THE EFFECT OF CARROT FEEDING ON THE SERUM PROTEIN CONCENTRATION OF THE RAT*

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In a previous paper (1) it was suggested that the eating of large amounts of carrots might be followed by a lowering of the plasma proteins and the indirect evidence favoring this hypothesis was analyzed. The present experiments were designed to test the question more directly.

Material and Methods

The methods used in the previous experiments were followed in the present work. In brief, young but mature white rats (150–220 gm.) previously on an adequate stock ration were placed on the experimental diet. After various intervals groups of four to six animals were exsanguinated under ether anesthesia, aliquot parts of serum from each were pooled and the total serum proteins were measured by the gravimetric method of Barnett, Jones and Cohn (2).

The Experimental Diet.—In order to reduce the problem to its simplest terms the diet consisted exclusively of carrots. The oxheart variety which is said to have a high content of carotene (3) was used although there was no special reason for this selection other than convenience. The carrots always came in fresh and had not been in storage. A liberal ration was placed in the cages every 2nd day and it was estimated that each rat ate 60–100 gm. or more daily. Denton and Kohman (4) studied the nutritive properties of carrots and pointed out that they contain approximately 1 per cent of protein. Each rat, therefore, ingested about a gram (or a little less) of protein daily; how much of this was digested and absorbed, and to what extent carrot protein is adequate for rats we cannot state. Carrots are said to contain considerable quantities of water-soluble and fat-soluble vitamins as well as salts (Na, Ca, Cl, P, Fe). Theoretically, therefore, they afford a ration not obviously inadequate from a nutritional standpoint, and in fact Denton and Kohman reported that rats on an exclusive carrot diet (except for the addition of salts) remained in apparently good health for as long as 16 weeks.

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RESULTS

The animals seemed to thrive on the carrot diet and they remained lively and ate well throughout the experiment even when ascites developed. Not a single animal died during the course of the observations which extended over 21 weeks. There were none of the usual evidences of vitamin deficiency; the hair, skin and nails and eyes remained normal, and there were no signs of gastrointestinal or respiratory tract disorder. The dystrophy of the hair which was previously noted in rats on a diet low in protein but otherwise adequate was not encountered in the present experiments.

There was a prompt and progressive loss of weight (Figs. 1 and 2) for the first 7 weeks, after which there was only a gradual decline. The significance of the figures is somewhat obscured first by the huge amount of gastrointestinal content of the carrot-fed rats together with an actual hypertrophy of the tract and later (see below) by the development, in some animals, of collections of fluid. The whole gastrointestinal tract (including contents) of the carrot rats weighed from 25–40 gm. as against 10–25 gm. in the controls. The contents weighed 10–15 gm. as against 3–6 gm. in the controls and the tract itself weighed 20–30 gm., an increase over normal of about 10 gm., probably due to work hypertrophy.

No gross pathological changes were evident in the viscera except that the livers, in a few of the rats with ascites, appeared to be fatty—they were pale and yellowish and the normal markings were indistinct. The mesenteric fat was well preserved in all but one animal.

No signs of subcutaneous edema were noted. After the first few weeks, however, the tissues (peritoneum, fascia, cut muscle) seemed moist in contrast to the controls. Five rats developed frank ascites and hydrothorax (see Table I).

Changes in the Serum Proteins

The total blood volume as measured by exsanguination decreased as the animals lost weight. Because of the complicating factors of fluid accumulation and variable gastrointestinal residue no accurate correlation with weight could be made. The changes in concentration of serum proteins are shown in Table II and Fig. 3. It is seen that there was a progressive lowering as time went by. The lowest figure

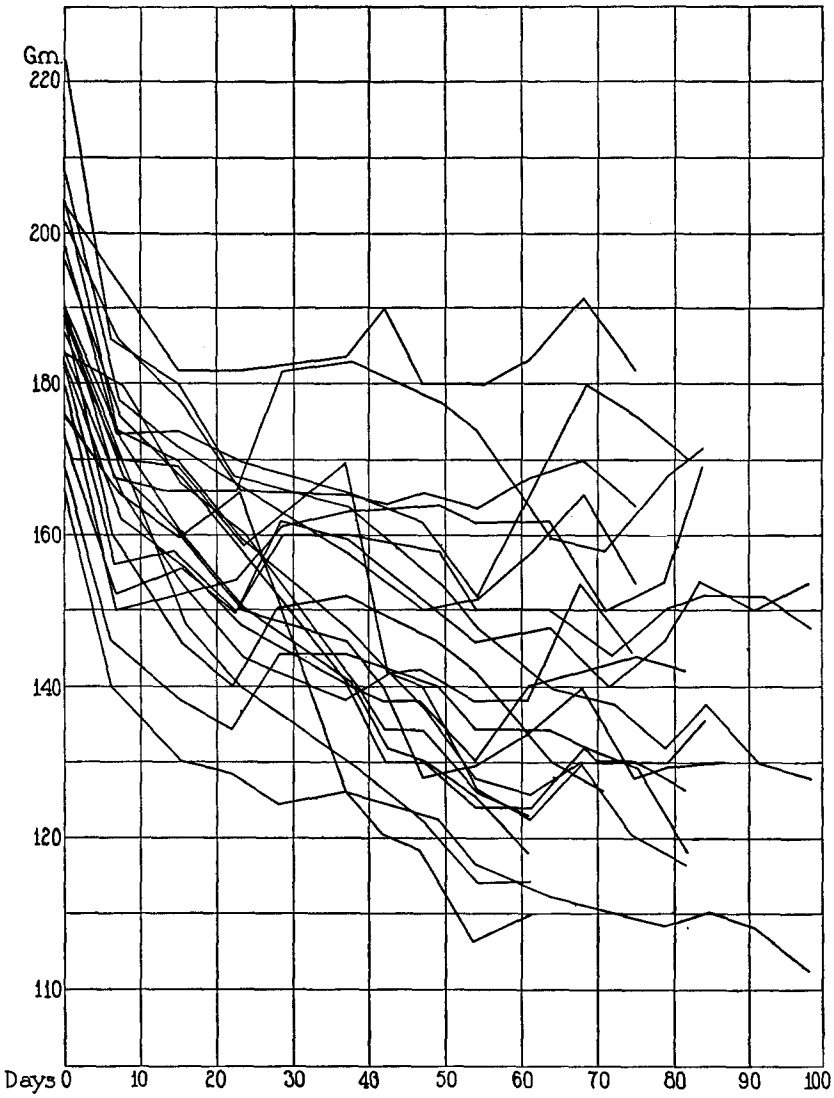

FIG. 1. Individual weight curves of twenty-four rats on a diet of carrots. Note the irregular shape of the curves which are probably influenced by varying gastro-intestinal content and accumulation of fluid.

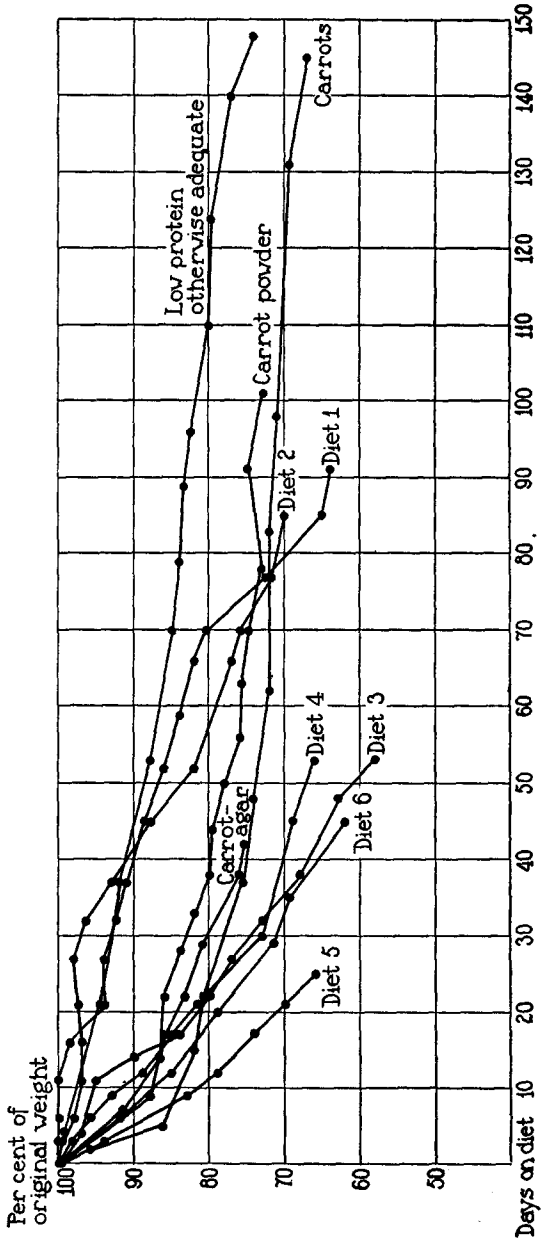

FIG. 2. Composite weight curves of rats on various diets

for pooled serum from a group of rats was 4.28 gm. per cent on the 145th day, but in individual animals (see Table I) values as low as 2.99 were obtained.

Explanation of the Changes

It may be accepted as a fact, then, that the concentration of serum protein falls in rats on an exclusive carrot diet and our next task is to attempt to explain this phenomenon. The point at issue, of course, is whether carrots exercise some positive serum protein-lowering influence, and clearly no such influence can be assumed until other and simpler explanations have been excluded.

TABLE I

The Development of Ascites in Rats on an Exclusive Carrot Diet

Rat No.	Duration of carrot feeding	Initial weight	Final weight	Corrected weight	Weight loss	Serum proteins	Remarks
	days	*gm.*	*gm.*	*gm.*	*per cent*	*gm. per cent*	
19163	82	186	170	130	30	—	Ascites = 20 cc. Hydrothorax = 5 cc.
19161	82	204	126	110	46	—	Ascites = 3 cc. Hydrothorax = 1 cc.
19160	61	180	120	105	42	4.00	Ascites = 3 cc.
19131	63	172	132	100	40	2.99	Ascites = 15 cc. Hydrothorax = 7 cc.
19190	145	190	134	109	43	—	Ascites = 17 cc. Hydrothorax = 8 cc.

Is the Hypoproteinemia Due to an Inadequate Protein Ration?—In a previous paper (1) we showed that rats kept on a diet which afforded not more than 0.2–0.3 gm. of protein daily showed no significant drop in serum protein concentration over a period of 5 months. With the carrot diet the daily protein ration per rat was about 1.0 gm. It seems unlikely, therefore, that lack of protein alone explains the drop of serum protein in the carrot rats although it is by no means

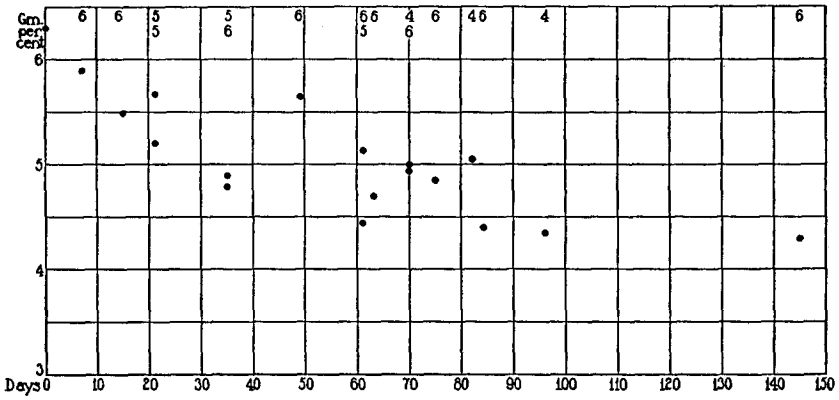

FIG. 3. Relation of serum protein concentration of rats to the duration of carrot feeding. The number of rats in each group is indicated at the top of the chart.

TABLE II

Serum Proteins of Groups of Rats on Exclusive Carrot Diet

Length of time on diet	No. of rats in group	Per cent of original weight	Per cent of original weight (corrected)	Total serum protein of pooled serum of group
days				*gm. per cent*
Untreated controls				6.30–6.50
7	6	93	87.2	5.90
14	6	93	87.1	5.49
21	5	90.2	84.5	5.67
21	5	85.6	80.6	5.21
35	6	84.0	79.0	4.80
35	5	83.8	77.9	4.88
49	6	88	82.9	5.66
61	5	62.9	56.0	4.44
61	6	82.6	77.4	5.13
63	6	68.0	60.6	4.72
70	6	75.5	70.4	4.95
70	4	74.5	69.4	5.00
75	6	78.2	73.2	4.86
82	4	83.8	78.2	5.06
98	4	71.2	67.0	4.34
145	6	67.0	50.5	4.28

certain that the animals actually absorbed an adequate amount of protein complete for their needs.

Is the Hypoproteinemia Due to Loss of Protein in the Urine?—12 hour total urines were collected from two lots of six rats each which had been on the carrot diet for 6 weeks. The average output of protein per rat for the two groups was 0.39 mg. and 0.67 mg. per 100 sq. cm. of body surface—values two to three times normal (5) but evidently inadequate to account for a significant lowering of serum protein.

Is the Hypoproteinemia Due to Vitamin Deficiency or to Some Other Factor Which Renders the Carrot Diet Inadequate?—Regardless of theoretical considerations as to its nutritional value, the exclusive diet of carrots was, in fact, inadequate in the present experiments as evidenced by the rapid loss of weight which the rats underwent. A study of Fig. 3, furthermore, shows that while the serum protein level bore a general relationship to the duration of carrot feeding there are rather gross discrepancies. Two lots of rats both killed on the 61st day, for example, showed serum protein values of 4.45 and 5.13 gm. per cent respectively. These discrepancies are even greater if individual rats are considered. One wonders, therefore, whether the fall of serum protein after carrot feeding is a specific phenomenon or whether the eating of any inadequate diet which leads to marked loss of weight will not result in a similar hypoproteinemia especially if the diet is low in protein.

To control this possibility rats were placed on various obviously inadequate diets (see below), the weight curves were studied and the serum proteins were measured. Weight loss was rapid in every case (Fig. 2) and since the experience of this laboratory has been that death occurs when the fat stores are depleted the animals were sacrificed when their weight fell to the vicinity of 100 gm., which was considered to be the danger zone. Aside from weight loss the animals appeared lively and normal and no lesions were found at autopsy. No visible edema or serous sac effusion developed in any of these rats in distinction to those fed on carrots.

Diet 1.—Starch-lard mixture (starch 75 per cent, lard 25 per cent) 90 per cent, casein 10 per cent. This diet is defective in protein and in vitamins. There was rapid loss of weight (Fig. 2). Four rats were killed on the 85th day and four on

the 92nd day. The serum proteins of the two groups were 4.98 and 5.46 gm. per cent—an average of 5.22.

Diet 2.—Starch-lard mixture 90 per cent, yeast 5 per cent, alfalfa 2 per cent, casein 3 per cent. This diet is defective in protein, but better supplied with vitamins than Diet 1. However, as with Diet 1 weight loss was rapid (Fig. 2). Five rats were killed on the 71st day and five on the 85th day. The serum proteins of the two groups were 5.26 and 5.20 gm. per cent respectively—an average of 5.23.

Diet 3.—Starch 70 per cent, lard 11 per cent, casein 5 per cent, salt mixture 4 per cent, cod liver oil 10 per cent. Weight loss was very rapid (Fig. 2) on this diet. The five animals were sacrificed on the 53rd day. The serum proteins were 5.57 gm. per cent.

TABLE III

Weight Loss and Serum Protein Level of Rats on a Diet of Starch and Lard

Length of time on diet	No. of rats	Weight (per cent of original)	Serum protein
days			*gm. per cent*
16	6	84.9	5.62
19	6	74.4	5.13
28	5	67.8	5.14
35	7	69.5	5.00
35	6	67.4	4.86
42	6	67.6	5.22
45	6	64.2	4.55

Diet 4.—Starch-lard mixture 95 per cent, casein 5 per cent. The weight curve is shown in Fig. 2. Ten rats were killed on the 47th day, five on the 53rd day. The serum proteins of the two groups were 5.44 and 5.55 gm. per cent.

Diet 5.—Starch-lard mixture 99 per cent, casein 1 per cent. The weight curve is shown in Fig. 2. Ten rats were killed on the 25th day. The serum proteins were 5.59 gm. per cent.

Diet 6.—Starch 75 per cent, lard 25 per cent. This diet was not only devoid of protein but grossly ill balanced and defective in regard to vitamins and salts. On it rats lost weight rapidly (Fig. 2) but there was no other evident departure from health. No edema developed. A good many animals were placed on this diet and the results are summarized in Table III.

In summary, then, rats on defective Diets 1 to 6 lost weight quite rapidly. In spite of the fact that they were not killed until death was imminent (as judged by weight loss) no animal in the whole series became edematous. Furthermore with the exception of some of the

rats on Diet 6 the serum proteins dropped little if at all below the level of about 5.25–5.50 gm. per cent which we regard as physiological for rats on any sort of low protein diet (1). In no case, with the exception of some of the rats on Diet 6, did the proteins fall to the levels commonly encountered in rats on the carrot diet.

It must be emphasized that Diets 1 to 6 do not serve in the true sense as controls for the carrot diet, since a proper control diet would be one on which the animals live as long and in as good condition as those on the test diet. From this standpoint the diet low in protein, but otherwise adequate which we previously studied (1), is our best control. On it there was no lowering of serum proteins comparable to that which occurred with the carrot regimen. With Diet 6 there was, to be sure, a considerable fall of the serum protein level but this diet

TABLE IV

Total Serum Proteins of Rats Fed Exclusively on Dry Carrot Powder

No. of rats	Length of time on diet	Total proteins of pooled serum
	days	*gm. per cent*
Control	0	6.30–6.50
9	57	5.12
6	84	5.53
4	101	5.76

is so inadequate from every standpoint that rats cannot be maintained on it for more than a few weeks, and hence no definite conclusion can be drawn as to whether the lowering of serum proteins was due to lack of protein or to general malnutrition.

Is the Hypoproteinemia Related to the Large Bulk of Water in the Carrot Diet?—Since the previous observations gave no definite answer to our question, further experiments were carried out in which rats were fed not fresh carrots, but dry carrot powder.

A commercial preparation was used which contained (by our own analysis) 391 mg. per cent of nitrogen which could account for a maximum protein content of 2.5 gm. per cent. Nineteen rats were used in this experiment. The carrot powder, and no other food of any sort, was offered, and water was allowed *ad lib.* Each rat ate on the average about 10 gm. per day containing not over 0.25 gm. of protein. The animals throve and when the last batch was sacrificed

on the 101st day they appeared entirely normal except for loss of weight. There was no suggestion of edema or ascites and the organs were small but they appeared normal. There was considerable loss of fat. The composite weight curve is shown in Fig. 2 and the serum protein estimations are given in Table IV.

It is seen that there was practically no lowering of serum protein on the carrot powder diet in contrast to that obtained with fresh carrots. Since the obvious difference between the two was water content it was decided to do further experiments with carrot powder,

TABLE V

Results of Feeding 10 Per Cent Carrot Powder Suspended in 3 Per Cent Agar-Agar

Rat No.	Duration of artificial carrot feeding	Ascites	Hydrothorax	Serum protein
	days			*gm. per cent*
24162	23	2.0 cc.	Few drops	
24166	23	11.0 cc.	2 cc.	
24175	23	Few drops	—	
24179	23	0.5 cc.	—	4.54
24264	23	2.0 cc.	Few drops	
24279	23	0.5 cc.	—	
24176	42	0.5 cc.	—	
24178	42	2.0 cc.	Few drops	
24154	42	2.0 cc.	—	
24153	42	2.0 cc.	Few drops	
24177	42	2.0 cc.	—	4.64
24186	42	2.0 cc.	—	
24163	42	1.0 cc.	—	
24161	42	0.5 cc.	—	

but to make the rats take as much water as they obtained with the whole carrot diet. To this end an artificial carrot was constructed as follows:

The best grade of agar-agar was made up in 3 per cent solution in distilled water, and after cooling to about 60°C., 10 gm. of carrot powder were added to each 100 cc. The mixture was stirred in a mechanical kitchen mixer so as to get uniform distribution of the powder and it was then allowed to solidify in deep dishes. After hardening it was cut into blocks and placed in the feed boxes. Each animal ate from 50–100 gm. of this carrot-agar ration daily and hence ingested a large quantity of water. The composite weight curve shown in Fig. 2

is similar to that of the rats fed on fresh carrots. There were no signs of the ordinary nutritional disorders which result from vitamin insufficiency. No recognizable subcutaneous edema was noted, but every rat in the series developed free fluid in the peritoneum and the blood proteins dropped rapidly to definitely subnormal values. The results on the whole paralleled those obtained with the fresh carrot diet but were more extreme and more constant. They are summarized in Table V. At autopsy, aside from decrease in peritoneal fat, the organs looked normal, though small. The gastrointestinal tract was hypertrophied as in the carrot rats and contained a huge agar residue.

These experiments show that addition of water (and agar) to the carrot powder serves to reproduce the effects which follow a diet of fresh carrots. It seems therefore that carrots contain no specific substance effective in lowering blood proteins and producing edema. Why the ingestion of water should produce these effects is not yet clear and further work on this point is in progress.

DISCUSSION

It has been shown that rats subsisting on a diet of carrots remain in good general condition for periods of as long as 21 weeks. There is, however, loss of weight, at first rapid, later more gradual, and a fall in the concentration of serum proteins. When this drop is extreme (4.50 gm. per cent or lower) ascites and hydrothorax are likely to develop. The response of individual rats varies greatly, however, even when they are all under similar conditions, so that loss of weight and drop of serum protein concentration occurs much more rapidly in some animals than in others.

The point at issue in the interpretation of the experiments is whether carrots contain some agent which has a positive disturbing effect on the blood protein-regulating mechanism or whether the drop in serum proteins is a non-specific effect of malnutrition. The fact that controls on a variety of low protein, ill balanced, vitamin-deficient diets failed to develop edema and suffered for the most part only a slight drop of serum proteins, if any, below the physiological level suggests that carrots actually exercise a positive deleterious influence. Further analysis however has shown that a diet of dried carrot powder leads neither to hypoproteinemia nor edema (ascites), whereas the forced addition of water by suspending the powder in agar reproduces all the effects of fresh carrot feeding. Water, therefore, seems to be the cru-

cial factor rather than some specific constituent of carrots. Incidentally it may be noted that the artificial carrot offers a simple and certain method of producing hypoproteinemia in an animal otherwise in good nutritive condition, and we propose to use this technique for the study of further aspects of the subject.

Finally it is of interest to correlate the carrot hypoproteinemia with clinical malnutritional disorders. It appears that a combination of factors is necessary in both cases; namely, an inadequate total caloric intake, an ill balanced diet, a defective protein ration and a large fluid intake. No one of these factors alone seems adequate to produce hypoproteinemia, at least with any constancy.

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