The University of Chicago

THE EXPERIMENTAL PRODUCTION OF EDEMA AS RELATED TO PROTEIN DEFICIENCY

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

BY

EMMA ANNA KOHMAN

Private Edition, Distributed By
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. LI, No. 2
March, 1920

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY,
Vol. 51, No. 2, March, 1920

THE EXPERIMENTAL PRODUCTION OF EDEMA AS RELATED TO PROTEIN DEFICIENCY

EMMA A. KOHMAN

From the Hull Physiological Laboratory, The University of Chicago

Received for publication January 2, 1920

INTRODUCTION

The shortage of food in general and of certain foodstuffs in particular, which occurred as one of the results of the recent war, has been the cause of a number of forms of malnutrition among which is that called "war edema" or "war dropsy." This is a form of malnutrition that has been recognized only recently, and the characteristics of the type of diet that has been the cause of its development have been a subject of considerable speculation and some research.

Budzynski and Chechowski (1) report a condition of dropsy among the inhabitants of Poland in 1915. The principal articles of food were potatoes (which were often of bad quality), soup and bread, with little fat and practically no milk or meat. Landa (2) reports a great number of cases of edema during recent war conditions in Mexico, 1915. The diet was made up largely of spinach and beets. Jurgens (3) in comparing conditions in various concentration camps of prisoners of war has studied the relation of an "edema disease" to the ordinary infectious diseases of prison camps and finds that the edema is not a part of the infectious syndrome as had been considered by some. He states that it results from a dietary deficiency and that where this was recognized and a more varied diet was given, the men soon recovered from the edema or its appearance was averted. He further states that often the full syndrome did not appear until the men were set to digging. Then 10 to 20 per cent of the men developed the edema disease. Wells (4), during his investigations in Roumania, 1917, observed a number of cases of dropsy among the people who were most seriously deprived of food. He describes this condition to the lack of fats, particularly the

lack of those fats containing the "fat-soluble A." He states: "We found, as far as we had time to observe it, that when the patients were given butter fat they were likely to get better. But, of course, when under treatment they always got milk, soup and other things." Park (5), who spent some time in war prisons, made a study of the prisoners who received war rations. He ascribes the condition to underfeeding, especially of fats and proteins, and to the large amounts of fluid ingested together with the increased amount of NaCl. Jaksche (6) describes an edematous condition due to the hunger conditions in Austria. He states that the edema develops when the calorie intake falls below 1400 calories per day. Knack and Neumann (7), Maase and Zondek (8) and Maase and Zondek (9) describe an edema mostly in men over forty. This edema they say is intermittent according to the diet. They state that this nutritive disorder is due to the combination of overfeeding of carbohydrates together with underfeeding of fat and an abnormally large intake of water from carbohydrates and soups. They further state that 100 grams of fat per day and a stay in bed is followed by improvement. Kraus (10) describes this condition of malnutrition occurring especially in the large cities and institutions of Germany. He states that the decrease in the quantity of food is undoubtedly the principal and perhaps the only cause of the increase in the death rate. The diet is described as a monotonous, coarse diet, not rich or tasty, with a lack of condiments and vitamines which led to a loss of appetite and this in turn to inanition. The intake of food was from 800 to 1300 calories per day where 15 per cent was indigestible carbohydrate, with very little fat and at the highest 50 grams of protein. This diet was used by men who were doing hard work. Guillermin and Guyot (11) report the same conditions in Poland and other war zones. McGarrison (12), describing the pathogenesis of deficiency disease, reports that inanition leads to a state of adrenal hypertrophy. He suggests that such a change may be held to account in great measure, through blood pressure changes, for the occurrence of "war edema." In an editorial (13) of the Journal of the American Medical Association on "what renders a diet inadequate," the question of the importance of the consideration of salts in the production and study of edema is discussed. Rubner (14), describing the food conditions and their effect on the race, describes war "edema" and refers to the lack of adequate protein in the diet more than any previous author. Beyerman (15) describes edema in the Netherlands where the symptoms disappeared promptly upon addition of fresh vegetables to the diet. Jansen (16) describes

the edema and suggests that the cause is probably an increase in the permeability of the capillary endothelium which alters the exchange of fluids and salts between the tissues and the blood. Vandervelde and Contineau (17) describe an edema of the lower limbs of patients who were deported from Germany. He states that there was a lack of food, deplorable hygienic conditions, and that there were disturbances in the cardio-vascular apparatus, accompanied by anemia and that dyspnea was brought on with little exertion. Therapeutic measures were dietary improvements and rest which were followed by the disappearance of all symptoms. Romano Tonin (18) reports a hunger edema in which polyuria is a constant accompaniment. Reach (19) observed that many subjects with edema had atrophic testes or cryptorchism. He states that in twenty-six cases of war edema where dietary changes were of no benefit, testicular apotherapy was tried and in fourteen cases the patients were cured. Denton and Kohman (20), while working on the dietary qualities of carrots, found that dropsy occurred in a large percentage of rats fed on a carrot diet, when the proportion of nitrogen had been reduced by the addition of some non-nitrogenous foodstuffs, such as fat and starch. In a preliminary note (21) of the present piece of work the writer describes the cure of rats which had developed edema on the low-protein-carrot-diet, by the substitution of 18 per cent pure casein for 18 per cent corn starch in the diet, all other factors remaining the same.

METHODS

Preparation of materials

Dried carrots. The carrots that were used in the dry diets were washed, trimmed and ground in an ordinary meat grinder, spread on glass trays which were supported in a drying oven through which air was passed which was heated to 37° to 40°C. The carrots were then stored until they were used in the diet, but were not left to stand more than one or two months.

Wet carrots. Carrots used in the wet diets were prepared as for drying but were used fresh in the diets.

Butter fat. Good quality of fresh butter was heated from 90° to 95°C. in order to separate the proteins and water from the butter fat which was decanted off.

Lard. Leaf lard was obtained from the market and rendered.

Casein. Skim milk was purchased in six-gallon lots. This was diluted with an equal volume of distilled water. The casein was precipitated with hydrochloric acid (250 cc. to 10 liters of H_2O) and filtered through cheesecloth. It was then ground with mortar and pestle and divided into six lots and each lot covered with 5 to 6 liters of distilled water slightly acidulated with acetic acid, and was left to extract for about 24 hours. The water was then filtered off through cheesecloth and the casein was ground in a mortar with pestle, diluted to 4 liters with distilled water and dissolved with sodium hydroxide (250 cc. of 50 per cent NaOH to 10 liters H_2O). When dissolved the solution was diluted to 6 liters or more and the casein was again precipitated, this time with glacial acetic acid. The water was filtered off through cheesecloth. The casein was again dissolved, as above described, and filtered through filter paper with the aid of a suction filter. The solution was again diluted and the casein precipitated with hydrochloric acid. The casein was then filtered out and washed with distilled water to remove the acid. The water was removed with the suction filter. The casein was then ground in 95 per cent alcohol, covered with 3 liters of alcohol and left to extract 48 hours. This extraction was repeated three times with 95 per cent alcohol and twice with ether, filtering the alcohol and ether off each time with the aid of the suction filter and mixing well the casein each time with the fresh extraction solution. The casein was then dried in air and stored.

This careful method for the preparation of casein with repeated extractions with alcohol and ether was used in order to be sure that the casein contained neither of the vitamines, "fat-soluble A" or "water-soluble B." In some previous work commercial casein was used after extended washings with dilute acetic acid and extractions with alcohol and ether. Animals fed on a diet in which this casein was used showed a retarded growth but none of the other symptoms commonly attributed to the absence of the fat-soluble vitamines.

Starch. Kingsford's cornstarch was used throughout the experiments.

"*Water-soluble B*" was extracted from wheat germ by two extractions, using 25 grams of wheat germ and 250 cc. of 95 per cent alcohol for each extraction. The alcohol containing the "water-soluble B" was mixed and dried with the other ingredients of the diets that were fed dry, and was dried on the proper amount of cornstarch which was used in the diets that were fed wet.

Salts

Salt II

	grams
Sodium chloride	0.50
Ca lactate	1.57
Ca $H_4(PO_4)_2$	0.60

Salt VI

Sodium chloride	0.50
Ca lactate	1.5507
Ca $H_4(PO_4)_2$	0.6232

Salt III

	grams
Sodium chloride	0.1739
Sodium sulphate	0.318
Sodium H_2PO_4	0.347
Potassium H_2PO_4	0.954
Ca $H_4(PO_4)_2$	0.540
Ca lactate	1.300
Fe lactate	0.118

Salt III is essentially the salt which was shown by McCollum (22) to supply all the necessary mineral for normal growth in rats when the diet was made up of purified food substances. Instead of $MgSO_4$, as used by him, Na_2SO_4 was used to supply the same amount of sulphur. The change was made to reduce the amount of magnesium as carrots have a high magnesium content. Salt II is used to supply the minerals that are deficient in carrots. The two salt mixtures were used in such proportions as to make the salt content of the diet as nearly as possible the same as that of milk.

Making up diets and feeding rats

Wet diets. The cornstarch with the extract of wheat germ, butter fat and salt was weighed, distilled water was added and the mixture boiled to a clear stiff paste, while stirring constantly. The starch was boiled only a minute or two in order to break up the starch cells and make them more easily digested. To this paste was added the proper weight of fresh carrots, ground in an ordinary meat grinder. This mixture was made up every two days and kept in the ice box between feedings. Fifty grams of this wet food mixture were weighed out, recorded and given to each rat each day and the amount scattered and left in the dish was estimated on the next day and recorded. The amount left was not weighed because considerable moisture would evaporate and this made weighing quite as subject to error as estimation. The difference of the two weights was used as the food consumption of the rat for that day. As some of the animals scattered their food a great deal, considerable more evaporation took place in the case of the food of some animals than of that of others. Although this evaporation was considered in estimating back what the rat had not eaten, there was, no doubt, some unavoidable error in the estimation of the food consumption of the rats on this diet. Still it was a

method, with some degree of accuracy, to compare the relative amount of food consumed and a means by which one would detect any great variation in the food consumption of an animal, or any great difference among different animals. Knowing the calorie value of the ingredients which make up a diet, and the weight of the completed diet, the calorie value per gram of diet was determined by dividing the total calorie value by the total number of grams in the mixture. From this figure and the food consumption record of each rat the daily calorie intake of each rat was determined.

Dry diets. The cornstarch, butter fat and salts were weighed out, distilled water was added and the mixture boiled to a stiff paste. To this the alcoholic extract of wheat germ was added and thoroughly mixed. The proper weight of previously dried carrots was then added and the entire mass well mixed, making a very stiff paste. This was then divided into equal portions by means of two tubes, one about 4 inches long and about 1 inch in diameter, and the other just enough smaller to fit into the larger one closely. The larger tube was filled with the stiff paste. This was pushed out by means of the smaller tube which had one end closed by means of a close-fitting cork. The column of paste was then divided into four equal parts with a knife. These cakes of paste were put on a glass tray and dried in the oven described above and stored for use. Knowing the value in calories and the dry weight of the bulk of food made up, the value of each small cake, in calories and grams, was determined by dividing the value of the entire quantity by the number of cakes into which the mass was divided. A record was kept of the number of cakes eaten by each rat, and from this record the food consumption of each rat for each week of the duration of the experiment was determined.

Care of the animals

The animals used for the experiments were raised in this laboratory from stock rats kept for that purpose. These rats were fed a variety of foods—milk, mixed grain, fresh carrots, bread and occasionally meat. The animals were weighed and their cages changed and sterilized once each week.

The animals used for experimentation were taken at from about 50 to 60 grams and only when they had shown normal growth previous to being put on the experimental diet. Each experimental animal was kept in a separate cage and a record was kept of his food consumption.

The cages used were made entirely of metal. The trays were covered with paper. The cages were changed and sterilized by live steam once a week for the rats eating dry diets, and twice a week or more, as thought necessary, for those rats kept on a wet diet. The animals on the dry diet were supplied with distilled water. Those on the wet experimental diet were given no water as the water content of the food was quite high.

RESULTS

General description of animals on a low protein diet. When normal growing rats are put on the low-protein carrot diet, they stop growing at once and very soon show a gradual loss in body weight, more rapid for some animals than for others. They may lose as much as 30 to 33 per cent of their original weight in nine or ten weeks and 46 per cent in sixteen weeks. Along with this loss in weight there is a decrease in activity, not marked at first but in later periods of the experiment very decided. The animal sits quietly in the cage most of the time with its back humped up, its eyes only partly open, and head bent down between its fore legs. It is usually asleep except while eating. When moving about it seems dull, moves slowly, the more so as the experiment progresses.

The coat soon becomes rough. The hair becomes fuzzy and dirty looking as compared to that of the normal rat. If the animal lives for a long time on this diet the hair comes out in patches until sometimes almost the entire body is bare. The skin becomes dry and scaly and the animal is very subject to lesions on the tail, ears and nose. The lesions are scaly red patches on the exterior surface of these parts, are never wet and do not contain pus but are slightly elevated.

There is a great decrease in general resistance. While the stock rats and rats on an adequate diet have never been troubled with lice, it is very difficult to keep an animal on this diet free from lice. Any exposure to cold also usually leads to death while the normal animal does not suffer from the same exposure. The animal shows a great muscular weakness with subsequent wasting. Anemia is indicated by the pallor of the ears as contrasted with the pink ears of a normal animal. One factor of this anemia seems to be a decrease in blood volume, for it is more difficult to draw blood from such an animal than from the normal animal.

The duration of life of rats on the low-protein-carrot-diet varies among different rats from a very short time (2 weeks) to 3 months and

in some cases more. Toward the end of this time there is usually extreme weakness and loss of appetite. At this stage quite a number of the animals die without showing any edema. In a larger percentage of cases, however, an edema develops, this percentage in the rats on the wet diet being higher (86 per cent) than in the rats on the dry diet (55 per cent). (See table 3). This edema is usually detected first about the face, especially about the eyes and cheeks. This may be noticed in some cases several weeks before any extreme edema occurs. A more extreme type is an edema about the chest and fore legs. This can first be detected by a thickening of the skin. The animal may die in this stage, or the edema may disappear and return intermittently until death, or a very extreme edema may develop where as much as 10 cc., or occasionally more, of fluid, either gelatinous or liquid, may collect under the skin of the chest in the form of a large "blister." Even this extreme type of edema may often disappear and return intermittently on this diet, but a gain in body weight has never been noticed in this partial recovery; on the contrary, there is a decided weakening from one time to the next which finally leads to death unless the diet is corrected soon enough. In some animals the edema is in the form of a general anasarca, a fluid being collected in the serous cavities, both pleural and peritoneal. Except in extreme cases this could not be detected with certainty until after the death of the animal when the cavities were opened and the fluid pipetted out and measured. The amount of fluid varies from 0.5 to 2 or 3 or occasionally more cubic centimeters. Sometimes there is an abnormal amount of fluid in both cavities and sometimes in either one or the other.

Post-mortem examinations were made of all the animals that died as a result of being fed the low-protein-carrot-diet. In all cases there was a depletion of fat in the body. A very common finding was pneumonic lungs. There was a marked decrease in size of the testicles in the male, which was easily detected in the living animal. There was usually a congestion of the lymph glands especially those in the neck.

EXPLANATION AND DISCUSSION OF TABLES AND RESULTS

The edema is not due to a deficiency of fats or fat-soluble vitamine. Wet diets. The lack of fat and "fat-soluble-vitamine A" has been emphasized as much or more than any other factor as the cause of edema in the regions which were most seriously deprived of food as a result of

the recent war. This idea, no doubt, developed from the fact that there was a great shortage of fats and dairy products in these regions, and because it has been previously shown that xerophthalmia, a dryness of the conjunctiva, which eventually leads to blindness, and an edema of the eyelids, occurs in rats as a result of being fed a diet entirely free from or low in its content of the fat-soluble vitamine as reported by Mendel (23), McCollum (24), Steenbock (25), Funk (26) and others. Edema as it has been produced in rats in this laboratory, however, is much more extensive and entirely distinct from that which occurs as reported in xerophthalmia for it may occur subcutaneously, covering large areas or almost the entire surface of the body of the rat, and may take the form of a general anasarca, with large amounts of fluid (5 to 6 per cent of the body weight) collected in the serous cavities.

My experimental results apparently prove that this edema as produced in rats in this laboratory is not a result of the lack of the fats or the fat-soluble-vitamine. The diet used in this work to produce edema in rats was made up largely of carrots, with additions of starch, fats and salts, carrots being the only source of protein. This diet was chosen because it was with such a diet that this edema was first produced in rats while studying the nutritive value of carrots (20).

Table 1 shows the results of feeding four groups of rats on diets in which the carrots and salt content was the same throughout and the caloric value was practically the same. The quantity of fat and starch was varied. The amount of each constituent of the diet is shown in grams and calories. In these diets fresh carrots were used, of which the calorie value is about 0.4 calorie per gram of fresh carrots. In diet I (X butter), 60 grams of butter fat were used, this supplying 1 gram of butter fat for every 63 calories of the diet. McCollum and Davis (22) have shown that 5 per cent butter fat in a diet of purified foodstuffs was sufficient for normal growth in young rats. This amount of butter fat used by them supplied 1 gram of butter fat for every 88 calories in the diet. The amount of fat supplied in X butter diet should be sufficient for rats.

In diet II (X lard) the fat was supplied in the form of lard, the same amount being used as was supplied as butter in the first diet. In diet III (no fat) no fat was supplied but the quantity of starch was increased. In diet IV (K + A − B) the butter was increased to twice the amount that was used in diet I (X butter). The calorie value was kept practically the same as in diets I and II by increasing the amount of starch where the fat was decreased. This increase of butter was made to

GENERAL EXPLANATION OF TABLES

The symbol "K" wherever it occurs in the tables denotes 550 grams (dry weight) of carrots, 228 grams of starch and 120 grams of fat.

"x" butter or "x" lard denote diets in which there are 60 grams of butter or lard respectively.

"A" represents fat-soluble-A in 120 grams of butter and "B" denotes the alcoholic extract of 50 grams of wheat germ. The presence or absence of these vitamines in the respective diets is indicated by the presence of a + or − sign before the letter. For example, K + A − B means that the standard diet K (550 grams of dried carrots, 228 grams of starch, 120 grams of fat) contained fat-soluble-A but was deficient in water-soluble-B.

TABLE 1

Table of wet diets with results of feeding

	I × BUTTER		II × LARD		III NO FAT		IV K + A − B	
	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories
Carrots	4500	1800	4500	1800	4500	1800	4500	1800
Starch	360	1440	360	1440	456	1824	228	912
	BUTTER		LARD				BUTTER	
Fat	60	540	60	540	0	0	120	1080
Salt II	19.2	0	19.2	0	19.2	0	19.2	0
Salt III	16.4	0	16.4	0	16.4	0	16.4	0
Total		3780		3780		3624		3792
Number of rats on diet	7		6		5		3	
Number of rats developing edema	3		3		2		3 only slight	
Per cent of rats developing edema	42⅞		50		40		100	

Table 1 shows the results of a study of the effect of certain fats and the fat-soluble vitamine on the production of edema in rats. Edema occurs in rats on all four of the diets so butter fat or the fat-soluble vitamine in butter does not prevent the edema, neither does it decrease the percentage of edema. Even when diet IV was fed with the butter fat content of 120 grams, all of these rats developed edema The table also shows that fat is not the cause of the edema, for it occurred with and without fat in the diet.

insure sufficient intake of butter fat and fat-soluble-vitamine by the rats as their food consumption seemed to be somewhat less than that of the normal rat and it was thought that possibly not enough of the fat was obtained in the smaller food consumption.

The lower part of table 1 shows the number of rats fed each diet, together with the number and percentage of them that developed edema. It is of interest to note that where the greatest amount of butter fat was used the greatest percentage of rats developed edema and that, although there is little difference, the lowest percentage of cases of edema developed on the diet in which there was no fat supplied. This shows that fat and fat-soluble-vitamine do not prevent edema from developing in rats if the protein content of the diet is very low.

The edema is not due to a deficiency of water-soluble vitamine or salts. Dry diets. Table 2 shows the results of a series of experiments in which dried carrots were used in making up the diets. The water used in making up the diet was also evaporated so the diet was administered in a dry form. The carrots dried to practically 11.8 per cent of the wet weight, making the calorie value of the dried carrots 3.33 per gram of dried carrots. The same constituents were used as those used in the diets given in table 1 with the addition of an alcoholic extract of wheat germ in some of the diets. Although carrots have a considerable amount of the water-soluble vitamine this addition of alcoholic extract was made to insure a sufficient intake of this vitamine, for the lack of this vitamine is known to cause beri-beri which is often accompanied by more or less edema (28).

Diets V (K + A + B) and VI (K + A − B) each contain the large amount of butter fat used in diet IV of table 1. In addition diet V (K + A + B) contains the alcoholic extract of 50 grams of wheat germ which supplied the extract of 1 gram of wheat germ for every 76 calories of the diet. McCollum (29) has shown that 3 per cent of wheat germ supplies sufficient water-soluble-vitamine for almost normal growth in young rats. This amount supplies one gram of wheat germ for every 133 calories in the diet. The amount of wheat germ used in the diets shown in table 2, together with the water-soluble-vitamine in carrots should supply enough of the water-soluble-vitamine to eliminate the lack of this vitamine as the cause of the edema which develops in these rats. This evidence is strengthened by comparing the percentages of edema on diets IX and X where 60 grams of butter fat are supplied in each but the water-soluble-vitamine is supplied only in X from which diet $33\frac{1}{3}$ per cent of the rats developed edema whereas only $16\frac{2}{3}$ per cent

TABLE 2

Table of dry diets with results of feeding

	V K+A+B		VI K+A−B		VII K−A+B		VIII K−Fat+B		IX ×Butter		X ×Butter+B		XI ×Lard		XII K+A+B+2×Salt	
	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories
Carrots (dry)	550	1815	550	1815	550	1815	550	1815	550	1815	550	1815	550	1815	550	1815
Starch	228	912	228	912	228	912	490	1960	360	1440	360	1440	360	1440	228	912
	Butter		Butter		Lard				Butter		Butter		Lard		Butter	
Fat	120	1080	120	1080	120	1080	0	0	60	540	60	540	60	540	120	1080
Salt II	19.2	0	19.2	0	19.2	0	19.2	0	19.2	0	19.2	0	19.2	0	38.4	0
Salt III	16.4	0	16.4	0	16.4	0	16.4	0	16.4	0	16.4	0	16.4	0	32.8	0
Extract of wheat germ	50	0	0	0	50	0	50	0	0	0	50	0	0	0	50	0
Total		3807		3807		3807		3775		3795		3795		3795		3807
Number of rats on diet	6		6		1		2		6		3		2		12	
Number of rats developing edema	3		2		0		0		1		1		0		2	
Per cent of rats developing edema	50		33⅓		0		0		16⅔		33⅓		0		16⅔	

Table 2 shows the results of a further study of edema in rats as produced by inadequate diets. The results given here are produced by feeding dry diets and supplying the rats with an unlimited amount of distilled water. In addition to a variation in the fats in these diets, the salts and the extract of wheat germ were varied. As in table 1, this table also shows that butter and the fat-soluble vitamine in butter do not alter the occurrence of edema in rats when there is a deficiency in protein. This table also shows that an increase in the salt content or the addition of the alcoholic extract of wheat germ does not influence or prevent the occurrence of edema.

developed edema on diet IX with no water-soluble-vitamine supplied. The percentage of rats developing edema as a result of the diets with lard or no source of fat, diets XII, VIII and XI, is again low but we cannot interpret this fact as meaning that fat is a cause of the edema, for in table 1 on diet III (no fat) 40 per cent of the rats developed edema. So the presence of "water-soluble B.' does not prevent edema from developing in rats on a low protein diet. McClugage and Mendel (30) report very poor utilization of salts where a diet is high in its content of indigestible cellulose from vegetables. In order to insure a greater assimilation of salts from the diet of carrots, which is very high in its content of indigestible matter, a diet was prepared in which the quantity of salt was doubled, diet XII (K + A + B + 2x salt) the same as diet V (K + A + B) except for its salt content. The percentage of edema was not markedly altered by the higher salt content in the diet. As the retention of salts by the tissues is a very well-known cause of edema, it is of interest to note that the percentage of edema developing on this diet of double salt content is not increased.

How does the water content of the diet influence the edema? Another factor in the production of edema is the water intake. The total percentage of edema developing from the diets of table 1 (wet diets) which were about 84 per cent water, is 52.4 per cent. The total percentage of edema developing from the diets of table 2 (dry diet) is 23.7 per cent. As the two series of experiments were conducted at different times the rats could not be selected from the same litters. In order to study this factor more carefully, another series of experiments was conducted, the results of which are recorded in table 3. In addition to this factor the salts were again varied to control this factor more completely, and a control diet was used in which casein was added as a source of protein.

In table 3 diets V (a) and XII (a) (dry diets) are the same as diets V and XII, table 2, respectively. Diets XIII and XIV (wet diets) are the same as V (a) and XII (a) table 3 respectively except for the water content. Eighty-six per cent of the rats on the wet diets developed edema whereas only 55 per cent of the rats on the dry diets developed edema. This percentage is much higher than that of the earlier work shown in tables 1 and 2. This is explained by the fact that the course of the edema was not so well understood in the early work. This edema is intermittent and may come on in a short time and disappear in a day. If no special notice was made of that particular animal at the time of the edema the case was overlooked. In the later work the animals were

watched more closely, hence no cases of edema were overlooked which accounts for the higher percentage of edema in the later experiments. In addition to this difference there is a decided difference in the length of time before the edema develops on the dry and wet diets. Rats on the wet diets develop edema and die in from 9 to 13 weeks, most of

TABLE 3

Table of dry and wet diets with results of feeding

	DRY				WET				DRY	
	V (a) K + A + B		XII (a) K + A + B + 2 × Salt		XIII K + A + B		XIV K + A + B + 2 × Salt		Control	
	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories	Grams	Calories
Carrots	550	1815	550	1815	4500	1800	4500	1800	550	1815
Starch	228	912	228	912	228	912	228	912	84	336
	Butter		Butter		Butter		Butter		Butter	
Fat	120	1080	120	1080	120	1080	120	1080	120	1080
Salt II	19.2	0	38.4	0	19.2	0	38.4	0	38.4	0
Salt III	16.4	0	32.8	0	16.4	0	32.8	0	32.8	0
Extract of wheat germ	50	0	50	0	50	0	50	0	50	0
Casein	0	0	0	0	0	0	0	0	144	576
Total		3807		3807		3792		3792		3807
Number of rats on diet	11		9		7		8		3	
Number of rats developing edema	5		6		7		6		0	
Per cent of rats developing edema	$45\frac{5}{11}$		$66\frac{2}{3}$		100		75		0	
	55				86					

Table 3 shows several very interesting and conclusive results. First, when the diet contains a high water content the percentage of edema in rats which are fed the low-protein diet, is markedly increased, an increase from 55 per cent to 86 per cent. Second, a variation in the quantity of salts in the diet had no effect on the occurrence or percentage of edema. With the single salt portion 12 rats out of 18 developed edema. With the double salt portion 12 rats out of 17 developed edema. Third, and very important, the table shows that when a sufficient amount of an adequate protein replaces an amount of cornstarch, of equal caloric value, no edema developed in rats as a result of feeding this carrot diet. Furthermore, on examination of figure 2, it will be noted that rats fed this diet, (the same as the low-protein-carrot-diet except for the protein content), grow and reproduce normally.

them being dead at the end of the 11th week. Quite a large number of the rats on the dry diets live 18 or even 30 weeks. However, a small number die as early as the 9th week. So an increased water intake increases the number of cases of edema developing in rats on a low-protein-diet.

In case of food shortages, therefore, when it is necessary to use only limited amounts of protein or to use proteins of a poor quality, it is of practical importance to use these foods in a relatively dry form rather than in the form of soups as is usually done to make the food more "filling."

The salt content of the diets in table 3 did not change the percentage of cases of edema. Out of seventeen rats on the double salt diet, twelve developed edema. Out of eighteen rats on the lower salt diet, twelve rats developed edema.

The edema is due to protein deficiency. In the control diet 144 grams of casein replaced 144 grams of starch, the caloric value, vitamine content, fat and salts remained the same. It should be noted that there were no cases of edema developing on this diet. Furthermore, the growth curves were normal (see fig. 1) for the rats on this diet. The females produced normally but showed some difficulty in rearing their young. However, rat 304 (see growth curve, fig. 1) gave birth to eight young weighing 35 grams at birth. After twelve days they had not shown normal growth and well-being. At this time the litter was reduced to four. At the age of six weeks the average weight of the four young was 55 grams and at the age of eight weeks, when they themselves ate of the control diet, the average weight of the four rats was 92 grams. They were active and in general good condition. This shows that the low-protein-carrot-diet used throughout the work to produce edema was adequate except for its protein content, for when sufficient adequate protein was added, no edema occurred and the rats grew and reproduced normally.

This control diet with adequate protein supplied by the addition of casein to the low-protein-carrot-diet was also used as a cure for rats that had developed edema on the low-protein-carrot-diet. In a number of cases a cure of the edema resulted when the edematous rat was put on this diet with adequate protein content, and the rat in question would become more active, would show a rapid increase in weight and also soon increase his food consumption. As long as the rat remained on this diet the edema did not return, but as soon as it was put back on the low-protein-carrot-diet it would lose weight, general symptoms

of malnutrition would appear and after a time the animal would die, some of the rats developing edema the second time. The most interesting and striking case is that of rat 165 whose weight curve is shown in figure 1.

Rat 165 was put on diet V (K + A + B), table 2, and after eating this diet for $11\frac{1}{2}$ weeks he had a large "blister" on the chest between the forelegs and showed a marked subcutaneous edema of the left

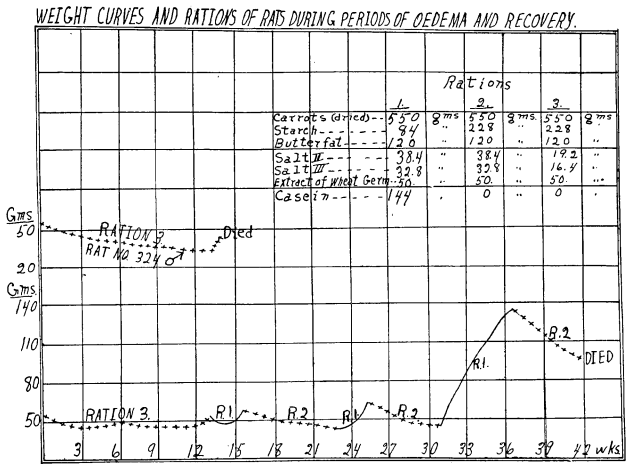


Fig. 1. The curve of rat 324 is a typical weight curve of rats kept on the low-protein-carrot-diet. There is a gradual loss in weight but when edema sets in a gain in weight occurs which is due to the accumulation of fluids in the tissues. No attempt was made to cure this rat.

The curve of rat 165 shows a gradual loss of weight while it was kept on the low-protein-carrot-diet, with an increase at the end of the 16th week due to the accumulation of fluid. Recovery followed a change in diet, the change consisting in giving adequate protein, with not much gain in weight, however. This animal developed a marked edema three times and was cured each time by a change to a diet containing adequate protein, a gain in weight and marked improvement in general condition resulting. During the last period of protein feeding, which was a relatively long period, the rat grew normally, its hair grew out perfectly over its entire body and it was very active. The rat finally died while on the low-protein-carrot-diet, without developing edema. At autopsy the lungs were found to be pneumonic, which was probably the cause of death.

side of the neck. After 3 days this edema had disappeared but after a few days it returned, the weight increasing from 45 grams to 52 grams due to the accumulation of fluid subcutaneously and in the tissues. At this time, 8 days after edema appeared for the first time, the rat was changed to the control diet (see table 3). In 2 days the edema about the chest and neck was entirely gone and only slight edema of the eyes remained, which cleared up in 2 more days. The weight of the rat had gone down to 47 grams. This finding is explained by the well-known observation of Bischoff and Voit (31) who noticed a decided loss in weight of a dog who had been kept on a diet of bread for 41 days, losing weight during this time; then when meat was administered in a quantity enough to cause a protein deposit, there was a decided loss in weight on the first day due to the loss of water.

After the above rat had been on this adequate diet a few days, increase in body weight followed but without any edema. This diet was continued for $2\frac{1}{2}$ weeks when the rat was put back on the low-protein-carrot-diet but with the double salt portion. At the end of $6\frac{1}{2}$ weeks the animal showed some slight signs of edema. This left but returned much more pronounced in a few days, again with a large "blister" on the chest. The rat was again returned to the control diet with adequate protein which was followed by disappearance of the edema in 14 hours. Again the animal became more active and showed rapid growth. His general appearance, however, was not normal for he had a great loss of hair and was not kept on the control diet with adequate protein long enough for new hair to grow. The animal was kept on this diet for 2 weeks during which time he gained 22 grams in body weight. He was again put back on the low-protein-carrot-diet with the double salt content. This was followed by the characteristic loss in weight and activity. During this period there was some growth of hair in the bare patches but the hair was not like the hair on the normal rat, it being very soft and fuzzy. After being on this diet for $5\frac{1}{2}$ weeks the rat developed edema for the third time, this time about the neck and face but without the large "blister." The control diet was given, and normal growth was resumed as shown in the weight curve in figure 1. In 2 weeks there was rapid growth of hair which was firm and normal. There were some patches of the fuzzy dirty hair left which resulted from the poorer diet, but this hair was gradually replaced by normal hair. The rat was kept on this diet for 6 weeks and at the end of this time was in splendid condition, active, had fine coat of hair and eyes in excellent condition. The rat was put back on

the low-protein-carrot-diet and lost weight again, this time more rapidly. At the end of about 4 weeks the animal died without showing any signs of edema. He had been quite weak for some time and seemed to breathe with difficulty. After death the lymph glands were slightly congested, the lungs were congested and pneumonic. The cause of death was probably pneumonia which was contracted due to his low resistance as a result of the diet deficient in protein.

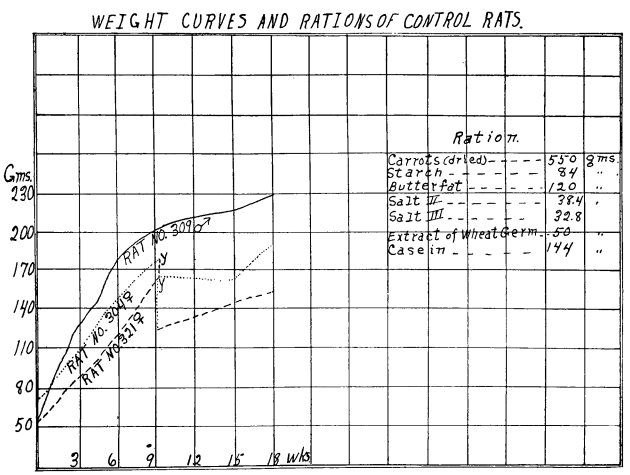


Fig. 2. The weight curves of the three control rats are given in this chart. These rats were fed the carrot diet containing an adequate amount of protein. (One is a male, the other two are females.) All showed normal growth and reproduction. The females gave birth to normal young (at *y*), but had some difficulty in rearing them. However, rat 304 succeeded in rearing a litter of young to the weaning stage and then the young ate the control diet and also grew normally with the control diet as their only source of food. These charts show that rats kept on a carrot diet with adequate protein do not develop edema.

Cure did not result in all cases of edema where the control diet was given to the rat. In a number of cases the rats were so feeble they refused to eat the food. The rats were usually left on the low-protein-carrot-diet until the edema had developed to an extreme degree and probably to such a degree that death would come before a sufficient

quantity of protein was assimilated. However, the writer feels confident that recovery would result in all cases if the diet were corrected soon enough, for in the control rats not only was edema averted, but none of the symptoms appeared that precede the edema where it develops.

In addition to the feeding done with the low-protein-carrot-diet, a lot of 3 rats was fed on a diet made up of 87 grams cornstarch, 5 grams butter fat, 4 grams of a complete salt mixture, 2 grams agar, 2 grams of casein and the alcoholic extract of 6 grams of wheat germ. This diet is adequate except for its protein content which makes up only 2 per cent of the diet. The general condition of the rats of this lot throughout the course of this experiment was about the same as that of the rats on the low-protein-carrot-diet, losing weight at about the same rate and having the same condition of hair and skin. One out of three of these rats developed edema just as those rats on the low-protein-carrot-diet did. The other two did not develop edema but showed the same signs of malnutrition.

The edema is not due to the low-calorie intake of the edema rats. Some observers have said that the edema that occurred in the war-stricken countries during the war was due to a low intake of calories. The food consumption records of my experimental animals show that the rats on the low-protein-carrot-diet did not consume as much of the diet as those who received adequate protein in their diets, although the same amount of food was given to each. Low calorie intake therefore had to be considered as a possible cause of the edema in these rats.

That low calorie intake is not the cause of the edema in the rats in this work is shown by figure 3, which gives the results of a study of this factor in the production of edema. Curve *I* is a composite weight curve of the three control rats which received 18 per cent of purified casein in their diet. Curve *III* shows the average weekly food consumption of these rats in calories. Curve *II* is a composite weight curve of six rats on the low-protein-carrot-diet. Curve *IV* shows the average weekly food consumption of these rats in calories. The only source of protein in the diet of the rats of curve *II* is the carrot in the diet. Curve *V* is a composite weight curve of five rats whose food consumption was limited in calories to that amount shown in curve *IV*. The diet of these rats was made up the same as that of the rats whose weight curve is represented by curve *II*, but with sufficient casein replacing starch in the diet to give each rat 1 gram of casein per day, which is approximately the amount of casein consumed by the control

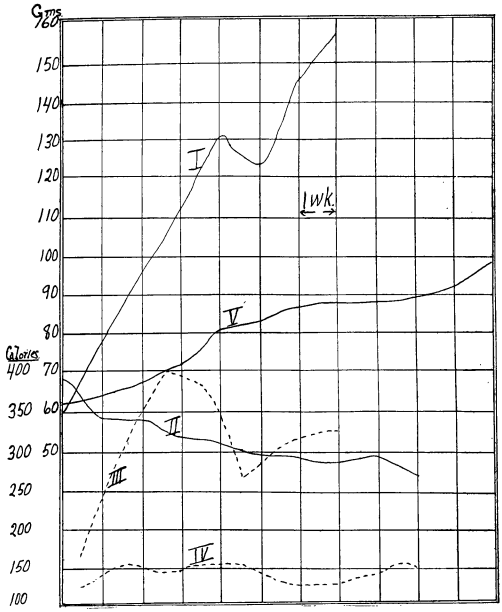


Fig. 3. The curves of figure 3 show that the low calorie intake of the rats used in this work is not responsible for the development of the edema.

Curve *I* is the composite growth curve of rats with 18 per cent casein in their diet. Curve *III* represents their average weekly food consumption in calories. Curve *II* is the composite weight curve of rats on the low-protein-carrot-diet. Curve *IV* represents their average weekly food consumption in calories. Curve *V* is the composite growth curve of rats whose food consumption is limited to that represented in curve *IV*, i.e., the same as the rats whose weight curve is shown by curve *II*, which means that the rats of curve *V* received a low-calorie diet; but the rats of curve *V* were fed adequate protein, 1 gram of casein per day being fed and cornstarch, of the caloric value of the gram of protein added, being withdrawn. So the rats in curve *V* received a low-calorie diet with adequate protein. These rats (curve *V*) did not show edema but slowly gained in weight. As pointed out before, the rats of curve *IV* showed a large percentage of edema. Which proves that the comparatively low-calorie intake of the edematous rats was not the cause of the edema, for edema did not develop in the rats (curve *V*) receiving adequate protein with low-calorie intake.

I. Composite weight curve of 3 rats on carrot + 18 per cent casein diet.

II. Composite weight curve of 6 rats on carrot—low-protein diet.

III. Curve showing average weekly food consumption in calories of rats of curve I.

IV. Curve showing average weekly food consumption in calories of rats of curve II.

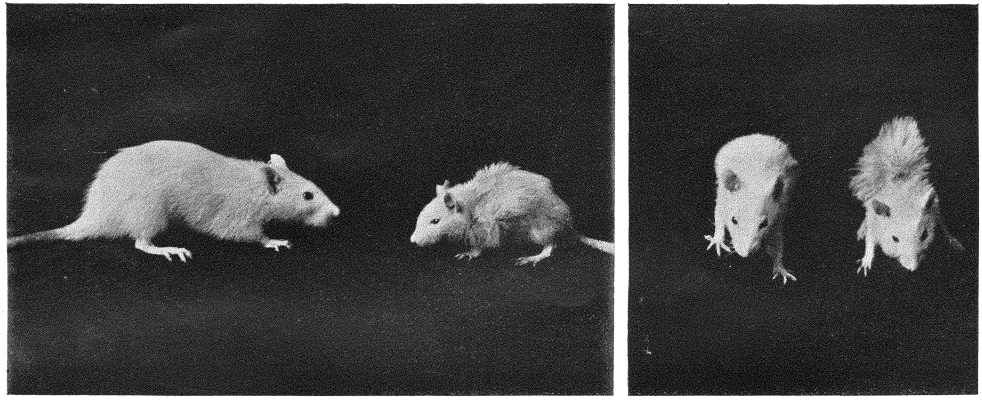
V. Composite weight curve of 6 rats on low calorie diet with adequate protein.

rats. There is a slow gradual gain in weight of these rats which was still continuing at the time the experiment was stopped. The rats showed a slight tendency to the lesions described in the rats kept on the low-protein-carrot-diet, which shows that these lesions are not specific for the absence of adequate protein in the diet, but due to an infection as a result of lowered resistance. These lesions could be cured by the application of vaseline. With the exception of the lowered resistance and the retarded growth, these animals appeared to be in excellent condition, the hair and eyes being normal as well as appetite and activity. If the low calorie intake was the cause of the edema in rats, these rats should have at least shown some edema at the time the experiment was stopped, for they had been kept on this low protein diet 14 weeks. The diet was fed in the wet form. When rats of the same age and weight had been fed the wet low-protein-carrot-diet (results shown in table 3) the rats were all dead at the end of 13 weeks and a large percentage of them had developed edema.

In additon to the work recorded in figure 3, some rats were fed a diet of even lower calorie value, the food consumption being reduced by 16 per cent, but each rat was still receiving 1 gram of purified casein per day. Some of these rats lost weight and died without showing edema and some were growing and in splendid condition when the experiment was stopped. So the low-calorie intake of the edematous rats is not the cause of the edema.

The edema is not due to some "toxic" effect of the carrots. The writer has been told that the people of Germany who used vegetables as such a large part of their diet and suffered from war edema are said to have called the disease "Rübenkrankheit." The low-protein-carrot-diets which were the cause of the development of edema in these rats had a very high carrot content and some might say that there may be some toxic product in the carrots that impairs the kidney or some other tissue of the body and makes it impossible to eliminate fluids from the body. This cannot be the case, for the control rats receive the same proportion of carrots in their diets and are perfectly normal. Furthermore one lot of three rats was fed on a low-protein-diet (2 per cent casein) with no carrots, but with the calories, usually supplied by carrots, supplied by cornstarch. The rats followed the same general course and one developed edema just as the rats on the low-protein-carrot-diet.

Why do not all of the rats develop edema and why is the edema intermittent? It is difficult to explain why some of the rats on this low-protein-diet develop edema and others do not. There is no relation



Rat 321 Rat 319 Normal rat Rat 319

Fig. 4. Rats 321 and 319 are from the same litter. The photograph was made after the rats had been feeding on experimental diets for eight weeks. Rat 321 received the control diet with adequate protein in the form of pure casein. It has grown normally (as shown by the photograph and by the growth curve in figure 2) and later reproduced normally. Rat 319 received the low-protein-carrot-diet and at the time the photograph was made was showing the early signs of edema, as shown in both the side view and front view of the rat. Its cheeks puff out and its eyes are partly closed. The normal rat was selected from the stock rats for a comparison of the normal shape of the face with the edematous face. It is a much younger rat than rat 319 but was selected because it was about the same size as No. 319. A comparison of the size of the eyes and the width of the face is quite striking.

between age or weight and the development of edema, neither is one litter more liable to edema than another. All of the rats on this diet grow weak and are quite similar in showing all the other symptoms of malnutrition. A muscular wasting is evident in all and an atrophy of the testicles of the male is quite evident. No doubt histological work would reveal atrophy in all the tissues. But why should the tissues of one rat undergo changes which cause them to retain fluids to this excessive degree and those of another rat not undergo these same changes, when both rats are from the same litter, have the same weight, and have the same environment and are receiving equal amounts of the same kind of food? Neither is there any relation between the amounts of food consumed and the development of edema; the food consumption varies among the rats that develop edema just as it varies among the rats that do not develop edema.

Another fact that is difficult to explain is the intermittency of the edema in many of the rats. This too is very irregular. Sometimes rats will show edema 6 or 8 weeks before their death. It disappears and may or may not return, or it may return and disappear several times. Very often rats will show an extreme edema, such as has been described as a "blister" on the chest and very often this disappears almost entirely in 12 to 24 hours, without any change in diet. Why do the tissues retain the fluid at one time and then change so suddenly? Could it be, perhaps, that the absence from the diet of sufficient amount of certain specific substances, whose absence is responsible for the development of the edema, causes the tissues generally or certain specific organs to undergo changes (as they do in actual starvation) which leads to the liberation of these specific substances and in turn to the temporary disappearance of the edema? No doubt a clearer knowledge of the tissue changes, that are responsible for the retention of the fluids, would throw some light on the subject.

How does the acid content of the diet influence the edema? An attempt was made to determine more accurately the changes that take place in the tissues to enable them to retain water to the striking degree that occurred in these edematous rats. As acidosis is a suggested cause of edema this was one of the first factors to be considered. The ordinary methods for determining this condition seemed difficult to apply in such small animals for it would be difficult to obtain sufficient blood without sacrificing the animal, which course did not seem practical in this stage of the work. As an indicative measure a diet was made using salt mixture VI instead of salt mixture II. Salt mixture VI has

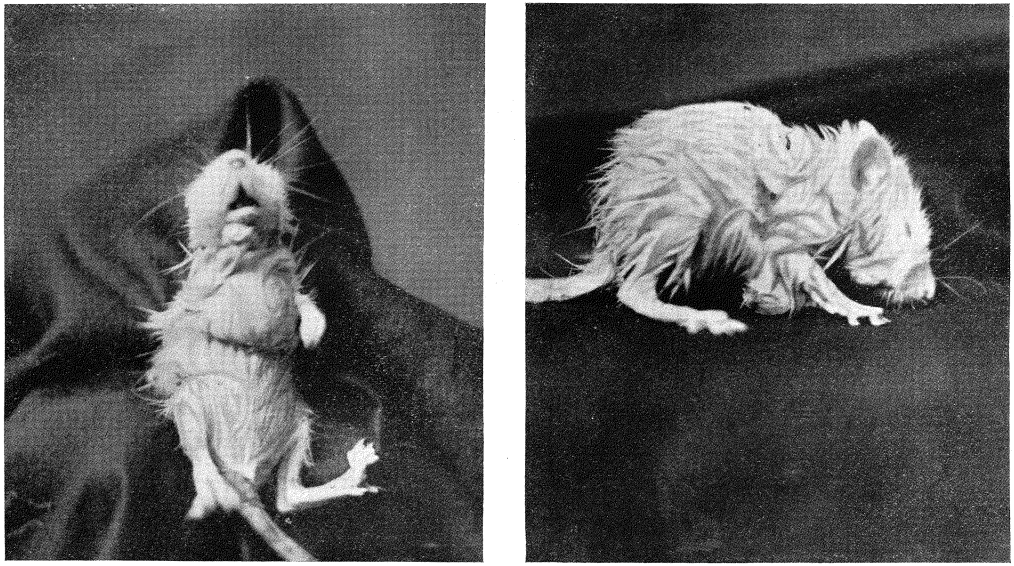


Fig. 5. Rat 302 is a typical case of the edema in the form of a "blister" on the chest. The hair of the rat was moistened with water and brushed down in order to give a better view of the large amount of fluid collected subcutaneously on the chest of the rat. This rat was very weak at the time the photograph was made and died about 12 hours later, but other rats with the same type of edema and of the same degree were cured by the administration of a diet of the same caloric but with an adequate protein content.

slightly more of the acid salt $Ca(H_2PO_4)_2$ in place of some of the calcium lactate as in salt mixture II. With the exception of this small change in the acid content this new diet was identical with diet XII (see table 3). As a control diet XII (a) was used. Of the rats fed this diet with increased acid content, 88 per cent (8 out of 9 rats) developed edema whereas on the control diet only 50 per cent (3 out of 6 rats) developed edema. It should be noted, however, that when diet XII (a) was fed earlier, as recorded in table 3, 66⅔ per cent of the rats developed edema, a somewhat greater percentage than occurred in this experiment. These results would indicate that the increased acid content of the diet so alters or injures the tissues as to make them more likely to retain water. That the acid itself is not the cause of the edema is shown by the fact that one of the rats that had developed edema on this more acid diet was cured by putting him on a diet with the same acid content but with 18 per cent pure casein replacing 18 per cent cornstarch. This rat was not only cured but in the succeeding 5 weeks increased in body weight 26 grams. This strengthens the evidence for the curative value of adequate protein in cases of edema of this type since it is here successful in curing the edema with the adverse condition of the higher acid content present.

The relation of the kidney to the protein deficiency. There is some indication that the kidney cells are injured and therefore fail to eliminate the water. Although very little work was done on the kidney tissues, sections were made of a few kidneys and some albumin found in the tubules. Attempts were made to test the urine. It was difficult to collect uncontaminated urine from the rats so that the urine was collected, after death, from the urethra (where urine remained in the bladder) by slight pressure on the bladder. Positive tests for albumin were obtained but when the normal rats were killed and the urine collected in the same manner and tested, positive results were also found which made the above experiments of no value. And yet it seems probable that the albumin in the tubules would follow the current of urine into the bladder.

In this connection it is interesting to note the work of Epstein (32), who recommends a diet high in well-selected proteins and low in carbohydrates and fats, in cases of edema in certain types of chronic nephritis. He further suggests that chronic parenchymatous nephritis might be genetically a disorder of nutrition. Allbutt (33) reports a case of renal dropsy cured by a high protein diet.

It is reported by Captain McCay (34) that renal disease is more common among the population of Bengal than among the Europeans who consume more protein than the Bengali.

One of the long recognized results of a high protein diet is the impairment of kidney cells. Chittenden (35) emphasizes the importance of limiting the "daily intake of protein food to as low a level as is consistent with the true needs of the body, in those cases where the kidneys are at all enfeebled or where it seems desirable to exercise due precaution as a possible means of prevention." From the evidence here given it would seem that there is equally as great danger of renal impairment from a low protein diet. This follows very reasonably for certainly there is a weakening of all the tissues, quite evident in the general muscular weakness of the animal which is certainly due to a specific protein starvation of the cells.

It is reported (3) that often edema did not occur among people when on the restricted war diet, until they did hard work. It is further reported that rest in bed along with dietary changes was very beneficial. This would indicate that muscular work is a predisposing factor to the edema, which seems very probable. If the tissues of the body are not receiving enough food, certainly muscular work would relatively diminish the amount of food. Furthermore, during muscular work lactic acid is formed and according to Fischer (36) acid renders the tissues able to hold more water.

CONCLUSIONS

1. When young rats are fed diets composed largely of carrots and with carrots as the only source of protein, a large percentage of the rats develop edema.

2. Fats or fat-soluble-vitamine do not prevent the occurrence or decrease the percentage of edema in rats, even if 10 per cent of the calorie value of the diet is made up of butter-fat.

3. The water-soluble-vitamine does not prevent the occurrence or decrease the percentage of edema in rats fed the low-protein-diet.

4. Salts do not play any appreciable rôle in the production of this type of edema, for even when the salt content is doubled there is no noticeable effect upon the occurrence of edema.

5. The water intake of the animal which is feeding on the low-protein-carrot-diet decidedly influences the development of the edema. Edema develops more frequently, is more severe and develops sooner on a wet diet than on a dry diet.

6. When a sufficient amount of an adequate protein is added to the low-protein-carrot-diet, in place of an equivalent amount of cornstarch, edema is not only averted in rats feeding on this diet but the rats show normal growth and reproduction.

7. That the edema does not develop as a result of some toxic substance in the carrots is shown by the fact that rats fed on the control diet with the same carrot content as the low-protein-carrot-diet but with adequate protein did not develop edema and showed normal well-being.

8. When the acid content of the diet was increased the percentage of rats developing edema was increased from 50 per cent to 88 per cent. That the acid in this diet was not the chief etiological factor of the edema is shown by the fact that one rat on this diet was cured and made to grow normally on the diet with no change in the acid content but with an adequate protein supply replacing an equivalent amount of corn starch in the diet.

9. The edema manifested in these rats is not due to simple starvation, or low caloric intake, for when rats are fed a diet, including 1 gram of adequate protein each day, of the same caloric value as the diet fed the edematous rats, they do not develop edema.

10. This edema can be successfully cured by supplying the diet with adequate protein. Edema was produced and cured three times in one rat by alternating low-protein-diet (edema-producing) with an adequate protein diet.

11. These findings warrant the general conclusion that if it is necessary to limit the amount of protein in a diseased condition or in a period of national economic stress (as was necessary in some of the European countries during the recent war), it is advisable to administer the low protein diet in a form free from excess of water and any acid producing foods. Symptoms of developing edema must be looked for and adequate protein supplied immediately to effect a cure.

Note 1. I wish to express my deepest gratitude to Dr. A. B. Luckhardt for his keen interest and enthusiasm for the work throughout its course and for the many helpful suggestions that were offered from time to time; and to Edgar C. Turner for his great interest and excellent assistance rendered in taking care of the animals.

Note 2. The wheat germ used in all of these experiments was furnished by the Washburn-Crosby Milling Co.

BIBLIOGRAPHY

(1) BUDZYNSKI AND CHECHOWSKI: Journ. Trop. Med. and Hyg., 1916, xix, 141.
(2) LANDA: Gaceta Med. de Mex., 1917, xi, 67; Physiol. Abstracts, 1918–19, iii, 124.
(3) JÜRGENS: Berl. klin. Wochenschr. Cit. Journ. Amer. Med. Assoc., 1916, lxvi, 1278.
(4) WELLS: Journ. Amer. Med. Assoc., 1918, lxxi, 954.
(5) PARK: Journ. Amer. Med. Assoc., 1918, lxx, 1826.
(6) JAKSCHE: Wien. med. Wochenschr., 1918, lxviii, 1030; Physiol. Abstracts, 1918–19, iii, 375.
(7) KNACK AND NEUMANN: Deutsch. med. Wochenschr., 1917, no. 29.
(8) MAASE AND ZONDEK: Berl. klin. Wochenschr., 1917, no. 36.
(9) MAASE AND ZONDEK: Deutsch. med. Wochenschr., 1917, no. 16.
(10) KRAUS: Berl. klin. Wochenschr., 1919, lvi, 3.
(11) GUILLERMIN AND GUYOT: Revue Med. d. la Suisse Romande, Geneva, 1919, xxxix, no. 3.
(12) MCGARRISON: Indian Journ. Med. Research. Cit. Brit. Med. Journ., 1919, i, 177.
(13) See editorial, Journ. Amer. Med. Assoc., 1914, lxiii, 1856.
(14) RUBNER: Report made in the Reichsgesundheitarat on December 20, 1917.
(15) BEYERMAN: Nederlandsch Tydschr. v. Geneeskunde, Amsterdam, 1919, i.
(16) JANSSON: Finske Läkaresällskapets Handlinger, 1919, lxi, 235.
(17) VANDERVELDE AND CANTINEAU: Cit. Journ. Amer. Med. Assoc., 1919, lxxiii, no. 16.
(18) TONIN: Gaz. d. Ospedali e. d. clihiche, Milan. Cit. Journ. Amer. Med. Assoc., 1919, lxxiii, 1095.
(19) REACH: Wien. klin. Wochenschr., 1919, xxxi, 1249. Cit. Endocrinology, 1919, iii, no. 3.
(20) DENTON AND KOHMAN: Journ. Biol. Chem., 1918, xxxvi, 249.
(21) KOHMAN: Proc. Soc. Exper. Biol. and Med., 1919, xvi, 121.
(22) MCCOLLUM AND DAVIS: Journ. Biol. Chem., 1915, xx, 657.
(23) OSBORNE AND MENDEL: Journ. Biol. Chem., 1914, xvii, 401.
(24) MCCOLLUM: Journ. Amer. Med. Assoc., 1918, lxxi, 939; Journ. Biol. Chem., 1919, xxxiii, 60.
(25) STEENBOCK, BONTWELL AND KENT: Journ. Biol. Chem., 1918, xxxv, 517.
(26) FUNK AND MCCOLLUM: Journ. Biol. Chem., 1916, xxvii, 52.
(27) MCCOLLUM AND DAVIS: Journ. Biol. Chem., 1915, xx, 657.
MCCOLLUM AND SIMMONDS: Journ. Biol. Chem., 1917, xxxii, 181.
(28) Hirsch's Handb. of Geog. and Hist. Pathol., ii, 56.
STEENBOCK: Sci. Monthly, August, 1918.
(29) MCCOLLUM AND SIMMONDS: Journ. Biol. Chem., 1917, xxxii, 181.
(30) MCCLUGAGE AND MENDEL: Journ. Biol. Chem., 1918, xxxv, 353.
(31) BISCHOFF AND VOIT: Gesetze der Nährung des Fleischfressas, 1860, 211.
(32) EPSTEIN: Journ. Amer. Med. Assoc., lxix, 444; Amer. Journ. Med. Sci., 1917, cliv, 638.
(33) ALBUTT: Brit. Med. Journ., 1918, ii, 395.
(34) MCCAY: Investigations on Bengal jail dietaries, Scientific memoirs of India, no. 37, 1910, 204.
(35) CHITTENDEN: The nutrition of man, 1907.
(36) FISCHER: Oedema and nephritis, 2nd ed., 1915.