SOME STUDIES ON THE RELATION BETWEEN VITAMIN B_{12} AND GROWTH HORMONE IN THE RAT

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SOME STUDIES ON THE RELATION BETWEEN VITAMIN B_{12} AND GROWTH HORMONE IN THE RAT

By

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INTRODUCTION.

INTRODUCTION

A number of factors other than inadequate dietary intake can cause malnutrition, including conditions that interfere with absorption or utilization of nutrients, or those that increase their requirement, destruction or excretion. "These 'conditioning factors' as Jolliffe terms them have one point in common: they increase body requirements for specific nutrients or precipitate nutritional deficiencies on diets that would otherwise be adequate were such factors not operative." (Ershoff, 1948). A general group of factors (stress factors) which increase body requirements for essential nutrients include physical exertion, fever, drugs, toxins, abnormal environmental conditions, pregnancy and lactation.

In recent years considerable information has accumulated concerning the vitamin requirements of animals subjected to stress brought about by marked changes in hormone levels within the body. It is a matter of some importance whether marked changes in the level of any particular hormone cause a concomitant increase or decrease in the need for various dietary nutrients. Such an effect of a particular hormone might conceivably be of aid in (1) helping to elucidate a possible interrelationship between the hormone and the dietary nutrient in normal metabolic processes, (2) differentiating between the ill effects caused directly by excessive amounts or deficiencies of certain hormones and those due to an accompanying increase or decrease in requirement of dietary nutrients and

(3) establishing the level of hormone secretion at which supplementation of a particular nutrient is required.

The most extensive investigations concerning the relation between endocrine secretions and dietary needs have been concerned with the thyroid. Hyperthyroidism has been shown to increase the need for calories, certain vitamins such as A, thiamin, riboflavin, pyridoxine, niacin, folic acid, ascorbic acid and minerals. Hypothyroidism presumably decreases the need for these nutrients. Hyperthyroidism has also been shown recently to increase the need for vitamin B_{12}. It has further been demonstrated that marked variations in estrogen or cortisone levels in the body can also increase the requirement for vitamin B_{12} and possibly other dietary growth factors in rats. Supplementation of the diets with vitamin B_{12} and antibiotics partially or completely prevented manifestation of dietary deficiencies induced by hormonal changes.

The present experiment was undertaken to determine whether or not the requirement for vitamin B_{12} could be altered under the stimulus to growth induced by administration of anterior pituitary growth hormone. It has already been shown that growth hormone increases the requirements for vitamin A (Ershoff and Deuel,1945; Margitay-Becht and Wallner, 1937) and pantothenic acid in rats (Lotspeich, 1950). It was postulated that if vitamin B_{12} were required for optimal growth, then the additional stimulus to growth incurred by the administration of growth hormone would result in an increased need for this vitamin. Such an increased need, it was believed, would be indicated by the precipitation of the symptoms of a vitamin B_{12} deficiency, the most obvious of which would be retardation of growth rate.

The actual data obtained in the experiments reported here indicate that growth hormone may not increase the need for vitamin B_{12}, and may even reduce requirements for this vitamin under certain experimental conditions.

REVIEW OF BIOLOGICAL EFFECTS OF GROWTH HORMONE

REVIEW OF BIOLOGICAL EFFECTS OF GROWTH HORMONE

I. Effects on Body Growth and Protein Metabolism.

As early as 1912 Cushing reported that removal of the anterior pituitary in growing animals stopped growth and precipitated a loss in body weight. Later, numerous reports indicated that replacement therapy, either by injection of the extract (Evans and Long, 1922; Fraenkel-Conrat et al., 1940; Marx, Simpson, Reinhardt and Evans, 1942) or by daily implantation of whole pituitary (Smith, 1923, 1927, 1930) resulted in a resumption of growth.

The administration of anterior pituitary extract or implantation into normal animals was shown to result in gigantism in young animals (Putnam et al., 1928; Smith, 1923, 1927, 1930). Similar treatment of adult animals gave results which were species dependent. In dogs but not in rats acromegaly occurred. However, in both species a marked visceromegaly occurred (Putnam et al., 1928).

One early report (Bryan and Gaiser, 1932) indicated that the ultimate body size of rats following anterior pituitary "growth hormone" injection was not larger than could be obtained by proper dietary means. The changes in body composition of animals after treatment with pituitary extracts causing accelerated growth have been studied by several workers (Downs, 1930; Wadelin, 1932; Bierring and Nielsen, 1932; Lee and Schafer, 1934). Lee and Schafer (1934, 1935) made the rather important finding that on a given quantity of the same food, animals treated with anterior pituitary

extract grew faster in weight than untreated controls. Their data indicated that (1) treated animals on an *ad libitum* diet consumed 10 to 15 percent more food than the non-treated controls, and (2) the treated animals gained more weight than the untreated, even if pair-fed.

The interpretation by these authors that the anterior pituitary growth hormone stimulated true growth was substantiated by Brues' work in 1936. His data showed a more rapid liver regeneration after partial hepatectomy in rats injected with anterior pituitary than occurred in untreated control animals. The liver of the injected rats contained more water, ash and protein than did the liver of the controls. It was also found that all visceral organs grew more rapidly following hormone injections than did the body as a whole.

In contrast to the results obtained by the above mentioned workers, Kleiber and Cole reported (1939) that they did not find any significant difference in the ash, fat or protein content of injected and control rats. Since true growth is generally interpreted as accumulation of protein, it was reasonable to expect that an important function of growth hormone would be the retention of nitrogen, which in turn increased the protein content of the body tissues.

Early reports (Teel and Watkins, 1929; Gaebler, 1933; Harrison and Long, 1940) have indicated that growth promoting pituitary extracts caused a reduction in both the blood non-protein nitrogen and urinary nitrogen. Later work (Marx, Magy, *et al.*, 1942; Fraenkel-Conrat *et al.*, 1942) with partially purified growth hormone have confirmed these results. In 1939 Houchin succeeded in reducing the alkali soluble protein components of the

liver following the injection of anterior pituitary extract and suggested the existence of a protein metabolizing hormone distinct from the lactogenic, thyrotropic, carbohydrate metabolizing, fat metabolizing and gonadotropic hormones.

The administration of anterior pituitary extract to dogs was reported to have a sparing effect on protein, i.e., there was a decrease in protein catabolism (Hirsky and Swadish, 1936). It was also demonstrated that if the extract were administered to dogs subsequently eviscerated, an increase in the rate of non-protein nitrogen accumulation resulted in these dogs as compared to the untreated eviscerated dogs. This was interpreted as indicating that the extract increased the rate of protein metabolism in muscles, but that the effect was not apparent in the presence of the abdominal viscera. The same workers reported that in the absence of the pancreas, the anterior pituitary increased rather than depressed the rate of nitrogen metabolism. It was postulated that a dual action of such extracts on protein resulted in (1) direct stimulation of protein catabolism in the muscles and (2) an indirect stimulation of protein anabolism, through stimulation of the pancreas.

Gaebler and Robinson (1942) reported that anterior pituitary extract could induce nitrogen retention in a dog lacking thyroid, pancreas and both adrenals. An earlier report by Gaebler and Bartlett (1939) indicated that the administration of anterior pituitary extract to normal dogs caused an increase in urine volume, water intake, body weight and oxygen consumption. A decrease in phosphate and nitrogen excretion in the urine was also noted.

In a review Soskin (1941) concluded that the anterior pituitary extract accelerates protein catabolism and gluconeogenesis from protein by the liver, but that in the presence of the pancreas the effects of the anterior pituitary are masked by the opposing action of insulin.

Harrison and Long (1940) demonstrated that anterior pituitary extract reduced urine nitrogen excretion, non-protein nitrogen of the blood and blood sugar, although ketonuria was increased. In addition, these workers made the important finding that the above effects persisted even after adrenalectomy.

Li and Evans (1947) in a review stated that although their early experiments were performed with crude or partially purified preparations, it appeared likely that the growth promoting action of the pituitary was accompanied by either an increase of protein anabolism, or a decrease of protein catabolism, or both. The work of Fraenkel-Conrat et al., (1943) demonstrated a decrease in liver arginase content in rats stimulated with a purified growth hormone preparation and seemed to indicate that protein catabolism had indeed been inhibited. Gaebler et al. (1949) studied the effect of growth hormone upon several enzyme systems, including liver and kidney D-amino acid oxidase and muscle succinic acid dehydrogenase. Their data indicated that, with respect to these particular enzyme systems, the concentration of the enzyme in the respective tissue was constant. They concluded that this indicated synthesis in direct proportion to tissue growth.

The effects of growth hormone upon body growth in general and nitrogen retention with accompanying protein synthesis in particular have been summarized by Li (1948) as follows:

(a) retention of urinary nitrogen

(b) lowering of blood amino acid concentration

(c) elevation of the protein content and a decrease in the fat content in the carcass and thymus

(d) elevation of the blood alkaline phosphatase and inorganic phosphate levels

(e) elevation of the ribonucleic acid content of the liver

(f) enhancement of amino acid uptake into protein in the skeletal muscle.

Through what mechanisms growth hormone performs these protein anabolic functions is as yet unknown.

II. Effects on Carbohydrate Metabolism.

The mechanisms by which growth hormone stimulates carbohydrate metabolism are as yet unknown; that such stimulation does occur can scarcely be doubted. The work of Mirsky and Swadish (1936) has already been cited as indicating a possible relationship between growth hormone and pancreatic activity.

Bennett and Li (1940) demonstrated that in rats made diabetic by alloxan and maintained on a constant food intake, a significant nitrogen retention and gain in weight were obtained upon injection of pure growth hormone. More evidence that growth hormone and the diabetogenic factor in anterior pituitary extracts may be the same has been provided by Houssay and Leloir (1939).

In a very recent report by Raben and Westermeyer (1952) evidence is offered that the diabetogenic factor of the pituitary is not identical

with growth hormone. The authors assert that growth hormone prepared from a glacial acetic acid extract was equal in growth promoting effects to other purified extracts, but did not produce diabetes. Russell (1938, 1942) after having done a considerable amount of work on the effects of growth hormone on carbohydrate metabolism, concluded that growth hormone administered to rats is concerned not only with the preservation of body carbohydrates during fasting, but also with the disposition of this substance when fed. In a later report, Illingworth and Russell (1951) reported that a single intraperitoneal injection of growth hormone in adult fasted rats caused an increased accumulation of glycogen in the gastrocnemius, heart and diaphragm muscles.

III. Effects on the Skeletal System.

The influence of the pituitary on the growth of the skeleton has been known for some time (Dott and Fraser, 1923; Handelsman and Gordon, 1930) but the specific effects of growth hormone on the epiphyseal cartilage of hypophysectomized animals have been described more recently (Freud et al., 1939; Ray et al., 1941). Kibrick et al., (1941) concluded that these effects were as follows: "Hypophysectomy rapidly initiates a loss in the dimensions of the epiphyseal plate, despite the fact that growth of the cartilage and bone may continue for a short time in the young animals after removal of the pituitary. This loss in thickness reflects the initial disturbance of the equilibrium that normally exists between chondrogenesis and osteogenesis. Administration of pituitary growth hormone rapidly restores the dimensions of the cartilage plate by

stimulating, first, chondrogenesis and then osteogenesis until an equilibrium is reestablished."

Becks et al., (1946) have shown that administration of growth hormone to hypophysectomized rats, even after postoperative intervals of a year or longer, was able to reawaken chondrogenic and osteogenic processes in the epiphyseal cartilage of the tibia to an extent comparable to that seen in normal, young, growing rats.

In early studies concerning the relation between the growth promoting effects of anterior pituitary and the thyroid, Evans, Simpson and Pencharz (1939) concluded that the growth effect obtained with anterior pituitary extracts were not dependent upon the thyroid but that they were greater if the thyroid were present. Marx, Simpson and Evans (1942) reported that purified thyroprotein did not itself possess a growth stimulating action, although it did exert a true synergism with growth promoting pituitary extracts in hypophysectomized rats. Such synergism is not mediated by the thyroid since it occurs even in the absence of the thyroid gland.

More recently, a great deal of information has been gained concerning the possible mechanisms involved in the action of growth hormone and of thyroxin on bone growth and differentiation. Most of the work in this area has been done with rats thyro-parathyroidectomized at birth. Several reports (Salmon, 1938a, 1938b; Scow and Simpson, 1945) indicate that such animals exhibit a marked retardation in growth and maturation. Later, several workers (Scow and Marx, 1945; Becks et al., 1948) showed that the administration of growth hormone caused an increase in rate of growth, both in body weight and length, without an increase in the rate of

maturation. Apparently the growth hormone is essential for growth, i.e., increased dimensions, but is not able to stimulate the process of differentiation or maturation in the thyro-parathyroidectomized animal. The thyroid was found to be necessary for the latter process.

It was suggested by Scow et al. (1949) that the mechanism by which thyroxin therapy corrects the retarded growth in thyro-parathyroidectomized animals was to restore in part at least the production of growth hormone by the pituitary. The non-endocrine viscera of such injected animals were maintained in proportion to body weight with the exception of brain and eyeball. The endocrine and reproductive viscera developed only in the rats receiving thyroxine.

Further evidence by Koneff et al. (1949) of the histological changes in the pituitary of thyro-parathyroidectomized animals tended to support the theory that thyroxin therapy caused the release of endogenous growth hormone. It was found that thyroxin administration alone was responsible for restoration of the acidophiles, the putative source of growth hormone. Histological evidence for the changes in individual bones also indicated that thyroidectomy markedly retards skeletal growth and differentiation, and that growth hormone stimulates growth without differentiation, (Becks et al., 1950). Thyroxin alone was demonstrated to stimulate growth and differentiation. There was no augmentation of either of these effects when both hormones were administered together.

These relationships between the effect of growth hormone and of thyroxin have been summarized by Asling et al. (1951) as follows: "It appears, that, in rats, growth hormone is a potent stimulus to skeletal

growth, but has little effect on maturation, while thyroxin directly advances maturation but stimulates growth only slightly. By administering these hormones concurrently in properly balanced dosages, a normal balance between growth and differentiation may be established. The necessity of both hormones for this balance is best shown by studying their actions in thyroidectomized-hypophysectomized rats."

Growth hormone has also been shown to be independent of the thymus gland, although thymus hypertrophy does occur in the intact animal upon the injection of growth hormone (Meinhardt, Marx and Evans, 1941). This may simply be due to an increase in protein anabolism causing an increase in thymus protein. One other action attributed to growth hormone may be mentioned, and that is the so-called "renotropic effect". It was reported by Selye and Jensen (1946) that crude extracts of the anterior pituitary caused pronounced enlargement of the kidneys, and that this renotropic effect was greatly enhanced by the simultaneous administration of thyroxine. The increase in size of the kidneys was much greater than could be expected from the growth hormone content of the extract, although other anterior pituitary preparations failed to exhibit any significant renotropic potency. Kochakian (1949) reported that androgens markedly increase the size of the kidney, while purified growth hormone does so only slightly. It seems possible that the crude extract used by Selye and Jensen (1946) contained sufficient gonadotrophic hormone to cause stimulation of androgen production, which in turn elicited the increase in kidney size.

VITAMIN B_{12} REVIEW

In 1948 Smith, Rickes et al. (1948a) announced the isolation of the anti-anemia factor in the semi-pure state and gave it the name vitamin B_{12} or erythrotin. Vitamin B_{12} was found by Shorb (1948) to be identical with one of the two unidentified growth factors required by Lactobacillus lactis Dorner, and both of these unidentified factors were present in refined liver extracts.

The subsequent work on the actions of vitamin B_{12} may be most conveniently divided into several categories. One main line of study has been the effects of vitamin B_{12} in the treatment of pernicious anemia. A second has been concerned with the effects of vitamin B_{12} on body growth and its specific role in metabolic processes, and a third has dealt with the relation between vitamin B_{12} and various endocrine secretions.

I. Effects of Vitamin B_{12} on Erythropoesis.

The work dealing with the effects of vitamin B_{12} in the treatment of pernicious anemia is primarily of a clinical nature, but many of the findings have contributed to the elucidation of its functions in various metabolic processes.

Crystalline vitamin B_{12} prepared from liver extracts gave a positive response when injected intramuscularly into patients with Addisonian pernicious anemia in doses as small as 3 µg (West, 1948), although the actual amount required to produce a maximum reticulocytosis response was

6 to 10 µg (Hall and Campbell, 1948; Ungley 1946a, 1948b). An average maintenance dose was 10 ug every two weeks (Ungley, 1949). Vitamin B_{12} was also shown to be effective in nutritional macrocytic anemia and tropical sprue (Patel 1948; Spies, Suarez et al., 1949). Vitamin B_{12} prepared from S. griseus cultures (Dunlop and Wilson, 1949, Miller and Moorhouse, 1949) and vitamin B_{12b} prepared from S. aureofaciens cultures (Lichtman et al., 1949) were apparently as effective in pernicious anemia as vitamin B_{12} prepared from liver extracts.

In a later report (Hausmann, 1949) it was indicated that vitamin B_{12} existed in certain substances in the form of conjugates, inactive in pernicious anemia until they had been digested with hogs' stomach mucosa or with pancreatic enzyme extract. It was further suggested that these conjugates might be identical with Castle's "extrinsic factor" (Castle 1929; Castle et al., 1930; and Watson and Castle, 1946). Ternberg and Bakin (1949) showed that the active principle in gastric juice long designated as the "intrinsic factor" was in reality a protein which combined with vitamin B_{12} (extrinsic factor) to form a complex which was resistant to the destructive changes wrought upon B_{12} itself by the digestive processes. It has been suggested that these interrelationships favored substitution of the term erythrotin for vitamin B_{12}, apoerythein for intrinsic factor, and erythein for the complex. Beerstecher and Edmonds (1952) have offered evidence for the existence of an apoerythein precursor in the gastric mucosa and suggested that it was probably identical with the intrinsic factor.

The availability of Co^{60} labeled-vitamin B_{12} has been of considerable aid in the study of the distribution of this substance under various

experimental conditions. Welch et al. (1952) using radioactive B_{12} have shown that the intrinsic factor is involved in the removal of vitamin B_{12} from the gastrointestinal tract.

Lang and Chow (1952) have studied the effect of age on retention of Co^{60} labeled-vitamin B_{12} and have found that in rats excretion is greater in young animals than in older ones. The rate of disappearance of Co^{60} labeled-vitamin B_{12} from various organs of the rat has also been studied (Harte, Chow, Barrows, 1952). Following the administration of a single dose of Co^{60} labeled-vitamin B_{12} more radioactivity was located in the kidneys than elsewhere after the first day, but this amount tended to decrease sharply with time. Lesser absolute amounts but much higher concentrations of activity were found in the pancreas. The amounts of activity in the liver remained essentially constant, or even increased with time. They concluded that the vitamin in liver must be associated with the protein moiety, since the total amount of radioactivity remained constant but the concentration rose when the liver glycogen was mobilized as a result of caloric deprivation.

II. Vitamin B_{12} and Body Growth.

The effects of vitamin B_{12} on the growth of animals have been widely studied in the past few years. Cary et al. (1946) and Hartman (1946) described a "factor X", the absence of which resulted in a decline in the growth rate of rats, a decline that became more marked when the protein content of the diet was increased. The factor appeared to be similar to

the so-called animal protein factor (APF), which increased the hatchability of hens' eggs (Nestler et al., 1938) and the growth rate of chicks maintained on an all vegetable protein ration (Hammond, 1944). It was also similar to a factor in cow manure that stimulated the growth of chicks (Rubin and Bird, 1946; Bird et al., 1946).

Crystalline vitamin B_{12} was found to exhibit animal protein factor activity for chicks fed soy bean meal as the sole source of protein; it was as effective as the cow manure factor in stimulating the growth of chicks (Ott et al., 1948; Lillie, Denton and Bird, 1948; Lillie et al., 1949). It also increased the growth rate of rats on a factor X-depleted diet, showing that vitamin B_{12} plays a fundamental role in the utilization of protein (Hartman, Dryden and Cary, 1949).

Some evidence has accumulated indicating that vitamin B_{12} is concerned with transmethylation. In the first place, on a diet complete in the known vitamins, the growth of chicks was improved by supplementation with choline or betaine; subsequent addition of a liver paste containing little choline was even more effective and further supplementation with choline or betaine then had little effect (Gillis and Norris, 1949). Crystalline vitamin B_{12} has also been shown to increase the growth rate of chicks on a diet low in choline. Renal injury in rats due to a low choline and methionine intake was minimized by the addition of vitamin B_{12} to the diet, and the gain in weight was also increased, but not when adequate amounts of choline were fed (Schaefer, Salmon and Strength, 1949).

It has been shown (Bennett, Joralemon and Halpern, 1952) that rats on a "labile methyl free" homocysteine diet containing folic acid are

able to maintain normal livers, and that without B_{12} such animals cease to grow and fatty livers result. Rats fed diets containing 15% casein showed signs of an acute choline deficiency in ten days, and the addition of vitamin B_{12} and folic acid reduced the liver fat and prevented kidney damage (Fischer and Hall, 1952).

Vitamin B_{12} had a marked lipotropic effect when injected into rats fed a high fat diet (Drill and McCormick, 1949). The administration of vitamin B_{12} to rats preceding acute carbon tetrachloride intoxication prevented liver injury (Popper et al., 1949).

Several investigators reported that vitamin B_{12} played a role in nitrogen retention and presumably in protein metabolism. It was reported that the circulating blood of chicks receiving 50 µg of vitamin B_{12} per kilogram of feed contained less non-protein nitrogen and less of each of seven amino acids than did the blood of vitamin B_{12}-deficient chicks. In addition, the birds fed the supplement grew more rapidly and utilized the feed more efficiently than did the B_{12}-deficient controls. The authors concluded that vitamin B_{12} appears to function in metabolism by enhancing the utilization of circulating amino acids for building fixed tissues (Charkey et al., 1950). Vitamin B_{12}-deficient chicks were less able to withstand the toxic effects of force fed glycine (Stern et al., 1951). Such an effect would tend to support the theory that vitamin B_{12} enhances the utilization of amino acids.

More recent work (Chow and Barrows, 1950) on rats raised on a corn soy bean diet indicated that the growth rate but not the efficiency of nitrogen utilization was increased by the addition of vitamin B_{12}. These

authors concluded that vitamin B_{12} does not enhance the biologic value of soy bean proteins, but may play an important role in carbohydrate or fat metabolism.

The carcass composition of rats fed on a corn-soy diet has also been investigated (Ling and Chow, 1952) and the results indicate a low fat, high water and normal protein content, while the sulfhydryl content of the blood cells was low, due primarily to a lowered glutathione content. The administration of vitamin B_{12} corrected these abnormalities. It was further demonstrated that a high carbohydrate diet, coupled with the injection of glucose could also cause a decrease in the glutathione content of the red blood cells. The authors suggested that the effect of the high carbohydrate diet could be due to an increased utilization or requirement of vitamin B_{12}. The fact that B_{12}-deficient rats had smaller carbohydrate reserves, and that intravenous injections of glucose were poorly utilized by these animals was offered as support for this theory.

III. Interrelations Between Vitamin B_{12} and Endocrine Secretions.

Several reports have been concerned with the relation between the level of various endocrine secretions and vitamin B_{12} metabolism. Rats fed rations containing thyroid-active materials required a factor present in liver, fish solubles and tomatoes for growth (Bethell and Lardy, 1949). The factor was present in anti-pernicious anemia active fractions, but whereas Bethell and Lardy (1949), Emerson (1949) and Meites (1950) found vitamin B_{12} to be active, Ershoff (1949) found it to be ineffective. Inconsistent results have been obtained when attempts were made to use

hyperthyroid rats for the assay of vitamin B_{12}. The assay was based on the assumption that hyperthyroidism induced in rats by feeding iodinated casein caused a depletion of vitamin B_{12} body stores. However, it has been claimed that the test is not specific for vitamin B_{12}.

Liver extracts active in pernicious anemia failed to stimulate the growth of thyrotoxic chicks, and crude liver extracts discarded from anti-pernicious anemia fractions were highly active (Nichol, Robbins, et al., 1949). Vitamin B_{12} replaced the animal protein factor of injectible liver preparations when tested on thyrotoxic chicks (Nichol, Dietrich, et al., 1949). In normal chicks, maximum growth was not obtained until other supplements were added (Stokstad et al., 1949).

Vitamin B_{12} was also shown to be less effective than 1:20 liver powder in counteracting the growth depression induced by feeding toxic levels of iodinated casein to normal male and to oophorectomized female rats (Watts et al., 1951). Little differences in response to these two supplements were noted in similarly fed normal females and castrated males.

A rapid depletion of the liver stores of vitamin B_{12} was reported to be accomplished by a diet containing sulfasuxidine and iodinated casein, but free of choline and vitamin B_{12} (Travers and Cerecedo, 1952). The growth of these rats was stimulated by the addition of vitamin B_{12} and of choline. Vitamin B_{12} was found to be without effect if choline were present in an adequate amount, and choline could prevent to a large extent the depletion of the liver stores of vitamin B_{12}.

Meites and Shay (1951) have shown that vitamin B_{12} is able to overcome the growth retarding effects of diethylstilbestrol and thyroprotein

when given individually or in combination to young rats. The vitamin increased the food consumption but did not alter the ability of either of the two hormones to depress the testes weight or the ability of diethylstilbestrol to increase the weight of the seminal vesicles. These authors also stated that vitamin B_{12} did not alter the turnover of thyroprotein in the rat, since the increase in the basal metabolism of rats given thyroprotein was unaffected by the administration of vitamin B_{12}. It has also been shown that desiccated whole liver or yeast can overcome the inhibition of ovarian development in immature rats fed huge doses of alpha-estradiol (Ershoff and Deuel, 1946; Ershoff and McWilliams, 1948).

Meites (1950a) has studied the effects of vitamin B_{12} on thiouracil action in rats. He concluded that vitamin B_{12} can overcome the growth-retarding action of thiouracil in female rats, even though the thiouracil depressed thyroid activity, as measured by the uptake of radioactive I^{131}. He believed that these findings suggested that vitamin B_{12} does not require the mediation of the thyroid to exert its anabolic effects on growth. Libby and Meites (1952) studied the effects of vitamin B_{12} and penicillin on thiouracil action in chicks and found that these two substances partially or completely counteracted the growth depression and reduction of appetite caused by thiouracil administration to young chicks. Neither the vitamin nor the antibiotic had any effect on the severe inhibition of comb growth induced by thiouracil. In a study on the effects of vitamin B_{12} on normal thyroid function, Meites (1950b) found that when the vitamin was included in the diet of normal or thyroprotein-treated rats, the growth rates of these animals was increased. He found, however,

that there was no significant effect on thyroid weight or uptake of radioactive I^{131}, and concluded that vitamin B_{12} does not alter normal thyroid activity in rats.

The effects of vitamin B_{12} on the survival and growth rates of young rats subjected to thyro-parathyroidectomy has been studied (Meites, 1952). Such animals supplied with 50 or 200 µg vitamin B_{12} per kilogram of diet showed an increase in survival and growth rates over similarly treated animals which did not receive vitamin B_{12} in the diet.

Wells and Kendall (1940) and Ingle (1950) have demonstrated that large doses of cortisone were able to inhibit both body and hair growth in rats, and that these effects have been partially attributed to the reduction in the availability of protein (Engel; Hoberman, 1950). Winter, Silber and Stoerk (1950) reported that large doses of cortisone reduced normal food intake in rats. In 1951, Meites reported that vitamin B_{12} and aureomycin were able to counteract the inhibition of body, hair and thymus growth induced by injecting immature rats with large doses of cortisone. Vitamin B_{12} was more effective than aureomycin and a combination of the two was more effective than either given alone. The actions of the vitamin and the antibiotic in overcoming the effects of the administered cortisone were accompanied by an increased food consumption and a greater efficiency in converting food into body weight gains.

While it is generally believed that a deficiency of a particular hormone decreases the need for various vitamins, there are several reports which indicate the reverse may be true, that is that low hormonal levels may actually increase the need for certain nutrients. As already noted

extreme hypothyroidism as well as extreme hyperthyroidism appears to increase the need for vitamin B_{12}. Dumm and Nalli (1948) have reported that in rats subjected to adrenalectomy, pantothenic acid increased survival and resistance to various stresses. Meites (unpublished observation) has also found that vitamin B_{12} and aureomycin increase survival time and body weight in adrenalectomized rats on a vitamin B_{12}-deficient diet. Shaw and Greep (1949) have demonstrated that in rats subjected to hypophysectomy, survival and growth rate have been enhanced by purified diets adequate in all known nutrients and apparently rich in vitamin B_{12}.

To summarize the actions of vitamin B_{12}, it has been demonstrated conclusively that the vitamin is identical with the antipernicious anemia factor and exerts a powerful stimulus to erythropoesis. Abundant evidence is also available that vitamin B_{12} is a powerful stimulus to growth in immature animals, and is doubtless identical with the so-called "animal protein factor". Though the mechanisms by which the vitamin exerts its influence on body metabolism are as yet not fully known, numerous reports indicate that it acts favorably on protein synthesis. In addition, several reports have indicated a possible role in fat metabolism. Finally, considerable work carried on by Meites and others seems to indicate that vitamin B_{12} can counteract some of the undesirable effects accompanying the administration of large quantities of hormones.

EXPERIMENT I. Relation Between Anterior Pituitary Growth Hormone and Vitamin B_{12} in the Growth-Plateaued Female Rat.

PROCEDURE:

Forty female albino rats of the Carworth strain, weighing 230 grams were placed on the following vitamin B_{12}-deficient diet (Zucker and Zucker, 1950) for three weeks:

Yellow corn meal	35% (by weight)
Ground wheat	25%
Linseed oil meal	10%
Soybean oil meal	20%
Alfalfa leaf meal	6%
Brewers yeast	3%
Sodium chloride	1%

During this period it was determined that the animals had reached a "growth plateau" and were gaining only three grams per week. At the end of this depletion period the animals were divided into five groups of eight animals each and were placed in individual cages.

The experimental diets consisted of the basal vitamin B_{12} deficient diet supplemented with crystalline vitamin B_{12}* in amounts ranging from 100 to 200 µg per kilogram of diet. Animals receiving growth hormone were injected subcutaneously each day with 1 mg. anterior pituitary growth hormone.** On the 26th day of the experiment, .01% (by weight)

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* Crystalline vitamin B_{12} was supplied through the kindness of Merck and Company, Inc., Rahway, New Jersey.

** The growth hormone was furnished by the kindness of Dr. I. M. Bunding of Armour Laboratories, Chicago, Illinois. One gram of this crude pituitary preparation GH-3 has an activity equivalent to approximately 40% of pituitary growth hormone standard 22 KH-2 and this material is contaminated to the extent of approximately 7% with thyroid stimulating hormone in terms of the new U. S. P. Standard.

Protamone* was added to the diets of the animals receiving growth hormone. Body weight and food intake were measured every two days.

After 40 days all animals were sacrificed and the kidneys, thymus, splean, adrenals and ovaries removed and weighed to the nearest tenth of a milligram on a Roller-Smith balance.

The following formula was used to calculate the standard error of the mean (S. E.) in this and the other two experiments:

$$S.\ E. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

RESULTS:

Figure 1 shows the average body weight plotted against time in days. Table I shows the average body weights at the beginning of the experiment, at 28 days, and at the termination of the experiment. Table I also shows the average daily food intake and the food intake per gram gain in body weight. The animals on the vitamin B_{12} deficient diet (group 1) gained as much as those receiving 200 µg of vitamin B_{12} per kilogram of food (group 2) and were somewhat more efficient in utilizing their food. Rats receiving growth hormone alone (group 3) or with vitamin B_{12} in the diet (groups 4 and 5) gained approximately 20 grams more weight and were more efficient in utilizing their food than animals on the vitamin B_{12} deficient diet and not receiving growth hormone (group 1).

Table II shows the weights of the tissues per hundred gram body weight. The weights of all organs from animals receiving growth hormone and thyroprotein are larger than those from animals either on the vitamin B_{12} deficient diet or on the diet supplemented with vitamin B_{12}.

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* An iodinated casein product containing thyroxine, manufactured by Ceroplyl Laboratories, Kansas City, Missouri.

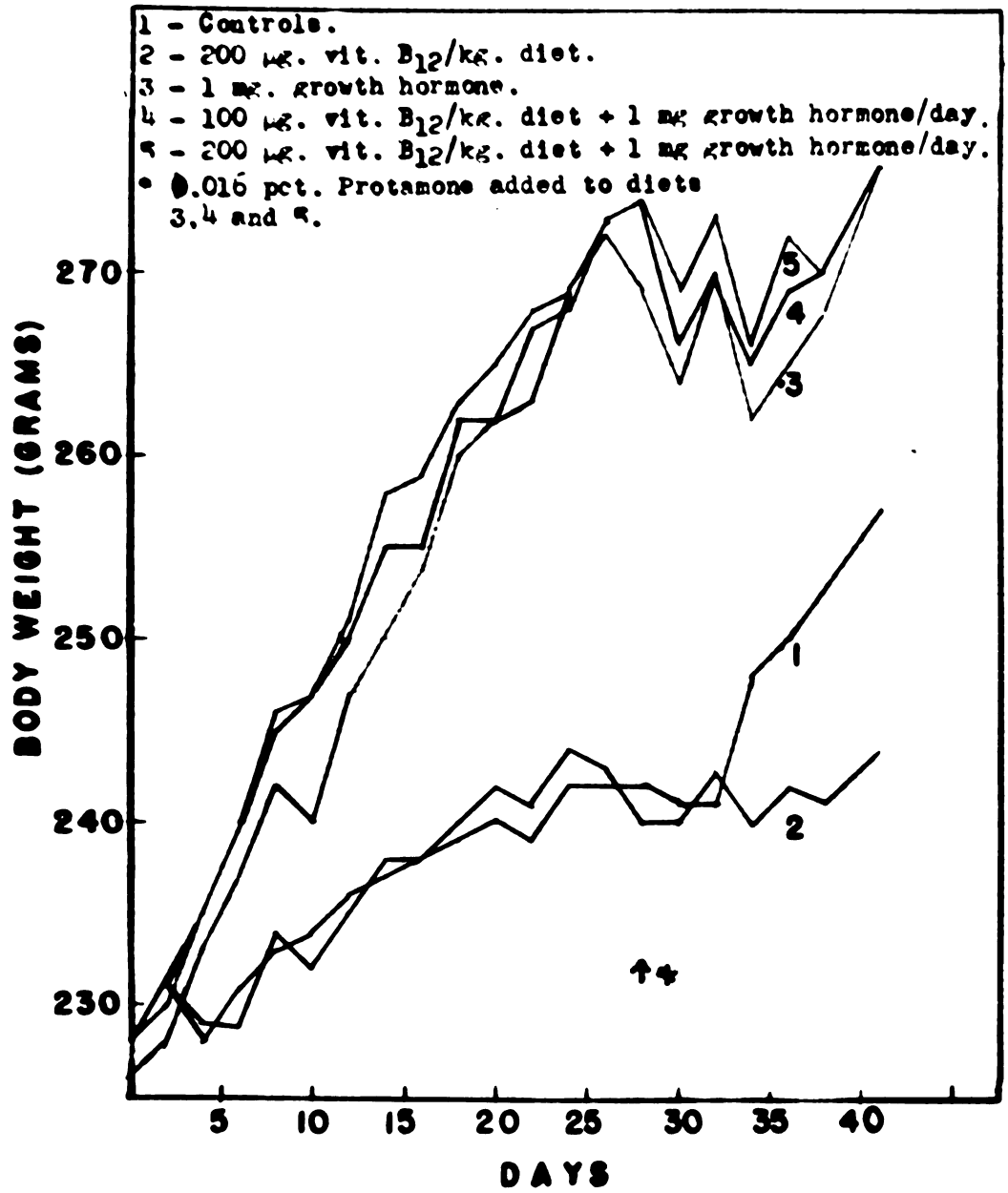

Figure 1
Effect of vitamin B_{12} and/or growth hormone on the body weight of mature female rats.

TABLE I

EFFECT OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON BODY WEIGHT AND FOOD INTAKE IN THE GROWTH PLATEAUED FEMALE RAT

Group	Treatment	Average Body Wt. (Gm.)			Average Food Intake (Gm.)	
		Original	At 28 Days	Final	Daily	Per Gram Gain in Body Wt.
1	Controls	226 ± 3	242 ± 4	257 ±6	14.7	22.4
2	200 µg. Vit. B_{12} per Kg. Diet	226 ± 3	240 ± 5	244 ±4	14.1	36.1
3	1 Mg. growth hormone per day	226 ± 3	269 ± 9	276 ±4	15.7	13.0
4	100 µg Vit. B_{12} per Kg. of diet + 1 mg growth hormone per day	229 ± 4	274 ± 9	276 ±7	15.3	13.0
5	200 µg Vit. B_{12} per Kg. of Diet + 1 mg. Growth Hormone per day	229 ± 4	274 ± 4	276 ±6	15.6	13.3

TABLE II

EFFECT OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON THE ORGAN WEIGHTS OF GROWTH-PLATEAUED FEMALE RATS

Group No.	Adrenal Mg/100 gm. Body Wt.	Thymus Mg/100 gm. Body Wt.	Kidney Mg/100 gm. Body Wt.	Spleen Mg/100 gm. Body Wt.	Ovaries Mg/100 gm. Body Wt.
1	16.19±.85*	64.6±2.4*	660±3.0*	195.0±3.1*	17.84±2.1*
2	17.46±1.0	63.9±1.6	750±2.0	214.9±3.1	15.26±2.5
3	23.55±1.8	73.4±7.9	840±3.0	234.5±5.5	26.09±5.7
4	22.76±1.1	75.9±2.6	810±3.0	31[illegible]±5.0	26.12±2.7
5	23.52±1.7	75.7±6.1	800±3.0	285.8±2.3	30.30±6.2

* Standard error of the mean.

EXPERIMENT II. Relation Between Anterior Pituitary Growth Hormone and Vitamin B_{12} in the Immature Male Rat.

PROCEDURE:

Fifty male albino rats of the Carworth strain and approximately twenty one days of age were fed for two weeks on the vitamin B_{12}-deficient diet used in Experiment I. At the end of this depletion period the animals were divided into six groups of eight each and placed on the experimental diets consisting of the basal vitamin B_{12}-deficient diet supplemented with crystalline vitamin b_{12} in amounts ranging from 20 to 40 µg per kilogram of diet. All animals in each group were marked by ear slits so that an individual record of weight gains and losses could be maintained. Body weight and food intake were measured every two days. Drinking water was available at all times. All animals were housed in a temperature controlled room maintained at 75° F.

Each of the animals which received growth hormone was injected subcutaneously each day with 1 mg. anterior pituitary growth hormone.* This was increased to 2 mg per day on the 13th day of the experiment and to 4 mg per day on the 24th day of the experiment.

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* Growth hormone preparation no. C-5-15 was supplied through the kindness of Dr. Robert W. Bates of E. R. Squibb and Sons, New Brunswick, New Jersey. This preparation causes a 15 gram gain in body weight in 2 weeks in hypophysectomized rats weighing about 100 grams when injected subcutaneously at rate of 0.1 mg per day. It contains less than 1% Prolactin and FSH, and may contain small amounts of thyrotropin and LH.

On the 29th day of the experiment 4 animals from each group were injected with 0.5 cc of Co^{60} labeled-vitamin B_{12}.* Each group of 4 animals was then placed in a metabolism cage and a composite urine sample collected for 2 hours. After this time a blood sample was taken from the dorsal aorta of each rat and the animals were then sacrificed with ether. The kidneys, liver, spleen and femoral muscle were removed and weighed to the nearest tenth of a milligram on an analytical balance. The tissues were then dried at 100° C for 48 hours and the dry weights and percent moisture determined. Following this the tissues were ashed at 500° C for two hours, weighed and the percent ash determined.

All tissues were analyzed for Co^{60} labeled-vitamin B_{12} by counting under a Geiger-Müeller counting tube. A self absorption experiment was conducted and all counts were corrected by use of the curve shown on page iii. The animals in each group which were not injected with Co^{60} labeled-vitamin B_{12} were also sacrificed at this time and the kidneys, liver, testes, spleen and thymus glands removed. All tissues were weighed to the nearest tenth of a milligram on a Keller-Smith balance.

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* The Co^{60} labeled-vitamin B_{12} was obtained through the kindness of Dr. L. F. Wolterink and was originally received from Merck and Co., Rahway, New Jersey. This material had the following specifications: 0.CoC mg. 5 ml., 3.5% methanolic Solution of vitamin B_{12} radioactive (Co^{60}) = 3.8 uc.

RESULTS:

The results of this experiment are shown in Figure 2 and Tables III through VII. Table III shows the average initial and final body weights, the daily food intake and food intake per gram gain in body weight. It can be seen that the animals on the vitamin B_{12}-deficient diet (group 1) gained less weight and were less efficient in the utilization of their food than any other group of animals. The two groups of animals receiving vitamin B_{12} in the diet (groups 2 and 3) gained approximately the same amount of weight and were about equally efficient in the utilization of their food. Rats receiving growth hormone but no vitamin B_{12} (group 4) apparently gained as much as the controls (group 1) ate a little more and were slightly more efficient in food utilization than animals on the vitamin B_{12}-deficient diet (group 1). Rats receiving growth hormone and either 20 or 40 µg of vitamin B_{12} per kilogram of diet (groups 5 and 6) gained more weight and utilized their food more efficiently than animals receiving the hormone alone (group 4) but no more than the animals which received the vitamin only (groups 2 and 3).

Table IV shows the weights of various organs expressed as either milligrams or grams per hundred gram body weight. Apparently there was no difference in the weights of the adrenal, thymus, kidney, liver, spleen or testes regardless of the diet or whether growth hormone was administered.

Table V shows the average Co^{60} labeled-vitamin B_{12} content of various tissues. The urine of animals on the vitamin B_{12}-deficient diet (group 1) contained less radioactive vitamin B_{12} than did that of animals receiving vitamin B_{12} in the diet (groups 2 and 3) and those receiving both vitamin B_{12} and growth hormone (groups 5 and 6).

The blood, kidney, liver and muscle tissues from animals on the vitamin B_{12}-deficient diet (group 1) contained more radioactivity than did similar tissues from animals receiving vitamin B_{12} in the diet (groups 2 and 3). There was no apparent difference in the spleen content.

The amount of radioactive B_{12} in the urine from animals on the vitamin B_{12}-deficient diet and receiving growth hormone (group 4) was less than that in any other group. The urine of animals receiving both vitamin B_{12} and growth hormone (groups 5 and 6) contained more radioactive B_{12} than that from animals on the deficient diet (group 1) but less than that from rats receiving vitamin B_{12} in the diet but not receiving growth hormone (groups 2 and 3).

The blood, kidney, liver, spleen and muscle tissues from animals receiving both vitamin B_{12} and growth hormone (groups 5 and 6) contained about the same amount of radioactive B_{12} as the tissues from animals receiving B_{12} but not receiving growth hormone (groups 2 and 3).

Tables VI and VII show the percent moisture and percent ash respectively of the various tissues and it will be noted that the values for all groups were very similar.

In general, it can be seen that the rats on the deficient diet (group 1) retained more radioactive vitamin B_{12} in the tissues than the rats which received either vitamin B_{12} alone or with growth hormone.

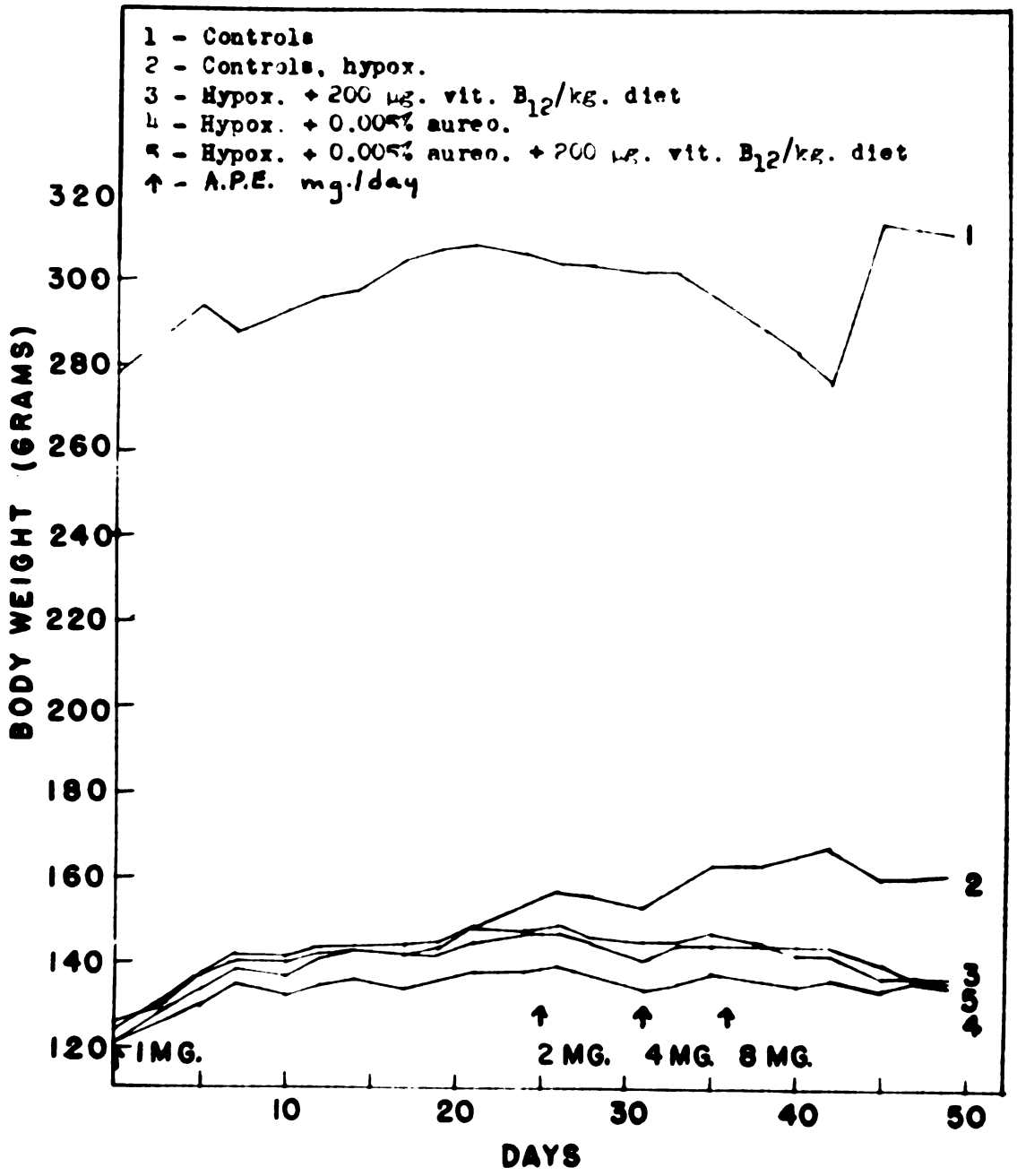

Figure 3
Effect of vitamin B_{12}, aureomycin and anterior pituitary extract alone or in combination on the growth of hypophysectomized rats.

TABLE III

EFFECTS OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON BODY WEIGHT AND FOOD INTAKE OF YOUNG MALE RATS

Group	Treatment	Average Body Wt. (Gm.)		Average Food Intake (Gm.)	
		Original	Final	Daily	Per Gram Gain in Body Wt.
1	Controls	86±4.0	181±6*	10.7	4.6
2	20 µg Vit. B_{12} per Kg. Diet	86±3.5	217±9	14.3	3.2
3	40 µg Vit. B_{12} per Kg. Diet	86±4.5	200±8	13.4	3.5
4	Growth Hormone	86±3.8	174±7	11.8	4.00
5	20 µg Vit. B_{12} per Kg. Diet + Growth Hormone	86±3.0	207±8	13.5	3.3
6	40 µg Vit. B_{12} per Kg. Diet + Growth Hormone	86±3.2	211±5	13.5	3.2

* Standard error of the mean.

TABLE IV

EFFECT OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON THE ORGAN WEIGHTS OF YOUNG MALE RATS

Group No.	Adrenal Mg/100 gm Body Wt.	Thymus Mg/100 gm Body Wt.	Kidney Mg/100 gm Body Wt.	Liver Gm/100 gm Body Wt.	Spleen Mg/100 gm Body Wt.	Testes Gm/100 gm Body Wt.
1	13.5±.5*	134.0±8.1*	907.5±37*	4.52±.12*	488.2±45*	1.5±.08*
2	12.3±.3	152.1±12.3	832.2±67	4.10±.001	288.0±40	1.3±.05
3	13.3±.8	169.7±6.5	802.8±38	3.48±.001	326.1±7	1.4±.05
4	15.0±1.3	133.0±15.4	829.5±46	4.16±.001	288.1±59	1.3±.006
5	14.9±.9	168.8±8.3	732.8±41	3.72±.51	476.8±61	1.1±.02
6	15.9±1.5	163.1±14.9	712.7±46	3.6035±.01	334.6±44	1.3±.035

* Standard error of the mean.

TABLE V

EFFECT OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON THE Co^{60} LABELED-VITAMIN B_{12} CONTENT OF TISSUES FROM YOUNG MALE RATS

Group	Urine*	Blood**	Kidney***	Liver**	Spleen**	Muscle**
1	56.9	80.8±5.7#	1.40±.18#	273.0±.02#	244.7±10.2#	91.1±.03#
2	69.0	50.5±6.5	1.20±.15	104.8±28.3	269.2±20.6	53.7±2.8
3	72.3	55.8±11.5	1.1±.08	117.3±5.8	259.5±18.2	62.8±11.0
4	30.7	54.6±4.4	1.5±.21	144.6±22.3	225.6±19.4	70.0±4.1
5	61.7	50.0±7.6	1.0±.02	132.7±4.1	144.7±16.0	64.2±10.4
6	58.6	64.3±4.6	1.1±.04	130.7±17.3	278.1±22.0	158.9±19.4

* Counts per sec. per ml. original volume.
** Counts per sec. per gm. ashed wt.
*** Counts per sec per mg. ashed wt.
\# Standard error of mean.

TABLE VI

EFFECT OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON THE PERCENT MOISTURE OF TISSUES FROM YOUNG MALE RATS

Group	Kidney	Liver	Spleen	Muscle
1	76.66%	69.07%	77.50%	76.88%
2	75.07	69.01	77.61	73.65
3	74.61	68.69	76.83	73.82
4	75.89	72.08	78.94	77.25
5	76.08	70.95	76.76	76.46
6	77.89	73.71	79.70	76.49

TABLE VII

EFFECT OF VITAMIN B_{12} AND/OR GROWTH HORMONE ON THE PERCENT ASH OF TISSUES FROM YOUNG RATS

Group	Kidney	Liver	Spleen	Muscle
1	1.48%	1.54%	1.80%	1.37%
2	1.58	1.73	1.77	1.32
3	1.64	1.59	1.80	1.37
4	1.49	1.62	1.98	1.37
5	1.65	1.70	1.90	1.34
6	1.31	1.76	1.92	1.44

EXPERIMENT III. Relation Between Vitamin B_{12} and Anterior Pituitary Extract in the Young Hypophysectomized Rat.

PROCEDURE:

Thirty hypophysectomized and 10 normal immature rats of the Sprague-Dawley strain were divided into five groups and placed on the following vitamin B_{12}-deficient diet:

Cerelose	62 gms.
Alcohol washed casein	25
Corn oil	5
Salt Mixture no. 2	4
Cod liver oil	5
Choline	100.0 mgm.
Calcium pantothenate	2.8
Niacin	1.0
Riboflavin	0.5
Thiamin	0.2
Pyridoxine	0.2
2, Methyl 1, 4 Naphthoquinone	0.04

The animals were housed in special cages in which cotton batting was placed to provide extra warmth.

The experimental diets consisted of the basal semi-synthetic diet to which was added 200 µg of crystalline vitamin B_{12} and/or 0.005% aureomycin. The hypophysectomized animals were injected subcutaneously daily with 1 mg. whole anterior pituitary extract* for 25 days and the dose was then increased to 2 mg. per day until the 31st day of the experiment. At this time the dose was further increased to 4 mg. per day for five days, after which time it was increased to 6 mg. per day until the experiment was terminated on the 49th day. Body weight and food consumption were measured every two days.

* Whole bovine pituitary extract, free of ACTH, supplied by Armour Laboratories, Chicago, Illinois.

RESULTS:

The results of this experiment are seen in Figure 3 and Table VIII. Figure 3 shows the body weight in grams plotted against time in days. It will be noted that all groups of hypophysectomized animals grew at a greatly retarded rate when compared with the intact controls. In addition, it can be seen that hypophysectomized animals receiving either vitamin B_{12}, aureomycin or a combination of the two not only failed to gain more than animals on the vitamin B_{12}-deficient diet, but actually gained slightly less.

Table VIII shows the average initial body weight, the average daily food intake and the average food intake per gram gain in body weight. It is apparent that the intact control animals were much more efficient in converting their food into body weight gains than the hypophysectomized animals.

Hypophysectomized animals on the vitamin B_{12}-deficient diet were more efficient in food utilization than were rats receiving either vitamin B_{12}, aureomycin, or a combination of the two.

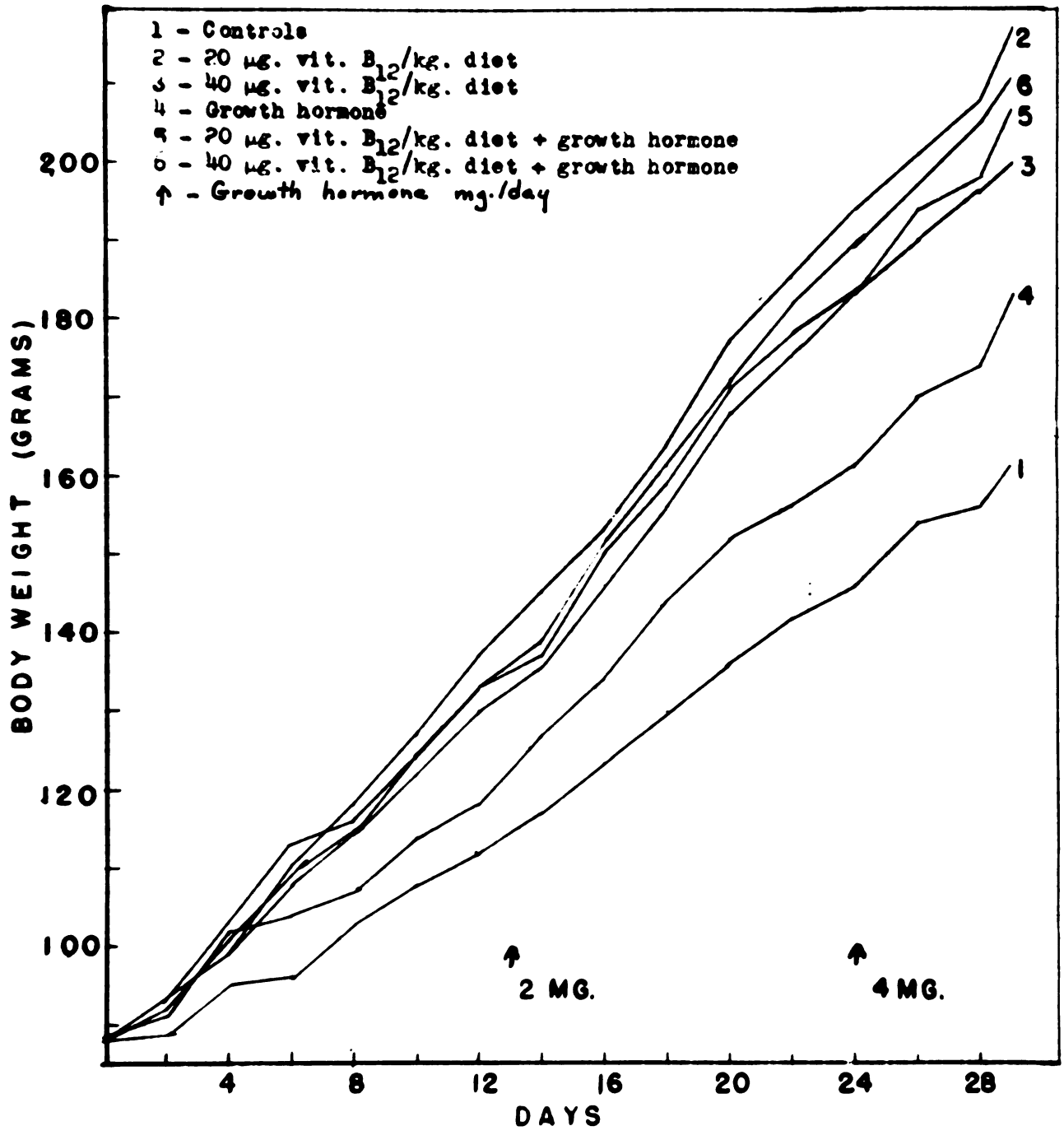

Figure 2
Effect of vitamin B_{12} and/or growth hormone on the body weight of immature male rats.

DISCUSSION

TABLE VIII

EFFECT OF VITAMIN B_{12} AND/OR ANTERIOR PITUITARY EXTRACT ON AVERAGE BODY WEIGHT AND AVERAGE FOOD INTAKE OF YOUNG HYPOPHYSECTOMIZED RATS

Group	Treatment	Average Body Wt.(Gm.) Initial	Average Body Wt.(Gm.) Final	Average Food Intake (Gm.) Per Day	Average Food Intake (Gm.) Per Gm Gain in Body Wt.
1	Controls, Intact	223±6*	310±3*	12.8	8.5
2	Controls, Hypox.	122±3	161±2	7.0	4.0
3	Hypox., 200 ug Vit. B_{12}/Kg Diet + A.P.L.**	124±2	137±1	.6	10.7
4	Hypox. 0.005% Aureo. + A.P.L.**	121±4	135±3	.6	9.4
5	Hypox., 200 ug Vit. B_{12}/Kg Diet + 0.005% Aureo. + A.P.L.**	126±3	136±2	.45	14.7

* Standard error of the mean.

** A.P.L. = Male anterior pituitary extract.

DISCUSSION

In these three experiments an attempt was made to study the relation between vitamin B_{12} and anterior pituitary growth hormone. In the first experiment, growth-plateaued female rats were used since it is the standard animal used for the assay of growth hormone. Unfortunately these animals apparently had good body stores of vitamin B_{12} and hence it was impossible to create a deficiency state. There is little doubt that the growth hormone was effective in the rats as can be seen from the increase in body weight and organ weights of animals receiving growth hormone over those of rats not receiving growth hormone. The data from this experiment, then, indicate that mature female, growth-plateaued animals stimulated with growth hormone will continue to grow even in the absence of vitamin B_{12} in the diet, presumably drawing upon adequate body stores of the vitamin for this continued growth. Register et al. (1949) have shown that a vitamin B_{12}-deficiency could not readily be induced in mature rats.

In the second experiment young male rats were used in order to insure attaining a deficiency of vitamin B_{12} in the animals. Animals of this strain and age have been used consistently in this laboratory for vitamin B_{12}-deficiency studies. These animals on a vitamin B_{12}-deficient diet grew at a retarded rate, gained less weight and were less efficient in utilizing their feed than similar animals fed a diet supplemented with vitamin B_{12}. The results of this experiment indicate that young rats on a vitamin B_{12}-deficient diet, injected with growth hormone, gained about

the same amount of weight as uninjected animals on a vitamin B_{12}-deficient diet. This is rather difficult to interpret, since if the growth hormone were exerting a real stimulus to growth in these animals, such a stimulus should have constituted a stress which would cause the animals to lose weight in the absence of an essential growth factor. Such a hypothesis seems logical when one considers the anabolic effect of growth hormone on protein synthesis and the many reports indicating that vitamin B_{12} is also involved in protein metabolism.

The possibility that the animals were not completely deficient in vitamin B_{12} must not be overlooked. There are several possibilities which arise. The diet might conceivably contain enough vitamin B_{12} for such animals to continue to grow at a reduced rate or the body stores of the animals may have been sufficiently good to buffer the extra requirement incurred by the injection of the growth hormone. In addition it is quite possible that the growth hormone was not exerting an effective stimulus to growth in these immature animals, since they were already secreting optimal amounts of this hormone. Such an effect has been reported previously (Evan, 1942). The latter possibility seems most plausible in the light of the data showing no effect of growth hormone on the organ weights, percent ash and percent moisture in the tissues of animals as compared to controls not receiving growth hormone. A general visceromegaly is one of the effects invariably noted with growth hormone injections.

The fact that rats receiving both growth hormone and vitamin B_{12} grew no more than those receiving only vitamin B_{12} further suggests that the growth hormone was not effective, although it is also possible that the

animals receiving only vitamin B_{12} were already growing at a maximum rate. In any case, the amounts of growth hormone used in this experiment did not appear to aggravate a deficiency of vitamin B_{12}.

The information concerning the distribution of radioactive vitamin B_{12} after a single subcutaneous injection of this compound seemed to indicate that animals maintained on a vitamin B_{12}-deficient diet retained more of this substance. Thus it was shown that animals on a vitamin B_{12}-deficient diet excreted less of the injected vitamin in the urine and retained more in the various tissues. The fact that animals receiving no vitamin B_{12} but getting growth hormone excreted less of the injected radioactive vitamin than those on the deficient diet suggests that the growth hormone might have caused an increased retention of the vitamin. This suggests then, that growth hormone may actually have a sparing rather than a depleting effect on vitamin B_{12} requirements of the body.

In the third experiment young hypophysectomized rats were used, and the growth curve and data on body weight indicate the inability of vitamin B_{12} and/or aureomycin to increase the rate of growth of such animals. In fact, the data indicate that animals on the deficient diet actually gained more and were more efficient in the utilization of their food than were rats which were furnished either or both of the supplements. This latter effect may conceivably be due to the large dose of vitamin B_{12} used which may have induced a vitamin imbalance and increased the need for one or several of the other B complex vitamins. In any case, the important observation remains that vitamin B_{12}, though a potent growth stimulus, was ineffective in the absence of the hypophysis.

It is apparent that further work must be done to elucidate the interrelationship between growth hormone and vitamin B_{12}. The main difficulty which has presented itself thus far is the inability to create a vitamin B_{12} deficiency and to secure an effective stimulus to growth by growth hormone administration in the same experimental animal. Thus in the experiments described it was possible to obtain a vitamin B_{12} deficiency in immature animals but growth hormone was ineffective as far as body growth is concerned. On the other hand, when mature animals were used the effects of growth hormone were readily apparent but it was impossible to create a deficiency of vitamin B_{12}. A possible solution to this problem may be the use of young rats from a colony which has been raised on a vitamin B_{12}-deficient diet for several generations.

Further work must also be done to determine whether growth hormone may not actually decrease rather than increase the requirements for vitamin B_{12} for body growth. On the whole, the experiments which have been described suggest that this is entirely plausible. If this latter idea can be borne out by further experiments, the growth hormone will then have been shown to be uniquely different from other hormones, most of which aggravate a deficiency of vitamin B_{12}.

SUMMARY

Summary

1. The effects of growth hormone on rats receiving diets supplemented or deficient in vitamin B_{12} were studied. All animals were placed on a vitamin B_{12}-deficient diet for a preliminary depletion period of approximately one month. Body and organ weights, food intake, and efficiency of food utilization were measured. Co^{60} labeled-vitamin B_{12} was administered in one experiment to study the effect of growth hormone upon the metabolism of this compound in rats fed diets either adequate or deficient in vitamin B_{12}.

2. In the first experiment, the effects of vitamin B_{12} and growth hormone alone or in combination on the growth of mature female rats of the Carworth strain were studied. It was shown that animals on the vitamin B_{12}-deficient diet gained as much as those receiving 200 µg of vitamin B_{12} per kilogram of diet. Rats receiving growth hormone alone gained more than the foregoing animals, while the addition of vitamin B_{12} did not result in any further increment in growth. The organ weights of all animals receiving growth hormone were about the same regardless of whether or not vitamin B_{12} was given, and were significantly greater than organs from animals not receiving growth hormone. It was concluded that in the growth-plateaued female rats, growth hormone is able to cause continued growth in the absence of dietary vitamin B_{12}, due presumably to the presence of adequate body stores of this vitamin.

3. In the second experiment immature male rats of the Carworth strain were used. The results of this study showed that young animals on a vitamin B_{12}-deficient diet grew at a slower rate, gained less total weight and were less efficient in the utilization of ingested food than were animals on a diet supplemented with vitamin B_{12}. It was further demonstrated that the administration of growth hormone to such rats, i.e., animals either deficient or supplemented with vitamin B_{12}, failed to cause an increase in growth rate or weight gained above that of animals not injected with growth hormone. The results of the work with radioactive vitamin B_{12} seemed to indicate a greater retention of this compound in the tissues from animals on a diet deficient in vitamin B_{12} than in those on a vitamin B_{12}-adequate diet. Growth hormone also appeared to increase retention of radioactive vitamin B_{12}. It was concluded that the growth hormone did not exert an effective stimulus in these rats in so far as body weight was concerned but that it may have altered the metabolism of vitamin B_{12}.

4. In a third experiment young hypophysectomized rats on diets either deficient or adequate in vitamin B_{12} and/or aureomycin were injected with whole anterior pituitary extract. The anterior pituitary extract induced more weight gain on the deficient diet than in animals given vitamin B_{12} alone or with aureomycin. This effect may have been attributable to the large dose of vitamin B_{12} used in this experiment (200 μg per kilogram of diet), since this may have created a vitamin imbalance. It was demonstrated, however, that vitamin B_{12} though a powerful growth stimulus, was ineffective in the absence of the hypophysis.

5. It is concluded that: (a) growth hormone administered to growth-plateaued female rats on a vitamin B_{12}-deficient diet will cause renewed and continued growth, (b) in the immature animal the effects of growth hormone on the requirements of vitamin B_{12} are not apparent from data on body and organ weights, although the possibility remains that this hormone does influence the metabolism of vitamin B_{12} as suggested by the distribution of injected Co^{60} labeled-vitamin B_{12} and (c) vitamin B_{12} alone and/or aureomycin supplied to hypophysectomized rats failed to cause an increase in body weight, showing that the hypophysis is essential for the growth action of these substances.

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APPENDIX

APPENDIX

Schweitzer and Stein (1950) have described several methods for correcting activity measured in samples of beta-emitting solids of varying thickness. The method used in this work was that originally described by Aten (1950). In this method a series of external absorbers were used to construct a graph from which the activity factor Am/At was determined.

The equation and the data from which the curve in figure 4 on page iii was calculated are shown below. All samples were corrected by use of this curve and the activity then expressed count/sec per milligrams or gram ash weight.

$$\frac{A_a}{A_m} = e^{-\mu x} \qquad \text{(equation 1)}$$

A_a = activity if no absorption occurred.

A_m = measured activity

μ = absorption coefficient

TABLE I

CALCULATION OF AVERAGE VALUE OF μ THE ABSORPTION COEFFICIENT

Absorber Thickness Mg/cm²	Counts Per Second	μ
0	278	--
1	248	.0770
2	221	.0726
3	193	.0779
4	170	.0782
5	150	.0728

Average μ = .0770

The various values of the ratio A_a/A_m shown in Table I were determined using equation 1, and substituting the value .0770 for μ and values ranging from 1 to 25 for x.

X(mg/cm^2)	A_a/A_m
1	92.66
2	85.71
3	79.36
4	73.40
5	67.73
6	62.98
7	58.35
8	54.03
9	49.28
10	42.70
15	31.53
20	21.40
25	9.99

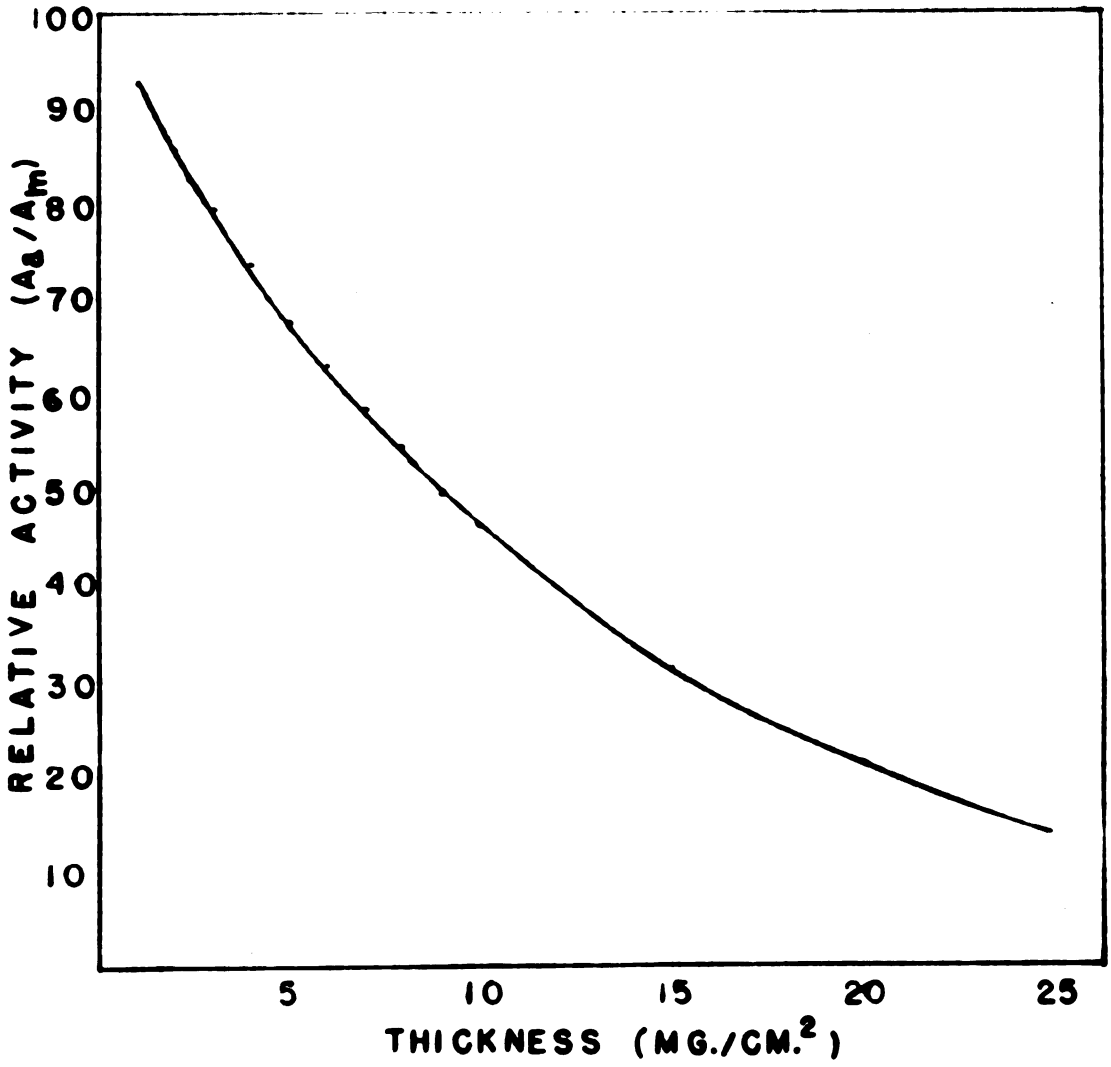

Figure 4
Self absorption curve for Co^{60} labeled vitamin B_{12}