THE PRODUCTION OF EDEMA AND SERUM PROTEIN DEFICIENCY IN WHITE RATS BY LOW PROTEIN DIETS.

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This study was undertaken in an effort to learn something of the mechanism of the production of malnutrition edema. It was decided to attempt to repeat the work of Kohman (1) who produced the condition experimentally by feeding low protein diets, and to study the effect of such diets on the serum proteins.

In 1895 Starling (2) first showed that the colloid osmotic pressure of the serum was proportional to the concentration of protein in it. He advanced the theory that decreased osmotic pressure in the serum was responsible for the deposition of water in the tissues. Disagreeing with Starling, Martin Fischer (3) claimed that edema resulted from alteration of the hydrogen ion concentration of blood and tissues which influenced the imbibition of fluid by the tissue proteins. Subsequent work has not supported Fischer's theory, but has increasingly substantiated that of Starling.

Govaerts (4) measured the colloid osmotic pressure of the serum of individuals, normal and otherwise, and found that an osmotic pressure lower than a certain figure was associated with a tendency toward edema (5). This lowered pressure could be caused by reduction in the total amount of protein, but especially by a loss of serum albumin (6). If the capillary pressure exceeded the osmotic pressure of proteins, water was deposited in the tissues. This held for edemas caused by stasis as well as others. Schade and Claussen (7) confirmed Govaert's work in all respects by their "onkometer" methods. In this country, Epstein (8) found lowered serum proteins in certain cases of renal disease, especially the type called nephro-

* The data in this paper are taken from the thesis of Dr. R. A. Frisch done in partial fulfilment of the requirement for the degree of Doctor of Medicine at Yale University School of Medicine.
sis, and suggested that, in order to relieve the edema, the serum protein be raised by increasing the protein intake. Peters (9) has emphasized the prevention of malnutrition in patients with renal disease by adequate dietary protein. He found (10) that in cases of malnutrition of any kind, the serum proteins were lowered and there was a tendency toward water retention.

During and just after the late war, edema was noticed in Central Europe where people were forced to subsist largely on turnips and other root vegetables for long periods of time. Schittenhelm and Schlecht (11) studied the condition in prison camps and decided that rest and increased protein and fat in the diet relieved the edema. They estimated serum proteins by refractometer methods and found that refractometric indices were low in 50 per cent of the cases. Finally the disease was attributed to a lack of protein and possibly fat in the diet. The symptoms were not those of a lack of vitamins. Jansen (12) found the condition similar to cachectic edemas reported by Govaerts in the reduction of serum protein, particularly of the albumin fraction.

In 1918 Denton and Kohman (13), while working on the dietary properties of carrots, noticed that some of their animals, which were fed on little else over extended periods, developed edema. In later work both Maver (14) and Kohman (1) found that a certain number of white rats developed edema when fed diets containing carrots, cornstarch, lard, and salts for periods of 8 to 12 weeks. Various food elements were added to the diets in an effort to bring them back to normal; but nothing succeeded that did not contain protein. Kohman (1) thought that the symptoms produced might be related to the famine edema of the war. Her diets eliminated from consideration every factor except the protein and she attributed the condition to a deficiency of that element. This work seemed worthy of repetition and further investigation. It was decided, first, if possible, to produce edema by the carrot diets and then learn a little more of the mechanism of its production by a study of the serum proteins.

EXPERIMENTAL.

The two diets whose composition is shown in Table I were chosen. They were the same except for the fact that in the second the carrots were dried at low temperature while, in the first, they were merely ground up fresh and mixed. The amounts of carrots fed gave an ample supply of vitamins. Kohman at first questioned the supply of vitamin B, but found that the addition to
the diet of wheat germ did not alter the experimental results. She could detect no difference when the salt content was varied. The only source of protein is that contained in the carrots.

Young, growing albino rats varying in weight from 50 to 70 gm., obtained from the Yale Department of Physiological Chemistry and Connecticut Agricultural Experiment Station strains, were used.

Blood samples for serum protein determinations were drawn by heart puncture without exposure to air (15). Because of the size of the animals it was impossible to get sufficient blood to determine protein fractions nor could the total amount of serum protein be corrected for the non-protein nitrogen. 0.5 to 1 cc. was the usual

| TABLE I.  
Composition of Diets. |
<table>
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<tr>
<td></td>
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<tr>
<td>Carrots</td>
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<tr>
<td>Starch</td>
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<tr>
<td>Lard</td>
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<tr>
<td>Salt mixture*</td>
</tr>
<tr>
<td>Carrots</td>
</tr>
<tr>
<td>Starch</td>
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<tr>
<td>Lard</td>
</tr>
<tr>
<td>Salt mixture*</td>
</tr>
<tr>
<td>Proteins</td>
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<td>Carbohydrates</td>
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amount of blood obtained and special tubes were made for centrifuging it. The gasometric micro-Kjeldahl method of Van Slyke (16) was used after it had been found to check excellently with the usual macro-Kjeldahl method. All determinations were made in duplicate on the diluted serum. The omission of non-protein nitrogen determinations cannot alter the deductions which have been made from the analytical data. The total amount of such nitrogen in normal rat blood is so small that its complete elimination from the blood as the result of the experimental procedure would have no important influence upon the reported serum protein changes.
In the first group of animals only those on the dry diet were given extra water. The animals on the wet diet could not eat enough to keep up to their normal caloric level and maintained a lower weight than the others, although their growth curves (Fig. 1) are essentially the same in character. At first there is a rapid decline in weight, no doubt due in large measure to adjustment to the diet; later there may be a slight recovery followed by a slow decline until the animal reaches a level at which it stays until a week or two before death, when there are fluctuations, more marked in some cases than others. Generally the life of a rat on the dry diet was longer than that of one on the wet. Toward the end the animals became very emaciated and their hair lost its
Frisch, Mendel, and Peters

smooth appearance. They presented no eye symptoms nor paralyses. Their deaths as observed in two cases were not marked by convulsions nor signs of spasticity.

Edema, unless considerable, was difficult to observe in the living animal. It was variable from day to day and slight puffiness about the face and neck could not be easily detected. The condition was usually most noticeable in the dependent parts; namely,

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet.</th>
<th>No. of animals</th>
<th>Average calories per day</th>
<th>No. of animals that died*</th>
<th>No. of animals that developed edema.</th>
<th>Total.</th>
<th>Demonstrable during life</th>
<th>No. of serum protein determinations.</th>
<th>Average serum protein, per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Wet diet without extra water.</td>
<td>6 20</td>
<td>3 Ex</td>
<td>2 Ex</td>
<td></td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3.2</td>
</tr>
<tr>
<td>II</td>
<td>Wet diet with extra water.</td>
<td>14 22</td>
<td>9 Ex</td>
<td>8 Ex</td>
<td></td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>3.8</td>
</tr>
<tr>
<td>III</td>
<td>Wet diet with protein.</td>
<td>5 45</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>6.9</td>
</tr>
<tr>
<td>IV</td>
<td>Dry diet without extra protein.</td>
<td>5 40</td>
<td>1 Ex</td>
<td>4 Ex</td>
<td></td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3.2</td>
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</table>

* Ex, died as a direct result of the experimental procedure. RI, died as a direct result of respiratory infection. HS, died as a direct result of heart puncture.

the anterior thoracic and abdominal walls. Sometimes when the skin was pricked with a needle, a drop of fluid would exude. Upon autopsy, general anasarca of varying degree was the most striking phenomenon. There was practically no gross fat visible and the muscles were small and pale. No other abnormalities were noted except in animals which died as a result of infection, when lung changes were observed.
The experimental results are epitomized in Table II.

Groups I and II in Table II show the result of the wet diet. Kohman did not supply extra water because of the amount in the diet, but in these investigations it was decided to allow the animals, except those of one group, to drink as much as they wished, as did those on the dry diet. However, they took very small amounts. Of six animals of Group I without extra water, edema appeared after about 10 weeks on the diet in two, one of whose serum proteins were 3.0 per cent. Those of one which showed no edema, but merely extreme emaciation, amounted to 3.4 per cent. Of Group II of fourteen animals, eight showed water in the tissues after 8 to 14 weeks, three of them to a most extreme degree. The average of six protein determinations was 3.8 per cent. The serum
protein content of those animals which did not develop edema was uniformly slightly higher than the proteins of those which did. Just before the animals were put on the experimental diet, blood samples for serum protein determinations were drawn from six of the same group of approximately the same weight and age. The average of the serum proteins of these six normal animals was 5.5 per cent. The average of five adult rats taken at random on adequate diets was about 8.0 per cent.

To determine the adequacy of the wet diet with respect to other factors than protein, five animals were put on a diet of the same materials and composition except that the starch up to 18 per cent of the total calories was replaced by vitamin-free casein (Harris). These animals presented a marked contrast to those of the previous

**Fig. 3.** Curve illustrating the fall of serum proteins on the dry diet. The solid points = individual observations; the circles = animals which developed edema.
groups. They continued to gain in weight at first as rapidly as those on a standard diet, later somewhat more slowly. Their appearance was always healthy, they showed no edema, and ate an increasing instead of decreasing amount of food. Fig. 2 is a composite growth curve of the group. Blood samples for serum protein determinations were drawn after 11 to 12 weeks on the diet. The results of these are shown in Table II, Group III.

The serum proteins were very slightly lower than those of the five normal adults, but considerably higher than those of the six young animals before the start of the experimental period.

Table II, Group IV, shows the results of animals on the dry diet. They were allowed to drink as they wished. The only animal of this group which did not show edema died at about 7 weeks of age as a result of a lung infection. The others all showed definite signs after 9 to 14 weeks on the diet. Serum protein determinations made on two animals averaged 3.2 per cent. The two survivors, one male and one female, one of which had blood drawn for analysis, were then put on a diet the same in every respect as before except that the starch up to 18 per cent of the total calories was replaced by vitamin-free casein (Harris). They lost their edema, gained weight rapidly, and were mated after 2 months, when the male weighed about 300 and the female 200 gm. A litter of eleven resulted, all of which lived and gained well until after weaning, when they were discarded.

In another group of animals the curve of the serum proteins throughout the experimental period was studied. Fig. 3 shows the results. The figures for the six normal animals determined previously were taken as a standard and samples drawn at intervals. There was a gradual decline in the amount of serum protein. The last three animals showed some degree of edema while living and post mortem, and all of these had serum protein values lower than 4.0 per cent.

**DISCUSSION.**

The number of animals used was too small to permit deduction as to the frequency with which edema will develop on these diets, but there is no question that it does appear as a direct result of the diets. In Kohman's work the animals on the wet diet showed edema more frequently than did those on the dry, while in this
Frisch, Mendel, and Peters

investigation the relation seems to be reversed. However, larger groups of animals would give a better comparison of the two diets. Those on the dry diet certainly ate more food and more protein calories. A better method of determining the degree of water retention than gross examination might give better figures for comparison as well as clarify some of the apparently negative results. Nevertheless an edema has been produced in healthy animals as the direct result of a nutritional deficiency.

Everything seems to point to lack of protein as the nutritional defect responsible for this condition. The animals on the wet diet ate less than 0.04 gm. of protein per gm. of rat per day and those on the dry diet less than 0.12 gm. This is definitely less than their protein requirement. Kohman could find no other factor responsible. She tried diets with greater amounts of fat, greater

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<tr>
<td>No. of determinations.............</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Average serum proteins, per cent.</td>
<td>8.0</td>
<td>6.9</td>
<td>5.5</td>
<td>3.6</td>
<td>3.0</td>
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</table>

and smaller amounts of salt, and extra vitamins, but her results remained unchanged until protein was added. Finally, with a synthetic diet complete in every respect except for protein, she produced edema in a small number of animals. In our studies the addition to the wet diet of casein alone caused the animals to run an entirely different course. This and the fact that two animals which had developed edema on the dry diet, after the mere addition of casein grew and reproduced normally, point strongly to the protein deficiency as the cause of the edema.

The determinations of serum proteins show a marked reduction in almost every case after a period of 2 to 3 months on the carrot diet. This fact is in keeping with previous experimental work on the relation of edema and serum protein deficiency. The serum proteins of these rats, in general, were lowered about 40 per cent from the figure at the start of the experimental period. However,
young rats seem to have less serum protein than the adult, a fact which indicates that the actual reduction is greater. Table III shows the average serum protein values of each of the groups studied.

There has not been a sufficient number of determinations to detect variations between the sexes, but there has been no indication of any great difference. Both sexes were used indiscriminately. The observed reduction in the serum proteins has been the direct result of low protein feeding. A similar reduction might occur with other dietary deficiencies, such as some vitamin lack. Certainly, where the problem has been studied, serum proteins have been found lowered in humans with malnutrition from any cause, but no report of any reduction produced experimentally has been published until the work of Leiter (17). He bled dogs, centrifuged off the plasma, mixed the cells with normal saline, and replaced them in the animals. By this means the serum proteins were lowered and an edema developed. His work has been confirmed by Darrow and Hopper (18) in the Yale Department of Pediatrics. In infants with digestive disorders, who were being fed on diets of flour or rice and water and developed the condition called Mehlnährschaden by Czerny, Gorter (19) noticed edema along with the other symptoms. If serum proteins had been determined they probably would have proved to be low as a result of the protein starvation, as was found in the famine edemas. These infants improved rapidly with added protein in the food and blood transfusion. Neither butter, salt, nor sugar added to the food relieved the condition.

The serum proteins are, of course, only partly responsible for the production of edema. Here, as in other conditions, they determine a tendency toward water retention which is influenced by other factors. The fluid intake is very important, for if it is reduced in any way, voluntarily or by some other means such as vomiting, no edema develops, even if the serum proteins are actually quite low. In the experiments described fluids were neither forced nor restricted, but the animals were allowed to drink as they wished. The rôle of salt has been studied a great deal, but its importance is not yet clear. More complete studies of the nitrogen metabolism, particularly the nitrogen equilibrium, in these animals might clarify the picture. Blood electrolyte
studies would help if they could be made. The basal metabolic rate probably would be found lowered as in malnourished humans. However, since Starling's first work, the lowering of the serum proteins has assumed increasing importance and appears to be the factor most concerned in the production of edema not caused by mechanical stasis.

SUMMARY.

A study has been made of the edema produced by Kohman in white rats by means of low protein diets. Kohman's work has been confirmed. Serum proteins were determined in normal rats and in rats on the experimental diets. They were found to be reduced in animals on the low protein diets, whether or not edema developed.

The authors wish to take this opportunity to express deep appreciation for the aid and advice of Dr. Anna J. Eisenman in the blood analyses.

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