

THE EFFECT OF HIGH PROTEIN DIETS ON THE KIDNEYS OF RATS.

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In recent years much interest has attached to the rôle of high protein diets in the production of chronic nephritis. In 1919 Newburgh (1) fed egg white, casein, and soy beans to three different groups of rabbits over relatively long periods of time and found evidence of renal injury of an acute, subacute, or chronic nature in a majority of his animals. Many of them, however, showed varying degrees of malnutrition and the pathological changes were not strictly those of chronic nephritis as seen in man.

In 1921 Squier and Newburgh (2) gave high protein diets to cases with essential hypertension and found that after varying periods of time (2 to 21 days) red cells and albumin appeared in the urine which had previously been free from these abnormalities. At the same time the blood urea rose. It is noteworthy, however, that in three of the five experiments, salt was added to the diet just prior to the appearance of the urinary abnormalities (thus introducing a complicating factor of unknown importance), and it is further noteworthy that the blood urea nitrogen tended to regain its previous level before the period of high protein feeding was over, indicating that the effects of the high protein diet might well have been temporary rather than permanent. In the same paper are described experiments on normal individuals who ate 1 to 1½ pounds of steak at one meal. Albumin did not appear as the result of this test but red cells were noted in all cases after the meal. But because a single large dose of a given substance produces a certain pathological result it does not by any means follow that smaller doses over a long period of time will produce the same result.

In 1923 Newburgh and Clarkson (3) described dilatation of the tubules and some scarring of the glomeruli in rabbits after diets of 27 to 36 per cent protein for periods of from 6 to 12 months. Again in 1923 Polvogt, McCollum, and Simmonds (4) kept rats on diets containing 31 to 40 per cent protein for periods of from 100 to 480 days. Growth was normal. Chronic nephritis was described as the result, but comparatively little pathological or clinical evidence is brought forward to support the conclusion.

Finally Osborne and Mendel (5) fed rats on extremely high protein (40 to 80 per cent) diets for from 3 to 14 months. Growth was normal. The gross and microscopical examination of the kidneys failed to reveal any changes of an inflammatory, degenerative, or proliferative nature, although the kidneys were nearly double the normal weight.

The evidence, therefore, that has so far been brought forward is of a somewhat conflicting nature. Rabbits are not ideal animals for such experimentation. They are normally herbivorous and are subject in varying degree to naturally occurring nephritis. Rats on the other hand are almost invariably free from spontaneous renal lesions and are normally omnivorous. Further, they are easily controlled, and Donaldson and others have supplied well established data for the normal state. It may be objected that rats are, perhaps, not susceptible to chronic nephritis and are not, therefore, any more suitable for such experiments than rabbits. But if in such a relatively immune animal pathological changes can be produced, the evidence is doubly important; and the mere rarity of spontaneous nephritis in rats in no way militates against the possibility of producing renal disease in them by appropriate means. Further, it should be understood that we do not consider our results with rats to be a repetition for purposes of confirmation or rejection of the results of Newburgh on rabbits; we believe, merely, that they may aid somewhat in solving the general problem of the relation of high protein diets to chronic nephritis.

With a view to further clarifying this important and interesting problem, a series of rats of known ancestry and pure, healthy stock was fed various diets for periods approximating a third of the animal's life.

The standard diet was made up of pure casein 20 per cent, pure arrowroot starch 56 per cent, creamery butter thoroughly washed

with water 15 per cent, salt mixture 4 per cent, and dried yeast 5 per cent.

Experimental Diet 1 was identical with the standard diet except that the casein was 76 per cent and there was no starch.

Experimental Diet 1a was the same as experimental Diet 1 except that there was added enough sodium bicarbonate to neutralize the sulfuric and phosphoric acids formed by the oxidation of the casein, thus preventing any disturbing factor of additional acidity in the diet.

In experimental Diet 2 pure egg albumin (Merck) was substituted for the starch. Otherwise, it was the same as the standard diet. Thus it contained 20 per cent casein and 56 per cent egg albumin or 76 per cent protein in all.

All rats were given water freely and each was confined to a cage by itself. All rats were given a small amount of fresh cabbage or carrot twice a week. Food was given to each animal each day slightly in excess of what he would actually eat. The amount actually eaten was carefully estimated each day. It was remarkable with what accuracy and uniformity the various rats ate. Those upon standard diet consumed, on the average, 0.99 gm. of protein per 100 gm. of body weight per day. Those on experimental Diets 1 and 1a (high protein and high protein plus alkali) consumed 3.90 gm. of protein per 100 gm. rat per day, and those on egg albumin ate 3.60 gm. Figured in calories this shows that the controls ate 21.8 calories per 100 gm. rat per day, those on high protein 22.8 calories, and the high egg albumin series 20.7 calories. In this connection it should be remembered that the rats varied greatly in weight and had always before them more food than they would eat. The figures become even more striking if the young growing rats are omitted, since they eat for a time very much more heavily than the adults. It is seen, therefore, that the high protein and albumin diet series consumed four times as much protein as the controls and that all diets were adequate calorically and practically efficient.

All rats on all diets did well, with the exception of one rat on high albumin diet which acquired a skin lesion and was killed. This rat is not included in the series reported.

The controls (three in number) averaged 260 gm. at the beginning of the experiment; at the end they averaged 298 gm. The

adult rats on high protein diet (three in number) averaged 220 gm. at the beginning and 265 at the end of the experiment. The young rats on this diet (two in number) averaged 54 gm. at the start of the feeding and 305 at the end. The rats on high proteins plus alkali (three in number) averaged 240 gm. at the start and 260 at the end. The rats on egg albumin (two in number) averaged 74 gm. at the start and gained to an average of 284 gm.

The rate of growth in each case was steady except during the hot month of August, when there was a loss of from 5 to 15 gm. in each rat, including the controls. Otherwise, the young rats gained rapidly and consistently and the old ones continued to gain slowly and became very fat.

During the progress of the experiment each rat was periodically placed in a metabolism cage and the urine collected and analyzed. All rats, so far as our experience goes, show slight albuminuria, the amount in each case being characteristic and uniform for that animal—usually from 2 to 20 mg. per day. Normal rats do not show casts or red cells.

In none of our rats did the albumin excreted increase during the period of the experiment, nor did the rats on experimental diets show more albumin than those on the standard diet. There was no evidence of nitrogen retention to be found in the urinary analyses. The amount excreted corresponded to the intake. Attempts were made to estimate the amount of amino acid excreted. Experience convinced us, however, that this could not be done with sufficient accuracy to warrant publication. It may be said, however, that on standard diet the amino acid nitrogen is about 1.5 per cent of the total. It does not increase proportionately with the total nitrogen; if the latter is quadrupled the amino acid nitrogen is not more than doubled.

Casts were not found in any of the specimens except rarely in one of the rats (No. 9) on high protein plus alkali. Red cells were never seen.

At the conclusion of the experiment each animal was etherized and the throat cut. The blood was collected and the non-protein nitrogen determined. The non-protein nitrogen of the control rats averaged 36.8 mg. per 100 ml. of whole blood; in the case of the high protein diets the average was 51.1. This is what is to be expected and is not in our opinion an indication of renal damage.

In general the non-protein nitrogen level of healthy individuals is proportional to the protein intake at the time. Further, it is extremely unlikely that the kidneys of all the high protein rats would have been injured with such complete uniformity with respect to their ability to excrete nitrogen.

Red counts were done shortly after the beginning of the experiments and again at the time of death. In no case did an anemia develop. All the red counts were between 8 and 9 million. The red cells appeared normal. In no rat of this series did the kidney lose its power to concentrate nitrogen. The marked diuresis in all the high protein rats was very striking and was of such an order as to keep the nitrogen concentration at about 4.5 gm. nitrogen per 100 ml.—exactly as on standard diet. The fairly constant pH values indicate that no marked disturbance of the neutrality mechanism was brought about by feeding excess acid-producing protein.

From a clinical point of view, therefore, we were unable to find any evidence of chronic nephritis.

The rats were autopsied and each tissue was studied microscopically by means of paraffin sections stained with eosin-methylene blue. In no case did we find either in the tubules or glomeruli or blood vessels of the kidneys any evidence of inflammatory, degenerative, or proliferative lesions. The kidneys of all rats appeared microscopically and grossly normal.

The kidneys of rats on high protein diets (Nos. 1, 1a, and 2) were all considerably hypertrophied, on the average 23.6 per cent heavier than the normal for the size rat (Donaldson (6)). There was less hypertrophy than other authors have found in similar studies, and this may in part be explained by the fact that all our rats were unusually fat and may have been "overweight," as the control kidneys were 5 per cent underweight according to Donaldson's figures.

All other organs were normal except in one control and one high protein animal in both of which there was found a curious pseudo-tuberculous lesion in the lungs. There was no evidence of arteriosclerosis.

There was, in short, no evidence obtained from pathological examination that the kidneys had been injured.

The table shows the average figures computed for the entire

TABLE I.

Rat No.	Sex.	Diet.	Age at start.	Age at finish.	Weight at start.	Weight at finish.	Months on diet.	Calories per 100 gm. per day.		Urine volume per 24 hrs.		Al- bumin per 24 hrs.	pH	Daily N intake.	Daily N per 100 gm.	Urine N daily.	Non-protein blood.	Weight of kidneys.	Per cent hypertrophy.
								gm.	cc.	gm.	gm.								
1	♂	Standard.	6	20	365	421	14	21.9	10.5	21.2	6.3	0.625	0.159	0.491	40.1	2.0	± 0		
2	♀	"	6	24	207	233	18	20.1	6.5	4.8	6.3	0.321	0.146	0.290	36.1	1.7	-10		
3	♀	"	6	16	207	240	10	23.8	8.5	11.6	6.0	0.388	0.173	0.377	34.2	2.8	+16.6		
4	♂	1	6	20	250	294	14	20.5	29.5	7.1	5.8	1.54	0.568	1.32	60.1	2.7	+28.5		
5	♀	1	6	20	216	256	14	20.3	25.5	16.2	5.9	1.33	0.560	1.12	50.2	2.7	+35		
6	♀	1	6	19	195	246	13	20.0	23.0	22.4	6.1	1.21	0.558	1.16	52.5	2.8	+16.5		
10	♂	1	1	16	48	305	15	25.8	29.0	14.4	6.2	1.26	0.712	1.15	58.1	2.4	+33		
12	♀	1	1	21	61	205	20	33.8	24.0	12.4	5.9	1.23	0.925	1.15	52.6	3.8	+46		
7	♂	1a	6	19	305	324	13	20.0	29.5	12.2	6.3	1.72	0.549	1.45	56.3	2.4	+26		
8	♀	1a	6	19	196	226	13	21.2	24.0	14.8	6.4	1.27	0.586	1.10	47.2	2.4	+26		
9	♀	1a	6	20	220	230	14	21.4	25.0	19.1	6.0	1.36	0.592	1.09	48.3	2.6	+8.3		
14	♀	2	1	13	83	296	12	21.8	22.0	4.2	6.1	1.14	0.504	1.00	43.3	2.2	± 0		
15	♀	2	1	16	66	273	15	19.8	20.0	18.2	6.1	0.93	0.546	0.90	43.3	2.2	± 0		

period for each rat. The calories figured per 100 gm. rat per 24 hours were based on the mean weight and the actual amount of food eaten over the entire period, each day's intake being calculated. In the column "Urine volume per 24 hrs." is given the average urine volume in cc. as actually determined for three 4 day periods at intervals throughout the experiment. The albumin is given as the average daily figure determined by phosphotungstic acid and centrifugalization. The pH was determined colorimetrically. The daily nitrogen intake is computed from the food actually eaten and given as total gm. of nitrogen per 24 hours. The column headed "Daily N per 100 gm." gives the average daily nitrogen intake per 100 gm. of body weight. The urinary nitrogen is based on average figures for three 4 day periods at intervals throughout the experiment and gives total nitrogen output per 24 hours. It is to be noted that the actual figures for the urinary nitrogen fall short of the calculated nitrogen intake. This discrepancy is to be explained partly by the fact that a certain amount of urine was lost in the metabolism cages, and partly by the fact that the rats ate somewhat less in these cages than they did in the open cages in the animal house. The non-protein nitrogen is given in mg. per 100 ml. of whole blood and was determined by Folin's method. The blood was taken after primary anesthesia immediately before autopsy. The percentage hypertrophy is the amount of increase of weight over the standard normal figure given by Donaldson.

CONCLUSION.

By feeding very high protein diets over a period of from 10 to 20 months or about a third of a rat's life, we have been unable to produce in these animals any recognizable nephritis.

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