Cobalamin: BIOCHEMISTRY AND PATHOPHYSIOLOGY
Edited by Dr. Bernard M. Babior
Published by John Wiley & Sons, Inc., 1975

INTRODUCTION

The History of Corrinoids

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EARLY CLINICAL OBSERVATIONS

History has the special fascination that with its help, from any moment in time-past the sequence of subsequent events may be foreseen as with the gift of prophecy. This is especially true of the often complex unfolding of scientific discovery. Thus we now can see that the story of corrinoids began in 1824 with Combe's (1) account of a fatal case of anemia in Edinburgh, provided as he suggested, that its cause was "some disorder of the digestive and assimilative organs." More certainly related to subsequent medical events is the description in 1855 of an "idiopathic anaemia" by Thomas Addison (2) of Guys Hospital in London. However, for the recognition of the particular kind of anemia of which he wrote somewhat incidentally in this famous monograph, *On the Constitutional and Local Effects of Disease of the Suprarenal Capsules,* we depend largely on the sagacity of medical contemporaries. Indeed, only 5 years later in the United States the elder Austin Flint, finding that autopsies of patients that he recognized as similar cases had disclosed no gross abnormalities, made a shrewd surmise. Aware that the microscopic examination of 100 stomachs by Handfield Jones had found atrophy of the secretory glands in 14, he predicted (3) that similar microscopic inspection would disclose similar findings in the idiopathic anemia described by Addison. A decade later Samuel Fenwick (4) of the London Hospital saw with the aid of the microscope the glandular atrophy of the stomach of a patient considered to have Addison's "idiopathic anaemia" and demonstrated the inability of acidified scrapings of the withered mucosa to digest hard-boiled white of egg. In 1886 Cahn and von Mehring (5) reported for the first time during life the typical lack of hydrochloric acid that in 1900 was found by Faber and Bloch (6) in 33 cases. The introduction of the fractional test meal and the flexible gastric tube of small diameter allowed in 1921 confirmation of the constancy of a lack of hydrochloric acid in over 100 cases (7); and following the introduction of the gastric biopsy tube by Wood et al. (8) in 1949 the progressive atrophic process was clearly shown during life to spare only the pyloric gland region (9).

It is understandable that the early suggestions (3, 4) that the anemia was due to defective nutrition were neglected in the era dominated by the concept of the infectious or toxic etiology of disease, so successfully illustrated by the discoveries of Pasteur, Koch, and their successors. Thus in 1900 William Hunter (10) emphasized the hemolytic features of the anemia and expressed his conviction concerning the septic origin of the disease. Shortly thereafter he claimed to have found streptococci as the

cause of the inflamed tongue and gastrointestinal symptoms. Indeed, as late as 1924 Arthur Hurst (11) presumed that the invariable achlorhydria permitted streptococcal colonization of the intestine with resulting elaboration of a hemolytic toxin. For this interpretation an analogy seemed to be found in several reports (12) after 1885 of a relation of the broad tapeworm to a form of pernicious anemia occurring in Finland and northern Russia that was cured when the parasite was expelled from its intestinal location.

CONCEPT OF NUTRITIONAL DEFICIENCY

It was only with the beginning of the twentieth century and the pioneer work of Eijkman in Java on beri-beri, followed by experiments with purified diets in animals in England by F. Gowland Hopkins and in this country by Osborne and Mendel, MacCollum, and others that a different concept of disease was established. Thus the idea gained credence that deficiencies of traces of accessory dietary factors, termed "vitamins" by Casimir Funk in 1913, might cause human disease under restricted dietary conditions. The idea that good food makes good blood is a lay belief of long standing. It was now to receive controlled experimental examination when George Whipple, working first at the Hooper Institute in San Francisco, began shortly before 1920 to study in chronically bled dogs the ability of various additions to a basal dietary regimen to enhance hemoglobin formation. In 1922, after his appointment as Professor of Pathology and Dean of the new medical school at Rochester, New York, Whipple (13) reported favorable effects with spinach, pork muscle, beef heart, and beef liver. By 1925 he and his principal research associate Dr. F. S. Robscheit-Robbins (14) with more prolonged experiments concluded that beef liver should head the list. Moreover it was fortunate that not until 1936 did it become apparent that the principal effective component of these items was their available iron content (15). Consequently, when these early results came to be known to the medical profession several clinicians tried dietary supplementation with meat and liver in various kinds of anemia in man including pernicious anemia (16, 17). Although it was found that a positive nitrogen balance could be achieved in that condition and some improvement in blood levels was observed to follow the feeding of such improved diets similar inexplicable "spontaneous remissions" were well known to experienced clinicians, despite the eventual fatal outcome of the disease. Consequently, when clinical improvement appeared it was not regarded as necessarily related to the modest amounts of meat or liver given; and the sage advice offered was not to abandon the well-established use of iron and arsenic (18).

On the other hand, George Minot was prepared to believe in the possible significance of Whipple's experiments for pernicious anemia. This Boston physician was Assistant Professor of Medicine at Harvard and had a special interest in diseases of the blood. While still a medical "house pupil" at the Massachusetts General Hospital he (19) had gained the impression from the cross-questioning of patients with pernicious anemia that they had often subsisted for some time on abnormal diets. Therefore, Whipple's work seemed to confirm his clinical suspicion; soon after 1922 Minot began to advise pernicious anemia patients to include liver in their diets. He became even more enthusiastic a little later when marked improvement occurred in the blood levels of one patient who really enjoyed eating liver in plentiful amounts. At that point he invited an assistant in private practice, Dr. William P. Murphy, to join him in an all-out trial of a special diet contaning "an abundance of food rich in complete proteins and iron—particularly liver—and relatively low in fat." As a result, in 1926 Minot and Murphy (20) were able to report consistent clinical improvement and gain in red blood cell levels in 45 patients who had successfully partaken of the special diet containing at least 120 to 240 g of liver daily. Shortly thereafter Minot consulted Edwin J. Cohn, Professor of Physical Chemistry at Harvard, as to the possibility of preparing a clinically effective extract of liver and of initiating a search for the identity of the active principle or principles involved. Protein of high biological quality was thought by Minot to be the basis of the therapeutic success of liver feeding. Therefore he presumably approached Cohn because of the latter's well-known research interest in proteins. A collaborative program was agreed upon: Cohn and his associates were to proceed with the preparation of chemical fractions of liver, each of which was to be tested for potency in an untreated patient with pernicious anemia. For this clinical evaluation daily percentage counts of newly formed red cells (reticulocytes) were made during successive contiguous 10-day periods, respectively, of uniform daily administration of the liver fractions to be tested. If, in a first period, no reticulocyte increase appeared, the fraction was provisionally judged to be inactive, until in a second period of administration of a different liver fraction a reticulocyte rise indicated its activity and so confirmed the lack of potency of the first liver fraction (21). In this way each patient was his own control and the necessity of waiting for substantial increases of circulating red cells was avoided.

SEARCH FOR THE ACTIVE PRINCIPLE IN LIVER

The pioneering fractionation procedures of Cohn and his associates (22) began with the extraction of minced liver with water at pH 5 and subsequent heating of the extract to 70°C. This coagulated most of the bulky and disagreeable-tasting proteins, which happily were found almost at once to be inert on clinical trial. Next, after extraction with ether, alcohol was added to 70% concentration and the resulting clinically inert precipitate discarded. When the concentration of alcohol in the filtrate was increased to 95% another precipitate formed, the so-called fraction G, and was found to be clinically active. At this point the help of Eli Lilly and Company was invited through collaboration with its medical director, Dr. G. H. A. Clowes, for the commercial production of fraction G. By 1928 this liver fraction in the form of a yellow powder known as Liver Extract 343 (Lilly) became available for testing in several clinics in this country and abroad under the auspices of a Harvard Committee (23). This preparation conserved most of the hematopoietic activity of 300 g of beef liver in a daily oral dose of 12.75 g.

Attempts in Cohn's laboratory to produce a more purified fraction for parenteral use encountered great losses of potency in getting rid of an active vasodepressor principle. In 1930 Gänsslen (24) in Germany described the surprisingly great clinical activity of a nearly protein-free extract of liver when injected in daily doses of material derived from only 5 g of liver and probably representing about 0.35 g of dry material. It was then found (25) that a sterile neutralized aqueous solution of Liver Extract 343 possessed on intravenous injection as much as 30 times the activity of the oral preparation in pernicious anemia. The vasodepressor effects (probably due to histamine) were evanescent and could be minimized by slow injection. This "crude liver extract" was also highly effective in the treatment of tropical sprue in Puerto Rico (26).

Progress in further fractionation of liver, by then also begun in other laboratories, was slow. The clinical testing required untreated patients with pernicious anemia who were not always available, especially as by now medical practitioners had available to them commercial liver extracts. Chemical techniques originally applied to the concentration of water-soluble vitamins were further utilized, among them metal salts to precipitate impurities (22). More useful was precipitation of activity with phosphotungstic acid and later with the help of Reinecke's salt, a method introduced by Dakin and West (27) in New York. Their work was also effectively aided by salting out techniques employing ammonium or magnesium sulfate. By 1935 this led to the development of a so-called purified liver extract commercially available for parenteral use of which

the daily dose of the dried material present had become as small as 0.015 g. A new impetus to progress came in 1936 when by means of initial extraction of minced liver with 50% acetone, followed by partition between water and phenol and adsorption on charcoal, LaLand and Klem (28) in Norway produced material active clinically in a single parenteral dose of 0.7 mg. In 1939 drops of an even more refined preparation, described as semicrystalline and orange-yellow, were placed on microscopic slides under sealed coverglasses. The German invasion interrupted their work, but 19 years later, when examined by others (29), the nearly forgotten slides displayed the deep red crystals by then known to be typical of vitamin B_{12}.

In 1945 Spies and his collaborators (30) briefly astonished the hematological world by demonstrating clear-cut activity in pernicious anemia with synthetic pteroylmonoglutamic (folic) acid in daily doses of 29 mg. This was an unexpected result because concentrates of purified liver extract were effective in much lower dosage and in fact by microbial analysis contained little or no folic acid. The explanation is now known to depend on the biochemical relationships of both folic acid and vitamin B_{12} to hematopoiesis to be discussed in another chapter.

The goal was now close at hand. In 1946 Emery and Parker (31) reported that a complete hematological remission could be obtained from a single injection of 1 mg of their material. In 1948 Randolph West (32), who from the early years had worked toward its isolation, was the first to demonstrate the clinical activity of crystalline vitamin B_{12} when injected in the form of cyanocobalamin in pernicious anemia patients. The precious material had been supplied to him by Karl Folkers and his associates (33) at the Merck Laboratories in the United States and was also isolated within a few weeks thereafter by Smith and Parker (34) at Glaxo Laboratories in England. In retrospect, according to E. Lester Smith (35) the reasons for the protracted effort were several. In the first place the losses in fractionation were so great that large quantities of starting material were required as well as the processing facilities of industrial laboratories. Preoccupation with the notion that pernicious anemia was a unique human disease limited testing of fractions to suitable patients and inhibited the search for methods of assay involving growth rates in laboratory animals or microbial species until almost the end. Finally, the classical methods of fractionating water-soluble substances were inadequate to the task. Only with the development of adsorption and partition chromatography could the necessary separations be effected. An illustration of this last point is to be found in the detailed accounts of the isolation procedures used by the Glaxo Laboratories team (36).

MICROBIOLOGICAL GUIDANCE

The main trunk of the tree of knowledge that flowered with the discovery of vitamin B_{12} was the search for the active principle of liver effective in pernicious anemia. However, other at first seemingly unrelated scientific explorations helped either to reach that goal or afterward to aid in understanding the biological origin and functions of vitamin B_{12} and other corrinoids. Thus belatedly study of the growth requirements of *Lactobacillus lactis* Dorner by Mary Shorb (37) in 1947 led to recognition that crude and slightly purified liver extracts contained an essential growth factor. This suggestive finding provided a biological assay of great assistance to the Merck scientists in the final stages of the isolation of vitamin B_{12}, which was indeed the factor required by this particular lactobacillus. Since then other and more specific assays have been devised. *E. coli* mutants were introduced for this purpose in 1950 (38). Lactobacilli, including *Lactobacillus leichmannii* used officially by the United States Pharmacopoeia in 1955, have the disadvantage for this purpose that they respond to thymidine as well as to other deoxyribonucleosides. In 1949 Hutner et al. (39) had found photosynthesis by the flagellate *Euglena gracilis* to require vitamin B_{12}; after heating to liberate the vitamin from binding proteins the assay of human serum by this organism (40) has only recently begun to be replaced by isotope dilution assays (41) using serum protein or other binders with charcoal or Sephadex to provide proportionate exclusion.

MECHANISM OF VITAMIN B_{12} DEFICIENCY

The success of liver feeding in 1926 made it clear that pernicious anemia was either the result of a nutritional defect or metabolic aberration. The clinical knowledge that achlorhydria was known sometimes to precede by many years the development of the anemia and that it persisted after liver therapy had corrected all other aspects of the disease suggested to Castle that the causative mechanism might be, as he defined it in 1929, "an inability to carry out some essential step in the process of gastric digestion" (42). This supposition seemed to be confirmed when it was shown by the use of the double reticulocyte response method described above that daily administration of 200 g of ground beef muscle was ineffectual unless given simultaneously with 150 cc of normal human gastric juice. The unknown component of the beef muscle was termed "extrinsic factor" and that of the gastric juice "intrinsic factor" (43). Because it contained both factors, dessicated hog stomach was found to be therapeutically active by Sturgis and Isaacs (44). Over the next 20 years the nature of each factor was repeatedly sought by various investigators and the conditions of their supposed interaction were defined to some extent purely by observations on patients. The natural supposition that the gastric instrinsic factor was an enzyme could not be substantiated. The clue as to a possible substrate provided by Reimann's (45) demonstration in 1931 that liver or oral liver extract could be potentiated when given with gastric juice was unfortunately not followed up until 1948. Then Berk et al. (46), after finding that small amounts of highly purified liver extract were enhanced in activity upon daily oral administration with gastric juice, showed the same thing to be true for as little as 5 μg of the newly isolated active principle of liver extract, vitamin B_{12}. In 1952 Glass and his associates (47) reported that the acid glandular mucoprotein of human gastric juice possessed intrinsic factor activity with vitamin B_{12}. The same year, after Rosenblum and Woodbury (48) had made available to investigators isotopically labeled ^{60}Co-cyanocobalamin, Heinle, Welch, and their co-workers (49) demonstrated that in pernicious anemia simultaneous administration of gastric intrinsic factor decreased fecal excretion of the label, shown the following year by Schilling (50) to be absorbed and in part excreted in the urine as the vitamin. It thus appeared that the function of intrinsic factor was simply to promote the assimilation of the vitamin in food without essential modification. However, when large amounts of vitamin B_{12} are fed, as with 240 g of liver, passive diffusion alone without intrinsic factor suffices for assimilation.

In 1955 Watson and Florey (51) demonstrated that removal of the secretory portion of the rat stomach abolished the animal's ability to assimilate ^{60}Co-B_{12}. This function could be restored by a source of rat, but not of human or hog, intrinsic factor. Subsequent observations by many authors using isotopically labeled vitamin B_{12} in patients, in gastrectomized animals, and in perfused or everted segments of their distal small bowel or with mucosal scrapings have defined the normal process of vitamin B_{12} assimilation as follows. In the stomach the native vitamin of animal food, mostly in the coenzyme form linked to its specific protein, is released by peptic digestion and is at once strongly bound to intrinsic factor (52). This binding phenomenon was first postulated by Ternberg and Eakin (53) in 1949 from the observed *in vitro* inhibitory effect of gastric juice upon the growth of bacteria requiring vitamin B_{12}. The intrinsic factor, a glycoprotein secreted as such exclusively by the gastric parietal cells in man (54), is partially protected in this bound form from intestinal enzymes and adventitious parasites (55). The complex, in which the nucleotide portion of the vitamin B_{12} molecule

relates structurally to the intrinsic factor (56), traverses the small bowel to the distal ileum where, by a specific but purely physical process (57), it becomes attached to the microvilli of the epithelial cells in the presence of calcium ion when, as normally, the local pH is 6.4 or above (58). Next, by an energy-requiring process, the vitamin is released into the intestinal cell from the intrinsic factor, which seems to remain active on the intestinal surface for a time (59). After a delay of some hours in the intestinal cell (60) the vitamin B_{12} enters the bloodstream where it is bound briefly to a plasma globulin, transcobalamin II. Later it is found attached to what may be a storage plasma globulin probably derived from leukocytes, transcobalamin I (61). A hereditary lack of transcobalamin II has recently been shown (62) to be associated with defective intestinal assimilation and to prevent transfer of the plasma vitamin to the erythropoietic cells. Significant knowledge of these plasma transport proteins began with the work of Hall and Finkler (63) in 1963.

The finding by Schwartz (64) in 1958 and the next year by Taylor (65) of anti-intrinsic factor antibodies in the serum of some patients with pernicious anemia at first seemed to provide an additional mechanism for vitamin B_{12} malabsorption. However, it is now recognized that this is significant only when such serum antibodies leak or are secreted into the alimentary tract. Instead, a suggestion of an immunological basis for the chronic atrophic gastritis was provided, especially when antiparietal cell antibodies were found in association with gastritis, sometimes preceding the anemia (66). More recent evidence that cellular rather than humoral immunity is more likely to damage tissue cells (67) and the increased incidence of pernicious anemia reported in younger subjects with hypogammaglobulinemia (68) first reported in 1961, seem to favor a chronic cellular immune reaction expressed by an infiltration of the gastric mucosa with lymphocytes and macrophages and reflected by the presence of circulating antibodies specifically directed against intrinsic factor and parietal cell cytoplasmic antigens.

ANIMAL PROTEIN FACTOR

Isolation from liver would never have provided a practical source of vitamin B_{12} for therapeutic purposes or of other corrinoids without industrial fermentations with streptomyces as initially employed in the production of antibiotic (69). This technique came to be used because of difficulties during World War II in obtaining satisfactory growth rates in hogs and in chickens fed vegetable rations lacking in the usual meat scrap supplementation. At maturity the hens laid eggs many of

which failed to hatch (70). It was soon discovered that these abnormalities could be corrected by the addition of animal or fish wastes to the vegetable rations, which were thus deemed to be lacking in an unknown "animal protein factor" (APF). Experiments with rats on similar restricted diets disclosed that "factor X" and "zoopherin" were identical with APF. When laying hens were raised under natural conditions, rather than the seemingly more hygienic way of being kept on wire mesh, egg hatchability improved. Rubin et al. (71) found that poultry droppings caused fermentation of spilled food and, like dried cow manure, supplied APF as liver extracts were already known to do. Fermentation of an artificial medium with an organism isolated from hen feces was found to yield APF; and after the isolation of vitamin B_{12} from liver this APF was shown to be effective in pernicious anemia (72).

COBALT DEFICIENCY IN RUMINANTS

A further unexpected relation to synthesis of corrinoids by fermentation emerged from the successful analysis of the cause of a wasting disease of sheep and cattle when pastured in certain localities in several parts of the world. Recognized by veterinarians under different names such as "bush sickness" and "pining," loss of appetite is followed by loss of weight and eventually anemia. In South Australia the so-called coast disease was at first found to be curable by commercial iron salts, but later not by pure iron salts. Eventually the curative impurity turned out to be cobalt, which could be supplied by oral administration or by addition in trace amounts to the soil of the grazing areas (73). The relative inefficacy of cobalt when given to sheep by injection led to consideration of its possible role as a growth requirement for the microorganisms of the rumen that play an essential role in ruminant nutrition by producing fatty acids from cellulose. No search for a cobalt-containing metabolite in rumen contents was made until after the isolation of vitamin B_{12} when rumen contents were found to be a good source of that vitamin and especially of related corrinoids (74). The discovery of cobalt in the vitamin B_{12} molecule then suggested an essential synthetic role for that element in rumen microbiology (75). Although this was rendered doubtful when the usual human doses of vitamin B_{12} were found to be ineffective in sheep upon parenteral injection, subsequently larger dosages were found to be fully effective in restoring appetite, growth, and blood formation (76). The explanation of this greater requirement is still uncertain.

METABOLIC FUNCTIONS OF VITAMIN B_{12}

Such were the diverse researches, termed "convergent" by E. Lester Smith (35), prior to the isolation of vitamin B_{12} in 1948. Since then much has been learned of its biogenesis, structure, and biological functions in the body in coenzyme forms. Much of this information has been derived from studies of its metabolic actions in bacteria requiring it for growth. The discovery of an orange-yellow, photosensitive coenzyme form of an analog of vitamin B_{12} was announced by Barker et al. (77) in 1958, as a result of studies of an unusual metabolic pathway for glutamate present in the organism *Clostridium tetanomorphum*. This led shortly to the discovery of the adenosyl coenzyme form of vitamin B_{12} itself. The complete spatial configuration of cyanocobalamin having been specified as a result of several years of chemical investigations culminating in the X-ray crystallographic work and exhaustive calculations of Dorothy Hodgkin's group in 1957 (78), she and Lenhert (79) were able to report the structure of the adenosyl coenzyme in 1961 only 3 years after its isolation. The vitamin B_{12} molecule was shown to consist of the nearly planar corrin ring joined through its central cobalt atom almost at a right angle to the so-called nucleotide portion of the molecule. In the coenzyme, the cyanide group, attached above the macrocycle to cobalt in cyanocobalamin, is replaced by the adenosine moiety attached by the C-5′ of the pentose.

The original supposition that vitamin B_{12} was involved in the synthesis of nuclear DNA was soon confirmed by Roberts et al. (80) in 1949. Within a decade attention had become focused upon the synthesis of the deoxyriboside moiety. Beck et al. (81) in 1962 proposed that the adenosyl coenzyme might be involved in the reduction of all four ribose nucleotides in man, as they showed to be the case in *L. leichmannii*, preparatory to DNA synthesis. On the other hand, there is suggestive evidence that in man, as in the mutant strains of *E. coli* requiring vitamin B_{12}, a different pathway is concerned in which B_{12} is only indirectly involved, and then as the methyl, not the adenosyl, derivative.

Early experiments on the growth of chicks and rats showed that vitamin B_{12} exerted a sparing effect upon their methionine requirement and could reduce somewhat their need for dietary choline (82). It was also discovered that a few mutant strains of *E. coli* need vitamin B_{12} for methionine synthesis unless supplied with 10,000 times as much of the amino acid. Around 1960 Woods' and Buchanan's groups independently concluded that the transfer of the methyl group from *N*-5-methyltetrahydrofolic acid, the principal form in serum, requires a different coenzyme of vitamin B_{12} than the adenosyl. When synthetic methylcobalamin became available it was shown that its methyl group was donated to convert homocysteine to methyl homocysteine (methionine), though in a somewhat complex system demonstrated by Foster et al. (83) in 1964. The cooperative role of methylcobalamin in transferring the one-carbon fragment from N-5-methyltetrahydrofolic acid to homocysteine in man was originally suggested in 1962 by Herbert and Zalusky (84) as an explanation of the "pile up" of the serum folate in clinical vitamin B_{12} deficiency. Experiments by Killman (85) in 1964 with human bone marrow deficient in vitamin B_{12} and by Metz et al. (86) in 1968 tend to confirm the suggestion that lack of vitamin B_{12} prevents the formation of the specific N-5,10-methylene form of tetrahydrofolate required for the methylation of deoxyuridylic to thymidylic acid in the synthesis of DNA. However, whether this or another system is involved in man remains to be determined.

A firmly established function of vitamin B_{12} in man is the involvement of the adenosyl coenzyme in the major pathway of propionate metabolism demonstrated by Beck and Ochoa in 1958. The final step involves the isomerization of methylmalonyl CoA to succinyl CoA and is catalyzed by the vitamin B_{12} coenzyme as was shown in 1961 by Marston et al. (87) in vitamin B_{12}-deficient sheep. In the human deficiency Cox and White (88) demonstrated the next year a large excretion of methylmalonic acid in the urine that was corrected by cyanocobalamin but not by folic acid. Recently this abnormality has been found to be associated with the synthesis of abnormal fatty acids with odd-numbered carbon chain lengths both *in vivo* (89) and *in vitro* (90) and so is possibly related to the specific demyelination of the spinal cord sometimes occurring in vitamin B_{12} deficiency.

BIOGENESIS AND SYNTHESIS OF VITAMIN B_{12}

The history of the discovery of the nature of the biogenesis of vitamin B_{12} and of corrinoid analogs is too complex a subject for detail here. British workers (91) engaged in research related to the dairy industry discovered in 1955 the existence of vitamin B_{12} analogs after microbial assays for vitamin B_{12} in rumen contents indicated less than the expected growth rates in vitamin-deficient chicks given such material. Further understanding has come largely from the use of labeled precursors in vitamin B_{12}- or analog-producing microorganisms. In 1950 Sahashi et al. (92) showed that preformed 5,6-dimethylbenzimidazole can be incorporated into the nucleotide portion of the vitamin B_{12} molecule. Fantes and O'Callaghan (93) found in 1955 than in *Streptomyces griseus* phenylene-

diamine was a precursor of an analog, whereas dimethylphenylenediamine increased the yield of vitamin B_{12} proper. Shemin and his associates (94) in 1956, utilizing ^{14}C-labeled glycine, δ-aminolevulinic acid, or porphobilinogen showed that the corrin ring is constructed in a fashion similar to that of the porphyrin ring of heme. Synthesis of the corrin ring was achieved by Eschenmoser in Zurich in 1964, and recently Eschenmoser and Woodward, working in collaboration, have effected total synthesis of the vitamin (95). So much for the historical highlights of biosynthesis. Man's ability to achieve with immense labor what the most primitive bacteria accomplish with ease is, judged by human standards, a superb achievement.

CONCLUSION

The history of the discovery of the corrinoids is a classical illustration of the seminal importance of clinical investigation to medical and biological science. The empirical discovery of the efficacy of liver feeding in pernicious anemia led eventually to the isolation, structural analysis, and synthesis of vitamin B_{12}. Without the first clinical steps, however, no one can say how long it would have been before the time came for the present elegant revelations of the nature and functions of the corrinoids. Indeed, at the service of the clinical investigator, as for no other biological scientist, is the boundless ingenuity of nature in providing the experiments created by disease. Once understanding of their proximate meaning reaches a certain point the torch can be passed to basic science for further and more rapid progress.

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