THE ETIOLOGY OF THE DEFICIENCY DISEASES.

BERI-BERI, POLYNEURITIS IN BIRDS, EPIDEMIC, DROPSY, SCURVY, EXPERIMENTAL SCURVY IN ANIMALS, INFANTILE SCURVY, SHIP BERI-BERI, PELLAGRA.

BY
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THE diseases mentioned above present certain general characters which justify their inclusion in one group, called deficiency diseases. They were considered for years either as intoxications by food or as infectious diseases, and twenty years of experimental work were necessary to show that diseases occur which are caused by a deficiency of some essential substances in the food. Although this view is not yet generally accepted, there is now sufficient evidence to convince everybody of its truth, if the trouble be taken to follow step by step the development of our knowledge on this subject. This article is written with the intention of giving a summary of the modern investigations, and by means of a careful selection of references to facilitate the research for anybody who wishes to read the original literature. This careful selection was absolutely necessary, for there is perhaps no other subject in medicine where so many contradictory and inexact statements were made, which instead of advancing the research retarded it by leadipg investigators in a wrong direction.

The deficiency diseases break out in countries where a certain unvarying diet is partaken of for long periods. When this food happens to be deficient in a substance which is necessary for the metabolism, we have the real conditions for the outbreak of this type of disease. From this point of view it is surprising to see peasants in Russia and in other countries, who live on potatoes, cabbage, and a little bacon nearly exempt from these diseases ; it will be seen later, however, that this one-sided food contains apparently all the protective bodies which are necessary.

All these diseases present some general characters, which may be sketched here. The most prominent symptoms are a general cachexia with an enormous loss of weight; marked nervous symptoms are often present, which are due probably to the

degeneration of the peripheral nervous system. It is now known that all these diseases, with the exception of pellagra, can be prevented and cured by the addition of certain preventive substances; the deficient substances, which are of the nature of organic bases, we will call " vitamines "; and we will speak of a beri-beri or scurvy vitamine, which means, a substance preventing the special disease. As regards the classification two different groups present themselves : the beri-beri group and the scurvy group. The investigations made on pellagra, however, have not yet resulted in a sufficient elucidation of its etiology to establish it as a deficiency disease and it is included here provisionally owing to its similarity in some respects to the other diseases mentioned.

THE BERI-BERI GROUP.

To this group, which is characterized by more or less distinct neuritis symptoms, belong beri-beri, polyneuritis in birds and epidemic dropsy. Beri-beri occurs in countries such as Japan, Malay States, Philippine Islands, Indo-China, &c., where rice is used as a staple diet. This diet, however, must be eaten for long periods (six to seven months) to produce the disease. The symptoms which are described in most of the textbooks of tropical diseases (1) present several distinct types, which can be shortly summarized as follows. In most cases the patients lose enormously iu weight, and very often suffer from oedema, contractions, paralysis and anaesthesia in the limbs. Pathological changes have been found such as degeneration in the nerves and heart. The disease, in most of the acute cases, terminates fatally. It has often been stated that sometimes the patients recover without any treatment. It is most probable in this case that the recovery is associated with some very important changes in diet. The investigators who have suggested a causal connection between beri-beri and an exclusive diet of rice are Wernich [2] and van Leenfc [3]. Acting on these ideas Takaki [4] was able by a change of diet, (addition of meat) practically to eradicate this disease from the Japanese navy, Eykman [5] and his collaborator Vordermann [6] (especially the latter) came to the conclusion, based on an investigation in 101 jails in Java made in 1895-1896, that the disease has a distinct relationship to the continuous consumption of decorticated (polished) rice. This statement was confirmed on a very large scale by Braddon [7], who found that natives (such as Indians, Tarniels)

who use parboiled (cured) rice, which has been previously steamed to remove the pericarp, were not in danger of contracting the disease. Finally Fraser and Stanton [8] confirmed the results of the previous workers especially [as to the harmlessness of parboiled rice.

The next step in Eykman's [9] investigation, established the important fact that birds (fowls, pigeons, ducks) when fed on polished rice developed a disease which he called polyneuritis gallinarum. The birds lose considerably in weight, and after three to four weeks, contract a disease which is very similar to human beri-beri. He also found that it was not possible to induce the disease with rice containing the pericarp or that part of the pericarp which is called the silver-skin by the Dutch authors. This most valuable observation facilitated enormously the further experimental research, and really started the experimental investigation. Eykman, in endeavouring to explain the origin of the disease, arrived at the conclusion that food rich in starch, like rice or starch itself, produced a substance in the intestine (pp. 526 and 527), which acted as a poison on the nerve-cells. He was, further, able to show that the silver-skin is richer in nitrogen than any other part of rice, and contains an antidote for the starch poison. He also stated [10] that the aqueous extract from rice-polishings (silver-skin) cures after the elimination of phytin, and that the protective substance dialyses and is not precipitated on alcohol. Gryns [11] confirmed Eykman's results, and to him belongs the credit of being the first worker to adopt the deficiency theory for the explanation of the etiology of this disease. He says clearly that the disease breaks out when a substance necessary for the metabolism of the peripheral nervous system is lacking in the food. He has discovered similar protective substances, in katjang-idjoe beans *(Phaseolus radiatus)* and meat, and showed that these foodstuffs lose their protective power when heated to 120° C. These heating experiments which were of great importance for the farther knowledge of deficiency diseases were confirmed by Eykman [10]. Breaudat [12] used rice-polishings as a remedy in cases of human beri-beri and obtained very good results. Fraser and Stanton [13] tried to characterize more closely the protective substance, and have found that it is soluble in strong alcohol and that this solution is effective after the elimination of alcohol-soluble proteins. From an analysis of different rices which induced beri-beri, they came to the

conclusion that a valuable practical indication as to whether the rice is harmful or harmless is its content of phosphorus. They found that the less the phosphorus content, the more liable it is to cause disease. On this basis Schaumann [14] constructed a theory, the phosphorus-deficiency theory, which he extended to other deficiency diseases such as scurvy and ship beri-beri, and which suggests that these diseases are due to a deficiency of certain organic phosphorus compounds in the food. With slight modifications he still holds this view.

During the last two years a quick succession of papers appeared which deal with the isolation of the protective substance from the different foodstuffs. Hulshoff Pol [15] found that an aqueous extract of Katjang-idjoe beans still retained its curative properties after the precipitate, obtained with lead acetate, was removed. Prom this filtrate he obtained a crystalline substance, called by him X-acid; he did not, however, show that this substance was actually capable of curing the disease. Schaumann [16], who added yeast to the series of curative substances, tried the different known constituents of yeast, such as yeast nucleic acid and lecithin, without, however, obtaining satisfactory results. Lately it was shown by Eykman [17] that the protective substance in yeast is soluble in 88 per cent, alcohol. Before this paper appeared the writer[1] tried to extract yeast completely with alcohol. The experiments failed, however, only a small part of this substance being extracted by this process, and even after repeated boiling for several hours with alcohol the extracted yeast still retained its curative properties. This shows that the bulk of the substance is present in the yeast in a combined state. Teruuchi [18] extracted rice-polishings with weak hydrochloric acid, and, after neutralizing to remove phytin, the solution was evaporated and extracted with alcohol. The solution obtained in this manner was active, although it contained only a small part of the total phosphorus content. Similar results were obtained by Chamberlain and Vedder [19], and Shiga and Kusama [20]. The latter have found that the protective substance of the rice-polishings is destroyed at 130° C. in 0.5 per cent, hydrochloric acid, or in 1 per cent, sodium carbonate solution, but not at 100° C. The rice-polishings being particularly rich in phytin, Aron and Hocson [21] performed some experiments

[1] Notes not yet published.

with this substance, and claim to have obtained satisfactory results. Their statements, however, are in discordance with those of Eykman (*loc. cit.* [11]), and could not be confirmed by Schaumann (*loc. cit.* [16]), and Cooper and Funk [22]. Kilbourne [23] suggested that the deficient substances might be salts, especially potassium salts, without, however, giving any experimental support to his theory.

In spite of all this experimental evidence which classifies beriberi beyond any doubt as a deficiency disease, Kohlbrugge [24] lately isolated a micro-organism from rice, which he calls *B. oryzæ*. The culture, when injected into fowls, was capable of producing polyneuritis in five days. The weak point of his theory is his explanation of the efficiency of rice polishings in preventing the disease. He states that the *B. oryzæ* produces a special kind of fermentation, which is stopped by the acid formed from rice-polishings. The epidemical occurrence of beri-beri suggested to many authors that infection is the real cause. It has been stated that by transferring patients from one pavilion to another they suddenly recovered. A careful inquiry showed, however, that this transfer was associated either with a change in diet or with a change in the cooking apparatus.

Summarizing our knowledge till 1911 of the chemical nature of the protective substance from the rice-polishings, we find the following well-established facts :—

(1) The substance is soluble in water, in alcohol, and in acidu lated alcohol.

(2) The substance is dialysable.

(3) It is destroyed by heating to 130° C.

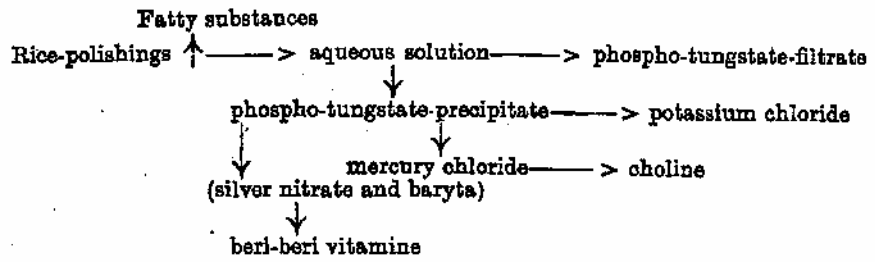
(4) Is neither a salt nor a protein.

We see, therefore, that our knowledge of the chemical nature of the substance was very small. This was the state of the subject when I began my experiments in the middle of 1911, first in collaboration with Cooper (*loc. cit.* [22]), and afterwards by myself. The first problem which had to be settled was to ascertain to what class of chemical bodies this peculiar substance belonged, and whether it was a complicated compound or one of a comparatively simple structure. Is it easily destroyed by chemical manipulations ? This last condition was a *sine qua non* for further chemical investigation.

By a series of experiments with yeast it was possible to show that dried, pressed yeast hydrolyzed for twenty-four hours with

20 per cent, sulphuric acid still retained its curative properties. Based on this remarkable stability in acid solution I came to the conclusion that the curative substance might be one of the nitrogenous substances, which are precipitated by phospho-tungstic acid, and that it is of a simple chemical nature. A systematic investigation of rice-polishings was tben started on these lines (*loc. cit.* [25]), and very satisfactory results were obtained. The different fractions separated during this investigation were tested on pigeons which were suffering from polyneuritis induced by a diet of polished rice. These pigeons presented very marked symptoms consisting of cachexia, weakness of the wings and legs, and a peculiar spastic paralysis of the neck, which in many cases caused the head to be bent back towards the tail of the animal. Left to themselves pigeons in this condition died as a rule in less than twelve hours. The various fractions obtained during the numerous manipulations were introduced by means of a syringe into the crop. The method used is shortly summarized as follows: The polishings were extracted on a shaking machine for several hours, with absolute alcohol partially saturated with gaseous hydrochloric acid. After removal of the alcohol *in vacuo* a fatty residue remained, which was melted and extracted with water on the water-bath; the aqueous fraction was then separated off in a separating funnel in the hot chamber at 37° C. This aqueous solution, which was capable of curing pigeons, was precipitated with phospho-tungstic acid. The separated phospho-tungstate precipitate was decomposed with baryta, and the solution, after removing the excess of baryta, tested on pigeons. Here the first experimental difficulty occurred. It was at first impossible to ascertain the quantity necessary for curing pigeons. In my first experiments too large a dose was administered, and the pigeons died in a few minutes with marked toxic symptoms. We will see later that this was due to the presence of choline. This difficulty can be avoided by calculating the dose on the quantity of polishings used. We know that 20 grm. of rice-polishings or the aqueous extract of this contains sufficient substance to cure pigeons. All the later fractions were administered in doses corresponding to -the amount of original polishings necessary to cure, a small allowance being made for unavoidable losses due to the necessary manipulation. The above-mentioned solution was totally free from phosphorus, proteins and carbohydrates. This fact simplified the chemical task considerably,

and indicated at the same time that the essential substance was of basic nature. This fact spoke against Schaumann's phosphorus deficiency theory, for it was clearly established for the first time that cures could be effected by solutions which contained no phosphorus. Since the filtrate from the phosphotungstic acid precipitate was shown to be entirely devoid of action, the whole of the curative substance must have been in the precipitate. This precipitate appeared to be very simple from the chemical standpoint, as only potassium and choline could be detected. By precipitation with gold- and platinic-chloride no other basic substances could be detected, and experiments showed that choline was devoid of action. After removing all the choline by means of platinic chloride in alcoholic solution, the filtrate still retained its curative properties. The presence of another basic substance was therefore inferred, and various reagents were used to precipitate the latter. After many trials it was found that the substance could be precipitated by means of silver nitrate and baryta. The silver salt obtained proved to be curative, and by decomposing a crystalline substance was obtained, which melted at 233° C. The analysis of this compound led to the formula $C_{17}H_{20}N_20_7$. The unsaturated nature of this compound would explain very well its physiological instability in heating experiments. So far the few experiments performed have shown that this substance seems to be the curative agent, and for purpose of simplicity I would propose to call it provisionally beri-beri vitamine. The method of fractionation is indicated in the following table:—



The yield of vitamine was extremely small. From 1 kg. polishings only ½ grm. of crystalline substance was obtained. This is the chief experimental difficulty in the research. It is hoped, however, that an extraction on a very large scale will give the opportunity of investigating this most interesting substance more closely.

The dose of the crystalline vitamine necessary for curing pigeons was very small. Such a minute dose as 40 mg. of the substance was not only sufficient to cure a pigeon in a very short time—often in three hours—but also maintained the cured animal in health for periods varying from seven to twelve days, when polished rice was used as food. After this time symptoms of the disease again manifested themselves.

Schaumann [26] expressed the idea that the curative agent acts only as an activator, and that besides this a substance of a phosphatide nature is necessary. He thinks that these minute quantities cannot be considered as food, but can only act as hormones. This can be settled by direct investigation. It is necessary to perform experiments on pigeons fed on polished rice with the addition of vitamine. These experiments will be performed when a sufficient quantity of pure substance is obtained. With the discovery of this substance it was possible to try to explain the action of these protective substances. It is surprising how rapidly the nervous symptoms disappear after the administration of vitamine. This naturally suggested that this substance is wanted for the metabolism of the nervous tissues and has some connection with cell lipoids. It was therefore thought useful [27] to compare the contents of nitrogen and phosphorus in the brains of pigeons fed on polished rice with that of normal pigeons. This investigation [28] showed that the brain of the polyneuritis pigeons was sensibly poorer in nitrogen and phosphorus compared with the normal ones, a fact which suggests a degeneration of the brain lipoids. Palladino [29] has shown recently that the same happens in the brain of starved dogs. It is interesting to mention here that Chamberlain, Bloombergh, and Kilbourne [30] have sometimes observed marked neuritic symptoms in starvation in birds. It is not surprising that in starvation the same deficiency occurs, but it is probable that very often the general weakness develops before the neuritic symptoms have time to appear. As a matter of fact, by using the voluntary feeding method instead of forced feeding I often noticed that the pigeons refuse to eat anything in the last period and soon show characteristic neuritic symptoms. Coming back to the chemistry of brain, I was surprised at the small normal nitrogen content of pigeons' brains. The relationship between the nitrogen content of the administered dose and the total nitrogen of the brain, or even of the total lipoid

fraction of the body, is so small that we might eventually consider the vitamine as a specific food supply for this special kind of tissue.

In search of foodstuffs which would give a better yield of vitamine other substances were investigated, and bodies capable of curing beri-beri could be detected in milk, yeast, ox-brain and lime-juice (see under Scurvy). The method[1] used here was analogous to that used in the case of rice-polishings. The cure with fractions obtained from ox-brain was so unexpectedly quick that it seems possible that the substance contained in brain is ready for use. It is obvious from the statements of previous workers that similar substances are present in vegetables, cereals and meat. It is interesting to note that the vitamine can be extracted from all these substances by means of alcohol. However, in the cases investigated by me the vitamine could be extracted from the alcoholic- residue with water.

Another problem as to the form in which the vitamine is present in the food presents itself here. Is it in the form of a lipoid and split off by simple contact with alcohol, or is it only absorbed to the lipoid fraction? This interesting question requires further consideration. Even if the vitamine is combined in a lipoid molecule, it is not necessary to accept Schaurnann's view. As long as the organism is in a healthy state it does not matter, according to the usually accepted conception of the breaking down of food into simpler products in the intestine, whether it gets a supply of a lipoid or of vitamine itself.[2] As the phosphoric acid is present in polished rice in large quantities and fatty acids can be eventually built from carbohydrates, the organism could easily synthetize its own lipoid. If the lack of vitamine occurs and the body is not able to synthetize this, and must get it ready from plants, then deficient metabolism ensues and death results. It ,was stated by Schaumann *(loc. cit.* [26]) that yeast cures better than anything else tried up to now. I suppose that in a state of general cachexia the synthetical power declines. Here the daily-supply of vitamine to the deficient food will elucidate this problem entirely.

The author's view on the cause of beri-beri may be summarized

[1] Not yet published.

[2] Compare here the paper by Fingerling [31] who finds that the organism is even able to use inorganic phosphates for the synthesis of lipoids.

as follows (the physiological importance of the vitamine will be discussed in the last chapter). Judging from the manifest nervous symptoms, the fatty degeneration in the nerve-cells, and the above-mentioned chemical changes, I am inclined to think that vitamine is necessary for the metabolism of the nervous tissue. The lack of vitamine in the food forces the animal to get this substance from its own tissues. The result is an enormous loss of weight. After this available stock begins to be scarce there is a consequent breaking down of the nervous tissue, with the result that nervous symptoms, such as are observed in beri-beri, manifest themselves.

In the beri-beri group of deficiency diseases we may include epidemic dropsy, which is called by the French authors the wet form of beri-beri. This disease occurs in India, and Grieg's [32] investigation suggests that it is due to the same cause as beri-beri—namely, to an exclusive diet of polished rice.

SCURVY GROUP.

(*Scurvy, Experimental Scurvy in Animals, Ship Beri-beri, Infantile Scurvy.*)

Scurvy.—As characteristic clinical symptoms of scurvy we find very often anaemia and general cachexia, a marked tendency for local haemorrhages and haemorrhagic inflammation, especially in the gums. In adults often hemorrhages in the periosteum and in the frontal endings of the ribs, with changes in the marrow of the bones, followed by a dislocation of the ribs from the cartilage. Lately cases with neuritis and anaesthesia of the limbs were observed by Holst [33].

It is now generally accepted that scurvy is caused by the food used. A diet consisting chiefly of starch, bread and tinned preserved food is especially liable to produce the disease. The remedy for this disease, as found by saifors in an empirical way, consists in good dietetical conditions, lime-juice, fresh fruits and vegetables, especially onions. Scurvy patients recover very quickly when these articles are available.

Just as in the investigation of beri-beri, the discovery of experimental scurvy by Holst [34] and his collaborator Frölich was of great importance. They found that many animals (guinea-pigs, rabbits, pigs, and dogs) kept on scurvy-producing diet, or on food known by experience to be capable of producing scurvy, or on

food autoelaved at high temperature, develop a disease which is closely analogous to human scurvy. These authors have observed that guinea-pigs fed on water and rye, or wheat bread (or oats, rye, wbeat; barley and rice-flour), or on barley (peeled grain), from oats, barley or rice, die after several weeks of this diet. If death occurs after three weeks the following changes were found constantly : The side teeth were loose, with hsemorrhages in the gums ; sometimes only a blue hyperaemia was observed, and in few cases ulceration ; haemorrhages were found constantly in the periosteum of the ribs.

For the investigation of scurvy Holst particularly recommends guinea-pigs, fed on oats and water. For such experiments only grown-up animals weighing over 350 grin, should be used. The animals should be weighed every few days, as they lose considerably in weight (about 40 per cent.). Similar experiments were made by Schaumann (*loc. cit.* [16]), who experimented especially on dogs and monkeys, by feeding them on food taken from sailing ships, where ship beri-beri broke out, or with food extracted previously with sodium carbonate. We will see further on the great similarity of the latter with scurvy.

The great experimental difficulty here was, that the scurvy protecting substance, or as we call it scurvy vitamine, is much less stable than the corresponding beri-beri vitamine (Holst) [35], As protective food in the experiments of Holst and Frölich fresh potatoes, apples, lime-juice, carrots, cabbage and leaves of lions-tooth (*Leontodon taraxacum*) were used. The addition of this food even protected guinea-pigs which had been fed already for three weeks on oats. However, only fresh food possesses the protective power ; dried material is completely valueless. This fact explains, largely, why food kept for a long time on ships produces scurvy. A guinea-pig fed on fresh potatoes dies after several months without scurvy, whereas one fed on boiled and dried potatoes dies after a few weeks with characteristic scurvy symptoms. No difference was observed if the drying process was performed in vacuo or in air. The same thing applies to carrots. Cabbage heated to 110° C. loses completely its protective power, and the juice obtained by pressing the cabbage was inactivated even at 60° or 70° C. The same occurs if the juice is kept for a couple of months, even with the addition of antiseptics.

Different in this respect is lime-juice. Although comparatively

poorer in vitamine, the latter seems to be much more stable; it is not destroyed even after boiling for one hour. A set of interesting experiments were described by Fürst [36] (from Holst's laboratory). He tried the effect of food, yielding an alkaline ash (it was often stated that scurvy is a result of a special kind of acidosis). This food failed to prevent scurvy, so did the addition of yeast. The last fact is remarkable, as it is known that yeast prevents beri-beri very well. This fact will be discussed in the following chapter. Fürst further describes some experiments with seeds. Grains which are known to prevent beri-beri such as oats, develop an antiscorbutic agent after they have been germinated. They lose this power when they are dried again, and in the presence of moisture develop the scurvy vitamine. In the process of germination seeds are known to develop all kinds of enzymes in order to utilize their food reserves. This fact suggested to Fürst that this substance might be produced by the action of enzymes, and he suggested that on board ship a store of dried grain should be kept, which after germination should be added to the diet on long voyages, when no fresh provisions could be provided. It is surprising that a chemical change, which can be performed by the enzymes of the seed, cannot be reproduced by the animal body, which seems not to be adapted for this kind of reaction. From this short *résumé* we see that the material which promises the best results in the way of chemical investigation with regard to vitamines is. lime-juice. The investigation of vegetables, owing to the feeble stability of their vitamine, do not promise good results. Some other points also suggested to me to work with lime-juice. It possesses the advantages of being easily obtained and contains substances in a soluble state, which makes the experimental work comparatively easy. Although the original commercial lime-juice does not cure polyneuritis pigeons (as was also stated by Chamberlain and Vedder, *loc. cit.* [19]) I was encouraged to persevere with it by its fairly high nitrogen content, which amounts to 0.35 per cent. This suggested to me that a fractionation, analogous to that done with rice-polishings, might give interesting results. The investigation is not finished yet and vill be repeated again on a large scale. The first fractionation was done on 50 litres and the results were very promising. Here, as in rice-polishings, the same difficulty occurs—the minute quantities of the vitamine present. The lime-juice was cleared by precipitation with lead acetate, and the filtrate, evaporated *in vacuo,* was precipitated

with phospho-tungstic acid. Although the original lime-juice was unable to cure polyneuritis pigeons, this was done by the decomposed phosphotungstate precipitate. However, the effect in some cases was not as good as with the similar fractions from rice-polishings; apparently here the question of dose enters. By means of silver nitrate and baryta two fractions could be obtained from this precipitate. The experimental work, which has to be repeated again, gave the following results : The first fraction, which could not be obtained crystalline, was effective in curing polyneuritis pigeons. I was surprised to see that the same fraction, after my return from holidays, lost its curative properties after six weeks. The second fraction yielded 0.4 grm. of a crystalline substance, which, administered to polyneuritis pigeons, kept them alive for seventy-two hours without, however, improving their condition. This minute quantity of substance was used up in two experiments on guinea-pigs fed on oats; into each guinea-pig a solution of this substance was injected subcutaneously every second day in the period, when they began to lose weight. No definite results have been obtained up till now. From these experiments I am inclined to think that scurvy vitamine is different from the beri-beri vitamine, although 'chemically belonging to the same class of substances.

Ship Beri-beri. — This disease, which somewhat resembles scurvy, was carefully investigated by Nocht [37], and takes an intermediate place between scurvy and beri-beri. As described by Nocht, this disease breaks out on board sailing ships on long voyages when fresh provisions begin to be scarce. Characteristic symptoms are ansesthesia, short breathing, and in fact death from heart weakness. After a supply of fresh food the patient recovers very quickly, in contrast to tropical beri-beri. Neuritis symptoms were described only in few cases. Holst and Fröhlich [34] consider this disease to be very closely related to scurvy, and it would appear that all that we know concerning the etiology of scurvy applies equally well to ship beri-beri.

Infantile Scurvy.—Infantile scurvy breaks out in children fed on, sterilized milk, with or without the addition of different flour preparations. For the first exact description of this disease we are jndebted to Sir Thomas Barlow [38]. The pathological changes were especially investigated by Fränkel [39], who describes changes in the bone-marrow, which loses and becomes pour in cells, as a result of the defective new formation of the bone;

further, he describes a separation of the epiphysis and alterations in the intermediate cartilage and the cartilages of the ribs. In children in which the teeth were present haemorrhages and affections of the gums were observed. This description indicates the similarity of these symptoms with those present in human scurvy cases, and especially with those described in the experimental scurvy of the guinea-pig. This close similarity of symptoms led Sir Thomas Barlow, Axel Holst [35] and Looser [40] to conclude that infantile scurvy and scurvy are identical. Recently Vortisch van Vloten [41] published a paper, in which he says that both diseases are identical and caused by a deficiency of nutritive salts, especially potassium and iron.

Clinical interest in this disease was considerably increased by the interesting communication of Neumann [42], who first showed that infantile scurvy had some connection with boiled milk. He found in a large number of cases that children fed on milk, previously heated in Soxhlet apparatus for ten to fifteen minutes, got infantile scurvy. His explanation was that during the boiling process toxic substances were formed in the milk. Heubner [43] was able to confirm these results. Corroboration of these statements has been furnished by data collected by A. Meyer [44]. Cases of infantile scurvy due to boiled milk were observed recently in this country by Brachi and Carr [45]. The patients can be cured either by an addition to the food of unboiled milk or of lime or fruit juices. As Holst and Fröhlich have found, these contain the antiscorbutic substance.

Is there any evidence that milk contains a substance which is destroyed by boiling? This question must be answered in the affirmative. Bordas and Raczkowski [46] have found that lecithin is partially destroyed during the boiling of milk, even at 60° C. a fair amount disappears, at 95° C, 28 per cent., and after heating for half an hour at 105° to 110° C. as much as 30 per cent, is destroyed.

Bartenstein [47] observed that guinea-pigs fed on boiled milk contract a disease which he considers to be identical with infantile scurvy. Fröhlich [48], who repeated Bartenstein's experiments, does not quite agree with the latter ; he states that guinea-pigs fed even on raw milk show, with the exception of slight tendency to a porosity of bones, a normal aspect, whereas those fed on milk previously heated for ten minutes at 100° C., thirty minutes at 100° C., and one hour at 112° C., show a high fragility of the bones,

which is a characteristic of scurvy, but without haemorrhages and changes in the ossifications.

Besides that, Frohlich (*loc. cit.*) [48] performed a series of experiments on the protective power of milk on guinea-pigs fed on oats. He found that pasteurized milk (heated at 70° C.) prevents scurvy, and milk heated for ten minutes at 98° C. loses its protective power completely.

Stepp [49] found that mice fed on food which was extracted with ether died, while the addition of the extract kept the animals in good health for a long time. Milk was shown to contain the necessary substance in small quantities and was inactivated by boiling. The animals could be kept also by an addition of ox-brain lipoid.

Some preliminary experiments done by the author[1] demonstrated the presence of the beri-beri vitamine in milk, its commercial preparation known as " Trumilk" was used for this purpose and extracted with alcohol and ether. After hydrolysis of the lipoid the vitamine was separated by means of phosphotungstic acid and silver nitrate and baryta. A small quantity of a nitrate of a base was thus obtained which was capable of curing polyneuritis pigeons. The scurvy vitamine could not be detected. The probable cause of this was that during the preparation of "Trumilk" the milk was exposed to a high temperature whilst being concentrated *in vacuo*.

This vast amount of evidence suggests that the real physiological difference between the raw and boiled milk is not in the destruction of enzymes, antibodies and changes in proteins, but in the content of vitamine. The scurvy vitamine in the milk seems to be a fairly stable one. It is not destroyed like that in cabbage at 60° to 70° C.; but resembles more that of lime-juice. The scurvy vitamine, as we know from other foodstuffs, is certainly destroyed at 120° C., but even at lower temperature the quantity of vitamine destroyed depends on two factors—the temperature used and the time of heating. Even in this case probably only a fraction of the vitamine is destroyed. The reaction of the milk, the natural content of the vitamines in cows' milk, which of course depepds entirely on the content of vitamines in ihe cows' food, are further factors of which we do not know the importance.

[1] Not yet published.

Recently Miss Lane-Claypone, in a report to the Local Government Board,[1] came to the conclusion, on the basis of the clinical evidence, that there is no real evidence to support the view that infantile scurvy is due to boiled milk. The reasons mentioned above—the influence of the time of heating and the vitamine content of the original milk—render the clinical observations of relative value only. In addition to this it is often impossible to perform exact experiments in the hospital, and therefore besides clinical evidence the results of experimental research on animals ought to be collected.

At present our knowledge does not go beyond the fact that milk is a food which provides all the constituents necessary for the growing organism, and must, of course, contain all kinds of vitamines. All investigations bear out this view. On the other hand, we know that these substances are very unstable and destroyed by heat. As a result of this I would suggest that it is safer not to heat the milk more than is strictly necessary.

THE RELATIONSHIP BETWEEN BERI-BERI AND SCURVY. Between these two diseases a close analogy undoubtedly exists. In the siege of Paris, cases of scurvy were described by Delpech [59], which arose from an exclusive diet of white rice. Similar observations were recorded by Bucquoy [50]. Experiments on animals show that rice produces in some animals (pigeons, fowls, ducks, &c.) polyneuritis, in others, such as guinea-pigs and dogs, scurvy. For instance, pigs under these conditions show symptoms of neuritis and scurvy (Holst, *loc. cit.* [33]). On the other hand, Holst describes cases of scurvy with marked neuritis. In spite of all this evidence both diseases have to be considered as entirely different. The common cause in both is a deficiency in diet, but a deficiency of two different substances. Many facts are known which speak in favour of this idea. We saw that the beri-beri vitamine is a much more stable substance than the scurvy vitamine. Different foodstuffs, which are known to prevent beri-beri and polyneuritis, like yeast, oats and barley (unhusked), are unable to prevent scurvy. On the other hand, grains develop the scurvy vitamine during germination. This fact suggests that the scurvy vitamine can be formed by enzyme action from the beri-beri vitamine, which is undoubtedly present in these seeds. This would

[1] Beports to the Local Government Board, New Series, No. 63,1912.

suggest a certain connection between the two substances. In favour of this view many facts are on record, but the problem can be easily solved by experiment. It is necessary to investigate whether this process goes hand in hand with the diminution of the beri-beri vitamine in the seeds. I intend to perform such experiments shortly. The complete investigation of lime-juice, in which both substances occur, may throw some light on this interesting problem. The nearer the chemical constitution of both substances the closer will probably be their physiological connection.

From the experiments on germinated seed, performed by Fürst (*loc. cit.* [36]) we saw that the animal organism is not able to perform the slight change which is brought about by enzyme action in the grain. From this it is obvious that the anti-scurvy substance must be supplied as such by plants. The outbreak of scurvy as a result of a diet which is known to contain the beri-beri vitamine suggests that the organism is not able to do this kind of transformation. But the opposite reaction (the formation of beri-beri vitamine from the scurvy vitamine) is possible, and there are no facts known which disprove this suggestion. In this case we would obtain a cycle, which may be sketched here :—

Beri-beri vitamine --------> plant enzymes -- ------> scurvy vitamine.
Scurvy vifcamine ------- > animal organism -- -----> beri-beri vitamine.

By this means the scurvy vitamine would prevent both diseases, whereas the beri-beri vitamine only beri-beri. As a matter of fact, this suggestion explains very well indeed the occurrence of ship beri-beri. When both substances are deficient, then the disease which we know under the name of tropical beri-beri breaks out. On ships the food gets poorer in scurvy vitamine quicker than in beri-beri vitamine. The result of this is an outbreak of scurvy or ship beri-beri. This, however, is only an attempt to explain ihe close relationship between these two disease.

In conclusion, we might say that beri-beri and scurvy are both caused by a deficiency of diet. The deficient substances, though different, are closely related, and transformable to a certain extent into each other. The influence of this deficiency on the general metabolism will be dealt with in the last chapter.

PELLAGRA.

The investigation of this disease moves now essentially on TDacteriological lines. From the history of beri-beri and scurvy

investigation we saw, however, that many years of careful study were necessary to establish the true etiology of this disease. The long struggle between the believers in the theories of infection and intoxication is worth mentioning, and still persists in spite of modern results. The idea that pellagra is due to some deficiency in the diet was expressed by several authors, but at present there is no positive evidence in favour of this theory, as against any other theory. A glance at all the existing theories suggests that an investigation of this disease on the lines mentioned above for beri-beri and scurvy might yield valuable results. Up to now the only evidence which speaks in favour of this view is its close analogy with beri-beri, and especially with scurvy. Research on this subject, which in the past has been very one-sided, is rendered more difficult by the impossibility of producing experimental pellagra in animals, and also by the lack of knowledge on the prevention of this disease by means of a change of diet.

Pellagra, which was known in Italy in the eighteenth century under the name of scurvy or Italian scurvy, occurs in Italy, Koumania, Austria, Spain, Portugal, Egypt, Algeria, United States, Mexico and Central America, and is strictly limited to districts where maize is used as the staple diet. This fact, along with the observation that the disease breaks out in countries where maize was recently imported, speaks clearly in favour of some relationship between pellagra and maize. This disease, which breaks out mostly in spring and autumn, shows nervous and psychic symptoms, and leads often to general cachexia, diarrhoea, and suicide. A very characteristic symptom is an erythema of the skin, which is caused by the sun and is localized in uncovered parts of the body, such as the hands, face, and bare feet.

In addition to such views as those expressed by Hodson [52], that pellagra is not a definite disease, there exist five distinct theories—namely, the intoxication, auto-intoxication, infection, photodynamic, and deficiency theories.

The *intoxication theory,* which expresses the view adopted in Italy for many years, was first suggested by Ceni [53] and Otto [54]. They observed that certain *Aspergillus* and *Penicillium* strains, which are constantly found in spoiled maize, secrete toxic substances, which, when isolated and administered to animals, provoked intoxication symptoms closely resembling those of pellagra. This theory was afterwards extended and energetically

advocated by Lombroso [55], followed by Gosio [56] and Gavina [57]. Lombroso especially performed a great number of experiments, and was able to isolate an alkaloid from these fungi which possessed a marked toxic effect resembling ergot poisoning. He considered the disease as a chronic toxaemia, due to toxic substances developed in maize by the action of micro-organisms, especially fungi. This theory is still accepted with slight modifications in Italy (Bertarelli) [58] and Antonini [59] and was recently expressed again by Camurri [60]. The latter accepts the view that besides an insufficiency of maize diet and lack of nutritive salts an intoxication takes place. He especially points out that ferments of maize, which he thinks capable of forming toxic substances, are able to act on maize, even after cooking. Acting on these views the Italian Government took measures to endeavour to eliminate spoiled maize from the market and to provide the population with drying apparatus: special hospitals for pellagra cases have also been established. All these measures seem to have very little effect, and the number of patients is still increasing.

The Auto-intoxication Theory.—Originated by v. Neusser [61] and de Giaxa [62], who stated that pellagra is due to toxic products formed in the intestine under the influence of certain bacteria, especially of *B. coli.*

The Infectious Theory.—An enormous amount of different micro-organisms have been credited with the power of causing pellagra, but only a few, which seem to have some claims based on a large amount of evidence, will be described here. Di Pietro [63] found a particular strain of *Penicillium glaucum,* to which he attributes the power of producing the disease. Ceni [64] isolated from the organs of pellagra patients *Aspergillus fiuorescens* and *A. fumigatus.* Tizzoni [65] and Tizzoni and Panichi [66] describe a microbe, isolated from the organs and faeces of pellagrins, which they call *Streptobacillus pellagræ*. The same germ could be detected in spoiled maize. The culture of this microbe inoculated into guinea-pigs was, however, unable to produce the disease, unless the animals were put, on a maize diet. Sambon [67], after a careful inquiry in Italy, came to the conclusion that pellagra is not necessarily associated with maize diet, and its topographical distribution (near to running streams) suggested to him that the disease may be caused by a protozoan transmitted by a biting fly from the genus *Simulium.* He was unable however to find the micro-organism.

Similar association with a Simulium fly was suggested in Georgia by Roberts [68].

Recently a very peculiar micro-organism was isolated from the cerebro-spinal fluid of the patients and described by the British Pellagra Commission [69], but has not been further investigated. Certain curative results obtained many years ago with arsenic, and recently with atoxyl, related by Babes and Vasiliu [70], and Babès, Vasiliu and Gheorghus [71] suggested a protozoological nature of the germ: good results with salvarsan were also recently related. The number of cured cases was relatively small and might be due to a change of diet.

Babès and Busila [72] were, however, unable to find a specific connection between the serum of the pellagrins and the micro-organisms isolated from the organs and faeces of the patients. The same negative results were obtained with extracts prepared from the" micro-organisms of pellagrins or of maize. Tizzoni [73] recently, in a preliminary communication, describes a specific precipitation obtained by *Streptobacillus pellagra* in the blood of pellagrins. Finally, Alessandrini [74], who observed a connection between pellagra and running water, in which larvae of nematodes were found, accepts the latter as the cause of the disease.

Nevius-Hyde [75] criticizes all the evidence brought forward and states that the disease is produced by all kinds of spoiled flour. In a recent American report on pellagra by Clarke, Hamill, Pollock, Curtis and Dick [76], the authors come to the conclusion that there is actually no basis for the infection theory. They were unable to infect monkeys with the blood of pellagrins. Further criticisms of these theories will be found in the paper of Raubitschek.

Photodynamic Theory.—Raubitschek [77], in his very interesting and critical paper, begins his research by investigating experimentally all the known theories, and comes to the conclusion that all these theories do not stand a careful experimental test. He found that polenta (a kind of maize-cake like that eaten in Italy) was almost sterile, and as a rule also the blood of pellagrins. The bacteriological examination, *post mortem*, gave no evidence whatever in favour of an infective etiology of pellagra, and the serodiagnostic examination of the blood did not show the presence of antibodies against maize proteins or germs contained in maize. Opposed to the view of many Italian authors, Raubitschek found

that extract of spoiled maize inoculated into animals gave no trace of symptoms which could be considered analogous to those in pellagra.

Based on the experiments of Aschoff [78], who admits the existence of sensitizing substances against light in the lipoids, he makes the hypothesis that spoiled maize produces a toxic substance which is able to sensitize the skin for sun rays. Analogous experiments were already performed by Hausmann [79]. Raubitschek's experiments were carried out in the following manner: White mice fed on maize and exposed to daylight developed, alter four weeks, a disease, with very marked loss of weight. After six to eight weeks the animals died, often with cramps and hyperaernia in the ears and mouth. White mice kept as controls in the dark or in light on a mixed diet, and dark-furred mice kept on maize in the light, did not show any trace of reaction. This disease is somewhat analogous to fagopyrismus, a disease which breaks out in white cattle fed on buckwheat. Eaubitschek found that the sensitizing substance is present in the alcohol-soluble fraction of the maize. Maize extracted previously with alcohol was proved, then, to be harmless to white mice exposed to light, whereas the extract, contained the toxic substance. The same results were obtained with spoiled maize (polenta) and rice. From the last fact Raubitschek concludes that beri-beri has a photo-dynamic origin. Similar observations were related independently by Horbaczewski [80] and Lode [81]. The results of these authors agree with those of Raubitschek. On the other hand, several objections were already made to this theory. Lavinder [82] was not able to confirm Raubitschek's results; Babès [83] does not believe that good maize is able to produce pellagra. Hausmann [84] points out that in time of intense sunlight the skin affection is less pronounced than in spring.

Raubitschek's experiments seem to apply only to albino mice, and do not show any analogies with the known experiments on the importance of lipoids or alcohol-soluble fraction for the life of animals (Stepp's experiments on white mice), in beri-beri and polyneuritis. Albino rats put on rice diet in daylight do not show any symptoms of disease for ten weeks.

The Deficiency Theory of Pellagra.—This short *résumé* shows very well that the present state of the pellagra investigation is the same as that of beri-beri ten years ago. One might be surprised

that the recent advances in our knowledge of beri-beri did not influence more the investigation of pellagra. It is beyond any doubt that pellagra has some close connection with maize diet. Even if cases of pellagra are known which have no relationship with maize, this would not speak against the deficiency theory, as long as the food used is also deficient. Unfortunately, I could not find many data about the diet in districts where pellagra occurs. Lombroso (*loc. cit.* [55]) and Camurri (*loc. cit.* [59]), however, describe such diet:—

LOMBROSO (p. 10).		CAMURRI.	
Maize	1,091 grm. a day.	Polenta	1,500 grm.
Beans	60 „ „	Milk	100 „
Bice and barley	67 „ „	Eice	100 „
Potatoes	67 „ „	Beans	100 „
Vegetables	250 „ „	Bacon	20 „
Pore meat and bacon	21 „ „	Vegetables	100 „
Olive oil	33 „ „	Potatoes	100 „
Fish	67 „ „	Cheese	50 „
Poultry	27 „ „	Olive oil	10 „

On the other hand, considerable changes in diet were observed during the different seasons. Here the following data would give an explanation of the occurrence of the disease in the spring and autumn. Lombroso (*loc. cit.*, [55] p. 11) reported about the change of diet in the Province of Ferrara:—

	Diet in eight winter months	Diet in four summer months
Polenta	1,000 grm	160 grra.
Milk	None	None
Eggs	Nearly none	Nearly none
Onions	One a day	Two a day
Bread from maize	50 grm.	400 grm.
Honie-inade bread	50 „	200 „
Meat	10 „	60 „
Cheese	5 „	20 „
Beans	150 „	40 „
Fish	20 „	Very little

This diet is very one-sided, and consists chiefly of starch, which is known to produce beri-beri. We know, however, from onr previous experience, that maize, beans, vegetables, milk and potatoes contain sufficient beri-beri and scurvy vitamine to prevent these diseases. We. must, therefore, conclude that pellagra is due, probably, to a. deficiency of a vitamine different from those men-

tioned above. Experiments carried out on animals with maize diet gave the following results :—

Bezzola [85] has shown that maize is not sufficient food to keep guinea-pigs in good health; they soon lose hair and get diarrhoea and die. No difference was observed whether good or spoiled maize was given. Lucksch [86] found that guinea-pigs fed on a good quality of maize lose their hair, show a hypersemia of the intestinal mucosa, and an increase in size of the suprarenal glands. Fed on a mixture of maize, flour and green food they lose hair and get paralysis of the hind legs and a catarrh of the small intestine. The maize diet was, then, proved too insufficient also for rabbits and dogs. In springtime the results were more marked than in autumn. The blood of these animals was examined and found sterile. Holst [87] points out the very frequent occurrence of scorbutic symptoms in pellagra, especially the porosity of the bones, which is one of the characteristic symptoms of scurvy. He repeated the experiments of Lucksch on guinea-pigs, and stated that he could prevent the disease described by Lucksch, which he considers to be scurvy, by an addition of fresh cabbage. Loss of hair and other symptoms were absent in his cases, although the experiments were carried out in springtime.

Schüffner and Kuenen *(Arch. f. Schiffs- und Tropenhyg.*, vol. xvi, p. 277, 1912) have found an erythema of the skin in 23 per cent, of the beriberi patients in Dutch India.

In order to ascertain definitely whether or not pellagra is a deficiency disease, exact investigation of food in pellagra districts are necessary. Further, it is important to know, whether it is possible to improve the condition of the patients by any change of diet. Lombroso (*loc. cit.* [54]) in his book states that a considerable improvement and even cure could.be obtained by a meat diet. These experiments, carried out on a large scale, with the elimination of possible errors, would probably give, some valuable .clues to the etiology of pellagra. Still more important would, be some means by which an experimental pellagra could be induced in animals. This would enable us to do research on this disease, independently of pellagra .districts.

THE RESULTS OF OUR KNOWLEDGE OF DEFICIENCY DISEASES. APPLIED TO ANIMAL METABOLISM AND NUTRITION.

The results of modern investigation of deficiency diseases seem, to be unknown to most physiologists. I noticed only one *résumé*

which draws attention to the deficiency diseases with regard to general metabolism; this is the *résumé* of Mandel on protein metabolism [88], The food was up to now valued only by its content in proteins, fats and carbohydrates, and calories value. From the time of the remarkable results of Emil Fischer on the chemistry of proteins a great number of different proteins of vegetable and animal origin were investigated. Each of the proteins was hydrolyzed and its content of amino-acids determined. The proteins themselves were used for metabolism experiments. It was shown that the proteins differ enormously in the quantity of their " building stones." The plant proteins especially, which are known to have an enormous content of glutaininic acid (up to 40 per cent.), differ from animal proteins, which contain on an average only 10 to 15 per cent. Some of these proteins, like gelatine, zein and gliadin, are known to be deficient in some of the amino-acids. Experiment has shown that proteins which are deficient in tyrosine, phenylalanine and tryptophane, are unable to keep the body in nitrogenous equilibrium. The relative quantities of amino-acids also constitute a very important factor. Michaud [89] has shown that by feeding dogs with their own proteins a relatively small amount of nitrogen suffices to keep the animals in nitrogenous equilibrium. Evidently an animal fed on proteins which differ in the quantity of amino-acids from the proteins of its own body is forced to use much more proteins and is unable to use these amino-acids, which are in larger proportion in the food than in its own body. The experiments of Abderhalden and Samuely [90], and Abderhalden, Funk and London [91], have shown that by feeding animals on gliadin, which contains 43 per cent. glutaminic acid, the composition of the proteins of the blood serum remains the same.

From this we can easily understand why Chittenden [92] finds that most people take in their food more nitrogen than is really necessary. In this food the organism is forced to find all the substances which are necessary for life, and which are present in this particular food in only small amount. By knowing all the substances which are necessary for life, it will be easy to choose a diet containing all those substances, and in this case the amount of nitrogen would probably be much below the figures obtained at present. At the same time this would solve the problem of choosing the cheapest food containing all the substances necessary

for life. We see from the present *résumé* that besides amino-acids small quantities of substances called vitamines must be contained in the food. Besides the vitamines described in this paper similar substances were found in milk. Osborne and Mandel [93], by feeding rats on different proteins, came to the conclusion that even proteins, which are probably complete in the number of amino-acids and contain the latter in a right proportion (casein), are capable of maintaining adult animals in nitrogenous equilibrium, but prevent normal growth in young animals. On adding to this diet milk freed from proteins the animals showed normal growth. Similar experiments were performed in this country by F. G. Hopkins.[1] I suppose that the substance facilitating growth found in milk is similar, if not identical, with the vitamines described by me.

In the metabolism experiments performed with the view of determining the nutritive value of different proteins the question of vitamines was not considered. We have seen, however, that animals kept on a diet deficient in vitamines lose enormously in weight, and do not maintain their nitrogen balance, even when the food is rich in nitrogen and calories. By an addition of small quantities of vitamines the animals kept on unchanged diet recover and are found to retain nitrogen. In future special care ought to be taken to supply the animal in metabolism experiments with sufficient quantities of the different vitamines. Only in this way can satisfactory and clear results be obtained. Abderhalden, in a regent paper [94], comes to the conclusion that the problem of artificial food is solved. Instead of synthetizing proteins it is possible to supply a mixture of amino-acids in the right proportion, and he thinks that all amino-acids and all necessary substances are already known. He fed dogs with meat which was broken up completely by ferments, and also with an artificial mixture of known amino-acids. His experiments were of short duration (seven days) and were performed after feeding for some time with broken-up meat, which contains, as was shown, all the protecting substances. An extension of his experiments for a longer period would undoubtedly result in his dogs getting scurvy. I agree with Abderhalden's general idea on this subject, but I think, however, that his conclusion is a little premature. The problem of artificial food cannot

[1] Private communication.

be solved, unless we know all the chemical constituents of our food. As has been shown in this paper, this is not the case, and many substances like vitamines await investigation ; the synthesis of these substances will take many years of work still.

I suppose that the present *résumé* has already convinced the reader of the physiological importance of the vitamines. It is quite possible that their importance is much more considerable than is indicated in this paper. A predisposition to many other diseases may be induced by the deficiency of these protective substances. Among these diseases rickets may be mentioned. The investigation of this disease has not made much progress. Besides an abnormal calcium metabolism, which is probably the result and not the cause of the disease, nothing of importance is known. I think that experiments with vitamines, which can at least do no harm, ought to be performed here in order to ascertain if a deficiency of the latter is not the real primary cause of the disease. We will see the whole importance of these protective bodies when we inquire for what purpose these small quantities of substances are required in the animal organism. It is obvious that the .minute amount necessary cannot be considered from the point of view of food. It is most probable that they are used as such or transformed into substances which are able to act in small quantities. Such substances in the body are known to be ferments, hormones and products of the secretion of internal glands. With the exception of adrenaline we know practically nothing about these substances. The secretions of thyroid and parathyroid glands, of pituitary and genital glands, are completely unknown and so are the enzymes and hormones. The further investigation of vitamines, the knowledge of their chemical composition, and their fate in the animal body, will probably help to elucidate these problems.

From the present *résumé* we can conclude that all the deficiency diseases can be prevented by a complete diet. A monotonous diet ought to be avoided, because in this case a deficient food is made use of for long periods and prepares the ground for the outbreak of the deficiency diseases. There is no doubt that as our knowledge of the relative value of different foodstuffs increases we will be able to prevent completely the outbreak of the latter.

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