THE GROWTH RESPONSE OF YOUNG ALBINO RATS TO GRADED AMOUNTS OF CRYSTALLINE VITAMIN B_1

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PURPOSE

The purpose of the study of the Growth Response of Young Albino Rats to Graded Amounts of Crystalline Vitamin B_1, was to establish a curve of response that could be used as a standard of reference for the Oregon State College colony. Graduated doses of from one to six micrograms of crystalline vitamin B_1 hydrochloride were fed daily.

REVIEW OF THE LITERATURE

History

Unprecedented advance in the investigation of the substance now known as vitamin B_1 began about one-half century ago.

The Orient began to report favorable treatment of endemic beriberi about 1887, when Takaki in an attempt to reduce the incidence in the Japanese Navy unknowingly introduced vitamin B_1 in natural foods into the rations. He was not conscious of the exact nature of the disease and attributed the disturbance to a lack of protein. He was among the first to discern the nutritional relationship existent in beriberi.

Eijkman, in 1897, observed "nutritional polyneuritis"

in fowls fed on polished rice and associated the disease with the lack of some substance found in the pericarp and embryo of the rice. He identified beriberi in man and birds as being a "deficiency disease". (12) (86)

Between 1901 and 1911 continued experimenting by Grijns and Vordermann in the Dutch East Indies; Branddon, Frazer, and Stanton in the Federated Malay States; and Vedder, Williams and Chamberlain in the Philippines, lent support to the nutritional nature of the entity. (84) (86) (12)

Eastern workers early began to use the extract of rice polishings as a source of the antineuritic substance for chemical determination. Frazer and Stanton, and Vedder, Williams and Chamberlain showed that the antineuritic substance was more stable in acid than in alkaline solution. (109) They proved it was not an amino acid or alkaloid, but thought it to be a nitrogenous base. Funk (26) used phosphotungstic acid and silver nitrate as reagents for precipitation for the polyneuritic substance in the foodstuffs then recognized as potent.

Repeated contributions to the chemical properties and physical characteristics, together with clinical observations, continued to strengthen the early work. The investigations proceeded in many laboratories throughout the world. Methods of assay assisted materially in providing a standard of reference and of comparison. While many controversial aspects are still to be solved, a climax in

the investigation was reached in the recent isolation and synthesis of crystalline vitamin B_1 hydrochloride.

Nomenclature

Funk introduced the use of the nomenclature "vitamine" to indicate the indispensable nature of the extract from rice polishings and brewer's yeast, on the ground that the nitrogen in basic combination was an amine. Later, when this was disproved, the "e" was dropped. Funk postulated the "vitamin hypothesis" in which he related the deficiency diseases to a diet lacking in some substance vital to life. The fact that there was more than one factor essential for nutritional well being was at that time undergoing research; and from the work of Holst and Frölich, Osborne and Mendel, and McCollum, the term vitamin embraced a much wider scope than Funk's original intent.

McCollum suggested the use of the term "water soluble-B" for the anti-beriberic factor. When Smith and Hendricks, Goldberg and others showed "B" to be of a complex nature, the British affixed the subscript and B_1 was used for the antineuritic factor. Jansen and Donath suggested the name "aneurin", while Peter's work reported on "torulin". Sherman suggested the use of "F" for the "B_1" fraction, but this was never accepted. Williams proposes the name of "thiamin".

The committee on vitamin nomenclature of the American

Society of Biological Chemists and the American Institute of Nutrition, in 1937, defined the antineuritic vitamin in the following words:

> "(1) That the vitamin which is recognizable as a specific in the prevention or cure of Beriberi in man and polyneuritis in experimental animals, and which has been identified as consisting of a ---- (2) methyl 4 amino pyrimidine group and a 4 methyl 5 beta hydroxyethyl thiazole group joined by a methylene group through carbon atom of the thiazole ring be designated as "B_1" and that the term "B" without a subscript be no longer used."

In this paper, the term B_1 will therefore be used to designate this substance.

Chemical Properties

Williams (108), in discussing the reactions of thiamin, states "that on titrating thiamin chloride with alkali we find a sharp rise when 1 mole is reached corresponding to the formation of the neutral, or non-acid salt; but from that point onward there is no further break until a total of three moles have been added. This peculiar potentiometric titration curve is due to the cyclic quartenary base using up hydroxyl ions and prevents the pH from rising. If the vitamin stands in an alkaline condition for some time, there is only partial recovery of the vitamin on acidification.

"The sensitivity of the vitamin to pH appears to depend greatly upon the nature of the medium. On accidental presence of sodium sulfite at pH 5 the vitamin splits. This

occurs with greater speed at higher temperatures. Concerning its thermostability, it is fairly stable. The solutions may be heated at pH 3.0 to 140° C. for one hour without appreciable loss under these conditions. At a pH of 7.0 about 50 per cent is lost.

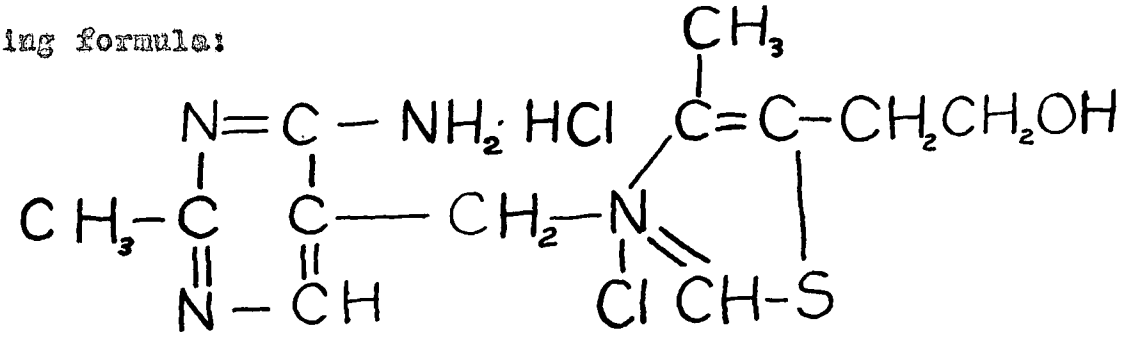
"The chemical nature of the substance shows it to be a rather strong base forming an acid chloride with melting point at 250° C.; it forms a picrolonate with melting point of 165° C. or 229° C.; a gold salt melting point of 198° C.; a rufianate with melting point of 291° C.

"It is adsorbed on charcoal or fuller's earth and eluted from charcoal by dilute hydrochloric acid and from the fuller's earth to some extent by alkalis, better by quinine salts or salts of other strongly basic alkaloids. It is precipitated by phosphotungstic acid, less fully by mercuric acetate or silver nitrate if these reagents are applied in a pH range greater than 5."

The thiazole nucleus and pyrimidine ring comprise two distinctive features in its chemical structure. The thiazole nucleus is unique in this biochemical compound. Makino and Imai, Japanese workers, were among the first to suggest a structure formulated on the basis that the thiazole cycle of the vitamin is joined to the pyrimidine moiety through an aliphatic methylene group. (50)

In 1911, Funk reported the isolation from rice-polish-

ings and from yeast, of a substance which he believed to be a salt of the antineuritic vitamin. It was not until 1926 that Jansen and Donath obtained about 1.3 milligrams of crystalline B_1 from 50 tons of rice polishings after going through a most laborious process. Many investigators contributed findings on the chemical nature and properties of the vitamin, but to Williams and his coworkers we owe the identification of the structure of B_1, which has the following formula:



The destruction of the vitamin is dependent on the time, temperature of heating, and pH of the solvent. It is considered to be thermolabile, but under certain conditions of dry heating other factors in the B complex are comparatively more easily destroyed, as reported by Keenan, Kline, and Elvehjem. (53) Oxidation is not an appreciable factor in its destruction. From a practical standpoint, solubility, mechanical refinement, and shift of pH to the alkaline side cause the largest loss of vitamin B_1.

Physiological Effects

Among the severe signs of vitamin B_1 deficiency are

beriberi in man and polyneuritis in animals. Polyneuritis was the first avitominosis to be studied experimentally.

Beriberi exists in two well recognizable forms, namely wet and dry. Dry beriberi was described by Bontius in 1642. Marshall described dry and wet beriberi as two separate diseases in 1812 (cited in 91). The wet form is associated with cardiovascular disturbances and generalized edema. The dry form manifests itself mainly in muscle-wasting and peripheral neuritis. (102)

Church (20) outlines the characteristic manifestations of beriberi or polyneuritis resulting from a lack of vitamin B_1 in the rat as changes "in muscular tonus, ataxia, disturbances of equilibrium, hyperexcitability, muscular tremors, and weakness ------ but true convulsions and paralysis are not a part of the beriberi syndrome". Williams (108) states that early stages of both forms of the disease are concurrent with loss of appetite and cessation of growth, fatigue, mental depression, and gastro-intestinal disturbances. However, Williams, Waterman, and Keresztesy (109) state that, "gross B_1 insufficiency is possible without manifestations of polyneuritis".

While the severe manifestations of the lack of vitamin B_1 in a restricted diet may be absent, other symptoms are well recognized as being associated with its subnormal intake, among these being anorexia. The presence of vitamin

B_1 furnishes appetite stimulation and its lack results in inanition and anorexia.

Cowgill noted (13) "that some relation exists in dogs between the desire to partake of food and the amount of the so-called water-soluble vitamin injected". On these dogs Karr (51) reported, "In all animals which refuse the whole or part of their diet, the desire to eat could be quickly restored by the injection of a substance containing water-soluble vitamin. The plan of the experiment was to feed the vitamin free food until the dogs refuse part or the whole of it for a number of days. Then feed the accessory substance and note the effect on the food intake. The preparation of water-soluble vitamin was given apart from the food after the meal had been offered in the morning; hence, it could not affect the palability of the rations."

Kon and Drummond (60) failed to show impaired motility of the digestive tract of pigeons. In describing the physiological effect of vitamin B_1 in the pigeon, they state, "It seems evident that starvation and wasting associated with it are largely, if not entirely, responsible for the pathological nerve changes observed in vitamin B deficiency exhibited by pigeons fed on diets deficient in vitamin B. These are direct consequences of the failure to consume sufficient food to maintain the body weight. Lack of appetite for the deficient food and acute nervous symptoms

are characteristic of vitamin B deficiency in the pigeons." McCarrison records intestinal lesions as pathological observations of vitamin B_1 deficiency, (cited in 86). Kruse and McCollum (61) point out: "owing to the lack of appetite the effects of water-soluble B deprivation should be regarded at least partly in the light of what happens in starvation and anhydremia". Sherman (85) says that the effect on the weight curve may be and usually has been the best basis for measurement, and the most characteristic effect of shortage of vitamin B_1 are failure of appetite and development of polyneuritis.

Williams (108) points out that, "animals eating voluntarily of a vitamin B_1-free diet progressively limit their intake of food. That is, what is normal feeding for a normal bird is over feeding for a partially depleted bird. Thus birds or rats eating voluntarily protect themselves to some degree from polyneuritis by eating less food. More food of starch variety means an increase of vitamin requirement." This is in accord with Funk, 1914, who showed that carbohydrate rich diets bring about a more rapid production of polyneuritis in pigeons than normal diets.

The abnormalities of carbohydrate metabolism brought about by vitamin B_1 deficiency have been described by Collazo and Bayo (cited in 40) and include accumulation of lactic acid in the brain of a pigeon. Gavrilescu and Peters

(34) found a subnormal oxygen uptake in pigeons *in vitro*.

Kinnersley and Peters (54) state that, "Pigeons in the terminal stages have an increased amount of lactic acid in the blood as compared with the normal. Fifteen seconds after death there is found in the normal approximately 55 milligrams per 100 grams tissue and in the avitaminiotic 95 milligrams. The increased lactic acid is not a consequence of the high blood sugar and tends to follow the blood lactic acid which is raised. The increased lactic acid is concomitant with the symptoms in avitaminoses B_1 and disappears with a short period after dosing with torulin." Williams offers the following explanation. "Since vitamin B_1 acts as a coenzyme it may have significance *in vitro* as a catalyst in oxidation-reduction systems. According to the recent work of Lohmann and Schuter, the pyrophosphoric ester of the vitamin is the prosthetic group of an enzyme which decarboxylates pyruvic acid. Without it pyruvic acid accumulates and poisons the tissues. Pyrophosphoric ester by hydrolysis with acid is converted into the phosphoric ester or with a phosphatase enzyme into free thiamin. The pyrophosphoric ester possesses antineuritic properties but acts more slowly than free thiamin. It is regarded as bringing about the destruction of pyruvic acid by converting it into acetaldehyde."

Evans (32) has shown for normal rats that there is de-

ferred sexual interest in males and delayed sexual maturity and definite disturbance in oestrous cycle in females.

Drury and Harris (25) report, "A true deficiency in rats is manifested by severe bradycardia, not ascribed to inanition and relieved at once by administration of vitamin B_1". Robertson and Doyle (77) found variations in the heart beats on the same rats.

Francis, Smith and Morse (cited in 40) were unable to alleviate the ill effects of high protein diets upon the kidney by increasing the allowance of the vitamin B complex. Williams states, "The proportion of protein in the diet cannot be said to be very important with respect to the B_1 requirement, but there is a great weight of evidence that carbohydrate raises the requirement." Sherman and Gloy (85) in studying the effect of vitamin B_1 varied the casein content of the diet to see the relation between the amount of protein to vitamin B_1 intake. Variations of protein from 12 to 54 per cent failed to show any difference in the response of the weight curves of experimental animals receiving uniform restricted allowances of vitamin B_1 in the form of orange juice. The results amply justify the conclusion that basal diets may vary widely in protein content and still yield interchangeable results in the testing of foods for vitamin B_1, and also that with a basal diet containing 18 to 20 per cent protein, the protein in-

take may be much changed by the feeding of either a high protein or a low protein food as a source of vitamin B_1, without affecting the validity and quantitative value of the test as an indication of the relative vitamin B_1 content of the food tested." Karr (51) (52) showed that nitrogen utilization in the digestive tract was "unaffective by the absence of water soluble vitamin B".

Other dietary components influence the metabolism of vitamin B_1 beside carbohydrate. Fat exerts a sparing action according to Evans and Lepkovsky (33) who report that large amounts of fat in the diet prevent or retard the on-set of polyneuritis when the B_1 supply is low. "When, with high intake of protein and vitamin G, fat acts to spare vitamin B_1, it could do so by slowing down the loss of vitamin B_1 from the rat's tissues. When rats are reared without vitamin B_1 the muscles of those on the low fat diet suffer a loss in vitamin B_1, both in absolute amount and in concentration per unit weight, but on the high fat diet though concentration per unit weight is reduced by about one-half, there is little loss in the absolute amount of the vitamin since the muscles weigh approximately twice as much." Softer fats are more effective than harder fats.

Whipple and Church (104) observed the same effects but attributed it to a favorable influence of fat on the growth in the intestine of micro-organism which are capable of

synthesizing vitamin B_1.

Individual and Species Variations

Irwin, Brandt, and Nelson (48) show individual variations in gain in weight of rats in response to vitamin additions to a diet are so great that relatively large numbers of individuals are required in order to secure statistically significant results.

Palmer (71) attributes this individual variation in gain in weight to the efficiency index. He states that it is the present custom in nutrition to assume that individual variations are reduced to a negligible proportion by using litter mates. Such an assumption is not accepted by geneticists unless the parents are homozygous for the factor in question. This is not very likely as to food utilization efficiency. There is a marked difference in the efficiency index of males and female rats, which appears to be a major factor in determining the difference in growth rates of the two sexes.

Light and Cracas (65) point out inherited strain differences in the vitamin B_1 requirement. Levene (64) plotted growth in various seasons and noted that the identical material seemed to produce better growth early in the fall than later in the year.

Assay Tests

Many tests have been devised to measure the relative

avitaminoses of experimental animals. Quantitative assaying tests are bacteriological, chemical, growth promoting, and curative.

An extensively used method of assay has been the rat growth method, developed by Chase and Sherman (17). A Sherman-Chase unit of vitamin B is the amount which, when fed as a daily allowance, results in a rate of gain of three grams a week in a properly standardized animal during an experimental period of four to eight weeks. (86) When all other dietary requirements of the rat are supplied in the basal diet except vitamin B_1, growth is, after depletion, proportional to the increased intake of vitamin B_1, according to Waterman and Ammerman (100); Chick and Roscoe (19); Sherman and Axtmayer (86); Bender and Supplee (4). The depletion period lasts until all gain in weight has ceased. While the unit gain in weight of three grams insures reasonable constancy of results for healthy test animals, yet it is far enough below the normal growth that there is ample room for response to an increased intake of the vitamin.

Birch and Harris (6) introduced the use of the bradycardia method. Arguments presented by Harris and Leong (42) for this method are that the irregularities and scatter of the "pigeon method" are obviated; the result of a single administration can be obtained in the course of a few days; the same rat can be used for repeated separate tests; the

error is no more than about 15 to 20 per cent.

Drury (25) had shown that rats suffering from vitamin B_1 deficiency had low heart rates. Since vitamin B_2 and other food constituents were without similar action, it was suggested that this action of the heart might form the basis of a very convenient, rapid and accurate method for estimating the vitamin. Electrocardiographic records are taken as soon as the normal heart rate falls on the B_1 deficient diet. Then single doses of the material to be tested are given and the animal replaced on the basal diet. The increase in the rate and time are roughly proportional to the dose given. Cures of four days are best for a comparative basis. Robertson and Doyle (77) could not get graded response with graduated doses of vitamin B_1 by this method.

The rat curative method is defined by Sampson and Keresztesy (81) as that amount of a substance which would produce complete relief of severe symptoms in 60 to 80 per cent of the test animals and which would keep these animals free from convulsions for four or more days.

The dose method is an amplified curative technique in that the dose is supposedly a means of measure of a proportion of the cure. Harris and Birch (6) found that the duration of the cure does not increase in proportion to the size of the dose so the dose method can be safely employed over a very limited range. The potency of a given preparation may appear less if large doses are employed than if

smaller doses are used. The duration of the cure influences the results.

Kinnersley and Peters and Reader (54) have extensively used duration of cure as means of evaluating the potency of antineuritic products. Their test animal is the pigeon and their "day dose" is obtained by dividing the dose given by the number of doses it takes for the bird to remain free from polyneuritis. With rats it is shown that the duration of cure is a function of the dose.

Weight maintenance in pigeons is used by Waterman and Ammerman (101) who found that the individual weights leveled off at each increment of vitamin B_1 administered.

Protection of birds against polyneuritis is another specific test. However, birds have not been standardized to the same extent that inbred colonies of rats have, therefore are not as reproducible in results of the protective tests as other methods used on rats.

Schopfer uses the mould Physomyces blakesleanus to determine the vitamin B_1 content of wheat germ preparations, and Meiklejohn, Phycomyes blakesleanus to estimate the thiamin content of blood. (Cited in 108) (83) Within certain limits the growth of the mould is directly proportional to the concentration of the vitamin when additions of a solution of the vitamin up to 0.01 micrograms in a synthetic medium is made. The bacteriological methods of assay quali-

tatively reveal the coenzyme action of thiamin.

Prebluda and McCollum (75) report a chemical method of assay. Certain derivatives of aniline or the naphthylamines after being treated with nitrous acid produce characteristic colored solutions when allowed to react under certain conditions with vitamin B_1. Since the coloration is of a permanent nature this method provides for the qualitative as well as the quantitative estimation of the vitamin.

Another method for the chemical determination of aneurin is through the formation of thiochrome. Jansen (49) worked out a method based on the thiochrome reaction, which consists in the oxidation of aneurin by potassium ferricyanide in alkaline solution to a compound showing a strong blue fluorescence (thiochrome), the intensity of which may be measured by means of a photoelectric cell attached to a galvanometer.

Westenbrink and Goudsmit (103) have used the thiochrome method for determination of vitamin B_1 in the urine.

Definition of a Unit

A unit of vitamin B_1 was considered by the League of Nations as being the antineuritic activity of 10 milligrams of the International Standard adsorption product as prepared by the method of Seidell. This method, described by Jansen and Donath, was to extract rice polishings in a sulphuric acid solution adjusted to a pH 4.5 and adsorb on

fuller's earth (18). This was set up in order to have a standard of reference for universal adoption in assay tests before the crystalline thiamin became available.

The Sherman-Chase unit was taken as that amount of vitamin B (B_1) which when fed daily would induce a gain of three grams per week during a test period of four to eight weeks.

The conversion of international units to Sherman-Chase units equivalents have been over a range of values as reported by different investigators.

The translating of the milligram of crystalline thiamin into international units has been met with conflicting results and in no case consistent findings.

Since the crystalline substance is now available the utility of the former standard of references is greatly minimized.

Waterman and Ammerman (100) reporting on growth in rats, found Sherman-Chase unit growth curves occurred between two and three gamma of the crystalline substance. Four gamma was the lowest which showed suggestion of deficiency disease, the growth being about one gram per day. Inconsistency in growth response was more conspicious over the lower range of doses and reflects itself in variations from animal to animal at any given level. The stimulating effect persisted up to and beyond a dosage of 160 gamma, an amount 80 to 160 times that

necessary for maintainence of life where a basal diet was constant and the addendum consisted of pure vitamin B_1 without the complications of other factors. All animals fed at the level of 0.5 gamma of crystalline vitamin B_1 . HCl died in less than 45 days with true paralysis. The behavior of these animals as contrasted to that of the control rats on the B_1 free diet which died without paralysis was regarded as evidence for the necessity of including small amounts of B_1 in diets designed for the production of typical polyneuritis. One gamma was the lowest dosage on which all of a single group of four rats survived the test period.

Birch and Harris (6) have also purposely used a depletion diet containing small amounts of vitamin B_1 in their injection assay. The best polyneutric diet would be one supplying a precisely controlled small amount equivalent to about 0.5 gamma per day of crystalline vitamin B_1.

Eckler and Chen (27) made rats definitely polyneuritic and treated them with various doses according to the technique of Smith. They followed Dann's suggestion of adding 0.4 per cent of brewer's yeast to the diet to reduce the incidence of death. Doses of 0.004 to 0.006 milligrams of synthetic vitamin B_1 given by mouth were sufficient to cure 77 to 85 per cent of the rats. The lowest curative dose was 0.004 milligrams in this experiment.

Waterman and Ammerman (100) using a rat growth assay

method found 200 international units per milligram as the potency of crystalline vitamin B_1 hydrochloride. Moll gives 500 international units per milligram by the rat curative method. Smith, using the rat curative method, finds approximately 500 international units per milligram. Kinnersley and Peters by the catatorulin test reports 500 international units per milligram (cited in 81). Knott and Schultz (59) in suggesting the improvement and standardization of the rat growth technique for short period assays of vitamin B_1 state that the international standard adsorbate furnishes in addition to vitamin B_1 some of the vitamin G complex.

Sampson and Keresztesy (81) report the apparent difference between international standard clay and thiamin as measured by the bradycardia method as due to partial unavailability. Healthy rats can elute the B_1 from clay more successfully than the polyneuritic animals. They show that in young rats on the Sherman-Chase diet, one unit of international standard was equal to four gamma of thiamin chloride.

Leong and Harris (63) using the bradycardia method state that one international unit of standard acid clay may be taken as approximately equivalent to three gamma (2.8 to 3.0) of the crystalline vitamin B_1 hydrochloride.

Heyroth (45) found the minimum curative dose for the rat is 1.2 times the pigeon day dose. The rat minimum

curative dose is 6.5 times the day dose required for the prevention of severe symptoms.

According to Waterman and Ammerman (101) the minimum dose of vitamin B_1 which cures polyneuritis in pigeons is approximately four gamma. Pigeons respond to curative doses more rapidly than do rats. Sampson and Keresztesy (81) state the curative dose of the international standard is 20 milligrams. Williams' method of eluting with quinine sulfate liberated approximately twice as much vitamin as by the Kinnersley and Peters baryta method or Smith alkaline methyl alcohol method. Curative dose by Williams' method took only 10 milligrams of acid clay.

Kinnersley and Peters (54) reported international unit of vitamin B_1 (10 milligrams of Jansen's clay) is approximately equivalent to two gamma of crystalline vitamin B_1 hydrochloride. Jansen (48 a) found from comparative assays on rats that one gram of the international standard preparation contains approximately 0.3 milligrams of aneurin hydrochloride. The international unit to him, therefore, represents the vitamin B_1 potency of three gamma of the crystalline vitamin B_1 hydrochloride.

Thus the value assigned to the international unit varies from two to five micrograms of the thiamin hydrochloride, although the original intent was to make the unit a basis of comparison. The criticism of Williams (108) must

be considered; "The object of adapting such a unit was the commendable one of facilitating a comparison of the results of various workers by providing a conversion factor suitable for common use". However, the time required to release it appears to vary with the degree of previous depletion of the animal and its consequent powers of assimilation.

Requirement

An all engaging question is what is the requirement of each species. Cowgill (12) thought the need to be a function of the caloric intake. Pregnancy, lactation, severe muscular exercise, metabolic excitants as endocrine disturbances and infections are among the reasons given for increased needs according to Straus (91), Kik, Smith and Walker (56), Evans (32), Williams (104), Theobald (95), Sure and Walker (93), Husseman and Hitler (47).

Cowgill (23) (12) estimated the vitamin requirement as 250 milligram equivalents per 100 calories for man. In this study a milligram equivalent was estimated to be one-fifth of a Sherman unit, that is, it takes one Sherman unit to make five milligram equivalents. Munsell (23) suggests 25 Sherman-Chase units per 100 calories for man. Rose (79 a) indicates 10 Sherman-Chase units per 100 calories. For the average adult this would be 250-400 Sherman-Chase units per day.

In 1936, the Council on Pharmacy and Chemistry of the

American Medical Association, outlined the adult need as 200 international units and the child's as 50 international units. Harris and Leong (63) showed that healthy adults varying in ages from 17 to 37 years on normal diet excreted daily 12 to 35 international units, or 30 to 90 gamma of vitamin B_1 hydrochloride. This was approximately five to eight per cent of the daily intake of vitamin B_1. They set the minimum standard allowance of vitamin B_1 for a 140 pound man as 200 International Units by their clinical observation of the urine excretion of vitamin B_1.

Knott (58) made a quantitative study of the utilization and retention of vitamin B_1 in children, and concluded the need to be 20 International Units per kilogram or 40 Sherman-Chase units per kilogram per day of vitamin B_1 for young children. Ross and Summerfeldt (80) showed the value of increased supply of vitamin B_1 and iron in the diet for the child.

Recent analyses by Baker and Wright (cited in 23) of large numbers of dietaries from regions where beriberi is prevalent showed that the vitamin B content of the diet fell in the range of 71 to 180 International Units per day. Protective diets might vary from 145 to 500 International Units, depending on the weight of the individual.

Theobald (95) successfully treated five cases of neuritis in pregnancy and found vitamin B_1 therapy relieved the "pins and needles" symptoms. Harris (40) (42) suggested

that liberal intake of vitamin B_1 is needed for successful lactation, but he attributes some of the efficacy to vitamin G as well. Rose (79 a) states the need during pregnancy as twice the normal; Williams (103), three to five times the normal; as do Baker and Wright (67 a). Kik, Smith and Walker (56) show vitamin B_1, in addition to stimulating the appetite, exerts its specific beneficial influence on the lactating animal organism, as evidenced by the lactation efficiency index. The influence of vitamin B_1 given to lactating females restricted in food and water as the litter mate controls showed a beneficial effect on lactation.

Kussoman and Hitler (47) and Sure and Walker (93) reiterate the increased need in pregnancy and lactation. Williams' (103) conclusion in terms of milligrams of crystalline thiamin rests at one milligram per day for normal and three to five milligrams for pregnancy.

So many factors influence the need for a given amount of this vitamin it is fallacious to speak of an absolute "vitamin B_1 requirement" for any species.

Rich sources of vitamin B_1 include yeast, whole seeds, whole grains, legumes and nuts. Pork ranks outstandingly high among the meats. Milk and citrus fruits because of the quantities consumed represent potent sources. While the distribution in nature is widespread, rich sources are suggested daily for optimal functioning of the organism.

EXPERIMENTAL PROCEDURE

The plan of this study was to use the rat growth method, feeding the Sherman-Chase vitamin B_1 free diet plus graduated amounts of crystalline vitamin B_1 for a four week experimental period.

Healthy, young albino rats weaned at 21 to 28 days old, weighing 39 to 55 grams, were raised in the stock colony of the Foods and Nutrition Department of Oregon State College. The stock animals were fed Ralston's Purina Dog Chow. This is an analyzed concentrated food, ample in all respects as determined by the presence of generous amounts of all known essential factors.

The animals were placed in individual large mesh, raised floor wire cages to prevent coprophagy, and supplied with the basal B_1 free diet and water ad libitum.

The Sherman-Chase B_1 free diet as fed consisted of:

Casein (purified)	18 gms.
Cornstarch	51 "
Osborne and Mendel salt mixture	4 "
Agar	2 "
Butter fat	8 "
Cod liver oil	2 "
Autoclaved yeast	15 "
	100 gms.

The purification of the casein was according to the method of Steenbach, Sell, and Nelson. Crude casein was extracted 11 times in 0.1 per cent glacial acetic acid solution, and dried and ground.

Butterfat was freed from curd and water after the method of Palmer and Kennedy, by filtering through absorbent cotton. Small quantities were prepared frequently and stored in a refrigerator.

Dried yeast was mixed with water, neutralized with sodium hydroxide as indicated by testing with bromthymol-blue, and autoclaved for six hours at 120° C. After drying at room temperature for a few days it was finely ground.

Depletion on the basal B_1 free diet lasted until stationary or declining weights were reached. It took about two weeks for this to occur.

At the beginning of the experimental period a negative control was selected from each litter receiving the basal diet with no supplement. Litter mate controls were daily given, in addition to the basal diet, graduated amounts of from one to six micrograms of crystalline vitamin B_1 hydrochloride solution by means of a tuberculin syringe. The solution was made up so that 1 c.c. contained 10 micrograms of the vitamin. The oral administration was aided by adding a slight amount of sucrose to the solution.

Weekly and mid-weekly weighings were made over the four week period.

The original intent of the study was to feed the vitamin at levels above six micrograms but a shortage of animals frustrated this plan.

At the end of the four week period all negative con-

trols had died, and the animals fed on the graduated amounts of vitamin B_1 hydrochloride were killed except animals numbers 402, 408, 427, 440 and 450. Numbers 402 and 408 were kept at the three microgram level for five and one-half weeks, and numbers 427, 440 and 450 for eight weeks with a doubled intake of B_1 over the last two weeks to determine the influence of a longer test period. No symptoms of deficiencies of other factors were noted, so it was assumed that the basal diet was adequate in all respects except for vitamin B_1.

RESULTS

Negative controls steadily lost weight and showed poor muscle tone, lack of appetite, incoordination and syncotic extremities. Death came within 28 to 45 days after starting on the basal vitamin B_1 free diet. No cases of convulsions or paralysis were noted. There was a mean loss of -22 grams for 15 negative controls.

Animals on the suboptimal amount of one microgram per day showed a progressive decline in weight. The gains ranged from -19 to +20 grams over the four week period.

Bisbey (6 a) also found a maximum variability of response when animals were fed a diet greatly deficient in vitamin B_1. The unsteady course occurred in animals fed the suboptimal amounts of one and two micrograms of vitamin B_1.

Animal number 512 was not included in the calculation due to the accident of being caught in the wire and held there for twenty-four hours. Death followed the next day. Some animals exhibited a reeling gait, muscular incoordination and poor muscle tone. Mean gain in weight for the 11 animals receiving was -4 $\pm$1.68 grams in four weeks.

Unit growth (i.e. 3 grams per week) was not reached on two micrograms daily. Mean gain in weight for 14 animals for the four week period was +11 $\pm$.10 grams with a range of -2 to +29. Only six animals showed a gain of three grams a

week. No cases of paralysis or convulsions were noted on this level.

Three micrograms was the first increment to cause a gain in weight above three grams a week. Mean gains for the 36 animals was +22 ±.17 grams during the experimental period, with a range of -1 to +46 grams.

Four micrograms given to 10 animals showed a mean gain of +26 ±.30 grams, with a range of +13 to +43 grams. This was the first level at which all animals showed a gain of at least three grams a week.

Five micrograms fed daily resulted in a mean gain of +36 ±.05 grams for 26 animals, with a +6 to +64 grams range.

Only two animals were available for experimentation on six micrograms so their mean gain of +39 grams is indicated in dotted lines in the chart. This level cannot be considered as having sufficient animals to justify any conclusions as to response.

Tables I to VIII give detailed records of growth of individual animals and Table IX the summary of all animals used in this investigation.

From these data Charts I and II were prepared. Chart III is the curve of response of this colony to graded amounts of vitamin B_1 and will be used in connection with determinations of vitamin B_1 values of food substances in this laboratory.

DISCUSSION

The curve of response made by plotting the mean gains in weight as ordinates against the micrograms of the vitamin B_1 fed, as the abscissas, can be used as a standard of reference for the colony of experimental rats of the Foods and Nutrition Department of Oregon State College. The importance of each laboratory establishing its own reference curve has been suggested by Eddy, (cited in 6 a) Irwin, Brandt and Nelson (48) and Palmer (71), Coward (11) and others.

This curve of response is of value in determining the vitamin content of food materials. When a food material is to be tested it is fed to groups of rats on one or two levels and at the same time one or two levels of the crystalline vitamin B_1 are fed to check the established curve. Since seasonal and individual variations are large factors even in well standardized test animals checks should constantly be run. The growth response of the feedings of the material being tested are plotted on the reference curve. From these points, lines are dropped to the axis of the abscissas and the vitamin values can be calculated. Coward (11) found a great deal of accuracy through the use of her curves of response in estimating the vitamin content of foods. This method is advantageous when only small quantities of the food are available (76 a).

Richardson and Mayfield (76 a) for their curve of response used the International Standard clay adsorbate. On the basis that 10 milligrams of the standard contain one international unit of vitamin B_1 which from the reports of Waterman and Ammerman (100) contains five gamma of the crystalline material, their results paralleled fairly closely the results obtained in this study, as do those of Waterman and Ammerman (100).

SUMMARY AND CONCLUSIONS

Healthy, young albino rats 21 to 28 days old, weighing 39 to 55 grams, of the Foods and Nutrition Department at Oregon State College, were placed on weaning on a Chase-Sherman B_1 free diet until stationary or declining weights were reached. Depletion was reached in 14 to 18 days. In the experimental period of four weeks litter mate controls were fed daily from one to six micrograms of crystalline vitamin B_1 hydrochloride by oral administration. A negative control was selected from each litter. Mean gains in weight over the experimental four week period were -22 grams for 15 negative controls; -4 $\pm$1.68 grams for 11 animals fed one microgram of vitamin B_1; +11 $\pm$.10 grams for 14 animals fed two micrograms of vitamin B_1; +22 $\pm$.17 grams for 36 animals fed three micrograms of vitamin B_1; +26 $\pm$.30 grams for 10 animals fed four micrograms of vitamin B_1; +36 $\pm$.05 grams for 26 animals fed five micrograms of vitamin B_1 and +39 grams for two animals fed six micrograms of vitamin B_1. Polyneuritic symptoms were noted on the suboptimal levels of one and two micrograms of vitamin B_1.

The curve of response established by plotting the mean gains in weight as ordinates against micrograms of the crystalline vitamin B_1 fed as abscissae will be used as a standard of reference for the Oregon State College rat colony of the Foods and Nutrition Department. By means of

this curve food material can be assayed with a comparable degree of precision.

Table I--Growth Response of Rats on Vitamin B_1 Free Diet

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	Survival on Vitamin B_1 Free Diet (days)
A505	M	43	55	50	48	43	34*	-21	45
A509	M	49	53	42	35	--	--	-22	31
A517	M	48	67	56	47	42	--	-27	39
A525	F	45	63	55	45	34	--	-29	38
A530	M	44	47	41	33	--	--	-19	39
A539	F	41	47	43	36	27	--	-20	35
A540	M	40	48	41	32	--	--	-16	28
A546	M	47	63	62	61	52	--	-20	41
A551	M	40	52	51	48	32	--	-20	35
A554	M	53	65	64	56	48	37	-28	41
A558	M	52	69	63	50	42	--	-27	35
A564	M	43	63	67	60	53	40	-23	42
A565	M	53	67	59	53	41	--	-26	35
A569	M	54	69	63	66	48	--	-28	34
A578	M	45	65	66	58	43	--	-22	40
Mean		46	59	55	47	43	37	-22	37

*Deceased

Table II--Growth Response of Rats Receiving One Microgram of Vitamin B_1 per Day

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.E.
B128	M	41	63	58	55	51	47	-16	
B129	M	41	68	69	62	55	58	-10	
A504	M	45	54	50	51	51	51	- 3	
A512*	F	47	50	43	43	36	--	-20	
A520	F	47	49	49	54	55	54	+ 5	
A522	M	47	64	60	55	52	48	-16	
A529	F	43	62	59	53	54	53	- 9	
A532	M	42	63	55	48	49	44	-19	
A556	M	42	51	56	54	55	54	+ 3	
A548	F	44	55	62	65	63	62	+ 7	
A562	M	46	55	61	74	78	75	+20	
A568	F	43	52	51	46	47	48	- 4	
Mean		43	58	57	56	55	54	- 4	±1.68

*not included in mean calculation

Table III--Growth Response of Rats Receiving Two Micrograms of Vitamin B_1 per Day

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.E.
B184	F	40	58	59	59	64	75	+17	
B198	F	43	66	74	80	90	95	+29	
B217	F	40	63	70	77	77	80	+17	
B202	F	49	71	72	74	75	76	+ 5	
B226	F	45	69	74	75	77	80	+11	
A506	F	43	49	45	52	58	65	+16	
A516	M	54	62	62	63	65	64	+ 2	
A521	F	46	57	56	56	53	55	- 2	
A524	M	44	57	54	51	56	61	+ 4	
A538	F	41	52	50	50	47	52	± 0	
A535	M	45	57	53	62	75	80	+23	
A550	M	48	56	58	61	60	62	+ 6	
A557	F	50	62	69	68	76	72	+10	
A561	M	47	61	74	76	80	74	+13	
Mean		45	60	62	65	68	71	+11	±.10

Table IV--Growth Response of Rats Receiving Three Micrograms of Vitamin B_1 per Day

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.B.
B35	F	39	55	64	72	88	97	+42	
B42	M	43	56	64	71	79	100	+44	
B53	M	44	68	77	82	94	101	+33	
B67	F	47	66	71	75	83	83	+17	
B68	F	47	62	67	67	74	79	+17	
B73	M	45	64	70	71	74	79	+15	
B81	M	44	55	63	68	73	77	+22	
B101	M	45	63	72	85	97	109	+46	
B111	M	42	74	86	98	105	106	+32	
B123	F	42	60	60	71	83	87	+27	
B130	F	42	63	71	75	76	93	+30	
B138	F	43	68	78	92	105	108	+40	
B142	F	48	86	100	110	111	112	+26	
B158	M	50	79	87	105	110	107	+28	
B183	F	41	58	69	79	88	97	+39	
B402	F	40	61	69	71	72	76	+15	
B427	F	42	60	64	68	75	78	+18	
B450	F	40	58	61	65	71	69	+11	
B408	M	45	67	71	68	65	66	- 1	
B440	M	41	55	62	62	64	71	+16	
B457	M	49	71	79	77	74	71	+ 0	
B474	F	40	55	61	66	74	87	+32	
B197	F	45	77	86	93	105	112	+35	
B469	F	43	65	65	66	66	69	+ 4	
B224	M	45	77	84	93	98	104	+27	
B485	M	55	77	81	88	91	91	+14	

Table IV--Growth Response of Rats Receiving Three Micrograms of Vitamin B_1 per Day

(Continued)

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.E.
A508	F	41	51	50	59	64	68	+17	
A510	M	45	56	52	57	56	60	+ 4	
A518	M	48	59	57	61	64	64	+ 5	
A526	F	45	58	58	60	61	67	+ 9	
A531	M	43	59	63	57	60	65	+ 6	
A533	F	42	60	58	62	73	84	+24	
A552	F	48	60	67	73	84	92	+32	
A559	M	51	68	78	77	79	86	+18	
A573	M	49	62	72	79	82	88	+26	
A575	F	43	70	75	82	87	88	+18	
Mean		45	64	67	73	78	86	+22	±.17

Table V--Growth Response of Rats Receiving Four Micrograms of Vitamin B_1 per Day

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.E.
A511	M	41	57	52	63	68	74	+17	
A513	F	46	49	50	58	64	72	+23	
A523	M	46	65	69	79	78	86	+21	
A527	F	44	60	62	63	68	73	+13	
A553	F	48	50	62	63	65	66	+16	
A547	F	47	61	74	83	93	99	+38	
A545	M	49	61	66	76	87	88	+27	
A560	M	50	68	75	84	92	99	+31	
A570	M	51	55	68	84	89	98	+43	
A574	F	46	53	62	69	84	88	+35	
Mean		47	58	64	72	79	84	+26	±.30

Table VI--Growth Response of Rats Receiving Five Micrograms of Vitamin B_1 per Day

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.E.
B530	F	41	45	52	63	70	71	+26	
B516	M	47	59	71	72	89	97	+38	
B499	F	42	56	68	77	82	82	+26	
B506	F	43	55	58	62	60	66	+11	
B511	M	46	49	67	77	91	96	+47	
B487	M	47	58	65	77	82	88	+30	
A515	F	43	48	48	55	58	68	+20	
A519	M	47	58	55	58	63	64	+ 6	
A528	F	44	65	74	77	86	94	+29	
A537	F	48	53	64	70	70	83	+30	
A534	M	45	61	66	76	86	107	+46	
B549	F	44	65	71	78	96	103	+38	
A555	M	48	58	74	87	97	100	+42	
B558	F	44	61	66	71	81	91	+30	
B567	F	41	69	82	103	115	114	+39	
B580	F	40	51	54	64	79	90	+39	
B585	M	40	56	64	74	89	103	+47	
A563	M	43	58	69	86	95	100	+42	
A567	F	49	56	65	65	70	77	+21	
A572	M	50	65	67	70	79	93	+28	
B629	M	44	58	69	85	94	99	+41	
B651	F	42	48	61	68	79	86	+38	

Table VI--Growth Response of Rats Receiving Five Micrograms of Vitamin B_1 per Day

(Continued)

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams	P.E.
B635	M	46	56	71	80	86	86	+30	
B656	F	44	55	69	83	100	113	+58	
B667	M	46	57	67	81	98	107	+50	
A579	M	45	62	69	81	104	126	+64	
Mean		44	57	62	75	85	93	+36	±.05

Table VII--Growth Response of Rats Receiving Six Micrograms of Vitamin B_1 per Day

No.	Sex	Initial Weight Grams	Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Gain in Weight Grams
A571	M	51	68	81	85	89	101	33
A577	M	47	67	75	82	94	111	44
Mean		49	67	78	84	92	106	+39

Table VIII--Summary of Growth of Male and Female Rats on Graded Amounts of Vitamin B_1

Vitamin B_1 in Micrograms	No. Cases	Mean Initial Weight Grams	Mean Depletion Weight Grams	Experimental Period: Weight at end of one week Grams	Weight at end of two weeks Grams	Weight at end of three weeks Grams	Weight at end of four weeks Grams	Mean Gain in Weight Grams
One	7M	43	60	58	57	56	54	- 6
	4F	44	55	55	55	55	54	- 1
Two	5M	48	58	60	63	67	68	+10
	9F	44	61	63	66	67	72	+11
Three	17M	46	53	71	71	74	85	+20
	19F	43	62	62	74	80	84	+23
Four	5M	47	61	66	77	82	89	+27
	5F	46	54	62	67	74	77	+25
Five	13M	46	58	67	77	87	97	+39
	13F	44	55	64	81	81	88	+33
Six	2M	49	67	78	84	92	106	+39

Table IX--Summary Table: Response of Rats to Graduated Amounts of Vitamin B_1

Daily Dose	No. Cases	Mean Initial Weight	Mean Depletion Weight	Mean Final Weight	Mean Gain in Weight	P.E.
Negative Controls	15	46	59	37	-22	
One Microgram	11	43	58	54	- 4	±1.68
Two Micrograms	14	45	60	71	+11	± .10
Three Micrograms	36	45	63	86	+22	± .17
Four Micrograms	10	47	58	84	+26	± .30
Five Micrograms	26	44	57	93	+36	± .05
Six Micrograms	2	49	68	106	+39	

CHART I

Gains in Weight of Rats on Graduated Amounts of Vitamin B_1

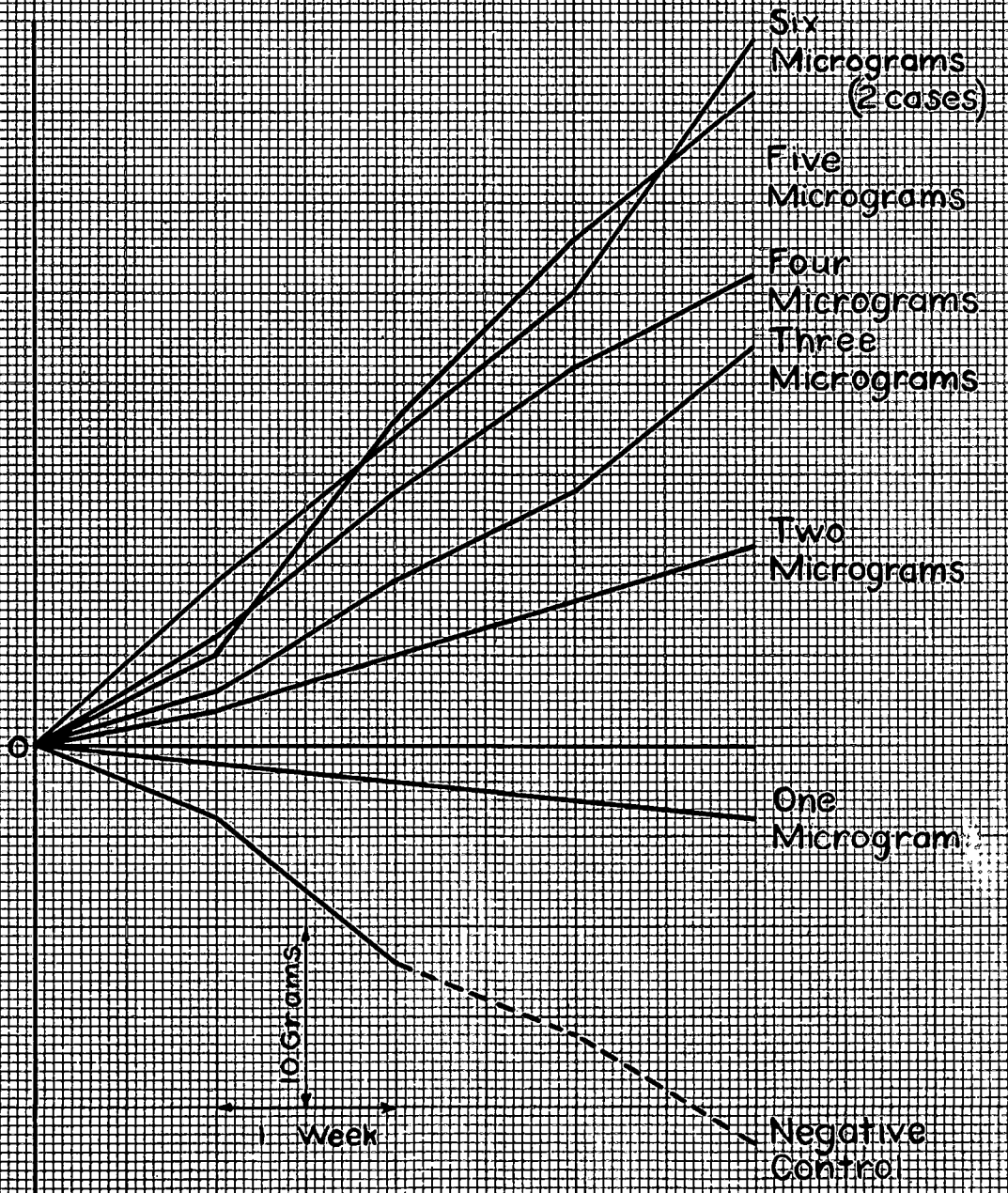


CHART II

Curve of Response of Males and Females on Graded Amounts of Vitamin B_1.

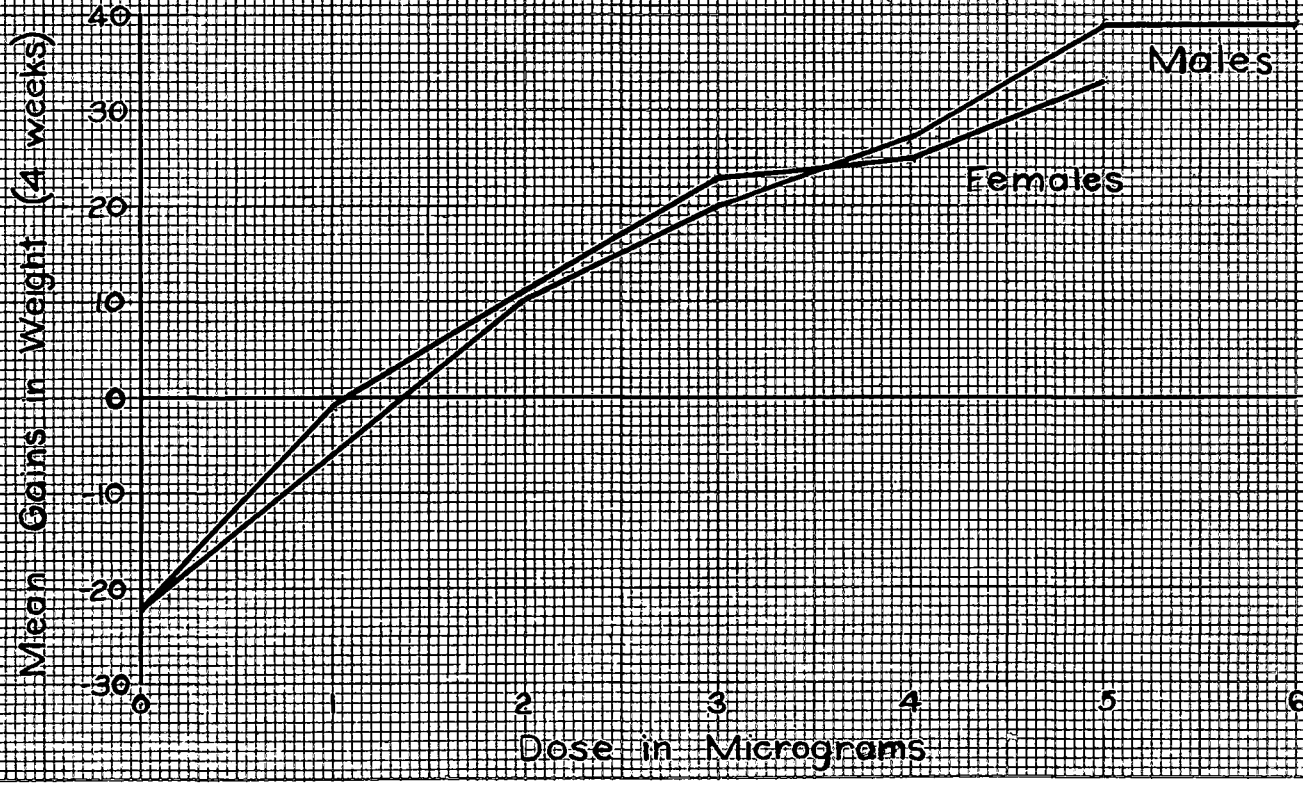
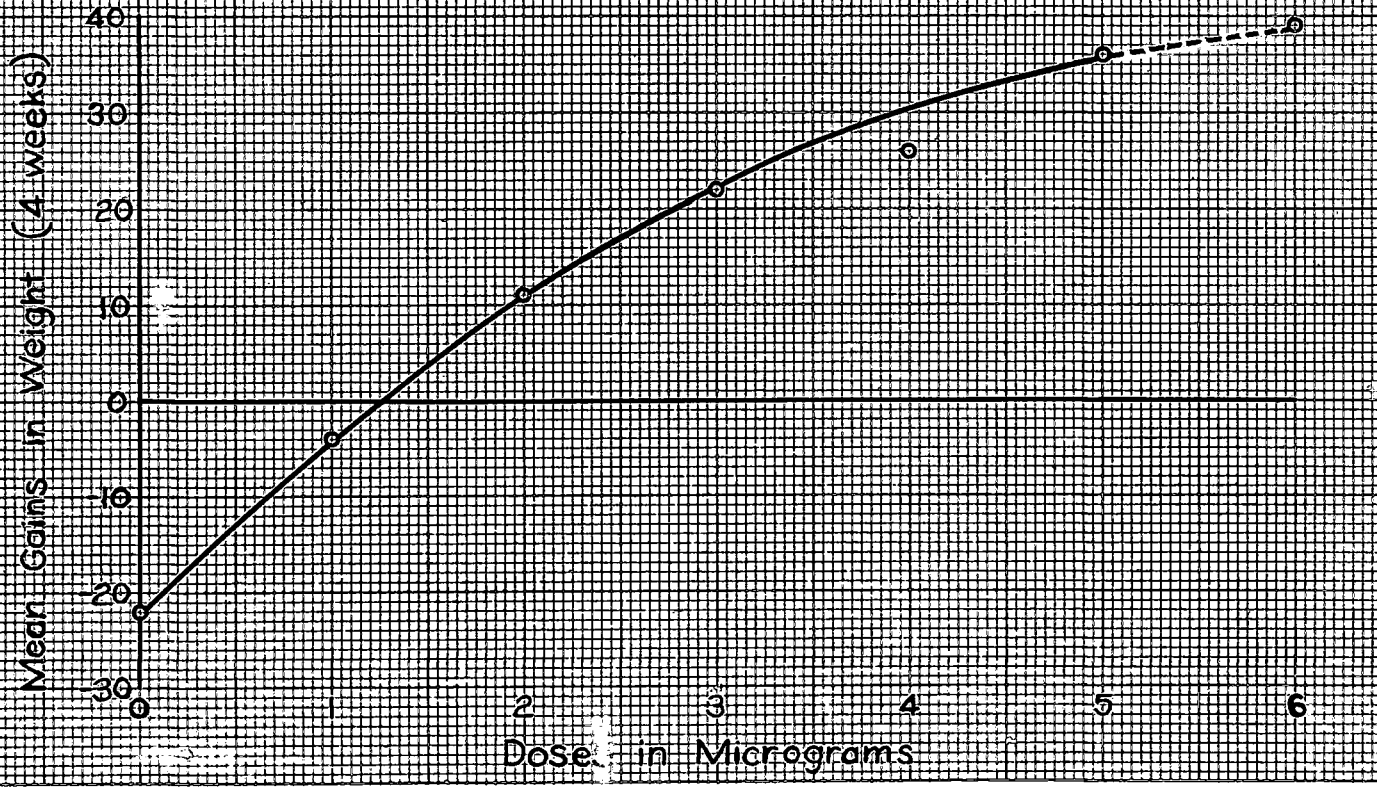


CHART III

Curve of Response of Rats to Graduated Amounts of Vitamin B_1



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