1929

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The University of Montana

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The Histological Effect of Diet Deficiency Diseases in White Rats

by

Helen Frances Griffin

Presented in partial fulfillment of the requirement for the degree of Master of Science Arts.

State University of Montana

1929

(Signed) W. J. Elrod
Chairman Exam. Com.
Introduction

In this research on the histological study of the diet deficiency diseases in white rats I am greatly indebted to Dr. R. T. Young, under whom this work was carried on, and to Dr. M. J. Elrod, the chairman of the department of Zoology, for the help and cooperation which I received at all times. Without their assistance, it would have been impossible for me to do this work. I am also much indebted to Miss Anne Platt of the Home Economics Department, who furnished the rats for the experiment and gave so freely of her experience at all times.

The general historical discussion of the recent work on vitamins and mineral deficiency was obtained in great measure from "The Newer Knowledge of Nutrition" by McCollum and Simmonds. 4

The study of the effect of those substances which may be lacking in the food supply is particularly important because of the wide range of diseases and pathological conditions which their absence may cause. The substances which have been studied in this problem are Vitamin A, Vitamin B, Calcium and Phosphorus. These substances are present only in small quantities in a few of the foods we use and hence we often do not obtain a sufficient supply for the proper body nutrition. Their lack leads to many diseases which are obscure although wide-spread such as beri-beri, pellagra, scurvy and others. The vitamins, particularly, are easily killed by heat, chemicals and other manipulations in the preparation of foods and hence are more difficult to obtain in sufficient quantities.

The study of deficient diets is comparatively new since scientists have been working definitely in this field only since the beginning of this century. It has long been known that a diet which was deficient in fresh fruits and vegetables produced certain diseases and sailors on long sea voyages were particularly susceptible. In 1834, a ship of the Japanese navy was sent on an eight months cruise with the usual diet which contained no fresh fruits or vegetables. On their return, another ship was sent over the same route, with a change in the quantity of fresh fruits and vegetables and a great improvement in the health of the men was noted. This led to the improvement of diet on ship board and from that time on, but little trouble with scurvy was observed.

In 1897, Eijkman, a Dutch doctor, produced artificial beri-beri in pigeons by feeding them on a polished rice diet and so determined that it, too, was a diet deficiency disease. Notwithstanding these two conclusive experiments, but little attention was paid to diets until the beginning of an experiment in Wisconsin in 1906. Believing that food stuffs from any cereal plant should be equally nutritive if they were chemically the same, an experiment was planned to study different feeds for cattle. Diets were made up from the entire plant of oats, wheat and corn. These diets were as nearly alike chemically as possible and were composed of the entire plant ground up together. Then for a control, a mixed diet from all the plants, also of the same chemical content was given. Much to the surprise of Hart and Humphrey, the experiment showed that these diets had far different effects. The animals which were fed on the corn plant grew and reproduced normally. The calves were normal in every respect. Those which were fed on the wheat plant were in very poor condition. The cattle were scrawny and undernourished and the calves were all...
premature and died immediately. They showed the effect of malnutrition. The animals which were fed on the oats plant were in medium condition and a few of the calves lived but were not strong and healthy. The most surprising part of the experiment came when it was found that that group of animals which was supposedly a control group—on the mixed diet—did not respond well. Their condition was also very poor and the calves died soon after birth. The investigators then came to the conclusion that there was something in these diets which made them different, although the chemical composition was as near alike as they could make them.

At the time that this experiment was going on, McCollum, the father of this branch of nutrition and the one who has been responsible for a great deal of the knowledge which we now possess, was trying an experiment on purified food stuffs. He fed domestic rats on a simple, purified diet in which the chemical composition could be determined exactly. He found that these animals did not do well and soon diet deficiency diseases were observed.

Then the study of phosphorus was begun. Some investigators claimed that the difficulty was with the use of inorganic phosphorus instead of the organic phosphorus which the body needed. It seemed very probable that organic phosphorus was needed by the body since phosphorus is such an important element in casein and egg yolk which are used for the nourishment of the young of most higher animals in the early stages. It was finally proved by McCollum that the body could use inorganic phosphorus compounds in synthesizing the organic substances containing phosphorus. It was also about this time that it was firmly established that these purified diets would not nourish rats perfectly and that some essential was still lacking.

Meanwhile the chemists had been busy studying foodstuffs and they discovered that there was a
difference in the proteins which could be used as foods. Some of the proteins contained all the amino acids while others contained only a few of them. This led to the conclusion that a complete protein would furnish all the necessary factors for proper growth and reproduction, but there, too, the investigators were to fail.

By 1913, a considerable list of known facts had been gathered and it seemed that the problem must soon be settled. It was known that the fat from butter or from egg yolk promoted growth but that from vegetable sources did not. It was also known that substances which had the same chemical composition on analysis would produce different results when fed to experimental animals. This led to the biological analysis as it is called—where food stuffs were fed to animals to see whether they contained this unknown substance which would produce growth. In 1914, Mendel stated that there must be two substances which are necessary for the proper growth of the animal body. In 1915, these two substances were definitely named as Vitamin A and B.

The reason for this naming dates back to the study of this group of substances by Funk in 1913. He called them "vitamines" or life-giving substances, but they were as yet hypothetical. Their chemical composition was unknown when they were discovered so algebraic terms were suggested which would have no meaning as to chemical composition. They were therefore called vitamins A and B. Later on, the spelling was changed to "vitamin" and the meaning enlarged to contain all the dietary essentials of this class. Since that time new principles have been discovered, among them being Vitamins C, D, and E with the prospect of more to be found.

The study of mineral deficiencies is one which has been investigated a great deal in the last few years. Calcium and phosphorus are two compounds which are of vital necessity for the growing animal...
and the proportion in which they are found in the diet is very important. Many of the effects of faulty nutrition can be directly traced to the deficiency of one or the other of these elements or an incorrect balance between them. The effects of calcium and phosphorus deficiency and Vitamin D show that there is a relationship between the mineral content of the diet, the Vitamin D content and the prevalence of rickets in both animals and humans. This relationship is one which is not well understood as yet and a great deal of investigation is being done in this field.

The pathology of these diet deficiency diseases was not studied until about 1915 when McCollum began to study the effect in the tissues of the body. From that time on, this new phase received considerable attention and some work on tissue changes has been done by various authors. In many cases, the experiments were not carefully controlled so that the normals might be compared or else the results were inconclusive or inconstant in many respects. Allen has studied the degeneration of rat testis due to a diet deficient in Vitamin A and found that the interstitial tissue was increased in quantity and the germ cells were degenerated.

1 Allen, Ezra, Degeneration in Albino Rat Testis Due to a Diet Deficient in the Water Soluble Vitamins, Anat. Rec. 16: 93-118, Apr. 1919.
Fujimaki, Kimura, Wada, and Shimada studied the changes in pavement epithelium in rats fed upon a Vitamin A free diet and found a marked change in only about 10% of the animals examined. This consisted in proliferation and hyperkeratosis of the epithelium. The scientist who has published the most results on the histological changes of tissues due to the diet deficiency diseases is McCarrison. His work is based on diet studies carried on in India and covers a great many diets and animals. He reports very decided changes in many of the tissues which he studied. In most cases, he used a diet which was deficient in many factors instead of just one--thus making it uncertain just which factor was responsible for any certain effect. His work has been criticized on this basis and a detailed discussion follows later.

My experiments were undertaken in an attempt to determine the histological and cytological effects of these deficiency diseases upon some of the organs of the body. It is a study of the pathological conditions present when but one factor can be responsible for the change and was carefully controlled from that viewpoint.


Method of feeding and care

In this experiment, two sets of white rats were used, set A being older at the beginning of the experiment than set B. The ages of the rats in set A averaged 43 days at the beginning of the experiment while those of set B were all 35 days old. The animals were kept in the same room under as nearly constant temperature and humidity as possible. The same types of cages and feeding pans were used and the animals were confined two to a cage in set A and one to a cage in set B. False bottoms were inserted in all of the cages so as to avoid excreta consumption. Only distilled water was used during the course of the experiment. In all there were twenty five rats used, fifteen in set A and ten in set B.

The diets were chemically pure and were made up in the laboratory from purified chemicals. A salt mixture consisting of the following was used in the control diets:

- Calcium carbonate ------- 134.8 g.
- Magnesium carbonate ------ 24.2 g.
- Sodium carbonate --------- 34.2 g.
- Potassium carbonate ------- 141.3 g.
- Phosphoric acid ---------- 103.2 g.
- Hydrochloric acid --------- 53.4 g.
- Sulfuric acid ------------- 9.2 g.
- Citric acid ------------- 111.1 g.
- Ferric citrate ----------- 6.34 g.
- Potassium iodide ---------- 0.02 g.
- Manganese sulfate -------- 0.079 g.
- Sodium fluoride --------- 0.248 g.
- Potassium alum ---------- 0.0245 g.

This mixture was thoroughly ground up, triturated with water and then evaporated to dryness and used in the food.

The food mixture for the control group was made
up in the following proportions:

- starch: 40 g.
- gelatine: 5 g.
- albumin: 20 g.
- dextrin: 20 g.
- butter fat: 10 g.
- sodium chloride: 1 g.
- salt mixture: 5 g.
- Fleischmann's yeast: 1 g.

These materials with the exception of the yeast were thoroughly ground and mixed together. The salt was added to increase the palatability of the food. Fresh Fleischmann's yeast was obtained every day and one gram was added to the ration for each rat. Filter paper was provided in the cages for roughage. No attempt was made to measure the amount of food utilized but liberal quantities were present in the cages at all times.

The animals on the Vitamin A free diet were given the same diet as those of the control group except that another fat was given in place of the butter fat. In the first part of the experiment olive oil was used for set A but later Mazola was substituted and used for the rest of the experiment. Set B received Mazola throughout the experiment.

The animals on the Vitamin B free diet also received the same as the control group with the exception of the yeast which was not given them.

The animals on the mineral free diets received a different salt mixture than the other groups. Those on the calcium deficient diet had a salt mixture made up without the calcium carbonate. Those on the phosphorus free diet had a salt mixture made up without the phosphoric acid. It was noteworthy that this salt mixture remained quite moist at all times, and it was impossible to evaporate it to dryness as was done with the other salt mixtures.
From set A, three animals were chosen for each diet, two being confined together and one separately. In set B, there were two animals for each diet and they were confined separately. The animals were weighed at the beginning of the experiment and every two weeks until the close of the experiment. The experiment with set A was begun first—on October 17th, and the second set of rats were started on November 14th. During this time, two rats died—a rat on a calcium free diet in set A and a rat on the Vitamin B free diet in set B. Part of the rats from each group were killed on December 12th and the rest on December 19th.
Rats on Control Diets
Rats on Vitamin H-Free Diet
Rats on Vitamin B-Free Diet
Rats on Calcium Free Diet
Rats on Phosphorus Free Diet

Composite Growth Curve of Rats on Experimental Diets

H Griffin
Rats on Control Diets
Rats on Vitamin A Free Diet
Rats on Vitamin B Free Diet
Rats on Calcium Free Diet
Rats on Phosphorus Free Diet

Set B
Composite Growth Curves of Rats on Experimental Diets

Griffin
Results as shown by physical condition

The results as shown by the weight tables are very interesting and show some rather peculiar conditions. The graphs shown in the table accompanying were made by averaging the weights of the rats in each diet group and thus creating a composite curve. This destroys the effect of the fluctuations of the records of individual rats and creates a smoother curve. It will be seen that the weights from set A may be divided roughly into three periods—(1) a period of living on the body tissues, (2) a period of rapid loss, (3) a period of slow loss which would have been terminated by death. In set B, there was a very short period of living on the body tissues followed by a period of rapid loss in weight. It is shown very strikingly in the accompanying graphs that the conditions of the supposedly normal group in set A were not what they should have been. This might have been due to a change in the food to which they were accustomed, followed by a period of increased growth as they became accustomed to it. The probable cause, however, is that pulmonary trouble which seemed to affect the whole colony at that time, also affected the rats on the experimental diets. The condition of the rat colony as a whole, was very poor for the greater part of the time during which the experiment was running and this probably accounts for the loss of weight shown by the control animals. This same condition affected the rats on the deficient diets, without doubt, but they did not show the same rate of increased growth, in spite of the handicap, that the control animals show.

Another set of tables which are very instructive
These show the fluctuations perhaps even more clearly than the graphs given. They were obtained by averaging the percent losses or gains of the individuals within each group and thus obtaining a composite figure. These show very clearly that there was a steady rate of increase in the animals in the control groups. It also shows that the control groups gained much more than the animals in the deficient diet groups. A deficient diet need not mean that the animal loses in weight but merely that the gain is not so
marked over a given length of time as in the normals. In the case of the calcium free rats in set A and the Vitamin B free, Calcium free, and Phosphorus free in set B, there was an actual loss of weight during the course of the experiment.

A very interesting feature to watch in the experiment was the external symptoms shown by the experimental animals. The animals on the normal diet were not afraid or nervous. They would play together and would not be disturbed when the cage was opened. The hair was smooth and glossy and very white. The eyes were alert and beady. The animals which were on the Vitamin A free diet seemed to grow quite well and the hair was in good condition. They were not very nervous when the cage was closed but were quite afraid if the cage was touched in any way. One rat kept trying to rush out when the cage door was opened and was very difficult to handle. The appetites of the rats lessened slightly as the experiment progressed. At the end of the sixth week in set A, an abnormal eye condition was noted. One rat had the left eye completely closed and the eyelid was reddened and badly swollen. This made the animal hold its head to one side in a very peculiar manner in the effort to see plainly. The other rats showed the same conditions but not so markedly.

There was a very decided effect in the rats on the Vitamin B free diet at the end of the first two weeks. They were very nervous and afraid and crouched in the corner of the cage whenever people were in the room. The hair was rough and of a peculiar color. When observing one of these rats, the eyes seemed rather glazed, and towards the end of the experiment the animal seemed to weave its head back and forth as it stood. They did not eat well and in the last week of the experiment the rats could be found sleeping in the food pans with their heads hanging down towards the food. There was a marked diarrhea.
The animals on the calcium free diet showed the most external changes. They were very nervous and threw themselves at the door of the cage whenever it was opened. Sometimes when the door of the animal room was opened, they would all squeal even though their cages were not approached. The hair was very yellowish and roughened. They habitually crouched either under the food pan or in the far corner of the cage. They also had a very marked diarrhea.

The animals on the phosphorus free diet did not show very many changes. They seemed to change very little in size and the hair was smooth and of normal color. If anything, they were easier to handle during the experiment than the normals--appearing to be rather sleepy and quiet. They seemed to be always hungry and would hurry to the fresh food but would refuse to eat very much after the first sample. There was very little diarrhea noted.
Autopsy Findings

The conditions at the autopsy were noteworthy in a few respects. The intestines of the experimental animals were bloated and full of gas, and the colon particularly was very hypertrophied. The material present in the intestines was yellow in color and very putrid. The stomach was ridged and folded, being atrophied, and some undigested food was present throughout the gastrointestinal tract.

The organ most changed in macroscopic appearance was the spleen. It showed very decided atrophy with an increase in pigmentation from that shown by the normals. This same condition was discovered by McCarrison in his study of diet deficiency diseases and he says, "It seems probable that the cells of the spleen are utilized for purposes of nutrition of more vital organs." Again, he says "The spleen provides a reserve of accessory food factors for use on occasions of metabolic stress. This reserve, however, is rapidly exhausted."

In one case, a Vitamin B deficiency rat from set A, the pancreas showed a very great degree of atrophy, it being almost impossible to distinguish the gland in the folds of the mesentery. In this case the rat died very soon after the beginning of the experiment and at all times showed a great loss in weight and poor body condition. There was probably some other cause for this abnormality besides the diet deficiency and this animal was not used in the results stated later in the summary.

The sex glands seemed perfectly normal in most respects when they were removed from the experimental animals. The testes were of normal size except in


3 Ibid
the case of the Vitamin B and the calcium free rats where they showed a slight degree of atrophy. The females showed recent ovulation and the ovaries seemed perfectly normal. This is opposite to the results of other authors. McCarrison\(^3\) states that the testes of rats on a Vitamin B deficient diet are only 1/11 the size of the normal rats. McCollum\(^4\) states the gonads of birds on a Vitamin free diet are greatly atrophied. A discussion of the abnormality of these results will follow later in this paper.*

In general, the autopsy findings were not exceedingly abnormal except in the case of the gastrointestinal tract and the spleen where the changes were very definite and easily seen. The remainder of the organs, while showing atrophy in many cases, showed very little definite and constant changes among the different experimental animals. The exact degree of weight change of the organs could only be found by using many more experimental animals than in this experiment and no attempt was made to ascertain these results.

\(^3\) McCarrison, Robert, Op. Cit.

\(^4\) McCollum, E. V. and Simmonds, Fina, Op. Cit.

* See page 2/
Methods of preparing tissues

The rats were killed by first giving chloroform in a small quantity and then bleeding the animals. They were autopsied immediately and the tissues were fixed in Zenker's, Regaud's and Champy's fluids. After complete fixation, small pieces of the tissue were dehydrated and imbedded in paraffin by the xylol method. The sections were cut 5 microns thick and stained with Iron Haematoxylin and counterstained with Eosin.
Results of tissue study - Adrenals, Fig. 1, 2, 3, 4, 5.

The first glands studied were the adrenals. It will be seen from the accompanying drawings that there was little or no change in the adrenal glands. There seemed to be some hypertrophy in the phosphorus free gland but it was very slight. The cortex and medullary cells were normal. The number of nuclei in the glands from the abnormal animals seemed to be normal and stained just like those from the normal animals. This agrees in general with the findings of McCarrison and other authors quoted by McCollum.

Kidneys - Fig. 6, 7, 8, 9, 10.

The kidneys were also examined and very little change was noticed there. The Malphigian corpuscles were normal in size and number and seemed to have a normal blood supply. One rat in the phosphorus free group showed a slight hemorrhage which is pictured in the section drawn. (Fig. 10) This, however, may be due to shock incident to the killing or other causes and is not conclusive proof of a hemorrhagic condition due to the faulty diet. The epithelium lining the renal tubules seemed perfectly normal and looked healthy. In general, it does not seem that the changes in the organism from a diet deficient in certain food factors impairs the renal function.

Liver - Fig. 11,12,13,14,15.

The liver seemed normal in size and showed a normal histological and cytological appearance upon microscopical examination. McCarrison, in feeding pigeons on a diet of milled and autoclaved rice found that the organ seemed of normal size but was really atrophied, this condition being masked by an intense congestion which was present in all cases. This was accompanied by karyolysis of the cells. He states that many of the liver cells lose their power of nuclear staining, due to the disappearance of the nuclear chromatin while the cell body becomes homogeneous or contains but a granular debris. In all cases, the liver condition was associated with septicemic conditions and the necrosis might have been due to this condition rather than to the actual effect of the deficiency disease. This effect, therefore, would not be shown in animals which were not suffering from a severe infection of this type. He has been able to demonstrate bacteria lying among the liver cells and detected them even by smears taken from the cut surface of the organ. This condition would, of course, lead to a far different pathological picture than the simple diet deficiency disease.

Pancreas, Fig. 16,17,18,19,20.

The pancreas was an organ which shows very varied changes. The pancreas of the normal rats showed small hemorrhagic areas, and a breaking up of the alveolar structure. This condition can be duplicated in those animals which are suffering from faulty diets. The experimental animals also

show pancreatic tissue which looks quite normal. In general, the cells of the experimental tissues show more vacuolization, but this too, is only relative. The control animals show many abnormal characteristics of the gastrointestinal tract and this may be the cause for the appearance of the pancreatic tissue. McCarrison, 3 in his work, found the same result in normal tissue. He says, "Having regard to the normal processes of degeneration and renewal of cells which go on in the pancreas, in health, the histological appearances presented by these diseases differed little from those of health." Miller 5 found that in his experiments, the changes were very inconstant, except for the change of form of the mitochondria from rods to spheres. From my observations, the normal rats of this colony do not have normal pancreatic tissue in all respects and the changes in it are due to perhaps the same factor that seems to have affected the gastrointestinal tracts of the animals. The one constant feature of the experimental animals is the constant atrophy of the pancreas over that showed by the normals.

Spleen, Fig. 21,22,23,24,25.

In the case of the spleen, the changes which were noted macroscopically give the clue to the microscopic appearance. There was a pronounced atrophy present and when the sections were examined, it was found that this atrophy was due to a disappearance of the spleen pulp and the lymphoid tissue. The fibrous tissue and the trabeculae are greatly


Increased, apparently because of the shrinkage of the spleen pulp. The fibrous coat of the gland was hypertrophied. There seemed a decrease in the number of Malphigian corpuscles although that could be confirmed only by an actual count of a great number of specimens. The cells of the spleen pulp present seemed perfectly normal in every case. There was an increase of pigment present in the organ. McCarrison suggests that this may be due to the decrease in other elements, thus showing the pigment present, or to the degenerative processes in the blood, thus causing an increase in pigment granules. The walls of the blood vessels in the experimental tissues seem a little thicker than in the control group which was also found by McCarrison.

Testes - Fib 26, 27, 28, 29, 30, 31.

The most perplexing group of tissues which was studied was testes. In all of the testes studied, there were normal spermatozoa present. In the case of the Vitamin A free rats, the spermatozoa were present in the lumen of the tubules in large quantities. The spermatids, spermatocytes, and spermatogonia were present and seemed very normal. They were dividing and showing the usual mitotic changes characteristic for each stage. In reviewing the literature on this subject, McCollum quotes Eckstein (1923) to the effect that a Vitamin A free

4. McCollum, E. V. and Simmonds, Nina.
diet did not interfere with ovulation in the female but did affect spermatogenesis in the male. The ovulation rate seemed to be perfectly normal, although the females resorbed the foetuses after fourteen days of pregnancy. However, reproduction in animals on a Vitamin A free diet is possible in the first generation at least, and the best animals to use in a study of this kind are the second or third generation. This would point to the supposition that the animals must exhibit normal fertility and the formation of sperms and ova. The animals which were on the Vitamin A free diet in this experiment showed perfectly normal condition of testis and the sperms looked as though they were functional but no study was made of the ovaries.

In the case of the Vitamin B free tissue (Figs. 28, 32) the sperms were also found in the tubules. This tissue was poorly prepared and the exact cell contents of the spermatogonia, spermatocytes, and spermatids could not be clearly seen, but they were present in large quantities as well as the mature sperm. According to the findings of Siperstein it would have been impossible for the animals to form these before the experiment started since they were too young when the diet was first given. Therefore, they must have been formed and complete spermatogenesis must have taken place during the time that the animals were on the deficient diet. McCarrison says "a lack of Vitamin B leads to the complete suppression of spermatogenesis, a thickening of the capsule of the gland,


and a lessening of the number and the diameter of the tubules which are lined with a single layer of cells. Allen\textsuperscript{1} states "Reduction in the quantity of water-soluble vitamin in the diet of rats results in the total degeneration of all the germ cells". These results are at total variance with the ones found in the experiment carried on here this year. There was the possibility of outside people entering the animal room and feeding those rats kept on the experimental diet which may have happened in spite of the utmost care. It may be also that some part of the food carried this vitamin in quantity sufficient to permit spermatogenesis but not general body development but this does not seem probable. The most that can be said is that the experiment in this respect is not conclusive and must be submitted to further study at a later time.

The testes of the rats on the calcium free and the phosphorus free diet were normal. This was to be expected since the fertility of animals suffering from these deficiencies is not appreciably affected. The offspring are not normal, but fertilization does take place in animals on these diets. There were references found dealing with the effect of mineral deficiencies on the gonads.

Intestine Fig. 33, 34, 35, 36, 37, 38, 39, 40, 41, 42.

The greatest changes were found in the gastrointestinal tract of the rats examined. This was expected, since the most damage to the organism would be caused by the abnormal food which was fed. All of the experimental animals showed a great increase in the glands of both the mucosa and the

\textsuperscript{1} Allen, Ezra. Op. Cit.
submucosa. This was especially marked in the Vitamin B free, calcium free and Vitamin A free animals. The crypts of Lieberkühn in the mucosa were enlarged and far more numerous than ordinary. The glands of Brunner in the submucosa were also more numerous and a far thicker layer was found. The tissues were from the duodenum close to the pyloric end of the stomach where these glands are more numerous. No reason for this increase is apparent and no references to changes in these glands can be found in the literature. It is therefore impossible to account for it at this time.

The epithelial cells were the most affected. The epithelium of the villi of the intestine of the Vitamin A free rats was very fragmented and in most cases was absent. It was very difficult to find an epithelial cell to draw under the oil immersion lens. (Fig. 39) Most of the epithelial cells, when found, were very abnormal, being badly broken and with misshapen nuclei. The epithelium in a few cases in the Vitamin B free tissues, showed a fairly normal condition, but was separated from the villi. In most of the cases it was disintegrated until it was almost impossible to distinguish the epithelial debris from reticular tissue under the oil immersion lens. The villi of the calcium free rats showed a more normal appearance than either of the preceding two. There were a few villi which showed an almost normal appearance but they were the exception rather than the rule. Most of the villi showed a complete disintegration of the cells with a gradual disappearance of the definite form of the nuclei. The condition of the phosphorus free tissue was most peculiar. The epithelium looked normal but was separated from the reticular tissue by a very small distance. Both the reticular tissue and the epithelium showed normal characteristics but the connection between the two had been broken.

The reticular tissue of the villi also showed great changes. In the Vitamin A group, there was
an increase in the number of leucocytes. The tissue had a retaining wall which held it in position and in proper shape even though the epithelium had separated. The reticular tissue in the villi of the rats suffering from Vitamin B deficiency was somewhat atrophied and showed fewer cellular elements with a corresponding increase in fibrous material. In the case of the calcium free tissues, the retaining wall, holding the reticular tissue was missing and hence the tissue was very scattered. There were very few cellular elements present. As was stated before, the reticular tissue of the animals on a phosphorus free diet was normal, except that there may have been a slight increase in the number of leucocytes.

Another very noteworthy change was the great atrophy of the muscular coats of the intestine, amounting to almost complete loss in some cases. This was forecast by the ballooning from the large quantities of gas which the intestines contained upon autopsy and was very noticeable on section. The circular coat of muscles seemed to be particularly affected.
Conclusion

In conclusion, there are some comments necessary. The most prolific writer and practically the only one who has done work in any quantity on this problem is McCarrison, an Indian army surgeon. His results have been greatly criticized on the ground that the diets which he fed were not properly balanced with respect to all the factors except the one which he expected to study. For example, practically all of the results which have been quoted in this paper, came from an experiment in which he fed a diet of milled and autoclaved rice alone. This would give a diet very high in carbohydrates and correspondingly low in fats and proteins. The food was deficient in calcium, phosphorus, sodium and chlorine as well as showing an excess of acid over basic radicals and a total lack of all the vitamins. With a diet of this type, many pathological conditions can be noted but can hardly be attributed to any one factor, in particular. McCollum states "Deprivation of chlorine quickly leads to inability of the stomach to secrete hydrochloric acid, and this alone would seriously interfere with digestion, and with the suppression of bacterial growth in the alimentary tract of which the normal intestine is capable in some degree. Diets so deficient in mineral salts might well lead to a state of depletion of the alkali reserve in the blood and may account for the air hunger which he (McCarrison) attributed to acidosis." Yet with a diet of this type, beri-beri is of course produced and the results were given as characteristic of beri-beri and the corresponding deficiency. This may account for some of the discrepancies found by me in study of the tissues.

Another reason for the discrepancy of results is that it is very difficult to make up a diet which contains all the factors necessary for life in the proper ratios and then remove one of them without affecting that balance in other ways. The incorrect ratios of calcium and phosphorus or fat, protein and carbohydrate in the diet will lead to pathological conditions which may well be taken for changes due to the one factor studied. It is also difficult to control the experiment so that normal rats may be compared. Temperature, humidity and other external conditions will affect the experimental animals as well as the controls.

Another factor which has been greatly neglected is the importance of bacterial infection due to lowered resistance and a breakdown of the tissues in the gastrointestinal tract. McCarrison found four chief organisms present in his experimental animals and it would be interesting to see if the same or similar organisms are present in a climate and conditions so far different from that in which he worked. It would also be interesting to study the changes in the intestinal flora during the course of the experiment and determine whether the pathogenicity of the normal flora is increased during the experiment. McCarrison demonstrated by slides that bacteria will invade the blood stream due to the discontinuity of the cell walls in the intestine and this may affect the tissues and be a secondary change, perhaps considered primary at the present time. The animals in this experiment did show evidences of bacterial infection but this was not studied.

Without a doubt the tissue change in the organs supposedly affected has been over estimated in many

cases. With such a decided change in the kind of food given the animal, a semi-starvation results. It has been noted by Siperstein in an experiment on semi-and total inanition with albino rats that conditions quite similar to those found in vitamin deficiencies are found. This would lead to an atrophy of the tissue and a corresponding change in the histological elements making up the tissue. This then is another secondary change instead of the primary one so often quoted. The unbalanced diet and the lack of appetite displayed by the animal without a doubt will affect the growth and development of the body organs and lead to atrophy in most instances.

An unfortunate condition in this experiment was that the animals used as controls were not normal in all respects. As was stated before, it has been very difficult to breed animals from the slides, not only of this experiment, but from some prepared by Dr. Young last year on supposedly normal animals, it would seem that the gastrointestinal tract is affected in some way. The epithelium in both cases is fragmentary and does not present a normal histological picture. This may be due to a bacterial infection present or it may be that the food has in some manner affected the rats. This abnormal condition has been referred to in the results of the study of the pancreas which also shares in this abnormality. Other tissues seemed to be normal.

The effect of the diet upon the intestine, therefore, is not so conclusive as might be wished. In no case, however, was the condition in the normal animals comparable with the pathological change which had taken place in the experimental groups. Some of that extreme breaking up of the villi and the atrophy of the muscular coats must have been due to the deficient diet. There also was a very great increase in the glandular elements over that in the control group.

Before the problem of the changes in the tissue elements of the body in these diet deficiency diseases is settled, there must be a great deal of change in the methods of experimenting and a great many more experiments done. The whole problem is very difficult, reference material being lacking and rather vague where found, and it presents a fertile field for further investigation. The problem is very closely associated with that of bacteriology, the chemical nature of digestion and absorption of food and the correct determination of the dietetic elements. A great many conflicting things have been noted in this paper, and it is by no means conclusive. The results have been presented as found but need further checking before final acceptance. Such a task must await a future date.
BIBLIOGRAPHY


EXPLANATION OF DRAWINGS

Plate I----Fig. 1----Adrenal of Normal Rat
Fig. 2----Adrenal of Vitamin A Free Rat
Plate II----Fig. 3----Adrenal of Vitamin B Free Rat
Fig. 4----Adrenal of Calcium Free Rat
Plate III----Fig. 5----Adrenal of Phosphorus Free Rat
Plate IV----Fig. 6----Kidney of Normal Rat
Fig. 7----Kidney of Vitamin A Free Rat
Plate V----Fig. 8----Kidney of Vitamin B Free Rat
Fig. 9----Kidney of Calcium Free Rat
Plate VI----Fig. 10----Kidney of Phosphorus Free Rat
Plate VII----Fig. 11----Liver of Normal Rat
Fig. 12----Liver of Vitamin A Free Rat
Plate VIII----Fig. 13----Liver of Vitamin B Free Rat
Fig. 14----Liver of Calcium Free Rat
Plate IX----Fig. 15----Liver of Phosphorus Free Rat
Plate X----Fig. 16----Pancreas of Normal Rat
Fig. 17----Pancreas of Vitamin A Free Rat
Plate XI----Fig. 18----Pancreas of Vitamin B Free Rat
Fig. 19----Pancreas of Calcium Free Rat
Plate XII----Fig. 20----Pancreas of Phosphorus Free Rat
Plate XIII----Fig. 21----Spleen of Normal Rat
Fig. 22----Spleen of Vitamin A Free Rat
Plate XIV----Fig. 23----Spleen of Vitamin B Free Rat
Fig. 24----Spleen of Calcium Free Rat
Plate XV----Fig. 25----Spleen of Phosphorus Free Rat
Plate XVI----Fig. 26----Testes of Normal Rat
Fig. 27----Testes of Vitamin A Free Rat
Plate XVII----Fig. 28----Testes of Vitamin B Free Rat
Fig. 29----Testes of Calcium Free Rat
Plate XVIII----Fig. 30----Testes of Phosphorus Free Rat
Plate XIX----Fig. 31----Testes of Normal Rat
Fig. 32----Testes of Vitamin B Free Rat

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Plate XX---Fig. 33--Intestine of Normal Rat
Plate XXI--Fig. 34--Intestine of Vitamin A Free Rat
Plate XXII--Fig. 35--Intestine of Vitamin B Free Rat
Plate XXII--Fig. 36--Intestine of Calcium Free Rat
Plate XXIII--Fig. 37--Intestine of Phosphorus Free Rat
Plate XXIV--Fig. 38--Intestine of Normal Rat
Plate XXV--Fig. 39--Intestine of Vitamin A Free Rat
Plate XXVI--Fig. 40--Intestine of Vitamin B Free Rat
Plate XXVII--Fig. 41--Intestine of Calcium Free Rat
Plate XXVII--Fig. 42--Intestine of Phosphorus Free Rat

(All drawings with the exceptions of Figs. 31, 32, 38, 39, 40, 41, and 42 are low power. These were made with an oil immersion lens. All the drawings were made with the aid of a camera lucida.)
Figure 5

Plate III
Figure 10

Plate VI
Figure 13

Figure 14

Plate VIII
Figure 18

Figure 19

Plate XI
Figure 21

Figure 22

Plate XIII
Figure 23

Figure 24

Plate XIV
Figure 25

Plate XV
Figure 30
Plate XVIII
Figure 31

Figure 32
Plate XIX
Figure 37
Plate XXII