PAPERS AND ORIGINALS

Experimental Approach in Chemical Pathology*

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In this lecture I shall try to give those who are not research workers some idea of what research in chemical pathology can be like, and how pure and applied research are interrelated. The lecture is not addressed primarily to other research workers, though I hope it will have some interest for them. It is very difficult to understand the nature of research without having had some experience of it. This is why research work seems to many medical students and doctors a dull and often incomprehensible activity. The easiest kind of research to appreciate, from the outside, is applied research in which there is a clearly defined objective. What is far less easy to understand is the value of basic research undertaken out of academic curiosity and how the results can contribute, sometimes quite unexpectedly, to the understanding of human disease. I am going to describe examples of both kinds of research from my own laboratory, and shall begin with the more basic type of investigation.

Protein Absorption

My first example comes from the field of protein absorption. Here I should like to take you back for a historical view. In H. G. Wells's novel *The Time Machine*, published in 1895, you will see that when the Time Traveller returns worn out and hungry from his harrowing experiences in the future he refuses to tell his story until fortified by mutton. "I won't say a word," he says, "until I get some peptone into my arteries." His remark accurately reflects our views on protein absorption in the late nineteenth century. It was known that proteins were digested by gastric and pancreatic enzymes to smaller fragments, peptones—or polypeptides as we would call them—but it was not realized that digestion went any further. Consequently it was thought that proteins were absorbed into the blood stream as peptones. In 1901, however, Cohnheim discovered that fluid collected from the intestinal lumen contained enzymes ("erepsin") which would hydrolyze peptides to amino-acids. On this foundation there was gradually built up what may be called the classical theory of protein absorption (Matthews and Laster, 1965). This was that proteins were completely hydrolyzed to free amino-acids within the lumen of the gut, and that these were absorbed by simple diffusion.

In the early 1950s it was shown that amino-acids were absorbed by stereochemically specific active mechanisms (Gibson and Wiseman, 1951; Wiseman, 1951; Matthews and Smyth, 1952). This stimulated a vast amount of work on the details of absorption of free amino-acids (Wiseman, 1968) but did not alter the central tenet of the classical theory of protein absorption—complete intralumen hydrolysis to free amino-acids. This theory, though open to serious criticism (Fisher, 1954), has dominated our thinking for more than 60 years. It was once so strong that no observation that was not compatible with it was accepted. Thus Messerli (1913) reported that "peptone" was absorbed more rapidly than the equivalent amino-acids. This could not happen if the classical theory were correct, and the work was ignored.

PEPTIDE ABSORPTION IN MAN

The breakdown of the classical theory did not begin until Newey and Smyth (1959, 1960, 1962) showed that several dipeptides, including glycylglycine, were hydrolyzed to amino-acids during absorption, and that this hydrolysis did not take place within the intestinal lumen but in association with the small-intestinal mucosal cells, and probably inside them. These very important experiments were not followed up until comparatively recently.

In 1967 my colleagues and I undertook an investigation of peptide absorption in man, in order to see whether it was impaired in small-intestinal disorders such as adult coeliac disease (Craft and Matthews, 1968; Craft *et al.*, 1968). We thought that by giving a peptide orally and following amino-acid concentrations in peripheral plasma we might be able to detect mucosal peptidase deficiency. To obtain control curves we gave the amino-acid glycine, the dipeptide glycylglycine, and the tripeptide glycylglycylglycine to normal volunteers. The doses we used contained the same number of amino-acid units whether glycine or one of the peptides was given. We expected

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that since the peptides would have to be hydrolyzed during absorption, absorption of glycine from the dipeptide and tripeptide would be somewhat slower than from the free amino-acid. What actually happened was so unexpected that at first we had some difficulty in believing our own results (Fig. 1). They indicated that glycine was absorbed most rapidly from the tripeptide, less rapidly from the dipeptide, and *least* rapidly from the free amino-acid.

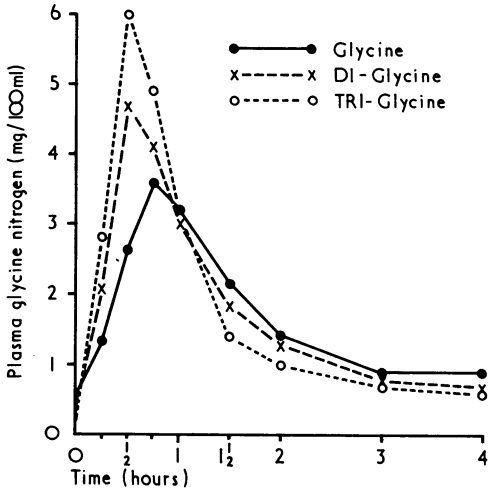

FIG. 1—Mean plasma glycine nitrogen concentrations in normal volunteers following equivalent doses of glycine, glycylglycine, and glycylglycylglycine by mouth. The doses (Gly 10 g, Gly-gly 8·8 g, Gly-gly-gly 8·4 g) each contained the same number of glycine units, so that after hydrolysis the doses of peptide would be identical with the dose of free glycine. Intralumen hydrolysis, therefore, could not possibly account for more rapid absorption from the peptides than from the free amino-acid. (From Craft *et al.*, 1968, by permission of the Editor of *Gut*.)

During this work a second interesting phenomenon was found—avoidance of competition for intestinal transport between amino-acids (Matthews *et al.*, 1969; Cheng *et al.*, 1971). The amino-acids fall into several "transport groups," the members of each group apparently sharing a common carrier (Matthews, 1968; Wiseman, 1968). When the small intestine is presented with a mixture of two amino-acids of the same transport group absorption of one is inhibited by the other. When these amino-acids are presented as a dipeptide, however, this inhibition is avoided (Fig. 2). In addition to suggesting that peptide uptake may be a more effective process than

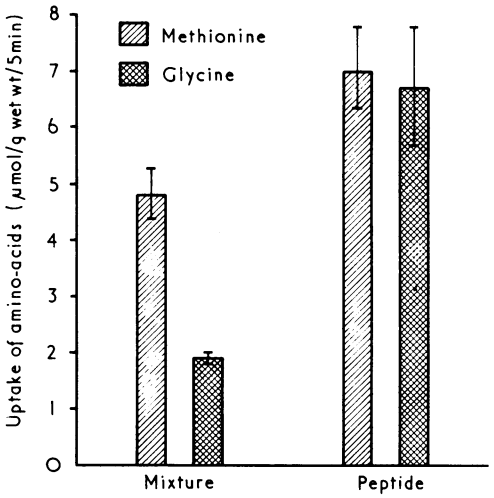

FIG. 2—Uptake of amino-acids from amino-acid mixture and the equivalent dipeptide by rings of rat small intestine in vitro. Mean ± S.E. of mean. (Reproduced by permission of the Editor of the *Journal of Clinical Pathology*.) (Data from Cheng *et al.*, 1971.)

This phenomenon, which has since been confirmed by other techniques and in other laboratories (Matthews *et al.*, 1968; Asatoor *et al.*, 1970a), could not possibly occur if peptides were hydrolyzed in the gut lumen. We had, in fact, a very simple demonstration that the intestinal mucosa took up intact peptides in man, as Newey and Smyth had shown in animals. In addition, we had found a new phenomenon—that absorption of amino-acid from peptide could be more rapid than from the equivalent free amino-acid. Here is an important feature of research—its unpredictability. In obtaining a series of control values for a clinical investigation, we were presented with a physiological finding interesting enough to provide a powerful stimulus for further research on the mechanisms of intestinal peptide uptake.

INTESTINAL PEPTIDE TRANSPORT IN ANIMALS

At this point the original purpose of the work became a relatively minor objective. The investigation in small-intestinal disease continued, and it was eventually shown that the rate of absorption from peptides could be limited by peptidase deficiency (Sadikali, 1971), but our main effort was now concentrated on "pure" research: finding out more about the mechanisms of intestinal peptide transport through animal experiments. First of all we showed, using small intestine in vivo and in vitro, that the phenomenon of more rapid absorption from peptides than from the equivalent amino-acids was not confined to the series glycine, glycylglycine, glycylglycylglycine, but also occurred with other dipeptides and tripeptides, including mixed peptides made up of two or more different amino-acids (Matthews *et al.*, 1969; Cheng *et al.*, 1971). Others have confirmed this, and it has now been shown for more than a dozen small peptides (Matthews, 1971). We also showed that it occurred in omnivorous, carnivorous, and herbivorous species (Lis *et al.*, 1971).

uptake of free amino-acids, this indicates particularly clearly that uptake of intact peptides by the cells precedes their hydrolysis. It cannot be explained by hydrolysis followed by uptake of free amino-acids, however near the cell surface hydrolysis take place. Further details of these and related investigations are described elsewhere (Craft *et al.*, 1968; Matthews *et al.*, 1968, 1969; Crampton *et al.*, 1970; Cheng *et al.*, 1971; Lis *et al.*, 1971).

At this stage it seemed to us important to attempt to answer the question of whether mucosal peptide uptake played a significant part in the whole process of protein absorption. This problem could not be approached through the study of individual peptides, since there are over 8,000 possible dipeptides and tripeptides alone. So we took a protein—lactalbumin—of completely known composition, so that we could make up an exactly equivalent amino-acid mixture. We digested the protein with pancreatic enzymes, producing a mixture largely composed of peptides of two or three amino-acid units, and compared the rate of absorption of this mixture from rat small intestine with that of the equivalent amino-acids. Absorption from the pancreatic digest was about twice as rapid as from the amino-acids. Similar findings were obtained with three other proteins (Crampton *et al.*, 1971). Much work remains to be done, but these experiments strongly suggest the importance of the part played by peptide uptake in protein absorption.

Hartnup Disease and Cystinuria

I have been talking for some time about pure research in intestinal transport. What possible application can this have to patients? In fact, patients came into the story at a comparatively early stage. In 1969 a woman of 23 was admitted to the Westminster Hospital with an acute psychiatric disorder and the skin rash of pellagra. Investigation showed that she was suffering

from the rare metabolic disorder of Hartnup disease. Hartnup disease is a disorder of renal and intestinal transport of neutral amino-acids. Apparently the carrier protein responsible for uptake of many amino-acids of this group is defective or lacking. The renal transport defect was discovered first. When Milne *et al.* (1960) and Milne (1964) showed that the transport defect was shared by the intestine this observation explained certain puzzling features of the disease, but at the same time created an additional puzzle. Why, if the patients could scarcely absorb several neutral amino-acids, including essential ones, were they comparatively well nourished, and why did they have no complaint of digestive disturbances after protein meals? This could not be adequately explained in terms of the views on protein absorption that were current at the time.

When this particular patient was admitted, by the good fortune that is so important in research, we had an unusually favourable combination of circumstances. We had the right patient, who was most helpful in volunteering for a series of tests when her acute illness was cured with nicotinic acid, and the right background of basic research and research interest in Hartnup disease. Professor Milne took the opportunity, and having established by an oral tolerance test that the patient could not absorb free L-histidine, which is one of the "affected" amino-acids in this disease, he gave the dipeptide carnosine, which is composed of L-histidine and the "unaffected" amino-acid β-alanine. From this dipeptide, L-histidine was absorbed relatively well (Navab and Asatoor, 1970). The patient was given two other dipeptides containing amino-acids which were poorly absorbed in the free state, and these amino-acids were also relatively well absorbed from the peptides (Asatoor *et al.*, 1970b). At this exciting stage there was a further stroke of good fortune. In spite of the rarity of Hartnup disease, my colleagues and I had the opportunity of investigating a second case, and the ability of the intestine to transport "affected" amino-acids from dipeptides was shown with two further peptides (see Table and Fig. 3) (Tarlow *et al.*, 1970).

Hartnup Disease: Amino-acids which are Poorly Absorbed when Free but Absorbed Relatively Well from Dipeptides

L-Histidine	from β-alanyl-L-histidine (carnosine)
L-Tryptophan	from glycyl-L-tryptophan
L-Tyrosine	from glycyl-L-tyrosine
L-Histidine	from glycyl-L-histidine
L-Phenylalanine	from L-phenylalanyl-L-phenylalanine

β-alanine and glycine are not affected by the amino-acid transport defect of Hartnup disease.

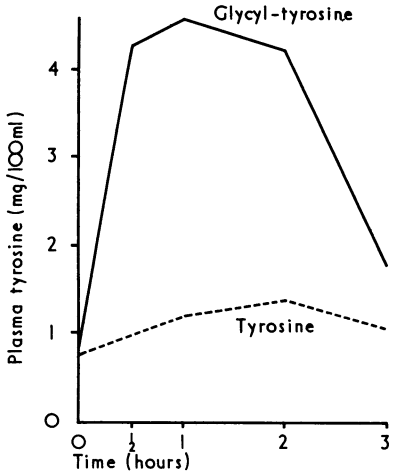

FIG. 3—Plasma tyrosine concentrations in a case of Hartnup disease following equivalent doses of free tyrosine and the dipeptide glycyltyrosine. (Data from case reported by Tarlow *et al.*, 1970.)

The results suggest that a long-standing puzzle of Hartnup disease is solved. We believe that the patients avoid intestinal disturbances and are able to maintain their general nutrition because they retain the ability to absorb "affected" amino-acids when they are presented in the form of peptides, which are abundant in the intestinal lumen during protein digestion. This shows how the application of basic research which is being carried on for its own sake can suddenly illuminate a problem in human disease. Now I should like to illustrate if I can how utilization of the opportunities presented by a clinical disorder can add in its turn to basic knowledge.

One of the most important theoretical questions about intestinal peptide uptake is this. Are dipeptides taken up from the intestinal lumen by amino-acid uptake mechanisms or do they have an independent uptake mechanism or mechanisms? The Table shows that whereas four of the five dipeptides given in Hartnup disease were mixed peptides consisting of one "affected" and one "unaffected" amino-acid, one of them—L-phenylalanyl-L-phenylalanine—consisted entirely of an "affected" amino-acid. This peptide was deliberately chosen to answer our theoretical question. The fact that "affected" amino-acids were absorbed from the mixed peptides could be explained by the hypothesis that the "unaffected" amino-acid in these peptides permitted uptake of dipeptide by the amino-acid uptake mechanisms that remain intact in Hartnup disease. However, the ability to absorb amino-acid from a peptide composed entirely of an "affected" amino-acid for which the intestinal carrier is lacking cannot be explained in this way. It is necessary to postulate that the mucosal cells possess a peptide uptake system that is independent of the uptake systems for free amino-acids (Asatoor *et al.*, 1970b; Matthews, 1971). This is of special interest in view of the fact that bacterial cells have been known for some years to possess independent peptide uptake systems (Payne, 1968), and the observation suggests that the biological distribution of these systems may be very wide.

Once it had been shown that the ability to absorb "affected" amino-acids from peptides was retained in Hartnup disease, it became clear that this phenomenon might be found in other types of amino-acid transport defect. It has in fact now been shown in the much commoner defect of cystinuria (Hellier *et al.*, 1970; Asatoor *et al.*, 1971). Thus the study of amino-acid transport defects in man has added to our understanding of the normal processes of protein absorption, providing information that would be difficult to obtain in any other way. It has confirmed in a striking way that uptake of intact peptides occurs in the human gut and has suggested that this mode of absorption is quantitatively important in man. It has also indicated that the uptake mechanism for dipeptides is independent of those for free amino-acids, and further research on this point is in progress in animals.

The recent accummulation of evidence that peptide uptake plays a significant part in protein absorption has nutritional implications that are by no means entirely academic. It is true that a healthy man can survive on diets containing only free amino-acids in place of the protein component (Winitz *et al.*, 1970). Nevertheless, it has now become clear that diets of this type, which have recently been made available commercially for use in metabolic disorders, are unphysiological and that in some circumstances they could be dangerous (Matthews, 1971). Their use in cases of amino-acid transport defects might have serious results.

Tobacco Amblyopia

In the remaining part of the lecture I shall deal, much more briefly, with a rather different kind of research. Whereas the work on peptide absorption was mainly a matter of ideas, and at first used simple and inexpensive methods, my second example, from the field of vitamin B_{12} metabolism, illustrates the potential of a new technique that was difficult and expensive to set up. It shows how a technical advance can suggest ideas for fresh lines of investigation, some of them quite unrelated to the purpose for which the technique was initially established. The investigation as originally conceived was applied research

with the definite and restricted objective of answering the following questions: Is there an excess of cyanocobalamin in the blood in tobacco amblyopia, and is this the cause of the condition?

Smith (1961) put forward the hypothesis that tobacco amblyopia might be the result of conversion of serum hydroxocobalamin (which he believed to be the active form of B_{12}) to cyanocobalamin by the cyanide in tobacco smoke. At the time the forms of B_{12} in blood were unknown, and we had no means of identifying them, so that Smith's hypothesis was unverifiable (Matthews, 1962), though Dr. John Wilson and I were later able to obtain some indirect evidence suggesting that smoking might interfere in some way with B_{12} metabolism (Matthews *et al.*, 1965; Wilson and Matthews, 1966; Linnell *et al.*, 1968). Some years ago it was shown that the active forms of B_{12} were neither hydroxocobalamin nor cyanocobalamin but two coenzyme forms, deoxyadenosylcobalamin and methylcobalamin (Toohey and Barker, 1961; Lindstrand and Ståhlberg, 1963; Lindstrand, 1964). Lindstrand and Ståhlberg (1963), using paper chromatography and bioautography, separated plasma cobalamins for the first time, and showed that the major form of B_{12} in plasma was methylcobalamin. Their work made it possible, in principle, to investigate Smith's hypothesis, and with Dr. Lindstrand's assistance we obtained some early chromatograms suggesting that the problem was worth pursuing (Lindstrand *et al.*, 1966).

Technique

The initial object of our work was to refine the technique of Lindstrand and Ståhlberg, which required great quantities of blood and gave only qualitative results, and place it on an approximately quantitative basis. The technical difficulties were formidable, owing to the light-sensitivity of several important forms of B_{12}, and the very small quantities of cobalamins (between 10^{-11} and $^{-12}$g) which had to be separated and estimated.

In the method we have developed the plasma extracts are run on thin-layer chromatograms with precautions against light, and the separated cobalamins, which are quite invisible, are identified by the growth-response of a B_{12}-dependent organism (Linnell *et al.*, 1969c, 1970, 1971). The resulting spots, stained crimson with a redox indicator, can thus be located, and estimated by photometric scanning. Normal values have been established and the method now gives complete separation of four different plasma cobalamins (Linnell *et al.*, 1970). The main component in healthy people and hospital control subjects is always methylcobalamin. Some deoxyadenosylcobalamin is also present, and some hydroxocobalamin. The proportion of cyanocobalamin is never more than a few percent even in the heaviest smokers (Linnell *et al.*, 1969b, 1969c, 1971; Wilson *et al.*, 1971). A chromato-bioautogram from a case of tobacco amblyopia is shown in Fig. 4. The proportion of cynaocobalamin in the plasma is considerably raised (up to about 30%) in many cases (Wilson *et al.*, 1971), so that part of our original question is answered. Smith's remarkable speculation was right in this respect. What is less certain is the significance of the finding. My colleagues and I believe that it probably plays no part in the pathogenesis of the condition, and is an incidental reflection of impaired ability to metabolize cyanide.

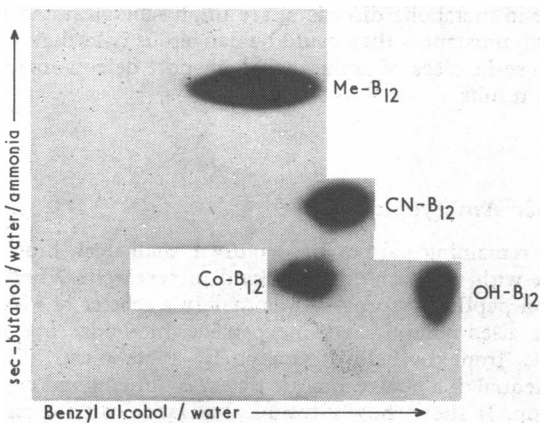

FIG. 4—Chromatography and bioautography of plasma from a case of tobacco amblyopia, showing a gross excess of cyanocobalamin. Co-B_{12} = deoxyadenosylcobalamin.

Similar results have been found in Leber's hereditary optic atrophy, a condition which may be associated with diffuse neurological disease and in which Wilson (1965) had previously found evidence of an error in cyanide metabolism. In collecting a control series for these cases, just as in the work on peptide absorption, we had a surprise. Patients with another type of hereditary optic atrophy, dominantly inherited optic atrophy, also showed an excess of cyanocobalamin in the plasma. Dominantly inherited optic atrophy is a condition that often occurs in children and in which there had been no reason to suspect any disturbance in cyanide or B_{12} metabolism. Recently we have also found a rise in plasma cyanocobalamin in some cases of optic atrophy of obscure origin (Linnell *et al.*, 1969d; Matthews and Linnell, 1971; Wilson *et al.*, 1971). It is too early to comment further on these findings, but we are working on the hypothesis that these optic atrophies are the result of errors in cyanide metabolism, the changes in cobalamins being secondary as in tobacco amblyopia.

Potential Value

The main point I should like to make about this research is that, however interesting the findings in neuro-ophthalmological disorders may be, it is now clear that they represent only a small part of what can be done with the technique that has been developed. Its possible applications are very much wider than originally envisaged (Matthews and Linnell, 1971), and the mere availability of the technique has suggested new lines of investigation, both basic and clinical.

The method is being used to follow the changes in individual cobalamins in an experiment on the effects of B_{12} deficiency and chronic cyanide toxicity in baboons. It can be applied to the study of the tissue and subcellular distribution of several individual forms of vitamin B_{12}, and should provide basic information in an area where very little is known. An obvious clinical application is in the study of changes in the proportion of plasma cobalamins in disease. In some conditions, including liver disease and the postgastrectomy state, we have found that abnormal levels of individual cobalamins can occur when there is a normal total B_{12} level (Linnell *et al.*, 1969a, 1971). One of the earliest findings was a quite unexpected abnormality in pernicious anaemia and other forms of B_{12} deficiency. This is a disproportionate reduction in plasma methylcobalamin, which often virtually disappears while the other coenzyme form, though reduced, is still present (Linnell *et al.*, 1969c, 1971). All these changes require further investigation. Their significance and potential diagnostic value have still to be explored. The technique can be used in the study of membrane transport of the various forms of B_{12} and in investigating their interconversions. Thus it has been shown for the first time that while some cyanocobalamin is absorbed from the intestine unchanged, some is converted to deoxyadenosylcobalamin during absorption (Hoffbrand *et al.*, 1970; Linnell *et al.*, 1971; Peters *et al.*, 1971).

We have investigated the forms of B_{12} in maternal and fetal blood and in milk, and found that the proportions of the coenzyme forms are different, which suggests either preferential binding of individual cobalamins or their interconversion during placental and mammary transport. It has been found that human milk contains both coenzyme forms of B_{12}, while cow's milk contains mainly hydroxocobalamin and prepared baby milks contain only hydroxocobalamin (Craft *et al.*, 1971). To carry out analyses of various milks may seem an excellent example of the collection of "useless" information. However, as I have already pointed out, one can never tell when informa-

tion acquired through academic curiosity will turn out to have clinical relevance, and the possible relevance of this will appear in a moment.

Very recently a number of cases of congenital errors in B_{12} metabolism have been described (Mudd *et al.*, 1969; Morrow *et al.*, 1969; Levy *et al.*, 1970). One of the most promising applications of the chromato-bioautographic method, which we certainly failed to anticipate when we set it up, is to assist in their diagnosis and investigation. For example, though the total plasma B_{12} may be normal in these conditions, the method can show that the proportions of individual cobalamins are grossly altered (Matthews and Linnell, 1971). In one of these metabolic disorders the child is unable to convert hydroxocobalamin to the two coenzyme forms of B_{12} in the normal way. If I may speculate, it is at least possible that such a child, fed on prepared milk containing only hydroxocobalamin, would be at a disadvantage compared with one fed by the mother. The breast-fed child would receive the coenzyme forms of the vitamin ready-made, whereas the bottle-fed child would not.

Conclusion

In this lecture I have given a personal account of the course of two pieces of research work, from their inception a few years ago to the present time (Figs. 5 and 6). I hope it has shown a little of what research is like, the unexpected turns it can take, and the parts played by experiment, observation, and hypothesis. I have tried to illustrate how close the relation can be between "pure" and "applied" research, and the value of following an interest for its own sake, even if it seems remote from everyday problems. If I have also been able to convey something of the fascination that there is in doing research, so much the better.

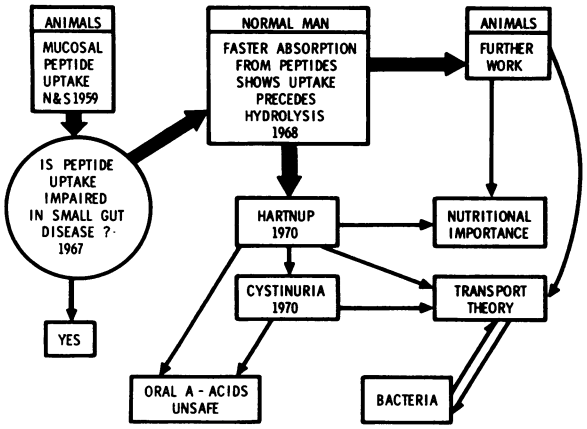

FIG. 5—Course of investigations on peptide absorption. The original "clinical" question (circled) was suggested by the physiological work of Newey and Smyth. The results obtained in normal control subjects directed the main course of the work (thick arrow) into further investigations in pure physiology, which provided the background for unexpected findings in amino-acid transport defects. The investigations in amino-acid transport defects have contributed in their turn to physiological knowledge.

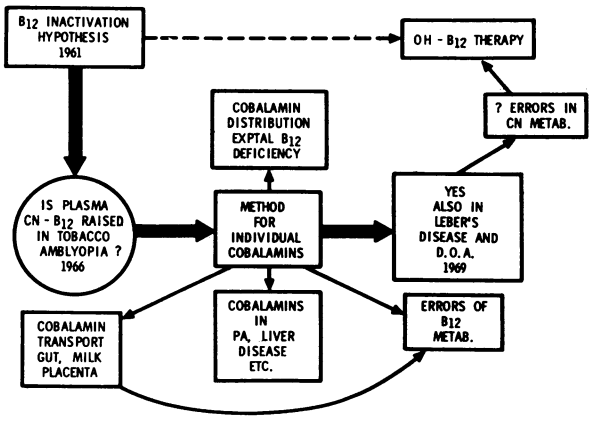

FIG. 6—Course of investigations using a technique for chromatography and bioautography of individual forms of vitamin B_{12}. The original "clinical" question (circled) was suggested by Smith's hypothesis. To answer this question a special technique had to be set up. The availability of this technique has made it possible to investigate many more problems than originally envisaged, some in clinical research and some more basic. Hydroxocobalamin therapy, which is used in tobacco amblyopia and Leber's disease in the belief that it assists the detoxication of cyanide, was originally suggested by Smith's hypothesis (dotted line), and its use is supported by the recent findings.

I am deeply indebted to those who have collaborated in the work described and helped in other ways, and am particularly grateful to Mr. Ian Craft for his invaluable help in the initial stages of the peptide research and to Mr. J. C. Linnell, who has been responsible for the development and application of the chromatographic and bioautographic method for individual cobalamins. I wish to thank Professor N. F. Maclagan for encouragement and provision of departmental facilities. Financial support has been provided by the Wellcome Trust, the Medical Research Council, the British Nutrition Foundation, and the Variety Club of Great Britain.

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Method of Removing Abnormal Protein Rapidly from Patients with Malignant Paraproteinaemias

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Summary

On 48 occasions large quantities of abnormal protein were removed from the blood of 11 patients with malignant paraproteinaemias (plasmacytomas) by exchanging their plasma with reconstituted blood bank plasma. The IBM continuous flow blood cell separator was used to exchange the plasma, a procedure that is safe, very rapid, and with clinical benefits which are much better than can be obtained by conventional plasmapheresis. In addition, the constituents of reconstituted plasma correct deficiencies in normal immunoglobulins found in these patients. Clearly for the management of some patients with paraproteinaemia a cell separator is essential.

Introduction

The therapeutic benefits of plasmapheresis in the management of patients with malignant paraproteinaemia are well established (Schwab and Fahey, 1960; Conway and Miles Walker, 1962; Skoog *et al.*, 1962; Lawson *et al.*, 1968), but such procedures are associated with certain disadvantages. They are time-consuming, technically difficult, and though removing abnormal protein also deplete the patients of normal proteins. Godal and Borchgrevink (1965) described the case of a patient with macroglobulinaemia developing pneumonia after plasmapheresis attributed to the removal of normal immunoglobulins, and Solomon and Fahey (1963), in a similar situation, noticed a reduction in serum albumin after the removal of 1 litre of plasma daily for four days, which was sufficient to cause ankle oedema.

The purpose of this paper is to show that the NCl IBM cell separator overcomes these difficulties. The separator exchanges the patient's plasma for reconstituted freeze-dried plasma, and large quantities of abnormal protein are removed not only quickly and easily but also with the special advantage of replacing the patient's own deficiencies of albumin and normal immunoglobulins.

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Materials and Methods

NCl IBM Cell Separator.—This is a machine designed for the collection of leucocytes from peripheral blood (Buckner *et al.*, 1969; Powles *et al.*, 1971). However, it can be used for plasma exchange. In the management of patients with paraproteinaemia the patient's plasma is exchanged for freeze-dried plasma. Blood is continuously drawn from the antecubital vein in one arm and separated into cells and plasma in a centrifuge bowl. The blood is exposed to a high gravitational force (100 *g*) so that platelets are removed from the patient's plasma before exchange occurs, and these platelets are returned to the patient with the other cells; this is important because the platelets in freeze-dried plasma are destroyed. The patient's plasma is then removed in exchange for reconstituted normal freeze-dried plasma which is mixed with the patient's separated cells and returned to a vein in another limb. Consequently whole blood with the abnormal protein greatly reduced is therefore returned to the patient. A dual-channelled auxiliary pump and special tubing sets (Avon Medical Ltd.) ensure that the volume of the patient's plasma removed equals the volume of reconstituted freeze-dried plasma returned to the patient.

If the patient is anaemic the donor red cells may be exchanged for the patient's plasma, correcting the anaemia without altering the total blood volume. About 2·5 litres of whole blood is processed every hour, and patients are treated for up to seven hours in a single session without undue discomfort. Heparin is used as an anticoagulant, the patient receiving 3,000 units intravenously before treatment starts and thereafter continuously 2,000 units every hour. Particular care is needed if the abnormal protein is a cryoglobulin. The centrifuge bowl is kept at about 33°C by means of radiant heaters, and extracorporeal fluids—that is, blood, plasma, and heparinized saline—are kept at 40°C.

Biochemical Estimations.—All biochemical estimations were performed on fresh serum collected and separated at 37°C and stored at −20°C. Viscosity was measured by forcing a constant volume of plasma at 37°C through a 1-mm aperture at constant pressure. The results were expressed in seconds and were compared with that of donor plasma.

Results

Eleven patients with paraproteinaemia were treated with plasma exchange on 50 occasions over a period of two years.

CLINICAL IMPROVEMENT

The clinical indications for plasma exchange in the patients studied are shown in Table I and the results are as follows: