DEFICIENCY DISEASES AND THE VITAMINS *

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I wish to acknowledge the very great honor you have accorded me in inviting me to deliver these lectures. When I consider this, and the calibre of the other lecturers whom you have previously invited, it is with some trepidation that I venture upon my task.

It was sufficiently clear to me that I could hope to tell you little or nothing new. But in addition to this function, lectures are often useful when they present a critical survey of a subject, especially when this survey is made from a more or less original point of view. This is what I shall attempt.

In the course of these lectures I have referred several times to conditions in Porto Rico. I wish to state here that I know nothing about Porto Rico from personal observation, and therefore my information is derived entirely from what I have been able to read. It is notoriously easy to acquire misconceptions in this way, and therefore I hope you will pardon any errors that I may make, and will attribute them to my very limited facilities. I am therefore delighted to have this opportunity to become better acquainted with you, and to learn as much about Porto Rico as is possible in the time allotted to me.

Nomenclature. The terms scurvy, beriberi, rickets and pellagra have the dignity of age and universal usage and probably will never be changed. The case is far otherwise with the vitamins, a term coined by Funk in 1911. Funk indeed used the word vitamine, indicating a vital amine; but it was soon recognized that the chemical radicle *amine* could not be appropriately applied to these substances, and the word was accordingly changed to vitamin. Other names have been suggested, as for instance, accessory food substances, which is clumsy, and auximones, which is objectionable in that it implies that they act as hormones. In 1921, I(1) suggested the name vitaliment as an appropriate term for such food principles as are necessary in infinitesimal amounts for the continuance of life, and as embodying no suggestion as to their chemical constitution or physiological action. Vitaliments, thus might include not only those organic compounds now known as vitamins, but also those

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inorganic compounds equally essential in minute amounts for the existence of human or mammalian life such as Iodine, iron and possibly copper which is apparently of importance in some way in the production of haemoglobin(2). Some such deficiency may ultimately prove to be the cause of that remarkable and mysterious blood disease, chlorosis. But this term, vitaliment, which I still think is the most appropriate name for such substances, attracted no general attention, and the term vitamin is now very generally used throughout the world.

About 1917, a prominent investigator who has made important contributions to our knowledge of diet and deficiency diseases, then believing that there were only two vitamins, called them fat-soluble A, and water-soluble B. This initiated the alphabetic designation of the vitamins which was continued although the better descriptive terms anti-neuritic and antiscorbutic had been in general use for some years previously. We now have a list of vitamins at least to G, and different investigators do not agree as to the proper letter that should be applied to each vitamin. The terminology is further complicated because the original B has been found to consist of at least two fractions which British students now call B_1 and B_2 and American students B and F or some other letter. Still further investigations strongly suggest a third fraction of B(3) which has been tentatively named B_3. It is certain that there are still further vitamins including very probably those whose deficiency is responsible for pernicious anaemia, sprue, and other diseases of whose causation we are now ignorant. The confusion that will arise should the number of vitamins reach X, Y, and Z can be readily imagined.

Those in favor of the alphabet(4) claim that while leading to confusion, such designation has the very great advantage of freedom from bias and from connotations inconsistent with anything which may be learned by further study of these substances. Thus, to call vitamin A the anti-xerophthalmic vitamin would be a misnomer because this is only one of several results, and probably not the most important one, arising from deficiency of this particular vitamin. A deficiency of this substance results in an increased incidence of respiratory disease, of skin, ear and sinus infections, of inflammations of the alimetary tract and genito-urinary tract, leading even to the production of vesical and renal calculi.

But it may be noted that the pathological change at the basis of all these diverse clinical manifestations is the same, namely a tendency toward the keratinization of all epithelial structures, followed by infection when the normal secretion of the tissue is no longer formed. The name anti-keratinization vitamin, while clumsy, would be a correct interpretation of the physiological and clinical significence of this vitamin.

The basic pathological changes produced by the deficiency of at least five of these vitamins are quite well known, as well as the specific diseases so caused, and many investigators used descriptive names such as anti-scorbutic, anti-neuritic and anti-rachitic even before the time of alphabetic terminology, which was initiated by the misconception that there was only one fat-soluble and one water-soluble vitamine. Whereas in fact there are at least three fat-soluble and three water-soluble. To me it seems obviously preferable that each vitamin should, as soon as possible, receive a descriptive term that will readily identify it, rather than an alphabetic designation that will mean nothing at all to most people and not the same thing even to investigators familiar with the subject. The alphabetic terminology should be restricted to those vitamins whose deficiency so far as known, produce no specific disease, or well defined pathologic changes.

We need not feel depressed by the fact that some of these names may not be the best possible, and may subsequently have to be changed. We should be encouraged by the example of the zoologists and bacteriologists who have not hesitated to change the name of some poor mosquito or micro-organism three or four times, not because the original name known to everyone was not a good name, but merely because it did not conform to certain rules of nomenclature. Eventually of course, the exact chemical structure of the various vitamins will be determined, and they can then be given a correct chemical designation. But this may be a matter of many years, and in the meantime we are obliged to discuss them not only in scientific papers, but with the laity. There will be less confusion if we do so by name.

The following table presents in alphabetic order the present known vitamins and some that are not as yet generally accepted, with the suggested name, the associated disease, and the pathologic change produced by its deficiency.

	Name	Chemical Composition	Disease produced	Characterictic Pathological Changes
A.	Anti-keratinic	Carotin?	Xerophthalmia etc.	Keratinization of epithelium
B.	Anti-neuritic.....	Organic base.....	Beriberi	Degeneration of nervous system
C.	Anti-scorbutic	Unknown.....	Scurvy	Degeneratión of intercellular cement substances
D.	Anti-rachitic.....	Irradiated Ergosterol	Rickets.....	Disturbance of calcium-phosphorus metabolism.
E.	Genetic or Anti-sterilic	Unknown, Fat-soluble, Isolate like A.	Sterility	Degenerative changes in the reproductive organs
F.	Anti-pellagric.....	Organic base.....	Pellagra	Characteristic lesions in epithelium
G.	Anti-anaemic.....	Organic base.....	Pernicious Anaemia	Failure of bonemarrow to produce red corpuscles
H.	Anti-psilotic.....	Unknown.....	Sprue.....	Characteristic lesions in the digestive tract

The Chemistry of the Vitamins. A. The anti-keratinic. Takahashi claimed in 1925 to have isolated this vitamin in a state of purity, called it Biosterin and gave it a formula of C_{27} H_{44} O_2. It is now generally recognized that this product was impure although the vitamin was present in a highly concentrated state. In brief, the process used by Takahashi and others is the saponification of the oil fat under precautions to prevent oxidation, which destroys the vitamin. A large part of the non-saponifiable fraction consists of cholesterol which is removed by crystalization from methyl alcohol at low temperatures, or precipitated by digitonin. The residue, consisting of a red brown oil is further purified by distillation with superheated steam in an atmosphere of nitrogen. Under these conditions the vitamin is carried over with other volatile products. This admittedly impure concentrate is non-nitrogenous, and a daily ration of 0.02 mg. is sufficient to supply all the needs of the rat.

About 1920, Steenbock and his associates noticed that this vitamin was closely associated with the yellow color of butter, corn, and vegetable leaves which contained it, and suggested that the vitamin was carotin. This was promptly denied by other investigators because rats supplied with carotin for the anti-keratinic vitamin failed to grow properly. But quite recently three Swedish investigators[8] claim that these earlier equivocal results from the use of carotin were due to lack of appreciation of the necessity for the anti-rachitic vitamin, also fat-soluble, in the ration used. They believe that carotin actually is the anti-keratinic vitamin because they obtain growth with as little as 0.005 mg. of crystalline carotin. Further this pig-

ment gives the blue color characteristic of this vitamin with antimony trichloride, a reaction that was devised by Rosenheim and Drummond and alleged to be specific for vitamin A. At present, therefore, we may say that either the anti-keratinic vitamin is carotin, or that it is some impurity present in traces in crystalline carotin. This second possibility cannot be dismissed from consideration, because carotin is a substance difficult to obtain in an undoubted state of purity even when crystallized, and because of the very minute amounts of vitamin known to be required by the rat. Less than 0.0001 mg. of anti-rachitic vitamin is adequate for this animal, and if the anti-keratinic were effectual in similar doses, one per cent of impurity in crystalline carotin might be sufficient to account for its physiological activity.

B. Anti-neuritic vitamin. From early investigations(6) it was learned that this vitamin was water soluble, alcohol soluble, ether insoluble, dialyzable and readily adsorbed. Later it was found that it is precipitated by phosphotungstic acid, picric acid, silver nitrate and other precipitants which clearly indicate that it is a simple nitrogenous organic base. It has been obtained many times in crystalline form, and lately Jansen and Donath(7) appear to have isolated it in a state of chemical purity, but its exact chemical constitution has not yet been determined.

C. Anti-scorbutic vitamin. Nothing has as yet been learned concerning the chemical constitution of this vitamin, owing to the fact that it is the most unstable of all the known vitamins, being readily oxidized especially in neutral or alkaline solutions, or when heated in the presence of oxygen. It has however been learned that it is soluble in water and alcohol, insoluble in ether, acetone, amyl and butyl alcohols. It is not precipitated by phosphotungstic acid or by neutral lead acetate, but may be precipitated by basic lead acetate. It is not adsorbable. With this information it is to be hoped that this vitamin may soon be obtained in crystalline form, at least in a state of chemical purity, since it is possible that this is a non-crystalline substance. At least all efforts up to the present time have failed to demonstrate crystals even in highly refined and potent concentrates.

D. The anti-rachitic vitamin. Hess and others had found that the antirachitic vitamin in cod liver oil was always in the non-saponifiable fraction, and the main constituent of this fraction in any oil is some sterol; cholesterol $C_{27}H_{46}O$ from animal sources, and a similar sterol, phytosterol, from plant sources. Supposedly pure

crystalline cholesterol was endowed with anti-rachitic properties by submitting it to ultra-violet irradiation.

But in 1925, Hess and Weinstock, and Windhaus and Hess([8]), by means of spectrum absorption tests, showed that the activity of the cholesterol was due to a minute amount of impurity which was found to be a closely related sterol, ergosterol, $C_{28}H_{43}OH$. Ergosterol may be prepared in considerable quantity from various vegetable cells, and is actually being made at the present time from yeast. This pure ergosterol is inactive, and must be irradiated by ultra-violet light to develop anti-rachitic potency. Spectroscopic study of this irradiated compound has shown that a chemical change is produced by this irradiation, and that this change is progressive. As radiation of a specific intensity is continued for from fifteen to twenty minutes, the anti-rachitic activity of the ergosterol increases very rapidly; but if irradiation is continued longer, the antirachitic potency again decreases([9]) as follows:

Minutes of radiation	Potency (cod liver oil being 100)
7½	150,000
15	225,000
22	250,000
30	200,000
120	50,000
3 hrs.	0

Examination of this product of irradiation has shown that at least two substances are produced by these rays from ergosterol, the unstable vitamin, and a second more stable and inactive substance which may be formed from ergosterol direct, or as a decomposition product of the anti-rachitic vitamin.

Irradiated ergosterol gives a violet color with fuchsin-sulphuric acid reagent, and will also reduce ammoniacal silver oxide to stable colloidal silver, while non-irradiated ergosterol gives neither of these reactions. These reactions indicate that irradiation has transformed a part of the ergosterol into an aldehyde or unsaturated ketone, and that this substance may actually be the antirachitic vitamin. At any rate, ergosterol is the mother substance or pro-vitamin.

E. The genetic or anti-prolific vitamin. Knowledge concerning this vitamin has developed since 1922, chiefly as the result of the work of H. M. Evans([10]) and his associates. Evans found that when rats were fed on certain diets containing a sufficiency of all known ingredients including vitamins A, B, and D (rats do not suffer from scurvy), they would grow and develop normally; but if the diet were continued, after a transitory period of fertility, complete sterility developed.

Evans and Burr[11] showed that this sterility is associated with characteristic changes in the reproductive organs. In the male, there is destruction of the germ cells and eventually of the entire seminiferous epithelium. In the female, the ovary and the process of ovulation remain normal; but during gestation, the embryo which at first develops normally, begins to show a retardation of development about the eighth day, and between the twelfth and twentieth days the foetus dies and is completely resorbed. This sterility may be prevented or cured by the addition of food containing the genetic vitamin, which is present in the largest quantity in wheat germ oil and to a lesser degree in the green leaves of plants such as lettuce, alfalfa, pea, etc. It is almost completely absent in cod liver oil, a fact that at once distinguishes it from the anti-keratinic and anti-rachitic vitamins although like these two, the genetic vitamin is fat soluble.

Using 6 kilos of wheat germ, Evans and his co-workers have extracted the germs with ether, and saponified the extract. The vitamin passes into the nonsaponifiable residue. The sterols are removed, and the residue further purified by vacuum distillation. A fraction boiling at 200 degrees and upward at 0.5 mm. pressure is found to contain the bulk of the active agent

This vitamin is remarkably stable to heat, light, oxidation and chemical reactions generally, a property possessed by none of the other fat-soluble vitamins. While it is obvious that very few people suffer from this deficiency, it remains possible that some hitherto unexplainable cases of human sterility may be due to this inadequacy in the diet.

F. The anti-pellagric vitamin. Little is known concerning the extract chemistry of this vitamin. It is contained in yeast, but is obviously not the anti-neuritic vitamin since it is not contained in many foods like beans and peas which are rich in anti-neuritic vitamin, and the latter is present in very small amounts in meat which is rich in antipellagric vitamin. Autoclaved yeast in which the anti-neuritic vitamin is destroyed, was found to be still effective in the prevention of pellagra. The fact that pellagra and beriberi so rarely occur together is further evidence with regard to the distinct identities of these vitamins.

That this vitamin is an organic base seems probable since it reacts chemically in a similar way to the anti-neuritic vitamin, and many of the concentrates of the latter have been mixed with the anti-

pellagric vitamin, and the two vitamins have been separated from such solutions by methods of selective adsorption.

G. The anti-anaemic vitamin. Pernicious anaemia has not yet been generally accepted as a deficiency disease, although this possibility has been suggested several times. The active substance in liver extract appears to be an organic base from the little that is known of its chemical reactions. It is soluble in water and seventy per cent in alcohol, but insoluble in absolute alcohol and ether It is precipitated by phosphotungstic acid, but not by basic lead acetate or saturated ammonium sulphate, and it gives no protein reactions.

H. The anti-psilotic vitamin. Nothing whatever is as yet known concerning this hypothetical vitamin, which is discussed further in the succeeding lecture.

Those who wish to learn more concerning the chemistry of the vitamins are referred to the recent text book of Gortner([12]) which contains an excellent chapter on this subject.

The physiological action of the vitamins. There has never been any generally satisfying explanation of the physiological action of the vitamins. It has been suggested that they act as food hormones. In addition to the fact that there is no proof of such action, this is an explanation that does not explain. For while we are very familiar with the action of important hormones like insulin, epinephrin, thyroxin and pituitrin, we have little or no information as to precisely how they produce their remarkable physiological effects.

It has also been suggested that the vitamins act as catalyzers, that is, promote certain chemical reactions without taking part ir them. It is possible that there may be some truth in this suggestion We know that ergosterol is itself inactive and does not produce calcification of bone in rickets until after it is irradiated; and that as the result of the application of this peculiar form of energy, a new compound of great potency is produced. It is therefore quite conceivable that this compound yields up chemical energy to the body thereby acting as a catalyst or activator regulating the deposition of calcium phosphate. This seems the more probable since it has been found that excessive doses of this vitamin (from 1,000 to 10,000 times the normal dose) may cause the death of experimental animals with excessive deposition of calcium in organs where it is not normally found. This is so far the only vitamin to produce injurious results in excessive amounts. This method of action seems

quite improbable in the case of these vitamins known to be organic bases.

Some investigators have claimed that the antineuritic vitamin, known to be an organic base, plays a part in carbohydrate metabolism because they believed that polyneuritis develop faster the higher the proportion of carbohydrate in the diet; and that a quantity of vitamin that would afford complete protection on a certain limited ration of carbohydrate, fails to protect when the amount of carbohydrate is considerably increased. This is of more than theoretical importance as it might have a very practical bearing on the formation of dietaries that will prevent beriberi.

But others, including myself([13]) have been unable to confirm this hypothesis, finding that the rapidity of development of polyneuritis bears no relation to the amount of carbohydrate consumed. Moreover metabolic studies conducted both on animals([14]) and on human cases of beriberi([15]), have failed to show that there was any alteration in the metabolic rate, respiratory quotient, or nitrogenous constituents of the blood in such animals or men suffering from a deficiency of the anti-neuritic vitamin. It is needless to say that if these facts are correct, as I believe they are, any connection of this vitamin with either carbohydrate or protein metabolism of food is extremely improbable to say the least.

It is almost certain that the vitamins play more than one role in the physiological economy, and it can hardly be supposed that the anti-rachitic vitamin will function in the body in the same way as do the organic bases. It has always been my working hypothesis that these latter act as essential building stones for certain tissues, just as protein metabolism cannot be maintained in the absence of certain amino-acids like tryptophane, cystine and lysine. The evidence in favor of this view may be summarized as follows:

1. The relative quantity of vitamin required by an animal and the rapidity of development of the corresponding disease on a deficient diet, are both closely related to the basal metabolic rate of the animal. The metabolic rate is proportional to the surface area, and the smaller the body, the larger is the surface area compared to the weight of the body. Thus the metabolic rate of the guinea pig is far higher than that of man, and guinea pigs develop scurvy in about twenty days, while it requires from four to seven months to produce the same result in man. Accordingly the guinea pig requires far more antiscorbutic vitamin weight for weight than does man. To protect completely a guinea pig of 250 grams from scurvy

5 c.c. of lemon or orange juice daily is required. If a man who weighs 300 times more, required lemon juice in proportion to his weight, he would be required to drink 1,500 c.c. daily, whereas it has been amply demonstrated that 30 c.c. will afford complete protection to men on an absolutely deficient diet.

Similarly the requirement for anti-neuritic vitamin is relatively higher for fowls than for man; higher for pigeons than for fowls, and higher for sparrows than for pigeons. The smaller the bird, the more rapid is the development of polyneuritis on a diet of polished rice. If we dismiss the metabolism of food, these facts indicate that these vitamins are concerned in the metabolism of the tissues.

2. That vitamins are concerned in the metabolism of tissues is also indicated by the fact that deprivation of any specific vitamin is followed by the degeneration of a corresponding specific tissue. Thus, deprivation of A, the anti-keratinic vitamin is followed by a widespread degeneration of keratinization of all epithelial structures, and all the diverse symptomatology produced by the deficiency of this vitamin depends upon this single pathological process.

Deprivation of B, the anti-neuritic vitamin is likewise followed by a very specific degeneration of the nervous system, a degeneration that is not confined to the peripheral nerves, but involves also the cells and nerve fibres of the brain and cord. It is true that we cannot explain all of the changes and symptoms found in beriberi as the results of this degeneration of the nervous system. But there is the possibility which has been suggested, that dry and wet beriberi are two separate conditions, caused by two different deficiencies, the second of which has not been so precisely determined as has the anti-neuritic vitamin. This subject will be discussed later.

The same thing is true of C, the Anti-scorbutic vitamin. Deprivation of this substance is followed by a widespread failure in the formation of the cement substance of connective tissue, and all the symptoms of scurvy are so explainable. The haemorrhages are due to failure of the cement substance between the endothelial cells of the capillaries, thus permitting blood to exude into the skin, muscles and intestine. The softening of the gums, teeth and bones are similarly caused.

Deprivation of E, the genetic vitamin, is followed by pathological changes in the reproductive organs: Deprivation of F, the anti-pellagric vitamin results in characteristic changes in the epithelium of the skin and of the digestive tract. Here again the epithelium is affected, but the character of the change is quite different from

that produced by the anti-keratinic vitamin. Deprivation of G, the anti-anaemic vitamin is followed by the failure of the haematopoietic bone marrow to produce erythrocytes.

This specificity of the vitamins for particular corresponding tissues is most remarkable. If it does not mean that these vitamins serve as building stones for these tissues, we have at least no other explanation that will account for these facts so satisfactorily.

3. The degeneration of the affected tissue begins practically at once when the corresponding vitamin is eliminated from the diet. Young rats of 35 grams weight placed on a diet deficient in anti-keratinic vitamin, develop symptoms of xerophthalmia in three to four weeks. Older rats of 55 grams, in about thirty to thirty-five days.(16)

Fowls fed on rice deficient in anti-neuritic vitamin develop clinical signs of polyneuritis after about thirty days, but obvious degeneration of the peripheral nerves has been demonstrated as early as seven days after the initiation of the deficient diet.(17)

Scurvy of 250 gram guinea pigs commences in from fifteen to twenty days after the initiation of the deficient diet. By following Hojer's technique, changes in the teeth may be demonstrated as early as ten days.(18) In all of these cases, the advanced changes found after the first clinical signs of the disease, simply cannot occur suddenly, but must have been a gradual process. It is certain that they must have commenced within at most a few days after the institution of the defective diet. Although we believe the action of the antirachitic vitamin to be different, its action is no slower, for young rats of from thirty-five to forty grams develop rickets in from eighteen to twenty days.

4. In all of these deficiency diseases, the curative dose of vitamin is much larger than the daily protective dose. In the only two diseases of which I can speak from personal experience, the curative dose is almost an exact multiple of the daily protective dose by the number of days the defective diet has lasted. Thus if 5 c.c. of a certain extract of rice polishings daily will protect a fowl from polyneuritis, and if another fowl has developed polyneuritis after thirty days on the deficient rice diet, it will require approximately 150 c.c. of the same extract to cure the second fowl. If a guinea pig that requires 5 c.c. of orange juice daily to protect it from scurvy, develops scurvy after twenty days on the deficient diet, it will require almost exactly 100 c.c. of orange juice, or a concentrate thereof, to produce a cure. On the other hand, should the daily

protective dose be cut down to one half of the protective dose (2½ c.c.) scurvy will develop after almost exactly forty days, and again it will require 2½ times 40 or 100 c.c. of lemon juice to produce a cure.

That is, it appears that the minimum daily protective dose of vitamin in both of these diseases is a fixed amount, absolutely required if the tissues are to be formed normally, and that any reduction in this amount will lead to a degree of specific pathologic change directly proportional to the degree of deficiency, and that a cure will not be effected until this fixed amount is provided.

Further, the amount of vitamin required varies with the age and size of the animal, and young growing animals require much larger amounts of vitamin than mature animals. This is so well recognized that all investigators insist upon young animals of a standard age and weight in testing the deficiency or sufficiency of a given quantity of food stuff for its specific vitamin content. It is a 250 gram guinea pig that requires 5 c.c. of orange juice as an anti-scorbutic. A guinea pig of from 500–700 grams will need less, and will develop scurvy much more slowly on a deficient diet. This seems to mean that growing tissue requires a definite amount of vitamin, and the reasonable deduction is that it is a component of that tissue.

5. Certain deficiency diseases are hastened or precipitated by factors that increase tissue metabolism. Exposure to fatigue and extremes of temperature appear to hasten the onset of beriberi, and on the same diet stokers are more susceptible than deck hands. Thus in one reported instance thirty-four cases of beriberi occurred among Chinese stokers and only three cases among the Chinese seamen. Williams and Johnston found that high temperature and fatigue hastened the onset of polyneuritis of fowls. The statistical evidence gathered by Siler, Garrison and MacNeal([19]) showed a greatly increased incidence of pellagra among women between seventeen to forty-five years of age, and that the incidence of pellagra among women giving birth to children during the summer months, that is during the pellagra season, was also greatly increased. Since there is little difference in the incidence of this disease in boys and girls of younger age, it would appear that the most reasonable explanation of the increased incidence among women of child-bearing age is the increased metabolism caused by this process. Such observations could be multiplied, but the above are sufficient to indicate that the demand of the body for vitamin is proportional to the degree of activity of tissue metabolism.

The objection has been raised against this hypothesis that the amounts of vitamin required are so infinitesimal that they cannot be of importance as tissue-building stones.

For at least one of the vitamins, the amounts present in food are larger than supposed, for in the case of the anti-neuritic vitamin it is known that the major portion is lost in the process of extraction and purification, and this may be the case with some of the other vitamins also. But entirely aside from this possibility, and admitting that the amounts required are infinitesimal, how can we argue that they may not be required as important ingredients of the tissues, when we know that 55 mg. of iron a day will supply all of that element required for the formation of haemoglobin, and that 3 mg. of iodine a month or 0.0001 gm. a day is sufficient for the maintainance of the normal activity of the thyroid, and of general health?

Depletion Periods: Both in man and experimental animals, a definite period of time elapses between the initiation of the defective diet and the appearance of the first clinical signs of the corresponding deficiency disease. The length of the depletion period may vary considerably in different individuals of the same age, and in experimental animals this has been shown to depend, at least partly, upon the vitamin content of the previous diet and upon the ability of the animal to store up a reserve. Reserve depots of vitamin are found notably in the liver, and to a lesser extent in other glandular organs. This depletion period of course varies for each vitamin and its respective deficiency disease in different animals.

The length of the depletion periods for experimental animals has been determined with considerable accuracy, and some of them have been given, but these periods cannot be applied to man, but a number of these periods have been fixed by clinical observation, experimentation, or both. As the result of the human feeding experiment of Fraser and Stanton,([20]) we know that three months is the minimum time in which men fed on an exclusive diet of deficient rice will develop beriberi. In most cases the period is somewhat longer.

It requires from four to seven months for men to develop scurvy. This period has been fixed by many old observation on sailors, at sieges, and more recently by Colonel Hehir([21]) at the siege of Kut in Mesopotamia during the late war. The minimum period is therefore four months.

Rickets is observed in children most often during the second half

of the first year and the first half of the second year. Many of these cases have existed for some months before they are seen by a physician. But cases are not frequent at five to six months, and probably few children are absolutely deficient either in anti-rachitic vitamin or sunlight, so that the real depletion period may be tentatively placed at from four to five months.

The depletion period for pellagra was partially learned from the human feeding experiment of Goldberger and Wheeler.([22]) In this case eleven convicts were fed on an experimental ration consisting chiefly of cereals. In this small number of men, the first appearance of the characteristic eruption of pellagra occurred five months after the inauguration of the defective diet.

It would seem unnecessary to point out that what we have learned concerning these depletion periods in man must be used in interpreting epidemiological observations on diseases like beriberi, scurvy and pellagra. Yet some emphasis upon this subject seems to be required because various observers are continually neglecting this hardly acquired but very definite information. Thus in a comparatively recent article,([23]) observers in Japan state that the view that beriberi is solely a deficiency disease is not tenable in view of its peculiar seasonal appearance in epidemic proportions. In Japan the people always use rice as the great food staple, but make up for its deficiency by the use of vegetables of various sorts. But these observers find that in the winter when the ground is frozen, much less vegetable food is available than in the summer, yet beriberi occurs and is epidemic in the hot months rather than in the winter. Upon such evidence as this, the great mass of facts that have been accumulated showing that beriberi is the result of a deficient diet, and never occurs when a proper diet is used, is to be discarded in favor of the theory that the disease must be caused by some infectious organism. Now if we only remenber that the minimum depletion period for beriberi is three months, and that it may be longer in many previously well fed individuals, and those still receiving a partial but inadequate amount of vitamin in the few vegetables eaten during the winter months, it will be apparent why beriberi in Japan does not appear in the winter when the food shortage commences, but in the following spring and summer. Since many people experience this deficiency at about the same time, the disease will assume "epidemic" proportions. The same kind of "epidemic" may be observed among a number of experimental animals after a similar sudden change of diet. This fact, which seems to these observers to disprove

the exclusive dietary origin of beriberi, is in fact very certain evidence of its truth, and the confusion has arisen simply because the well known facts as to the length of the depletion period in man were ignored.

The fact that pellagra occurs in the spring and summer when vegetable food is abundant, rather than during the winter when the deficiency occurs, is similarly explainable.

At this point, I cannot forbear from commenting upon the apparently immortal theory of bacterial infection as the explanation of the deficiency diseases. It pops up again and again after everyone thought it was dead. It has been claimed quite recently that bacteria are the cause of Xerophthalmia, of beriberi, of scurvy and of pellagra. Apparently rickets is the only disease that has not its infection enthusiasts, and I am sure I do not know why, for if the stools of rickety children were examined carefully enough, I am sure some hitherto undescribed organisms that would give an agglutination reaction or a complement fixation reaction could be found.

All of these diseases have been, and can again and again be produced by the proper dietary deficiency. It is most unscientific to assume additional causes (bacteria) to explain what has already received an adequate explanation. The simplest cause that will account for all the phenomena of the disease is the real cause. Bacteria can of course be found in all of these diseases, as well as in all healthy people, and the fact that we do not as yet know all of these organisms is no evidence of their pathogenicity when we encounter them in a diseased condition readily accounted for and produced and cured experimentally by another method.

The Effects of Partial Depletion. Mention has been made of the fact that certain pathological changes may be demonstrated in experimental animals suffering from certain deficiencies, long before clinical signs of the characteristic disease appear. Moreover some experiments have indicated that while a certain minimum of vitamin may ensure the animal's growth and reproduction, it does not promote maximum growth and well being which is only attained by a more bountiful supply of vitamin. Such observations have led to the consideration of the possibility that large numbers of people may be suffering from a partial deficiency and may be decidedly below par, and more subject to various infections, even though they suffer from no obvious deficiency disease. This in turn has led to the very undesirable advertising campaign to induce the entire pop-

ulation to eat yeast for that tired feeling, constipation, or what have you.

There can be no serious vitamin shortage without the production in time of the corresponding deficiency disease. On the other hand, there is indeed ground for the belief that many individuals are poorly nourished even in a country like the United States, and although they suffer from no recognizable deficiency disease. We are therefore upon safe ground in recommending that the diet should contain a profusion rather than a strict minimum of animal proteins, milk products, fresh fruits and vegetables. These are valuable additions to the diet for many other reasons in addition to their recognized vitamin content. It is quite unnecessary for anyone to eat yeast. For although yeast does contain a rich supply of the two vitamins formerly included in B, these are quite as easily supplied in a reasonable supply of meat, and a dish of peas or beans, and when this fact is recognized, the superiority of real food over yeast is sufficiently apparent.

The bearing of this fact upon your immediate dietary problem is also obvious. I have understood that beriberi is relatively infrequent in Porto Rico in spite of the fact that one of the staple foods is rice. But beans also constitute a staple food, and beans are among the most efficient beriberi-preventing foods that we possess. But no one would claim that a diet of rice and beans with occasional codfish ([24]) on which I understand many of your poor people subsist, is a sufficient or adequate diet. The desirability of improving economic conditions so that the entire population can acquire a sufficient and varied diet needs no emphasis, and although such economic improvements are notoriously difficult of attainment, I hope that the time for such improvement is not far distant. The United States should assist in every legitimate way to secure a market for your money crops, but I believe dietary conditions will be definitely improved by a more diversified agriculture.

Experimental animals. All animals do not suffer from all deficiency diseases. Neither the fowl nor the rat ever develop scurvy although another rodent, the guinea pig is the most highly susceptible animal known to this disease. Similarly, the rat is relatively insusceptible to the deprivation of the anti-neuritic vitamin and is able to grow to maturity on very minimal amounts of this substance. Birds are far more susceptible to this deficiency although they are immune to scurvy. The explanation of this anomaly is perhaps that although most animals cannot synthesize the vitamins and are de-

pendent for them upon plants, both birds and rats can synthesize the antiscorbutic vitamin.

It would seem that it would be logical to select the most susceptible animal for experimental work on any dificiency disease, and particularly for determining the relative value of protective foods. Yet this is far from an invariable rule, and the majority of the experiments that have been performed upon the so-called vitamin B have been performed on rats; and some investigators have gone so far as to claim that the rat is the most suitable animal for such experimentation and should be adopted as a standard.

The rat possesses many qualities that make it an exceptionally convenient experimental animal. They are hardy, suffer from few natural diseases, and can be kept in large numbers with a minimum of inconvenience and expenses and in a relatively small space. In addition to these merits, they are probably the most suitable animal for work on the anti-keratinic and anti-rachitic vitamins since they are very sensitive to both deficiencies. Their fatal defect as a test animal for vitamin B is their relative insusceptibility to this deficiency, or their ability to thrive on a very minute amount. Thus it has been assumed that because a small amount of milk furnishes sufficient vitamin B for the maintainance of the rat, that milk is rich in vitamin B. But when tested on susceptible birds on a rice diet, it has been found that milk has very little anti-neuritic vitamin as compared with leguminous foods, and that relatively large amounts failed to prevent the development of polyneuritis. Since milk has thus been shown to be relatively poor in anti-neuritic vitamin, and by the guinea pig test is equally poor in anti-scorbutic vitamin, and is also relatively high priced and notoriously difficult to obtain in tropical countries, it cannot be compared with the cheap and homely bean as a beriberi preventive, or the orange as an anti-scorbutic. The general importance of milk in the diet of adults has been greatly overestimated, however desirable it may be for infants. The merit of milk as a diet for growing children consists far more in its high calcium content than upon its vitamin content.

So most of the foods listed in tables with their vitamin content, which have been tested for B by the rat test, give little information as to their anti-neuritic content, and cannot be used as efficient beriberi preventives for men living on a staple rice diet, although when consumed in large bulk in a well mixed diet, they may afford protection.

Only this past year a serious proposal was made to abandon the

use of the undermilled rice which has eliminated beriberi as a diagnosis from the Filipino Scouts for the past seventeen years, substituting certain vegetables and milk which had been shown sufficient by rat test. It is needless to say that we are not willing to assume such a responsibility.

Fowls are difficult to keep and require a great deal of room, and even pigeons are troublesome as compared with rats. If a standard bird is desired for assaying the anti-neuritic content of foods, I would suggest the sparrow. They are even more sensitive than pigeons, twenty of them can be kept in a single cage on a single test, they are inexpensive for they can be caught in traps, and we are doing the community a service in eliminating them. I gave these birds a comparative test in Manila, and should I do any further work on the anti-neuritic vitamin, sparrows will be my choice.

REFERENCES

(1) Vedder: The Etiology of Scurvy. (1921) The Military Surgeon **47**: 133.

(2) Krause: The Supplementary Value of Inorganic Iron and Copper. (1929) J. Dairy Sci. **12**: 242. Also Waddell Steenbock and Hart. (1929) J. Biol. Chem. **83**: 243, and 251.

(3) Williams and Eddy: Physiological Functions of Vitamins. (1927–28) Carnegie Inst. Yearbook, **27**: 375.

(4) Sherman: The Meaning of Vitamin A. (1928) Science **21**: 619.

(5) Euler, von Euler and Helström: A-Vitaminwirkungen der Lipochrome, Biochem. Zeitschrift, (1928) 203.370. See also, Moore: A Note on Carotin and Vitamin A. (1929) The Lancet, **216**: 499.

(6) Chamberlain and Vedder: Contributions to the Etiology of Beriberi. (1911) Philippine Jour. Scs. **6**: 251; **6**: 395; and (1912) **7**: 39.

(7) Jansen and Donath: Isolation of Anti-beriberi Vitamin. Mededeelingen van den Dienst der Volksgezondheid in Ned-Indie, (1927) Part I.

(8) Windhaus and Hess: Contaminating Substances as a Factor in Activating Cholesterol by Irradiation. (1927) Proc. Soc. Exper. Biol. and Med., and Development of Marked Activity in Ergosterol Following Ultra-violet Irradiation. Ibid, (1927) **24**: 461.

(9) Blunt and Cowan: Irradiated Foods and Irradiated Ergosterol. (1929) Jour. Am. Med. Assoc. **93**: 1301.

(10) Evans and Bishop: On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction. Science, (1922) **56**: 650.

(11) Evans and Burr: The anti-sterility vitamin Fat-Soluble E. (1925) Proc. Nat. Acad. Sci., **11**: 334.

(12) Gortner: Outlines of Biochemistry. (1929) John Wiley and Sons.
(13) Vedder: Is the Neuritis-preventing Vitamin Concerned in Carbohydrate Metabolism? (1918) Jour. Hyg., 17: 1–9.
(14) Mattill: The Utilization of Carbohydrate by Rats deprived of Vitamin B. (1923) Jour. Biol. Chem., 55: 717–727.
(15) Fleming: Metabolic Mechanism in Beriberi. (1923) Philippine Jour. Sc., 23: 407.
(16) McCollum: The Newer Knowledge of Nutrition. (1923) The Macmillan Co. 2nd Ed. p. 268.
(17) Vedder and Clark: A Study of Polyneuritis Gallinarum. (1912) Philippine Jour. Sc. 7: 423.
(18) Eddy: An Improvement in the Quantitative Assay of the Anti-scurvy Vitamin. (1929) Am. Jour. Pub. Health, 19: 1309.
(19) Siler, Garrison and MacNeal: (1917) Third Report of the Robert M. Thompson Pellagra Commission.
(20) Fraser and Stanton: An Inquiry Concerning the Etiology of Beriberi. Studies from the Institute for Medical Research, Federated Malary States, No. 10.
(21) Hehir: Mesopotamia Commission Report, Appendix III, (1917) See also, Scurvy, (1919) Indian Jour. Med. Research, 6: 79 and Effects of Chronic Starvation during the Siege of Kut. (1922), Brit. Med. Jour. 1: 865.
(22) Goldberger and Wheeler: The Experimental Production of Pellagra in Human Subjects by Means of Diet. (1920) Hygienic Lab. Bul. 120.
(23) Matsumura, Kakinuma, Kawashima, Ochiai, Miyata, Fujisaki, Kanao, Noguchi, Aoki, Sato, Ito, and Suzuki: The Etiology of Beriberi. (1929) Jour. Am. Med. Assoc. 92: 1325.
(24) Cook and Rivera: Rice and Beans as an Adequate Diet. (1929) The Porto Rico Jour. Pub. Health & Trop. Med. 5: 16.