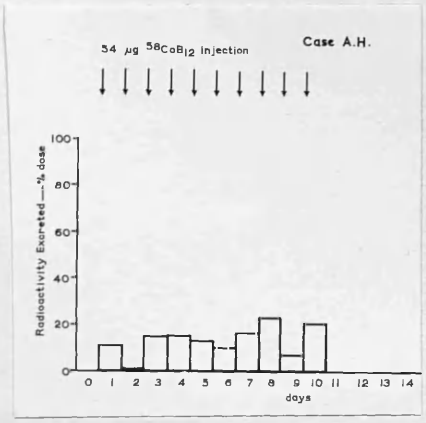


Fig. 8

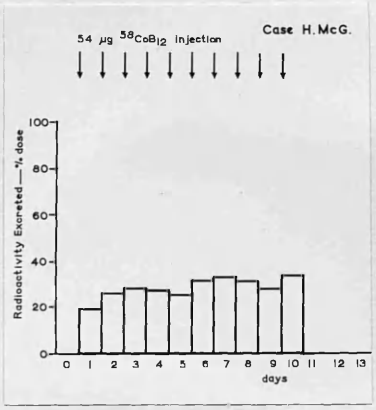


Fig. 9

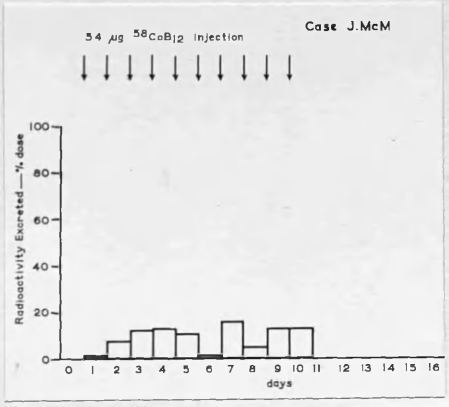


Fig. 10

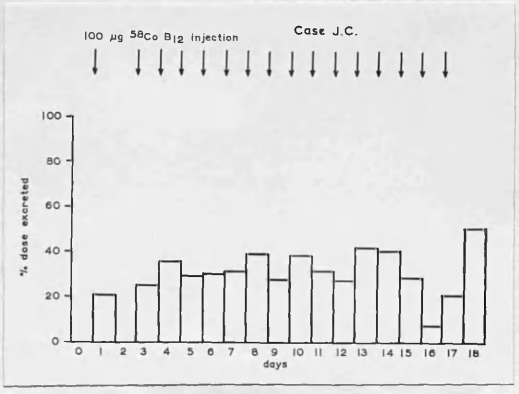


Fig. 11

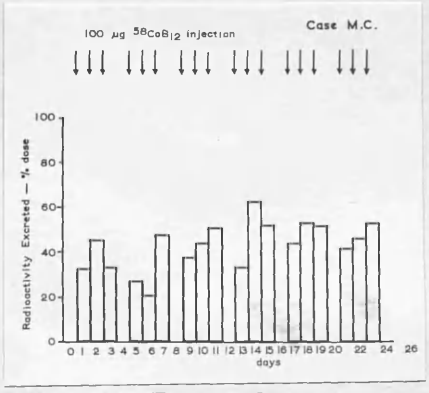


Fig. 12

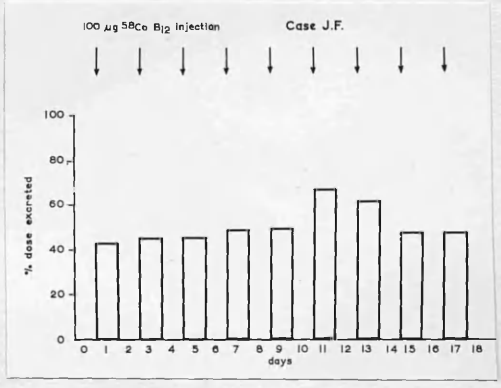


Fig. 13

Figs. 8–13: Showing amounts of radioactivity excreted by patients given repeated injections of 54 ug or 100 ug ^{58}Co vitamin B_{12} (see Tables A.2–4, 6–8).

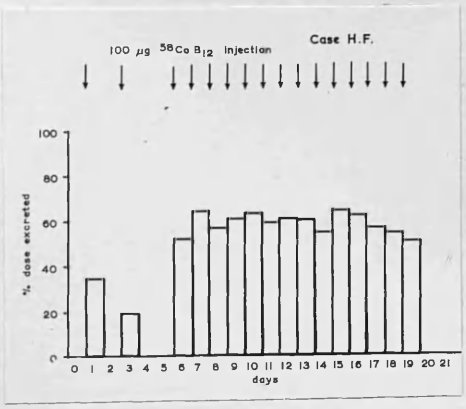


Fig. 14

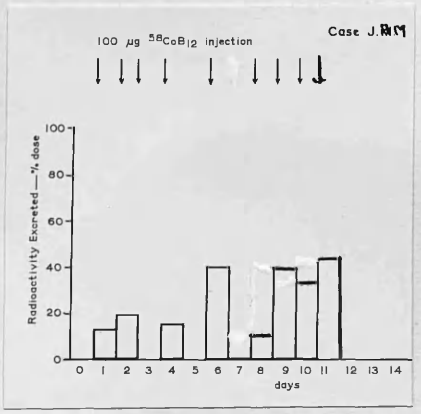


Fig. 15

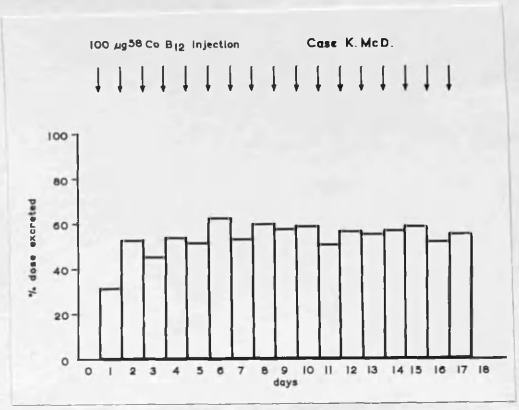


Fig. 16

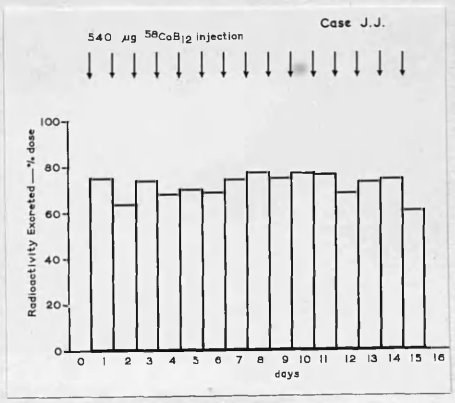


Fig. 17

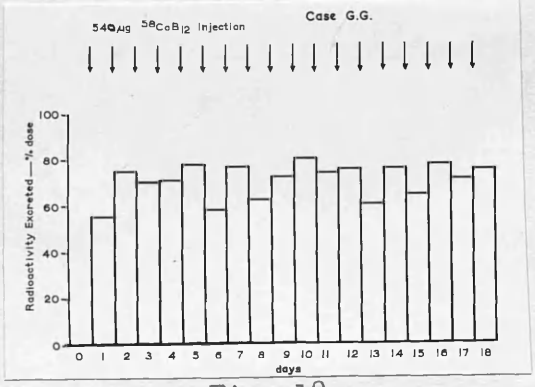


Fig. 18

Figs. 14–18: Showing amounts of radioactivity excreted by patients given repeated injections of 100 ug or 540 ug ^{58}Co vitamin B_{12} (see Tables A.9, 10, 14, 15 & 29).

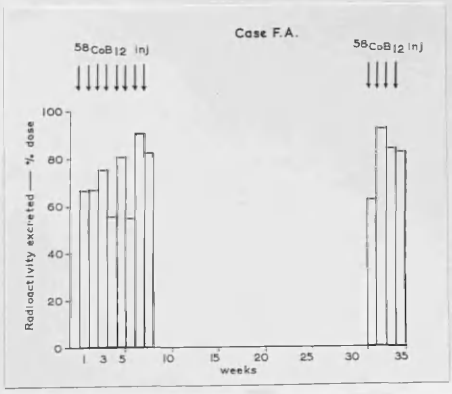


Fig. 19

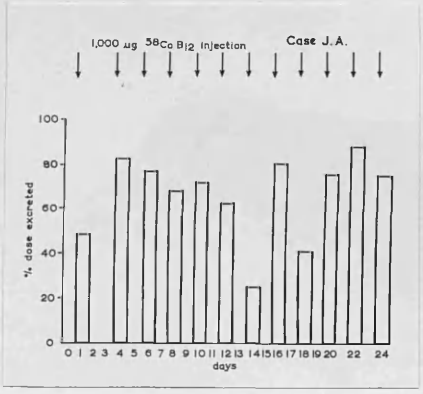


Fig. 20

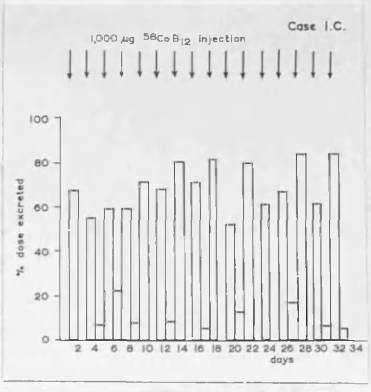


Fig. 21

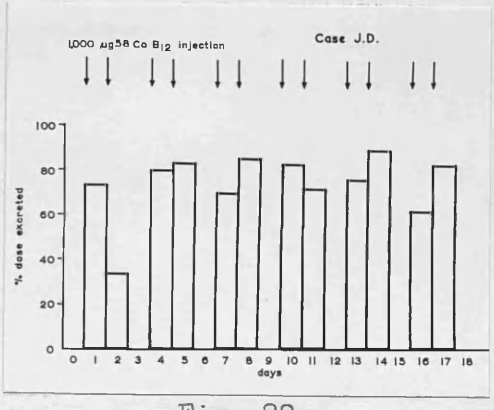


Fig. 22

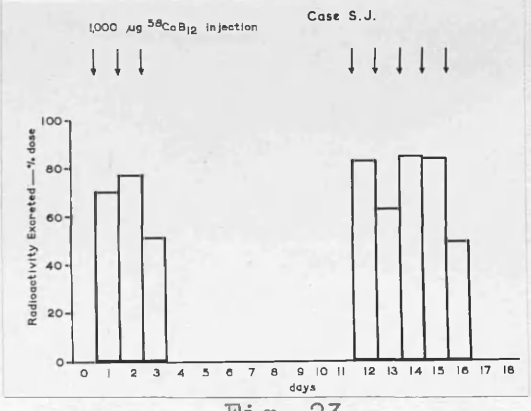


Fig. 23

Figs. 19-23: Showing amounts of radioactivity excreted by patients given repeated injections of 1000 ug ^{58}Co vitamin B_{12} (see Tables A.17 & 18, A.30-32).

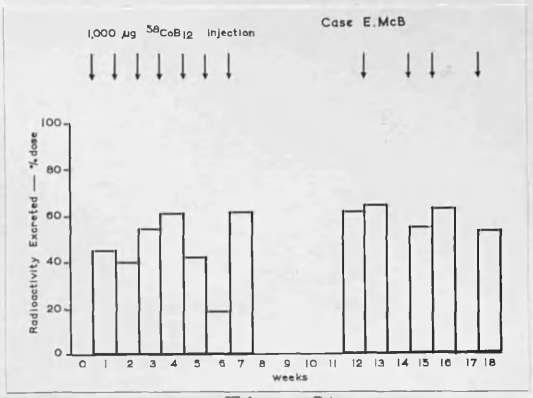


Fig. 24

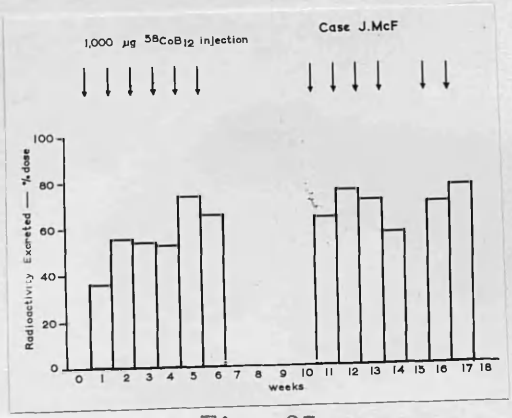


Fig. 25

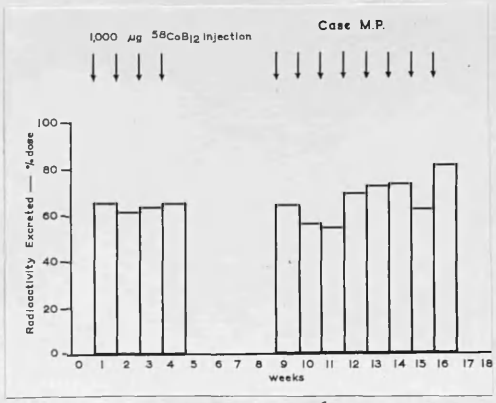


Fig. 26

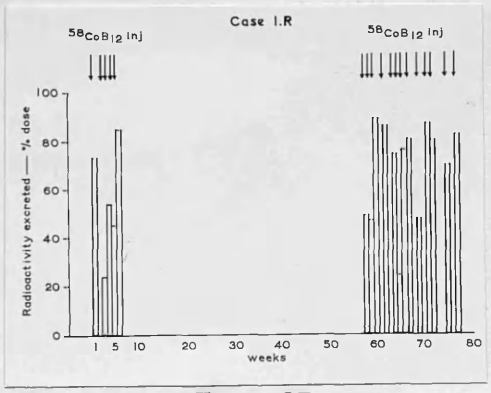


Fig. 27

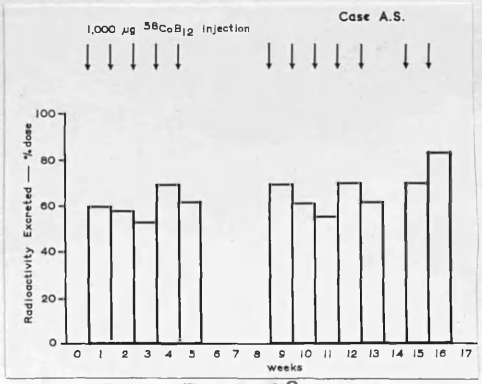


Fig. 28

Figs. 24-28: Showing amounts of radioactivity excreted by patients given repeated injections of 1000 ug ^{58}Co vitamin B_{12} (see Tables A.33-37).

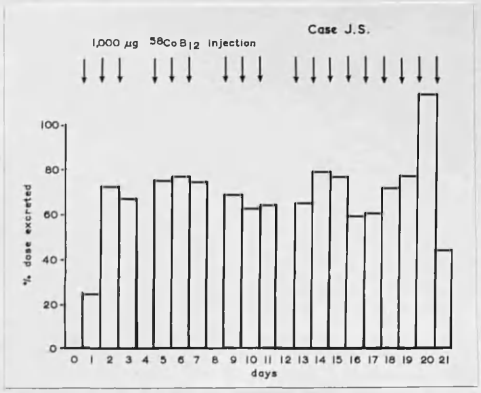


Fig. 29

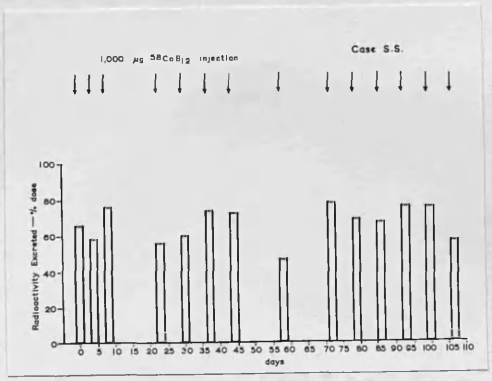


Fig. 30

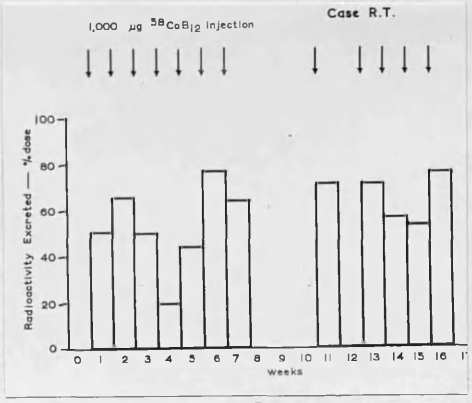


Fig. 31

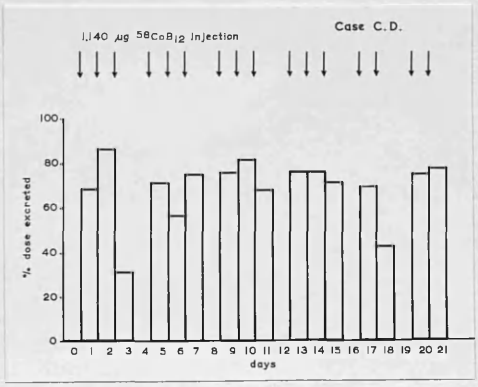


Fig. 32

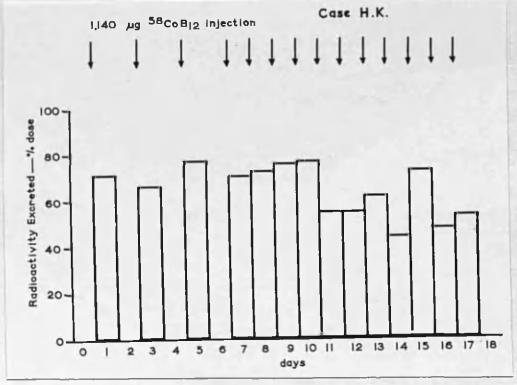


Fig. 33

Figs. 29-33: Showing amounts of radioactivity excreted by patients given repeated injections of 1000 ug or 1140 ug ^{58}Co vitamin B_{12} (see Tables A.19, 21, 22, 38 & 39).

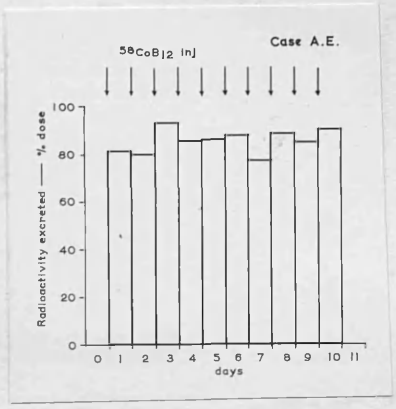


Fig. 34

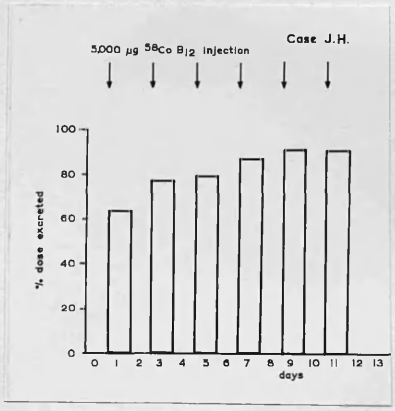


Fig. 35

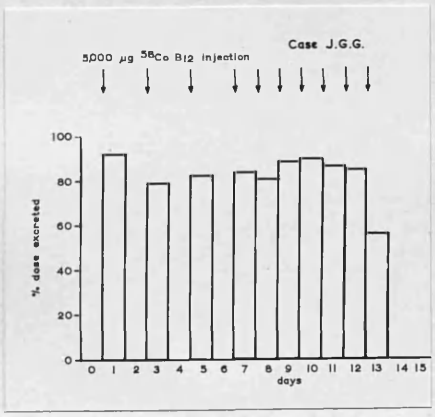


Fig. 36

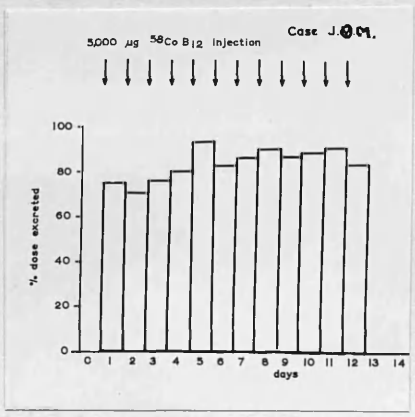


Fig. 37

Figs. 34-37: Showing amounts of radioactivity excreted by patients given repeated injections of 5000 ug ^{58}Co vitamin B_{12} (see Tables A.40-43).

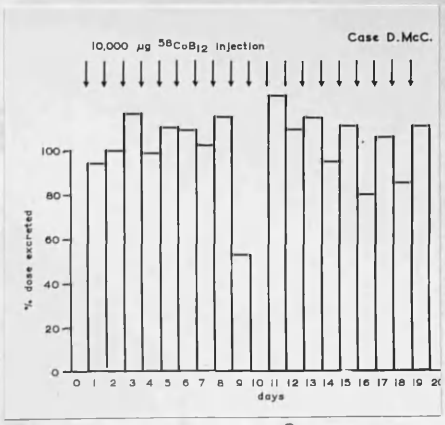


Fig. 38

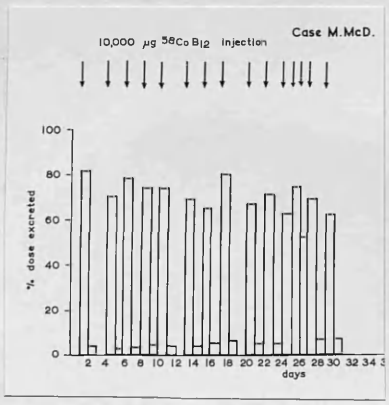


Fig. 39

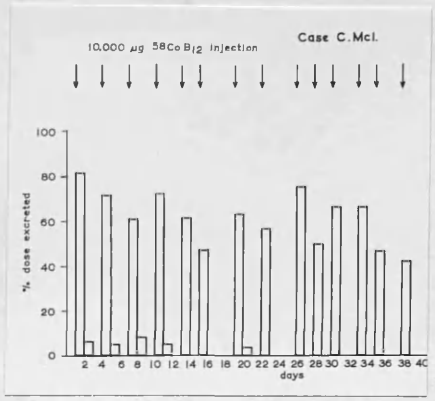


Fig. 40

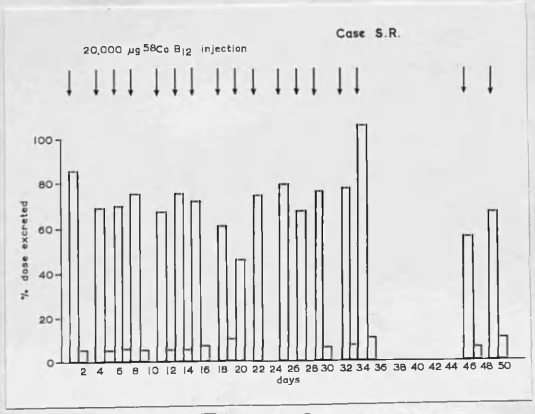


Fig. 41

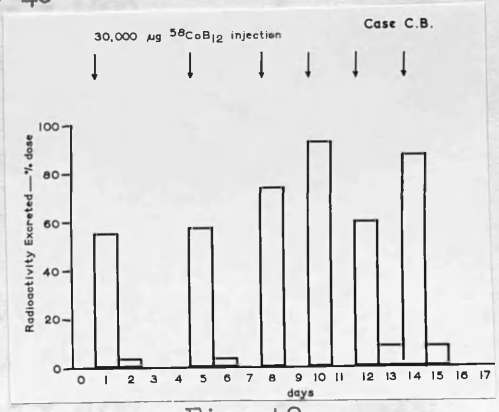


Fig. 42

Figs. 38–42: Showing amounts of radioactivity excreted by patients given repeated injections of 10,000 ug, 20,000 ug or 30,000 ug ^{58}Co vitamin B_{12} (see Tables A.44–48).

CASE	^{58}Co VITAMIN B_{12} INJECTIONS ug	TOTAL ^{58}Co B_{12} GIVEN ug	CALCULATED ^{58}Co B_{12} RETAINED ug
A.H.	10 x 54	540	466
H.McG.	10 x 54	540	401
J.McM.	10 x 54	540	494
J.C.	17 x 100	1700	1185
M.C.	18 x 100	1800	1042
J.F.	9 x 100	900	451
H.F.	16 x 100	1600	726
J.A.M.	8 x 100	800	585
K.McD.	17 x 100	1700	790
G.G.	18 x 540	9720	2731
J.J.	15 x 540	8100	2292
F.A. *	12 x 1000	12000	4195
J.A.	12 x 1000	12000	4104
I.C.	16 x 1000	16000	4044
J.D.	12 x 1000	12000	3132
S.J.	8 x 1000	8000	2272
E.McB.	12 x 1000	12000	5760
J.McF. *	12 x 1000	12000	4469
M.P.	12 x 1000	12000	4116
I.R. *	18 x 1000	18000	6229
A.S. *	12 x 1000	12000	5271
J.S.	18 x 1000	18000	5598
S.S.	14 x 1000	14000	4718
R.T. *	12 x 1000	12000	5007
C.D.	16 x 1140	18240	5727
H.K.	14 x 1140	12960	5681
A.E.	10 x 5000	50000	7125

CASE	^{58}Co VITAMIN B_{12} INJECTIONS ug	TOTAL ^{58}Co B_{12} GIVEN ug	CALCULATED ^{58}Co B_{12} RETAINED ug
J.G.G.	10 x 5000	50,000	9500
J.H.	6 x 5000	30,000	5760
J.O.M.	12 x 5000	60,000	8460
D.McC.	18 x 10000	180,000	6480
M.McD.	15 x 10000	150,000	43500
C.McI.	14 x 10000	140,000	50820
S.R.	17 x 20000	340,000	83300
C.B.	6 x 30000	180,000	41760

Table 4: showing amounts of ^{58}Co vitamin B_{12} given and amounts retained calculated by subtracting the amounts excreted in urine from amounts injected in the cases from which the results in figs. 8 - 42 were obtained. The cases are listed in alphabetical order in each dose group. Cases marked with an asterisk all received vitamin B_{12} either before or during the observation period.

DISCUSSION

First experiment

Although there is general agreement in the literature that normal and vitamin B_{12} deficient subjects do not excrete significantly different amounts of injected vitamin B_{12} this conclusion, either stated or implied, is based on flimsy evidence in most publications. The reports by Chow et al (1950), Conley et al (1950) and Krevans et al (1951) are based on results obtained from the same patients, three of whom were vitamin B_{12} deficient and two of whom were normal; those by Conley et al (1951) and Unglaub et al (1954) from results from two vitamin B_{12} deficient and two normal subjects while Chesterman et al (1951) drew their conclusions from observations on one vitamin B_{12} deficient patient and ten normal subjects. Acceptable evidence justifying the conclusion that there is no significant difference in the amounts excreted by vitamin B_{12} deficient and normal subjects was provided by Sokoloff et al (1952) who studied six vitamin B_{12} deficient

/patients and six normal subjects receiving injections of vitamin B_{12} in the dose range 42 - 211 ug, by Mollin & Ross (1953a) who studied five vitamin B_{12} deficient patients and five normals in the dose range 40 - 1000 ug and by Reizenstein (1959b) who studied six vitamin B_{12} deficient subjects and fifteen normals in the dose range 0.3 - 1.0 ug. When evaluating these results it must be noted that no allowance was made for the fact that renal function may be severely impaired by chronic anaemia (Bradley & Bradley 1947). These results therefore form a basis not for comparing the amounts excreted by normal and by vitamin B_{12} deficient subjects but only for comparing amounts excreted by normal and by vitamin B_{12} deficient subjects with renal insufficiency due to chronic anaemia. It was for this reason that a third group of patients who were chronically anaemic but had normal serum vitamin B_{12} levels was included in the present investigation. Ideally a group of patients with low serum vitamin B_{12} levels and other stigmata of

/vitamin B_{12} deficiency but who were not anaemic, should also have been studied: such patients have been described by Adams (1957a) and Killander (1957b) but are uncommon and a sufficient number of results from such patients could not be obtained. The alternative of treating vitamin B_{12} deficient patients with folic acid or blood transfusion to eliminate the effect of anaemia on renal function, was not considered justifiable.

When the results obtained in the investigations reported in Chapter 1 were analysed, it was noted that the mean values of radioactivity excreted by the vitamin B_{12} deficient subjects did not apparently differ greatly from those excreted by normal subjects given the same dose of ^{58}Co vitamin B_{12}. It was appreciated that a detailed analysis of these results would not have yielded a valid result, as, amongst other things, the population of, as opposed to the number of results obtained from, the vitamin B_{12} deficient patients in each dose group was small. The present method of

/investigation was therefore chosen to clarify the point and it is clear from the results that the amounts of radioactivity excreted after injections of 100 ug and 1000 ug ^{58}Co vitamin B_{12} are not affected by chronic anaemia or by chronic anaemia and vitamin B_{12} deficiency: it seems extremely unlikely therefore that vitamin B_{12} deficiency in the absence of anaemia would affect the amounts of radioactivity excreted.

It would not have been surprising to find that the amounts excreted by the vitamin B_{12} deficient groups of patients were significantly less than the amounts excreted by the normal groups of patients, suggesting that depleted tissues 'soaked up' the injected ^{58}Co vitamin B_{12} but this is plainly not the case. The reason for this is not clear. Several factors may be involved and the rate of excretion of injected ^{58}Co vitamin B_{12}, the rate of binding of injected ^{58}Co vitamin B_{12} to tissues, the capacity of the tissues to bind injected ^{58}Co vitamin B_{12} and the blood level of injected

/^{58}Co vitamin B_{12} must all be considered. If the renal excretion of injected Co vitamin B_{12} were very rapid it is conceivable that a proportion could be swept out on the renal tide before it could be bound to tissues whether depleted or not. Although the renal excretion of injected ^{58}Co vitamin B_{12} is rapid (see Chapter 4) it does not occur at such a rate as to provide such an explanation for the results unless it were also shown that the process of binding of injected ^{58}Co vitamin B_{12} to tissues took some time. This is very unlikely in view of the rapid clearance of injected Co vitamin B_{12} from the blood stream, (Miller et al 1957, Estren et al 1958, Hall 1960) and the rapid uptake by tissues as shown by external counting (Estren et al 1958, Glass 1959, Brody et al 1960). Apart from the rate of binding of injected Co vitamin B_{12} to tissues there is the possibility that the capacity of the tissues to bind injected Co vitamin B_{12} is limited whether the tissues are depleted or not. This factor alone is unlikely to be of much importance as

/the amount of ^{58}Co vitamin B_{12} retained after an injection of 50 ug is about 40 ug whereas about 800 ug is retained in the same period after an injection of 10,000 ug: it might be a major factor however, if the amount bound were also related to the concentration of free injected ^{58}Co vitamin B_{12} in the blood stream. The blood concentration is greater after injection of a large dose and as a general rule the actual amount, as opposed to the percentage of the dose retained, increases as the dose injected is increased. If the binding capacity and amount bound were related to the blood concentration one might expect a greater amount to be retained after an intravenous dose than after intramuscular administration of the same dose. This does not occur with doses of 100 ug and 1000 ug at least (Chapter 3) but this is not necessarily evidence against this theory as the rate of excretion is faster after intravenous than after intramuscular administration leaving less injected vitamin available for binding. Other factors too may be involved. It has been suggested that vitamin B_{12}

/does not exist as such in the body and that injected vitamin B_{12} is converted to an active form (Chapter 1. Adams 1961a). It would be reasonable to expect depleted tissues to retain more injected active form than normal tissues but not necessarily reasonable to expect a difference when an inactive form or precursor was injected. The fact that the inactive form is converted to the active form in, or after the process of binding is not evidence against this explanation. When the active form or forms of vitamin B_{12} are established beyond doubt it will be important to study the amounts excreted by normal and by vitamin B_{12} deficient subjects as was done in this study to determine if this is a relevant factor.

From this discussion it is obvious that no single factor can explain the results and that the answer probably lies in a complex inter-relation of several factors all operating simultaneously. Further investigations on this problem are in progress.

/Second experiment

It has been shown that vitamin B_{12} deficient subjects in haematological relapse do not excrete significantly different amounts of injected ^{58}Co vitamin B_{12} from normal subjects at least as far as the first injection of ^{58}Co vitamin B_{12} given to vitamin B_{12} deficient subjects is concerned. This does not imply that the proportion of injected vitamin B_{12} which is excreted will not increase as the body stores are augmented by repeated injections and this, in fact, has been reported to occur in vitamin B_{12} deficient patients (Sokoloff et al 1952, Mollin & Ross 1953a), in normal subjects (Chesterman et al 1951, Sokoloff et al 1952, Estrada et al 1954), in one leukaemic patient (Mollin & Ross, 1953a) and in one rat (Harte et al 1953). In all but one of these investigations the technique adopted was to measure the urinary loss, after a single injection of vitamin B_{12}, by microbiological assay, to give 'saturating' intramuscular doses ranging from 20 ug daily for two weeks (Chesterman et al 1951) to 1000 ug daily for seven days

/(Mollin & Ross 1953a) and then to measure the urinary loss after a second injection. The conclusion that the proportion excreted increased as the body stores were increased was based on the finding of an increased amount excreted after the second injection. In only one investigation was the possibility considered that the amount excreted by any one patient might vary from day to day. Mollin & Ross (1953a) measured the daily excretion after injections for four days and then, finding that there was no significant variation, 'saturated' the patient and then measured the amounts excreted after two further injections and found that the amounts excreted were greatly increased. It is obvious from the results shown in Figs. 8 - 42 that this could have occurred by chance and it is likely that this is the explanation for the findings of the other authors who have investigated this problem.

In a few cases studied in this series there was an apparent trend to excretion of greater amounts with repeated injections of

/^{58}Co vitamin B_{12}: in a few others the reverse appeared to be the case. From the overall picture, however, it seemed likely that these apparent trends would have disappeared had the observation period been extended. The results were submitted to Dr. R. A. Robb who advised that they were such clear evidence against a constant trend to the excretion of greater or smaller proportions of the amounts injected that statistical analysis was unnecessary. The results also show that it is very unlikely that there is any stage of tissue saturation with ^{58}Co vitamin B_{12} or its active form at which the amount excreted increases in a plateau like manner. Had there been such a stage it might not have been obvious when larger doses alone were given but would have been obvious when smaller doses were given. The overlap of total amounts of injected ^{58}Co vitamin B_{12} retained in the body shown in Table 4 is additional evidence in this connection. The amounts of ^{58}Co vitamin B_{12} retained were calculated by subtracting the amount excreted in the urine from the amount

/injected and range from values much less to very greatly in excess of those found in normal tissues by microbiological assay (see Chapter 7). To secure retention of amounts in excess of the normal stores it was necessary to give large doses of ^{58}Co vitamin B_{12}: such doses were given to patients with vitamin B_{12} deficiency states in haematological relapse and for reasons already given in Chapter 1 it can be assumed with confidence that the radioactivity excreted was a true measure of the amount of ^{58}Co vitamin B_{12} excreted.

The extraordinary capacity of vitamin B_{12} deficient subjects to retain very large amounts of injected ^{58}Co vitamin B_{12} has not been described previously. As the amounts retained were often greatly in excess of those present in normal tissues it seems probable that this capacity is also enjoyed by normal subjects. If this is the case it is surprising that this phenomenon has not been utilised by nature. It may be that the extent of the body stores of vitamin B_{12} or its active form is limited by

/the small amounts which can be absorbed from the intestinal tract and that the amounts present in normal tissues represent a nice balance between intake and expenditure allowing for all eventualities other than complete deprivation.

The ease with which very large amounts of ^{58}Co vitamin B_{12} can be stored in tissues is particularly interesting in relation to the treatment of patients with vitamin B_{12} deficiency states and the findings suggest that it would be a practical therapeutic proposition to treat patients by massive injection therapy, the so called 'stosstherapy', to ensure retention of enough vitamin B_{12} to meet the demands for years. No toxic effects were observed in any patient in this series at any time and all have been followed up for at least one and a half years and the majority for longer periods. One obvious problem with massive injection therapy however, lies in the difficulty of knowing how much vitamin B_{12} any one patient will retain from an injection or series of injections. It is clear from the results that the proportion

/of each dose which is excreted varies not only from patient to patient but also in the same patient at different times. Unless the amounts excreted were measured, and the amount retained known, such a method of treatment would be of limited value. To some extent this problem has been overcome (see Chapter 6) but other factors such as the utilisation of injected vitamin B_{12} must also be considered. This form of therapy is discussed again in conjunction with observations on these factors in Chapter 8.

SUMMARY

The amount of vitamin B_{12} excreted after intramuscular doses of 100 ug and 1000 ug has been studied in normal subjects, in vitamin B_{12} deficient patients in haematological relapse and in patients with chronic anaemia and normal serum vitamin B_{12} levels using the direct isotope method of measuring the ^{58}Co vitamin B_{12} excreted in the urine. No significant difference was found between the mean values obtained from the three groups of patients in each dose group. The findings and possible explanations for the findings are discussed.

A series of vitamin B_{12} deficient patients in haematological relapse and a smaller series of patients who had been treated with parenteral vitamin B_{12} were given repeated injections of ^{58}Co vitamin B_{12} in doses ranging from 54 ug to 30,000 ug and the urinary excretion of radioactivity was measured. There was no constant trend to an increase or decrease in amounts excreted with repeated injections as might have been expected. The amounts of

/^{58}Co vitamin B_{12} retained in the body ranged from 400 ug to 83,000 ug. The findings are discussed, particular reference being paid to the significance of the findings in relation to massive therapy with vitamin B_{12}.

CHAPTER III

OBSERVATIONS ON THE EFFECT OF SOME DRUGS AND THE ROUTE OF ADMINISTRATION ON THE URINARY EXCRETION OF INJECTED ^{58}Co VITAMIN B_{12} AND ON THE DIURETIC EFFECT OF INJECTED ^{58}Co VITAMIN B_{12}.

From the therapeutic and economic aspects the urinary loss of injected vitamin B_{12} represents a waste which is considerable when large doses are given. It seemed worthwhile therefore, investigating the effect of some drugs on the renal excretion of injected ^{58}Co vitamin B_{12} in order to find out if they affected the amounts excreted and thus to gain information relating to the mode of excretion and to any possible means of reducing the amount excreted. At the same time it seemed logical to compare the effect of intravenous and intramuscular injections on the excretion of the injected vitamin and to investigate the claims that vitamin B_{12} has a diuretic action.

PATIENTS, MATERIALS & METHODS

The effect of drugs or different routes of administration was studied in thirteen patients. All came under observation with a macrocytic anaemia and megaloblastic erythropoiesis seen on marrow biopsy smears and all had other diagnostic criteria of vitamin B_{12} deficiency states. Nine patients were studied when they

/were first seen in haematological relapse; four (Cases F.A., J.McF., I.R. & A.S.) had been treated and were in haematological remission when the investigation was begun. Details of the patients are given in the Case Reports in the Appendix. The study of Cases I.R. & F.A. was interrupted and during this time they received 1000 ug vitamin B_{12} intramuscularly each month. All patients were cognisant of the nature and objects of the experiments and all cooperated willingly.

The method of investigating the effect of the drugs was to compare the mean value of the amounts of radioactivity excreted in 24 hour collections of urine after injections of ^{58}Co vitamin B_{12} with the mean value of the amounts of radioactivity excreted by the same patient in the 24 hours after injections of ^{58}Co vitamin B_{12} given with the drug being tested. Similarly the method of investigating the effect of differing routes of administration was to compare the mean values of amounts of radioactivity excreted in the urine in the

/24 hours after intramuscular administration with those obtained in the 24 hours after intravenous administration. During the investigation, injections, or a series of injections of ^{58}Co vitamin B_{12}, were given with or without the drug or by different routes of administration alternately or at random. Each patient received at least twelve injections of ^{58}Co vitamin B_{12} of which half were given without the drug and half were given with the drug or by a different route. The drugs studied were probenecid (Benemid - Merck, Sharp & Dohme), mersalyl (Mersalyl Injection B.P. - British Drug House), chlorothiazide (Saluric - Merck, Sharp & Dohme) and vasopressin tannate (Pitressin Tannate in Oil - Parke Davis). The doses of ^{58}Co vitamin B_{12} used were 100 ug 0.2 uc, 1000 ug 0.2 uc and 1140 ug 0.2 uc.

The effect of probenecid was studied in five patients. Cases F.A. and A.S. were given probenecid orally 0.25 g. q.i.d. for 48 hours prior to, and 24 hours after, intramuscular injections of ^{58}Co vitamin B_{12} which were given

/at intervals of at least one week. Cases M.C., J.S. & C.D. received short courses of probenecid 0.25 g. orally q.i.d. during which time two or three intramuscular injections of ^{58}Co vitamin B_{12} were given at 24 hour intervals: each course of probenecid began 24 hours before the first injection and ended 24 hours after the last injection of the series.

The effect of mersalyl was studied in three patients. The drug was given intramuscularly in a dose of 2 ml. at a different site but at the same time as the intramuscular injection of ^{58}Co vitamin B_{12}.

The effect of chlorothiazide was studied in two patients, both of whom received 2.0 g. orally one hour prior to the intramuscular injection of ^{58}Co vitamin B_{12}.

The effect of vasopressin tannate was studied in one patient who was given 2 ml. intramuscularly one hour before the intramuscular injection of ^{58}Co vitamin B_{12} at a different site.

The excretion of ^{58}Co vitamin B_{12} after intramuscular and intravenous administration was

/studied in four patients; the effect of drugs on the excretion of ^{58}Co vitamin B_{12} given intramuscularly was also studied in two of these patients (Cases M.C. & J.S.)

The diuretic effect of vitamin B_{12} was investigated by analysing the values for volumes of urine excreted in 24 hour periods by vitamin B_{12} deficient subjects in haematological relapse who were given repeated injections of ^{58}Co vitamin B_{12} in doses ranging from 100 ug 0.2 uc to 30,000 ug 0.1 uc at intervals of 24 hours or longer. The mean values of urine volumes excreted in the 24 hours after injections of ^{58}Co vitamin B_{12} were compared to the mean values of 24 hour urine volumes excreted on days when no radioactivity was found in the urine and to mean values of 24 hour urine volumes for the periods 24 - 48 - 72 hours after injection when some radioactivity was excreted.

The preparation of ^{58}Co vitamin B_{12} for injection and other technical methods used are described in the Appendix. A t test was used to compare two mean values and an analysis of

/variance to compare three mean values obtained from the same patient.

RESULTS

The results of the investigation into the effect of drugs or differing routes of administration on the renal excretion of injected ^{58}Co vitamin B_{12} are shown diagramatically in Figs. 43 - 55 and the essential statistical results are set out in Tables 5 & 6. Full details of individual results are given in Tables A.7, A.9, A.10, A.18, A.19, A.21, A.30, A.31, A.33, A.34, A.36 - 38 in the Appendix. None of the drugs tested had any significant effect on the amounts of injected ^{58}Co vitamin B_{12} excreted and no significant difference was found between the mean values excreted after intramuscular and after intravenous administration.

The results of the investigation into the diuretic effect of vitamin B_{12} are given in Table 7 with the discussion of the findings. Details of individual results are given in Tables A.8, A.17, A.31, A.41 & 42, A.45 - 48. No significant diuretic effect was found.

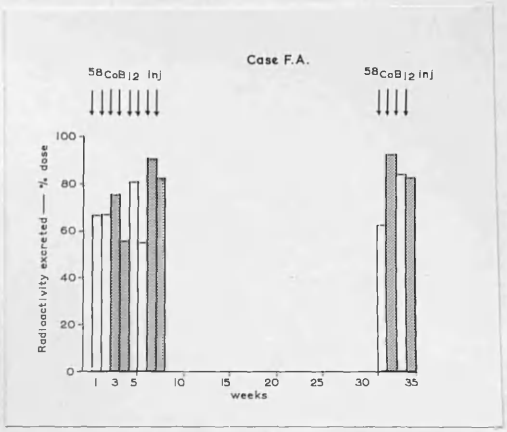


Fig. 43

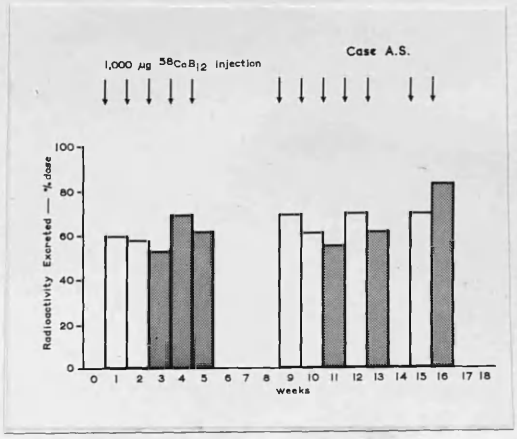


Fig. 44

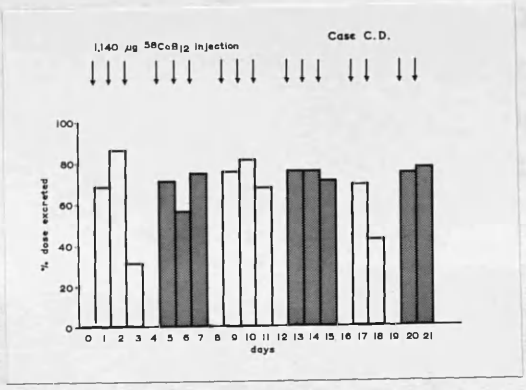
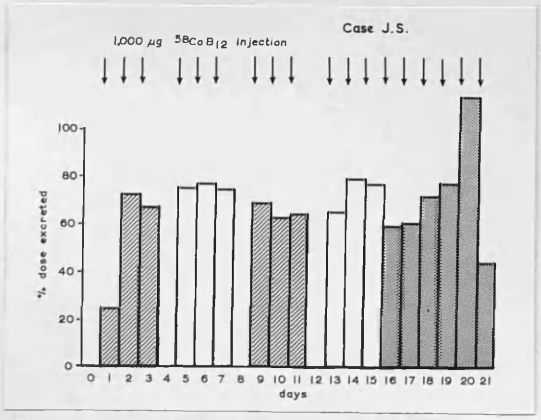
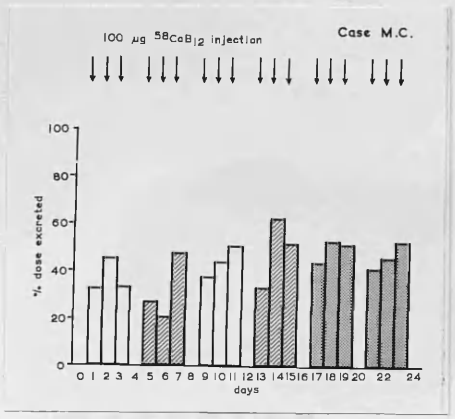


Fig. 45

Figs. 43 - 45: showing amounts of radioactivity excreted by three patients given repeated injections of ^{58}Co vitamin B_{12} alone or with probenecid. Cases F.A. and A.S. were given 1000 ug and Case C.D. 1140 ug ^{58}Co vitamin B_{12} intramuscularly (see Tables A.21, 30 & 37).

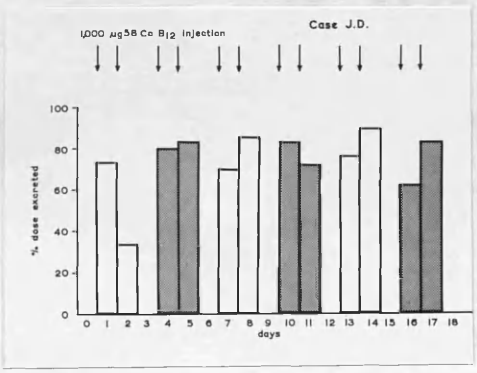
The plain rectangles indicate the amounts excreted after administration of ^{58}Co vitamin B_{12} only and the hatched rectangles the amounts excreted when oral probenecid was also given.

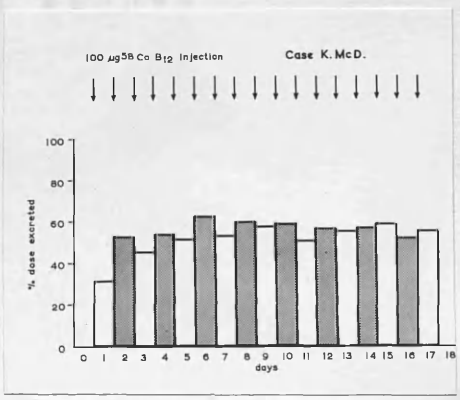




Figs. 46 & 47: showing amounts of radioactivity excreted by two patients given repeated injections of ^{58}Co vitamin B_{12} alone or with probenecid (see Tables A.7 & A.19).

The plain rectangles indicate the amounts excreted after intramuscular ^{58}Co vitamin B_{12} only, the hatched rectangles the amounts excreted after intramuscular ^{58}Co vitamin B_{12} and oral probenecid, the stippled rectangles after intravenous ^{58}Co vitamin B_{12} only.





Figs. 48 & 49: showing amounts of radioactivity excreted by two patients given repeated injections of ^{58}Co vitamin B_{12}. (see Tables A.10 & A.31).

The plain rectangles indicate the amounts excreted after intramuscular administration and the hatched rectangles the amounts excreted after intravenous administration.

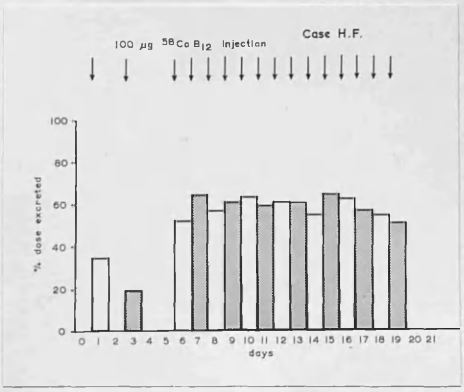


Fig. 50

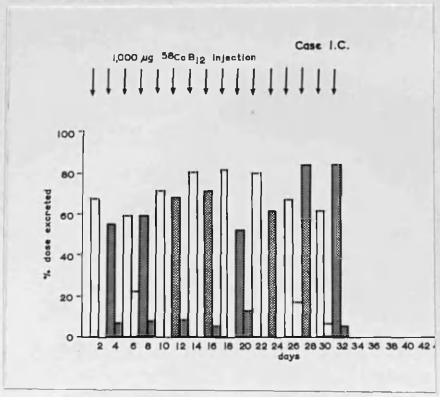


Fig. 51

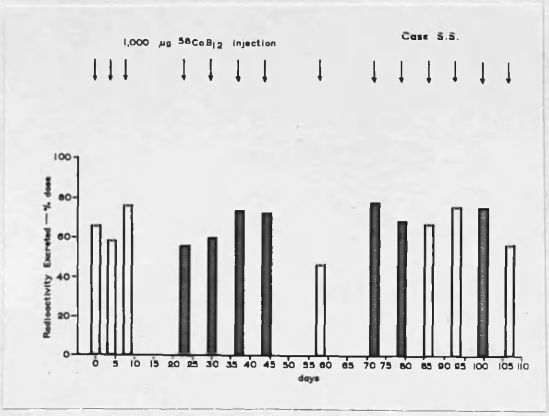


Fig. 52

Figs. 50 - 52: showing amounts of radioactivity excreted by three patients given repeated injections of ^{58}Co vitamin B_{12} intramuscularly, alone or with mersalyl. (see Tables A.9, A.18 & A.38).

The plain rectangles indicate the amounts excreted after intramuscular ^{58}Co vitamin B_{12} only and the hatched rectangles the amounts excreted when mersalyl was given also.

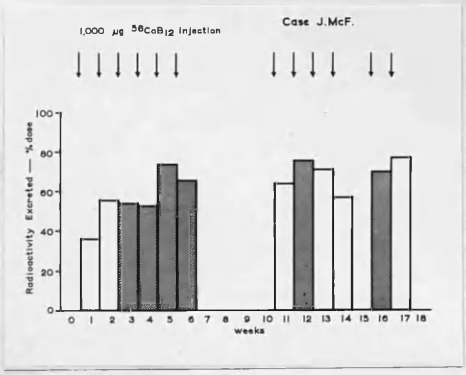


Fig. 53

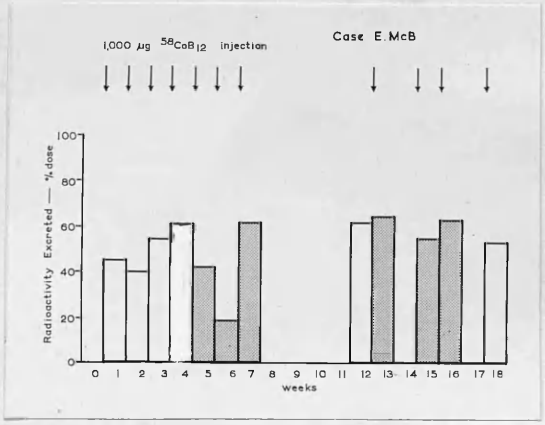


Fig. 54

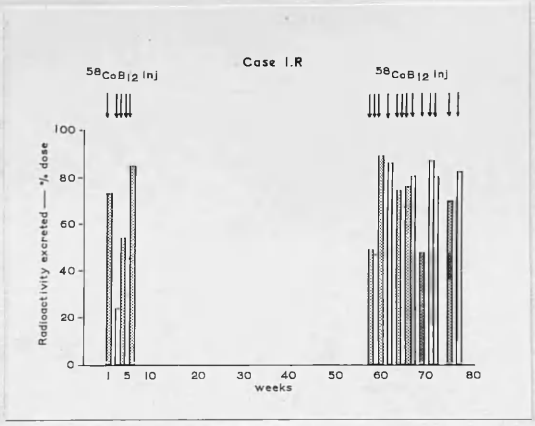


Fig. 55

Figs. 53 - 55: showing the amount of radioactivity excreted by three patients given repeated intramuscular injections of 1000 ug ^{58}Co vitamin B_{12} (see Tables A.33, 34, & 36).

The plain rectangles indicate the amounts excreted after injections of ^{58}Co vitamin B_{12} only and the hatched rectangles the amounts excreted when oral chlorothiazide was also given to Cases J.McF. and I.R. and when intramuscular vasopressin was also given to Case E.McB.

CASE	^{58}Co VITAMIN B_{12} DOSE	DRUG	WITHOUT DRUG		WITH DRUG		t
			No. OF DOSES	MEAN RADIOACTIVITY EXCRETED % dose	No. OF DOSES	MEAN RADIOACTIVITY EXCRETED % dose	
M.C.	100 ug im	probenecid	6	38.7	6	40.1	0.01
F.A.	1000 ug im	"	6	68.6	6	79.7	1.59
A.S.	"	"	6	64.8	6	63.9	0.17
J.S.	"	"	6	74.9	6	60.2	1.07
C.D.	1140 ug im	"	8	65.1	8	72.0	0.96
H.F.	100 ug im	mersalyl	8	54.8	8	54.4	0.07
I.C.	1000 ug im	"	8	70.7	8	66.8	0.73
S.S.	"	"	7	63.8	7	68.7	0.94
J.McF.	1000 ug im	chlorothiazide	6	60.1	6	65.3	0.72
I.R.	"	"	9	61.9	9	68.8	0.71
E.McB.	1000 ug im	vasopressin	6	52.7	6	51.3	0.13

Table 5: showing essential statistical results obtained from patients given intramuscular ^{58}Co vitamin B_{12} with and without various drugs. The difference between means is not significant in any case ($P => 0.05$).

CASE	^{58}Co VITAMIN B_{12} INJECTED	INTRAMUSCULAR ADMINISTRATION		INTRAVENOUS ADMINISTRATION	
		No. OF INJECTIONS	MEAN RADIOACTIVITY EXCRETED % DOSE	No. OF INJECTIONS	MEAN RADIOACTIVITY EXCRETED % DOSE
M.C.	100 ug	6	38.73	6	47.6
K.McD.	"	9	51.1	8	56.3
J.D.	1000 ug	6	70.9	6	76.8
J.S.	"	6	74.9	6	71.6

Table 6: showing essential statistical results obtained from four patients given ^{58}Co vitamin B_{12} intravenously and intramuscularly, no drugs being given on either occasion. The standard error of a single measure in Case M.C. is 10.94 and in Case J.S. 17.54. The value of 't' is less than 1.0 in Cases K.McD. & J.D. The difference between mean values for the same patient is not significant in any case ($P > 0.05$)

DISCUSSION

In the study of the effect of various drugs and different routes of administration on the excretion of injected ^{58}Co vitamin B_{12} any information gained would have been particularly relevant to the treatment of patients with vitamin B_{12} deficiency states and for this reason it was decided to confine the investigations to patients who required treatment with vitamin B_{12}. The results reported in Chapter 2 had made it clear that there was no constant trend to an increase or decrease in the amounts of injected ^{58}Co vitamin B_{12} excreted with repeated injections, whether the patients were in haematological remission or relapse when treatment was begun, and the precaution of randomizing or alternating injections with or without drugs was therefore unnecessary although probably desirable on general principles. The study of the diuretic effect of vitamin B_{12} was confined to patients receiving vitamin B_{12} being in the nature of a preliminary investigation. The results made it clear that further investigations in other

/patients were unlikely to be of value.

Effect of drugs

The lack of effect of probenecid is disappointing from the practical aspect. This drug is best known as a uricosuric agent, its effect being due to inhibition of resorption of filtered urate but is also inhibits tubular excretion of a wide variety of chemically unrelated substances (see 'Benemid' Annotated Bibliography 1957). Detailed investigation of the effect of this drug on the excretion of injected vitamin B_{12} was clearly desirable in view of its unique properties and it was disappointing to find that it was without effect. Had it been found that probenecid reduced the amount of injected ^{58}Co vitamin B_{12} excreted it would have been reasonable to assume that injected ^{58}Co vitamin B_{12} is, in part at least, excreted by the tubules; however, the fact that probenecid does not affect the excretion of injected ^{58}Co vitamin B_{12} cannot be held as conclusive evidence that there is no tubular component to the excretion of vitamin B_{12}, particularly as it has been shown

/that probenecid does not affect all tubular secreting mechanisms (Beyer et al 1951).

When treating patients with vitamin B_{12} deficiency states in haematological relapse with repeated injections of ^{58}Co vitamin B_{12} it was noted that the amount of ^{58}Co vitamin B_{12} excreted was not related to the urine volume, (see Tables A.2 - 4, A.6 - 10, A.14, A.15, A.17 - 19, A.21, A.22, A.29 - 48). This did not, of course, mean that a drug-induced diuresis would necessarily fail to affect the amount excreted and it was necessary to study this point in detail. The mode of action of chlorothiazide and mersalyl is not fully understood but it is likely that their main action is to interfere with tubular function causing decreased resorption of water and electrolytes (Lewis 1961, Spencer 1961) the former acting on the proximal tubule and the latter on both the proximal and the distal tubule (Lewis 1961). Part of the action of chlorothiazide is due to carbonic anhydrase inhibition but there is evidence that its diuretic effect is not solely due to this action (Matheson

/& Morgan 1958) and there is also evidence that while it shares a method of action with mersalyl it also has an action via a further mechanism which is not related to carbonic anhydrase inhibition (Ford 1957). The amount of injected ^{58}Co vitamin B_{12} excreted was not significantly affected by chlorothiazide or by mersalyl and it appears therefore that the excretion of injected ^{58}Co vitamin B_{12} is unaffected by the common mechanisms by which a diuresis is provoked.

In view of the results obtained using probenecid and the diuretics it seemed unlikely that vasopressin, which causes tubular resorption of water, would affect the amount of injected ^{58}Co vitamin B_{12} excreted and this proved to be the case.

It has already been noted that the failure of probenecid to affect the amount of injected ^{58}Co vitamin B_{12} excreted is not, in itself, evidence against the presence of a tubular component in the excretion of injected vitamin B_{12}. This also applies to the lack of effect of the other drugs. Taken separately these negative

/results are of little value. Taken together however, the results at least give rise to a suspicion that the amount of injected vitamin B_{12} excreted is predominantly a function of the glomerular mechanism with little or no tubular component. The fact that the glomerular filtration rate may be reduced by chlorothiazide is not important here as this effect is small in the absence of renal disease (Spencer 1961).

If it is the case that the urinary loss of injected vitamin B_{12} is a function of glomerular filtration only, it follows that the only method of reducing the loss is by reducing the blood flow through the kidney. The only practicable method of doing this at present is by using hypotensive drugs and it is clear that the attendant risks far outweigh any advantages which might be gained by their use. It seems reasonable therefore to conclude that at present there are no practicable methods of reducing the loss of injected vitamin B_{12} in urine other than by reducing the amount which is presented to the kidney for excretion. This might be achieved

/by very slow administration over a long period or by the use of 'slow release' depot preparations. The former method can scarcely be called practicable and has not been investigated; the value of the latter method has been investigated and some comments will be made on the value of a depot preparation in Chapter 6.

Effect of differing routes of administration

It is usually the case that when a drug is given intravenously the amount excreted is greater than when it is given intramuscularly. This is clearly not the case with ^{58}Co vitamin B_{12} in the doses used and it is of some practical importance to know that the amount lost in the urine is no greater when the intravenous route of administration is preferred to the intramuscular route. The most likely explanation for this finding is simply that absorption from an intramuscular site is very rapid and there is evidence that this is the case (Glass et al 1955, Glass 1959). Whether this is the complete explanation for the finding is not clear. Other factors may well be involved but their nature and significance

/is obscure and unimportant at this stage.

The diuretic effect of vitamin B_{12}

A diuresis often occurs when patients with pernicious anaemia in haematological relapse are treated with vitamin B_{12}. This is usually ascribed to an enforced reduction in the circulating plasma volume as a result of the increase in circulating red cell mass. A diuretic effect, as distinct from this phenomenon, has been claimed for vitamin B_{12} by Barnard & Weitzner (1949) who attributed it to the effect of the cobalt ion on the renal vascular tree. This claim was refuted by Jalili (1950) and Bedford (1951) who also studied normal subjects and patients with a variety of diseases not including vitamin B_{12} deficiency states in haematological relapse. Support for vitamin B_{12} as a diuretic however, still exists (Mascherpa et al 1957).

The problem was investigated by analysing results obtained from patients with vitamin B_{12} deficiency states in haematological relapse given repeated injections of ^{58}Co vitamin B_{12} on

CASE	^{58}CO B_{12} INJECTIONS ug.	24 HOUR URINE COLLECTIONS						t	F
		NO RADIOACTIVITY EXCRETED		RADIOACTIVITY EXCRETED					
				0–24 hours post B_{12}		24–48–72 hours post B_{12}			
		No.of Results	Mean Vol. ml.	No. of Results	Mean Vol. ml.	No. of Results	Mean Vol. ml.		
J.F.	100 im	10	1066.5	9	1207.7	-	-	0.6	-
J.A.	1000 im	14	1765.3	12	2016.2	-	-	0.9	-
J.D.	1000 im	7	1471.1	6	1663.3	-	-	-	0.4
	1000 iv			6	1581.6	-	-	-	
J.G.G.	5000 im	5	1960.0	10	2109.0	-	-	0.8	-
J.H.	5000 im	7	632.8	6	663.3	-	-	0.3	-
M.McD.	10,000 im	5	990.0	15	1277.4	12	1197.5	-	0.2
C.McI.	10,000 iv	20	1479.5	15	1545.0	5	1031.0.	-	1.8
S.R.	20,000 im	14	1549.2	17	1566.4	13	1273.8	-	2.3
C.B.	30,000 im	9	918.8	6	1113.3	4	936.2	-	0.4

Table 7: showing mean values of urine volumes excreted in 24 hour periods related to injections of ^{58}Co vitamin B_{12}.

/alternate days or at longer intervals. The post treatment diuresis was clearly seen in some cases but these values are still suitable for analysis. The results in Table 7 show that the mean urine volumes for the 24 hours after injections of ^{58}Co vitamin B_{12} were always greater than those which did not contain radioactivity but this difference is not statistically significant ($P = > 0.05$). The conclusion that ^{58}Co vitamin B_{12} given parenterally in doses ranging from 100 ug to 30,000 ug, has no significant diuretic effect in patients with vitamin B_{12} deficiency states is therefore unavoidable. When these findings are considered together with those of Jalili (1950) and Bedford (1951) it seems very unlikely that vitamin B_{12} has any significant diuretic effect in any circumstance.

SUMMARY

The loss of vitamin B_{12} in urine after parenteral injections may be considerable. As a method of blocking or reducing this loss would probably be of therapeutic value the effect of some drugs on the renal excretion of injected ^{58}Co vitamin B_{12} was studied to find out if the drugs affected the amounts excreted and to gain information about the mechanism of excretion.

The effect of probenecid (Benemid), chlorothiazide (Saluric), mersalyl and vasopressin (Pitressin Tannate in Oil) was studied by comparing the amounts of radioactivity excreted after injections of ^{58}Co vitamin B_{12} given with and without these drugs. No drug had a significant effect on the amount of radioactivity excreted. The findings suggest that injected ^{58}Co vitamin B_{12} may be excreted by glomerular filtration only. Methods of reducing the urinary loss of injected vitamin B_{12} are discussed in the light of the findings. It is concluded that the only practicable method of reducing the loss is by the use of depot preparations.

/ The effect of intramuscular and intravenous administration on the amount of injected ^{58}Co vitamin B_{12} excreted was studied by a similar method. It was found that the mean amounts excreted were not significantly different. Reasons for this finding are discussed.

The claims that vitamin B_{12} has a diuretic effect were investigated by comparing the values for 24 hour urine volumes excreted after injections of ^{58}Co vitamin B_{12} with those when little or no radioactivity was present. No significant difference was found implying that ^{58}Co vitamin B_{12} has no significant diuretic effect in the doses used.

CHAPTER IV

OBSERVATIONS ON THE MODE AND RATE OF EXCRETION OF INJECTED ^{58}Co VITAMIN B_{12} BY THE NORMAL KIDNEY AND BY AN ARTIFICIAL KIDNEY.

The failure of various drugs to affect the amount of injected ^{58}Co vitamin B_{12} excreted in urine stimulated some further enquiries into the mode of excretion of injected vitamin B_{12} by the kidney. It was convenient to investigate the rate of excretion of injected vitamin B_{12} at the same time.

Observations on the mode and rate of excretion of injected ^{58}Co vitamin B_{12} by the human kidney and by an artificial kidney are reported in this chapter.

PATIENTS, MATERIALS AND METHODS

Observations were made on five patients admitted to hospital with a macrocytic anaemia, megaloblastic erythropoiesis seen on marrow biopsy smears and low serum vitamin B_{12} level. Details of these patients are given in the Case Reports in the Appendix. With one exception all had been treated with parenteral ^{58}Co vitamin B_{12} prior to the present study. The doses of ^{58}Co vitamin B_{12} given to these patients in this study ranged from 100 ug 0.2 uc to 1140 ug 0.2 uc and were the same as each patient had received

/previously. Case J.G.G. had not been treated prior to the present study and he was given 5000 ug 0.2 uc ^{58}Co vitamin B_{12}.

Urine was collected for 24 hours prior to the injection of ^{58}Co vitamin B_{12} and for hourly or half-hourly periods for the next 6 - 8 hours and from then until 24 hours after the injection. One patient (Case M.W.) had a catheter in situ for reasons unconnected with the experiment: in the others urine was voided naturally during, and at the end of, each collection period. All patients were encouraged to drink as much as possible during the observation period. The patients were cognisant of the nature and objects of the study and all cooperated willingly.

The preparation of ^{58}Co vitamin B_{12} for injection, and other technical and statistical methods are described in the Appendix.

The excretion of vitamin B_{12} by an artificial kidney was studied during haemodialysis performed to relieve uraemia in six patients with anuria due to renal failure. Details of these patients (Cases AK 1 - 6) are given in the Case Reports

/in the Appendix. A Kolff twin coil artificial kidney unit was used: operating instructions and details of this machine are given in the Appendix. Samples of blood and bath fluid were obtained before injecting 1000 ug vitamin B_{12} into the cannula connecting the machine outflow to the patient, that is, intravenously. Samples of bath fluid were taken at intervals thereafter and the vitamin B_{12} content was estimated by microbiological assay. All samples were assayed together in at least two different assay batches and the results given are the mean values of the assays. Recovery experiments were done on the bath fluid to detect the presence of inhibitors to the growth of Euglena Gracilis.

RESULTS

The results are shown in Figs. 56 - 61 and the essential statistical results are given in Tables 8 & 9. Detailed results obtained in individual cases are given in the Appendix in Tables A.49 & A.50. No radioactivity was found in any pre-injection collection of urine.

Recovery experiments with bath fluid taken

/from the artificial kidney at the beginning and the end of the dialyses did not show any evidence of inhibitors to the growth of Euglena Gracilis.

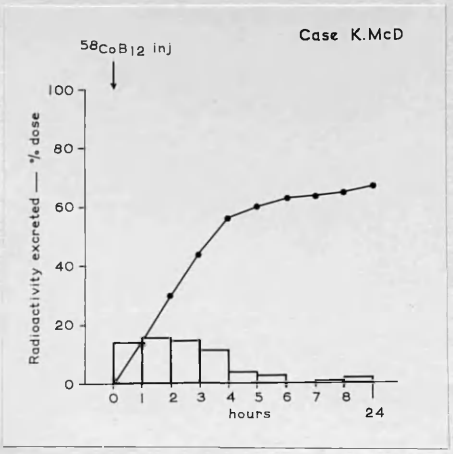


Fig. 56

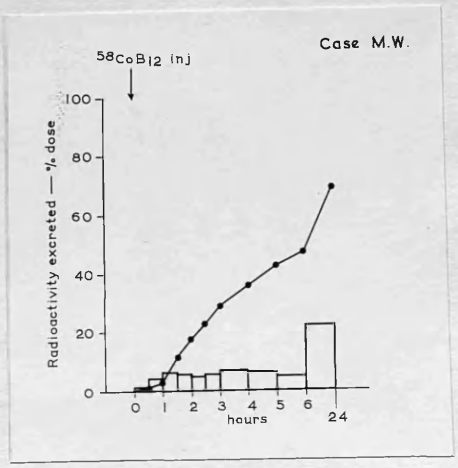


Fig. 57

Figs. 56 & 57: showing amounts of radioactivity excreted by two patients at intervals after intramuscular administration of ^{58}Co vitamin B_{12}. Case K.McD. received 100 ug and Case M.W. 1000 ug. The amounts excreted in hourly or half hourly periods are shown by plain rectangles and the cumulative amounts excreted by solid circles joined by continuous lines (see Table A.49).

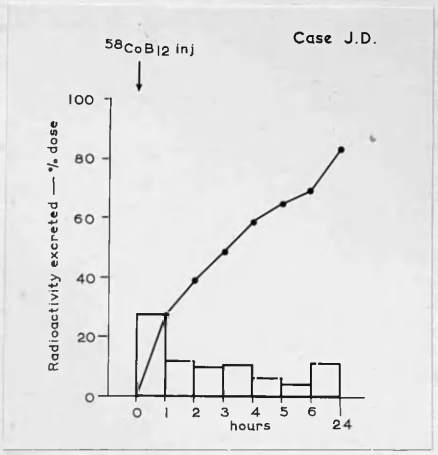


Fig. 58

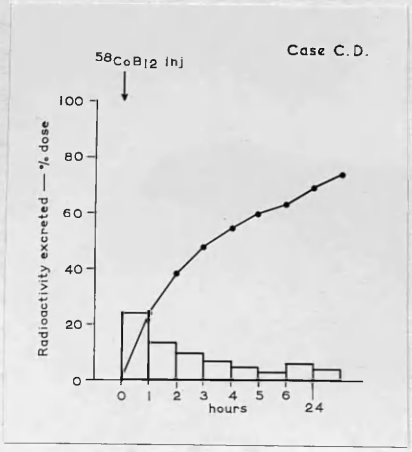


Fig. 59

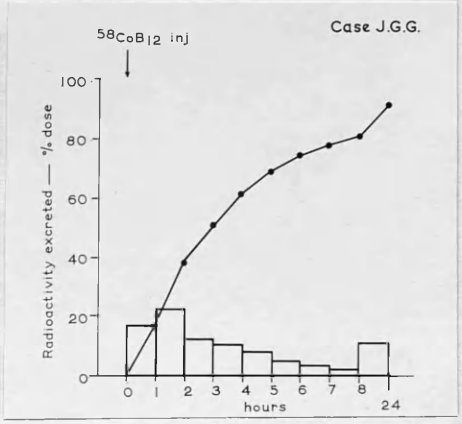


Fig. 60

Figs. 58 - 60: showing amounts of radioactivity excreted by three patients at intervals after administration of ^{58}Co vitamin B_{12}. Case J.D. received 1000 ug intravenously, Case C.D. 1140 ug intravenously and Case J.G.G. 5000 ug intramuscularly. The amounts excreted in hourly periods are shown in plain rectangles and the cumulative amounts excreted by solid circles joined by continuous lines (see Table A.49).

CASE	TIME AFTER INJECTION (hours)	No. OF OBSERVATIONS n	REGRESSION OF y (% DOSE EXCRETED) ON x (LOG TIME)	COEFFICIENT OF CORRELATION r	P
K.McD.	0 - 8	8	Y = 14.6987 - 13.4645x	-0.8609	< 0.02
	0 - 24	9	Y = 14.1994 - 11.7983x	-0.8065	< 0.01
M.W.	0 - 6	9	Y = 4.4222 + 3.1602x	+0.7867	< 0.02
	0 - 24	10	Y = 3.9479 + 8.5906x	+0.7435	< 0.02
J.D.	0 - 7	6	Y = 19.3199 - 20.8195x	-0.9594	< 0.01
	0 - 24	7	Y = 15.0854 - 5.5518x	-0.44	> 0.05
C.D.	0 - 7	7	Y = 17.7592 - 18.0979x	-0.9775	< 0.01
	0 - 24	8	Y = 16.7402 - 14.1973x	-0.9189	< 0.01
J.G.G.	0 - 8	8	Y = 17.3369 - 14.8511x	-0.8500	< 0.01
	0 - 24	9	Y = 15.8889 - 10.0156x	-0.6831	< 0.05

Table 8: showing essential statistical results obtained in the study of the excretion of injected ^{58}Co vitamin B_{12} by patients given doses of 100 ug intramuscularly (K.McD.), 1000 ug intramuscularly (M.W.), 1000 ug intravenously (J.D.), 1140 ug intravenously (C.D.) and 5000 ug intramuscularly (J.G.G.).

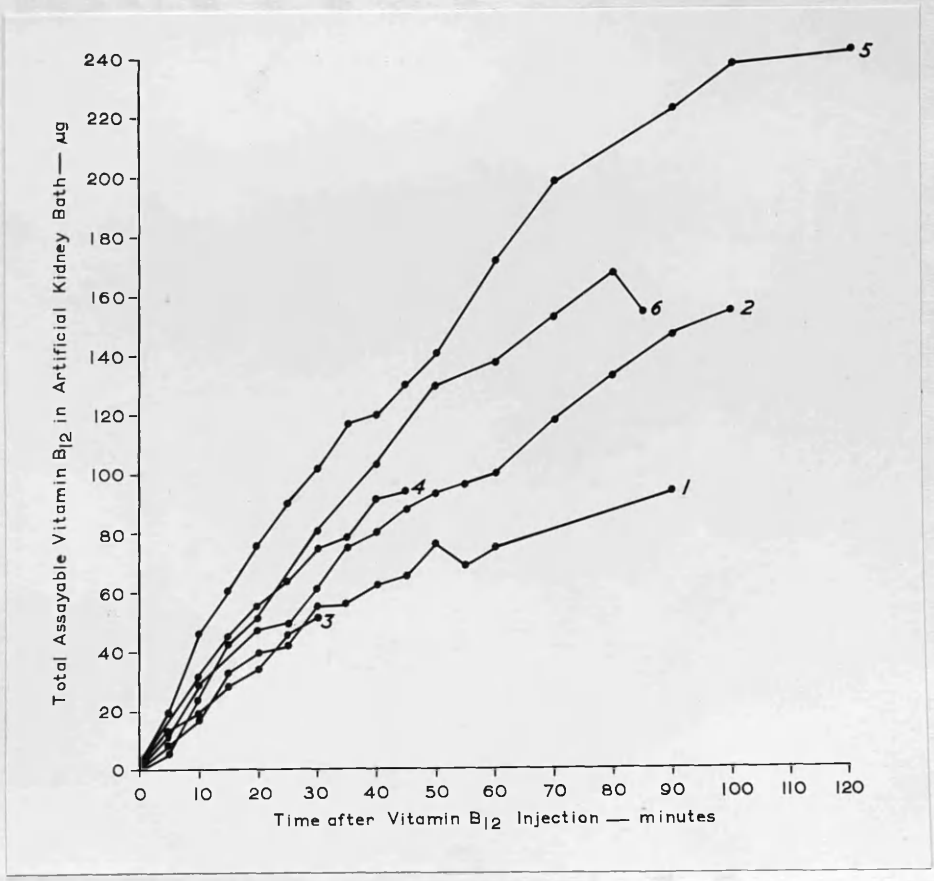


Fig. 61: showing amounts of assayable vitamin B_{12} in the artificial kidney bath fluid found at intervals after intravenous injection of 1000 ug vitamin B_{12} to six anuric patients undergoing haemodialysis (see Table A.50).

CASE	No. OF OBSERVATIONS n	REGRESSION OF y (VITAMIN B_{12} IN BATH) ON x (LOG TIME)	COEFFICIENT OF CORRELATION r	P
AK 1.	13	Y = 69.9495x - 48.8732	.9881	< 0.001
AK 2.	16	Y = 112.6270x - 91.9828	.9574	< 0.001
AK 3.	6	Y = 55.4182x - 34.0053	.9785	< 0.001
AK 4.	8	Y = 98.3690x - 70.3791	.9887	< 0.001
AK 5.	16	Y = 179.5250x - 144.4910	.9785	< 0.001
AK 6.	13	Y = 138.9915x - 113.8951	.9715	< 0.001

Table 9: showing essential statistical data from observations on the excretion of vitamin B_{12} by the artificial kidney. All patients were given 1000 ug vitamin B_{12} intravenously.

DISCUSSION

It was obvious in both experiments that the amount of injected vitamin B_{12} excreted per unit time was greatest soon after injection and that it diminished later (Figs. 56 - 61). This suggested that there might be a correlation between the logarithm of time and the amount of vitamin B_{12} excreted.

In the case of the results obtained from the vitamin B_{12} deficient patients, analyses showed that there was a coefficient of correlation, which differed significantly from zero in all cases, between the amount excreted in each collection period, expressed as a percentage of the dose given and the logarithm of the mid point of the collection period i.e. log. 3.5 in the case of the collection from the end of the third hour to the end of the fourth hour after injection. The coefficient of correlation differed significantly from zero in all the analyses when the values for the final collection period to 24 hours after injection were excluded and was significantly different from zero in all but one of the analyses

/when the values for the final collection period were included (Table 8). It is obvious that this correlation would also apply if the amounts excreted were expressed in absolute terms instead of as percentages of the dose of radioactivity excreted.

In the case of the results obtained from the artificial kidney experiment a coefficient of correlation differing significantly from zero was obtained in all cases between the total assayable vitamin B_{12} in the bath expressed in microgrammes and the logarithm of the time in minutes at which the samples were taken (Table 9).

The relationships are not exponential in the true mathematical sense but are of a type commonly referred to as exponential in biological work and will be referred to as such for the sake of brevity.

Significance of results in relation to the mode of excretion of injected vitamin B_{12}

The artificial kidney can be regarded conveniently as an atubular nephron in which excretion occurs by filtration only. Because

/the excretion of vitamin B_{12} by the human kidney is exponential, as is the excretion of vitamin B_{12} by the artificial kidney, it does not necessarily follow that the excretion of vitamin B_{12} by the human kidney also occurs by a process of filtration only. The results show that the two patients, Cases J.D. & C.D., who were given 1000 ug and 1140 ug ^{58}Co vitamin B_{12} intravenously, excreted 276 ug (27.6 and 24.2% of the dose respectively) in the first hour after injection (Table A.49) whereas the artificial kidney excreted 75, 100.5, 138 and 172 ug in the same period after an injection of 1000 ug vitamin B_{12} given by the same route (Table A.50): as there is no question of resorption of excreted vitamin B_{12} by the artificial kidney and as the 'dialysing area' of the artificial kidney is somewhat greater than that of the human kidney these results appear to imply that the human kidney excretes injected ^{58}Co vitamin B_{12} not only by glomerular filtration but also by tubular excretion. It must be appreciated however, that the blood flow through the type of artificial kidney used

/is much less than through the human kidney. Unfortunately it was not possible to measure the flow rate through the artificial kidney during the experiments as flowmeters were not available: the only method of measuring the flow rate was to drain the machine outflow into a measuring cylinder at the end of the dialysis: by this crude method flow rates in the region of 250 ml. per minute were obtained and subsequent experience with the same machine fitted with a flowmeter has shown that it is extremely unlikely that the flow rate would have exceeded 400 ml. per minute during any of these dialyses. It will be appreciated that a flow rate of even 400 ml. per minute is almost certainly very considerably less than that through the kidneys of the non anuric patients studied. The fact that some of the non anuric patients were anaemic is not important here as this has been shown to be without effect on the amount of injected ^{58}Co vitamin B_{12} excreted in the urine (Chapter 2). With these facts in mind the results are more in accord with the concept that vitamin B_{12} is excreted by

/glomerular filtration only, rather than by glomerular filtration and tubular excretion, in keeping with the finding that drugs which are known to act on the tubules do not affect the excretion of injected ^{58}Co vitamin B_{12} (Chapter 3).

The pattern of excretion with different routes of administration

The greatest amount of injected ^{58}Co vitamin B_{12} excreted in any collection period was found in the first hour after injection when this was intravenous, as in Cases C.D. & J.D. (Figs. 58 & 59) and later when this was intramuscular as in the other cases (Figs. 56, 57 & 60). This is probably due to flooding of the blood stream and presentation of a large amount of ^{58}Co vitamin B_{12} to the kidney after intravenous administration whereas after intramuscular administration the delay in absorption, short though it is, (Glass et al 1955, Glass 1959) entails a delay in the presentation of the injected ^{58}Co vitamin B_{12} to the kidney so that the greatest amount excreted is found

/in the second hour after injection.

Excretion in the 24 hours following injection

It has been stated by Smith (1960) that an appreciable proportion of an injection of vitamin B_{12} is excreted in the first 8 hours after injection but that little more appears after 8 hours. There is good evidence for this in the report by Mollin & Ross (1953a) as far as doses of 40 - 80 ug are concerned. It is obvious from the results presented here however, that this is not the case with injections of the order of 1000 ug or more. The facts that excretion is exponential and that the proportion excreted increases as the dose injected is increased (see Chapter 6) are the main reasons why significant amounts should be excreted in the 8 - 24 hour period after large injections but not after small injections. Another factor which may influence the amount excreted in the 8 - 24 hour period after large injections is the route of administration: the delay in absorption attending intramuscular administration will naturally prolong the entire process. It is interesting to

/note that the amounts excreted in the 6 - 24 hour period after the intravenous administration of 1000 ug to Case J.D. and 1140 ug to Case C.D. were 13.9% and 10.6% of the dose respectively whereas the amount excreted by Case M. W. in the same period after intramuscular administration of 1000 ug was 22.5% of the dose.

The fact that significant amounts are excreted in the 8 - 24 hour period must be appreciated in any investigation of the amounts of vitamin B_{12} excreted after parenteral therapy. Had an 8 hour collection period been used when investigating the effect of different routes of administration on the amount excreted it is likely that an erroneous conclusion would have been reached - probably that greater amounts were excreted after intravenous injection. The formula relating the amount excreted to the amount injected put forward by Chesterman et al (1951) was based on amounts excreted in the 0 - 8 hour period after injection only and will therefore underestimate the amount excreted when large

/doses are given. This point will be referred to again in Chapter 6.

Excretion later than 0 - 24 hours after injection

It is convenient to consider the question of excretion of injected vitamin B_{12} in the period following the 24 hours after injection at this stage. During the investigations reported in Chapters 1 & 2 many patients were given injections at intervals of one or more days. When injections were given daily the final collection period was usually the 24 - 48 hour period after the last injection: when injections were given at intervals of more than one day the amount of radioactivity, and in some cases the amount of assayable vitamin B_{12} excreted, was measured in several 24 - 48 hour periods. It is not possible to summarise the findings but reference to Tables A.2 - 4, A.6 - 10, A.14 & 15, A.17 - 19, A.21 & 22, A.29, 31 & 32 will show that significant amounts of radioactivity were rarely found in the 24 - 48 hour period when doses of 54 - 1140 ug ^{58}Co vitamin B_{12} were given and reference to Tables A.7 - 9, A.17 - 19, A.21 & 22

/will show that the amount of assayable vitamin B_{12} excreted in this period was very small. From these results it is clear that excretion after injections of up to 1140 ug is complete within 24 hours for practical purposes in the vast majority of cases. Note however should be taken of one exceptional case: on nine occasions after sixteen injections of 1000 ug ^{58}Co vitamin B_{12} Case I.C. excreted in the 24 - 48 hour period, amounts of up to 22% of the dose injected (Table A.18). No obvious reason for this was apparent. The injections were given in the usual manner and there was no reason to doubt the accuracy of the urine collections. Poor absorption from the injection site is an obvious factor but different sites were used. No explanation can be given other than suggesting that this constitutes a biological abnormality not previously described.

From what has been said one might expect the excretion of significant amounts of injected ^{58}Co vitamin B_{12} in the 24 - 48 hour period when large doses were given. Reference to Tables

/A.40 - 48 will show that this does occur but the amounts excreted in this period, or later, after doses of from 5000 to 30,000 ug were very small and insignificant in most cases.

The fact that excretion may although rarely, continue for more than 24 hours is relevant to some other investigations reported in this thesis. Firstly, in relation to the study of the amounts excreted by patients given repeated injections of ^{58}Co vitamin B_{12} reported in Chapter 2. A false impression of a constant trend to the excretion of increasing amounts might have resulted from the excretion of significant amounts in the period 24 - 48 hours after injection in some cases although it is unlikely that this could have been 'carried over' from day to day to such an extent as to have caused misleading results. It is obvious that this did not occur. Secondly in relation to the calculation in Chapter 6 of average daily loss by patients given repeated injections of large doses of ^{58}Co vitamin B_{12}: in all cases the collections were continued until significant amounts of radioactivity were no

/longer excreted so that the results presented are accurate.

The effect of renal disease on the excretion of injected vitamin B_{12}

It is convenient to mention this point here. It is known that the Schilling test may give fallacious results in the presence of renal disease, (Rath et al 1957, Dunn et al 1958) particularly when the blood urea is greater than 100 mg.% (Bull et al 1956).

The excretion of injected ^{58}Co vitamin B_{12} was studied in two patients known to have gross renal disease. The first patient, a female aged 27, was in the terminal stage of chronic nephritis; in the 24 hours after an intravenous injection of 100 ug ^{58}Co vitamin B_{12} she excreted 1.6% of the dose of radioactivity in 450 ml. of urine. The blood urea at the time of the test was 348 mg.%. The second patient, a male aged 51, also had chronic nephritis; after an intramuscular injection of 1000 ug ^{58}Co vitamin B_{12} he excreted 4.4% of the dose of radioactivity in the first 24 hours (urine volume

/985 ml.) and 2.8% in the second 24 hours (urine volume 735 ml.). A second injection of the same amount was given five days after the first and no radioactivity could be detected in the following 24 hour urine collection (volume 450 ml.). The blood urea at the time of the first test was 328 mg.% and at the time of the second test 376 mg.%. As will be shown later, and indeed is obvious from the results already presented, the amounts excreted by these patients are grossly subnormal. The exact mechanism whereby the chronic nephritic kidney fails to excrete injected vitamin B_{12} is not clear and it would be superficial to relate it to a reduced glomerular filtration rate only and unwise to compare the excretion of injected vitamin B_{12} with that of other solutes. The results raised the possibility that a test of renal function, based on the amount of injected ^{58}Co vitamin B_{12} excreted in the 24 hours after an injection, might be of some value in the assessment of the severity of renal disease. Preliminary investigations suggest that such a

/test is only likely to be of diagnostic value by the time that conventional tests show marked damage and would therefore be of little clinical value: further studies in this subject are in progress.

SUMMARY

The mode and rate of excretion of injected ^{58}Co vitamin B_{12} by the human kidney was investigated in five patients by collecting the urine at intervals after injection. A linear correlation with a coefficient of correlation differing significantly from zero was found between the amount of ^{58}Co vitamin B_{12} excreted, expressed as a percentage of the dose injected and the logarithm of time.

The excretion of injected vitamin B_{12} by an artificial kidney was studied on six occasions during haemodialysis. A linear correlation with a coefficient of correlation differing significantly from zero was found between the amount of assayable vitamin B_{12} excreted and the logarithm of time.

The significance of the findings in relation to various aspects of the renal excretion of injected vitamin B_{12} is discussed in detail. It is considered that the findings are in keeping with the concept that the excretion of injected vitamin B_{12} is a function of glomerular filtration only. The different patterns of excretion after

/intramuscular and intravenous administration are considered to be due to flooding of the bloodstream after intravenous administration. It is shown that excretion continues for more than eight hours particularly with doses of the order of 1000 ug or more but that it is usually complete in 24 hours in the majority of cases. Note is taken of one patient who excreted significant amounts later than 24 hours after injection. The effect of renal disease on the amount of injected ^{58}Co vitamin B_{12} is considered with reference to results obtained from two patients with chronic nephritis.

CHAPTER V

OBSERVATIONS ON THE BILIARY AND FAECAL EXCRETION OF INJECTED ^{58}CO VITAMIN B_{12}

Until recently little attention had been paid to the excretion of injected vitamin B_{12} in bile and faeces. Recent work on the biliary and faecal excretion following injections of 0.5 ug ^{58}Co vitamin B_{12} in normal and vitamin B_{12} deficient subjects raised the possibility that there might be a considerable loss by this route when larger doses of vitamin B_{12} were given. This possibility did not appear to have been investigated and, since it was relevant to the theme of the present investigations, it was decided to investigate it using ^{58}Co vitamin B_{12} in doses commonly used in clinical practice.

PATIENTS, MATERIALS AND METHODS

The faecal loss of injected ^{58}Co vitamin B_{12} was studied in nine patients who had come under observation with a macrocytic anaemia, megaloblastic erythropoiesis seen on marrow biopsy smears and low serum vitamin B_{12} level and one patient, Case W.R., who had an iron deficiency anaemia following partial gastrectomy and whose serum vitamin B_{12} level was normal. Details of these patients are given in the Case

/Reports in the Appendix. The vitamin B_{12} deficient patients were treated exclusively with parenteral ^{58}Co vitamin B_{12} and Case W.R. received six daily injections of ^{58}Co vitamin B_{12} before iron therapy was begun. The doses of ^{58}Co vitamin B_{12} given ranged from 54 ug 0.3 uc to 20,000 ug 0.1 uc. All patients received at least three injections of ^{58}Co vitamin B_{12} before the faecal collections were begun and most patients received at least five injections: the injections were continued while the faecal collections were made. Great care was taken to ensure that the faeces were not contaminated with urine. With one exception collections were made for three days. The faeces were homogenised and the radioactivity measured by the method described in the Appendix.

The biliary loss of injected ^{58}Co vitamin B_{12} was studied in seven patients who had undergone biliary tract surgery at least four days previously. All had a normal serum vitamin B_{12} level. Biliary drainage, by a T-tube inserted into the common bile duct, had been established

/at operation for purely surgical reasons. Bile was collected for 24 hours prior to the injection of ^{58}Co vitamin B_{12} and for at least 24 hours after injection. The doses of ^{58}Co vitamin B_{12} given ranged from 1000 ug 0.2 uc to 5000 ug 0.2 uc. The radioactivity in the collections was measured by the method described in the Appendix.

All patients were aware of the nature and objects of the experiment and all cooperated willingly.

RESULTS

The detailed results of the faecal excretion study are given in Table 10 and of the biliary excretion study in Table 11.

CASE	^{58}Co VITAMIN B_{12} INJECTIONS	DURATION OF FAECAL COLLECTION	TOTAL RADIOACTIVITY IN COLLECTION % of the radioactivity in a single injection	DAILY FAECAL LOSS % of the radioactivity in a single injection
H.McG.	54 ug im daily	3 days	2.4	0.8
W.R.	"	"	2.8	0.9
G.G.	540 ug im daily	"	3.6	1.2
J.J.	"	"	3.7	1.2
J.J.	"	"	1.9	0.6
J.D.	1000 ug im 2 days out of 3	"	1.9	0.6
S.J.	1000 ug im daily	"	1.7	0.6
A.E.	5000 ug im daily	"	< 3.0*	< 1.0*
J.G.G.	"	"	5.4	1.8
J.H.	"	"	< 3.8*	< 1.3*
S.R.	20,000 ug im every 2 - 3 days	random stools	< 1.0*	< 1.0*

Table 10: showing results obtained in the faecal excretion study. A significant number of counts was not obtained in three samples and the results in these cases, indicated by an asterisk, are the maximum possible values.

CASE	AGE	SEX	SERUM VITAMIN B_{12} uug/ml	TIME AFTER OPERATION days	^{58}Co VITAMIN B_{12} INJECTED ug	PREINJECTION 24 HOUR BILE		POST INJECTION 24 HOUR BILE	
						Volume ml.	Radio-activity % dose	Volume ml.	Radio-activity % dose
1	50	F	400	4	1000 im	260	0	280	< 1%
2	57	"	380	5	"	180	0	310	< 1%
3	67	"	390	5	"	300	0	400	< 1%
4	60	"	600	7	2000 im	210	0	350	< 1%
5	56	"	240	4	"	275	0	240	< 1%
6	62	"	610	6	5000 im	315	0	(1) 450	1%
								(2) 250	< 1%
7	58	"	510	6	"	215	0	(1) 250	< 1%
								(2) 330	< 1%

Table 11: showing results obtained in the biliary excretion study. In cases 6 & 7 collections were made for the periods 0-24 hours and 24-48 hours after the injection of ^{58}Co vitamin B_{12}. Significant count rates were obtained in only one sample (Case 6.0-24 hour collection) and the values given are maximum possible values.

DISCUSSION

Direct measurement, by microbiological assay, of the vitamin B_{12} excreted in bile and faeces following a parenteral injection is not a reliable procedure as large amounts of vitamin B_{12} are normally present in the faeces (Bethell et al 1948, Callender & Spray 1951, Pennington 1951, Girdwood 1956a) while bile, in addition to vitamin B_{12}, (Grasbeck & Okuda 1957, Okuda et al 1958) also contains substances which inhibit the growth of Euglena gracilis (Reizenstein 1959a).

The isotope technique was therefore employed although there is no direct evidence that the radioactivity in bile and faeces after parenteral ^{58}Co vitamin B_{12} is in the form of ^{58}Co vitamin B_{12} rather than other ^{58}Co. containing compounds. From the evidence marshalled by Reizenstein (1959c) however, and from the finding that the radioactivity in urine following parenteral ^{58}Co vitamin B_{12} is a direct measure of the amount of assayable vitamin B_{12} present (Adams 1961a, Chapter 1) it can be assumed with reasonable confidence that any radioactivity found in bile

/and faeces following parenteral ^{58}Co vitamin B_{12} is in fact due to intact ^{58}Co vitamin B_{12} molecules.

The results give a clear indication of the magnitude of the biliary and faecal loss after parenteral ^{58}Co vitamin B_{12} in the doses used. It is obvious from the results in Table 10 that the faecal loss, that is the total loss from the gastro-intestinal tract, is negligible from the therapeutic aspect. There are reasons for believing that the results may in fact exaggerate the faecal loss. If the amounts of radioactivity excreted were proportional to the ^{58}Co vitamin B_{12} blood levels then the amounts excreted by the patients in this series would be unduly high due to repeated injections given before and during the faecal collections. That this may be the case is suggested by the fact that, although the percentage of the dose of radioactivity excreted in the faeces is much the same in each dose group, the absolute amount excreted is, of course, much greater following large doses when the ^{58}Co vitamin B_{12} blood levels would be higher than after smaller doses. It should be noted that there

/is some evidence which has been interpreted as suggesting that the faecal loss of injected vitamin B_{12} may be greater in patients with pernicious anaemia than in normals. Grasbeck et al (1959) found that gastrectomised rats excreted more radioactivity in faeces after parenteral ^{58}Co vitamin B_{12} than normal rats and they attributed this to lack of intrinsic factor and hence to diminished resorption of ^{58}Co vitamin B_{12} excreted in bile. The limit to the amount of vitamin B_{12} which can be absorbed with the aid of the intrinsic factor mechanism, as opposed to simple diffusion, is so great however, that it is unlikely that the presence of intrinsic factor would have materially altered the amounts excreted in faeces by the patients, particularly those given large doses of ^{58}Co vitamin B_{12}, in this series all of whom had deficient secretion of intrinsic factor as judged by the Schilling test, except Case W.R. who was not tested. An alternative explanation for the results obtained by Grasbeck et al (1959), and not considered by them, is that

/gastrectomy may affect the faecal loss of injected vitamin B_{12} by some mechanism independent of a reduction in intrinsic factor secretion, or in association with a reduction in intrinsic factor secretion. The greatest faecal loss in the present series was observed in Case J.G.G. who had previously undergone high partial gastrectomy; on the other hand Case W.R. who had undergone a standard partial gastrectomy did not have a greater faecal loss than the patients with intact stomachs. The position is complicated by the facts that the doses of ^{58}Co vitamin B_{12} given to these patients differed greatly and that the ability of Case W.R. to secrete intrinsic factor was not measured: it would therefore be unwise to draw any conclusions from these results. Interesting though these points are it is clear that the faecal loss is trifling under conditions when it might be expected to be greatest and further investigations have seemed to be of academic value only and have not been pursued. There is no particular reason to believe that the faecal loss could be affected

/by anaemia and similar studies in non anaemic patients whether capable of secreting intrinsic factor or not, were not considered necessary particularly as the biliary excretion studies on non anaemic patients served to check the results obtained in the faecal excretion study.

Theoretically the faecal loss might be due to biliary excretion alone, to excretion from or seepage through the intestinal wall, to desquamation of intestinal epithelium or to a combination of these factors. This point has been studied by Grasbeck et al (1957, 1958) and by Reizenstein (1959b) using small (0.5 ug) parenteral doses of ^{56}Co vitamin B_{12}. They have produced evidence that the faecal loss is in part biliary and in part intestinal. In addition they have shown that the biliary excretion is much greater than the faecal excretion and from this they conclude that there is considerable reabsorption of the vitamin B_{12} excreted in bile. The biliary excretion studies summarised in Table 11 were not undertaken with a view to studying the question of an enterohepatic

/circulation but were done simply as a check on the intestinal loss after large parenteral doses of ^{58}Co vitamin B_{12} in man. Ideally these studies should have been done on patients with pernicious anaemia as the object of the experiments was to determine whether there was any significant loss from the therapeutic aspect. For obvious reasons this was not possible and 'normal' patients, that is, those with normal serum vitamin B_{12} levels had to be studied. The large doses used were resorted to in an attempt to demonstrate a biliary loss when it might have been expected to be greatest and the assumption was made that with the doses used there was no equilibration of injected ^{58}Co vitamin B_{12} with the body stores. The results in Table 11, when compared to those in Table 10, do not appear to support the view that there is an entero hepatic circulation but it should be noted that Grasbeck et al (1957, 1958) and Reizenstein (1959ab) allowed for the fact that bile collected by drainage of the common bile duct or by duodenal lavage does not

/represent the total daily output of bile and calculated their values for biliary excretion accordingly. Furthermore, they studied the biliary and faecal excretion in the same patient. It should also be noted that even with a T-tube in the common bile duct a significant amount of bile passes to the duodenum and the values for ^{58}Co vitamin B_{12} in such collections will therefore be an underestimate of the total excreted in bile. The value of the biliary studies reported here is simply that they confirm the view, obtained from the faecal studies, that the intestinal loss of parenteral ^{58}Co vitamin B_{12} in the doses used is trivial from the therapeutic aspect.

SUMMARY

The loss of injected ^{58}Co vitamin B_{12} in faeces was studied in ten patients receiving frequent injections of ^{58}Co vitamin B_{12} in the dose range 54 - 20,000 ug by measuring the radioactivity in faecal collections. By this technique the amount lost ranged from 0.6 - 1.8% of each dose injected. Reasons are given for believing that these values may be an over-estimate of the faecal loss of injected ^{58}Co vitamin B_{12}.

The biliary loss of injected ^{58}Co vitamin B_{12} was studied in six patients given injections of ^{58}Co vitamin B_{12} in the dose range 1000 - 5000 ug by collecting bile via a T-tube inserted into the common bile duct. The radioactivity in the collections was usually less than 1% of the dose injected.

The results are discussed and it is concluded that the loss of injected ^{58}Co vitamin B_{12} in bile and faeces is negligible from the therapeutic aspect.

CHAPTER VI

OBSERVATIONS ON THE RELATIONSHIP BETWEEN THE AMOUNT OF ^{58}Co VITAMIN B_{12} INJECTED AND THE AMOUNT EXCRETED IN THE URINE.

In the course of investigations carried out during a period of four years a large number of patients were given ^{58}Co vitamin B_{12} parenterally in doses ranging from 0.5 ug to 30,000 ug. In nearly all cases the urinary excretion of radioactivity had been measured for at least 24 hours after the injection. Inspection of the values of radioactivity excreted showed that there was clearly no correlation between the amounts of ^{58}Co vitamin B_{12} injected and the amounts of radioactivity excreted, expressed as a percentage of the dose of radioactivity injected. It seemed possible, however, that there might be a correlation between the logarithms of the amounts of ^{58}Co vitamin B_{12} injected and the amounts of radioactivity excreted. Because of the obvious practical and academic importance of such a correlation it was decided to collect and analyse all results.

MATERIALS AND METHODS

Values relating to amounts excreted after injections of 30,000, 20,000, 10,000, 5000, 1140, 1000, 540, 100, 54, 50, 5, 2, 1 and 0.5 ug

/were available for analysis. Only two patients had received injections of amounts greater than 10,000 ug and the urinary excretion of radioactivity had been measured in only a few of the cases given doses of less than 50 ug. It was therefore decided to restrict the investigation to results obtained in the dose range 50 - 10,000 and to pay particular attention to the results obtained in the dose range 50 - 1000 ug as this is the range commonly used in current clinical practice.

It has already been shown that normal and vitamin B_{12} deficient subjects do not excrete significantly different amounts of radioactivity expressed as a percentage of the dose of ^{58}Co vitamin B_{12} injected, after injections of 100 ug and 1000 ug (Chapter 2). It has also been shown that there is no constant trend to an increase or decrease in the amount of radioactivity excreted when vitamin B_{12} deficient subjects are given repeated injections of ^{58}Co vitamin B_{12} in the dose range 54 - 30,000 ug (Chapter 2). It was acceptable therefore to include in the analyses all results obtained from normal and vitamin B_{12}

/deficient subjects in the dose range 100 - 1000 ug. As it seemed a reasonable assumption that these findings would also apply in the case of 50 ug, 54 ug, and 1140 ug doses, all results from these dose groups were also included. The results relating to 5,000 and 10,000 ug injections were obtained from vitamin B_{12} deficient patients who had low serum vitamin B_{12} levels prior to the injection(s) of ^{58}Co vitamin B_{12} and it was valid to include these results for reasons given in Chapter 1. It has also been shown that the route of administration of ^{58}Co vitamin B_{12} does not affect the amount excreted after injections of 100 ug and 1000 ug (Chapter 3) and it was assumed that this also applied to other dose groups. The vast majority of results were obtained from patients over the age of 50 and inspection of the results did not show any obvious age-wise differences. Results from all age groups were therefore included in the material to be analysed. Results obtained from patients known to have renal disease were excluded for reasons given in Chapter 4.

/ The technical methods and statistical techniques used are described in the Appendix.

Results available for analysis

In all, 727 results, obtained from 176 patients, were available for analysis. Many patients had received more than one injection of the same dose and several had received injections of different doses.

DOSE INJECTED ug.	NUMBER OF PATIENTS IN EACH DOSE GROUP*	NUMBER OF RESULTS IN EACH DOSE GROUP
50	16	32
54	22	54
100	63	171
540	16	47
1000	66	270
1140	34	62
5,000	8	44
10,000	3	47

Table 12: showing disposition of results available for analysis. As noted in the text several patients appear in two or more dose groups: the true total of patients is 176.

/Further selection and grouping of results

Several problems presented when further selecting or grouping the results available for analyses.

The first problem was whether to limit the investigation to values known to be total amounts excreted or to include values for the amounts excreted in the first 24 hours only after an injection. Nearly all the results in the 5000 ug and 10,000 ug dose groups and some in the 1000 ug, 100 ug and 50 ug dose groups were obtained from continuous 24 hour urine collections from patients receiving daily injections and therefore account for all the radioactivity excreted. In the case of many of the results in the 1000 ug and lower dose groups the results were obtained from collections made for the first 24 hours after injection only; at such dose levels however, excretion is usually complete in this period (Chapter 4) and it seemed reasonable to accept these results representing as the total amount excreted. From observations made in Chapter 4 it is obvious that any underestimate

/of the total amount excreted by inclusion of the results for amounts excreted in the first 24 hours after injection only is so small as to be negligible. All available results were therefore included in the material to be analysed.

The second problem was whether to include all results obtained from patients who were given different doses on different occasions or to limit the results obtained from such patients to only one dose group. There seemed to be no good reason for not including all the results obtained from the same patient and this policy was adopted.

The third problem related to the grouping of results for analysis and was more difficult to solve. Three courses were open:

(1) to analyse only the mean values of all results obtained in each dose group.

(2) to analyse all results obtained in all dose groups.

(3) to analyse all results from patients given single injections of one dose and all the mean values of results from patients given multiple

/injections of the same dose in each dose group.

There did not appear to be any clear cut solution to this problem, although the last method seemed more logical and the results were therefore analysed by each method with the following results:

(1) analysis of mean values of all results obtained in each dose group 50 - 10,000 ug inclusive, the dose groups being 50,54, 100, 540, 1000, 1140, 5000 and 10,000 ug.

$$Y = 27.1929x - 19.0316$$

$$(n = 8,\ r = 0.9465,\ P = < 0.001)$$

where x = logarithm of the dose injected in ug and y = the percentage of the dose of radioactivity excreted.

(2) analysis of all results obtained in all dose groups 50 - 10,000 ug inclusive.

$$Y = 26.6873x - 16.3952$$

$$(n = 727,\ r = 0.7512,\ P = < 0.001)$$

(3) analysis of all results from patients given single injections of one dose and all mean values of results from patients given multiple injections of the same dose in the dose groups 50 - 10,000 ug

/inclusive.

$$Y = 26.6511x - 14.6840$$

$$(n = 228,\ r = 0.6795,\ P < 0.001)$$

From these analyses it is clear that there is a highly significant correlation between the logarithm of the amount of ^{58}Co vitamin B_{12} injected and the amount of radioactivity excreted expressed as a percentage of the dose given, in the dose range 50 - 10,000 ug regardless of the method of grouping the results for analysis. From the findings reported in Chapter 1 this implies that there is a significant correlation between the logarithm of the amount of vitamin B_{12} injected and the amount of assayable vitamin B_{12} excreted in the dose range 50 - 10,000 ug.

Attention was then focused on the results obtained in the dose range 50 - 1000 ug, that is, the range encompassing the doses commonly used in clinical practice. A total of 574 results obtained from 136 patients was analysed and the following results obtained:

(1) analysis of mean values of all results

/obtained in each dose group 50 - 1000 ug inclusive, the dose groups being 50, 54, 100, 540, and 1000 ug.

$$Y = 37.1001x - 39.8523$$

$$(n = 5,\ r = 0.9521,\ P = < 0.02)$$

where x = logarithm of the dose injected in ug and y = the percentage of the dose of radioactivity excreted.

(2) analysis of all results obtained in all dose groups 50 - 1000 ug inclusive.

$$Y = 30.3092x - 24.8588$$

$$(n = 574,\ r = 0.7349,\ P = < 0.001)$$

(3) analysis of all results from patients given single injections of one dose and all mean values of results from patients given multiple injections of the same dose in dose groups 50 - 1000 ug inclusive.

$$Y = 30.1899x - 27.8132$$

$$(n = 183,\ r = 0.7805,\ P = < 0.001)$$

It is again clear that in this limited dose range there is a highly significant correlation between the logarithm of the amount of ^{58}Co vitamin B_{12} injected and the amount

/excreted, regardless of the method of grouping. the results for analysis.

Further investigations showed that the regression coefficients of the six equations did not differ significantly from each other ($t = <1.9$, $P = >0.05$ in all cases).

The findings are shown in Figs. 62 & 63.

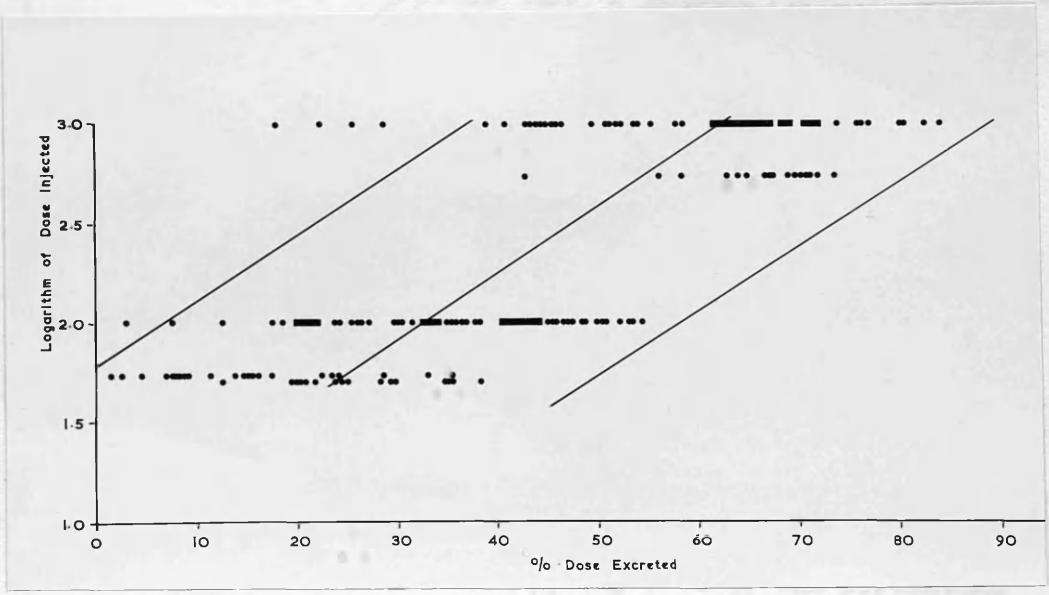


Fig. 62: showing the relationship of the logarithm of the dose of ^{58}Co vitamin B_{12} injected to the percentage of the dose of radioactivity excreted in the dose range 50 - 1000 ug. with the regression line and 95% confidence limits. Single results and mean values of multiple results are shown by solid circles. Rectangles indicate areas where many values coincide or overlap.

The regression equation is Y = 30.1899x - 27.8132 where x is the logarithm of the dose injected and y is the percentage of the dose of radioactivity excreted.

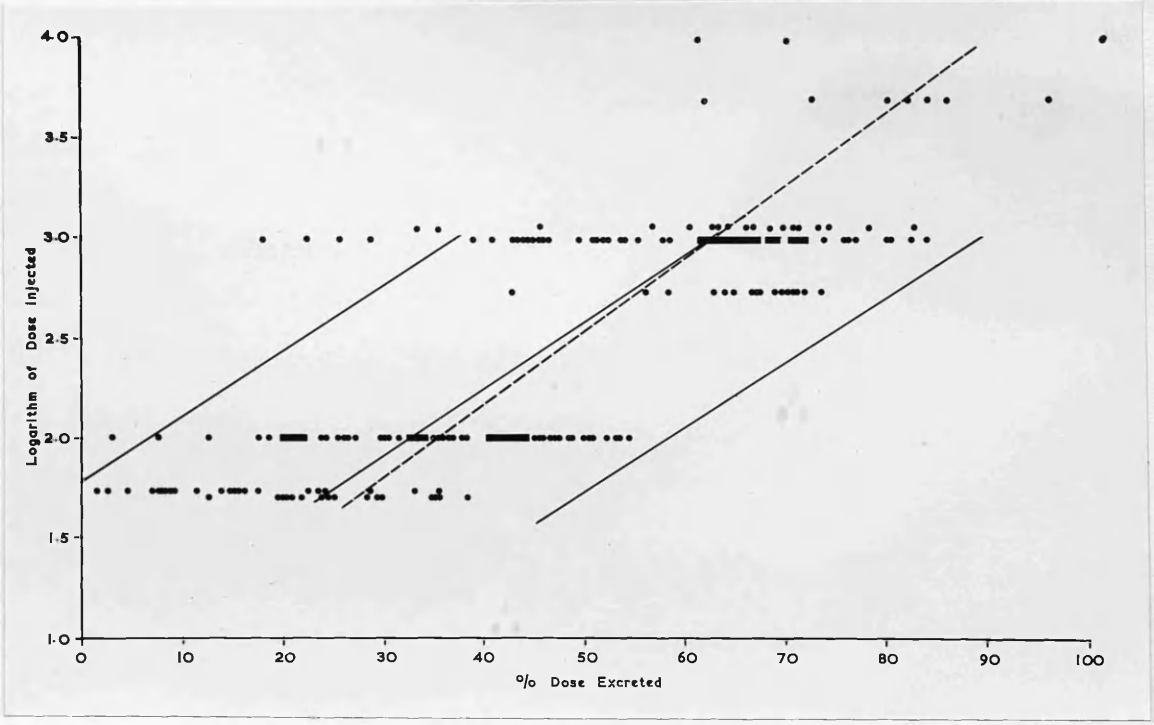


Fig. 63: showing the relationship of the logarithm of the dose of ^{58}Co vitamin B_{12} injected to the percentage of the dose of radioactivity excreted in the dose range 50 - 10,000 ug. The continuous lines are the regression line and 95% confidence limits for the dose range 50 - 1000 ug and the broken line the regression line for the dose range 50 - 10,000 ug. Single results and mean values of multiple results are shown by solid circles: rectangles indicate areas where many values coincide or overlap.

The regression equation of the 50 - 10,000 ug series is $Y = 26.6511x - 14.6840$ where x is the logarithm of the dose injected and y is the percentage of the dose of radioactivity excreted.

DISCUSSION

Significance of the findings

The value of the findings is obvious. They establish a relationship between the amount of vitamin B_{12} injected and the amount excreted and they enable predictions to be made with confidence about the amount of vitamin B_{12} which will be excreted and hence, as intestinal excretion can be disregarded for practical purposes (Chapter 5) the amount which will be retained, after any injection within the dose ranges studied. In addition a range of normal values, hitherto lacking, is now available. It must be emphasised that predictions from the equations will refer to a rather abstract mathematical value. It is obvious from Figs. 62 & 63 that the statistical range (mean ± 2 SD) obtained in each dose group is considerable. This however, does not detract from the value of the findings. The majority of patients with pernicious anaemia receive many injections of vitamin B_{12}; initially at short intervals and later at longer intervals. Over a period therefore the mean value excreted

/for several injections will approximate reasonably closely to the value obtained from the equations. Significant differences between mean values obtained from patients given repeated injections might be expected to occur, and have in fact been demonstrated formally by analyses of variance, but these values do not differ significantly from the value calculated from the equation. The extent of the range in mean values from patient to patient is shown in Table 13.

Interest particularly centres round the dose group 50 - 1000 ug as this is the range in which most doses used in clinical practice are found. The most satisfactory equation for this dose group is probably:

$$Y = 30.1899x - 27.8132$$

where x = logarithm of dose injected and
y = percentage of dose excreted i.e. the equation obtained from analysis of all results obtained from patients given single injections of one dose and all mean values of results from patients given multiple injections of the same dose in each dose group. This equation tends to even out

CASE	No. OF INJECTIONS	MEAN PERCENTAGE DOSE EXCRETED
F.A.	13	71.2
J.A.	13	66.5
C.B.	6	66.0
I.C.	16	74.5
J.D.	12	73.9
J.O.G.	9	62.9
J.O.H.	6	62.6
S.J.	10	70.5
W.L.	6	39.0
E.McB.	12	52.0
J.McF.	12	62.7
M.P.	13	67.4
I.R.	18	65.3
J.S.	24	71.3
A.S.	12	64.4
S.S.	14	66.1
R.T.	12	58.2

Table 13: showing the mean percentage of the dose excreted by seventeen patients each given at least six injections of 1000 ug ^{58}Co vitamin B_{12}. The value calculated from the equation $Y = 30.1899x - 27.8132$ where x is the logarithm of the dose injected and y is the percentage of the dose of radioactivity, is 62.7.

/any bias introduced by including all results obtained from every patient particularly as many patients had received many injections of the same dose and is more precise than the equation calculated from the mean value of all results in each dose group only. Using this equation the amounts excreted and retained after injections of 50 - 1000 ug are shown in Tables 14 & 15. As shown in Chapter 5 the amount of injected ^{58}Co vitamin B_{12} lost in the faeces is trivial and can be disregarded for practical purposes; the values for retained doses were therefore calculated on the assumption that all injected ^{58}Co vitamin B_{12} not excreted in the urine was retained in the body.

Previous literature

It is relevant to review the literature on this subject with these results in mind. There are several reports concerning the amount of vitamin B_{12} excreted in the urine after parenteral injection and in all cases the amount excreted was measured by microbiological assay. The literature is summarised in Table 16.

DOSE INJECTED ug	CALCULATED DOSE EXCRETED		
	% DOSE	ug	MEAN $\pm$ 2 SD ug
50	23	12	0 - 25
100	33	33	7 - 58
200	42	83	32 - 135
250	44	111	47 - 168
300	47	141	63 - 219
400	51	203	100 - 306
500	54	268	150 - 397
600	56	336	182 - 491
700	58	407	226 - 587
750	59	442	242 - 636
800	60	479	272 - 685
900	61	552	320 - 784
1000	63	628	369 - 886

Table 14: showing results obtained by calculating from the formula Y = 30.1899x - 27.8132 where y = percentage of dose excreted and x = logarithm of injected dose in the dose range 50 - 1000 ug. The results were calculated to the fourth decimal place and rounded off to whole numbers for the sake of clarity.

DOSE INJECTED ug	CALCULATED DOSE RETAINED		
	% DOSE	ug	MEAN $\pm$ 2 SD ug
50	77	38	25 - 50
100	67	67	42 - 93
200	58	117	65 - 168
250	56	139	82 - 203
300	53	159	81 - 237
400	49	197	94 - 300
500	46	232	103 - 350
600	44	264	109 - 418
700	42	293	113 - 474
750	41	308	114 - 508
800	40	321	115 - 528
900	39	348	116 - 580
1000	37	372	114 - 631

Table 15: showing results calculated from formula $Y = 30.1899x - 27.8132$ where x = logarithm of dose and y = percentage of dose excreted in the dose range 50 - 1000 ug and assuming that all the injected vitamin B_{12} not excreted in the urine is retained im the body. The results were calculated to the fourth decimal place and rounded off to whole numbers for the sake of clarity.

/Estimations of the amounts excreted after other intermediate doses have been reported by Sokoloff et al (1952) and Mollin & Ross (1953a).

In some of these papers it is clear that the authors appreciated that the amount excreted could vary considerably not only from patient to patient but also in the same patient: in others however, this point does not appear to have been fully, if at all, appreciated and for this reason, as well as on the findings reported in Chapter 2 the conclusion reached by Sokoloff et al (1952) that the proportion excreted increases with repeated injections is suspect. Likewise, the conclusion reached by Lang et al (1953) that patients with diabetic neuropathy excrete less than normal subjects, by Estrada et al (1954) that the Mexican excretes significantly less than the North American and by Baker et al (1956b, 1958) that patients with hepatic disease excrete less than 20% of an injection of 50 - 60 ug as opposed to the normal 50 - 80% may require revision in the light of the wide range of results

PARENTERAL DOSE ug	AMOUNT EXCRETED % DOSE	
50	6.4 - 8.2	(Chesterman et al 1951)
	15.4 - 22.0	(Lang et al 1953)
	8.8 - 29.4	(Watkin et al 1953)
	7.6 - 28.0	(Estrada et al 1954)
	14.5 - 31.5	(Unglaub et al 1954)
	1.0 - 76.0	(Baker et al 1956a)
	9.8 - 19.5	(Sheely et al 1957)
	0 - 25.0	(present series)
100	50 - 68	(Chow et al 1950)
	19 - 49	(Conley et al 1951)
	40	(Krevans et al 1951)
	51 - 98	(Reisner & Weiner 1952)
	30 - 37.8	(Unglaub et al 1954)
	7.0 - 58	(present series)
1000	33 - 47	(Chesterman et al 1951)
	38.5 - 67	(Mollin & Ross 1953a)
	51 - 98	(Reisner & Weiner 1952, 1953)
	36.9 - 88.6	(present series)

Table 16: showing estimates of amounts excreted following parenteral injections of vitamin B_{12} in man. The range for the present series is obtained from the mean value calculated from the equation $Y = 30.1899x - 27.1832 \pm 2$ SD where y = percentage of dose excreted and x = logarithm of the injected dose in the dose range 50 - 1000 ug.

/presented here. Mention should also be made of the formula $E = D - 1.2D^{0.89}$, where D = dose and E = amount excreted, propounded by Chesterman et al (1951) from 20 results in the dose range 5 - 1000 ug and measuring excretion for 8 hours after injection only, a procedure which, as already noted (Chapter 4) will lead to an underestimate of the amount excreted, particularly with large doses. Using this formula the calculated amount excreted after 50 ug is 11 ug, after 100 ug is 27.7 ug and after 1000 ug is 439 ug. values much lower than those found in this investigation.

Application of the results

The value of the results in defining a normal range has already been mentioned. A more practical value is easily seen. It is known that patients presenting with vitamin B_{12} deficiency states in haematological relapse have only a trivial amount of assayable vitamin B_{12} in their livers and as the liver is the main storage organ for vitamin B_{12}, it is reasonable to believe that the tissues of these patients are grossly depleted

/of vitamin B_{12} or its active principle. It seems logical therefore that the initial treatment of such patients should be directed to restoration of the depleted body stores to normal. If the normal body stores are known then a satisfactory plan for initial treatment can be established on the basis of the results presented in this chapter. This point will be discussed further in the next chapter.

Economic aspects of the results

It is often stated that the use of large doses of vitamin B_{12} in clinical practice is wasteful and uneconomic because of the greater urinary loss. Although it is true that the amount excreted increases as the dose is increased it should be noted that the absolute amount retained also increases (Table 15). It is pertinent to examine the economic aspects of this phenomenon. Calculating from the basic N.H.S. cost of single ampoules of vitamin B_{12} ('Cytamen' - Glaxo) and from the values for retained doses after injections of 50 - 1000 ug given in Table 15 and similarly for retained doses

/after injections of 5000 and 10,000 ug, it appears that the best value in terms of microgrammes of vitamin B_{12} retained per penny spent is obtained by using doses in the range 250 - 1000 ug (Table 17). On these results alone there seems to be a case for using doses in this range in routine clinical practice in preference to larger or smaller doses. However, in these crude calculations no account is taken of the number of injections which will be required in a period of say a year and this factor amongst others, must be considered when discussing the economic aspect of maintenance therapy with vitamin B_{12}. The matter is taken up again and allowance made for this and other factors in Chapters 7 & 8.

<u>The results in relation to the use of depot preparations of vitamin B_{12}</u>

The calculated loss of vitamin B_{12} in urine after an injection of 1000 ug is 628 ± 259 ug. It is logical to believe that an effective method of reducing this loss would be of therapeutic and possibly of economic value.

DOSE INJECTED ug	COST pence	CALCULATED AMOUNT RETAINED ug	'VALUE' ug retained/pence
50	3.5	38	10.1
100	5	67	13.5
250	7	139	19.8
500	14(2 x 7)	232	16.5
1000	20	372	18.6
5,000	100(5 x 20)	720	7.2
10,000	200(10 x 20)	808	4.0

Table 17: showing 'value' of various doses of vitamin B_{12} in terms of microgrammes retained per cost of vitamin B_{12} injected. The cost is the basic N.H.S. cost of single ampoules of vitamin B_{12} (Cytamen - Glaxo) no allowance being made for reductions in bulk purchasing etc.

/The loss is unaffected by several drugs acting on the kidneys (Chapter 3), and it was concluded that the only practicable method of reducing the amount excreted is by the use of slow release depot preparations (Chapter 4). It has been claimed that, by 'complexing' vitamin B_{12} with zinc and tannic acid, absorption from an injection site is delayed and excretion drastically reduced (Thompson & Hecht 1959, Davis et al 1959). A brief trial of such a preparation was carried out.

The daily urinary loss of vitamin B_{12} following an intramuscular injection of 1000 ug vitamin B_{12} zinc-tannic acid complex ('Depinar' - Armour) was measured by microbiological assay in three patients. It would have been simpler and more accurate to use a radioactive preparation but the manufacturers were unable to supply this. Two patients had pernicious anaemia in haematological relapse and the third (Case M.J.S.) had an iron deficiency anaemia and normal serum vitamin B_{12} level. The results shown in Table 18 certainly give evidence of continued absorption (and excretion) of vitamin B_{12} from the injection site for longer periods after injection than occurs

DAY	ASSAYABLE VITAMIN B_{12} EXCRETED PER DAY - ug		
	CASE J.S.G.	CASE J.T.	CASE M.J.S.
0	0.06	0.06	0.15
1	112.4	73.1	691.0
2	159.6	5.1	12.2
3	114.3	57.1	10.7
4	46.5	15.6	15.9
5	1.7	0.6	33.5
6	0.7	0.4	16.0
7	0.5	0.3	4.5
	435.7	152.4	783.9

Table 18: showing amounts of vitamin B_{12} excreted in the urine by three subjects after intramuscular injection of 1000 ug vitamin B_{12} zinc tannic acid complex ('Depinar' - Armour).

/with conventional preparations but are at variance with the manufacturers claim that less than 2% of the injected dose is excreted in the urine in the week after administration. There was no obvious reason why these patients should have excreted more than 2% of the dose apart from inconsistencies in the manufacture of the preparation and three different batches, obtained from the hospital pharmacy and stored as advised by the manufacturers were used. In its present form therefore, there do not seem to be any grounds for advising the use of this preparation in routine clinical practice, (Adams 1961b) and further investigations into this product have not been considered worth while.

It is convenient to note a different approach to the problem at this point. In a search for a long acting vitamin B_{12} preparation various cobalamins in various media and in various modifications were studied. It was found by Glass et al (1961), and confirmed by Killander & Schilling (1961) that the amount of hydroxocobalamin (vitamin B_{12a} or B_{12b}) excreted

/is much less than the amount of vitamin B_{12} excreted after injections of the same dose. In addition it has been shown that blood levels attained and sustained for at least three weeks after an injection, are greater following an injection of hydroxocobalamin than after an injection of the same dose of vitamin B_{12}. As hydroxocobalamin exerts a haemopoietic effect equivalent to that of vitamin B_{12} (Lichtmann et al 1949, Schilling et al 1951) these findings suggest that there may be a good case for using hydroxocobalamin in preference to vitamin B_{12} provided that the cost of treatment is not increased. The place of hydroxocobalamin in the treatment of patients with pernicious anaemia is being investigated at the present time. The explanation for the greater retention of hydroxocobalamin appears to be that tissues which bind vitamin B_{12} will bind much greater quantities of hydroxocobalamin apparently by a different mechanism (Bauriedel et al 1956).

SUMMARY

The relationship between the amount of vitamin B_{12} injected and the amount excreted in the urine has been investigated by analysing a large number of results from patients given ^{58}Co vitamin B_{12} in doses of 50 - 10,000 ug.

A linear correlation between logarithm of the amount injected in ug and the percentage of the dose of radioactivity excreted was found. The coefficients of correlation of the regression equations did not differ significantly when the dose ranges 50 - 1000 ug and 50 - 10,000 ug were considered separately.

On the basis of the findings a normal range of values for amounts of injected ^{58}Co vitamin B_{12} excreted in urine have been calculated and, as the amount of injected ^{58}Co vitamin B_{12} excreted in faeces is trivial, a normal range of values for amounts of injected ^{58}Co vitamin B_{12} retained in the body has also been calculated.

The literature is reviewed in the light of the results and comments are made on the economic

/aspects of parenteral vitamin B_{12} therapy, on the initial treatment of patients with vitamin B_{12} deficiency states and on the urinary losses with depot preparations of vitamin B_{12}.

CHAPTER VII

OBSERVATIONS ON THE BODY STORES OF ASSAYABLE VITAMIN B_{12} IN NORMAL AND VITAMIN B_{12} DEFICIENT SUBJECTS.

With Notes on the Significance of Low Serum Vitamin B_{12} Levels and on the Initial Treatment of Patients with Vitamin B_{12} Deficiency States.

When planning the treatment of patients with vitamin B_{12} deficiency states it is obviously desirable to have some knowledge of the total amount of vitamin B_{12}, or its active principle, stored in the tissues of normal subjects. Such knowledge is also desirable for more academic purposes such as calculations of vitamin B_{12} turnover in man. It is relatively easy to measure the assayable vitamin B_{12} in biopsy material or in tissues obtained at operation or post mortem examination but the value of such results is limited. The obvious procedure of homogenising corpses and measuring the vitamin B_{12} content of the homogenates is distasteful, scarcely practicable and probably illegal.

A previously undescribed method of measuring the total assayable vitamin B_{12} in the body was devised. The basic assumption of the method is that when a subject is given Co vitamin B_{12} parenterally the proportion which is retained in the body is distributed throughout the tissues in proportion to the amounts of non radioactive vitamin B_{12} or its active principle already present

/in these tissues. Thus if 50% of the retained dose of radioactivity were found in the liver this would imply that 50% of the total body stores were present in that organ: if the total assayable vitamin B_{12} content of the liver was found to be 2000 ug then one would assume that the total body stores were 4000 ug.

An experiment based on these concepts, was planned with the object of measuring the total assayable vitamin B_{12} in man and the results are reported and discussed in this chapter. In addition, observations on tissues from two patients who had evidence of abnormal vitamin B_{12} metabolism, are also reported and discussed.

PATIENTS, MATERIALS AND METHODS

The 'normal' patients studied had been admitted to a hospital in the terminal stages of disease. In all cases the disease was one in which deranged vitamin B_{12} metabolism is not a recognised feature and in most the primary lesion was "anatomical" rather than "metabolic". Details of these patients are given in Table 19. After blood had been taken for serum vitamin B_{12}

/estimation an injection of Co vitamin B_{12} was given intravenously. Urine was collected for 24 hours after injection from the two patients who were given doses greater than 1.0 ug. The time elapsing between the injection and death was noted as accurately as possible. The liver was secured at post mortem examination, weighed, and a generous amount was homogenised as described in the Appendix. The radioactivity in an aliquot of the homogenate was measured by the technique described in the Appendix and the total hepatic radioactivity expressed as a percentage of the dose of Co vitamin B_{12} given was calculated. Suitable dilutions of the homogenate were assayed by the technique described in the Appendix. The values for total assayable body vitamin B_{12} were calculated from the values for the total hepatic radioactivity and the total hepatic assayable vitamin B_{12}. In two patients the observations were extended to include other tissues.

Recovery experiments were done on the liver homogenates obtained from patients who died with evidence of metabolic upset such as uraemia.

Observations on tissues from two patients with abnormalities of vitamin B_{12} metabolism who came to post mortem examination were made using the same techniques.

The clearance of intravenously injected ^{58}Co vitamin B_{12} and the hepatic uptake of radioactivity as determined by surface, or external, counting was studied in two patients; details of these patients are given in the case reports in the Appendix. Blood was taken from a vein in the opposite arm before, and at intervals after, an intravenous injection of Co vitamin B_{12} and the radioactivity in 5 ml. aliquots of plasma or blood haemolysed by the addition of saponin, was measured as described in the Appendix. The total blood volume was measured by the Evans blue dye (T 1824) technique during the experiment and the total blood, or plasma, volume radioactivity expressed as a percentage of the dose given, was calculated using this value. External counting of hepatic radioactivity was done by the technique described in the Appendix.

RESULTS

The results obtained from the normal subjects are shown in Table 19.

The results from the subjects with abnormalities of vitamin B_{12} metabolism are more conveniently given together with a discussion of these results at a later stage

Recovery experiments on liver homogenates did not show the presence of inhibitors to the growth of Euglena gracilis.

CASE	AGE	SEX	SERUM B_{12}	INTRAVENOUS Co VITAMIN B_{12}	TIME INTERVAL INJECTION - DEATH	CAUSE OF DEATH	LIVER				CALCULATED TOTAL ASSAYABLE B_{12} IN BODY
							WEIGHT	RADIOACTIVITY % DOSE	ASSAYABLE B_{12} µg/g.	µg/organ	
1	73	F	566	4.0 µg 4.0 µc ^{58}Co	21 hours	DEGENERATIVE HEART DISEASE	1600	36.6	0.765	1224	3344
2	68	F	1466	2.0 µg 2.0 µc ^{58}Co	4 days	HYPERTENSIVE HEART DISEASE	1300	47.2	0.604	786	1672
3	17	M	630	1.0 µg 1.0 µc ^{58}Co	17 days	ANURIA	1250	29.1	0.594	739	2541
4	64	F	340	1.0 µg 1.0 µc ^{58}Co	11 hours	MYOCARDIAL INFARCTION	1700	36.6	0.423	719	1965
5	52	F	260	1.0 µg 1.0 µc ^{58}Co	15 days	BRONCHIAL CARCINOMA	1750	60.0	1.091	1911	3185
6	71	F	390	1.0 µg 1.0 µc ^{58}Co	5 days	CEREBRAL HAEMORRHAGE	1750	28.7	0.302	530	1847
7	57	F	720	0.5 µg 0.5 µc ^{57}Co	2½ days	RETICULOSARCOMA	1400	35.1	0.627	880	2504
8	61	M	700	0.5 µg 0.5 µc ^{57}Co	6 days	COR PULMONALE CEREBRAL EMBOLISM	900	42.4	0.811	730	1723
9	51	M	900	0.5 µg 0.5 µc ^{57}Co	5 days	AORTIC ANEURYSM	1650	53.9	0.311	514	953
10	79	F	640	0.5 µg 0.5 µc ^{57}Co	11 hours	PNEUMONIA	1270	31.9	0.408	519	1629
11	37	M	760	0.5 µg 0.5 µc ^{57}Co	18 hours	ANURIA	1745	31.4	0.774	1351	4304
12	65	M	530	0.5 µg 0.5 µc ^{57}Co	5½ days	AORTIC STENOSIS	1200	31.8	0.490	588	1845
13	64	M	420	0.5 µg 0.5 µc ^{57}Co	2 days	CEREBRAL HAEMORRHAGE	1150	33.0	0.801	921	2792
14	81	F	295	0.5 µg 0.5 µc ^{57}Co	30 days	ARTERIOSCLEROSIS	1520	50.1	0.456	694	1385
15	70	M	205	0.5 µg 0.5 µc ^{57}Co	17 hours	MYOCARDIAL INFARCTION	1750	38.5	0.463	771	2002
16	61	F	500	0.5 µg 0.5 µc ^{57}Co	19 days	ACUTE LEUKAEMIA	2120	48.3	0.505	1071	2218
17	77	F	270	0.5 µg 0.5 µc ^{57}Co	56 days	SENILITY	1600	62.1	0.718	1150	1852

Table 19: showing details of cases and results obtained in the study of the body stores in 'normal' subjects.

DISCUSSION

Review of the literature

The method used appears to be original and no comparable study has been reported apart from that by Adams (1961d) on which this chapter is based. The only relevant papers are those by Estren et al (1958) and Brody et al (1960) both of whom mention finding 50% of the dose of radioactivity in the liver of a patient who died one week after an injection of Co vitamin B_{12}. It appears that both papers refer to the same patient. The vitamin B_{12} content of the liver of this patient was not measured.

Method and techniques

Considerable caution must be exercised when interpreting the results. Obviously not one of these patients could be regarded as normal in the accepted sense, even if the assumption that there was no upset of vitamin B_{12} metabolism is correct. Study of the truly normal subject however, presents almost insuperable difficulties. It is possible to obtain enough hepatic tissue by biopsy to allow accurate assay measurements

/but this alone gives no information about the total body stores. To combine the assay and isotope techniques in the study of biopsy material is impossible as the amount of radioactivity in the sample of tissue obtained would be too small to be detected unless enormous doses of radioactivity were injected. A combination of biopsy to obtain material for assay and external counting to evaluate the hepatic radioactivity appeared to be a technique worth investigating and this was done in two patients. The first, Case T.H., was not anaemic but had a low serum vitamin B_{12} level (40 uug/ml) when studied. He was given 4.0 ug 4.0 uc ^{58}Co vitamin B_{12} intravenously and the hepatic count rate reached a plateau in a few seconds and remained there for the subsequent two hours during which time frequent observations were made: in that period the amount of radioactivity in the blood declined from 47% of the dose at 2 minutes after injection to 8% of the dose at 120 minutes after injection (Fig. 64). These results are good evidence that external counting, at least by the

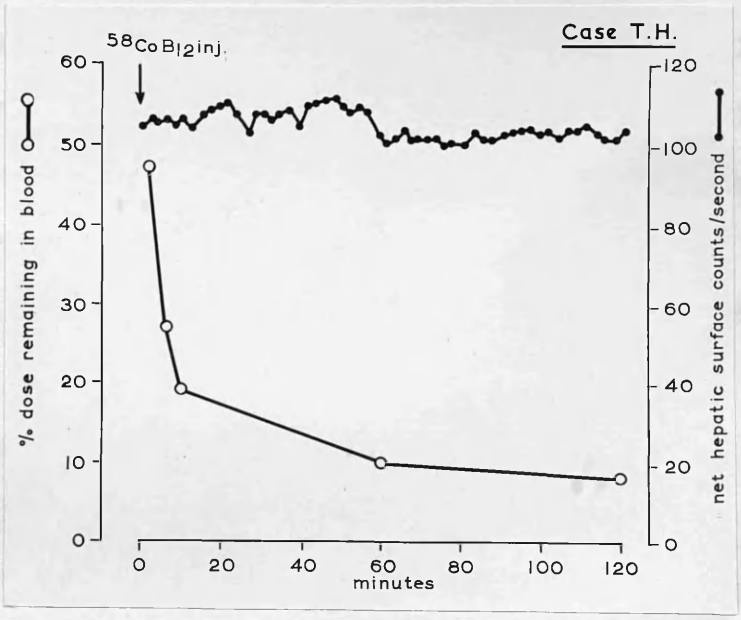


Fig. 64

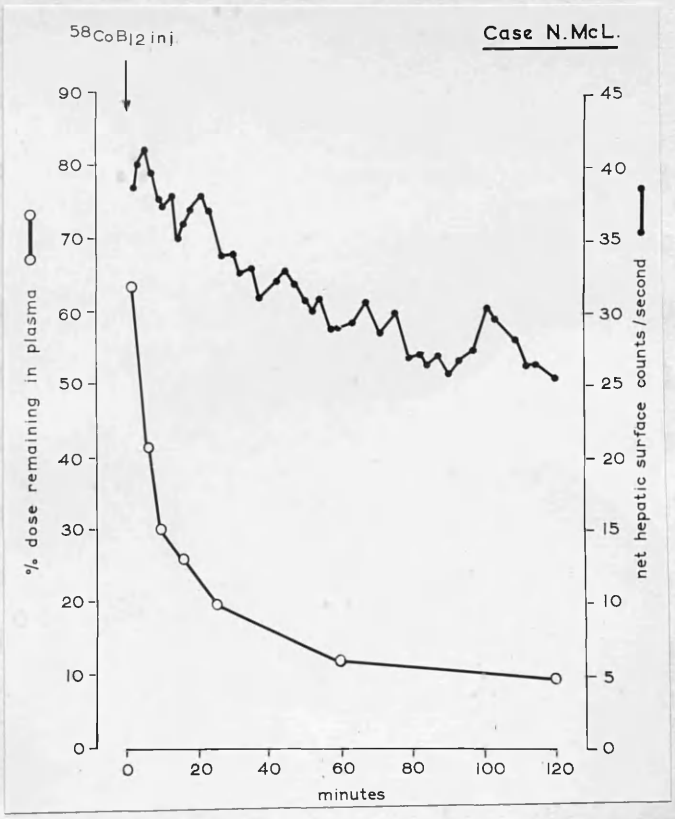


Fig. 65

Figs. 64 & 65: showing clearance from the blood or plasma and uptake by the liver of ^{58}Co vitamin B_{12} given intravenously.

/technique used, does not give a quantitative measure of the Co vitamin B_{12} accumulated in liver unless one postulates that 53% of the dose was taken up by the liver in the two minutes after injection, and that 39% was taken up entirely by other tissues in the period 2 - 120 minutes after injection.

The second patient, Case N.McL., was not anaemic and had received 1000 ug vitamin B_{12} intramuscularly each month for over a year, the last injection being three days before the intravenous injection of 4.0 ug 4.0 uc ^{58}Co vitamin B_{12}. The hepatic count rate reached a peak five minutes after injection and declined over the next two hours. During this period the plasma radioactivity fell from 64% of the dose at 2 minutes after injection to 9% of the dose at 120 minutes after injection (Fig. 65). Unless one also postulates that in this case there was a discharge of accumulated ^{58}Co vitamin B_{12} from the liver in the period 5 - 120 minutes it is impossible to avoid the conclusion that external counting, at least by the technique used, is of

/little value in giving a quantitative measure of the ^{58}Co vitamin B_{12} accumulated in the liver. A probe inserted into liver substances might enable the uptake to be measured quantitatively but this is not a practicable proposition in man at present.

The assay technique used appears to be acceptable. Ross & Mollin (1957) have shown that the results obtained using Euglena gracilis in the assay of tissues are comparable to those obtained with Ochromonas malhamensis which is more certainly specific for vitamin B_{12} than other assay organisms (Ford 1953). Large masses of liver and large volumes of homogenate were used to avoid errors inherent in the use of small samples. Whole homogenates or dilutions of whole homogenates were used: this method is stated to give a higher yield than using extracts (Ross & Mollin 1957). Digestion of tissues with papain or other enzymes, advocated by Shenoy & Ramasarma (1954) when using Lactobacillas leichmannii as a test organism, was not used as the author has not found that this procedure gives a higher yield when using

/Euglena gracilis.

In many cases the general condition of the patient made it difficult to collect urine with accuracy in the post injection period and in all but two 'normal' cases it was assumed that no radioactivity had been lost in the urine. This assumption seems justified on the findings of Mollin et al (1956), Miller et al (1957), Brody et al (1960) and Hall (1960) who reported that the amount of radioactivity excreted after an injection of up to 4.0 ug Co vitamin B_{12} is small, usually less than 2% of the dose. Even if as much as 5% were excreted it is obvious that the calculations would be substantially unaffected. The urinary loss in the 24 hours after injection was measured in Case 1 , who received 4.0 ug and was found to be 0.19% of the dose and in Case 2 who was given 2.0 ug and was found to be 0.44% of the dose.

It is unlikely that the age of the patients is of importance as Swendseid et al (1955) have shown that hepatic assayable vitamin B_{12} is unaffected by ageing. It should be noted that

/no patient had cirrhosis of liver, a condition in which the assayable vitamin B_{12} content of the tissue is lower than normal (Swendseid et al 1957).

Ideally two or more organs should have been analysed to ensure accuracy in the calculation of total body vitamin B_{12}. Detailed study of two cases however, showed that the amount of radioactivity in tissues, other than the liver, was very small. (Table 20). It was therefore decided to use the liver as a single reference organ.

A point of great importance is whether the time which elapsed between the injection of the Co vitamin B_{12} and death was adequate to allow complete clearance from the blood stream and distribution to tissues. It is known that the initial clearance of injected Co vitamin B_{12} from the blood stream is rapid and the results obtained from Cases T.H. and N.McL. are similar to those reported by Miller et al (1957), Estren et al (1958), Brody et al (1960) and Hall (1960). The rate of clearance has been studied in detail by Brody et al (1960) who found that in normal

CASE	RADIOACTIVITY IN ORGANS - % DOSE INJECTED						
	LIVER	SPLEEN	HEART	BRAIN	LUNGS	KIDNEY	PANCREAS
1	36.6	3.8	0.9	0.5	5.3	2.1	0.7
2	47.2	1.1	0.9	0.9	1.8	1.2	1.2

Table 20: showing radioactivity in various organs after an injection of ^{58}Co vitamin B_{12}.

/subjects there was an initial rapid loss of plasma radioactivity in the 30 minutes after injection, followed by slower loss in the next 30 - 90 minutes and a final phase of slow loss with an average half time of disappearance ($T\frac{1}{2}$) of approximately 24 hours: their results obtained from 10 'normal' patients given 0.5 - 1.0 ug Co vitamin B_{12} intravenously showed that 2 - 5% of the injected dose was still detectable in the plasma at 8 hours after injection and the average amount present in plasma at 24 hours was 3% of the dose. These results suggest that it would be unwise to assume that complete clearance from the blood stream occurs before at least 48 hours after injection.

Even when complete clearance from the blood has occurred it cannot be assumed that the distribution of injected Co vitamin B_{12} to tissues is complete and final and in proportion to the non-radioactive stores. The possibility of selective uptake in one or more organs and subsequent slow redistribution to other tissues must be considered - the problem of 'relocation'.

/This problem was originally raised by Glass (1959) who noted a peak of hepatic radioactivity by external counting on the fifth day after an injection of Co vitamin B_{12} and ascribed this to relocation. The observations on Cases T.H. and N.McL. already reported suggest external counting is of little value as a quantitative measure and it is doubtful if Glass' (1959) evidence justified his conclusions. There is however, other evidence that redistribution of injected Co vitamin B_{12} may occur. If all the vitamin B_{12} or its active form in the body were in one pool or compartment then injected Co vitamin B_{12} would equilibrate with the stores rapidly. There is evidence however, that the vitamin B_{12} in the body is in at least three compartments each of which has a separate turnover rate (Reizenstein 1959c). With an open type multicompartment system isotopic equilibrium may be attained quickly if internal transfer rates are rapid: if internal transfer rates are slow then equilibrium may never be attained. Further studies by Reizenstein et al (1961) using an analogue computer to analyse

/data obtained by whole body counting have led to the conclusion that with vitamin B_{12} internal transfer rates are slow and that isotopic equilibrium of injected Co vitamin B_{12} and the tissue stores does not occur in a convenient time period. At first sight this conclusion seems to be at variance with the demonstrably rapid uptake of injected Co vitamin B_{12} by liver (cf. Figs. 64 & 65) but this is not necessarily so. It appears therefore that the basic assumption in the study, that the injected Co vitamin B_{12} retained in the body equilibrates with the tissue store, is not valid and this casts doubts on the value of the results. The assumption may not be valid but the results are none the less of some value for several reasons. In a multicompartment system with slow internal transfer rates the specific activity will be higher initially in the compartment with the most rapid turnover and later higher in the compartment with the slowest turnover reaching a peak value in each compartment at some time. The plasma compartment, which is minute in terms of vitamin B_{12} content, has a rapid turnover: the liver,

/which is a vast compartment in terms of vitamin B_{12} content, has a slow turnover, about the same as the body as a whole (see Chapter 8). If the results were significantly affected by slow internal transfer rates one would expect a steady rise in values for hepatic radioactivity over a long period: the extent of this rise, and later fall, would give some indication of the errors resulting from the methods used. To examine this point the results were analysed allowing for increasing time intervals between injection and death, and the results are set out in Table 21. It will be seen that there is a rise in mean hepatic radioactivity from 43.4% of the dose at 48 hours by which time clearance of injected Co vitamin B_{12} from the blood stream would be virtually complete to 49.9% of the dose at 15 days or more. This rise is relatively small and suggests that the results, although not as precise as would be preferred, do give an acceptable indication of the body stores.

The values for assayable vitamin B_{12} are in keeping with most previous estimates which are

TIME INTERVAL INJECTION TO DEATH	No. OF CASES	MEAN HEPATIC RADIO-ACTIVITY % dose injected	MEAN HEPATIC ASSAYABLE VITAMIN B_{12} ug.	MEAN CALCULATED TOTAL VITAMIN B_{12} IN BODY ug.
> 11 hours	17	40.9	888	2221
> 1 day	12	43.4	876	2043
> 2 days	12	43.4	876	2043
> 3 days	10	45.3	871	1922
> 4 days	10	45.3	871	1922
> 5 days	9	45.1	880	1949
> 6 days	6	48.6	1049	2150
> 15 days	5	49.9	1112	2236

Table 21: showing values for mean hepatic radioactivity, mean hepatic assayable vitamin B_{12} and mean calculated total body vitamin B_{12} allowing for increasing time intervals between injection of ^{58}Co vitamin B_{12} and death.

/summarised in Table 22. They are higher than those reported by Pitney et al (1955) ~~and Nelson & Doctor (1958)~~ and lower than those reported by Ross & Mollin (1957)/ AND NELSON + DOCTOR (1958) The reason for this is not obvious. The increase in mean hepatic assayable vitamin B_{12} found in patients who survived six or more days after injection is an odd finding and is probably fortuitous.

The results suggest that the liver contains about half of the assayable vitamin B_{12} in the body and that the total body stores range from 1000 - 4000 ug with a mean of about 2000 ug. This value is lower than previous estimates of 3000 ug (Estren et al 1958) or 3900 ug (Grasbeck et al 1958) which were obtained by microbiological assay of tissues and by multiplying the mean tissue values by the average weights of the tissues. It is difficult to decide which method gives a more accurate result both having obvious faults and sources of error. Not unnaturally the author prefers the method used in the present investigation.

HEPATIC ASSAYABLE VITAMIN B_{12}	No. OF CASES	REFERENCE
0.14 - 0.90 ug/g	5	Drouet et al (1951)
200 - 250 ug/organ	2	Haussman (1951)
0.47 - 1.14 ug/g	-	Wolff et al (1951)
0.24 - 0.74 ug/g	3	Girdwood (1952)
0.28 - 0.74 ug/g	2	Heinrich & Lahann (1954)
0.11 - 0.47 (mean 0.28) ug/g	10	Pitney et al (1955)
0.54 - 1.40 ug/g	25	Swendseid et al (1955)
0.4 - 2.6 (mean 1.1) ug/g	-	Blum & Heinrich (1957)
486 - 4330 (mean 1500)ug/organ	13	Ross & Mollin (1957)
0.17 - 1.41 (mean 0.7) ug/g	122	Swendseid et al (1957)
0.5 - 1.14 ug/g	-	Wolff (1957)
1.41 - 2.58 (mean 1.94) ug/g	13	Nelson & Doctor (1958)
0.302 - 1.091 (mean 0.588) ug/g	17	Adams (1961d) (Present Series)
514 - 1910 (mean 888)ug/organ	17	

Table 22: values for assayable vitamin B_{12} in hepatic tissue obtained by biopsy or at post mortem examination from 'normal' subjects.

OBSERVATIONS ON VITAMIN B_{12} DEFICIENT SUBJECTS

Results from two patients are presented and discussed under this heading. Although neither patient was vitamin B_{12} deficient at the time of death the title is a convenient one.

CASE REPORTS

Case M.S., female, aged 68, was admitted to hospital with a macrocytic anaemia, Hb. 44%, megaloblastic erythropoiesis was seen on marrow biopsy smears, serum vitamin B_{12} level 42 uug/ml and a histamine fast achlorhydria. She was given a single intramuscular injection of 1000 ug ^{58}Co vitamin B_{12} and 61.0% of the radioactivity was excreted in the subsequent 24 hours. An optimal reticulocyte response was observed and progress was uneventful until the 12th day after injection when she died, suddenly and unexpectedly, in acute left ventricular failure. Post mortem examination disclosed a large myocardial infarct, presumed to be the cause of the cardiac failure; the gastric mucosa was atrophic. The liver contained 9.22% of the dose of radioactivity injected, equivalent to

/92.2 ug ^{58}Co vitamin B_{12}. By assay the total vitamin B_{12} content of the liver was found to be 112.1 ug. The possibility that the injected ^{58}Co vitamin B_{12} retained in the body was not distributed to the tissues in proportion to the non radioactive stores does not affect the conclusion from these findings that the liver contained only 20 ug assayable vitamin B_{12} or 0.015 ug/g prior to treatment, values which are in close accord with those previously reported (Table 23). When an allowance was made for the amount of radioactivity excreted in the urine in the 24 hours after injection the radioactivity in the liver was calculated to be 23% of that retained in the body. This value is much less than that found in 'normal' subjects who died about the same time after injections of Co vitamin B_{12}. The significance of this finding is not clear. In view of the conclusions reached by Reizenstein et al (1961) and the fact that this patient received a large amount of Co vitamin B_{12} whereas the 'normal' subjects received a small amount it would be unwise to draw conclusions from it.

HEPATIC ASSAYABLE VITAMIN B_{12}	No. OF CASES	REFERENCE
about 1.0 ug/organ	1	Haussman (1951)
0.023 - 0.140 ug/g	5	Drouet et al (1951)
0 ug/g	1	Girdwood (1952)
0 ug/g	1	Heinrich & Lahann (1954)
0.10 ug/g	2	Swendseid et al (1957)
10 ug/organ	1	Ross & Mollin (1957)
0.030 ug/g	3	Nelson & Doctor (1958)
0 - 0.085 ug/g	3	Girdwood (1960b)

Table 23: showing values for assayable vitamin B_{12} in hepatic tissue obtained by biopsy or at post mortem examination from untreated vitamin B_{12} deficient subjects in haematological relapse.

/It cannot of course be assumed that the 20 ug calculated to be present in the liver prior to treatment represented 23% of the body stores and the exact extent of the pretreatment body stores cannot be estimated.

Case T.H. had undergone total gastrectomy for carcinoma of stomach six years before he died. He came under observation two years after operation when he was not anaemic but had a low serum vitamin B_{12} level (100 uug/ml) and megaloblastic erythropoiesis was seen on marrow biopsy smears. He was given parenteral vitamin B_{12} but attended for maintenance treatment irregularly. Five years after operation he was readmitted to hospital with symptoms suggestive of a myocardial infarction and this diagnosis was confirmed by electrocardiography. A routine chest X-ray showed secondary deposits in the lung fields. His serum vitamin B_{12} level was subnormal and after the clearance study reported earlier no further vitamin B_{12} was given. He made a satisfactory recovery from his cardiac lesion and was discharged and resumed work. Nine

/months later he was readmitted in the terminal stages of neoplastic disease. He was not anaemic and the serum vitamin B_{12} level was still subnormal. Treatment with nitrogen mustard conferred little benefit and he died six years after operation. Three days before death his peripheral blood values were Hb. 83%, P.C.V. 41%. Samples of serum obtained in the ten months before death were assayed in the same batch and the results are shown in Table 24. Post mortem examination confirmed the presence of secondary deposits in the lungs but no deposits were found in the abdominal cavity or organs. The total assayable vitamin B_{12} in the liver was 356 ug or 0.254 ug/g. These values for total assayable vitamin B_{12} in liver are less than any obtained in the 'normal' subjects studied by the same methods (Table 19) and greater than those reported for vitamin B_{12} deficient subjects in haematological relapse (Table 23).

DISCUSSION

Significance of results

The results obtained from the 'vitamin B_{12} deficient' subjects are relevant to the problem

DAYS BEFORE DEATH	SERUM VITAMIN B_{12} uug/ml
302	70
218	42
86	35
37	37

Table 24: showing serum vitamin B_{12} levels in Case T.H. in the ten months preceding death. All samples were assayed together in three separate assays and the value given is the mean of the three estimations.

/of the significance of a low serum vitamin B_{12} level. Mollin & Ross (1953b) and Pitney & Beard (1955) suggested that the serum vitamin B_{12} level only falls to an unequivocally low level, less than 80 uug/ml. by the method used (Mollin 1960), when the tissue stores are depleted. This view has been challenged by Booth & Spray (1960) who found that the fall in serum vitamin B_{12} levels in rats after total gastrectomy was more rapid than the fall in hepatic assayable vitamin B_{12} and suggested that the serum vitamin B_{12} level may not provide an index of the body stores. The findings in Case M. S. are in keeping with the theory of Mollin & Ross (1953b) and Pitney & Beard (1955) as are the findings of others who have analysed tissues from patients with megaloblastic anaemia in haematological relapse (Table 23). The findings in Case T.H. however, are in keeping with the observations on rats and do not support the belief that a low serum vitamin B_{12} level necessarily implies tissue depletion. Although the amount of assayable vitamin B_{12} found in the liver was less than that

/found in any 'normal' subject, being just under half the mean value for this group, it cannot be regarded as insignificant and representing tissue depletion. The amount found was much greater than that observed in the livers of patients with megaloblastic anaemia in haematological relapse (Table 23) the highest value found in such patients being 0.14 ug/g. (Case 5 Drouet et al 1951) and it may be relevant that this patient had also previously undergone gastrectomy for carcinoma. Although there is no particular reason to believe that the neoplastic process, treatment with a cytotoxic drug a month before death or altered intestinal anatomy are factors which might have upset a normal balance between tissue and serum vitamin B_{12}, the coincidence of the circumstances of this case and Case 5 of Drouet et al (1951) suggest that it would be unwise to disregard them. It should also be noted that Case T.H. was not anaemic at any time and this raises the further possibility that a low serum vitamin B_{12} level may in fact reflect tissue vitamin B_{12} depletion but only when there is coincident megaloblastic

/anaemia. Further study of this problem is clearly desirable and analysis of liver biopsy material from patients in the pre-anaemic stages of pernicious anaemia (Adams 1957a) might be particularly rewarding in resolving this problem.

Practical application of the results

Whether the serum vitamin B_{12} level does, or does not, provide an index of the body stores it is virtually certain from the findings summarised in Table 23 that the tissues of patients with pernicious anaemia in haematological relapse are grossly depleted. It seems logical to believe that the initial treatment of such patients should be the administration of enough vitamin B_{12} to ensure retention of the amount normally present in the body as suggested by Mollin & Ross (1953b). Taking the normal body store as 2000 ug and calculating from the formula relating dose of vitamin B_{12} injected to the percentage of the dose excreted (Table 14, Chapter 6) the number of injections of any one dose required to replenish the body stores can easily be calculated. The results of such calculations considering

/doses used in current clinical practice are shown in Table 25. Although it might be possible to replenish the body stores by a single massive dose a series of not less than five injections is probably preferable in view of the variation in amount excreted not only from patient to patient but in each patient. The most convenient routine for initial treatment therefore appears to be a daily injection of 1000 ug vitamin B_{12} for five days, a routine scarcely different from that suggested by Mollin & Ross (1953b). This routine should be regarded as the minimum treatment required to restore body stores to normal and there is no reason why more injections should not be given although it is probably unnecessary and wasteful to give more than twelve daily injections of 1000 ug which should ensure retention of nearly 4,500 ug which is the maximum value for total body vitamin B_{12} found in the 'normal' subjects.

There is no obvious reason why these conclusions should be modified in respect of treatment of patients with vitamin B_{12} deficiency states other than pernicious anaemia. The deficiency

DOSE INJECTED ug.	CALCULATED DOSE RETAINED ug.	No. OF INJECTIONS REQUIRED
50	38	53
100	67	30
250	139	40
500	232	9
1000	372	5

Table 25: showing number of injections of varying doses of vitamin B_{12} required to ensure retention of about 2000 ug. The values for calculated dose retained are taken from Table 16, Chapter 6.

/is unlikely to be more marked in other conditions and ill effects do not result from retention of very large amounts of injected vitamin B_{12}.

Calculation of the cost of initial treatment taking into account only the basic N.H.S. cost of an ampoule of vitamin B_{12} (Cytamen - Glaxo), shows that, in theory, the most economic routine is fourteen injections of 250 ug or five injections of 1000 ug (Table 26). When other costs, notably the cost of giving an injection in terms of material and labour, are considered, it is obvious that the method of choice is injections of 1000 ug. The most impressive feature of these calculations is the trivial cost of the dramatic benefits which result from vitamin B_{12} therapy in suitable cases.

DOSE INJECTED ug	COST pence	No. OF INJECTIONS REQUIRED	COST
50	3½	53	15/5½
100	5	30	12/6
250	7	14	8/2
500	14(2 x 7)	9	10/6
1000	20	5	8/4

Table 26: showing cost of treatment designed to ensure retention of about 2000 ug. No allowance has been made for the actual cost of the injection, the cost being the basic N.H.S. cost of an ampoule of vitamin B_{12} (Cytamen - Glaxo) only.

SUMMARY

A method of determining the total assayable vitamin B_{12} in the body was devised. The assumption was made that the amount of injected vitamin B_{12} which is not excreted is distributed throughout the body in proportion to the body stores. By measuring the radioactivity and estimating the assayable vitamin B_{12} content of liver obtained at post mortem examination, the total assayable vitamin B_{12} in the body can be calculated.

Results from a series of sixteen 'normal' patients are reported. The techniques used and the problems apparent in such an investigation are discussed in detail. The results are analysed to allow for clearance of injected Co vitamin B_{12} from the blood stream and 'relocation' in tissues. The findings suggest that the liver contains about half of the total assayable vitamin B_{12} in the body and that the mean value of the total assayable vitamin B_{12} in the body is in the region of 2000 ug. Observations made on tissues obtained from patients with abnormalities of vitamin B_{12}

/metabolism are reported and discussed particularly in relation to the concept that the serum vitamin B_{12} level only falls to a subnormal level when the tissue stores are depleted.

The initial treatment of patients with vitamin B_{12} deficiency states is discussed in the light of the findings and certain conclusions about the treatment of such patients are drawn.

CHAPTER VIII

THE RELATIONSHIP OF THE AMOUNT OF VITAMIN B_{12} RETAINED IN THE BODY FOLLOWING PARENTERAL INJECTION TO THE DURATION OF EFFECT.

With Some Notes on the Treatment of Vitamin B_{12} Deficiency States and on Utilisation of Vitamin B_{12} in Man.

At present there is general agreement that the treatment of choice of vitamin B_{12} deficiency states is parenteral vitamin B_{12}. Reference to the standard textbooks of medicine and haematology and also to the literature on vitamin B_{12} metabolism in man however, show that there is doubt as to what constitutes adequate treatment.

It was decided therefore, to study the duration of therapeutic effect of varying doses of vitamin B_{12} given intramuscularly to patients with vitamin B_{12} deficiency states with the object of defining principles on which the treatment, particularly the maintenance treatment, of such patients should be based.

THEORETICAL CONSIDERATIONS

Patients with pernicious anaemia absorb little, if any, of the vitamin B_{12} in their diet and from the therapeutic point of view the amount of vitamin B_{12} which they absorb from a normal diet is negligible. For practical purposes therefore, their 'intake' of vitamin B_{12}

/is that given by injection. The loss after injection is almost entirely by excretion in the urine in the subsequent 24 hours, the loss in faeces being trivial (Chapters 4 & 5). It is very unlikely that the vitamin B_{12} which is excreted in the urine after parenteral administration has any therapeutic effect. Thus, for practical purposes, the amount of vitamin B_{12} which exerts a therapeutic effect in such patients is the amount injected minus the amount lost in the urine in the 24 hours after injection - the retained or effective dose. It seems reasonable to believe that the duration of effect of this retained or effective dose is the time after injection for which the serum vitamin B_{12} level remains within normal limits. Until fairly recently the objects of treatment of patients with pernicious anaemia were simply the restoration and maintenance of normal peripheral blood levels and the avoidance of signs or symptoms suggestive or indicative of vitamin B_{12} deficiency such as glossitis or neuropathy. It is now obvious that peripheral blood values alone are only a

/crude index of the efficacy of treatment as normal, or near normal, peripheral blood values have been found in the presence of megaloblastic erythropoiesis, subnormal serum vitamin B_{12} levels and even neuropathy (Thomson 1944. Bastrup-Madsen 1954, 1956ab, Girdwood 1956b, Victor & Lear 1956, Adams 1957ab, Killander 1957b, 1958abd, Pedersen et al 1957, Adams & Timbury 1959). Under these circumstances it is entirely reasonable to believe that one of the objects of therapy, possibly the main biochemical object, is the maintenance of a normal serum vitamin B_{12} level.

With these considerations in mind three experiments were devised with the object of investigating the relationship, if any, between the effective dose of injected vitamin B_{12} and the duration of effect of this dose.

Each experiment will be described, the results presented and a brief comment made on the results. The results will then be analysed and the findings and their significance discussed in detail.

All except two patients studied had pernicious

/anaemia. One (Case J.McQ.) came under observation with a post gastrectomy megaloblastic anaemia and in one (Case C.D.) the final diagnosis was the loop syndrome. All patients were aware of the nature and objects of the experiments and all cooperated willingly. It was found convenient to use some patients for more than one experiment. The methods of preparing ^{58}Co vitamin B_{12} for injection, other technical methods and the statistical techniques used are described in the Appendix.

FIRST EXPERIMENT

The object of the experiment was to find out if the duration of effect of the first injection of ^{58}Co vitamin B_{12} given to a vitamin B_{12} deficient patient in haematological relapse was similar to that obtained from a second injection of the same dose given when the serum vitamin B_{12} level was higher.

Five patients were studied and the doses of ^{58}Co vitamin B_{12} given ranged from 50 ug 0.3 uc to 1000 ug 0.2 uc. Prior to treatment a 24 hour collection of urine was made. After the first injection of ^{58}Co vitamin B_{12} the amount excreted was established by measuring the urinary excretion of radioactivity for at least 24 hours. The serum vitamin B_{12} level was estimated frequently and when it fell to about the lower limit of normal, 140 uug/ml, a second injection of ^{58}Co vitamin B_{12} of the same dose was given and the cycle repeated. At the end of the observation period all samples of serum obtained from each patient were assayed in at least two different assay batches; the results given are the mean

/values of the different assays. The results are shown in Figs. 66 - 70 and Table 27. Details of individual results are given in Tables A.51 - 55 in the Appendix.

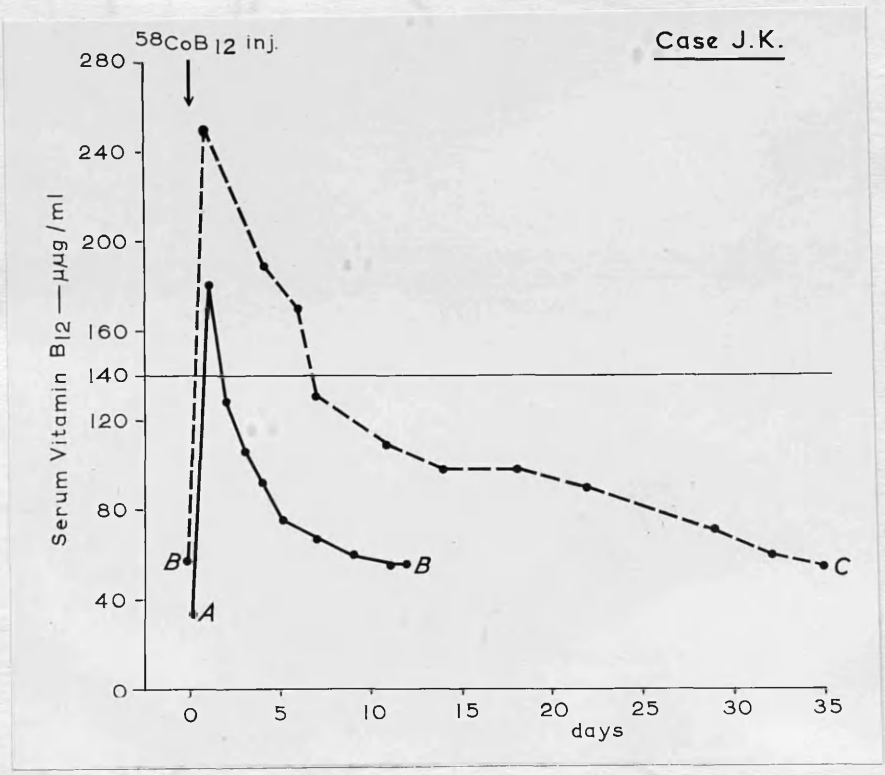


Fig. 66: showing serum vitamin B_{12} levels after injections of ^{58}Co vitamin B_{12} in Case J.K. For clarity the levels after the second injection joined by the interrupted line BC have been superimposed on the levels after the first injection joined by continuous line AB. See Table A.51. Figs. 67 - 70 also follow this convention.

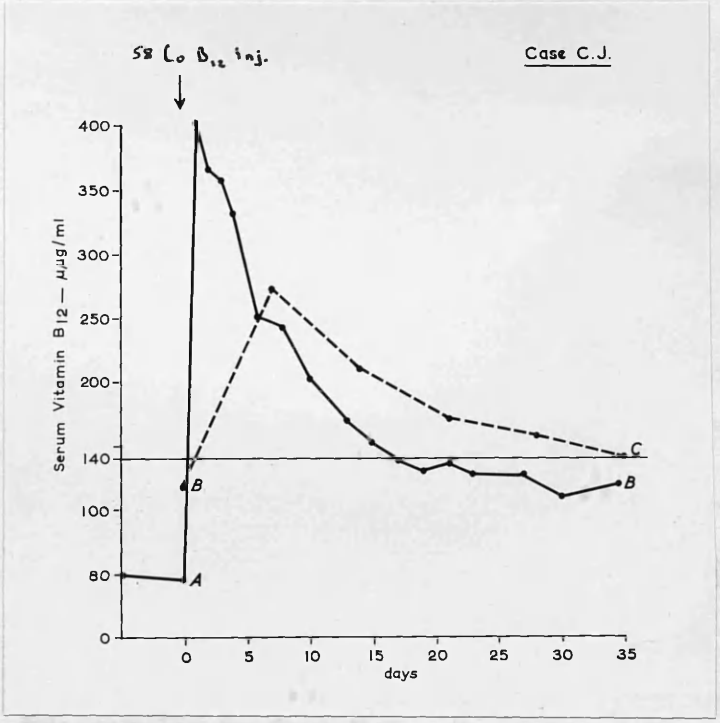


Fig. 67: results in Case C.J. See Table A.52.

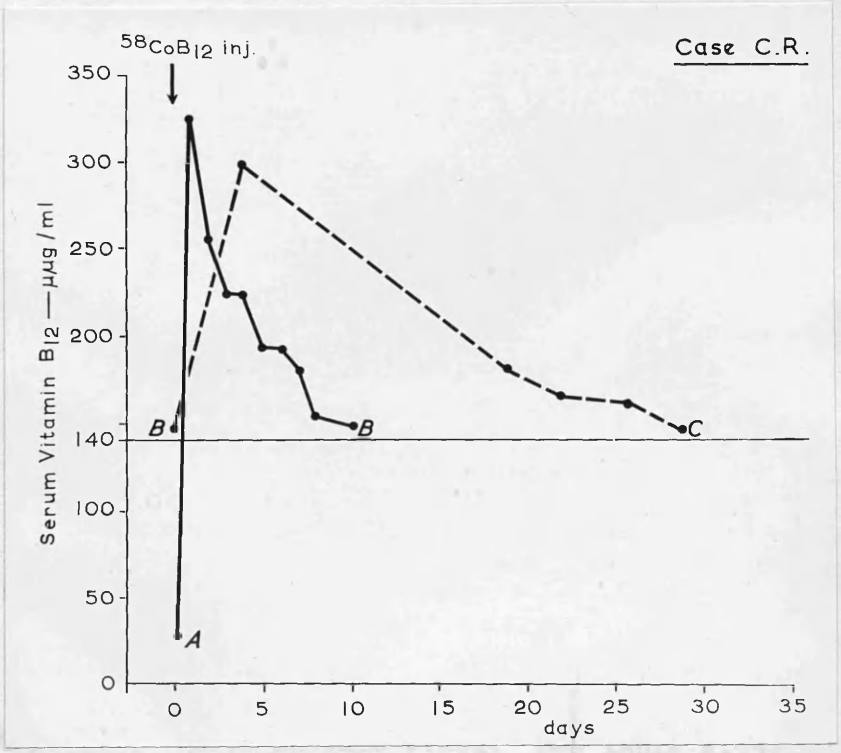


Fig. 68: results in Case C.R. See Table A.53.

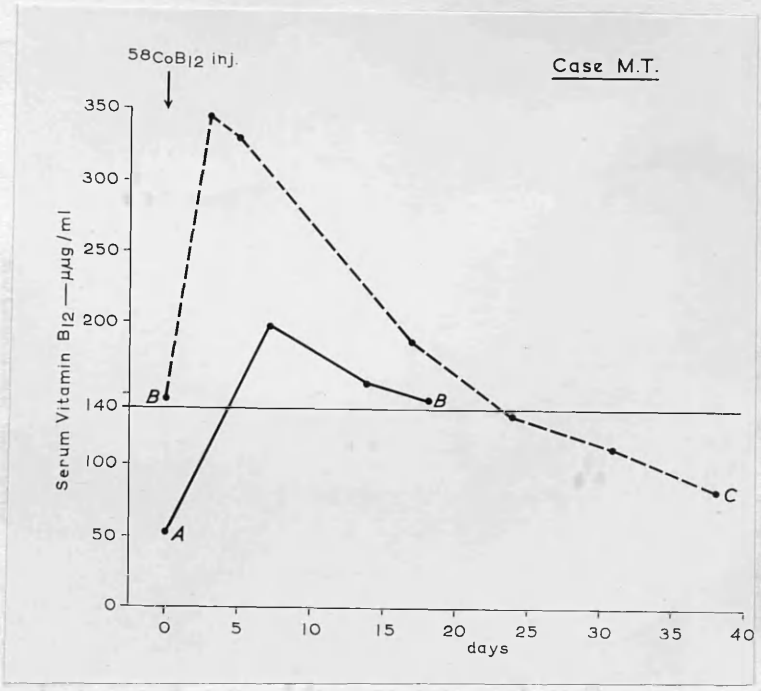


Fig. 69: results in Case M.T. See Table A.54.

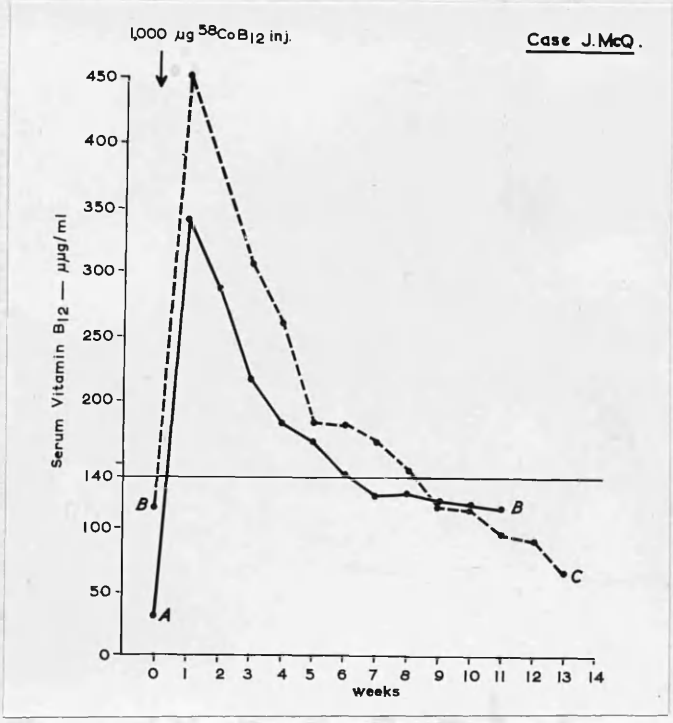


Fig. 70: results in Case J.McQ. See Table A.55.

CASE	FIRST INJECTION			SECOND INJECTION		
	INJECTED DOSE ug	RETAINED DOSE ug	DURATION OF EFFECT days	INJECTED DOSE ug	RETAINED DOSE ug	DURATION OF EFFECT days
J.K.	50	41.5	1.75	50	39.5	7
C.J.	100	80.0	17.5	100	78.0	35
C.R.	100	72.0	10	100	80.2	29
M.T.	100	81.2	18	100	85.0	24
J.McQ.	1000	324.0	42	1000	485.0	59

Table 27: showing details of results in first experiment.

COMMENTS

In all five patients the duration of effect, in terms of maintaining a serum vitamin B_{12} level above 140 uug/ml, the lower limit of normal by the method used (Mollin 1960) was markedly less after the first injection than after the second injection. In four cases this was clearly not related to a larger effective dose after the second injection and this was probably not a factor in the other case. Unfortunately the number of cases studied was too small for statistical analysis to be carried out with confidence. This is particularly regrettable as there is a marked variation in the duration of effect with the same effective dose even when the injections are given to the same patient at the same serum vitamin B_{12} level as will be shown later. The possibility arises therefore that the observed difference in duration of effect may be no greater than might occur by chance. Careful scrutiny of the results however, leaves a strong impression that the duration of effect of the first injection is likely to be constantly

/less than that obtained with a subsequent injection given when the serum vitamin B_{12} level is higher. It seems unlikely that any alteration in peripheral blood values is a relevant factor. It is probably unduly simple to ascribe the increased duration of effect to the raised serum vitamin B_{12} level alone but the available evidence permits no more profound conclusion. An increase in tissue stores may be a factor but it would be most unwise to attempt to correlate this with an increase in serum vitamin B_{12} levels in view of the findings reported in Chapter 7.

SECOND EXPERIMENT

The object was to compare the duration of effect of different doses of ^{58}Co vitamin B_{12} in the same patient. Treatment was witheld from patients who had already been treated and were in full haematological remission. The serum vitamin B_{12} level was estimated frequently and when it fell to an arbitrary level, usually the lower limit of normal, an injection of ^{58}Co vitamin B_{12} was given and the urinary loss of radioactivity in the subsequent 24 hours was measured. Following the injection the serum vitamin B_{12} level was estimated frequently and when it again fell to about the original preinjection level a further injection was given and the cycle repeated. At the end of the observation period all samples of serum obtained from each patient were assayed in at least two different batches. The results given are the mean values of the different assays. Twenty two patients were studied, some on more than one occasion, and the doses of ^{58}Co vitamin B_{12} used ranged from 50 ug 0.3 uc to 1000 ug 0.2 uc. In some cases

/two injections of the same dose were given at an interval of 24 hours.

The results are shown in Figs. 71 - 92, details of individual results being given in Tables A.53 & 54, 56 - 75 in the Appendix. In the figures the retained dose is given in parentheses after the injected dose.

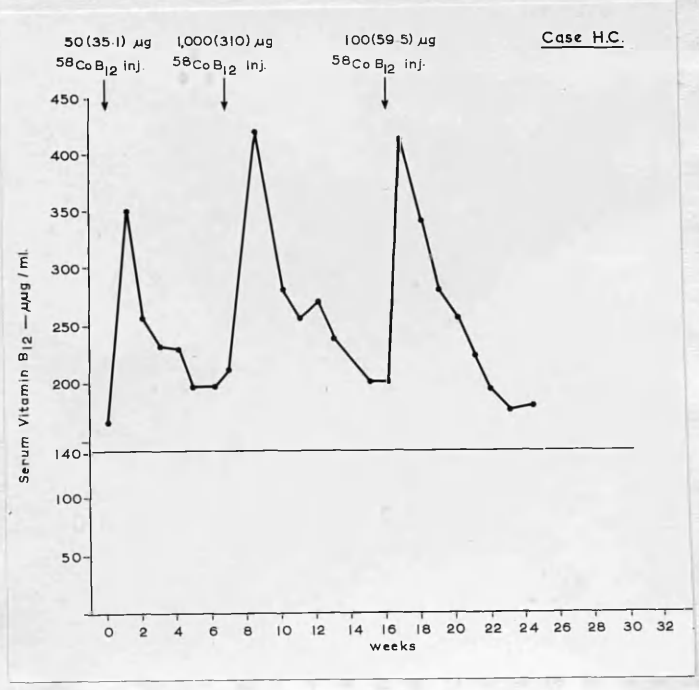


Fig. 71: results in Case H.C. See Table A.56.

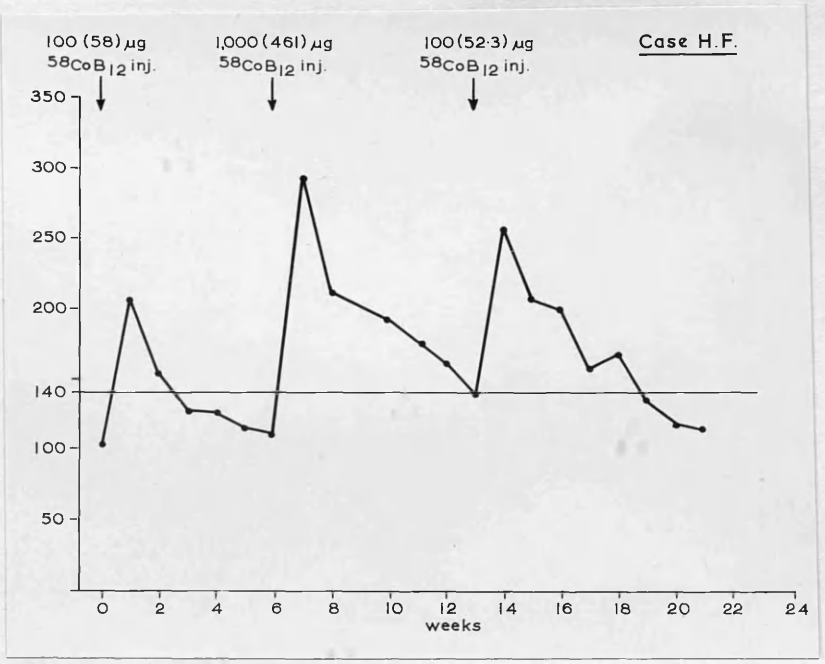


Fig. 72: results in Case H.F. See Table A.57.

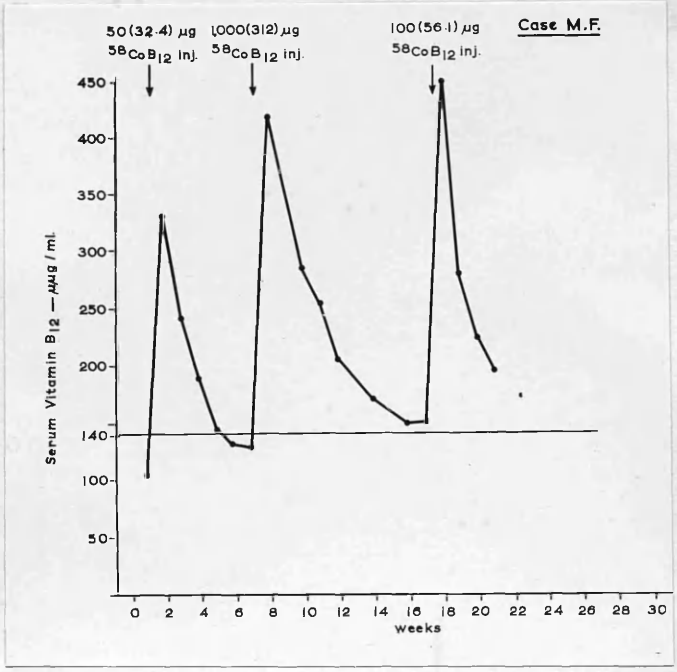


Fig. 73: results in Case M.F. See Table A.58.

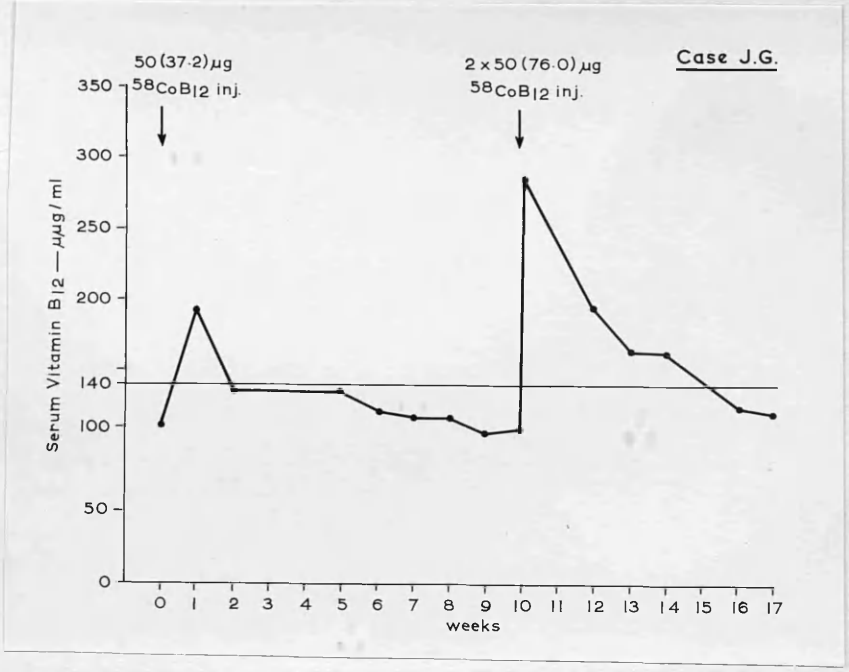


Fig. 74: results in Case J.G. See Table A.59.

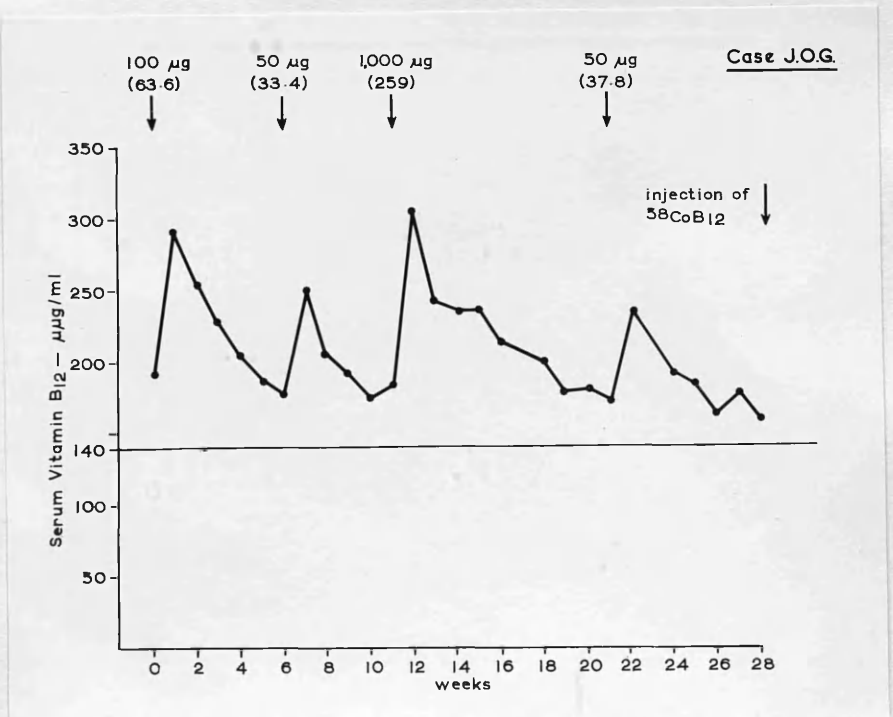


Fig. 75: results in Case J.O.G. See Table A.60.

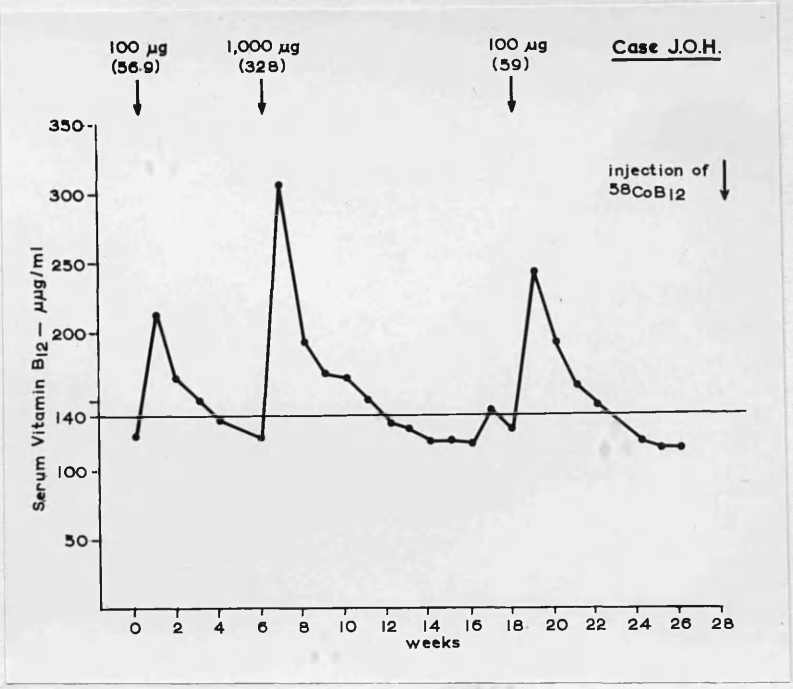


Fig. 76: results in Case J.O.H. See Table A.61.

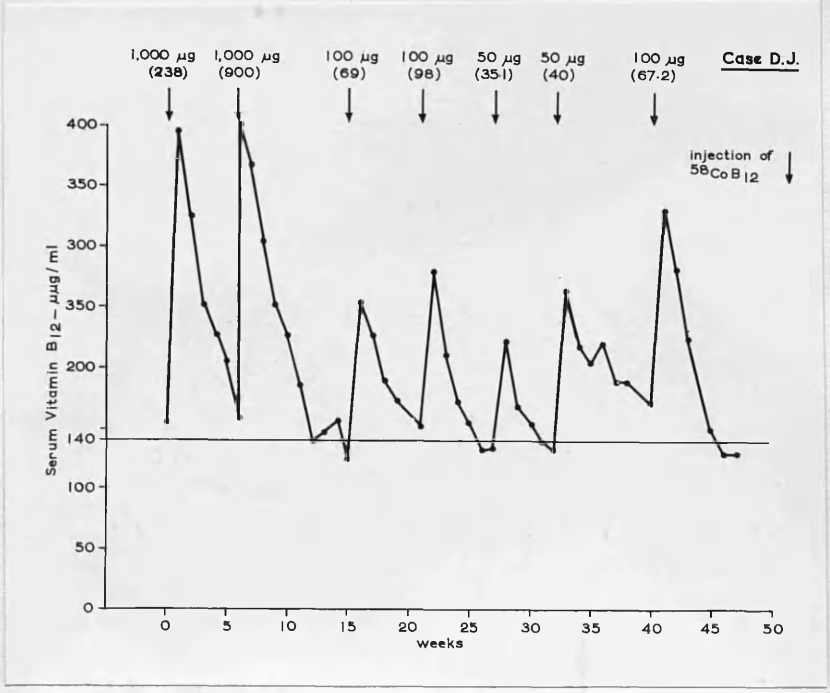


Fig. 77: results in Case D.J. See Table A.62.

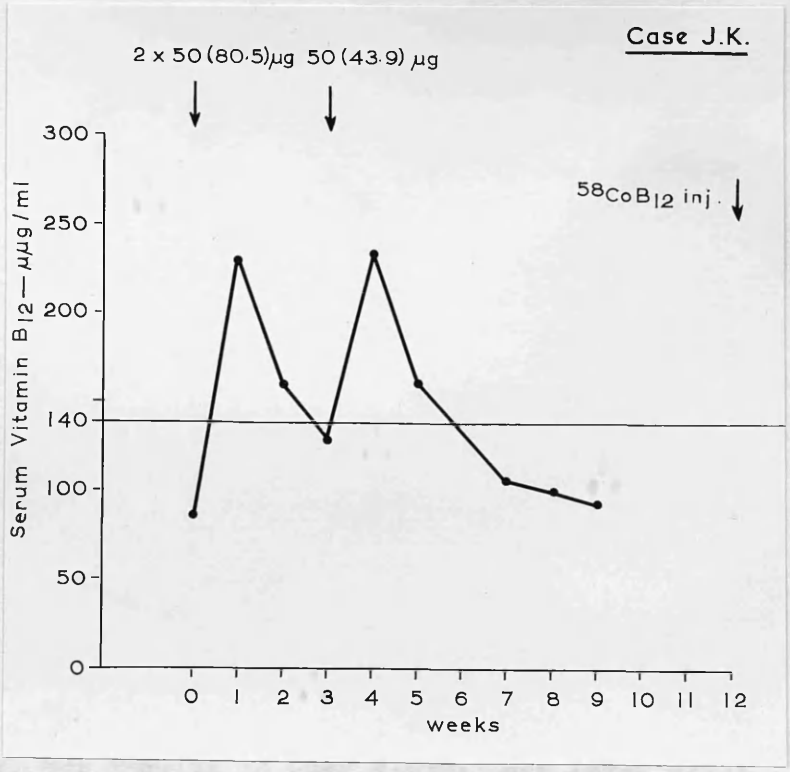


Fig. 78: results in Case J.K. See Table A.63.

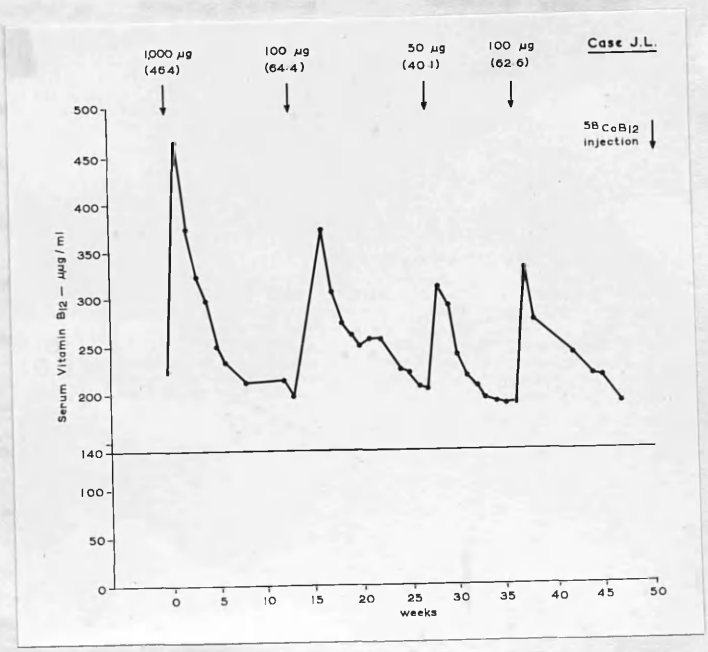


Fig. 79: results in Case J.L. See Table A.64.

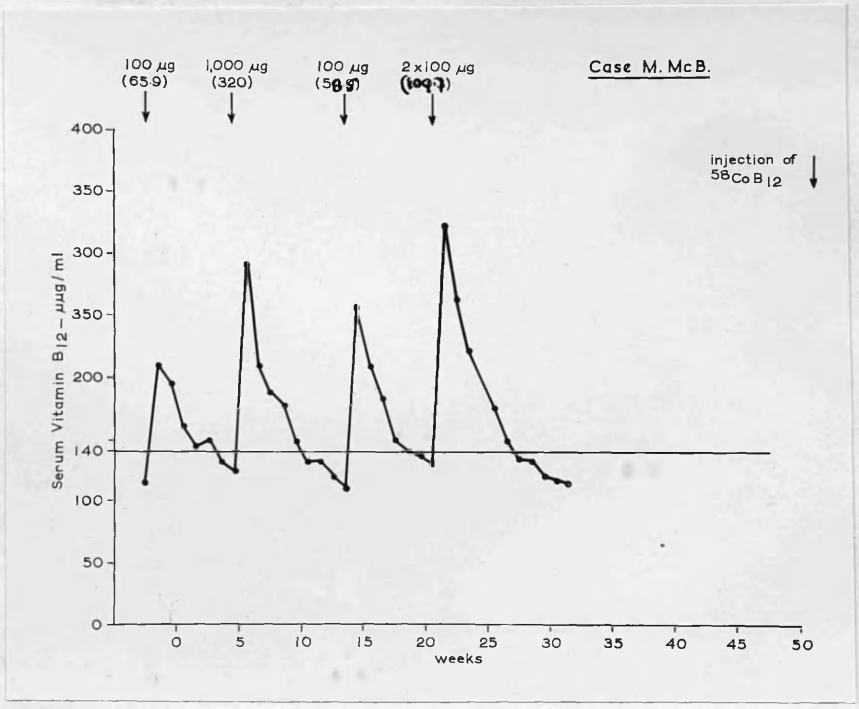


Fig. 80: results in Case M.McB. See Table A.65.

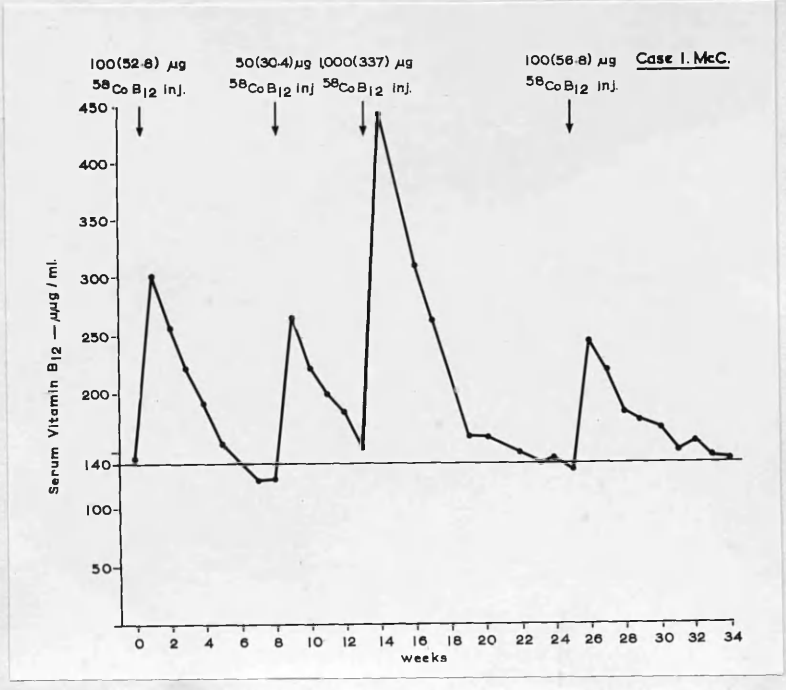


Fig. 81: results in Case I.McC. See Table A.66.

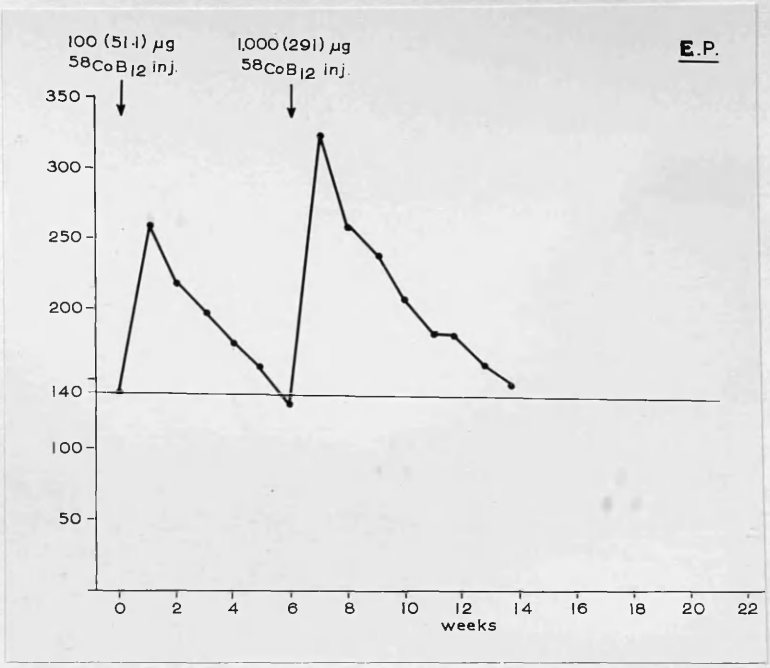


Fig. 82: results in Case E.P. See Table A.67.

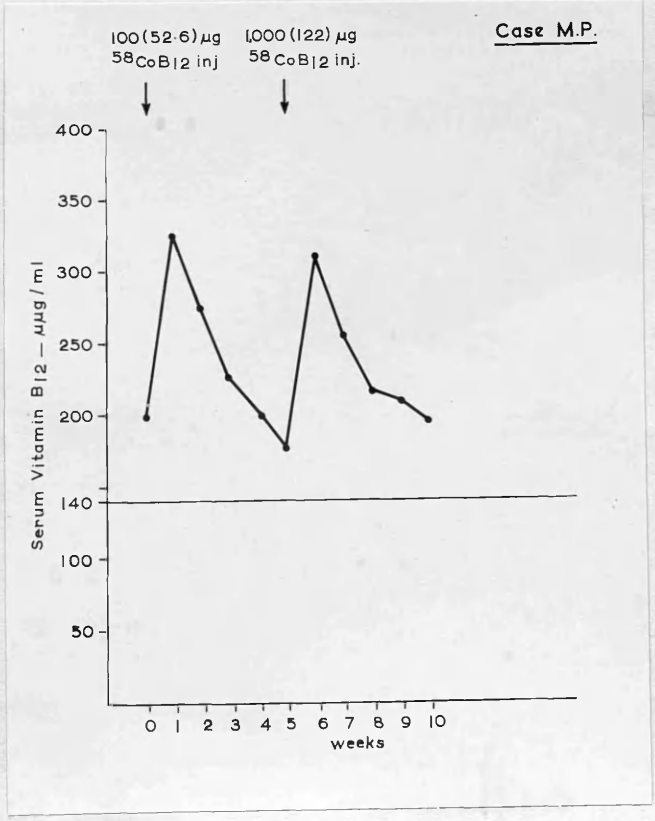


Fig. 83: results in Case M.P. See Table A.68.

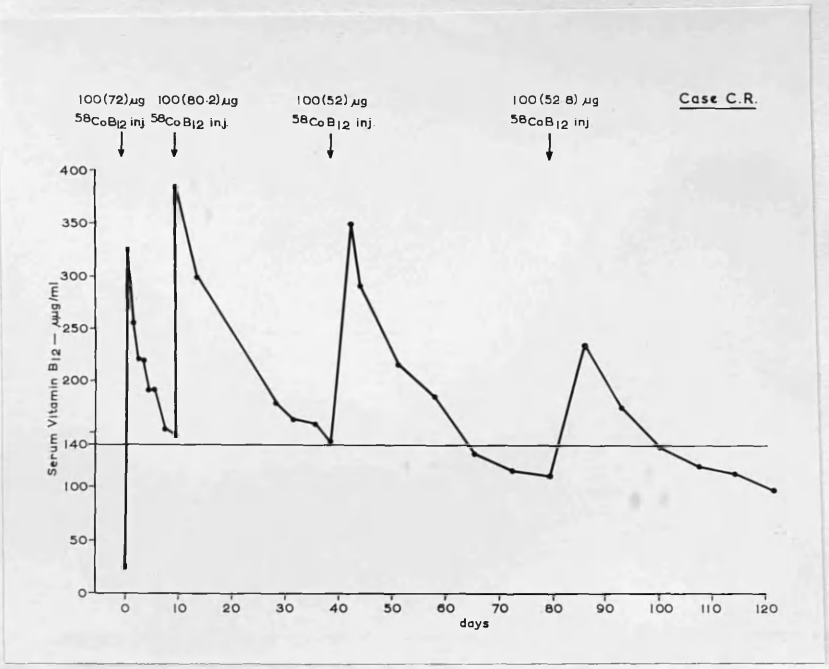


Fig. 84: results in Case C.R. See Table A.53.

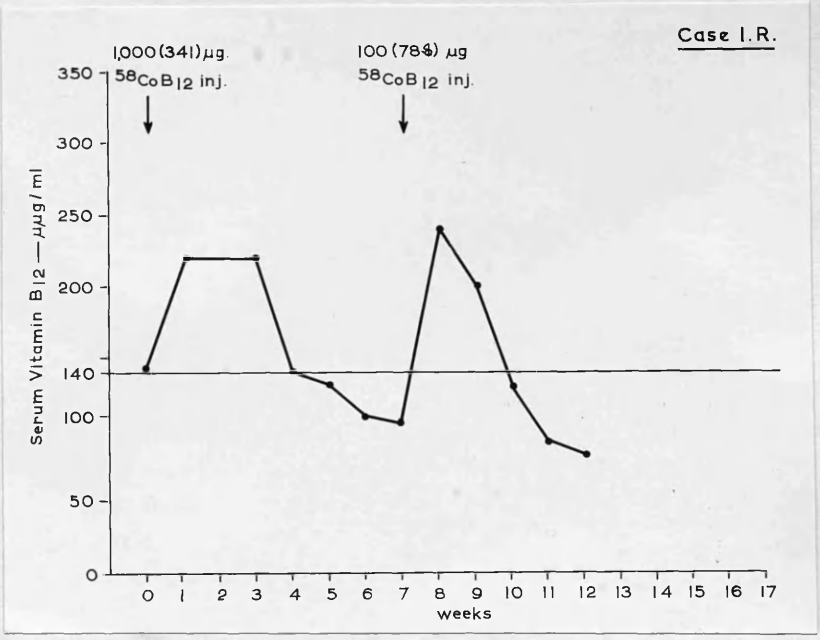


Fig. 85: results in Case I.R. See Table A.69.

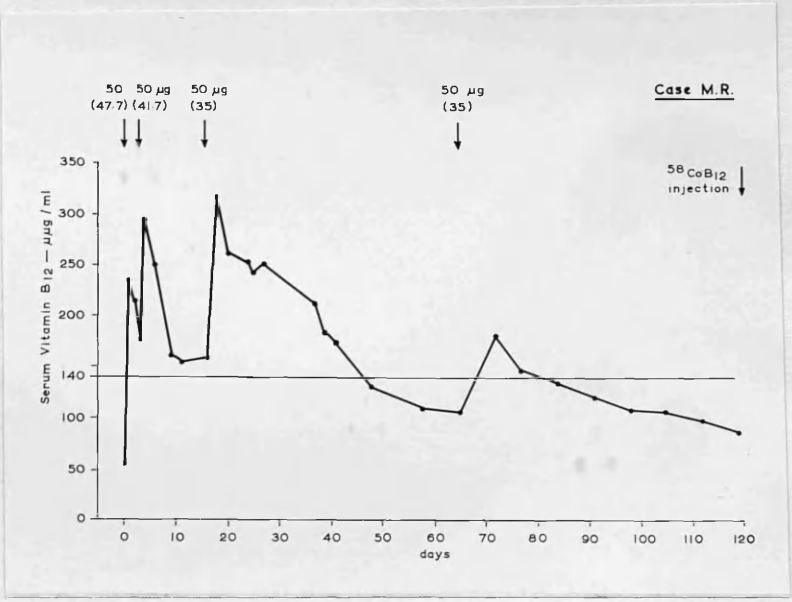


Fig. 86: results in Case M.R. See Table A.70

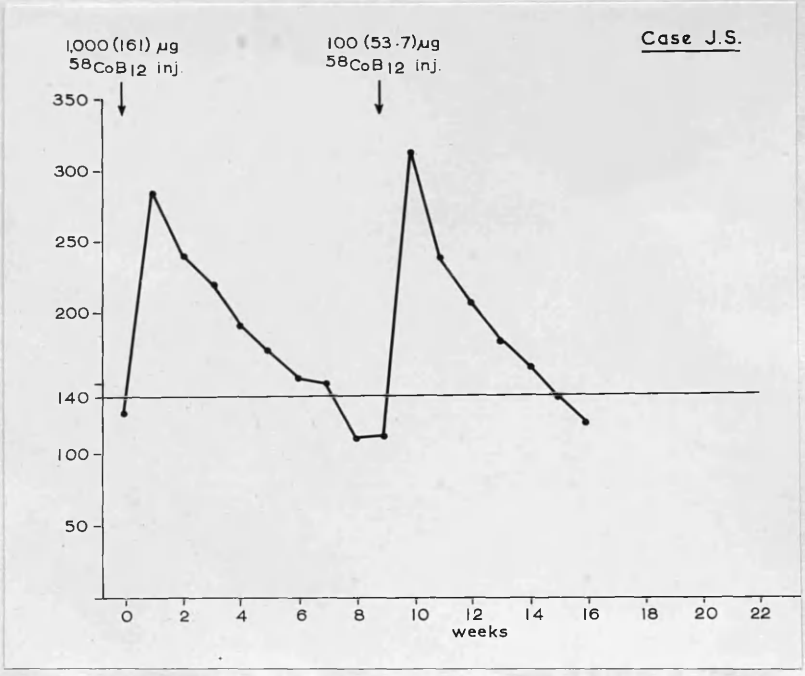


Fig. 87: results in Case J.S. See Table A.71.

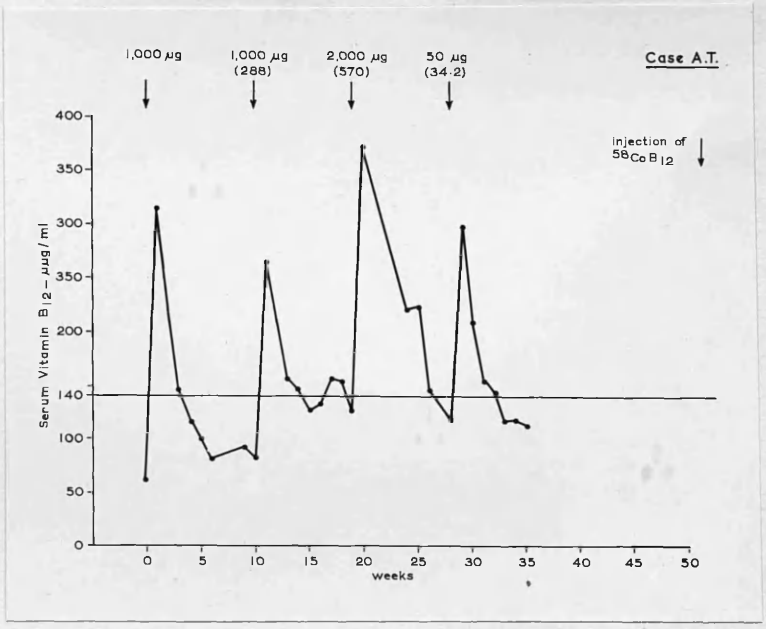


Fig. 88: results in Case A.T. See Table A.72.

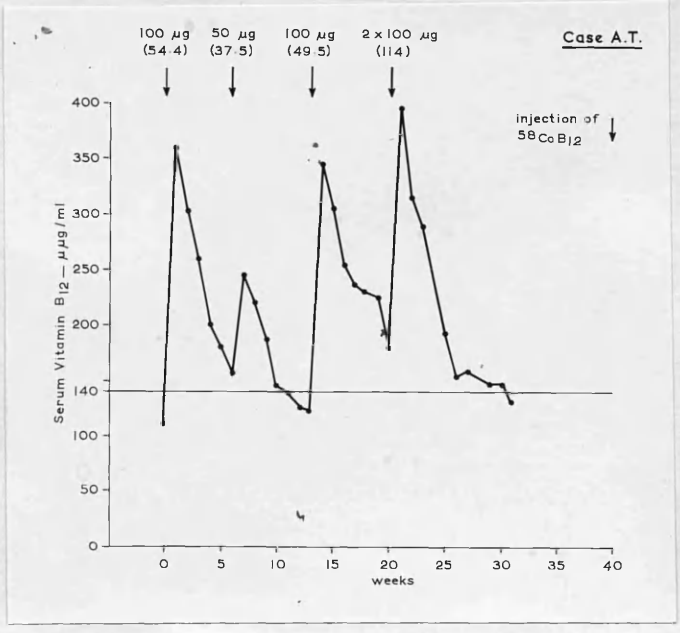


Fig. 89: results in Case A.T. See Table A.73.

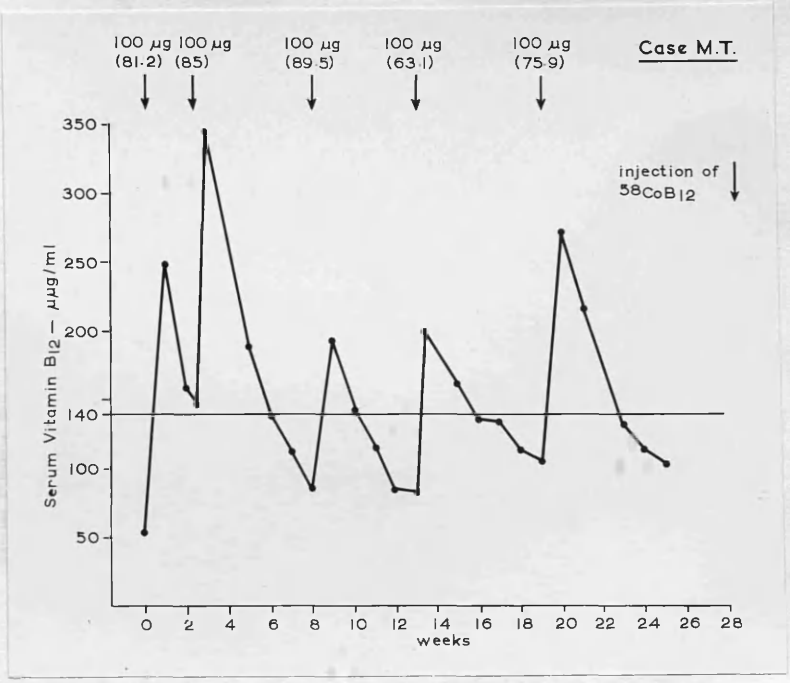


Fig. 90: results in Case M.T. See Table A.54.

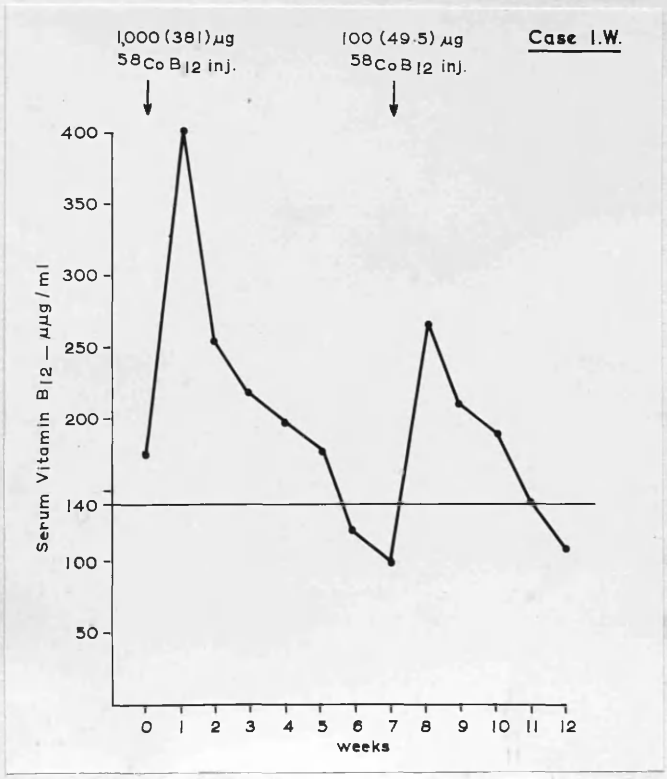


Fig. 91: results in Case I.W. See Table A.74.

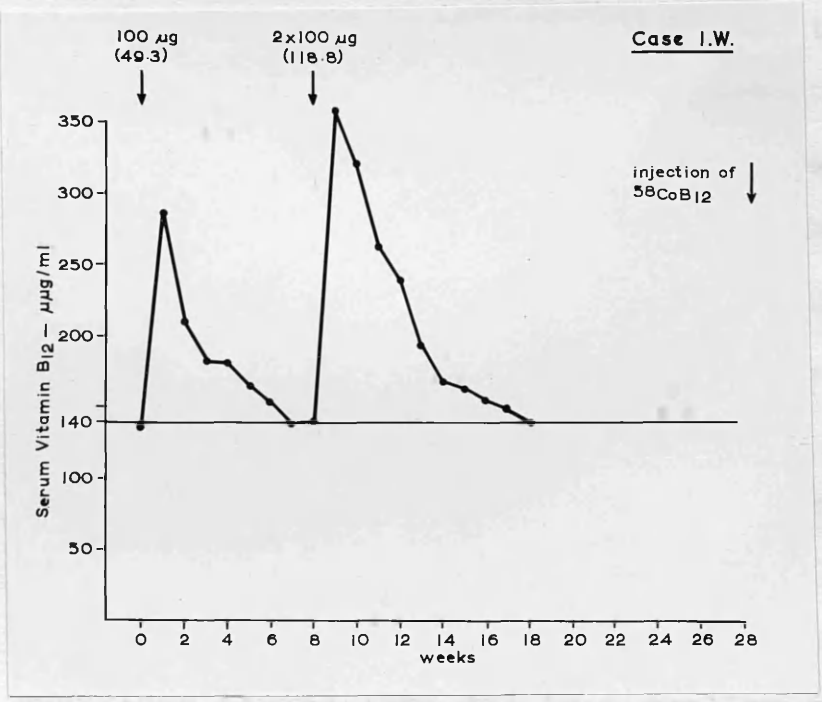


Fig. 92: results in Case I.W. See Table A.75.

COMMENTS

The striking feature, apparent in every case, was that the duration of effect of an injection of 1000 ug was only rarely greater than twice that obtained with injections of 50 ug or 100 ug in spite of the fact that the amount of ^{58}Co vitamin B_{12} retained in the body after the

/larger injection was from three to ten times greater than the amount retained after the smaller injection. In other words the duration of effect was clearly not directly proportional to the amount of ^{58}Co vitamin B_{12} retained. This phenomenon was observed whether the duration of effect was calculated in terms of maintaining the serum vitamin B_{12} level above the lower limit of normal (140 uug/ml) or above a higher or lower value. There was no doubt about these findings which have been reported previously on only two occasions (Adams 1961 ce) in a smaller series. As far as was possible the injections were given at the same serum vitamin B_{12} level in each patient and the same pattern was seen whether the larger injection was given before or after the smaller injection. In one or two cases it appeared that the phenomenon might be related to a difference between the serum vitamin B_{12} levels at the time of the injections as was noted in the first experiment but this did not obtain in all cases and in some the reverse appeared to be the case. The phenomenon cannot be explained on the basis

/of significant loss of injected ^{58}Co vitamin B_{12} in faeces (Chapter 5) and investigations showed that it was not due to continuing urinary excretion after 24 hours. It is known that ^{58}Co vitamin B_{12} may deteriorate in vitro, (Smith 1959) and the possibility that this was in some way a factor was also investigated: deterioration of ^{58}Co vitamin B_{12} was not observed by weekly microbiological assay with any of the batches used and two batches were found to have retained their potency between the time of injection and the time the sera from patients given that batch were assayed. The repetition of the same pattern in every case suggested that the phenomenon was not due to any technical error but was biological.

THIRD EXPERIMENT

The object was to determine the duration of effect of a series of injections of ^{58}Co vitamin B_{12} given in a short period to patients in haematological relapse. After a 24 hour collection of urine had been completed several injections were given and the loss in 24 hour collections of urine was measured until at least 48 hours after the last injection. Each patient received the same dose of ^{58}Co vitamin B_{12} at each injection. After the treatment period the serum vitamin B_{12} level was estimated frequently until it fell to the lower limit of normal. All samples of serum obtained from each patient were then assayed in at least two different assay batches. The results given are the mean values of the different assays. Eight patients were studied. The total amounts of ^{58}Co vitamin B_{12} given to and retained by each patient are shown in Table A.85, details of individual cases being given in Tables A.57 & A.76 - 82 in the Appendix. The results are shown in Fig. 93.

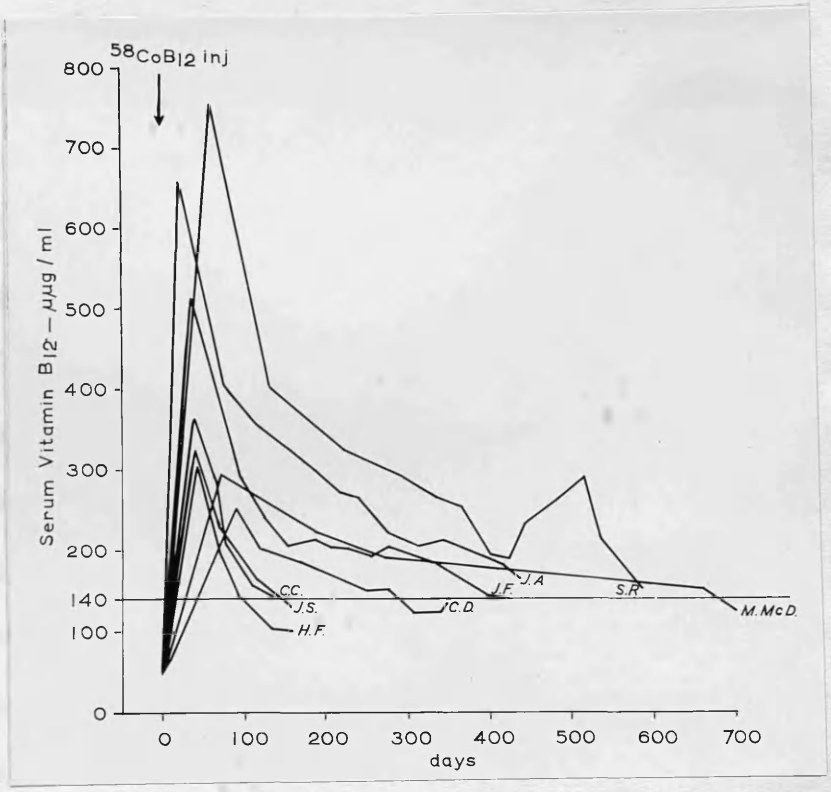


Fig. 93: results in Cases given massive therapy. See Table A.85.

COMMENTS

The duration of effect of the various retained doses varied very greatly from case to case. The most striking feature was that the duration of effect in nearly all cases was much less than might have been expected from the findings in the second experiment.

FURTHER INVESTIGATIONS - ANALYSIS OF RESULTS

It is a well established principle in pharmacology that if a drug is excreted in the urine in an exponential manner and if the clearance of the drug from the blood stream is also exponential in form, that the duration of effect of the drug is proportional to the logarithm of the dose of the drug (Wilson & Schild 1959). Analysis of results obtained from experiments on the urinary excretion of parenteral ^{58}Co by the normal and by an artificial kidney had shown that there was a linear relationship between the amount of vitamin B_{12} excreted and logarithm of the time, the coefficient of correlation differing significantly from zero. (Chapter 6). The fall in serum vitamin B_{12} levels after an injection of vitamin B_{12} also appeared to conform to this pattern. With these factors in mind it seemed worthwhile analysing the results obtained in the present experiments to find out if there was a relationship between the logarithm of the amount of vitamin B_{12} retained in the body and the duration of its effect

/in days.

In selecting cases for analysis certain problems became apparent. The first was whether to take the duration of effect as being the period for which the serum vitamin B_{12} level remained above the preinjection level or whether to take it as the period for which the serum vitamin B_{12} level remained above the lower limit of normal (140 uug/ml). It was decided to adopt the latter alternative for two reasons: in the first place it seemed undesirable, on general grounds, to analyse data in which the 'end points' varied widely from case to case; in the second place it appeared more likely that results relevant to the treatment of patients with vitamin B_{12} deficiency states could be obtained by this technique. It was also apparent that if the end point was to be 140 uug/ml then only results in which the preinjection level was also 140 uug/ml also should be studied. A review of the results showed that there were only a small number of cases which satisfied these rather rigid criteria. As it was obvious that a large number of results would be

/needed for a satisfactory analysis it was decided to include cases in which the preinjection serum vitamin B_{12} level deviated to a small degree from the figure of 140 uug/ml. It seemed likely that any errors accruing from this would cancel each other out in a sufficiently large series. A further difficulty in the selection of results was whether to include results obtained from patients given multiple injections of ^{58}Co vitamin B_{12} whether these were simply two injections of 50 or 100 ug given at a 24 hour interval or whether they were repeated injections given over a period of several days.

In the first place a series of 40 results obtained from eighteen patients was selected, mainly from those obtained in the second experiment. Results relating to multiple injections were excluded and, for reasons already given, results relating to the first injection of ^{58}Co vitamin B_{12} given to patients in haematological relapse were also excluded. Analysis of these values, which are detailed in Table A.83, showed a linear correlation between the logarithm of the

/ retained dose and the duration of effect in days, the coefficient of correlation differing significantly from zero. The regression equation was:

$$Y = 17.0886x - 0.4270$$

$$(n = 40,\ r = 0.5276,\ P < 0.001)$$

where y = the duration of effect in days and x = the logarithm of the retained dose. The results are shown in Fig.94.

When these 40 results were augmented by 5 obtained in the second experiment from patients given two injections of 50 or 100 ug ^{58}Co vitamin B_{12} at 24 hour intervals (Table A.84) the relationship was little changed, the regression equation being:

$$Y = 18.7895x - 2.0727$$

$$(n = 45,\ r = 0.4714,\ P < 0.01).$$

and the coefficients of correlation were not found to differ significantly ($t < 1.0$, $P > 0.05$).

There were obvious objections to including results obtained in the third experiment to those already analysed. The main objection was that in the third experiment all patients were treated

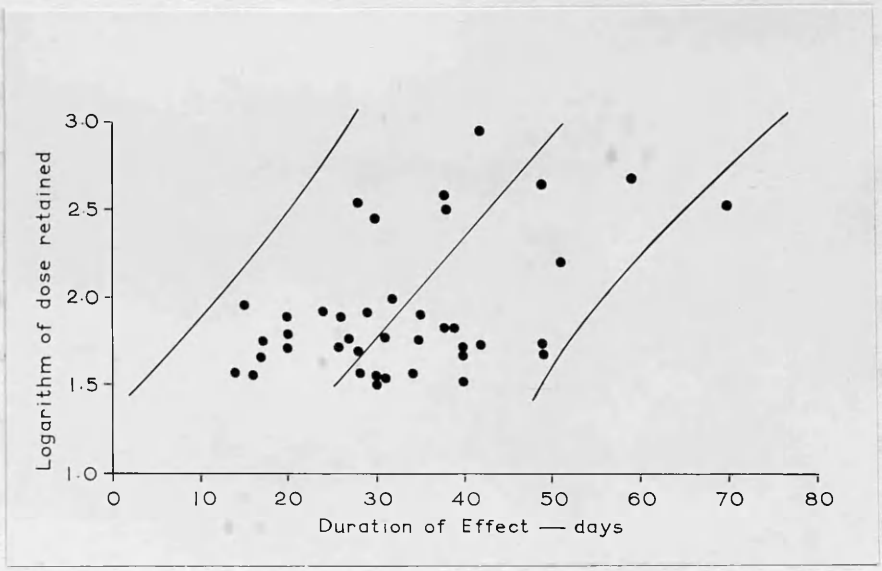


Fig. 94: showing relationship of the logarithm of the retained dose to the duration of effect in days. Forty observations are plotted and the regression line and 95% confidence limits are also shown. The regression equation is Y = 17.0886x - 0.4270 where x = the logarithm of the retained dose and y = the duration of effect in days in terms of maintaining the serum vitamin B_{12} level about 140 uug/ml.

/when in haematological relapse with low serum vitamin B_{12} levels whereas in the second experiment they were treated when in haematological remission with serum vitamin B_{12} levels at the lower limit of normal. There was no reason why the results obtained in the third experiment should not be analysed alone although the small number of observations made it obvious that the value of a correlation, if any, would be limited. In fact an analysis of six suitable values selected from those in Table A.85 showed that the coefficient of correlation did not differ significantly from zero ($r = 0.52$, $n = 6$, $P = > 0.05$).

DISCUSSION

As far as can be ascertained the finding of a significant correlation between the logarithm of the retained dose and the duration of effect has only been reported on one occasion (Adams 1961a). The finding is of considerable practical and academic importance, although not entirely unexpected, and its significance will be discussed in detail.

Practical application of the results

The immediate practical value of the correlation between the logarithm of the retained dose and the duration of effect is that it enables maintenance therapy with parenteral vitamin B_{12} for patients with pernicious anaemia to be planned on a rational basis. The inclusion in the analysis of results from one patient with a post gastrectomy vitamin B_{12} deficiency state does not affect the value of the findings as absorption tests with orally administered ^{58}Co vitamin B_{12} showed that this patient had a defect of absorption as complete as that found in patients with pernicious anaemia.

By calculating from the equation relating the dose of ^{58}Co vitamin B_{12} injected and the percentage of the dose excreted in the urine (Chapter 6) for doses up to 1000 ug and by further calculating from the equation

$$Y = 17.0886x - 0.4270$$

where y = the duration of effect in days and x = the logarithm of the effective dose, the calculated duration of effect of doses of vitamin

/B_{12} commonly used in clinical practice can be obtained (Table 28). The equation Y = 17.0886x is used in preference to Y = 18.7895x - 2.0727. Although there is little difference between them the second equation was obtained by analysis of results from patients treated dissimilarly and it is preferable to use the first equation which was obtained by analysis of results from patients treated under comparable conditions. The results of the calculations, shown in Table 28, form the basis for dose schedules for maintenance treatment of patients with pernicious anaemia where the object of treatment is to maintain the serum vitamin B_{12} level at, or above, the lower limit of normal.

It should be stressed that the values in Table 28 are calculated mean values. It is not proposed to discuss the results in terms of statistical ranges as this is scarcely necessary when considering the results in relation to long continued maintenance therapy. The statistical ranges (mean $\pm$ 2 SD) are large for several reasons. Firstly, because the measurement of duration of effect is essentially a measurement by

DOSE INJECTED ug.	CALCULATED DOSE RETAINED ug.	CALCULATED DURATION OF EFFECT days
50	38	27
100	67	31
200	117	35
250	139	36
300	159	37
400	197	39
500	232	40
600	264	41
700	293	42
750	308	42
800	321	42
900	348	43
1000	373	43

Table 28: showing calculated duration of effect for calculated doses retained after injections of vitamin B_{12} commonly used in clinical practice. The calculated dose retained is taken from Table 15, Chapter 6. The calculated duration of effect was found from the equation $Y = 17.0886x - 0.4270$ where y = duration of effect in days and x = logarithm of retained dose. The values were calculated to the fourth decimal place and rounded off to whole numbers for the sake of simplicity.

/microbiological assay and the results will vary widely from individual to individual thereby; secondly because the preinjection serum vitamin B_{12} levels varied from case to case and finally because there is likely to be a variation from individual to individual in any such biological system.

The findings in Table 28 suggest that safe economical maintenance therapy will be achieved by an injection of 50 ug every 3 weeks, 100 ug every 4 weeks, 250 ug every 5 weeks or 1000 ug every 6 weeks in the vast majority of patients with pernicious anaemia.

There are no comparable studies reported in the literature with which the findings can be compared. The technique used does not appear to have been employed before probably because of the difficulty of measuring the urinary loss of injected vitamin B_{12}. Mollin & Ross (1953b) observed the duration of effect of varying doses of vitamin B_{12} given intramuscularly in terms of maintaining the serum vitamin B_{12} level above 100 uug/ml which was then regarded as the lower limit of normal. However, most of their cases

/were in haematological relapse when first studied and the urinary excretion of vitamin B_{12} was not estimated. Their results are summarised in Table 29. On the basis of these results they suggested that adequate maintenance therapy could be achieved by giving 40 ug intramuscularly every 10 days or 160 ug every 21 days. It is not permissible to extrapolate to obtain a value for duration of effect for a 40 ug dose, the smallest dose used in the present study being 50 ug which gave a mean duration of effect of 26 days; the calculated mean duration of effect of a 160 ug dose is 33 days . These values differ somewhat from those suggested by Mollin & Ross (1953b). It is likely that the main reason for the difference is that most of their patients were in haematological relapse when first studied. It has already been suggested on the basis of the results obtained in the first experiment that the duration of effect resulting from the first injection of vitamin B_{12} given to a patient in haematological relapse is much less than that obtained by subsequent injections of the same dose. Another factor may

DOSE ug.	NUMBER OF OBSERVATIONS	DURATION OF EFFECT IN DAYS
20	7	6 - 18 +
40	6	2 - 22
80	9	11 - 35 +
160	7	11 - 48
320	3	39 - 98
1000	3	34 - 58 +

Table 29: results relating the amount of vitamin B_{12} injected and the duration of effect obtained by Mollin & Ross (1953b).

/be their use of 100 uug/ml as the 'end point' as opposed to the 140 uug/ml 'end point' used in this study, although one might expect that the use of a lower end point would give a greater duration of effect.

The results are also interesting in relation to the findings of Kinloch (1960) who tested the value of 1000 ug vitamin B_{12} given intramuscularly every 12 weeks as routine maintenance therapy and to the studies by Killander & Werner (1961) who used doses of 500 ug and 1000 ug every eight weeks. From the calculations in Table 28 these doses should not have provided effective maintenance therapy and the finding that they did so would have cast grave doubts on the validity of the results and their interpretation reported here. In fact these dose schedules, as assessed by frequent peripheral blood counts and estimations of serum vitamin B_{12} levels were unsatisfactory.

Comparison of the calculated dosage schedules and those tested critically by Meyer et al (1953), Meacham & Heinle (1953), Lear & Castle (1956) and

/Will et al (1959) is not feasible because the doses used by these authors were small. There is however, nothing in these papers which causes one to doubt the value of the calculated dose schedules - the reverse is in fact the case.

Although the dose schedules derived by calculation should prove economical and adequate it must not be inferred that these schedules represent completely satisfactory maintenance therapy. The schedules should prove satisfactory in maintaining a serum vitamin B_{12} level just above the lower limit of normal and should therefore be regarded as the minimum effective schedules and not as the ideal. On general grounds it is probably desirable to aim at maintaining the serum vitamin B_{12} level at, or near, the mean normal level rather than just above the lower limit of normal. It is not possible to calculate 'ideal' dose schedules which would achieve this purpose from the results obtained in this study. The effect of giving 1000 ug ^{58}Co vitamin B_{12} intramuscularly every 28 days was studied in six patients with vitamin B_{12} deficiency states and

/the results are shown in Fig. 95. The urinary losses after injection were measured and were in the range already observed. It will be seen that the serum vitamin B_{12} levels rose during the observation period and such a dose schedule may be near the ideal treatment.

Economic aspects of maintenance therapy

Mention should be made of the economic aspects of the suggested dose schedules. The annual cost of each dose schedule is shown in Table 30 and it appears that, in theory, the most economical dose schedule is an injection of 50 ug every 3 weeks. The calculations however, do not take into account dispensing fees, price reductions for bulk buying, the cost of materials such as syringes whether disposable or resterilised and the cost of labour and other relevant factors. When these are considered it is probable that the most economical dose schedule is an injection of 250 ug every 5 weeks. The most striking feature of the calculations is the trivial cost of treatment with regard to the benefits it confers.

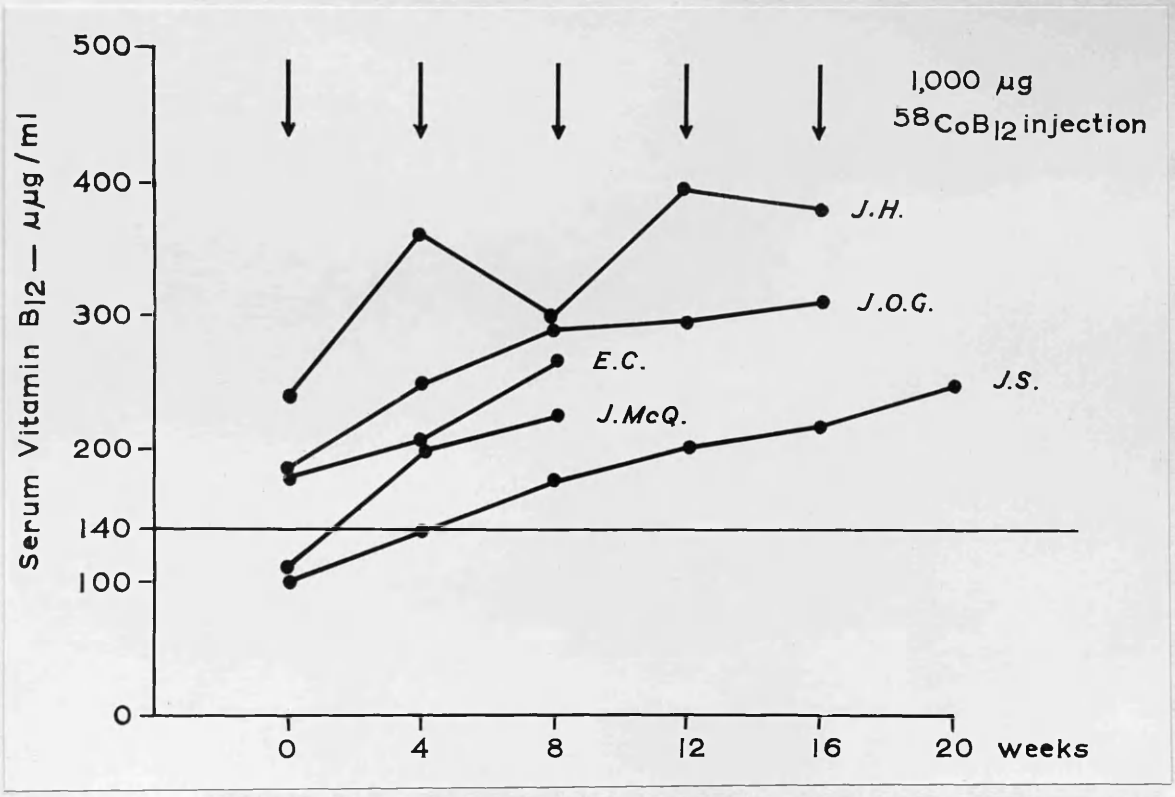


Fig. 95: showing serum vitamin B_{12} levels in six patients given 1000 ug ^{58}Co vitamin B_{12} intramuscularly every 4 weeks. All samples of serum from each patient were assayed together in at least two different assays and the results shown are mean values.

DOSE SCHEDULE	COST OF VITAMIN B_{12} pence	COST OF TREATMENT PER YEAR
50 ug every 3 weeks	3½	5/1
100 ug every 4 weeks	5	5/5
250 ug every 5 weeks	7	6/1
500 ug every 5½ weeks	14 (2 x 7)	11/-.
1000 ug every 6 weeks	20	14/5
1000 ug every 4 weeks	20	21/8

Table 30: showing cost of minimum effective maintenance therapy with vitamin B_{12} per year according to various dose schedules and also the cost of treatment per year with 1000 ug vitamin B_{12} intramuscularly each month. The cost of the vitamin B_{12} is the basic N.H.S. cost of ampoules of vitamin B_{12} (Cytamen - Glaxo) only.

/The place of massive therapy

It is obvious from the results that massive therapy - stosstherapy - whether in the form of single injections of very large doses or in the form of a series of injections given in a short space of time, has a very limited place in maintenance therapy. With single injections of very large doses the bulk is excreted rapidly and the duration of effect of the retained dose is little greater than that obtained with much smaller doses. For example, with injections of 1000 ug the mean retained dose is 372 ug and the calculated duration of effect is 43 days: with injections of 10,000 ug the mean retained dose is 808 ug and the calculated duration of effect is 49 days - an increase of only 14% in duration of effect for a tenfold increase in dose injected. It is possible that there is some place for multiple injections given in a short space of time. It has not been established that there is a correlation between the logarithm of the total retained dose and the duration of effect with this form of therapy but it seems likely that this would be obtained

/if a sufficient number of cases were studied. If this were the case it might be possible to construct dose schedules with treatment consisting of a series of injections of say 1000 ug given at intervals of six months or longer but it is obvious that such a method of maintenance therapy would be almost as uneconomical as single massive doses and unlikely to appeal to a patient or doctor on the grounds of convenience alone.

The findings in relation to the treatment of vitamin B_{12} deficiency states other than pernicious anaemia.

The value of the results and findings in planning treatment, particularly maintenance treatment for patients with pernicious anaemia has been discussed and it is pertinent to consider whether the conclusions should be modified for other conditions requiring treatment with vitamin B_{12}.

In theory at least there should be no need for continued treatment of vitamin B_{12} deficiency states due to inadequate dietary intake. In

/practice however, it may be necessary to institute maintenance therapy by dietary supplements or by parenteral therapy. Absorption of orally ingested vitamin B_{12} is normal in such patients, and a dietary supplement sufficient to ensure the absorption of 2 ug daily should meet basal need: the factors leading to this value will be discussed later. The optimal requirement is greater and a supplement ensuring absorption of 10 ug daily should meet this requirement. If parenteral therapy is preferred the dose schedules should prove adequate: the defect in such patients is comparable to that in pernicious anaemia in that the amount actually absorbed is trivial although for vastly different reasons. The fact that vitamin B_{12} is obtained by biosynthesis by Streptomyces griseus does not appear to have contra-indicated its use by even the strictest ultravegetarian - presumably it is not regarded as animal produce.

It is not likely that the suggested dose schedules need be revised for any of the many conditions (Girdwood 1956b, Herbert 1959) in which

/malabsorption is the cause of vitamin B_{12} deficiency. The lesion which leads to malabsorption and deficiency in pernicious anaemia is irreversible and the block to absorption of the vitamin B_{12} normally present in the diet is virtually complete. There is some evidence that the block to absorption of vitamin B_{12} which may occur in adult coeliac disease or after partial gastrectomy is incomplete and variable (Adams & Seaton 1961, Adams & Cartwright 1962) suggesting that these patients might need less injected vitamin B_{12} than patients with pernicious anaemia. The variability of absorption however, cannot be assumed and prudency dictates that such patients should be treated as if the block were complete and final.

Increased utilisation of vitamin B_{12} or its active principle would be an indication for altering the dose schedules. This is an uncommon cause of vitamin B_{12} deficiency probably by reason of the large body stores. There is some evidence that hyperutilisation occurs in pregnancy and thyrotoxicosis. The need for vitamin B_{12} appears

/to be increased in pregnancy, both in normal subjects (Heinrich 1954, Okuda et al 1956, Boger et al 1957, Izak et al 1957) and in patients with pre-existing pernicious anaemia (Adams 1958) and this has been ascribed to hyperutilisation rather than to foetal parasitisation as the foetus has only a small amount of vitamin B_{12} in its tissues (Ross & Mollin 1957). Nevertheless megaloblastic anaemia during pregnancy solely due to hyperutilisation of vitamin B_{12} is an exceptional event. From the data given by Adams (1958) it seems likely that 1000 ug vitamin B_{12} intramuscularly each month should be adequate treatment. The association of thyroid disease and megaloblastic anaemia due to vitamin B_{12} deficiency is now well established (Tudhope & Wilson 1960, McNicol 1961) but the precise part, if any, played by hyperutilisation is uncertain. There seems to be a prima facie case for incriminating some degree of hyperutilisation when a vitamin B_{12} deficiency megaloblastic anaemia is associated with untreated thyrotoxicosis in which case the logical course would be to

/treat the megaloblastic anaemia by the usual methods and to exhibit antithyroid drugs concurrently. In myxoedema malabsorption of vitamin B_{12} is said to occur (Leithold et al 1958) but this was not confirmed by Tudhope & Wilson (1960) and there is no evidence for hyperutilisation in this condition. Hyperutilisation of vitamin B_{12} might play a part in the megaloblastic anaemia associated with hepatic cirrhosis (Movitt 1950, Krasnow et al 1957) but upsets of folic acid metabolism are more likely to be aetiological factors (Jandl & Lear 1956, Herbert 1959) while Movitt (1950) and Davis & Brown (1953) stress the part played by dietary deficiencies. There does not appear to be hyperutilisation of vitamin B_{12} in the megaloblastic anaemias associated with malignant disease, leukaemia, haemolysis or myelofibrosis which are usually due to folic acid deficiency (Herbert 1959) nor does there appear to be hyperutilisation in the megaloblastic anaemia of scurvy (Brown 1955), Di Guglielmo's disease (Adams & Seaton 1960) or anticonvulsant megaloblastic anaemia although low serum vitamin B_{12} levels

/have been reported in a few cases (Kidd & Mollin 1957, Spray & Witts, 1958, Montgomery & Craig 1958).

An excessive loss of vitamin B_{12} in urine or faeces might be an indication for altering the dose schedules. Such a condition would imply an inability to bind vitamin B_{12} and to date only one such case has been described (Horrigam & Heinle 1952) and the exact nature of the upset in vitamin B_{12} metabolism in this case was never defined (Herbert 1959).

The place of parenteral vitamin B_{12} in maintenance therapy

Earlier it was stated that parenteral vitamin B_{12} is the treatment of choice in pernicious anaemia. This statement should be discussed briefly. It can be said, without fear of contradiction, that other methods of treatment have never proved as satisfactory as parenteral vitamin B_{12}.

It is now believed that the haemopoietic activity of liver extracts, often clinically unsatisfactory (Mollin 1950), is entirely due to

/their vitamin B_{12} content and as this is variable, and often minute (Girdwood et al 1950, Adams & Timbury 1959) these preparations should be eschewed. A further objection to their use is the development of sensitivity which was becoming a major problem prior to the introduction of vitamin B_{12}, (Noren 1950, Davis & Brown 1953). This problem is very rarely encountered with pure vitamin B_{12}. A study of thirty eight patients with clinical or latent allergy to liver extracts showed that none were sensitive to vitamin B_{12} suggesting that vitamin B_{12} is non allergic (Noren 1950). The case of sensitivity to vitamin B_{12} reported to Gillhespy (1955) however was also sensitive to liver extracts but not to proteolysed liver given orally. The author has investigated a patient who had an allergic reaction after an injection of vitamin B_{12} but detailed study excluded vitamin B_{12} as the cause of the reaction.

The place of folic acid in the treatment of pernicious anaemia is limited to 'starting' a response in the very rare cases which will not

/respond to parenteral vitamin B_{12} alone (Girdwood 1959). There is some evidence that folic acid in therapeutic doses may accelerate the utilisation of vitamin B_{12} and that long continued folic acid therapy in pernicious anaemia may not only be unnecessary but inadvisable (Lear & Castle 1956).

Maintenance therapy with vitamin B_{12} given other than parenterally has only occasionally proved satisfactory. Early optimistic reports about the use of vitamin B_{12} and heterologous intrinsic factor (Glass & Boyd 1953, Lowther et al 1954, Meulengracht 1954, Bastrup-Madsen & Paulsen 1955) were not confirmed by results of long-term trials (Blackburn et al 1955, 1959, Adams 1957b, Kristensen et al 1957, Lowenstein et al 1957, Killander 1958bc). The failure of these preparations to sustain a haematological remission was shown to be due to an acquired block to the absorption of vitamin B_{12} when this was given with heterologous intrinsic factor although absorption was still restored to normal when homologous intrinsic factor was given (Schwartz

/et al 1957, Killander 1957c, Stokes & Pitney 1958). Further studies resulted in the demonstration of an inhibitor to heterologous intrinsic factor in the sera of patients who had been treated with oral vitamin B_{12} and heterologous intrinsic factor and also in the sera of some patients who had not been so treated (Taylor 1959, Schwartz 1960) and it was suggested that this inhibitor was an antibody. Agreement has not yet been reached on the precise mechanisms involved in acquired resistance to heterologous intrinsic factor but it is now quite clear that these preparations have no place in the routine treatment of patients with pernicious anaemia. Oral vitamin B_{12} alone in doses as small as 5 ug per day may induce a remission in pernicious anaemia (Estren & Wasserman 1956) and a daily oral dose of 100 ug appears to be an effective form of treatment (Chalmers & Shinton 1958, Hemsted & Mills 1958) although there are conflicting opinions about the reliability of a dose of 1000 ug every week or fortnight (Reisner et al 1955, Conley & Krevans 1955, McIntyre et al 1960).

/Inhalation therapy, (Monto et al 1953, Monto & Rebuck 1954, Israels & Shubert 1954) appears to be effective but has never attained popularity in therapeutic circles. The vitamin B_{12} peptide complex introduced by Heathcote & Mooney (1958) met with considerable criticism (Castle 1958, Glass 1958, Herbert et al 1958) and has not found a place in routine treatment. Of all these methods of treatment the only reliable one appears to be oral vitamin B_{12} alone in a dose of 100 ug daily and even those who report favourably on this type of therapy state that parenteral vitamin B_{12} is preferable (Chalmers & Shinton 1958).

It is evident therefore, that at present, the treatment of choice is parenteral vitamin B_{12}. The results and conclusions reported in this chapter therefore have a strong practical bias.

<u>The findings in relation to the time taken to develop overt vitamin B_{12} deficiency after deprivation</u>

It must be stated clearly that the findings cannot be used as a basis for calculating the time interval which will elapse between deprivation of

/vitamin B_{12}, such as occurs when maintenance therapy in pernicious anaemia is discontinued or when total gastrectomy is performed, and the appearance of overt vitamin B_{12} deficiency in the form of neuropathy or megaloblastic anaemia. In the first place it is well known, although the mechanism is not clear, that an unequivocally low serum vitamin B_{12} level may exist for a considerable time before clinical stigmata appear. In the second place the findings in this investigation are based on results relating to serum vitamin B_{12} levels about 140 uug/ml, the lower limit of normal, whereas in overt vitamin B_{12} deficiency the serum vitamin B_{12} levels are usually unequivocally low, being less than 80 uug/ml (Mollin 1960) and the difference between these levels relative to time is very great, probably representing a late slow phase of clearance such as observed by Brody et al (1960) in the clearance of small doses of Co vitamin B_{12} from the blood stream. It is not even possible to use the findings to calculate the time taken for the serum vitamin B_{12} level to fall to the lower limit of normal after a total

/gastrectomy as the findings relate to retained doses considerably less than the normal body stores and extrapolation would be unwise, the more so when different groups of patients are being considered.

Results in relation to the need for and utilisation of, vitamin B_{12}

Mention must be made of the implication of the results in relationship to the metabolism of vitamin B_{12} or its active principle, by man.

Considerable confusion still exists about the question of the daily need for vitamin B_{12} by man and different experimental approaches, exemplified by those adopted by Darby et al (1958), by Grasbeck (1959) and by Reizenstein (1959cd) have given apparently widely dissimilar results.

Darby et al (1958) treated patients with pernicious anaemia by daily injections of 0.5 to 2.0 ug vitamin B_{12} and observed the effect not only on peripheral blood values but on red cell size. In spite of earlier reports to the contrary (Larsen 1948, 1951, 1952, Owrens 1951) the red cell size in treated pernicious anaemia

/is normal (Adams 1956) and macrocytosis is now regarded as an early sign of vitamin B_{12} deficiency (Herbert 1959). The patients studied by Darby et al (1958) did not have macrocytosis and maintained normal peripheral blood values and the authors concluded that a daily injection of 0.5 to 2.0 ug was adequate maintenace therapy and therefore that this figure was also the daily need for vitamin B_{12} in man.

Grasbeck (1957) adopted a different approach. He assumed the elimination of vitamin B_{12} followed first order kinetics and by noting the time taken to develop clinical evidence of vitamin B_{12} deficiency following total gastrectomy and assuming that the body stores had by then dropped to less than a tenth of normal, he calculated that the velocity constant (k) was 0.0015 - 0.0030 $days^{-1}$ giving a utilisation of 0.15 - 0.30% of body stores per day. Taking the normal body stores to be 3900 ug this was 5.9 - 11.7 ug/day.

Reizenstein (1959c) obtained his value of 7.0 ug/day for normal utilisation by analysis of

/data obtained from patients who had been given small (0.5 ug) doses of Co vitamin B_{12} and whose urinary and faecal excretion had been measured over long periods.

The link between these apparently discrepant findings may lie in the finding of a correlation between the logarithm of the retained dose and its duration of effect in terms of maintaining a serum vitamin B_{12} at, or above, the lower limit of normal. Such a correlation implies that utilisation does not occur at a constant rate (as it would if the correlation were between dose, as opposed to logarithm of dose, retained, and duration of effect) but at a rate proportional to the concentration (van Gemert & Duyff 1950). The correlation only applies to one compartment, the plasma, of a multicompartment system. It would not be unexpected to find that it applied to other compartments and there is already some evidence that this occurs in the liver (Glass et al 1958, Schloesser et al 1958) which may be the major compartment. If it did so then there could be no such thing as a 'daily requirement

/for vitamin B_{12}' as the amount utilised each day would depend on the amount in the body. It would however, be quite permissible to speak of a 'minimum daily requirement', that is, an amount just sufficient to prevent the appearance of signs of deficiency from appearing but this value should not be confused with the amount utilised under normal conditions. The calculation of 'utilisation values' obtained by dividing the amount of vitamin B_{12} retained by the duration of effect in days (Adams 1961c) giving values of about 5.5 ug/day after a 1000 ug injection and about 1.7 ug/day after a 50 ug injection cannot be accepted as mathematically valid in view of the fact that the duration of effect as measured by the time taken for the serum vitamin B_{12} level to fall to the preinjection value has since been shown to be proportional to the logarithm of the effective dose as opposed to the effective dose but at least it illustrates the point, which can be expressed succintly - high logarithm concentration - high utilisation: low logarithm concentration - low utilisation.

/ Extrapolation from the equation $Y = 17.0886x - 0.4270$ where y = duration of effect in days and x = logarithm of dose retained to find a retained dose with an effect of one day would give a value for minimum daily need to maintain a serum vitamin B_{12} level at or above 140 uug/ml but it would obviously be unwise to do this. However, it should be noted that after an injection of 50 ug, the smallest dose used, the calculated retained dose is 38 ug and that the calculated duration of effect of this retained dose is 26 days suggesting that the minimum daily need, that is, the amount just sufficient to maintain the serum vitamin B_{12} at or above 140 uug/ml and to prevent the appearance of signs of deficiency, is probably in the region of that suggested by Darby et al (1958).

As already noted it is not possible to use the results to calculate dose schedules which would ensure normal utilisation of vitamin B_{12} by patients with pernicious anaemia. Grasbeck (1959), working on the assumption that utilisation

/follows first order kinetics, has suggested that a retained dose of 300 ug each month would ensure normal utilisation. A dose schedule of 1000 ug intramuscularly each month, originally tested on a purely empirical basis (Fig. 95) would certainly ensure retention of the amount suggested by Grasbeck (1959) and should probably be regarded as more physiologically acceptable than the minimum effective dose schedules already suggested.

The results in relation to the turnover of Vitamin B_{12} in plasma

The correlation of the logarithm of the retained dose and the duration of effect probably applies to the plasma compartment only as the duration of effect was measured only in this compartment. Information about the turnover in this compartment can therefore be obtained by analysing the data further. From the results and findings already given it can be calculated by the methods described by Dawes (1956) that the biological half life ($T\frac{1}{2}$) of injected ^{58}Co vitamin B_{12} is 5.14 days and that the velocity

/constant (k) is 0.1347 days^{-1}. These values correspond closely to Reizenstein's (1959c) estimate of $T\frac{1}{2} \simeq 6$ days. The closeness of the estimates is satisfying particularly as they were obtained by totally different methods. Co vitamin B_{12} was used in both investigations and it might appear that recent work by Reizenstein et al (1961) on the equilibration of injected Co vitamin B_{12} with the tissue stores casts doubts on the validity of the results. This is unlikely to be so, at least with the results presented here. The calculation of biological half life and velocity constant depend on the slope of the regression line obtained from the regression equation and the values used in calculating these are independent of the problems of equilibration, Co vitamin B_{12} being used solely to measure excretion in the 24 hours after injection, which has been shown to be a valid procedure, and the duration of effect being measured essentially by microbiological assay and not dependent, as in Reizenstein's (1959c) studies, on tissue radioactivity or continuing urinary or faecal

/excretion of radioactivity. The short biological half life of injected vitamin B_{12} in the plasma compartment contrasts sharply with that in the liver, probably the main storage organ, and the body as a whole which have been calculated to average about 12 months, (Schloesser et al 1958, Glass 1958, Grasbeck 1959, Reizenstein 1959cd).

SUMMARY

A series of experiments were planned with the object of defining principles on which maintenance therapy for patients with pernicious anaemia should be based.

The common feature of the experiments was the determination of the duration of effect of an injection of ^{58}Co vitamin B_{12} by estimating the time for which the serum vitamin B_{12} level remained above the lower limit of normal after the injection. The amount of ^{58}Co vitamin B_{12} which produced this effect was estimated by measuring the urinary loss after injection and assuming that this proportion had no therapeutic effect.

In the first experiment the duration of effect of injections of ^{58}Co vitamin B_{12} given to patients with low serum vitamin B_{12} levels in haematological relapse was compared to the effect of subsequent injections of the same dose given when the serum vitamin B_{12} levels were higher. The findings suggest that the effect of the first injection is less than the

/effect of the second injection and that this effect is not related to a greater amount of vitamin B_{12} retained in the body after the second injection.

In the second experiment the duration of effect of different doses of ^{58}Co vitamin B_{12} was observed in the same patients, the methods used being similar to those in the first experiment. The results show that the duration of effect of a large dose is less than might be expected compared to a small dose and that the difference is not due to a greater amount retained after the large injection.

In the third experiment in which a small number of vitamin B_{12} deficient patients in haematological relapse were given a series of injections of ^{58}Co vitamin B_{12} and the duration of effect established, it was clear that the duration of effect was less than might be expected from the amount of ^{58}Co vitamin B_{12} retained.

Analysis of selected results obtained mainly in the second experiment disclosed a linear relationship with a coefficient of correlation

/differing significantly from zero between the logarithm of the retained dose of ^{58}Co vitamin B_{12} and the duration of effect.

By calculating from the equation relating the logarithm of the dose of ^{58}Co vitamin B_{12} injected and percentage of dose excreted in urine (Chapter 6) and further calculating from the equation relating the logarithm of the retained dose to the duration of effect, various dose schedules were constructed. Such schedules constitute minimum effective treatment, that is treatment which should succeed in maintaining the serum vitamin B_{12} level at or above the lower limit of normal. The value of these schedules is discussed and the place of ideal or optimal dose schedules is also discussed.

The literature on the subject of maintenance therapy is reviewed in the light of the findings and the economic aspects of treatment and the place of massive parenteral vitamin B_{12} therapy are also reviewed. The value of the suggested dose schedules in the treatment of vitamin B_{12} deficiency states, other than pernicious anaemia,

/is discussed and the place of other forms of vitamin B_{12} therapy is considered.

The findings are discussed in relation to the utilisation of vitamin B_{12} and the normal need for vitamin B_{12}. The half life of vitamin B_{12} in the plasma compartment was estimated from the results and the value obtained approximates closely to a value obtained by other methods.

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THE
PRINCIPLES AND PRACTICE
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VITAMIN B_{12} THERAPY

VOLUME 2

TABLES A.1 - A.85

^{58}Co VITAMIN B_{12} INJECTIONS ug	VITAMIN B_{12} DEFICIENT (CONTROL) GROUP			BORDERLINE GROUP		NORMAL GROUP	
	PATIENTS	NO. OF RESULTS		NO. OF PATIENTS	NO. OF RESULTS	NO. OF PATIENTS	NO. OF RESULTS
54	A.H.	10					
	H.McG.	10	30	-	-	18	18
	J.McM.	10					
100	J.C.	17					
	M.C.	18					
	J.F.	17	77	26	26	31	31
	H.F.	16					
	K.McD.	9					
540	G.G.	18					
	J.J.	15	33	-	-	14	14
1000	J.A.	12					
	I.C.	16	46	-	-	15	15
	J.S.	18					
1140	C.D.	16					
	H.K.	14	30	17	17	15	15

Table A.1: showing disposition of patients into various experimental groups in the studies reported in Chapter 1.

CASE A.H. CONTROL PATIENT - 54 ug SERIES

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug.
0	-	630	0	0.015
1	54 ug i.m.	1290	11.5	3.0
2	"	550	1.3	0.3
3	"	600	15.6	6.3
4	"	1660	15.8	4.0
5	"	1800	13.1	3.9
6	"	2000	10.9	2.7
7	"	1350	17.0	7.0
8	"	2350	23.4	9.3
9	"	1370	7.5	2.0
10	"	1450	21.0	5.3
11	-	1750	0	-

Table A.2 - showing detailed results obtained from Case A.H. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

CASE H.McG. CONTROL PATIENT - 54 ug SERIES

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug.
0	-	1650	0	< 0.04
1	54 ug i.m.	1170	19.6	8.0
2	"	2190	26.0	12.9
3	"	1440	28.1	12.3
4	"	2380	27.3	13.6
5	"	2350	25.7	10.9
6	"	2390	31.4	13.7
7	"	2310	32.4	15.6
8	"	2380	31.4	14.9
9	"	2150	28.0	13.4
10	"	2350	33.5	15.7
11	-	2330	0	-

Table A.3 - showing detailed results obtained from Case H.McG. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

CASE J.McM. CONTROL PATIENT - 54 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	1680	0	<0.04
1	54 ug i.m.	800	1.4	0.2
2	"	1350	7.9	2.8
3	"	1440	12.1	4.3
4	"	1030	12.9	4.1
5	"	1060	10.3	3.6
6	"	660	1.0	0.1
7	"	1110	15.8	6.3
8	"	740	4.6	0.7
9	"	1040	12.8	3.7
10	"	1130	12.5	4.4
11	-	770	0	-

Table A.4 - showing detailed results obtained from Case J.McM. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

NORMAL PATIENTS - 54μμg ^{58}Co VITAMIN B_{12} (MATCHED SERIES)

CASE NO.	AGE	SEX	DIAGNOSIS	SERUM VITAMIN B_{12} μμg/ml	PREINJECTION 24 HOUR URINE			POSTINJECTION 24 HOUR URINE		
					VOL mls.	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.	VOL mls.	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.
1	75	F	MYOCARDIAL INFARCT	250	1470	0	0.044	1750	11.3	3.8
2	56	M	"	315	1100	0	0.116	960	14.7	6.0
3	64	F	"	330	1140	0	0.125	800	15.6	6.0
4	51	F	HIATUS HERNIA	370	1780	0	0.210	600	35.6	16.2
5	29	M	HEADACHE	485	1700	0	0.120	1000	8.8	3.3
6	40	M	MITRAL STENOSIS	575	1600	0	0.113	1250	6.8	2.0
7	35	F	HYPERTENSION	250	1260	0	0.100	1210	23.2	9.6
8	59	F	EPILEPSY	210	1180	0	0.068	860	8.6	2.8
9	21	M	DIABETES	350	1920	0	0.352	2000	32.8	15.8
10	47	F	NEUROSIS	345	410	0	0.217	350	7.4	2.5
11	16	M	CYST OF LUNG	300	1730	0	0.155	1550	1.3	0.4
12	22	M	ANEURYSM OF SCALP	400	2400	0	0.254	2240	15.1	5.9
13	48	M	POST PROSTATECTOMY	460	3200	0	0.384	2370	22.3	8.5
14	17	M	POST APPENDICECTOMY	370	1430	0	0.120	300	4.6	1.3
15	44	F	DISSEMINATED SCLEROSIS	340	1190	0	0.119	900	16.1	7.1
16	25	M	HAEMOPHILIA	290	1620	0	0.191	1310	7.5	1.4
17	68	M	GASTRIC ULCER	345	750	0	0.063	2270	17.2	10.7
18	67	M	GANGRENE OF TOE	335	1200	0	0.084	920	5.2	1.6

Table A.5. - showing details of, and results obtained, from normal group of patients - 54 ug series.

CASE J.C. CONTROL PATIENT - 100 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	1050	0	< 0.04
1	100 ug i.m.	1110	20.9	26.6
2	-	no collection		
3	100 ug i.m.	1950	25.2	22.4
4	"	2090	35.1	32.9
5	"	2070	28.8	26.9
6	"	1730	29.7	31.1
7	"	2515	30.5	25.6
8	"	2150	38.7	37.9
9	"	1620	27.2	17.8
10	"	1520	36.8	33.7
11	"	1270	30.5	29.6
12	"	1410	26.5	17.8
13	"	1970	40.9	44.8
14	"	2150	39.5	41.3
15	"	1480	27.5	27.7
16	"	620	7.7	4.6

CASE J.C. (contd.)

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
17	"	1000	20.2	14.7
18	"	2020	51.0	52.5
19	-	1560	0	-

Table A.6 - showing detailed results obtained from Case J.C. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

CASE M.C. CONTROL PATIENT - 100 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	0	550	0	0.016
1	100 ug i.m.	400	32.4	30.0
2	"	600	45.6	48.6
3	"	660	33.1	36.7
4	-	1020	0	0.6
5	100 ug i.m.*	1240	27.2	24.7
6	" *	620	20.0	19.4
7	" *	1160	47.1	57.2
8	-	600	0	0.1
9	100 ug i.m.	970	27.5	26.8
10	"	790	43.5	48.1
11	"	910	50.3	53.9
12	-	910	0	0.3
13	100 ug i.m.*	630	33.2	40.0
14	" *	910	62.0	50.9
15	" *	800	51.3	58.0
16	-	400	0	0.1

CASE M.C. (contd.)

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
17	100 ug i.v.	520	43.5	52.5
18	"	810	52.3	61.5
19	"	1300	51.5	79.7
20	——	510	0	0.3
21	100ug i.v.	650	41.2	51.1
22	"	1000	45.2	55.0
23	"	1360	52.1	69.3
24	-	1250	0	-

Table A.7 - showing detailed results obtained from Case M.C. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

*** Probenecid was given orally as described in Chapter 3.**

CASE J.F. CONTROL PATIENT - 100 ug SERIES

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	760	0	0.019
1	100 ug i.m.	820	42.0	33.3
2	-	625	0	0.1
3	100 ug i.m.	815	44.7	42.2
4	-	950	0	0.2
5	100 ug i.m.	920	45.1	47.8
6	-	1310	0	0.2
7	100 ug i.m.	1110	48.0	50.7
8	-	1130	0	0.1
9	100 ug i.m.	730	49.2	50.3
10	-	830	0	0.1
11	100 ug i.m.	1395	66.6	54.7
12	-	1570	0	0.1
13	100 ug i.m.	1890	60.9	63.8
14	-	1460	0	0.1
15	100 ug i.m.	1890	46.2	45.3

CASE J.F. (contd.)

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
16	-	860	0	0.8
17	100 ug i.m.	1300	47.2	57.8
18	-	1170	0	0.1

Table A.8 - showing detailed results obtained from Case J.F. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

CASE H.F. CONTROL PATIENT - 100 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug.
0	-	710	0	0.001
1	100 ug i.m.	1230	34.0	34.4
2	-	1700	0	0.1
3	100 ug i.m.*	1900	18.3	15.5
4	-	1360	0	0.1
5	-	no collection		
6	100 ug i.m.	950	52.1	52.4
7	" *	2610	64.4	70.9
8	"	720	56.7	59.9
9	" *	1960	60.4	62.9
10	"	890	62.9	65.4
11	" *	1670	59.8	63.8
12	"	890	61.3	66.7
13	" *	1730	60.3	64.9
14	"	820	55.1	61.5
15	" *	1620	64.6	69.6
16	"	950	62.8	65.8

CASE H.F. (contd.)

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug.
17	" *	1770	56.6	64.9
18	"	900	54.2	59.4
19	" *	1910	51.0	53.9
20	-	1700	0	-

Table A.9 - showing detailed results obtained from Case H.F. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

*Mersalyl was given intramuscularly as described in Chapter 3.

CASE K.McD. CONTROL PATIENT - 100 ug SERIES

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	670	0	0.14
1	100 ug i.m.	495	31.8	32.4
2	" i.v.	650	52.5	57.2
3	" i.m.	430	45.2	49.4
4	" i.v.	615	53.6	55.9
5	" i.m.	825	51.9	51.0
6	" i.v.	1020	62.0	71.0
7	" i.m.	800	53.7	52.8
8	" i.v.	660	59.8	54.3
9	" i.m.	780	57.8	59.0
10	" i.v.	980	58.8	56.1
11	" i.m.	830	50.5	51.6
12	" i.v.	970	55.9	54.8
13	" i.m.	1480	55.7	58.4
14	" i.v.	985	56.3	54.1
15	" i.m.	970	58.2	58.2

CASE K.McD. (Contd.)

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
16	" i.v.	670	51.8	47.3
17	" i.m.	910	55.2	50.0
18	-	900	0	-

Table A.10 - showing detailed results obtained from Case K.McD. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the first decimal place.

BORDERLINE SUBJECTS - 100 ug SERIES

CASE	SERUM VITAMIN B_{12} uug/ml.	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
H.C.	199	1700	40.5	41.6
H.F.	151	1000	43.9	42.3
J.O.G.	192	1360	36.4	38.3
M.G.	172	1380	33.2	31.1
J.O.H.	130	1820	41.0	43.6
J.O.H.	126	1470	43.1	41.7
D.J.	124	980	31.3	35.6
D.J.	151	900	2.0	0.3
J.L.	198	690	35.6	44.1
J.L.	180	2250	37.4	48.3
M.McB.	131	1220	46.3	58.5
M.McB.	109	1245	41.5	31.1
M.McB.	114	1500	34.1	35.6
I.McC.	120	1170	42.3	46.8
I.McC.	145	1000	47.2	45.5
I.McC.	133	1430	43.2	51.0
E.P.	149	1215	42.7	40.0
E.P.	147	1300	48.9	55.9
I.R.	106	1130	21.2	24.6
A.T.	122	2390	50.5	52.6
A.T.	179	1200	45.9	47.4
A.T.	112	1650	45.6	46.5

BORDERLINE SUBJECTS (Contd.)

CASE	SERUM VITAMIN B_{12} uug/ml.	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
M.T.	104	740	24.1	18.7
I.W.	142	710	47.2	59.2
I.W.	177	1650	50.3	46.8
I.W.	137	1320	50.7	49.2

Table A.11 - showing detailed results obtained from 'borderline' group of patients in the 100 ug series. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: the serum vitamin B_{12} level was estimated on a sample taken immediately prior to the injection of 100 ug ^{58}Co vitamin B_{12} intramuscularly. Values have been rounded off to the first decimal place.

NORMAL PATIENTS - 100μg ^{58}Co VITAMIN B_{12} (MATCHED SERIES)

CASE NO.	AGE	SEX	DIAGNOSIS	SERUM VITAMIN B_{12} $\mu\mu$g/ml	PREINJECTION 24 HOUR URINE			POSTINJECTION 24 HOUR URINE		
					VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.	VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.
1	46	M	MYOCARDIAL INFARCT	215	1280	0	0.384	1010	35.6	30.3
2	58	M	"	405	770	0	0.223	1250	37.8	40.0
3	40	M	"	410	1300	0	0.161	1500	30.0	32.0
4	41	M	"	230	975	0	0.085	1310	34.1	42.5
5	39	M	EPILEPSY	200	2650	0	0.143	2420	45.3	44.1
6	66	M	BRONCHIAL NEOPLASM	240	850	0	0.146	1010	26.3	17.4
7	45	M	DUODENAL ULCER	215	1530	0	0.116	1710	43.9	39.3
8	34	M	GALLSTONES	480	2000	0	0.276	2110	50.5	50.2
9	50	M	HYPERTENSION	365	1520	0	0.106	1590	24.3	19.0
10	25	F	"	375	2120	0	0.186	2080	23.7	8.7
11	39	M	LYMPHOBLASTOMA	390	570	0	0.060	1590	19.9	10.5
12	41	F	DISSEMINATED SCLEROSIS	740	2200	0	0.277	1710	35.5	16.6
13	70	M	DIABETES	156	950	0	0.066	1100	33.8	29.3
14	44	M	CUSHINGS DISEASE	200	1820	0	0.131	1450	42.3	39.8
15	25	M	HODGKINS DISEASE	295	990	0	0.202	1100	21.6	12.7
16	50	M	HYPERTENSION	240	2300	0	0.124	1830	43.3	44.3

Table A.12 - showing details of, and results obtained from, normal group of patients - 100 ug series.

NORMAL PATIENTS - 100ug ^{58}Co VITAMIN B_{12} (MISCELLANEOUS SERIES) - CONTINUED

CASE NO.	AGE	SEX	DIAGNOSIS	SERUM VITAMIN B_{12} $\mu\mu g/ml$	PREINJECTION 24 HOUR URINE			POSTINJECTION 24 HOUR URINE		
					VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} $\mu g.$	VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} $\mu g.$
16	46	M	MYOCARDIAL INFARCT	215	1280	0	0.384	1010	35.6	30.3
17	58	M	"	405	770	0	0.223	1250	37.8	40.0
18	40	M	"	410	1300	0	0.161	1500	30.0	32.0
19	41	M	"	230	975	0	0.085	1310	34.1	42.5
20	39	M	EPILEPSY	200	2650	0	0.143	2420	45.3	44.1
21	66	M	BRONCHIAL NEOPLASM	240	850	0	0.146	1010	26.3	17.4
22	45	M	DUODENAL ULCER	215	1530	0	0.116	1710	43.9	39.3
23	34	M	GALLSTONES	480	2000	0	0.276	2110	50.5	50.2
24	50	M	HYPERTENSION	365	1520	0	0.106	1590	24.3	19.0
25	25	F	"	375	2120	0	0.186	2080	23.7	8.7
26	39	M	LYMPHOBLASTOMA	390	570	0	0.060	1590	19.9	10.5
27	41	F	DISSEMINATED SCLEROSIS	740	2200	0	0.277	1710	35.5	16.6
28	70	M	DIABETES	156	950	0	0.066	1100	33.8	29.3
29	44	M	CUSHINGS DISEASE	200	1820	0	0.131	1450	42.3	39.8
30	25	M	HODGKINS DISEASE	295	990	0	0.202	1100	21.6	12.7
31	50	M	HYPERTENSION	240	2300	0	0.124	1830	43.3	44.3

Table A.13 - showing details of, and results obtained from, normal group of patients - 100 ug series.

CASE G.G. CONTROL PATIENT - 540 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	1415	0	< 0.035
1	540 ug i.m.	910	55.8	346
2	"	1350	75.3	432
3	"	910	70.3	398
4	"	1100	70.8	376
5	"	1020	77.8	440
6	"	1450	58.0	301
7	"	1800	76.9	427
8	"	1750	62.4	346
9	"	1110	72.7	383
10	"	1290	80.4	416
11	"	1730	74.4	409
12	"	1350	75.8	408
13	"	1230	60.2	320
14	"	1290	75.8	422
15	"	1500	64.5	355

CASE G.G. (contd.)

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
16	"	1470	77.9	448
17	"	1200	71.1	414
18	"	1320	75.6	452
19	-	1230	0	-

Table A.14 - showing detailed results obtained from Case G.G. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

CASE J.J. CONTROL PATIENT - 540 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	400	0	< 0.01
1	540 ug i.m.	1910	75.4	406
2	"	2380	63.9	351
3	"	1980	74.0	349
4	"	1940	68.0	383
5	"	2370	69.7	376
6	"	2310	68.3	364
7	"	2370	74.3	421
8	"	2310	77.1	439
9	"	2370	75.0	344
10	"	1710	77.7	432
11	"	1750	76.2	470
12	"	1480	68.1	381
13	"	2380	73.5	405
14	"	2230	74.1	393
15	"	1690	60.8	341
16	-	2340	0	-

Table A.15 - showing detailed results obtained from Case J.J. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

NORMAL PATIENTS - 540μg ^{58}Co VITAMIN B_{12} (MATCHED SERIES)

CASE NO.	AGE	SEX	DIAGNOSIS	SERUM VITAMIN B_{12} μμg/ml	PREINJECTION 24 HOUR URINE			POSTINJECTION 24 HOUR URINE		
					VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.	VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.
1	48	M	MYOCARDIAL INFARCT	375	1910	0	0.391	1590	68.8	382
2	63	M	"	186	2100	0	0.105	1970	73.6	453
3	51	M	"	310	2570	0	0.128	2480	70.3	397
4	69	M	"	355	1430	0	0.105	1590	64.1	358
5	41	M	"	400	1220	0	0.195	830	67.2	394
6	43	F	SARCOID	400	2350	0	0.716	3500	67.4	385
7	39	F	HYPERTENSION	335	1550	0	0.207	2080	58.2	349
8	40	M	RHEUMATOID ARTHRITIS	375	1350	0	0.099	1360	71.1	418
9	43	F	AURICULAR FIBRILLATION	330	1070	0	0.066	1040	67.3	380
10	24	F	VOMITING	335	1050	0	0.080	1450	42.8	239
11	70	M	OESOPHAGEAL NEOPLASM	410	620	0	0.026	870	56.2	324
12	39	F	GASTRIC ULCER	320	850	0	0.065	1100	70.8	402
13	34	M	SUBARACHNOID HAEMORRHAGE	455	425	0	0.011	1340	63.1	366
14	54	F	TABES DORSALIS	540	730	0	0.097	1300	65.2	375

Table A.16 - showing details of, and results obtained from, normal group of patients - 540 ug series.

CASE J.A. CONTROL PATIENT - 1000 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug.
0	-	600	0	0.04
1	1000 ug i.m.	1070	48.2	486
2	-	2085	0	2
3	-	2970	0	0
4	1000 ug i.m.	3040	80.0	729
5	-	2400	0	5
6	1000 ug i.m.	2920	76.9	757
7	-	1800	0	2
8	1000 ug i.m.	2125	68.0	841
9	-	1930	0	4
10	1000 ug i.m.	1900	71.5	887
11	-	2360	0	9
12	1000 ug i.m.	2390	62.3	788
13	-	2460	0	16
14	1000 ug i.m.	2090	25.4	363
15	-	2000	0	2
16	1000 ug i.m.	2260	79.5	861
17	-	1200	0	8
18	1000 ug i.m.	1340	40.7	442
19	-	2090	0	0
20	1000 ug i.m.	2100	75.0	917
21	-	910	0	1

CASE J.A. (contd.)

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
22	1000 ug i.m.	1630	88.0	929
23	-	850	0	1
24	1000 ug i.m.	1330	74.4	911
25	-	1060	0	3

Table A.17 - showing detailed results obtained from Case J.A. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

CASE I.C. CONTROL PATIENT - 1000 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	1380	0	0.045
1	1000 ug i.m.	550	67.4	843
2	-	475	4.1	32
3	1000 ug i.m.*	1980	55.2	746
4	-	450	6.9	85
5	1000 ug.i.m.	690	59.2	645
6	-	550	22.0	253
7	1000 ug i.m.*	2860	59.0	772
8	-	810	8.2	75
9	1000 ug i.m.	1350	71.5	924
10	-	1570	0	4
11	1000 ug i.m.*	2060	67.9	817
12	-	650	8.2	49
13	1000 ug i.m.	1400	80.0	942
14	-	1240	0	32
15	1000 ug i.m.*	2250	71.3	778
16	-	730	5.5	28
17	1000 ug i.m.	1225	81.2	938
18	-	960	0	28
19	1000 ug i.m.*	2050	52.3	660

CASE I.C. (contd.)

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
20	-	780	12.7	42
21	1000 ug i.m.	1170	78.6	877
22	-	1420	0	35
23	1000 ug i.m.*	2300	60.4	795
24	-	650	0	22
25	1000 ug i.m.	920	67.2	763
26	-	1570	17.0	135
27	1000 ug i.m.*	2510	84.5	820
28	-	900	0	20
29	1000 ug i.m.	690	60.9	569
30	-	750	6.6	24
31	1000 ug i.m.*	2300	84.1	897
32	-	670	5.5	9

Table A.18 - showing detailed results obtained from Case I.C. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

*Mersalyl was given intramuscularly as described in Chapter 3.

CASE J.S. CONTROL PATIENT - 1000 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	1680	0	0.04
1	1000 ug i.m.*	510	24.2	262
2	" *	1210	72.2	814
3	" *	1110	67.1	739
4	-	1230	0	1
5	1000 ug i.m.	1850	75.7	875
6	"	1150	76.5	866
7	"	1755	75.3	948
8	-	2350	0	8
9	1000 ug i.m.*	1885	69.4	777
10	" *	2030	63.3	853
11	" *	1970	65.4	857
12	-	2000	0	6
13	1000 ug i.m.	1950	65.9	836
14	"	1410	79.7	973
15	"	2360	76.3	1097
16	1000 ug i.v.	1760	59.5	695
17	"	1960	61.6	852
18	"	1680	72.7	907

CASE J.S. (contd.)

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
19	1000 ug i.v.	1750	77.6	936
20	"	2170	114.0	1497
21	"	1500	44.2	543
22	-	1680	0	-

Table A.19 - showing detailed results obtained from Case J.S. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

* Probenecid was given orally as described in Chapter 3.

NORMAL PATIENTS - - 1000μg ^{58}Co VITAMIN B_{12} (MISCELLANEOUS SERIES)

CASE NO.	AGE	SEX	DIAGNOSIS	SERUM VITAMIN B_{12} $\mu\mu$g/ml	PREINJECTION 24 HOUR URINE			POSTINJECTION 24 HOUR URINE		
					VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.	VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.
1	53	M	MYOCARDIAL INFARCT	770	520	0	0.108	1210	44.2	645
2	57	M	"	400	1190	0	0.099	1400	67.2	994
3	65	M	"	460	1440	0	0.100	1250	45.3	737
4	45	M	DUODENAL ULCER	940	2120	0	0.534	2375	67.5	1068
5	61	M	"	630	925	0	0.199	1450	80.3	965
6	50	M	CEREBRAL THROMBOSIS	640	2720	0	0.209	1630	80.0	826
7	54	M	CHRONIC BRONCHITIS	690	1420	0	0.176	970	62.4	921
8	40	M	HODGKINS DISEASE	810	1800	0	0.24.	1170	41.3	643
9	34	M	SINUSITIS	610	1600	0	0.188	1870	55.5	860
10	47	M	BRONCHIAL NEOPLASM	270	520	0	0.058	550	25.7	423
11	69	M	CEREBELLAR THROMBOSIS	880	1450	0	0.171	680	51.0	918
12	44	F	DISSEMINATED SCLEROSIS	380	300	0	0.070	230	28.7	391
13	53	M	"	460	2050	0	0.213	1810	52.4	577
14	42	F	AURICULAR FIBRILLATION	450	1350	0	0.167	1150	77.2	891
15	50	F	HYPERTENSION	400	1680	0	0.262	1445	76.0	780

Table A.20 - showing details of, and results obtained from, normal group of patients - 1000 ug series.

CASE C.D. CONTROL PATIENT - 1140 ug SERIES

DAY	^{58}Co B_{12} INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	1330	-	< 0.03
1	1140 ug i.m.	1170	68.0	795
2	"	1260	86.0	869
3	"	500	31.1	320
4	-	770	0	6
5	1140 ug i.m.*	1080	71.2	815
6	" *	510	56.0	698
7	" *	1050	75.2	887
8	-	900	0	6
9	1140 ug i.m.	1020	75.3	882
10	"	1200	81.5	1068
11	"	750	67.7	878
12	-	1100	0	7
13	1140 ug i.m.*	1700	75.5	943
14	" *	2280	75.6	942
15	" *	2230	70.9	937
16	-	1730	0	4
17	1140 ug i.m.	2020	69.4	969
18	"	1250	42.1	513
19	-	1340	0	4

CASE C.D. (contd.)

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
20	1140 ug i.m.*	1180	74.5	920
21	" *	1050	77.3	935
22	-	600	0	1

Table A.21 - showing results obtained from Case C.D. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

* Probenecid was given orally as described in Chapter 3.

CASE H.K. CONTROL PATIENT - 1140 ug SERIES

DAY	$^{58}Co\ B_{12}$ INJECTION	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
0	-	580	0	0.493
1	1140 ug i.m.	830	71.6	963
2	-	no collection		
3	1140 ug i.m.	780	66.5	858
4	-	no collection		
5	1140 ug i.m.	1630	77.6	978
6	-	1610	0	4
7	1140 ug i.m.	1780	70.7	912
8	"	1400	72.1	959
9	"	1700	76.3	1062
10	"	2250	77.0	913
11	"	950	55.0	618
12	"	2280	55.5	611
13	"	1850	61.8	738
14	"	1950	44.2	585
15	"	1620	72.8	907
16	"	1230	47.8	901
17	"	1110	52.8	402
18	-	1200	0	-

Table A.22 - showing detailed results obtained from Case H.K. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number.

BORDERLINE SUBJECTS - 1140 ug SERIES

CASE	SERUM VITAMIN B_{12} uug/ml	24 HOUR URINE COLLECTION		
		VOLUME ml.	RADIOACTIVITY % dose	TOTAL ASSAYABLE VITAMIN B_{12} ug
E.C.	90	1710	74.8	872
E.V.C.	178	970	64.9	747
H.C.	178	1900	79.2	846
T.C.	144	1540	72.6	929
H.F.	197	950	77.9	924
J.G.	120	880	76.1	898
J.O.G.	158	1700	71.1	774
J.O.H.	115	1700	65.3	592
D.J.	131	1020	56.6	669
J.K.	93	530	65.3	816
J.L.	191	1430	76.7	909
I.McC.	145	910	76.7	910
T.McG.	138	570	70.4	862
E.P.	190	620	71.4	930
I.R.	160	1040	75.2	880
M.R.	88	1575	70.5	977
M.T.	160	700	44.7	529

Table A.23 - showing details of results obtained from borderline group of patients 1140 ug series. Each value for total assayable vitamin B_{12} is the mean of at least two estimations: values have been rounded off to the nearest whole number. The serum vitamin B_{12} level was estimated on a sample taken immediately before the intramuscular injection of 1140 ug ^{58}Co vitamin B_{12}.

NORMAL PATIENTS - 1140μg ^{58}Co VITAMIN B_{12} (MATCHED SERIES)

CASE NO.	AGE	SEX	DIAGNOSIS	SERUM VITAMIN B_{12} μμg/ml	PREINJECTION 24 HOUR URINE			POSTINJECTION 24 HOUR URINE		
					VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.	VOL mls	RADIO ACTIVITY % DOSE EXCRETED	TOTAL ASSAYABLE VITAMIN B_{12} μg.
1	32	M	DUODENAL ULCER	370	910	0	0.060	2130	82.7	1044
2	53	M	"	300	1160	0	0.139	1360	66.7	830
3	51	M	"	345	900	0	0.126	1280	56.7	678
4	55	M	"	600	600	0	0.432	670	63.2	777
5	70	M	CARDIAC FAILURE	620	2930	0	0.079	1190	62.9	762
6	54	M	MYOCARDIAL INFARCT	415	1100	0	0.127	960	74.3	931
7	56	M	"	375	480	0	0.110	900	71.3	936
8	47	F	HYPERTENSION	350	2890	0	0.078	2250	36.5	392
9	54	M	"	340	1330	0	0.090	2060	70.9	834
10	32	F	"	280	1580	0	0.104	1620	66.1	949
11	23	M	DISSEMINATED SCLEROSIS	500	470	0	0.108	540	33.8	410
12	50	M	CEREBELLAR THROMBOSIS	260	1100	0	0.125	1700	76.8	998
13	20	F	THYROTOXICOSIS	510	1130	0	0.176	1260	73.3	806
14	63	M	CERVICAL SPONDYLOSIS	790	950	0	0.269	850	45.4	527
15	55	M	SPINAL ARTERY THROMBOSIS	370	1230	0	0.177	1350	60.5	662

Table A.24 - showing details of, and results obtained from, normal group of patients - 1140 ug series

CASE	AGE	SEX	Hb. %	SERUM B_{12} uug/ml.	DIAGNOSIS	RADIO-ACTIVITY % dose
B.C.	65	F	44	40	pernicious anaemia	39.7
J.C.	77	M	41	38	"	20.9
M.C.	65	F	56	74	"	32.4
H.F.	55	F	42	30	"	34.0
J.F.	72	F	38	52	"	42.0
J.McC.	63	M	35	68	"	25.9
K.McD.	47	F	37	50	"	31.8
J.McG.	67	M	54	32	loop syndrome	25.4
J.O.T.	50	M	35	62	pernicious anaemia	35.2

Table A.25: showing details of, and results obtained from, chronically anaemic patients with low serum vitamin B_{12} levels given 100 ug ^{58}Co vitamin B_{12} intramuscularly.

CASE	AGE	SEX	Hb. %	SERUM B_{12} uug/ml.	DIAGNOSIS	RADIO-ACTIVITY % dose
1	78	F	40	590	not known	26.9
2	72	M	52	320	iron deficiency cause not known	13.7
3	67	F	40	620	iron deficiency gastric polyp	28.8
4	74	F	43	490	iron deficiency cause not known	48.1
5	66	M	47	350	rheumatoid arthritis	21.3
6	55	M	47	390	iron deficiency post gastrectomy	33.1
7	55	M	46	200	reticulosis	20.0
8	55	M	56	400	reticulosis	24.9

Table A.26: showing details of, and results obtained from, chronically anaemic patients with normal serum vitamin B_{12} levels given 100 ug ^{58}Co vitamin B_{12} intramuscularly.

CASE	AGE	SEX	Hb. %	SERUM B_{12} uug/ml.	DIAGNOSIS	RADIO-ACTIVITY % dose
J.A.	59	M	26	30	pernicious anaemia	48.2
I.C.	70	F	53	58	"	67.4
J.D.	59	M	41	45	"	72.9
S.J.	47	F	42	25	B_{12} dietary deficiency	70.0
W.L.	78	M	38	25	pernicious anaemia	50.0
E.McB.	51	F	36	37	pernicious anaemia	45.5
J.McQ.	54	M	56	25	post gastrectomy B_{12} deficiency	67.6
M.P.	48	F	72	60	pernicious anaemia	65.3
J.S.	51	F	58	50	"	24.2
M.S.	68	F	44	42	"	61.0
S.S.	54	M	37	56	"	64.4
M.W.	79	F	72	30	"	68.1

Table A.27: showing details of, and results obtained from, chronically anaemic patients with low serum vitamin B_{12} levels given 1000 ug ^{58}Co vitamin B_{12} intramuscularly.

CASE	AGE	SEX	Hb. %	SERUM B_{12} uug/ml.	DIAGNOSIS	RADIO-ACTIVITY % dose
1	70	F	58	700	iron deficiency carcinoma of caecum	38.4
2	27	F	60	315	iron deficiency reticulosis	57.9
3	79	F	50	270	iron deficiency cause not known	39.6
4	70	M	59	280	chronic lymphatic leukaemia	57.6
5	46	F	48	430	iron deficiency duodenal ulcer	75.7
6	23	F	41	370	iron deficiency gastric ulcer	47.4
7	41	F	65	480	iron deficiency duodenal ulcer menorrhagia	52.7
8	66	M	53	265	iron deficiency duodenal ulcer	68.3
9	19	F	50	225	iron deficiency cause not known	55.3

Table A.28: showing details of, and results obtained from, chronically anaemic patients with normal serum vitamin B_{12} levels given 1000 ug ^{58}Co vitamin B_{12} intramuscularly.

TABLE A.29 - CASE J.A.M.

DAY	^{58}Co VITAMIN B_{12} INJECTION	24 HOUR URINE COLLECTION	
		VOLUME	RADIOACTIVITY EXCRETED
	ug.	ml.	% dose
0	-	520	0
1	100 im	920	13.2
2	"	990	20.1
3	"	sample lost	
4	"	1040	15.4
5	-	750	0
6	100 im	1040	40.4
7	-	780	0
8	100m	450	10.2
9	"	370	40.0
10	"	740	33.7
11	"	1440	42.7
12	-	1000	0

Table A.29: showing detailed results obtained from Case J.A.M., given repeated injections of ^{58}Co vitamin B_{12}, at intervals of one to two days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.30 - CASE F.A.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug.	POST INJECTION 24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 im	1630	66.2
2	"	1900	66.7
3	" *	1900	75.0
4	" *	1650	55.7
5	"	2330	80.3
6	"	2180	54.9
7	" *	2140	90.4
8	" *	2180	82.5
32	"	1630	62.2
33	" *	1400	92.4
34	"	1430	81.8
35	" *	1550	82.4

Table A.30: showing detailed results obtained from Case F.A. given repeated injections of ^{58}Co vitamin B_{12} at intervals of at least one week. The patient had been treated with vitamin B_{12} and was in full haematological remission and not excreting radioactivity prior to this study.

* Oral probenecid was given with the injections on weeks 3, 4, 7, 8, 33 & 35 and vitamin B_{12} between weeks 8 & 32 as described in Chapter 3.

TABLE A.31 - CASE J.D.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug.	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	900	0
1	1000 im	1260	72.9
2	"	980	33.1
3	-	1230	0
4	1000 iv	1520	79.9
5	"	1600	83.3
6	-	2000	0
7	1000 im	1800	69.8
8	"	2000	84.8
9	-	1530	0
10	1000 iv	1900	82.7
11	"	2030	70.9
12	-	1770	0
13	1000 im	1550	75.9
14	"	1900	89.0
15	-	1120	0
16	1000 iv	1230	61.9
17	"	1700	82.6
18	-	1750	0

Table A.31: showing detailed results obtained from Case J.D. given repeated injections of ^{58}Co vitamin B_{12} at intervals of one to two days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.32 - CASE S.J.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	680	0
1	1000 im	720	70.0
2	"	740	77.4
3	"	620	51.2
4	-	1000	0
12	1000 im	1230	83.0
13	"	1150	63.3
14	"	680	84.8
15	"	940	84.5
16	"	870	59.3
17	-	900	0

Table A.32: showing detailed results obtained from Case S.J. given repeated injections of ^{58}Co vitamin B_{12} at intervals of one to two days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.33 - CASE E.McB.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug.	POST INJECTION 24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 ug im	1770	45.5
2	"	1520	40.0
3	"	1670	54.3
4	"	1730	61.2
5	" *	715	42.4
6	" *	930	19.3
7	" *	860	62.0
12	"	1440	61.6
13	" *	840	64.5
15	" *	1700	55.8
16	" *	830	63.8
18	"	1500	53.9

Table A.33: showing detailed results obtained from Case E.McB. given repeated injections of ^{58}Co vitamin B_{12} at intervals of at least one week. The patient was in haematological relapse and was not excreting radioactivity prior to the study. * Vasopressin was also given on weeks 5, 6, 7, 13, 15 & 16 as described in Chapter 3.

Table A.34 - CASE J.McF.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug.	POST INJECTION 24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 im	1500	36.0
2	"	1510	55.6
3	" *	2450	54.3
4	" *	2540	52.8
5	" *	1975	73.9
6	" *	2355	65.0
11	"	1550	64.1
12	" *	1900	75.7
13	"	1750	71.2
14	"	1210	56.7
16	" *	2690	70.5
17	"	1230	77.3

Table A.34: showing detailed results obtained from Case J.McF. given repeated injections of ^{58}Co vitamin B_{12} at intervals of at least one week. The patient had been treated with vitamin B_{12} and was in full haematological remission and not excreting radioactivity prior to this study. * Oral chlorothiazide was given with the injections on weeks 3, 4, 5, 6, 12, & 16 as described in Chapter 3.

TABLE A.35 - CASE M.P.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 im	1460	65.3
2	"	1070	61.5
3	"	1115	63.9
4	"	1090	65.0
9	"	1020	64.3
10	"	900	55.9
11	"	1750	54.6
12	"	1310	69.0
13	"	950	72.4
14	"	1060	72.8
15	"	1370	62.3
16	"	1420	81.5

Table A.35: showing detailed results obtained from Case M.P. given repeated injections of ^{58}Co vitamin B_{12} at daily intervals. The patient was in haematological relapse with a low serum vitamin B_{12} level and not excreting radioactivity, prior to this study.

TABLE A.36 - CASE I.R.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug	POST INJECTION 24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 im *	2500	73.4
2	"	1640	24.1
3	" *	2175	54.5
4	"	950	45.6
5	" *	1550	85.1
57	" *	2340	48.9
58	"	1680	46.8
59	" *	2320	88.8
61	"	1500	86.7
63	" *	1980	71.5
64	"	1600	24.7
65	" *	2650	76.1
66	"	1700	80.1
68	" *	2040	51.1
70	"	1840	86.9
71	"	2010	79.9
74	" *	2300	69.8
76	"	1350	83.1

Table A.36: showing detailed results obtained from Case I.R. given repeated injections of ^{58}Co vitamin B_{12} at intervals of at least one week. The patient had been treated with vitamin B_{12} and was in full haematological remission and not excreting radioactivity prior to the study. * Oral chlorothiazide was given on weeks 1, 3, 5, 57, 59, 63, 65, 68 & 74 and vitamin B_{12} between weeks 5 & 57 as described in Chapter 3.

TABLE A.37 - CASE A.S.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug	POST INJECTION 24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 im	780	59.8
2	"	900	58.0
3	" *	1210	53.1
4	" *	1370	69.3
5	" *	1130	61.5
9	"	1130	69.8
10	"	750	61.2
11	" *	1720	55.2
12	"	1280	70.0
13	" *	1360	61.5
16	"	1120	70.3
16	" *	1160	83.2

Table A.37: showing detailed results obtained from Case A.S. given repeated injections of ^{58}Co vitamin B_{12} at intervals of at least one week. The patient had been treated with vitamin B_{12} and was in full haematological remission and not excreting radioactivity prior to the study. * Oral probenecid was given on weeks 3, 4, 5, 11, 13 & 16 as described in Chapter 3.

TABLE A.38 - CASE S.S.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	1350	0
1	1000 im	1750	64.4
5	"	1930	56.8
9	"	1580	76.0
25	" *	3220	56.1
32	" *	2630	60.4
39	" *	2000	74.7
46	" *	1920	64.4
60	"	960	47.0
74	" *	1920	78.5
81	" *	2715	69.3
88	"	1205	67.1
95	"	1070	76.7
102	" *	2490	76.0
109	"	1040	57.1

Table A.38: showing detailed results obtained from Case S.S. given repeated injections of ^{58}Co vitamin B_{12} at intervals of four to fourteen days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0. * Mersalyl was given on days 25, 32, 39, 46, 74 & 102 as described in Chapter 3.

TABLE A.39 - CASE R.T.

WEEK	^{58}Co VITAMIN B_{12} INJECTION ug	POST INJECTION 24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
1	1000 im	1990	50.1
2	"	1950	65.6
3	"	2040	49.7
4	"	1980	19.8
5	"	2100	44.0
6	"	1880	77.0
7	"	2090	64.0
11	"	2040	71.3
13	"	1840	71.3
14	"	1760	56.6
15	"	1770	53.5
16	"	1400	76.4

Table A.39: showing detailed results obtained from Case R.T. given repeated injections of ^{58}Co vitamin B_{12} at intervals of one to four weeks. The patient had been treated with vitamin B_{12} and was in full haematological remission and not excreting radioactivity prior to the study.

TABLE A.40 - CASE A.E.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	560	0
1	5000 im	660	80.9
2	"	1700	80.5
3	"	1370	92.9
4	"	1170	86.0
5	"	1780	86.0
6	"	1470	88.0
7	"	1800	77.8
8	"	1190	89.2
9	"	2070	85.7
10	"	1800	90.5
11	-	1870	0

Table A.40: showing detailed results obtained from Case A.E. given repeated injections of ^{58}Co vitamin B_{12} at intervals of one day. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.41 - CASE J.G.G.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	2000	0
1	5000 im	3200	91.9
2	-	2020	0
3	5000 im	1940	79.4
4	-	2400	0
5	5000 im	2230	82.4
6	-	1900	0
7	5000 im	1870	83.6
8	"	2050	80.4
9	"	2040	88.3
10	"	2070	88.9
11	"	2380	86.5
12	"	1660	85.1
13	"	1650	54.8
14	-	1480	0

Table A.41: showing detailed results obtained from Case J.G.G. given repeated injections of ^{58}Co vitamin B_{12}, at intervals of one to two days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.42 - CASE J.H.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug.	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	600	0
1	5000 im	510	63.1
2	-	480	0
3	5000 im	530	76.4
4	-	710	0
5	5000 im	700	78.0
6	-	550	0
7	5000 im	730	86.4
8	-	520	0
9	5000 im	730	90.7
10	-	530	0
11	5000 im	780	90.3
12	-	1040	0

Table A.42: showing detailed results obtained from Case J.H. given repeated injections of ^{58}Co vitamin B_{12} at intervals of two days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.43 - CASE J.O.M.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	1050	0
1	5000 im	930	75.4
2	"	1000	71.1
3	"	1350	76.0
4	"	1130	80.2
5	"	2070	93.4
6	"	1120	83.4
7	"	1200	86.6
8	"	1130	90.1
9	"	1300	87.9
10	"	1630	88.9
11	"	1220	90.4
12	"	1450	84.1
13	"	920	0

Table A.43: showing detailed results obtained from Case J.O.M. given repeated injections of ^{58}Co vitamin B_{12} at intervals of one day. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.44 - CASE D.McC.

DAY	^{58}Co VITAMIN B_{12} INJECTION µg	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	2070	0
1	10,000 iv	2380	94.0
2	"	1070	100.0
3	"	1530	116.8
4	"	1345	98.6
5	"	2320	110.8
6	"	2285	109.4
7	"	1990	103.1
8	"	2125	114.8
9	"	925	53.1
10	"	specimen lost	
11	"	2040	124.2
12	"	1935	108.9
13	"	2125	114.2
14	"	1280	94.4
15	"	2225	112.2
16	"	1360	79.4
17	"	1560	105.5
18	"	1535	84.4
19	"	1650	110.0
20	"	1715	0

Table A.44: showing detailed results obtained from Case D.McC. given repeated injections of ^{58}Co vitamin B_{12} at intervals of one day. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.45 - CASE M.McD.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	450	0
1	10,000 im	815	81.6
2	-	865	3.7
3	-	1005	0
4	10,000 im	1610	70.6
5	-	1110	3.1
6	10,000 im	1065	78.5
7	-	1395	4.6
8	10,000 im	810	74.1
9	-	895	4.3
10	10,000 im	1660	74.0
11	-	1220	3.7
12	-	1455	0
13	10,000 im	1380	69.1
14	-	850	4.2
15	10,000 im	790	65.2
16	-	1050	5.6
17	10,000 im	1100	80.0
18	-	1170	6.0
19	-	790	0
20	10,000 im	1530	66.5
21	-	1400	5.3
22	10,000 im	1520	71.7
23	-	1260	5.5
24	10,000 im	1380	62.5

CASE M.McD. (contd.)

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
25	10,000 im	1235	75.0
26	10,000 im	1415	52.2
27	10,000 im	1445	69.4
28	-	1355	7.5
29	10,000 im	1400	62.1
30	-	1800	8.0
31	-	1250	0

Table A.45: showing detailed results obtained from Case M.McD. given repeated injections of ^{58}Co vitamin B_{12} at intervals of two to three days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.46:- CASE C.Mcl.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml	RADIOACTIVITY % dose
0	-	490	0
1	10,000 iv	560	81.0
2	-	370	6.5
3	-	250	0
4	10,000 iv	880	71.6
5	-	835	5.3
6	-	1870	0
7	10,000 iv	1770	60.9
8	-	1945	8.4
9	-	1220	0
10	10,000 iv	1940	72.5
11	-	1215	5.7
12	-	1025	0
13	10,000 iv	1650	61.7
14	-	1740	0
15	10,000 iv	750	47.4
16	-	1950	0
17	-	1850	0
18	-	1230	0
19	10,000 iv	1115	63.3
20	-	790	3.9
21	-	1500	0
22	10,000 iv	1460	56.5
23	-	1620	0
24	-	1790	0

CASE C.McI. (contd.)

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
25	-	1040	0
26	10,000 iv	2260	75.8
27	-	1735	0
28	10,000 iv	1470	50.0
29	-	1850	0
30	10,000 iv	1540	66.3
31	-	870	0
32	-	2345	0
33	10,000 iv	1920	66.8
34	-	1970	0
35	10,000 iv	1850	46.8
36	-	1675	0
37	-	2410	0
38	10,000 iv	1570	42.2
39	-	1600	0

Table A.46: showing detailed results obtained from Case C.McI. given repeated injections of ^{58}Co vitamin B_{12} at intervals of two to four days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.47 - CASE S.R.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	750	0
1	20,000 im	1125	84.3
2	-	1080	5.3
3	-	690	0
4	20,000 im	1240	67.5
5	-	1770	5.1
6	20,000 im	1745	69.1
7	-	1430	5.6
8	20,000 im	2260	74.7
9	-	1470	4.9
10	-	1360	0
11	20,000 im	1750	66.4
12	-	950	5.1
13	20,000 im	2020	74.7
14	-	960	5.4
15	20,000 im	1290	71.0
16	-	1360	6.6
17	-	1190	0
18	20,000 im	760	60.0
19	-	1660	10.0
20	20,000 im	1840	45.1
21	-	1860	0
22	20,000 im	1650	73.5
23	-	1870	0
24	-	2470	0

CASE S.R. (contd.)

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
25	20,000 im	1720	78.5
26	-	1790	0
27	20,000 im	1570	66.3
28	-	2000	0
29	20,000 im	1595	75.0
30	-	1000	5.5
31	-	2200	0
32	20,000 im	1750	76.4
33	-	1190	6.3
34	20,000 im	1530	104.8
35	-	1230	9.9
36	-	1375	0
37	-	1770	0
46	20,000 im	1625	55.4
47	-	1210	5.8
48	-	1125	0
49	20,000 im	1160	65.6
50	-	1250	10.0
51	-	1240	0

Table A.47: showing detailed results obtained from Case S.R. given repeated injections of ^{58}Co vitamin B_{12} at intervals of two to twelve days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TABLE A.48 - CASE C.B.

DAY	^{58}Co VITAMIN B_{12} INJECTION ug	24 HOUR URINE COLLECTION	
		VOLUME ml.	RADIOACTIVITY % dose
0	-	720	0
1	30,000 im	735	56.5
2	-	865	3.6
3	-	800	0
4	-	690	0
5	30,000 im	785	57.5
6	-	820	4.0
7	-	860	0
8	30,000 im	740	74.4
9	-	560	0
10	30,000 im	890	93.2
11	-	1500	0
12	30,000 im	2190	60.0
13	-	1460	9.5
14	30,000 im	1340	88.0
15	-	600	8.9
16	-	950	0
17	-	1060	0
18	-	1130	0

Table A.48: showing detailed results obtained from Case C.B. given repeated injections of ^{58}Co vitamin B_{12} at intervals of two to four days. The patient was in haematological relapse with a low serum vitamin B_{12} level on day 0.

TIME AFTER INJECTION hrs.	RADIOACTIVITY EXCRETED - % DOSE (URINE VOLUME IN ML. IN BRACKETS)				
	K.McD.	M.W.	C.D.	J.D.	J.G.G.
0 - ½	14.0 (890)	1.5 (225)	24.2 (100)	27.6 (140)	16.9 (85)
½ - 1		4.2 (240)			
1 - 1½	15.7 (170)	6.2 (310)	13.9 (360)	11.7 (300)	22.3 (315)
1½ - 2		5.9 (280)			
2 - 2½	15.3 (400)	4.8 (220)	10.0 (250)	9.9 (215)	12.2 (210)
2½ - 3		5.7 (180)			
3 - 4	11.4 (260)	7.3 (340)	7.1 (130)	10.5 (320)	10.3 (110)
4 - 5	3.6 (180)	6.6 (490)	5.0 (170)	6.1 (110)	8.0 (350)
5 - 6	2.7 (200)	5.0 (175)	3.3 (60)	4.1 (70)	4.7 (90)
6 - 7	1.2 (170)	22.5 (1080)	6.1 (260)	13.9 (1750)	3.7 (270)
7 - 8	1.2 (200)		4.5 (1350)		2.7 (150)
8 - 24	2.5 (525)				11.1 (1120)

Table A.49: showing amounts of radioactivity excreted at intervals after injections of ^{58}Co vitamin B_{12} to five patients. The doses and routes of administration are described in Chapter 4.

TIME AFTER INJECTION minutes	TOTAL ASSAYABLE VITAMIN B_{12} IN ARTIFICIAL KIDNEY BATH FLUID - ug					
	CASE AK 1	CASE AK 2	CASE AK 3	CASE AK 4	CASE AK 5	CASE AK 6
0	0	0	0	0	0	0
5	8.5	11.0	7.7	-	19.0	7.3
10	17.4	29.0	19.5	31.4	46.0	24.8
15	32.8	34.0	28.5	45.0	60.4	44.5
20	39.5	47.0	34.0	55.0	76.4	52.0
25	42.0	49.5	45.0	63.0	90.0	64.5
30	55.5	61.0	52.0	75.0	102.0	81.0
35	56.0	75.0		78.0	117.0	88.0
40	62.5	80.0		92.0	120.0	103.0
45	66.0	88.0		94.5	130.0	-
50	76.5	94.0			141.0	130.0
55	69.0	96.0			-	-
60	75.0	100.5			172.0	138.0
70	-	118.0			199.0	153.0
80	-	133.0			220.0	168.0
85	-	-			-	155.0
90	94.5	147.0			223.0	
100		155.0			238.0	
120					242.0	

Table A.50: showing amounts of assayable vitamin B_{12} found in the artificial kidney bath fluid at intervals after intravenous injection of 1000 ug vitamin B_{12} to six patients undergoing haemodialysis.

CASE J.K.

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	33	50 (41.5 ug retained)
1	181	
2	127	
3	107	
4	90	
5	77	
7	67	
9	59	
11	57	
12	56	50 (39.5 ug retained)
13	250	
16	189	
18	169	
19	130	
23	108	
26	98	
30	97	
34	90	
41	71	
44	61	
47	55	

Table A.51: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.K. The patient was in haematological relapse and had not been treated prior to day 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in batch.

CASE C.J.

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	45	100 (80.0ug retained)
1	402	
2	367	
3	357	
4	332	
6	250	
8	242	
10	202	
13	168	
15	152	
17	139	
19	130	
21	136	
23	126	
27	126	
30	109	
34	118	100 (78.0ug retained)
41	272	
48	210	
55	170	
62	157	
69	140	

Table A.52: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case C.J. The patient was in haematological relapse and had not been treated prior to day 0. Each value for serum vitamin B_{12} is the mean of the estimations: all samples were assayed in the same batch.

CASE C.R.

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	25	100 (72.0ug retained)
1	327	
2	255	
3	222	
4	222	
5	193	
6	193	
7	180	
8	156	
10	148	100 (80.2 ug retained)
14	299	
29	180	
32	165	
36	161	
39	144	100 (52.0 ug retained)
43	352	
45	292	
52	217	
59	180	
66	132	
73	115	
80	109	100 (52.8 ug retained)
87	235	
94	173	

CASE C.R. (contd.)

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
101	137	
108	119	
115	111	
122	94	

Table A.53: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case C.R. The patient was in haematological relapse and had not been treated prior to day 0. Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE M.T.

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	54	100 (81.2 ug retained)
7	198	
14	158	
18	147	100 (85.0 ug retained)
21	345	
23	332	
35	189	
42	137	
49	112	
56	87	100 (89.5 ug retained)
62	193	
69	143	
77	115	
84	86	
91	83	100 (63.1 ug retained)
95	199	
105	162	
112	136	
119	136	
126	114	
133	104	100 (75.9 ug retained)

CASE M.T. (contd.)

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
140	272	
147	217	
163	139	
170	113	
177	104	

Table A.54: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case M.T. The patient was in haematological relapse and had not been treated prior to day 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.McQ.

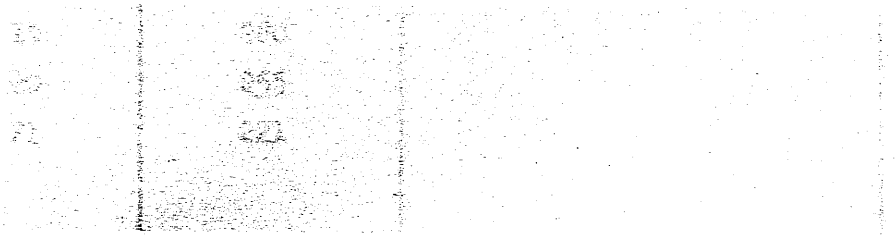
TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	29	1000 (324 ug retained)
1	340	
2	287	
3	215	
4	182	
5	167	
6	141	
7	124	
8	127	
9	121	
10	117	
11	115	1000 (485 ug retained)
12	452	
14	305	
15	260	
16	183	
17	178	
18	166	
19	143	
20	117	
21	114	
22	94	

CASE J.McQ. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug.
23	89	
24	64	

Table A.55: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.McQ. The patient was in haematological relapse and had not been treated prior to day 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.



CASE H.C.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	166	50 (35.1 retained)
1	350	
2	257	
3	231	
4	228	
5	197	
6	197	
7	212	1000 (310 retained)
8	418	
9	380	
10	282	
11	255	
12	270	
13	237	
15	200	
16	199	100 (59.5 ug retained)
17	410	
18	340	
19	280	
20	255	
21	221	

CASE H.C. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug.
22	192	
23	174	
24	178	

Table A.56: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case H.C. The patient had been treated and was in full haematological remission on Week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE H.F.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	30	16 x 100 ug ^{58}Co B_{12} i.m. (726 ug retained)
6	350	
10	200	
14	147	
17	100	
23	102	100 ug ^{58}Co B_{12} i.m. (58.0 ug retained)
24	205	
25	152	
26	127	
27	125	
28	115	
29	111	1000 ug ^{58}Co B_{12} i.m. (461 ug retained)
30	292	
31	210	
32	207	
33	192	
34	175	
35	161	
36	137	100 ug ^{58}Co B_{12} i.m. (52.3 ug retained)
37	257	
38	205	

CASE H.F. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
39	198	
40	154	
41	167	
42	133	
43	117	
44	113	

Table A.57: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case H.F. The patient was in haematological relapse and had not been treated prior to week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE M.F.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	105	50 (32.4 ug retained)
1	330	
2	242	
3	189	
4	140	
5	132	
6	128	1000 (312 ug retained)
7	417	
8	332	
9	285	
10	255	
11	206	
13	170	
15	149	
16	151	100 (56.1 ug retained)
17	452	
18	280	
19	225	
20	197	

Table A.58: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case M.R. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.G.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug.
0	112	50 (37.2 ug retained)
1	195	
2	135	
5	135	
6	121	
7	117	
8	117	
9	105	
10	108	2 x 50 (76.0 ug retained)
11	283	
12	192	
13	164	
14	163	
16	124	
17	120	

Table A.59: showing relationship of serum vitamin B_{12} levels to injections of ^{58}Co vitamin B_{12} in Case J.G. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.O.G.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	192	100 (63.6 ug retained)
1	292	
2	252	
3	227	
4	204	
5	187	
6	177	50 (33.4 ug retained)
7	250	
8	206	
9	192	
10	173	
11	181	1000 (259 ug retained)
12	302	
13	242	
14	235	
15	237	
16	212	
17	194	
18	200	
19	177	
20	181	

CASE J.O.G. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug.
21	171	50 (37.8 ug retained)
22	235	
23	210	
24	190	
25	182	
26	162	
27	177	
28	158	

Table A.60: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.O.G. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.O.H.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	126	100 (56.9 ug retained)
1	212	
2	166	
3	150	
4	138	
6	123	1000 (328 ug retained)
7	307	
8	193	
9	170	
10	168	
11	152	
12	135	
13	130	
14	121	
15	121	
16	120	
17	143	
18	130	100 (59.0 ug retained)
19	245	
20	189	
21	160	

CASE J.O.H. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug.
22	147	
24	121	
25	116	
26	115	

Table A.61: showing relationship of serum vitamin B_{12} levels to injections of ^{58}Co vitamin B_{12} in Case J.O.H. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE D.J.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	156	1000 (238 ug retained)
1	397	
2	327	
3	252	
4	227	
5	206	
6	157	1000 (900 ug retained)
7	370	
8	307	
9	252	
10	227	
11	186	
12	138	
13	147	
14	156	
15	124	100 (69.0 ug retained)
16	255	
17	227	
18	188	
19	173	
21	151	100 (98.0 ug retained)
22	277	
23	212	

CASE D.J. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
24	174	
25	157	
26	131	
27	134	50 (35.1 ug retained)
28	223	
29	166	
30	153	
31	139	
32	132	50 (40.0 ug retained)
33	263	
34	220	
35	204	
36	222	
37	189	
38	185	
40	171	100 (67.2 ug retained)
41	332	
42	277	
43	225	
45	148	
46	130	
47	131	

Table A.62: showing relationship of serum vitamin B_{12} levels to injections of ^{58}Co vitamin B_{12} in Case D.J. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.K.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	86	2 x 50 (80.5 ug retained)
1	232	
2	161	
3	129	50 (43.9 ug retained)
4	235	
5	163	
7	106	
8	101	
9	93	

Table A.63: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.K. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.L.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	222	1000 (464 ug retained)
1	465	
2	372	
3	322	
4	300	
5	250	
6	232	
8	212	
14	215	
15	198	100 (64.4 ug retained)
18	367	
19	307	
20	275	
21	262	
22	250	
23	257	
24	257	
26	225	
27	222	
28	207	
29	204	50 (40.1 ug retained)
30	312	
31	292	
32	240	
33	218	

CASE J.L. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
34	207	
35	195	
36	192	
37	179	100 (62.6 ug retained)
38	332	
39	277	
43	242	
45	220	
46	217	
48	191	

Table A.64: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.L. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE M.McB.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	114	100 (65.9 ug retained)
1	209	
2	193	
3	162	
4	145	
5	149	
6	132	
7	125	1000 (320 ug retained)
8	292	
9	208	
10	188	
11	178	
12	147	
13	132	
14	131	
15	119	
16	109	100 (58.5 ug retained)
17	257	
18	210	
19	184	
20	150	
21	141	
22	136	
23	131	2 x 100 (109.7 ug retained)
24	325	

CASE M.McB.(contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
25	267	
26	222	
28	175	
29	149	
30	136	
31	132	
32	121	
33	117	
34	114	

Table A.65: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case M.McB. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE I.McC.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	145	100 (52.8 ug retained)
1	302	
2	257	
3	222	
4	191	
5	156	
6	139	
7	125	
8	127	50 (30.4 ug retained)
9	265	
10	222	
11	200	
12	184	
13	153	1000 (337 ug retained)
14	445	
15	340	
16	312	
17	262	
18	215	
19	165	
20	164	
22	149	
23	140	
24	144	

CASE I.McC. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
25	133	100 (56.8 ug retained)
26	247	
27	220	
28	183	
29	175	
30	169	
31	149	
32	159	
33	146	
34	145	

Table A.66: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case I.McC. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE E.P.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	147	100 (51.1 ug retained)
1	262	
2	220	
3	199	
4	177	
5	162	
6	134	1000 (291 ug retained)
7	327	
8	260	
9	240	
10	210	
11	186	
12	186	
13	166	
14	149	

Table A.67: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case E.P. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE M.P.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	200	100 (52.6 ug retained)
1	327	
2	275	
3	227	
4	200	
5	176	1000 (122 ug retained)
6	310	
7	255	
8	217	
9	210	
10	194	

Table A.68: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case M.P. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE I.R.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	144	1000 (341 ug retained)
1	220	
3	220	
4	140	
5	120	
6	108	
7	106	100 (78.8 ug retained)
8	240	
9	200	
10	128	
11	92	
12	84	

Table A.69: showing serum vitamin B_{12} levels in relation to ^{58}Co vitamin B_{12} injections in Case I.R. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE M.R.

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	56	50 (47.7 ug retained)
1	235	
2	216	
3	176	50 (41.7 ug retained)
4	297	
6	200	
9	160	
11	153	
16	156	50 (35.0 ug retained)
18	317	
20	262	
24	252	
25	242	
27	252	
37	212	
39	181	
41	172	
48	130	
58	108	
65	106	50 (35.0 ug retained)
72	176	
77	146	
84	134	
91	120	

CASE M.R. (contd.)

TIME days	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
98	107	
105	105	
112	97	
119	85	
126	88	

Table A.70: showing relation of serum vitamin B_{12} levels to injections of ^{58}Co vitamin B_{12} in Case M.R. The patient had not been treated and was in haematological relapse on day 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.S.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTED ug
0	129	1000 (161 ug retained)
1	282	
2	240	
3	220	
4	191	
5	172	
6	153	
7	150	
8	111	
9	113	100 (53.7 ug retained)
10	312	
12	237	
13	205	
14	178	
15	161	
16	139	
17	122	

Table A.71: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.S. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE A.T.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug.
0	60	1000 ug vitamin B_{12}
1	316	
2	220	
3	144	
4	100	
5	100	
7	80	
9	92	
10	80	1000 (288 ug retained)
11	264	
12	208	
13	156	
14	144	
15	124	
16	132	
17	156	
18	152	
19	124	2000 (570 ug retained)
20	376	
24	220	
25	224	
26	144	
28	116	50 (34.2 ug retained)
30	300	
31	208	

CASE A.T. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
32	152	
33	144	
34	116	
35	118	
36	112	

Table A.72: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case A.T. The patient was in haematological relapse on week 0 when an injection of vitamin B_{12} was given.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE A.T.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	112	100 (54.4 ug retained)
1	360	
2	302	
3	260	
4	200	
5	185	
6	157	50 (37.5 ug retained)
7	245	
8	220	
9	187	
10	145	
11	138	
12	126	
13	122	100 (49.5 ug retained)
14	345	
15	305	
16	252	
17	237	
18	230	
19	225	
20	179	2 x 100 (114.0 ug retained)
21	377	
22	315	

CASE A.T. (contd.)

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
23	290	
25	192	
26	153	
27	159	
29	148	
30	148	
31	130	

Table A.73: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in case A.T. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE I.W.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	176	1000 (381 ug retained)
1	400	
2	254	
3	216	
4	196	
5	178	
6	120	
7	100	100 (49.5 ug retained)
8	264	
9	208	
10	188	
11	138	
12	108	

Table A.74: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case I.W. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE I.W.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	137	100 (49.3 ug retained)
1	287	
2	210	
3	182	
4	182	
5	166	
6	153	
7	138	
8	142	2 x 100 (118.8 ug retained)
9	362	
10	322	
11	257	
12	240	
13	193	
14	168	
15	163	
16	155	
17	149	
18	142	

A.75: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case I.W. The patient had been treated and was in full haematological remission on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.A.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	66	12 x 1000 (4104 ug retained)
5	660	
11	405	
17	352	
23	322	
27	300	
31	270	
35	265	
39	220	
45	202	
49	212	
53	202	
59	177	
61	158	

Table A.76: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.A. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE C.D.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	50	16 x 1140 (5727 ug retained)
13	255	
18	203	
25	184	
31	195	
32	191	
33	177	
36	156	
40	164	
44	122	
49	127	
50	134	

Table A.77: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case C.D. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.F.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	52	9 x 100 (451 ug retained)
6	515	
10	387	
14	290	
18	240	
22	205	
27	215	
30	203	
33	203	
37	193	
40	202	
41	222	
48	181	
53	161	
57	142	
61	140	

Table A.78: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.F. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE G.G.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	40	18 x 540 (2731 ug retained)
6	360	
9	290	
12	210	
16	157	
20	141	

Table A.79: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case G.G. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE M.McD.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	40	15 x 10,000 (43,500 ug retained)
9	292	
27	222	
39	192	
95	148	
100	127	

Table A.80: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case M.McD. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE S.R.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	25	17 x 20,000 (83,300 ug retained)
9	765	
19	405	
25	327	
42	290	
48	265	
52	257	
57	194	
61	180	
64	237	
69	260	
74	290	
77	217	
83	149	

Table A.81: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case S.R. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE J.S.

TIME weeks	SERUM VITAMIN B_{12} uug/ml.	^{58}Co VITAMIN B_{12} INJECTION ug
0	50	18 x 1000 ug (5598 ug retained)
6	332	
10	231	
18	159	
24	129	

Table A.82: showing serum vitamin B_{12} levels in relation to injections of ^{58}Co vitamin B_{12} in Case J.S. The patient was in haematological relapse on week 0.

Each value for serum vitamin B_{12} is the mean of two estimations: all samples were assayed in the same batch.

CASE	PREINJECTION SERUM VITAMIN B_{12} LEVEL uug/ml.	DOSE INJECTED ug	DOSE RETAINED ug	DURATION OF EFFECT ABOVE 140 uug/ml. days
H.F.	102	100	58.0	17
"	111	1000	461	49
"	137	100	52.3	40
M.F.	105	50	32.4	30
J.G.	112	50	37.2	14
J.O.H.	126	100	56.9	27
"	123	1000	328	40
"	130	100	59.0	31
C.J.	118	100	78.0	35
D.J.	157	1000	900	42
"	151	100	98.0	32
"	134	50	35.1	28
"	171	100	67.2	38
J.K.	129	50	43.9	17
M.McB.	114	100	65.9	39
"	125	1000	320	38
"	109	100	58.5	35
I.McC.	145	100	52.8	42
"	153	1000	337	70
J.McQ.	115	1000	485	59
E.P.	147	100	51.1	40
C.R.	148	100	80.2	29
"	144	100	52.0	26
"	109	100	52.8	20

CASE	PREINJECTION SERUM VITAMIN B_{12} LEVEL uug/ml.	DOSE INJECTED ug	DOSE RETAINED ug	DURATION OF EFFECT ABOVE 140 uug/ml. days
I.R.	144	1000	341	28
"	106	100	78.8	20
M.R.	156	50	35.0	31
"	106	50	35.0	16
J.S.	129	1000	161	51
"	113	100	53.7	49
A.T.	80	1000	288	30
"	116	50	34.2	30
"	157	50	37.5	34
M.T.	147	100	85.0	24
"	87	100	89.5	15
"	83	100	63.1	20
"	104	100	75.9	26
I.W.	137	100	49.3	49
"	176	1000	381	39
"	100	100	49.5	28

Table A.83: showing details of results selected for analysis relating the amount of ^{58}Co vitamin B_{12} injected and retained after parenteral administration to the duration of effect.

CASE	PREINJECTION SERUM VITAMIN B_{12} LEVEL uug/ml.	DOSE INJECTED ug	DOSE RETAINED ug	DURATION OF EFFECT ABOVE 140 uug/ml. days
J.G.	108	2 x 50	76.0	35
J.K.	86	"	80.5	18
M.McB.	131	2 x 100	109.7	47
A.T.	179	"	114.0	73
I.W.	142	"	118.8	70

Table A.84: showing details of results selected for analysis relating the amount of ^{58}Co vitamin B_{12} injected and retained after two injections of ^{58}Co vitamin B_{12} at 24 hour intervals to the duration of effect.

CASE	PREINJECTION SERUM VITAMIN B_{12} LEVEL uug/ml.	DOSE INJECTED ug	DOSE RETAINED ug	DURATION OF EFFECT ABOVE 140 uug/ml. days
J.F.	52	9 x 100	45	427
H.F.	30	16 x 100	726	100
G.G.	40	18 x 540	2731	140
J.A.	66	12 x 1000	4104	>427
J.S.	50	18 x 1000	5598	152
C.D.	50	16 x 1140	5731	290
M.McD.	40	15 x 10,000	43500	679
S.R.	25	17 x 20,000	83300	> 581

Table A.85: showing details of amounts of ^{58}Co vitamin B_{12} injected and retained and the duration of effect in eight patients given a series of injections in a short space of time. The results from Cases J.A. & S.R. were not included in the analysis as the serum vitamin B_{12} level had not fallen to 140 uug/ml by the end of the observation period.

TECHNICAL MATERIALS AND METHODS

MICROBIOLOGICAL ASSAYS

ISOTOPE MATERIALS AND METHODS

OTHER TECHNICAL METHODS

STATISTICAL METHODS

MICROBIOLOGICAL ASSAYS

METHOD

MEDIUM

STOCK CULTURES

PROCEDURE

COLLECTION AND PREPARATION OF SAMPLES

RECOVERY EXPERIMENTS

PREPARATION OF GLASSWARE

The technique used was modified from that described by Ross (1952), Hutner et al (1956) and Ross et al (1957) using Euglena gracilis 3 strain as the test organism. The organism was supplied by Dr. G. I. M. Ross. Apart from the preparation and sterilisation of the medium which was carried out in the Departments of Biochemistry and Bacteriology, Western Infirmary, all work was done entirely by the author.

Medium

The medium used was that described by Hutner et al (1956). A large volume was made up: single strength medium was dispensed in 500 ml. aliquots in glass bottles sealed by a rubber bung and metal screw cap and double strength medium in 20 ml. aliquots in smaller glass bottles similarly sealed. After dispensing the solutions were sterilised by autoclaving, at 10 lbs. per square inch for 20 minutes.

Stock Cultures

Stock cultures of the organism were maintained in single strength basal medium containing 0.05% tryptone and 30 - 40 uug

/vitamin B_{12} per ml. Stock cultures were subcultured after washing, every two weeks.

Procedure

This differed slightly from that described by Ross (1952), Hutner et al (1956) and Ross et al (1957) the modifications used being employed to assist a single worker to deal with a large number of samples in a limited time. The principal modification lay in the use of single strength medium throughout. Solutions for assay containing final concentrations of 0, 1.25, 2.5, 5, 10, 15, 25 and 50 uug vitamin B_{12}/ml. were prepared by adding suitable volumes of standard solutions of vitamin B_{12} in single strength medium to appropriate volumes of single strength medium to a total volume of 4 ml. The standard solutions were made by adding commercially available vitamin B_{12} to single strength medium, diluting as required to obtain final concentrations of 100 and 20 uug/ml. The solutions for assay with final concentrations of 1.25, 2.5, 5.0 and 10.0 uug/ml. were made by adding 0.25, 0.5, 1.0 and 2.0 ml. of the

/20 uug/ml. stock solution to 3.75, 3.5, 3.0 and 2.0 ml. single strength medium respectively and the solutions with final concentrations of 10, 15, 25 and 50 uug/ml by adding 0.4, 0.6, 1.0 and 2.0 ml. of the 100 uug/ml stock solution to 3.6, 3.4, 2.0 and 1.0 ml. of single strength basal medium respectively.

Samples for assay were prepared by adding the appropriate volume of test solution to the appropriate volume of single strength medium dispensed by automatic burette to a total volume of 4 ml.. Information on the preparation of samples for assay is given elsewhere. The dilution of test sample in medium was 1.20, 1.40 or 1.80 depending on the vitamin B_{12} content. Dilutions of 1.20 and 1.40 were commonly used for serum and 1.40 for other samples.

All samples were assayed in aluminium capped 6" x 5/8" Pyrex glass tubes in duplicate. Triplicate or quadruplicate estimations were not found to be superior to duplicate estimations and were therefore discontinued. After preparation all solutions were heated at 100°C

/in a water bath for 15 minutes. After cooling the samples were inoculated and incubated. The inoculum was prepared from stock cultures which had been incubated for two weeks: they were washed three times with single strength medium and resuspended in a suitable volume of single strength medium before use. The incubation period was five, occasionally six, days in a perspex water bath illuminated from below by two 30 watt fluorescent strip lights. A faint pink tint was found to assist growth and was achieved by adding a few drops of Edicol supra-rose solution to the water in the bath. A few crystals of copper sulphate were also added to discourage the growth of moulds. An even bath temperature of 29°C. was maintained by two 150 watt "Queensborough" aquarium heaters connected by a Sunvic type 102/4 relay to a mercury contact thermometer and by a "Handilab" stirrer. Readings were made in a Unicam SP 300 GP colorimeter and the values for the unknown samples were calculated from a curve constructed from the values obtained for the standards.

/Optical cells with a light path 2.5 mms. thick were used instead of the usual 10 mm. thick cells, allowing readings to be made without diluting the samples. The modifications, i.e. the use of single strength medium, the dispensing of medium by an automatic burette and the use of thin optical cells were all approved by Dr. G. I. M. Ross. When the modified method was tested against the original methods using Euglena gracilis 3 strain described by Hutner et al (1956) and Ross et al (1957) it gave the same results. An aliquot of a large volume of pooled serum was assayed in every batch. To ensure accuracy many samples were assayed in more than one assay batch as noted in the text.

Collection and preparation of samples

Treatment with cyanide or papain was never used and no preservatives were added to any sample.

Serum: Blood was collected by clean venepuncture into 20 ml; sterile acid washed bottles and allowed to clot at room temperature. After centrifugation at 2000 - 3000 r.p.m. the serum was decanted into 5 ml. sterile acid

/washed bottles and stored at -20°C.

Urine: Collections were made directly into acid washed dark glass bottles via a large filter funnel to avoid contamination by faeces. No preservatives were used. The total volume was noted. Urine collected after an injection of vitamin B_{12} was diluted at once and aliquots of the original and the diluted samples were stored in sterile, acid washed 5 ml. or 20 ml. bottles at -20°C till assayed. Assays were performed as soon as possible after collection. As general rule urine samples collected after an injection of 1000 ug vitamin B_{12} were diluted 1:1000 by diluting 10 ml. urine with 990 ml. water and further diluting by adding 10 ml. of this solution to 90 ml. water. Similarly samples collected after injections of 500 ug vitamin B_{12} were usually diluted 1.500 and samples collected after injections of 100 ug and 50 ug were usually diluted 1.100 and 1.10 respectively.

Liver: The liver was removed at autopsy and weighed after removal of the gall bladder. A generous sample was taken, weighed and homogenised with distilled water in a Kenmix '55' homogenisor to a known volume. Aliquots of the homogenate were diluted serially and stored in sterile acid washed bottles at -20°C.

Artificial kidney bath fluid: Samples were taken with an acid washed pipette and stored in sterile acid washed glass bottles at -20°C. till assayed.

Recovery experiments

The presence of inhibitors to the growth of Euglena gracilis in test samples was investigated on several occasions. The method employed was to set up in the same assay batch tubes containing:

(1) single strength basal medium only.

(2) single strength basal medium with the test sample in a concentration of (usually) 1.40.

(3) single strength basal medium with vitamin B_{12} in a concentration of 2.5, 5.0, 10.0 and

/15.0 uug/ml.

(4) single strength basal medium with vitamin B_{12} in a concentration of 10 uug/ml and also the test sample in the same concentration as in (2), the total volume being 4 ml. in each tube. Estimations were made in duplicate or triplicate. The solutions were heated as usual, cooled, inoculated with the standard inoculum and incubated for five days. The vitamin B_{12} content of (2) and (4) was calculated as usual. Inhibitors were assumed to be absent when the value of (4) - (2) = 10 uug/ml. $\pm$ 1 uug/ml.

Tissue homogenates, artificial kidney bath fluids and urines containing drugs or their end products were investigated by this method. Collections of urine were made for 24 hours, the patient taking the drug for at least 24 hours before the collection period and continuing to take it during the collection period. Usually the urines were not diluted prior to assay. The drugs taken were acetylsalicylic acid, aluminium hydroxide, amylobarbitone sodium, butobarbitone, chlorothiazide, digitalis,

/ferrous sulphate, Imferon, insulin soluble and IZS, magnesium trisilicate, mersalyl, penicillin, phenindione, phenobarbitone sodium, probenecid, propantheline bromide, reserpine and tetracycline. Normal therapeutic doses were given. In many cases the author was the subject of the study. No inhibitory effect on the growth of Euglena gracilis was detected.

Preparation of glassware

All glassware used in assays or in the preparation or storage of samples for assay was cleaned by immersion in potassium dichromate sulphuric acid mixture for at least one hour, washing at least six times with vitamin B_{12} free water and sterilising by dry heat or in an autoclave.

ISOTOPE MATERIALS AND METHODS

Co vitamin B_{12}

- Source
- Purity
- Preparation and storage of stock solutions
- Stability of stock solutions
- Doses
- Preparation of doses for injection

Isotope counting methods

- Collection and preparation of samples
- Counting equipment
- Counting techniques

Source

All supplies of Co vitamin B_{12} were obtained from the Radiochemical Centre, Amersham. The ^{58}Co containing vitamin was commonly used: on occasions the ^{57}Co containing vitamin was used, usually when ^{58}Co vitamin B_{12} was not available.

Purity

The radiochemical purity of the products was always greater than 90% as established by dilution analysis carried out at the Radiochemical Centre.

Preparation and storage of stock solutions

Until mid 1959 the isotopes were received in aqueous solution: after that date they were received freeze-dried in sealed glass ampoules. Soon after receipt stock solutions were made, containing 0.5 ug with a specific activity of 0.5 uc in 5 ml. 0.9% saline. These solutions were dispensed in acid washed, dark glass bottles stoppered with a rubber diaphragm and screw cap, autoclaved at 5 lbs. per square inch for 30 minutes and stored in the dark at $+4^{\circ}C$ when not in use. Each bottle contained 80 to 100 ml.

/of the stock solution.

Stability of stock solutions

The vitamin B_{12} content of each batch was determined by microbiological assay each week the batch was in use. Deterioration was never observed.

Doses

The doses of radioactivity given were kept as small as possible compatible with obtaining acceptably accurate results. The doses used are given in the text. As a general rule the doses of radioactivity given parenterally were 0.3 uc (50 ug), 0.2 uc (100-1000 ug), 0.2 or 0.1 uc (1000-30,000 ug). In many cases the radioactivity of a dose is actually over-rated as no correction was made for decay e.g. a dose of 2.0 ml. of the stock solution which had a specific activity of 0.2 uc when freshly prepared was still regarded and listed as having a specific activity of 0.2 uc one month after receipt when the specific activity would have declined appreciably. This procedure was adopted for convenience and also served to ensure that

/the total amount of radioactivity given to each patient was well within the limits permitted by the Medical Research Council's Advisory Panel on the Allocation of Radioactive Isotopes for Clinical Use.

Preparation of doses for injection

Two methods of preparing solutions of ^{58}Co vitamin B_{12} for injection were used. In both cases solutions were prepared by the author. The first method, used for the 'matched' series reported in Chapter 1 was as follows. The appropriate volumes of stock solution of ^{58}Co vitamin B_{12} were added aseptically to ampoules containing appropriate volumes of non radioactive vitamin B_{12} using tuberculin syringes, a suitable number of ampoules being prepared at the beginning of each experiment. The tuberculin syringes were calibrated by weighing on an Oertling balance when empty and when filled with distilled water and were found to contain 1.00, 1.08 and 1.14 ml. The necessary corrections resulted in the apparently unusual choice of doses of ^{58}Co vitamin B_{12}

/used in the studies reported in Chapter 1,viz. 54, 540, and 1140 ug. The second method, often used for other studies, was to draw up the desired volume of stock Co vitamin B_{12} solution into a 5 ml. syringe and then to draw up the desired volume of vitamin B_{12} solution and mix the solution in the barrel of the syringe. The volumes were measured from mark to mark in the barrel of the syringe. Some air was left in the syringe and injected after the solution to ensure that no solution remained in the nozzle of the syringe or needle.

Collection and preparation of samples

(1) Urine: Collections were made as described previously. The total volume was noted and 450 ml. aliquots were taken into screw-capped, wide mouthed plastic bottles. If the total volume was less then 450 ml. as occurred when hourly collections of urine were made the volume was made up to 450 ml. with water.

(2) Bile: Collections were made into acid washed dark glass bottles via a T-tube inserted

/into the common bile duct. The same procedure was followed as with urine samples.

(3) Faeces: Stools were collected into washed stainless steel bed pans care being taken to avoid contamination with urine. Samples were transferred to waxed paper cartons and the collection homogenised to a known volume with water and a 450 ml. aliquot of the homogenate taken for counting.

(4) Liver: The liver was homogenised as described previously and a 450 ml. aliquot of the undiluted homogenate taken for counting.

(5) Other tissues: Were treated in the same way as liver.

(6) Blood: 5 ml. aliquots of heparinised venous blood haemolysed by the addition of saponin were pipetted into suitable glass tubes.

(7) Plasma: 5 ml. aliquots of plasma were taken into suitable glass tubes after centrifugation of heparinised venous blood.

/It was established that adsorption of radioactivity from samples and standards onto the walls of plastic bottles or glass tubes did not occur.

Counting equipment

Three different counters were used: the first to count the radioactivity in aliquots of urine, bile, faeces and tissue homogenates. The second to count the radioactivity in aliquots of blood or plasma. The third for external counting of hepatic radioactivity.

(1) The radioactivity in 450 ml. aliquots of urine, bile, faeces or tissue homogenates contained in screw-capped, wide-mouthed, plastic bottles was measured by an end-on method in a suitably modified EKCO scintillation counter N 550 with a thallium activated sodium iodide crystal 3.81 cm. diameter and 2.4 cm. deep shielded by three inches of lead and an EKCO automatic scaler type N 530 A. A standard containing 0.2 uc Co vitamin B_{12} with 2000 ug vitamin B_{12} as a carrier in 450 ml. water was used.

(2) The radioactivity in 5 ml. aliquots of blood or plasma contained in glass test tubes was

/measured with an EKCO type N 664 universal scintillation counter fitted with well type thallium activated sodium iodide crystal N 597 shielded by four inches of lead and an EKCO automatic scaler type N 530 A. A standard containing 0.05 uc Co vitamin B_{12} in 5 ml. water was used.

(3) The uptake of hepatic radioactivity was measured by an EKCO type N 599 c scintillation counter fitted with a thallium activated sodium iodide crystal type N 566 A. A wide angle collimator was used. The scaler was an EKCO automatic type N 530 A.

Counting techniques

With the exception of a few samples all counting was done by the author in the Isotope Laboratory, Gardiner Institute, Western Infirmary. When 450 ml. volumes were being counted the background count was established over 300 seconds after a suitable time had been allowed for the equipment to "warm up". The background count rate over the period in which the studies reported here were done ranged from

/1.49 - 4.95 counts per second and was usually 2.00 counts per second. The standard was then counted until at least 10,000 counts were recorded. Samples were generally counted for at least 300 seconds or until 5000 counts had been recorded. In the experiments reported in Chapter 1 all samples in the 1140, 1000 and 540 ug series were counted until 10,000 counts had been recorded giving a counting error of ± 1% and all samples in the 100 ug and 54 ug series were counted until 5000 counts had been recorded giving a counting error of ± 2%. The range of results obtained was typical of that obtained in other experiments and is shown overleaf.

	Net Standard counts/second	Net Sample counts/second
1140ug series (matched)	145.78-187.79	10.58-86.61
1000ug series (misc.)	56.21-193.25	1.82-58.41
540ug series (matched)	147.89-176.76	15.09-60.53
100ug series (misc.)	94.00-225.70	3.40-80.93
54ug series (matched)	175.94-195.94	2.02-67.05

The net dose count was the same as the net standard count except in the 54 ug series where the net dose count ranged from 263.86 - 293.91 counts per second.

When the gross counts per sample were less than twice the background the radioactivity in the sample was regarded as insignificant and a value of zero was recorded. Knowing the background and standard count rates the lowest detectable amount of radioactivity in a sample could be calculated. The coincidence of a large sample volume, a high background count and a low standard count could, in theory, result in a recorded value of 0% when in fact the actual

/value was as much as 5%. This never occurred with urine samples and values recorded at 0% dose excreted never represent true values of more than 1% dose excreted. With samples of bile and faeces (see Chapter 5) significant count rates were often not obtained and the only solution was to record the result as $< x\%$ dose excreted as calculated above. In such cases it seemed wrong to adopt the alternative of increasing the dose of radioactivity in the hope of obtaining more accurate results.

The counting of 5 ml. samples of blood or plasma was conducted on similar lines. A background count was established over 300 seconds, the standard count until 10,000 counts had been recorded and the sample count over at least 300 seconds.

The accumulation of hepatic radioactivity was measured by establishing the background count over the liver for 300 seconds and counting for periods of 100 seconds every 2 minutes or longer after

/the injection of Co vitamin B_{12}. No standard was used in this technique. The counter was placed over the anterolateral projection of the liver in contact with the skin and remained in position for the duration of the experiment.

OTHER TECHNICAL METHODS

HAEMOGLOBIN ESTIMATION

MARROW BIOPSY SMEARS

DETERMINATION OF GASTRIC ACIDITY

^{58}Co VITAMIN B_{12} ABSORPTION TESTS

SERUM IRON ESTIMATION

FAECAL FAT ESTIMATION

XYLOSE EXCRETION TESTS

INTESTINAL BIOPSY

ARTIFICIAL KIDNEY

Haemoglobin

Estimations were made photoelectrically on venous blood using Sequestrene as an anticoagulant. Values are expressed as percentages, 100% = 14.8 g./100 ml.. Estimations were made in the Haematology Departments, Western Infirmary and Vale of Leven Hospital.

Marrow biopsy smears

Sternal marrow biopsy was performed under local anaesthesia and preparations made by the method of Davidson et al (1943) and stained with May-Grunwald and Giemsa or with Leishmann. All blood and marrow smears were inspected by the author.

Gastric acidity

The presence or absence of gastric acid was established by the augmented histamine test (Kay 1953) in all patients presenting after January 1956 the pH of the aspirate being determined with pH papers and usually checked by the author. Histamine fast achlorhydria was diagnosed if the pH did

/not fall below 6.0. Prior to 1956 various stimulants to acid secretion and various pH indicators were used.

Co vitamin B_{12} absorption tests

The capacity to absorb orally administered Co vitamin B_{12} was determined by the method of Schilling (1953) as modified by Adams & Seaton (1961) using an oral dose of 0.5 ug 0.5 uc. The normal value was taken as $>7.5\%$ dose excreted. Tests were carried out after the experiments reported in this thesis had ended and intervals of up to three years elapsed between the patient coming under observation and tests being done. Tests with intrinsic factor were not carried out on patients who had received commercial preparations of vitamin B_{12} and heterologous intrinsic factor until at least a year after such treatment had been stopped: when necessary tests with homologous intrinsic factor were done on such patients. Heterologous intrinsic factor was obtained from several drug firms and the dose varied

/but was always considered to be optimal. All tests were conducted and the samples counted by the author.

Serum iron

The serum iron concentration was estimated by the method of Ramsay (1957) in the Biochemistry Department, Western Infirmary.

Faecal fat

The amount of fat in the faeces was estimated by the Soxhlet method of Harrison (1947). Three day collections on a ward diet were usually submitted for examination and the normal value was taken as < 5.0 g./day or $< 25\%$ of the dried weight of the sample. Estimations were made in the Biochemistry Departments, Western Infirmary and Vale of Leven Hospital.

D-Xylose absorption

The amount of d-xylose excreted in the urine in the five hours after oral administration of 25.0 g was estimated by the method of Roe & Rice (1948) in the Biochemistry Department, Western Infirmary.

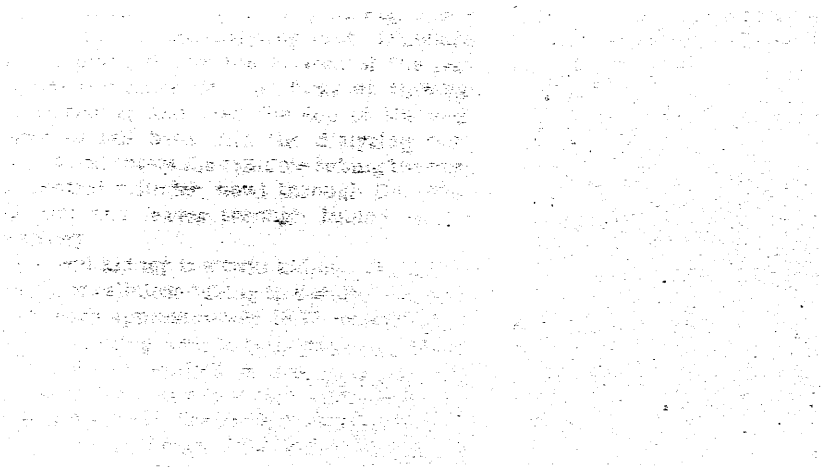
/The normal value was taken as > 5.0 g.

Gastric and intestinal biopsy

Samples of gastric or intestinal mucosa were obtained by an intestinal biopsy tube (Shiner 1956) or capsule (Crosby & Kugler 1957) passed under radiological control. The samples were fixed within five minutes and were stained by haematoxylin and eosin.

Artificial kidney

Details of the machine and operating instructions are given overleaf.



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DISPOSABLE COIL KIDNEY

The disposable coil kidney produced by Travenol Laboratories, Inc., is based on developmental work of Willem J. Kolff, M.D. of the Cleveland Clinic, Cleveland, Ohio. The principle by which the kidney operates and its clinical application has been reported by Kolff et al.[1,2,3] The kidney consists of permanent equipment (container, tank and pumps) and expendable equipment (coil dialyzing unit, arterial and venous sets).

DESCRIPTION

A schematic cross section of the coil is shown in Fig. 1. Two cellulose tubes (A) are enveloped in fiberglass screens (B). The coil is wrapped around a central cylinder. In operation, blood flows through the cellulose tubing and dialyzing fluid is pumped up through the screen.

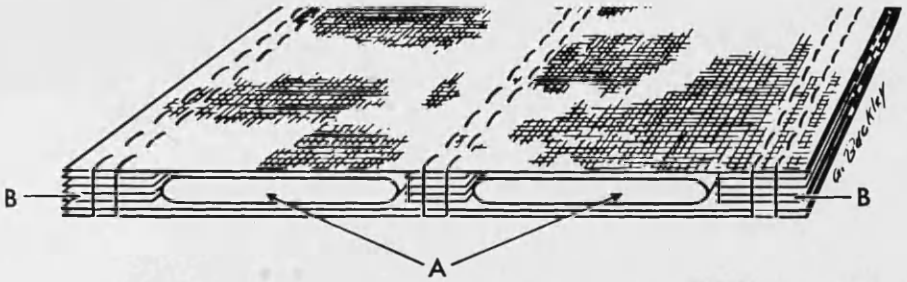

Fig. 1

The complete circuit of dialyzing fluid and blood is shown in Fig. 2. (A) in Fig. 2 is a cross section of the dialyzing unit. Dialyzing fluid is pumped into the bottom of the permanent container (B) and flows up through the screening and over the top of the container to fall back into the dialyzing fluid tank. Blood enters the cellulose tubing through the central cylinder, flows through the cellulose coil and leaves through tubing at the periphery.

The coil kidney is a twin kidney. It has two lengths of cellulose tubing in parallel arrangement; each approximately 10.75 meters long.*

The dialyzing area is approximately 19,000 sq. cm. Blood required for priming is approximately 1200 ml. At a flow rate of 200-400 ml. of blood per minute, the average amount of urea removed in a dialysis of 5-6 hours' duration is greater than 70 gms. There is a wide range in urea clearance and this is attributable to the wide variance in the initial levels of patient's blood urea, body weight and blood flow. The amount of urea clearance is 100-300 ml. per minute. Dialyzing fluid flow should be 3-5 liters per minute. The pump is designed to produce this rate of flow. The ultrafiltrate is approximately 300 ml./hour (2-6 lbs. weight loss during dialysis). If the outflow pressure is increased to 250 mm. Hg., then the loss is approximately 700 ml. per hour (6-8 lbs.).

Patient's blood is pumped from the radial artery into two tubes of the arterial set which are placed in parallel in the blood pump, see Fig. 2(E), so that a substantially equal flow of blood is obtained in each tube as it passes through the coil.

Blood leaves the coils through tubes in the periphery and flows through two filter chambers of the venous set (F), where air bubbles are trapped and blood flow can be observed. Blood passes through the filter before it returns to the patient's vein.

Dialyzing fluid is contained in a 100 liter tank (C) beneath the disposable unit. Temperature of the fluid is maintained at 39°C. (102°F.) and 90% O_2 plus 10% CO_2 is bubbled into it continuously through inlet (D).

*The cellulose tubing has a wall thickness of 1/1000 of an inch. When lying flat, it has a width of 4.5 cm.

Permanent equipment consists of:

1. Pump for blood (Sigmamotor pump, Model TL, available from Travenol).
2. A 100 liter tank for dialyzing fluid. The tank is mounted on wheels and provided with:
   a. A clear plastic cover and lamp.
   b. A platform for the blood pump.
   c. A container for the coil dialyzing unit.
   d. An opening in the tank through which O_2 and CO_2 can be admitted into the dialyzing fluid.
   e. A stand for hanging set and blood bottle.
   f. A central panel with electrical control switches and lights for the equipment g, h and i.
   g. Automatic temperature regulator set at 39°C. (102°F.) and dial thermometer.
   h. Dialyzing fluid pump which circulates fluid through the coil unit.
   i. Exhaust pump with attached hose which will drain the dialyzing tank in 5 minutes.

DIRECTIONS FOR ASSEMBLING

1. Remove Coil unit* from plastic bag and fasten in place in permanent container (B) with the closed end of the plastic core down. Blood inflow (arterial) tubes have clear vinyl plastic ends with cotton plugs. Outflow (venous) tubes are attached to the periphery and terminate in connectors. Corresponding inflow and outflow tubes from one of the cellulose coils are identified by a yellow band.
2. Cut off part of plastic ends on Coil inflow tubes to remove cotton plug. Remove protectors from plug-ins on arterial set and insert into cut ends of inflow tubes (G).

   CAUTION: *Avoid kinks in tubing between the arterial catheter and the pump tubes.*
3. Cut connecting tube on venous set in center and insert plug-ins on outflow tubes of coil unit (H).
4. Clamp side arms (L) on each filter chamber.
5. Hang filter chambers in upright position (filter at bottom) on stand using green plastic ring (I).
6. Put tap water in the 100 liter tank (C) and circulate it through the screening of the coil unit (A) for about 10 minutes.
7. Remove tap water from tank and fill with dialyzing fluid prepared as follows.
8. Start flow of 90% O_2 and 10% CO_2 into tank.

*NOTE: Should the coil unit show evidence of having been water soaked prior to use, DO NOT USE.

PREPARATION OF DIALYZING FLUID

Dialyzing fluid of the following composition is effective. Fluid of other electrolyte composition may be used.

Component	Gm. per 100 L.	Milliequivalents per liter					
		Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-
NaCl[1]	570 =	97	—	—	—	97	—
$NaHCO_3$	300 =	36	—	—	—	—	36
KCl	40 =	—	5	—	—	5	—
$CaCl_2$[2]	28 =	—	—	5	—	5	—
$MgCl_2$[3]	7.5 =	—	—	—	1.5	1.5	—
	Total	133	5	5	1.5	108.5	36

Invert Sugar (TRAVERT) 0.4%.[4]

Lactic acid to adjust pH to approx. 7.4

To maintain the pH during dialysis, 90% O_2 with 10% CO_2 is bubbled through fluid.

1. Dissolve NaCl, $NaHCO_3$ and KCl in a separate container of hot water.
2. Pour into dialyzing fluid tank and then add one liter of 40% TRAVERT (Invert Sugar).
3. Fill tank with warm tap water until 100 L. mark is reached. Temperature should be 39°C. (102°F.).

[1]When high serum sodium levels are present, 600 Gm. NaCl (138 mEq. of Na^+) is used in the first bath, to prevent a rapid shift in serum sodium concentration.

[2]15 or 30 Gm. KCl (1.8 to 3.7 mEq. of K^+) may be used, depending upon initial serum potassium levels.

[3]When $MgCl_2 \cdot 6H_2O$ is used, 15 Gm. is required to provide 1.5 mEq. per liter of Mg^{++} in the rinsing fluid.

[4]40% Travert (Invert Sugar) available in 1000 ml. pour bottle from Travenol Laboratories, Inc.

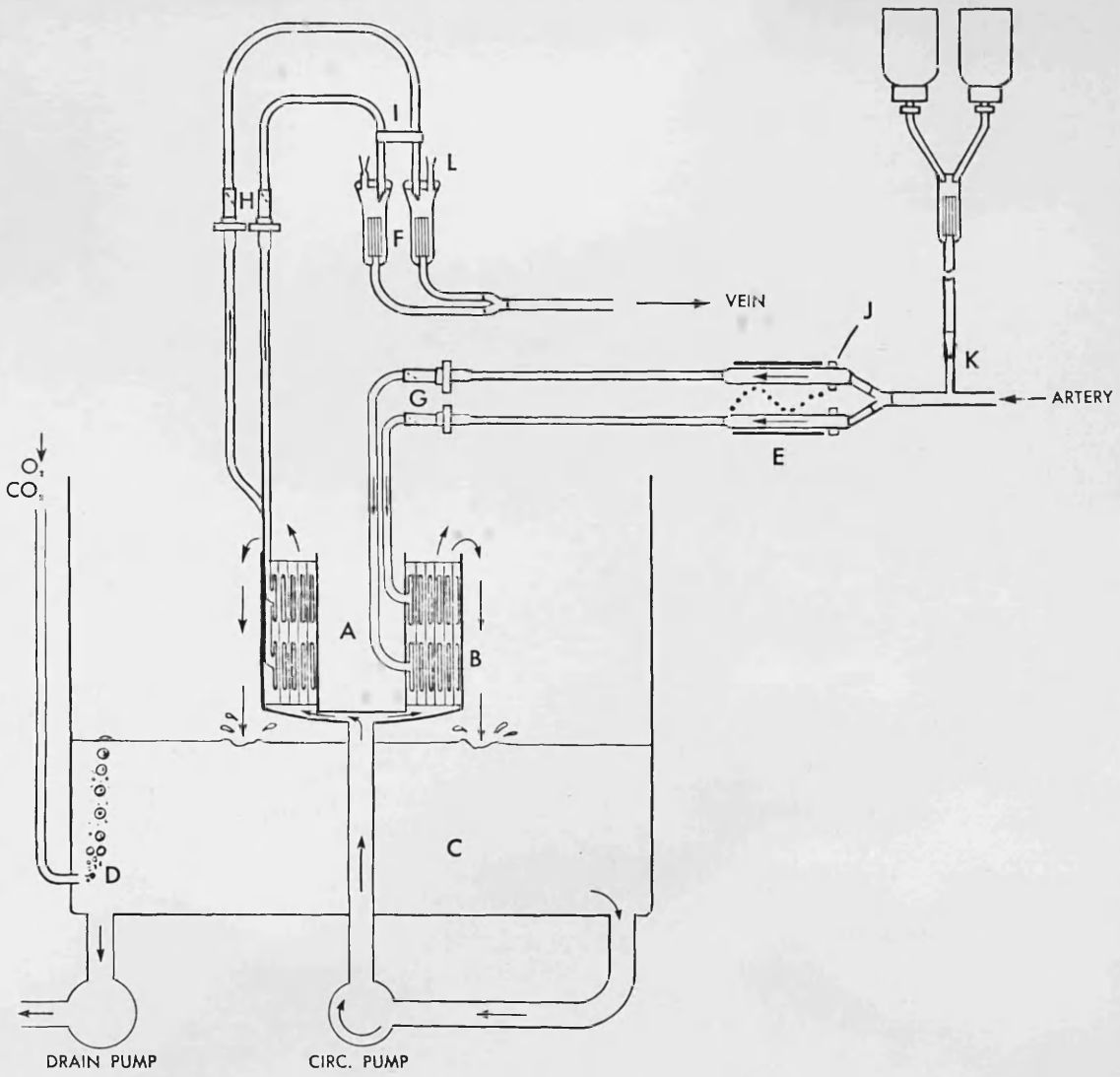

Fig. 2

4. Add lactic acid until pH is approx. 7.4.
5. $CaCl_2$ and $MgCl_2$ can now be added without formation of precipitate.
6. As a final check prior to testing the Kidney, spin a test tube of dialyzing fluid with some blood. If hemolysis does not occur, the solution is isotonic and satisfactory for use. However, if hemolysis occurs, the solution should be discarded.

If the patient has a serum potassium higher than 6 mEq./L., one-half the usual potassium concentration is used in dialyzing fluid. If patient is dehydrated, replacement

with parenteral fluid may be indicated because considerable ultrafiltrate is removed per hour.

To replace fluid during dialysis of patient:

1. Keep blood pump running.
2. Drain dialyzing fluid from tank with exhaust pump.
3. Stop recirculating pump before all the fluid is out of the tank and do not restart until the new batch of dialyzing fluid is prepared and in tank. If dialyzing fluid is pumped up through the Coil before the solution is made isotonic by dilution, hemolysis will occur in the cellulose tubes of the Coil.

IMPORTANT: Dialyzing fluid must be prepared immediately prior to dialysis since Ca and Mg precipitate at an increasing rate upon standing at body temperature. If precipitation occurs, the solution will become cloudy. Precipitation may also occur if the temperature of the water used in preparing the solution is over 110°F.

TESTING KIDNEY

1. Switch on circulating pump. Circulate dialyzing fluid through screen of coil unit. *Never test kidney before coil unit is wet.*
2. Place pump tubes of arterial set in Sigmamotor with plastic stops (J) just outside of pump housing on inflow side of pump. Care should be taken to properly position the pump tubes in the holes of the pump housing and that the tubing is not pinched when it is closed.
3. The male Luer adapter of standard administration sets may be plugged into the female Luer adapter at the end of the side arm on the arterial set (K). A Y-type administration set with filter is recommended for connecting to saline and blood bottles. Pump at least six liters of sterile saline solution through the unit using Sigmamotor pump. CAUTION: *Start pumping slowly. Stop Sigmamotor before bottles are empty to avoid introducing air into the system.*
4. Remove trapped air from pump tubes of arterial set by bending outflow ends of tubes upward.
5. Pump 100 ml. of heparinized blood through the unit. Follow this with sterile saline to which 250 USP heparin units per liter have been added. At this point, dialyzing fluid must be isotonic to prevent hemolysis.
6. Leaks can be detected from the appearance of blood in the dialyzing fluid.
7. Observe flow in filter chambers (F) to be sure that flow is substantially equal through both coils of the unit.
8. Calibrate blood pump* (E) with sterile saline by collecting outflow from kidney into graduate. Adjust flow to about 5% above average desired rate. (Flow rate will gradually decrease during dialysis.)

*NOTE: UA-11A—Model TL blood pump may be calibrated prior to use. See Instructions for the UA-11A pump.

PREPARATION OF THE PATIENT

Two units of fresh blood, preferably not older than 24 hours, must be available on the day of dialysis. Additional units not older than 3 days should be on hand in case of emergency. These bloods must be cross-matched with the patient's blood and with each other.

To prepare the patient:

1. A light breakfast is allowed if the patient desires, but should not be forced.
2. Shave both arms from above elbow to below wrist and both lower legs to the ankle.
3. Surgical cut-down on the patient's vessels should be done about one hour prior to connection so that the wounds have opportunity to clot. If poor blood flow is anticipated from the radial artery, it is advisable to insert a catheter high into the saphenous vein until its tip lies in the inferior vena cava.
4. Bring patient to artificial kidney and insert venous catheter. Catheters are filled with saline containing 1000 USP heparin units (approx. 10 mg.) per 100 ml.
5. Heparinize patient with 50 to 100 USP units (approx. 0.5-1.0 mg.) per kilogram body weight.
6. Insert arterial catheter.

When both catheters are in place, the artificial kidney is primed with blood.

PRIMING AND OPERATING KIDNEY

Approximately 2 units of blood are required to prime the kidney. Additional units should be available should a fall in blood pressure occur after connecting the patient to the apparatus. As pointed out, these bloods should be cross-matched with the patient's blood and with each other. Most patients require more than the initial 2 units of blood used to prime the kidney to prevent hypotension. *Prepare to observe this fall within the first hour.*

1. Add 1000 to 2000 USP heparin units (approx. 10-20 mg.) to each unit of fresh blood.
2. Pump blood into kidney through administration set connected to side arm (K) on Arterial Set. Fill the cellulose tubes and venous set with blood.

   NOTE: *It is advisable to create some additional pressure in the outflow line to make cellulose tubing distend. This may be done by briefly holding the outflow end of the venous set 100 cm. above the level of the kidney.*
3. Shut off blood pump (E). Clamp inflow line between side arm and blood pump and fill remainder of arterial tubing with blood.
4. To establish blood level one to two inches above filter in each chamber, carefully release clamp on side arms (L) to bleed air out. When desired level is attained, clamp securely. If level is too high, a syringe may be connected to adapter on side arm after removing protector and air forced in to reduce blood level. Medication may be added if desired by using a syringe in the same manner or preferably by injection into the rubber sections on the inflow or outflow tubes of the coil unit.
5. When blood appears at the outflow end of the unit, the catheters are attached to the venous and arterial sets.

Duration of treatment is 5-7 hours, during which time the patient needs continuous observation.

1000 USP heparin units (approx. 10 mg.) are injected into the inflow (arterial) line approximately every hour during the first hours of dialysis. Clotting time on blood drawn from the blood outflow line should be done hourly and should be maintained between 15 to 20 minutes (Lee-White glass tube method. End point—first evidence of clotting in tube). When clotting occurs within 20 minutes, 2000 USP heparin units are given to the patient.

No additional heparin is given during the last two hours of dialysis as long as the clotting time is longer than 10 minutes. When there is danger of occurrence of actual bleeding at the end of the dialysis, 1 mg. protamine sulfate, for each 100 USP heparin units used, is slowly given intravenously.

PRECAUTIONS

1. At a flow rate of 200-400 ml. of blood per min., a patient may bleed to death in a few minutes if a broken tube or loose connection is not noticed. Operator of the apparatus should keep the unit under observation at all times. Careful check and control of the patient's blood pressure and general condition should be made during the period of dialysis.

2. In patients with low blood pressure or small arteries, the arterial wall may be sucked into the opening of the catheter, a vacuum created and air sucked in through puncture holes in the tubes. Therefore, an open buret or inverted blood bottle with air vent is provided on the side arm of the arterial set. Vacuum may be broken by infusing a small amount of blood. If arterial flow is small, it may be necessary to hang the buret or blood bottle at a certain level and adjust the blood pump so that this level stays constant and no vacuum is produced. When blood flow is abundant, the buret or blood bottle should be clamped off.

3. Perforation of a vein with the outflow catheter may cause enormous hematomas. The return vein should be checked carefully during the first minutes of operation.

4. If the lower part of the saphenous vein is used for blood return, resistance may be so high that high back pressure builds up in the kidney. This also may occur if adhesive tape or position of the arm obstructs the return flow. These difficulties can be recognized by observing the pressure in the filter chambers.

5. Empty containers of electrolytes should be kept as proof for correct composition of the dialyzing fluid.

6. Temperature of the dialyzing fluid must be watched carefully at all times.

7. Blood levels in the filter chambers must be maintained throughout the dialysis.

8. Carefully position the pump tubes of the arterial set in the proper openings making sure that they are not pinched by the housing of the Sigmamotor pump.

9. The lamp should be placed no closer to the plastic cover than 8 inches.

10. Condensation on the plastic cover can be reduced by application of silicone to the inside surface by means of commercially available silicone impregnated cloths.

11. Dialyzing fluid must be isotonic before starting circulating pump to prevent hemolysis of blood in the cellulose tubes.

12. Heating element in tank *must be off* when it is not completely covered with solution.

EQUIPMENT

All of the items below are available through TRAVENOL LABORATORIES, INC.—MORTON GROVE, ILLINOIS.

EXPENDABLE EQUIPMENT

CATALOG LIST NO.	
U200	DISPOSABLE COIL KIDNEY (COMPLETE)
	This Unit Consists of Three Elements
	U200A COIL DIALYZING UNIT
	U200B INLET (ARTERIAL) SET
	U200C OUTLET (VENOUS) SET
U510	TAPERED CATHETERS FR 14 TO FR 5—12/BOX

NONEXPENDABLE EQUIPMENT

CATALOG LIST NO.	
UA-10	100 LITER DIALYZING PUMP-TANK UNIT—60 CYCLE
FUA-10	100 LITER DIALYZING PUMP-TANK UNIT—50 CYCLE
UA-11A	SIGMAMOTOR BLOOD PUMP, MODEL TL—60 CYCLE
FUA-11A	SIGMAMOTOR BLOOD PUMP, MODEL TL—50 CYCLE

FOR DIALYZING FLUID

CATALOG LIST NO.	
TG114	1000 ML. 40% TRAVERT (INVERT SUGAR) SOLUTION IN POUR BOTTLE

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TRAVENOL LABORATORIES, INC.

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STATISTICAL METHODS

SYMBOLS

t TEST

ANALYSIS OF VARIANCE

REGRESSION EQUATION

COEFFICIENT OF CORRELATION

COMPARISON OF REGRESSION COEFFICIENTS

CONFIDENCE LIMITS

The statistical techniques used were all standard methods and only a brief description is necessary. Frequent reference was made to works by Bernstein and Weatherall (1952), Fisher and Yates (1957) and Fisher (1958) and considerable guidance and tuition was given by Dr. R. A. Robb, Mitchell Lecturer in Statistics, University of Glasgow. All calculations however, were made by the author using a Facit calculating machine and the author accepts responsibility for the choice of methods, calculations and interpretation of results.

The following symbols were used:

x and y = when two values of the same sort were studied.

x, x_1, x_2 = when three values of the same sort were studied.

Y = the value of y corresponding to a given value of x obtained from the regression equation relating y to x.

S(x) = the sum of all the values of x.

S(X) = the sum of $S(x) + S(x_1) + S(x_2)$.....

/$S(x^2)$ = the sum of all the values of (x^2).

$(Sx)^2$ = the square of S(x).

n = the number of observations.

N = the sum of $n + n_1 + n_2 \ldots\ldots\ldots\ldots$

$(\bar{x})$= the arithmetic mean of S(x).

(xy) = the product of x and y.

S(xy) = the sum of all the values of (xy).

S(x) S(y) = the product of S(x) and S(y).

V = degrees of freedom

r = coefficient of correlation.

sd = standard deviation.

se = standard error.

t = a statistic calculated from the mean and the standard error.

F = a statistic calculated as the ratio of two estimates of variance.

P = the probability of a complex event occurring.

t TEST

The t test was used to test whether the means of two samples x and y differed significantly.

The standard deviation of all values was calculated from the equation

$$(sd)^2 = \frac{S(x - \bar{x})^2 + S(y - \bar{y})^2}{(n_x + n_y - 2)}$$

and the standard error of the difference between means from the equation

$$(se)^2 = (sd)^2 . \left(\frac{1}{n_x} + \frac{1}{n_y}\right)$$

The value of t was then found from the equation

$$t = \frac{(\bar{x} - \bar{y})}{se}$$

Where t was equal or greater than $t_{0.05}$ the means were significantly different at the 5% level of significance and where t was equal or greater than $t_{0.01}$ the means were significantly different at the 1% level of significance. The values for $t_{0.05}$ and $t_{0.01}$ were obtained from the table of the t distribution with $(n_x + n_y - 2)$ degrees of freedom.

ANALYSIS OF VARIANCE

This technique was used to test whether the means of say three samples differed significantly. The following values were calculated:

(1) The total sum of squares for three samples (A):

$$A = S(x^2) + S(x_1^2) + S(x_2^2) - \frac{(SX)^2}{N}$$

(2) The sum of squares between groups (B):

$$B = (Sx)^2 + (Sx_1)^2 + (Sx_2)^2 - \frac{(SX)^2}{N}$$

(3) The residual sum of squares was calculated by subtracting B from A and also independently from the equation:

$$S(x - \bar{x})^2 + S(x_1 - \bar{x}_1)^2 + S(x_2 - \bar{x}_2)^2$$

(4) The degrees of freedom between groups (V_1):

$$V_1 = \text{No. of groups} - 1.$$

(5) The total degrees of freedom (V_3):

$$V_3 = (N - 1)$$

(6) The residual degrees of freedom (V_2)

$$V_2 = V_3 - V_1$$

The values were then analysed as follows:

Source of Variation	Degrees of Freedom	Sum of Squares	Variance Mean Square	F
Between Groups	V_1	B	$B/V_1 = C$	C/D
Residual	V_2	A - B	$A-B/V_2 = D$	
TOTAL	V_3	A		

The probability of a difference between the means was found by comparing F with the values of $F_{0.05}$ and $F_{0.01}$ in the tables the degrees of freedom V_1 and V_2 corresponding to the greater and lesser mean square respectively.

The probability of a difference between $(\bar{x}_1)$ and $(\bar{x}_2)$ say was found by a t-test, t being found by dividing the difference of the means by the standard error which was obtained by taking the square root of the product of the residual mean square (D) and $(\frac{1}{n_1} + \frac{1}{n_2})$.

REGRESSION EQUATIONS

The linear regression of one variate y on another variate x was calculated from the equation:

$$Y = \bar{y} + b\,(x - \bar{x})$$

where $b = \dfrac{S(x - \bar{x}) \cdot (y - \bar{y})}{S(x - \bar{x})^2}$

i.e. $Y = \bar{y} + x \left(\dfrac{S(x - \bar{x}) \cdot (y - \bar{y})}{S(x - \bar{x})^2} \right) - \bar{x} \left(\dfrac{S(x - \bar{x}) \cdot (y - \bar{y})}{S(x - \bar{x})^2} \right)$

COEFFICIENT OF CORRELATION

The coefficient of correlation (r) was calculated from the equation:

$$r^2 = \frac{S(x - \bar{x})\ (y - \bar{y})}{S(x - \bar{x})^2} \cdot \frac{S(x - \bar{x})\ (y - \bar{y})}{S(y - \bar{y})^2}$$

$S(x - \bar{x})\ (y - \bar{y})$ being calculated from $S(x)$, $S(y)$, $S(xy)$ and n thus:

$$S(x - \bar{x})\ (y - \bar{y}) = \left\{S(xy) - \left(\frac{S(x)S(y)}{n}\right)\right\}$$

and $S(x - \bar{x})^2$ being calculated from $S(x)^2$ and n thus:

$$S(x - \bar{x})^2 = S(x^2) - \frac{(Sx)^2}{n}$$

and $S(y - \bar{y})^2$ similarly.

COMPARISON OF REGRESSION COEFFICIENTS

The method of testing whether two regression coefficients differed significantly was as follows. The following values were calculated for each group:

$$A = S(y - \bar{y})^2 - \left(\frac{(S(x - \bar{x})(y - \bar{y}))^2}{S(x - \bar{x})^2} \right)$$

$$V = (n - 2)$$

The standard error (se) of the difference of the regression coefficients was then obtained from the equation:

$$(se)^2 = \left(\frac{A + A_1}{V + V_1} \right) \cdot \left(\frac{1}{(S(x - \bar{x})^2} + \frac{1}{S(x_1 - \bar{x}_1)^2} \right)$$

The value of t was then found by dividing the difference of the regression coefficients by the standard error. The regression coefficients were not significantly different from each other at the 5% level of significance if the value of t was less than $t_{0.05}$ as found in the table of the t-distribution.

CALCULATION OF 95% CONFIDENCE LIMITS

The 95% confidence limits were calculated by multiplying the standard error of the regression line at various values of x by $t_{0.05}$ obtained from the table of the t distribution for (n - 2) degrees of freedom. The standard error was obtained from the equation.

$$se = \sqrt{\left\{\frac{S(y-\bar{y})^2}{n-1} \cdot 1+\frac{1}{n}\right\}+\left\{\frac{(x-\bar{x})^2}{S(x-\bar{x})^2}\right\}} \cdot \sqrt{\frac{(n-1)}{(n-2)} \cdot (1-r^2)}$$

calculating $\left(\frac{S(y-\bar{y})^2}{n-1}\right)$, $\left(1+\frac{1}{n}\right)$, $\left(\frac{(x-\bar{x})^2}{(S(x-\bar{x})^2)}\right)$ and

$\left(\left(\frac{(n-1)}{(n-2)}(1-r^2)\right)\right)$ separately.

CASE REPORTS

The reports are listed in alphabetical order. Particular attention is paid to diagnostic investigations. The age given is the age on admission to hospital unless otherwise stated. Abbreviations have been used freely, e.g. W.I.G. for Western Infirmary, Glasgow, V.L.H. for Vale of Leven Hospital, Alexandria, HFA for histamine fast achlorhydria as judged by the augmented histamine test (Kay 1953), IF for intrinsic factor and so on. Achlorhydria implies absence of gastric acid as demonstrated by methods other than the augmented histamine test. The investigations in some cases were not as complete as were desired for a variety of reasons and the diagnosis given for each case is based on the available evidence.

Case F.A. female, aged 58. Admitted W.I.G. May 1956. Macrocytic anaemia, Hb. 41%. Megaloblastic erythropoiesis. HFA. Treated with parenteral vitamin B_{12}. Reticulocytes 12% on 5th day. Schilling test 5.4%: with IF 24.5%.

Diagnosis: pernicious anaemia.

Case J.A. male aged 59. Admitted W.I.G. September 1958. Macrocytic anaemia, Hb. 26%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 30 uug/ml. Serum iron 140 ug/100 ml. Treated with 1 pint blood transfusion and parenteral ^{58}Co vitamin B_{12}. Serum iron 5 ug/100 ml on 2nd day: reticulocytes 33% on 6th day. Barium meal and follow through normal. Schilling test 0%: with IF 12.0%.

Diagnosis: pernicious anaemia.

Case C.B. male aged 65. Admitted W.I.G. August 1958. Dietary history poor. Macrocytic anaemia, Hb. 34%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 59 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 12% on 7th day. Barium meal and follow through normal. Xylose excretion 5.8 g. Faecal fat

/4.0 g/day (3 x 3 day collection). Schilling tests - 13.8%, 19.5%, 20.9%, 13.6%, 10.4% and 12.5%.

Diagnosis: dietary vitamin B_{12} deficiency.

Case B.C. female aged 65. Admitted W.I.G. November 1960. Macrocytic anaemia, Hb. 44%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 40 uug/ml. Serum iron 110 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 25 ug/100 ml on 3rd day: reticulocytes 19% on 7th day. Schilling test 0%: with IF 9.4%.

Diagnosis: pernicious anaemia.

Case E.C. female aged 61. Admitted V.L.H. February 1959. Macrocytic anaemia, Hb. 38%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 48 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 25% on 6th day. Schilling test 3%: with IF 12%.

Diagnosis: pernicious anaemia.

Case E.V.C. female aged 59. Admitted V.L.H. December 1958. Macrocytic anaemia, Hb. 32%. Megaloblastic erythropoiesis. HFA. Serum

/B_{12} 25 uug/ml. Treated with parenteral vitamin B_{12}. Reticulocytes 20% on 7th day. Schilling test 0%:with IF 13%.

Diagnosis: pernicious anaemia.

Case H.C. female aged 57. Admitted W.I.G. 1951. Macrocytic anaemia, Hb. 40%. Megaloblastic erythropoiesis. Achlorhydria. Treated with parenteral vitamin B_{12}. Reticulocytes 40% on 5th day. Later given oral B_{12} and IF but failed to maintain normal serum B_{12} levels and parenteral vitamin B_{12} recommenced 1958. Schilling test 4.4%: with IF 9.5%.

Diagnosis: pernicious anaemia.

Case I.C. female aged 70. Admitted W.I.G. October 1958. Macrocytic anaemia, Hb. 53%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 58 uug/ml. Serum iron 115 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 10 ug/100 ml on 2nd day: Reticulocytes 12% on 3rd day. Schilling test 0%: with IF 26.5%.

Diagnosis: pernicious anaemia

Case J.C. male aged 77. Admitted V.L.H. March

/1959. Macrocytic anaemia, Hb. 41%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 38 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 16% on 5th day. Schilling test 1.3%: with IF 11.1%. Diagnosis: pernicious anaemia.

Case M.C. female aged 65. Admitted W.I.G. April 1959. Macrocytic anaemia, Hb. 56%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 74 uug/ml. Serum iron 98 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 45 ug/100 ml on 3rd day. Reticulocytes 17% on 6th day. Schilling test 0%: with IF 10%. Diagnosis: pernicious anaemia.

Case T.C. male aged 22. Admitted W.I.G. after a haematemesis 1954. Partial gastrectomy. Recurrences of bleeding 1955, 1957, 1958. Laparotomy 1958 - angiomatous lesion in gastric remnant - total gastrectomy. Serum B_{12} fell to 140 uug/ml 14 months after operation and parenteral vitamin B_{12} begun. Schilling test 0%: with IF 8%. Diagnosis: IF deficiency following total

/gastrectomy.

Case C.D. male aged 55. Admitted W.I.G. March 1959. Macrocytic anaemia, Hb. 33%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 50 uug/ml. Serum iron 35 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 5 ug/100 ml on 2nd day: reticulocytes 35% on 5th day. Barium meal and follow through normal. Faecal fat 8.8 g./day (3 day collection). Repeated Schilling tests < 5%: with IF < 5%: after tetracycline 11.7%. Diagnosis: loop syndrome.

Case J.D. male aged 59. Admitted W.I.G. January 1960. Macrocytic anaemia, Hb. 41%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 45 uug/ml. Serum iron 160 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 15 ug/100 ml on 2nd day: reticulocytes 26% on 7th day. Schilling test 2.8%: with IF 23.4%.
Diagnosis: pernicious anaemia.

Case A.E. female aged 31, when diagnosed as having pernicious anaemia 1940. Treated with parenteral liver extract and later with

/vitamin B_{12} until 1957. Developed Addison's disease 1959 and treated with salt DOCA and later with Cortisone. Anaemia recurred and readmitted W.I.G. February 1960. Macrocytic anaemia, Hb. 50%. Megaloblastic erythropoiesis. HFA. Serum vitamin B_{12} 120 uug/ml. Serum iron 5 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 8% on 5th day: Faecal fat 2 g./day (3 day collection). Schilling test 0%: with IF 15%.

Diagnosis: pernicious anaemia. Addison's disease.

Case H.F. female aged 55. Admitted W.I.G. February 1959. Macrocytic anaemia, Hb. 42%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 30 uug/ml. Serum iron 150 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 45 ug/100 ml on 1st day: reticulocytes 21% on 5th day. Schilling test 0.7%: with IF 11.0%.

Diagnosis: pernicious anaemia.

Case J.F. female aged 72. Admitted W.I.G. January 1959. Macrocytic anaemia, Hb. 38%. Megaloblastic erythropoiesis. HFA. Serum

/B_{12} 52 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 23% on 5th day. Schilling test 4.2%: with IF 16.1%.

Diagnosis: pernicious anaemia.

Case M.F. female aged 66. Admitted W.I.G. 1959. Macrocytic anaemia, Hb. 54%. Megaloblastic erythropoiesis. Achlorhydria. Treated with parenteral vitamin B_{12} and later with oral vitamin B_{12} and IF. Failed to maintain normal serum B_{12} levels and parenteral vitamin B_{12} recommenced 1958. Schilling test 0%: with IF 8%.

Diagnosis: pernicious anaemia.

Case G.G. male aged 60. Admitted W.I.G. August 1959. Macrocytic anaemia, Hb. 60%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 40 uug/ml. Serum iron 225 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 160 ug/100 ml. on 2nd day. Reticulocytes 15% on 5th day. Schilling test 0%: with IF 8.7%.

Diagnosis: pernicious anaemia.

Case J.G. female aged 76. Admitted W.I.G. April

/1956. Macrocytic anaemia, Hb. 47%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 26 uug/ml. Treated with parenteral vitamin B_{12}. Reticulocytes 13% on 6th day. Schilling test 1.0%: with IF 15%.

Diagnosis: pernicious anaemia.

Case J.G.G. male aged 45. High partial gastrectomy for carcinoma of stomach May 1951. Readmitted W.I.G. October 1959. Macrocytic anaemia, Hb. 35%. Megaloblastic erythropoiesis. Serum vitamin B_{12} 36 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 34% on 5th day.

Diagnosis: presumed IF deficiency following high partial gastrectomy.

Case J.O.G. male aged 60. Admitted W.I.G. August 1956. Macrocytic anaemia, Hb. 73%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 45 uug/ml. Treated with parenteral vitamin B_{12}. Reticulocytes 14% on 7th day. Barium meal and follow through normal. Schilling test 1.5%: with IF 14.5%.

Diagnosis: pernicious anaemia.

Case J.S.G. male aged 73. Brother of Case J.O.G. Admitted W.I.G. March 1961. Macrocytic anaemia, Hb. 68%. Megaloblastic erythropoiesis. HFA. Serum vitamin B_{12} 40 uug/ml. Serum iron 130 ug/100 ml. Treated with parenteral vitamin B_{12} zinc tannic acid complex. Serum iron on 2nd day 23 ug/100 ml: rise in peripheral blood to normal. Schilling test 0%: with IF 22.7%.

Diagnosis: pernicious anaemia.

Case M.G. female aged 66. Admitted V.L.H. April 1956. Macrocytic anaemia, Hb. 38%. Megaloblastic erythropoiesis. HFA. Serum vitamin B_{12} < 50 uug/ml. Treated with parenteral vitamin B_{12}. Rise in peripheral blood to normal. Schilling test 0%: with IF 11.4%.

Diagnosis: pernicious anaemia.

Case A.H. male aged 48. Admitted W.I.G. September 1959. History of treatment of anaemia by liver injections 1929. Macrocytic anaemia, Hb. 42%. Megaloblastic erythropoiesis. Free gastric acid. Serum

/vitamin B_{12} 25 uug/ml. Serum iron 165 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 12% on 5th day. Faecal fat 6 g/day (3 day collection). Barium meal and follow through normal. Schilling tests 6.5%: with IF 1.9% and 1.5%.

Diagnosis: malabsorptive disease.

Case J.H. female aged 66. Admitted W.I.G. June 1959. Macrocytic anaemia, Hb. 26%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 73 uug/ml. Serum iron 250 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 55 ug/100 ml. on 2nd day: Reticulocytes 65% on 6th day. Schilling test 0%: with IF 14.7%.

Diagnosis: pernicious anaemia.

Case J.O.H. male aged 67. Admitted W.I.G. September 1956. Macrocytic anaemia, Hb. 49%. Megaloblastic erythropoiesis. HFA. Serum B_{12} < 50 uug/ml. Treated with parenteral vitamin B_{12}. Reticulocytes 12% on 5th day. Barium meal and follow through normal. Schilling test 1%: with IF 16.8%.

Diagnosis: pernicious anaemia.

Case T.H. male aged 45. Admitted W.I.G. June 1955. Total gastrectomy with splenectomy, partial pancreatectomy and oesophago-jejeunal anastomosis for carcinoma of stomach. June 1957 - Hb. 87%. Macrocytic film. Megaloblastic erythropoiesis. Serum B_{12} 100 uug/ml. Schilling test 0%: with IF 12.3%. Diagnosis: intrinsic factor deficiency following total gastrectomy.

Case C.J. female aged 53. Admitted W.I.G. March 1956. Macrocytic anaemia, Hb. 57%. Megaloblastic erythropoiesis. Diagnex test meal showed gastric acid. Faecal fat 2 g./day (4 day collection). Treated with parenteral vitamin B_{12} and oral folic acid and discharged on oral folic acid only. Readmitted November 1959. Macrocytic anaemia, Hb. 56%. Megaloblastic marrow. Serum B_{12} 45 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Schilling test 0%: with IF 34.1%. Later developed thrombocytopenia. Readmitted after a haematemesis October 1960. At laparotomy a malignant gastric polyp was

/found and partial gastrectomy performed as well as splenectomy. Gastric mucosa atrophic: jejeunal mucosa normal.

Diagnosis: pernicious anaemia.

Case D.J. male aged 68, on coming under observation in 1957 while being treated with oral B_{12} and IF. Diagnosis of pernicious anaemia previously established on macrocytic anaemia, megaloblastic erythropoiesis and achlorhydria. Failed to maintain normal serum B_{12} level on oral therapy and parenteral therapy instituted. Schilling test 1.9%: with IF 10.0%.

Diagnosis: pernicious anaemia.

Case J.J. male aged 40. Admitted W.I.G. August 1959. Macrocytic anaemia, Hb. 54%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 34 uug/ml. Serum iron 120 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 60 ug/100 ml on 2nd day: reticulocytes 10% on 6th day. Schilling test 0%: with IF 10.3%.

Diagnosis: pernicious anaemia.

/Case S.J. female aged 47. Vegetarian since childhood. Admitted W.I.G. September 1959. Macrocytic anaemia, Hb. 42%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 25 uug/ml. Serum iron 172 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 57 ug/100 ml on 2nd day: reticulocytes 26% on 4th day. Schilling tests 22%, 10.7%, 13.1%, 14.0%, 2.7%, 9.4%, 14.9%, 15.3%. Xylose excretion 1.6, 3.9 g. Faecal fat 3 - 4 g./day (3 day collections). Barium meal and follow through normal. Gastric biopsy normal. Jejeunal biopsy atrophic. Diagnosis: vitamin B_{12} deficiency probably due to deficient dietary intake in a patient with evidence of malabsorptive disease.

Case A.K. 1 male aged 56. Transferred W.I.G. with anuria and uraemia due to paralytic ileus following removal of gangrenous appendix. Dialysed with fall in blood urea from 596 mg.% to 290 mg.%. Clearance of injected vitamin B_{12} studied in 5th and 6th hours of dialysis. Died suddenly shortly after dialysis.

/Case A.K. 2 female aged 30. Transferred W.I.G. with anuria and uraemia following intra-abdominal haemorrhage after Caesarean section. Dialysed with fall in blood urea from 380 mg.% to 158 mg.%. Clearance of injected vitamin B_{12} studied in 5th and 6th hours of dialysis. Spontaneous diuresis with complete recovery.

Case A.K. 3 female aged 41. Transferred W.I.G. with anuria and uraemia. Brief history of pyrexia and oedema of legs. Dialysed on four occasions in next 30 days. Clearance of injected vitamin B_{12} studied during first dialysis. Renal biopsy showed appearances suggestive of chronic nephritis. Died in uraemic coma 47 days after admission. Diagnosis of chronic nephritis confirmed at autopsy.

Case A.K. 4 female aged 52. Admitted W.I.G. with polyarthritis, skin rash, oedema of all tissues, anuria, uraemia and later delirium and coma. Dialysed with fall in blood urea from 420 mg.% to 180 mg.%. Eventual recovery.

Case A.K. 5 female aged 43. Known mental

/defective. Admitted to neurosurgical unit for investigation of signs suggestive intracranial space occupying lesion. Cortical atrophy diagnosed on ventriculography. Became comatose and oliguric and found to have hyperglycaemia and uraemia. Transferred to W.I.G. Hyperglycaemia controlled by insulin but blood urea rose. Dialysed with fall in urea 520 mg.% to 120 mg.%. Clearance of injected B_{12} studied in 5th and 6th hours of dialysis. Died of pneumonia 13 days later.

Case A.K. 6 female aged 30. Known case of pulmonary tuberculosis with non functioning left kidney. Transferred to W.I.G. with anuria and uraemia. Dialysed with fall in urea 392 mg.% to 170 mg.%. Clearance of injected vitamin B_{12} studied in 4th and 5th hours of dialysis. Spontaneous diuresis and recovery.

Case H.K. female aged 62. Admitted W.I.G. March 1959. Macrocytic anaemia, Hb. 60%. Megaloblastic erythropoiesis. HFA. Serum

/vitamin B_{12} 50 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 15% on 6th day. Barium meal and follow through normal. Schilling test 4.0%: with IF 16.0%.

Diagnosis: pernicious anaemia.

Case J.K. male aged 68. Admitted V.L.H. November 1958. Macrocytic anaemia, Hb. 31%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 36 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 36% on 4th day. Schilling test 0%: with IF 15%.

Diagnosis: pernicious anaemia.

Case J.L. male aged 50. Admitted W.I.G. July 1956. Macrocytic anaemia, Hb. 33%. Megaloblastic erythropoiesis. HFA. Treated with parenteral vitamin B_{12}. Reticulocytes 11% on 5th day. Barium meal and follow through normal. Schilling test 3%: with IF 10.6%.

Diagnosis: pernicious anaemia.

CASE W.L. male aged 78. Admitted V.L.H. April 1958. Macrocytic anaemia, Hb. 38%.

/Megaloblastic erythropoiesis. HFA. Serum B_{12} 25 uug/ml. Treated with parenteral vitamin B_{12}. Rise in peripheral blood values to normal. Readmitted after cerebrovascular accident one year later and died in hospital. Gastric mucosa atrophic at post mortem.

Diagnosis: pernicious anaemia

Case J.A.M. female aged 80. Admitted W.I.G. May 1959. Macrocytic anaemia, Hb. 43%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 120 uug/ml. Serum iron 150 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 105 ug/100 ml on 2nd day. Reticulocytes 15% on 6th day. Schilling test 16%: with IF 6%. Barium meal and follow through - hiatus hernia. Faecal fat 25% of dried specimen.

Diagnosis: malabsorptive disease.

Case J.O.M. female aged 68. Admitted W.I.G. February 1960. Macrocytic anaemia, Hb. 51%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 42 uug/ml. Serum iron 135 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}.

/Serum iron 10 ug/100 ml on 2nd day. Reticulocytes 10%, on 6th day. Schilling test 1.6%: with IF 17.2%.

Diagnosis: pernicious anaemia.

Case E.McB. female aged 51. Admitted V.L.H. May 1958. Macrocytic anaemia, Hb. 36%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 37 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 18% on 5th day. Barium meal and follow through normal. Schilling test 1.5%: with IF 13.7%.

Diagnosis: pernicious anaemia.

Case M.McB. female aged 56. Admitted V.L.H. August 1956. Macrocytic anaemia, Hb. 39%. Megaloblastic erythropoiesis. HFA. Treated with parenteral vitamin B_{12}. Rise in peripheral blood values to normal. Schilling test 1.5%: with IF 12.1%.

Diagnosis: pernicious anaemia.

Case D.McC. male aged 52. Admitted W.I.G. June 1959. Macrocytic anaemia, Hb. 74%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 54 uug/ml. Serum iron 165 ug/100 ml.

/Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 45 ug/100 ml. on 2nd day: reticulocytes 17% on 5th day.

Diagnosis: pernicious anaemia.

Case I.McC. female aged 67. Admitted W.I.G. November 1956. Macrocytic anaemia, Hb. 41%. Megaloblastic erythropoiesis. HFA. Gastroscopy atrophic mucosa. Serum B_{12} 30 uug/ml. Treated with parenteral vitamin B_{12}. Reticulocytes 30% on 5th day. Schilling test 1%: with IF 17.2%.

Diagnosis: pernicious anaemia.

Case J.McC. male aged 63. Admitted W.I.G. December 1960. Macrocytic anaemia, Hb. 35%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 68 uug/ml. Serum iron 114 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 23 ug/100 ml on 2nd day. Reticulocytes 19% on 7th day. Schilling test 6%: with IF 20.5%.

Diagnosis: pernicious anaemia.

Case K.McD. female aged 47. Admitted W.I.G. June 1959. Macrocytic anaemia, Hb. 37%.

/Megaloblastic erythropoiesis. HFA. Serum B_{12} 50 uug/ml. Serum iron 115 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 70 ug/100 ml on 2nd day: reticulocytes 19% on 6th day. Barium meal and follow through normal.

Diagnosis: pernicious anaemia.

Case M.McD. female aged 69. Admitted W.I.G. April 1958. Macrocytic anaemia, Hb. 33%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 40 uug/ml. Serum iron 175 ug /100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 17 ug/100 ml on 4th day: reticulocytes 26% on 6th day.

Diagnosis: pernicious anaemia.

Case J.McF. female aged 68. Admitted W.I.G. April 1958. Macrocytic anaemia, Hb. 60%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 110 uug/ml. Treated with parenteral vitamin B_{12}. Reticulocytes 12% on 6th day. Barium meal and follow through normal. Schilling test 1%: with IF 17.2%.

Diagnosis: pernicious anaemia.

/Case H.McG. male aged 40. Admitted W.I.G. September 1959. Macrocytic anaemia, Hb. 45%. Megaloblastic erythropoiesis. HFA. Serum B_{12} < 25 uug/ml. Serum iron 250 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 75 ug/100 ml.on 2nd day: reticulocytes 16% on 5th day. Barium meal and follow through normal. Schilling test 3.8%: with IF 18.4%

Diagnosis: pernicious anaemia.

Case J.McG. male aged 67. Laparotomy November 1960 - intestinal diverticulosis. Admitted W.I.G. December 1960. Macrocytic anaemia, Hb. 54%. Megaloblastic erythropoiesis. Free gastric acid. Serum B_{12} 32 uug/ml. Serum iron 30 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 6% on 5th day. Faecal fat 9 g./day (3 day collection). Xylose excretion 4.7 g. Barium meal and follow through - diverticulosis of small bowel. Barium enema normal. Jejeunal biopsy normal. Schilling test 4%.

Diagnosis: loop syndrome - small intestinal

/diverticulosis.

Case T.McG. male aged 65. Admitted W.I.G. September 1956. Macrocytic anaemia, Hb. 33%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 51 uug/ml. Treated by one pint blood transfusion and parenteral vitamin B_{12}. Reticulocytes 19% on 6th day. Schilling test 2.1%: with IF 16.4%.

Diagnosis: pernicious anaemia.

Case C.McI. female aged 63. Admitted W.I.G. January 1958. Macrocytic anaemia, Hb. 52%. Megaloblastic erythropoiesis. HFA. Gastroscopy - atrophic mucosa. Serum B_{12} 40 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 30% on 5th day. Barium meal and follow through normal. Died of cerebrovascular accident ten months later. Gastric mucosa atrophic at post mortem.

Diagnosis: pernicious anaemia.

Case N.McL. male aged 47. Admitted W.I.G. 1944 with corrosive gastritis subsequent to drinking solder flux. Total gastrectomy with oesophagojejeunal anastomosis,

/October 1944. Readmitted June 1950 with macrocytic anaemia, Hb. 32% and megaloblastic erythropoiesis. Treated with parenteral vitamin B_{12} and oral folic acid with complete response. Later defaulted. Readmitted June 1957 - macrocytic anaemia, Hb. 41%. Megaloblastic erythropoiesis. Serum B_{12} < 60 uug/ml. Treated with parenteral vitamin B_{12}. Rise in peripheral blood values to normal. Schilling test 0.3%: with IF 19%. Diagnosis: IF deficiency following total gastrectomy.

Case J.McM. male aged 58. Admitted W.I.G. May 1955. Total gastrectomy for carcinoma of stomach. Readmitted September 1959 - macrocytic anaemia, Hb. 51%. Megaloblastic erythropoiesis. Serum B_{12} 68 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Rise in peripheral blood values to normal. Schilling test 4%: with IF 15.4%. Diagnosis: intrinsic factor deficiency following total gastrectomy.

Case J.McQ. male aged 54. Admitted W.I.G. 1949. Partial gastrectomy for duodenal ulcer. Admitted V.L.H. August 1959. Macrocytic

/anaemia, Hb. 56%. Megaloblastic erythropoiesis. HFA. Serum B_{12} < 25 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 20% on 6th day. Schilling test 3.0%: with IF 14%.

Diagnosis: intrinsic factor deficiency following partial gastrectomy.

Case E.P. female aged 71. Admitted W.I.G. February 1956. Macrocytic anaemia, Hb. 47%. Megaloblastic erythropoiesis. HFA. Treated with parenteral vitamin B_{12}. Reticulocytes 11%, on 6th day. Schilling test 0%: with IF 11%.

Diagnosis: pernicious anaemia.

Case M.P. female aged 48. Admitted V.L.H. May 1958. Macrocytic anaemia, Hb. 72%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 60 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Rise in peripheral blood values to normal. Schilling test 1.6%: with IF 13.1%.

Diagnosis: pernicious anaemia.

Case C.R. female aged 69. Admitted W.I.G. August 1959. Macrocytic anaemia, Hb. 39%.

/Megaloblastic erythropoiesis. HFA. Serum B_{12} < 25 uug/ml. Serum iron 138 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 0 ug/100 ml on 3rd day: reticulocytes 30% on 5th day. Barium meal and follow through normal. Schilling test 0%: with IF 14.4%.

Diagnosis: pernicious anaemia.

Case I.R. female aged 71. Admitted W.I.G. September 1956. Macrocytic anaemia, Hb. 44%. Megaloblastic erythropoiesis. HFA. Treated with parenteral vitamin B_{12}. Reticulocytes 14% on 6th day. Barium meal and follow through normal. Schilling test 0%: with IF 18.2%.

Diagnosis: pernicious anaemia.

Case M.R. female aged 53. Admitted W.I.G. May 1958. Macrocytic anaemia, Hb. 19%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 62 uug/ml. Treated with one pint blood transfusion and parenteral ^{58}Co vitamin B_{12}. Reticulocytes 20% on 5th day. Schilling test 5.6%: with IF 13.2%.

Diagnosis: pernicious anaemia.

Case S.R. male aged 70. Admitted W.I.G. June 1958. Macrocytic anaemia, Hb. 39%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 25 uug/ml. Serum iron 125 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 0 ug/100 ml on 3rd day: reticulocytes 30% on 5th day. Barium meal and follow through normal. Schilling test 0%: with IF 14.4%.

Diagnosis: pernicious anaemia.

Case W.R. male aged 51. Admitted W.I.G. 1946. Partial gastrectomy for duodenal ulcer. Readmitted September 1959. Microcytic anaemia, Hb. 60%. M.C.H.C. 25.7%. Normoblastic erythropoiesis. Serum iron 87 ug/100 ml. Iron binding capacity 410 ug/100 ml. Serum B_{12} 172 uug/ml. Given parenteral ^{58}Co vitamin B_{12} prior to iron therapy.

Diagnosis: iron deficiency following partial gastrectomy.

Case A.S. female aged 67. Admitted W.I.G. 1952. Macrocytic anaemia, Hb. 42%. Megaloblastic erythropoiesis. Achlorhydria. Treated with

/parenteral vitamin B_{12} with full response. Barium meal normal. Schilling test 2.8%: with IF 15.5%.

Diagnosis: pernicious anaemia.

Case J.S. female aged 51. Admitted V.L.H. April 1959. Macrocytic anaemia, Hb. 58%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 50 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 30% on 5th day. Barium meal and follow through normal. Schilling test 0%: with IF 12%.

Diagnosis: pernicious anaemia.

Case M.S. female aged 68. Admitted V.L.H. October 1959. Macrocytic anaemia, Hb. 44%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 42 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 22% on 5th day. Died suddenly in acute cardiac failure on 12th day. P.M. - myocardial infarct: oedematous lungs: gastric mucosal atrophy.

Diagnosis: pernicious anaemia.

Case M.J.S. female aged 67. Admitted W.I.G. February 1961. Microcytic anaemia, Hb.44%,

/M.C.H.C. 23%. Normoblastic erythropoiesis. Serum iron 20 ug/100 ml. Serum B_{12} 390 uug/ml. Given vitamin B_{12} zinc tannic acid complex parenterally prior to iron therapy. Barium meal - duodenal diverticulum. Barium enema normal.

Diagnosis: iron deficiency anaemia (? cause).

Case S.S. male aged 54. Admitted W.I.G. April 1958. Macrocytic anaemia, Hb. 37%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 56 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 16% on 5th day. Schilling test 1.3%: with IF 15.7%.

Diagnosis: pernicious anaemia.

Case A.T. female aged 64. Investigated at OPD V.L.H. 1958. Macrocytic anaemia, Hb. 79%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 60 uug/ml. Treated with parenteral vitamin B_{12} with full response. Barium meal and follow through normal. Schilling test 0%: with IF 22%

Diagnosis: pernicious anaemia.

Case J.T. female aged 61. Admitted W.I.G. January 1961. Macrocytic anaemia, Hb. 50%. Megaloblastic erythropoiesis. HFA. Serum B_{12} <25 uug/ml. Serum iron 90 ug/100 ml. Treated with parenteral vitamin B_{12} zinc tannic acid complex. Serum iron on 3rd day 35 ug/100 ml: reticulocytes 23% on 5th day. Diagnosis: pernicious anaemia.

Case J.O.T. male aged 50. Investigated at OPD W.I.G. December 1960. Macrocytic anaemia, Hb. 35%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 62 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}. Reticulocytes 18% on 5th day. Schilling test 0%: with IF 13.9%. Diagnosis: pernicious anaemia.

Case M.T. female aged 59. Admitted V.L.H. October 1958. Macrocytic anaemia, Hb. 56%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 50 uug/ml. Treated with parenteral ^{58}Co vitamin B_{12}: reticulocytes 17% on 5th day. Schilling test 3.7%: with IF 12.5% Diagnosis: pernicious anaemia.

Case R.T. male aged 78. Admitted W.I.G. March 1958. Macrocytic anaemia, Hb. 31%. Megaloblastic erythropoiesis. HFA. Serum B_{12} 34 uug/ml. Serum iron 125 ug/100 ml. Treated with parenteral vitamin B_{12}. Serum iron on 4th day 60 ug/100 ml: reticulocytes 28% on 5th day. Schilling test 0.7%: with IF 12.7%

Diagnosis: pernicious anaemia.

Case I.W. female aged 41. Admitted W.I.G. August 1956. Macrocytic anaemia, Hb. 42%. Megaloblastic erythropoiesis. HFA. Treated with parenteral vitamin B_{12} with full response. Schilling test 0%: with IF 18.6%.

Diagnosis: pernicious anaemia.

Case M.W. female aged 77. Admitted W.I.G. April 1955. Macrocytic anaemia. Megaloblastic erythropoiesis. Achlorhydria. Treated with parenteral vitamin B_{12}. Discontinued maintenance treatment. Readmitted April 1959. Macrocytic anaemia, Hb. 72%. Megaloblastic erythropoiesis.

/HFA. Serum B_{12} 30 uug/ml. Serum iron 172 ug/100 ml. Treated with parenteral ^{58}Co vitamin B_{12}. Serum iron 52 ug/100 ml on 2nd day: reticulocytes 9% on 4th day. Schilling test 0%.

Diagnosis: pernicious anaemia.

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