Vitamin B_{12} Conference

Robert A. Harte and Bacon F. Chow

Research Laboratories, Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pennsylvania, and
Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland

A SYMPOSIUM on the current status of research on vitamin B_{12} was held at the School of Hygiene and Public Health of The Johns Hopkins University on June 5 and 6 with Bacon F. Chow as chairman. Sixty scientists, representing government, university, hospital, and industrial research laboratories, as well as the American Medical Association and the National Vitamin Foundation, attended. Twenty of these presented summaries of their current work and led discussions of hypotheses that will, it is hoped, serve as springboards for future researches.

More and more, nutritionists have come to recognize the vital importance of the interrelationships of all the elements of diet. It is no longer sufficient to deal with gross effects such as weight gain, reproduction, or even nitrogen balance, if the role of an essential nutrient, such as vitamin B_{12}, is to be appreciated. Investigations of a substance so protean in character as B_{12} must call on scientists with special interests in fields as diverse as nuclear physics, hematology, enzymology, endocrinology, and nutrition, as well as internal medicine and ophthalmology.

The subject matter of the conference may be divided among the major headings Metabolic Roles, Hematopoiesis, and Renal Function. The original concept that vitamin B_{12} is identical with Castle's extrinsic factor remains unchanged, but current research tends to extend our ideas of its general importance far beyond this limited role. The availability of B_{12} tagged with radioactive Co^{60} has facilitated studies of its absorption and excretion pathways and has pinpointed its loci of storage in the host following oral or parenteral administration. The promise of future availability of tagged vitamin with even higher specific activity, and improvements in counting techniques, as with the scintillation counter, will permit extension of these studies at true physiologic levels. This will go far toward eliminating the errors almost inevitably introduced in extrapolating conclusions drawn from data obtained under nonphysiologic conditions.

Both *in vitro* and *in vivo* experiments were reported which suggest that B_{12} plays an important role in the synthesis of nucleic acids and in the metabolic pathways of such compounds as glycine, serine, methionine, and choline. Growth studies, although necessarily of limited specificity, emphasize the importance of adequate supplies of B_{12} in the metabolism of carbohydrate and fat, including not only the conversion of carbohydrates (and the carbon skeletons of amino acids) to fat but also the metabolism of fat itself.

The presence or absence of adequate amounts of dietary B_{12} has significant effects in modifying the concentration of sulfhydryl-containing compounds of physiologic importance, among which would be included glutathione and coenzyme A. If the hypothesis that, in the absence of sufficient B_{12}, the reduction of the —S—S— form of coenzyme A to the —SH | form fails to proceed can be substantiated, then the role of B_{12} in fat metabolism becomes clearer. This intriguing thesis finds support in the gross observation of fat accumulation in chicks and rats subsisting under conditions of chronic B_{12} deprivation.

There seems to be no evidence to indicate that B_{12} plays a significant role in overall nitrogen balance in the rat. Both paired-feeding and forced-feeding experiments have shown substantially identical nitrogen balances to obtain, regardless of the presence or absence of vitamin in the diet. There are reports of increases in nitrogen balance in rabbits under the influence of the vitamin, but possibly these observations reflect increased food intake and not changes in the efficiency of utilization of dietary protein.

These and other reported clues to the varied metabolic transformations involving B_{12} await clarification and extension through detailed studies of isolated enzyme systems. Despite their significant implications, major interest continues to focus on the role of B_{12} in the therapy of Addisonian (pernicious) anemia and on the interrelationships between the vitamin and Castle's intrinsic factor.

Pernicious anemia must, in the light of present knowledge, be regarded as the result of a tissue deficiency of B_{12} rather than a dietary lack. The failure of dietary B_{12} to reach the tissues appears to reflect a hypofunctioning of certain secretory cells in the gastrointestinal tract, leading to a deficiency, of greater or less severity, of intrinsic factor. This concept, which stems from Castle's postulation of intrinsic factor in 1929, has resulted in wide acceptance of the thesis that successful oral therapy of Addisonian anemia calls for an adequate exogenous source of intrinsic factor. Since that time, much research effort, at best, only partially successful, has been concerned with the isolation and characterization of intrinsic factor.

This background of almost a quarter century underlines the significance of clinical studies reported by C. Lockard Conley and his associates. They have observed that pernicious anemia patients in relapse could be brought into remission and maintained without an

exogenous source of intrinsic factor by the oral administration of large doses of vitamin B_{12}. Using a regimen that calls for initial oral administration of a single 5000-μg dose followed by 1000-μg doses once each week, more than 15 patients in relapse have been brought to remission and maintained symptom-free for periods up to 2½ yr; over 100 patients have been followed for at least a year. This schedule probably does not represent minimal therapy, but is safe in that there have been no failures. When the same total amount of B_{12} was given in divided doses, the clinical results were not as good. The quantities of B_{12} involved appear large when compared with the generally accepted idea that the usual daily intake in the dietary of clinically healthy individuals is only 1–2 μg, but they are still small in absolute amount. The extraordinary potency of B_{12} as an essential factor in erythropoiesis is thus again affirmed.

If continued observation of these patients over an additional period of years confirms the finding that the recommended regimen will maintain pernicious anemia patients asymptomatic, then it would appear that intrinsic factor plays only an accessory role and that the mass-action effects of large enough doses of B_{12} readily replace it in the therapy of the disease. Until studies of this character, where patients are under continuing scrutiny of competent investigators for long periods of time, establish the adequacy of the proposed regimen in avoiding the insidious complications encountered under therapy that corrects only the hematologic picture, treatment of choice for Addisonian anemia remains the periodic injection of crystalline vitamin B_{12}. Nevertheless, these reports emphasize and support the thesis that the fraction of orally ingested B_{12} absorbed is capable of fulfilling the important physiologic functions of the vitamin.

These and other reports to the conference raise the overall question of the possible existence of other manifestations of B_{12} deficiency. There was extended discussion of the existence, in varying degrees, of B_{12} deficiencies associated with conditions other than pernicious anemia. Data were presented that indicate highly significant variations in the degree of tissue saturation with respect to B_{12} in diabetics with and without retinopathy, compared with each other and with clinically healthy individuals of the same age and sex. These variations in saturation states in the two types of diabetics are associated with striking changes in the excretory patterns for adrenocortical hormones, suggesting some relationship between B_{12} and these steroids.

Variation in the extent of tissue saturation with respect to B_{12} appears also to be related to age among clinically healthy subjects. In this connection one is reminded of the diminishing secretion of acid from the parietal cells of the gastric mucosa among the aged. The observed B_{12} saturation differences thus may be explained as reflecting possible progressive changes in absorptive mechanisms with increasing age.

In part at least, these results with diabetics and amoung various age groups, as well as other data comparing populations maintained over long periods of time on strikingly different dietaries (Mexicans on mainly vegetable diets compared with Americans on highly nutritious diets), find partial explanation in known biochemical facts. Full elucidation of the extent and gravity of B_{12} deficiency states among people not suffering from pernicious anemia must await further surveys and additional clinical and biochemical studies.

From studies on laboratory animals, strong indications have emerged to suggest that the kidney is a focal point in the metabolism of B_{12}. By far the largest portion of an administered dose is concentrated in the kidney and remains detectable in that organ over long periods of time. Experiments with humans indicate that, while much of administered B_{12} is promptly excreted, the quantity retained is directly correlated with the administered dose. Tissue saturation can be achieved, therefore, only through adequate dosage.

Although the scope of the conference was limited to the subjects discussed above, no summary of the varied roles of B_{12} would be complete without mention of its effects on growth in children. Several papers in the literature report studies in this direction. Superficially, the findings may appear to disagree. Nevertheless, no studies meeting the criteria laid down in one of the first reports have failed to support the thesis that oral supplementation with B_{12} has a beneficial effect on child growth. These criteria are: (*a*) previous history of nutritional stress, including B_{12} deficiency; (*b*) ad libitum access to food during the study period; and (*c*) adequate nutrients and calorie distribution in the diets offered.

In addition to providing an up-to-date summary of important researches in progress in this field as well as the current thinking of some of its leading workers, the meeting served to emphasize once more the value of bringing together outstanding students of the varied disciplines that contribute to the progress of such complex investigations.