EVALUATION OF THE HEALTH ASPECTS OF VITAMIN B\textsubscript{12} AS A FOOD INGREDIENT

1978

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
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NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshaling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or non-governmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB

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I. INTRODUCTION

This report concerns the health aspects of using vitamin B₁₂ as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), * which summarizes the world's scientific literature from 1920 through 1973. In addition, the Select Committee was provided with a review prepared by LSRO (1a)* of more recent literature on the health aspects of vitamin B₁₂ as a food ingredient. To assure completeness and currency as of the date of this report, this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of June 13, 1978 (43 FR 25487-25489) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using vitamin B₁₂ as a food ingredient. The Select Committee received no requests for such a hearing on vitamin B₁₂.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321 (s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The documents (PB-241 966/1) for reference 1 and (PB-275 755/7WJ) for reference 1a are available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that these conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on vitamin B_{12} and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

In 1926, Minot and Murphy (3) demonstrated that pernicious anemia could be controlled by a diet rich in whole liver. It was not until 1948 that two groups, one in England (4) and one in the United States (5) almost simultaneously isolated the active therapeutic principle which in microgram amounts had the clinical effectiveness of large quantities of liver. Rickes et al. (5) termed this potent substance vitamin $B_{12}$. The structure of the vitamin (Figure 1) was elucidated in 1956 (6) and its total laboratory synthesis reported in 1977 (7).

The vitamin $B_{12}$ form found naturally in the liver contains the 5' deoxyadenosyl group linked with the cobalt atom. However, this linkage is unstable and was replaced by cyanide in the original isolation of vitamin $B_{12}$ to yield a stable compound termed cyanocobalamin. Although other forms of the vitamin exist, the term cyanocobalamin has become entrenched in the literature as the generic term for all the cobalamins active in man. In an attempt to clarify the somewhat confusing terminology surrounding the vitamin $B_{12}$ members, the International Union of Pure and Applied Chemistry (8) adopted the following nomenclature rules: Cyanocobalamin is a permissive (semi-systematic) name for vitamin $B_{12}$. The term "vitamin $B_{12}$" used without qualification refers exclusively to cyanocobalamin. The term cobalamin describes vitamin $B_{12}$ minus the cyanide group. Coenzyme vitamin $B_{12}$ means only 5'deoxyadenosylcobalamin, but vitamin $B_{12}$ coenzyme refers to any form of vitamin $B_{12}$ with coenzyme activity. The predominant forms in animal tissues are protein-bound coenzyme vitamin $B_{12}$ and methyl-cobalamin (9).

Cyanocobalamin forms dark red crystals or may exist as an amorphous red powder. In the anhydrous form, it is very hygroscopic and when exposed to air may absorb about 12 percent of water. It is sparingly soluble in water, soluble in alcohol, but insoluble in acetone, chloroform and ether. The U.S. Pharmacopeia (10) specifies that cyanocobalamin must contain not less than 96.0 percent nor more than 100.5 percent of $C_{83}H_{88}CoN_{14}O_{14}P$ calculated on a dry basis.

The National Formulary (11) specifies cobalamin concentrate as the dried, partially purified product resulting from the growth of selected Streptomyces cultures or other cobalamin-producing microorganisms. It may contain presumably harmless diluents and stabilizing agents. Each gram of concentrate must contain not less than 500 $\mu$g of cobalamin. The concentrate occurs as pink to brown granular fine powder. Upon drying, the concentrate must not lose more than 5 percent of its weight. The pH of its solution (1 g concentrate in 200 ml) must be between 4.0 and 8.0.
FIGURE 1

Structure of Vitamin B₉₉

R = CN for cyanocobalamin
-OH for hydroxocobalamin
-CH₃ for methylcobalamin

for 5’deoxyadenosyl-cobalamin (B₁₂ coenzyme)
No specifications for vitamin B₁₂ are listed in the Food Chemicals Codex (12) or in the Handbook of Food Additives (13). The latter reference calls attention to the degradation of the vitamin upon exposure to light and to the adverse effects of high concentrations of ascorbic acid, thiamin and niacinamide on vitamin B₁₂ stability in liquid multivitamin preparations. Ferrous salts cause rapid destruction of the vitamin, but ferric salts stabilize it in solution.

Vitamin B₁₂ is considered GRAS as a nutrient and/or dietary supplement [21 CFR 182.5945](2). The Food and Drug Administration considers the use of vitamin B₁₂ with intrinsic factor* concentrate as a dietary supplement for distribution only under prescription (14).

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*Intrinsic factor is discussed in Section IV.
III. CONSUMER EXPOSURE DATA

It is generally stated that vitamin \( B_{12} \) can be synthesized only by microorganisms and that its presence in higher plants and animals depends directly or indirectly on these sources (15). However, coenzyme \( B_{12} \) has been isolated from the livers of germ-free mice (16) and the synthesis of vitamin \( B_{12} \) by mouse mammary tumors has been reported (17). The richest dietary sources are animal organs (liver, kidney, brain, heart), some shellfish (clams, oysters) and egg yolk. Intermediate sources include muscle meats, dairy products, fish, shrimp, and lobster. Vegetables, cereals and egg white are extremely poor sources (15). The daily intake of vitamin \( B_{12} \) may vary over a hundredfold range (from 1 to 100 \( \mu \)g), but the "average" diet in the United States is estimated to supply between 5 and 15 \( \mu \)g per day (18). A breast-fed infant receives approximately 0.3 \( \mu \)g daily (19). Commercial milk-based infant formulas marketed in the United States contain 1 to 2.5 \( \mu \)g vitamin \( B_{12} \) per liter, while certain special formulas may contain as much as 6 \( \mu \)g per liter (20).

According to a survey of food manufacturers by a National Research Council subcommittee (21), vitamin \( B_{12} \) was added only to some products in the food categories of breakfast cereals, grain products (such as pasta or rice dishes), milk products, snack foods, nonalcoholic beverages, reconstituted vegetable proteins, baby food baked goods, and baby food formulas. The level of addition to these categories is shown in Table I. An entry in Table I does not mean that all or a majority of the foods in a category contain added vitamin \( B_{12} \).

Estimates of the per capita daily intake of vitamin \( B_{12} \) of various age groups were calculated on the basis of the \( B_{12} \) content of these various food categories, on the frequency of consumption of these foods from surveys by the Market Research Corporation of America, and on the mean portion size as determined by the U.S. Department of Agriculture. Results of these calculations suggest a possible average intake for individuals over 2 years of age of approximately 0.1 mg per day. In these calculations, it is assumed that all food products within a category contain vitamin \( B_{12} \) at the levels shown in Table I. The NRC subcommittee recognizes that such an assumption is likely to lead to overestimates of intake, often by a considerable margin.

A more realistic estimate of the average intake (17 \( \mu \)g per day) was obtained from the amount of vitamin \( B_{12} \) added to foods in 1970 by the food processors (an estimated 1260 kg) (21). It is believed that this value also overestimates the actual consumption of the vitamin, for no allowance is made for the vitamin \( B_{12} \) added to food but not consumed; i.e., for wastage or destruction. About 8 percent of the vitamin in liver (22) and 30 percent in
TABLE I

Addition of Vitamin $\text{B}_{12}$ to Foods by Food Category (21)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Weighted mean* mg per kg (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast cereals</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Grain products, such as pastas or rice dishes</td>
<td>2</td>
</tr>
<tr>
<td>Milk products</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Snack foods</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Reconstituted vegetable proteins</td>
<td>2</td>
</tr>
<tr>
<td>Baby food baked goods</td>
<td>40</td>
</tr>
<tr>
<td>Baby food formulas</td>
<td>3</td>
</tr>
</tbody>
</table>

*The weighted mean was calculated from the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean, see Section X and Exhibit 50 of reference 21.
milk are lost by boiling at 100°C for 5 minutes. Milk pasteurized for 2 to 3 seconds loses 7 percent of its vitamin B₁₂ (23).

The Select Committee concludes that the daily intake of vitamin B₁₂ from its addition to food is probably not more than 50 μg.

In addition to the amounts of vitamin B₁₂ consumed in food, unknown but significant amounts may be taken in the form of oral or parenteral preparations. The Physicians' Desk Reference for 1977 (20) lists 31 separate proprietary vitamin B₁₂ products as well as numerous forms of vitamin mixtures and dietary supplements. The total vitamin B₁₂ represented by the use of these preparations is unknown.

Requirements

Evidence from three different types of studies has been used to estimate the daily vitamin B₁₂ requirements: 1) the amounts needed to prevent or cure megaloblastic anemia resulting from vitamin B₁₂ deficiency; 2) comparison of blood and liver levels in normal and deficient subjects; and 3) body stores and turnover rates of the vitamin.

Intramuscular injection of as little as 0.1 μg daily in patients with pernicious anemia produces a suboptimal reticulocyte response and a slow increase in the hemoglobin concentration (24). Parenteral doses of 0.5 to 1.0 μg per day seem necessary to maintain patients with pernicious anemia in complete hematologic and neurologic remission (9). When given with intrinsic factor (see Section IV), 1 μg of vitamin B₁₂ by mouth also causes good hematologic response in deficient patients (25).

The total body stores of vitamin B₁₂ in normal subjects have been estimated to be between 1 and 5 mg with a mean of about 2 mg. Megaloblastic anemia occurs on average of 4 years (about 1500 days) after total gastrectomy. This suggests a loss of about 1 μg vitamin B₁₂ daily (19). Whole body turnover studies using labeled vitamin B₁₂ in patients with early or impending hematologic relapse showed a loss of 0.25 to 1.05 μg per day (26).

On the basis of these studies, the Joint FAO/WHO Expert Group in 1970 recommended a daily intake of 2 μg for the normal adult (19). The Food and Nutrition Board of the National Academy of Sciences in 1968 had recommended a daily intake of 5 μg for adults and adolescents (27). Upon review of the existing data in 1974, the Board reduced its recommendation to 3 μg per day (18).

Breast-fed infants normally receive about 0.3 μg daily and show no signs of vitamin B₁₂ deficiency (19). This level has been adopted by both
the Food and Nutrition Board (18) and the FAO/WHO Expert Group (19) as the recommended daily intake for infants during their first year of life.

Few data are available on which to base the allowance for pregnant women. The fetal demand on maternal stores has been estimated to be 0.3 \( \mu g \) per day and the elevated metabolic demands of pregnancy an additional 0.3 \( \mu g \) daily (19). The FAO/WHO Expert Group recommends 3.0 \( \mu g \) per day for pregnant women and the Food and Nutrition Board 4 \( \mu g \) per day. The daily intakes recommended by these two bodies are summarized in Table II.

### TABLE II

Recommended daily intakes of Vitamin B\(_{12}\)

<table>
<thead>
<tr>
<th>Age or status</th>
<th>FAO/WHO Expert Group (1970)(^a)</th>
<th>Food and Nutrition Board (1974)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-12 months</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>1-3 years</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>4-9 years</td>
<td>1.5</td>
<td>1.5-2.0(^c)</td>
</tr>
<tr>
<td>10 years and over</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Nursing mothers</td>
<td>2.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

\(^a\) Reference 19.
\(^b\) Reference 18.
\(^c\) 4-6 years, 1.5; 7-10 years, 2.0.
IV. BIOLOGICAL STUDIES

Absorption

The vitamin B₁₂ that occurs naturally in foods exists largely in coenzyme form, bound to cellular protein constituents. Upon ingestion, the bound vitamin B₁₂ is liberated by the proteolytic enzymes in the gastric and intestinal fluids. The free vitamin B₁₂ then forms a tightly bound complex in the stomach with a substance known as the intrinsic factor (9). This factor has not been completely characterized, but it is known to be a glycoprotein probably existing as a monomer and a dimer with molecular weights of 55,000 to 60,000 and 110,000 to 120,000, respectively (28). It is secreted in man by the gastric parietal cells (29). The intrinsic factor-vitamin B₁₂ complex is carried by peristalsis to the ileum and normally survives the transit through the proximal regions of the small intestine without being digested. Breakdown of the complex may occur, however, in persons with a heavy infestation of the fish tapeworm (Diphyllobothrium latum), which secretes a releasing enzyme that enables it to unbind vitamin B₁₂ from the intrinsic factor and to utilize it. Since the vitamin B₁₂ requirements of the tapeworm are high, a vitamin B₁₂ deficiency may be induced in the host (30).

The intrinsic factor-vitamin B₁₂ complex attaches itself to receptors located on the epithelial cell surface of the distal small intestine. The receptor sites appear to be on the microvillous membranes and brush borders. The uptake of intrinsic factor bound vitamin B₁₂ by the microvillous membranes was tenfold greater per milligram tissue nitrogen than was the uptake by homogenates of whole intestinal mucosa (31). The composition of the receptor for the intrinsic factor-vitamin B₁₂ complex is unknown, but MacKenzie and Donaldson (32) speculate that it is a glycoprotein or a mucopolysaccharide and that the attachment may resemble that of mucopolysaccharide blood group antigens with their gamma globulin antibodies. Vitamin B₁₂ is then released from its complex by an unknown mechanism and passes through the ileal epithelial cell into the portal venous blood. They suggested but did not demonstrate, that the complex is digested by a "releasing factor" present within or on the surface of the intestinal cell. Some support for this concept comes from the preliminary results of Peters and Hoffbrand (33) who fed a double-labeled complex (¹⁷⁷⁰-Cyanocobalamin, ¹³¹⁻¹-intrinsic factor) to guinea pigs. Significant amounts of both isotopes were detected at the brush border membrane 30 minutes after feeding, but after one hour only the radioactive vitamin B₁₂, and not the labeled intrinsic factor, was present in the mitochondrion. This suggests that the complex was split at the brush border and only the vitamin B₁₂ gained entry into the enterocyte.

Whatever the cellular mechanics involved in the passage of vitamin B₁₂ through the ileal epithelial cell, the absorbed vitamin is rapidly attached to a specific transport protein, transcobalamin II, as it enters the portal
system (9). The primary function of transcobalamin II, also known as the vitamin B$_{12}$-binding beta globulin, is to transport the vitamin to the tissues. A second protein binder, transcobalamin I (or vitamin B$_{12}$-binding alpha globulin) functions primarily as a storage protein for the vitamin, with a possible minor delivery function. A third binding protein has also been found in serum in lesser amounts than transcobalamins I and II.

The transcobalamins rapidly deliver vitamin B$_{12}$ to the liver, bone marrow, reticulocytes, and possibly to other tissues as well (9, 34). The delivery mechanism is remarkably similar to that by which the intrinsic factor conveys vitamin B$_{12}$ to the ileal mucosal cells. The immature red cells, in common with the gut enterocyte, possess "receptor sites" on their surface, specific for the complex of vitamin B$_{12}$ and the binding beta globulin. Both systems require a transport glycoprotein, a pH greater than 6, ionic calcium and a receptor site on the cell surface for the glycoprotein-vitamin B$_{12}$ complex (9).

In addition to the absorptive mechanism just described, requiring the mediation of the intrinsic factor, a small amount of vitamin B$_{12}$ may be absorbed by simple diffusion. This is a very inefficient process and only about 1 percent of the free vitamin is absorbed (9). In contrast with intrinsic factor assisted absorption which is confined to the ileum, "diffusion absorption" may occur along the entire length of the small intestine. Although less reliable than parenteral administration, this mechanism makes possible oral therapy in pernicious anemia.

**Metabolism**

The metabolic roles of vitamin B$_{12}$ and of folic acid are intimately entwined and a deficiency of either may ultimately result in megaloblastic anemia. A plausible explanation for the overlapping metabolic effects of folate derivatives and of vitamin B$_{12}$ has been advanced by Larrabee and coworkers (35). Tetrahydrofolate, which is required for the synthesis of thymidylate (and thus of DNA) is generated in a cyclic process from methyl tetrahydrofolate. Normally, the methyl group of this compound is transferred to homocystine to produce methionine. This methyl transfer is catalyzed by coenzyme vitamin B$_{12}$. The absence of vitamin B$_{12}$ causes the accumulation of methyl tetrahydrofolate and the consequent depletion of the essential tetrahydrofolate. Herbert (9) believes this "folate trap" hypothesis may explain in large part the similarity of the hematologic pathology in folate and vitamin B$_{12}$ deficiencies.

Vitamin B$_{12}$ is also involved in a number of other metabolic conversions (36). It provides the cofactor for methylmalonyl-CoA mutase in the conversion of methyl malonate to succinate, an intermediate in both fat and carbohydrate metabolism. Pernicious anemia patients excrete large amounts
of methyl malonic acid and its precursor, propionic acid. Vitamin B₁₂ also acts as a cofactor in the metabolism of glutamate and lysine, the formation of methane, the synthesis of acetate and the dehydrogenation of diols and triols.

**Excretion**

Physiological amounts of free vitamin B₁₂ are rapidly bound in the blood by specific binding globulins. Only small amounts of the bound vitamin can pass the renal barrier, but the unbound vitamin is rapidly filtered through the glomerulus. Injected vitamin B₁₂ in excess of the serum binding capacity is excreted into the urine (30). Quantities in excess of 50 µg are almost completely excreted. This forms the basis of the Schilling test which is the most widely used assay for vitamin B₁₂ deficiency (37). A dose of 0.5 µg of labeled vitamin B₁₂ is given by mouth, followed by the injection of a massive dose (1 mg) of the unlabeled vitamin which saturates the binding protein of the blood. The absorbed radioactive vitamin B₁₂ remains in the free state in the blood and is excreted by the kidney. Normal subjects excrete 10 to 30 percent of the administered dose in 24 hours, whereas pernicious anemia patients usually excrete less than 2 percent.

Relatively large amounts of vitamin B₁₂ are secreted into the bile from hepatic stores (38). The amount secreted daily in this manner may be greater than that in the total blood volume. However, an efficient entero-hepatic circulation allows the reabsorption of most of the biliary vitamin B₁₂, so that the fecal loss is small (9). This explains why deficiency signs may not appear for several years after total gastrectomy or for decades among vegetarians consuming almost no vitamin B₁₂.

**Acute toxicity**

Acute toxicity data are scarce. Traina (39) reported that the intraperitoneal injection into mice of 1.5 mg per kg killed two of 10 animals and that 3 mg per kg, both intraperitoneally and subcutaneously, caused the death of all mice tested. Intraperitoneal injection of 0.75 mg per kg produced no toxic signs. Traina used a commercial preparation of the vitamin in his study.

These results were challenged by Winter and Mushett (40) who found no toxicity after administering much larger doses. They injected up to 1600 mg per kg of crystalline vitamin B₁₂ in solution, both intraperitoneally and intravenously, into mice. They also injected up to 100 mg per kg intraperitoneally into rats and guinea pigs. In no instance were there deaths or toxic manifestations. Some mice were observed for a week while others were sacrificed 48 hours following treatment. Weight gain after vitamin injection was normal and necropsy revealed no gross pathological changes. Since Winter and Mushett employed doses more than 500 times that used by
Traina (39) with no untoward effects, they concluded that the latter's preparation was contaminated, or contained toxic impurities.

In man, Berlin et al. (41) studied the absorption of vitamin B₁₂ in the absence of intrinsic factor mediation. They administered massive doses of the vitamin by mouth to pernicious anemia patients. As much as 100 mg were given in a single dose, with no evidence of discomfort or untoward effects.

Although Traina (39) failed to demonstrate allergenicity of vitamin B₁₂ after parenteral administration to guinea pigs, Lipton and Steigman (42) successfully sensitized guinea pigs to crystalline B₁₂ by prior intradermal injections of the vitamin in Freund's adjuvant. Human sensitivity to vitamin B₁₂ administered parenterally has been reported in several studies (43-50) and at least five fatalities have occurred following intramuscular injection (48-50). Sensitivity to oral administration has not been reported.

**Short-term studies**

Most of the extensive studies on vitamin B₁₂ are in the areas of vitamin B₁₂ deficiency, or exploration of possible therapeutic effects of large doses of vitamin B₁₂ for a variety of conditions. Few provide relevant information regarding the effect of adding vitamin B₁₂ to food, except in affirming the lack of its toxicity at dose levels far in excess of nutritional requirements. Daniel et al. (51) fed female rats 200 μg per kg of vitamin B₁₂ (about 20 μg per kg body weight per day), in their diets and found no evidence that their milk was toxic to the offspring. Richardson and Brock (52) reported somewhat similar results in reproductive studies in which various amounts of vitamin B₁₂ were added to purified diets. Female rats were fed basal diets to which 10 to 1000 μg vitamin B₁₂ per kg diet were added (about 1 to 100 μg per kg body weight daily). In one group each female received 100 μg vitamin B₁₂ (about 500 μg per kg body weight) once weekly by subcutaneous injection rather than in the diet. The females were mated and the percentage of litters, the number of offspring, the weaning rate and the average weaning weight were determined. No difference was detected among any of the groups receiving the vitamin B₁₂ supplementation. The authors conclude that large doses of the vitamin (at least 100 times that required for satisfactory reproduction) can be given to the mother without any ill effect upon the offspring.

Weanling male and female rhesus monkeys born in captivity were given intramuscular injections of 500 μg vitamin B₁₂ (about 250 μg per kg) at approximately monthly intervals (53). The duration of treatment was not stated. The growth rate of the treated animals from weaning until their prepubertal growth spurt was 50 to 200 percent greater than the untreated animals.
Long-term studies

Patients with pernicious anemia have remained in clinical remission for years by repeated administration of vitamin B\textsubscript{12} either orally or intramuscularly. Brody (54) reported a 68-year-old woman with pernicious anemia who took 50 \( \mu \text{g} \) vitamin B\textsubscript{12} orally three times daily for 10 years. She remained in clinical remission although her body stores as measured by liver biopsy remained low.

In an extensive study on the oral treatment of pernicious anemia, Berlin et al. (41) reported 64 cases in which oral vitamin B\textsubscript{12} had been given for periods of 10 to 70 months. These patients received 500 to 1000 \( \mu \text{g} \) daily without any side reactions or other unfavorable effects from the long-term therapy. The authors recommended that 2000 \( \mu \text{g} \) be given twice daily during the first month to replenish the body stores and 1000 \( \mu \text{g} \) daily subsequently. They stated that oral treatment with large doses of vitamin B\textsubscript{12} was introduced in Sweden in 1964 and is now in widespread use with no reported negative experiences.

No data are available on the administration of large amounts of vitamin B\textsubscript{12} to normal individuals for extended periods.

Special studies

Carcinogenesis. Knowledge that vitamin B\textsubscript{12} is involved in nucleic acid metabolism has stimulated investigations into its possible role in carcinogenesis. A number of studies have shown that tumors take up and accumulate relatively large amounts of vitamin B\textsubscript{12}. Injection of vitamin B\textsubscript{12} into chicks caused an increased growth in Rous sarcoma (55). The labeled vitamin B\textsubscript{12} was taken up rapidly by osteosarcomas in mice and the radioactivity was localized to the actively growing parts (56). Miller and colleagues (57) reported vitamin B\textsubscript{12} uptake by Walker carcinoma in rats and methylcholanthrene-induced sarcoma in hamsters. Cooper and Paranchych (58) observed an increased specific uptake of vitamin B\textsubscript{12} in vitro by Erlich ascites tumor cells and HeLa cells. An active uptake is generally believed to indicate an involvement by the vitamin in the growth of the neoplasm. Rigby and Bodian (59), however, demonstrated that despite a high specific uptake of vitamin B\textsubscript{12}, C1300 tumors in mice showed a marked diminution of their growth rate. Bodian (60) reported a marked regression of neuroblastoma in children to whom vitamin B\textsubscript{12} was administered. However, the effectiveness of this therapy could not be confirmed by other investigators (61, 62).

Contrary to the reports indicating an increased uptake of vitamin B\textsubscript{12} by tumor cells, there are as well studies reporting a decreased uptake. Thus, Stein et al. (63) gave \( ^{60} \text{Co} \)-labeled vitamin B\textsubscript{12} to hepatoma-bearing
rats and found the radioactivity of the tumor cells to be considerably lower than normal liver tissue. In fact, Stein (64) in a later report proposed a test for the detection of liver tumors, based upon a reduced uptake of radioactive vitamin. Cooperman (65) found the neuroblastoma tissue of an infant to have both a low concentration of vitamin B_{12} and a low uptake of radioactivity. Liver, pancreas, and adrenal tumors all had considerably lower vitamin B_{12} concentrations than the adjacent normal tissues. Thus, neuroblastoma tissue can proliferate and presumably synthesize nucleic acids in the presence of low concentrations of vitamin B_{12}. Cooperman suggests that, in some cases at least, the metabolic role of vitamin B_{12} in malignant cells differs from that in the proliferating normal cells.

In a survey of patients with various neoplastic diseases, those with myelocytic leukemia generally had high serum vitamin B_{12} levels but most nonleukemic patients had normal levels (66). Four of five patients with chronic, and six of nine patients with acute, myelocytic leukemia had elevated serum vitamin B_{12} levels. The vitamin B_{12}-binding capacities are also elevated in patients with myelocytic leukemia and these estimations are often used as diagnostic tests for this condition (67). However, Carmel and Coltman (68) caution against this practice, for similar elevations can be observed in nonleukemic leukocytosis.

A strong correlation was also noted between hepatic metastases and elevated serum vitamin B_{12} levels (66). Of 12 nonleukemic cancer patients with markedly elevated vitamin B_{12} values, 10 had abnormal liver function tests and eventual autopsy of eight of these confirmed hepatic involvement. Patients with cirrhosis of the liver (69) and with acute hepatitis (70) have also been reported to have high vitamin B_{12} values.

No reports are known attributing carcinogenic properties to vitamin B_{12} alone. However, it has been reported to enhance hepatoma formation in rats fed a methionine-deficient diet containing p-dimethylaminoazobenzene (DAB) (71). Rats fed on this diet for 170 days had an incidence of 17 percent hepatomas. When the diet was supplemented with 50 µg vitamin B_{12} per kg (about 5 µg per kg body weight daily), hepatomas appeared in 78 percent of the rats. A control group receiving the same basal diet with vitamin B_{12} but without DAB showed no hepatic tumors.

Vitamin B_{12} has also been reported to accelerate the development of methylcholanthrene-induced sarcomas (72). Cobalt in metallic form or as salts have been shown to produce malignant tumors at the site of subcutaneous injection (73, 74).

**Mutagenesis.** No reports of studies on the mutagenicity of vitamin B_{12} have come to the attention of the Select Committee (75).
Teratogenesis. There is a progressive increase in the amount of vitamin B_{12} transferred to the fetus during gestation. In rats, the amount transported per g of placenta increased tenfold from day 10 to day 19 (76). This transfer occurs against a concentration gradient, for maternal plasma levels late in pregnancy are considerably lower than the corresponding fetal levels (77).

Richardson and Brock (52) maintained five generations of female rats on synthetic diets supplemented with 10 to 1000 μg vitamin B_{12} per kg diet (about 1 to 100 μg per kg body weight daily). Over 90 percent of all the offspring reached normal weaning weight. There was no evidence that large amounts of vitamin B_{12} (more than 100 times the required amount) in the diet of the mother had any toxic effects on the offspring.

Newberne (78) treated four groups of female rats as follows: 1) depleted of vitamin B_{12} from weaning; 2) received 30 μg vitamin B_{12} per kg diet (about 3 μg per kg body weight daily); 3) received 500 μg vitamin B_{12} (about 2500 μg per kg) by injection daily from day 10 of gestation through term; and 4) received 30 mg vitamin B_{12} per kg diet (about 3 mg per kg body weight daily). The periods during which groups 2 and 4 were fed their respective diets were not stated. All groups were mated to normal males. Tissues of the newborn rats of groups 3 and 4 were heavier and reached maturity earlier compared with groups 1 and 2. No pathological changes or abnormalities were reported.

Other studies

Foulds et al. (79) successfully treated with hydroxocobalamin, two patients suffering from the rather rare disease, Leber's hereditary optic atrophy. This condition is believed to be a genetically determined sensitivity to certain constituents of tobacco smoke, perhaps cyanides or cyanogens, resulting from a defect in their detoxification. The effectiveness of hydroxocobalamin was presumably due to its strong affinity for cyanide. Cyanocobalamin, on the contrary, is stated to have aggravated the condition when it was substituted for hydroxocobalamin (80).

Findlay (81) claimed that subcutaneous injection of 0.5 μg vitamin B_{12} increased the tensile strength of scar tissue and accelerated the healing of experimental wounds in rats.

Vitamin B_{12} deficiency is frequent in psychiatric patients and in various forms of central nervous system disturbances. As Herbert and Tisman (82) point out, unless recovery is complete with vitamin B_{12} therapy, it is difficult to determine whether the condition is due to vitamin B_{12} deficiency or to other causes.

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V. OPINION

Vitamin B₁₂ has shown no toxicity to animals at several thousand times their nutritional requirements. In man, pernicious anemia patients have received daily doses for years 10 to 20 times that of the highest estimate of average daily consumption. The only reaction to vitamin B₁₂ so far demonstrated in man is the development of sensitivity that can become manifest as allergy or anaphylaxis after parenteral administration of relatively high doses.

Vitamin B₁₂ absorption is specific and limited, so that only a very small proportion of vitamin B₁₂ given orally becomes physiologically available and active. The body stores are depleted very slowly because of enterohepatic recirculation.

The addition of vitamin B₁₂ to food, in amounts far in excess of need or of absorbability appears to be without hazard.

On the basis of available evidence, the Select Committee concludes that:

There is no evidence in the available information on vitamin B₁₂ that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current and in the manner now practiced, or that might reasonably be expected in the future.
VI. REFERENCES CITED


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