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THE PRODUCTION OF BRIGHT'S DISEASE BY FEEDING HIGH PROTEIN DIETS *

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For nearly a century the etiology of Bright's disease has been the subject of much medical thought and investigation. But in spite of this, the clinician is still unable to find a cause for the disease in the great majority of cases seen by him. The present investigation was undertaken with the hope of throwing some additional light on this difficult subject.

The chief business of the kidneys is to rid the organism of the end products of protein metabolism. This task is accomplished to a considerable extent by active secretion on the part of certain portions of the tubular epithelium. These cells will not only remove normal waste, but they will also remove from the blood stream substances which are foreign to it even though they may be severely injured by the poisons passing through them. Thus investigators¹ have shown that when the kidney eliminates such substances as mercury, or the salts of uranium and chromium, a high grade tubular nephritis may be produced; and that the acute tubular injury may be followed by a chronic nephritis.

The substances dealt with by this group of investigators do not play any considerable rôle in the causation of clinical nephritis. But the experiments demonstrate a fact of great importance, namely, that the tubular epithelium may suffer any degree of injury including death as a result of its efforts to remove certain abnormal substances from the blood.

The degree of injury produced by the nephrotoxic substances is largely dependent on their concentration in the blood. Thus mercury in therapeutic doses usually has no detectable effect on the kidney; although when its use is long continued, albumin and casts may appear in the urine. But when swallowed in large amounts in soluble form, acute fatal nephritis may result. Whether mercury will injure the

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1. McNider, W. deB.: J. M. Research **34**:177 (May) 1916.

kidney, is determined not alone by certain qualities inherent in mercury, but also by the quantity of mercury which passes through the kidney in a unit of time.

May this same principle be applied to the nitrogenous bodies habitually secreted by the renal cells? May it not be true that the kidneys can secrete a certain amount of nitrogenous matter for an indefinite period without suffering harm, but that they will be injured if the quantity of some or all of the nitrogenous substances secreted be increased and kept at the higher level for some time?

The experiments which form the substance of this paper were undertaken with the intention of discovering whether nephritis will be produced when the kidneys have been eliminating an unusually large amount of nitrogenous material over a considerable period of time.

To attain this end, rabbits were fed diets abnormally high in protein. The experiments fall naturally into three groups determined by the type of protein used.

Group A includes all those animals whose nitrogen metabolism was increased by means of egg white.

Group B consists of rabbits that lived on a diet high in protein because of the addition of casein.

The animals in group C were fed soy beans.

GROUP A.—THE EGG WHITE EXPERIMENTS

All the animals in this group received egg white, but the diets were of three different sorts, each planned with a different object in view. The group may accordingly be subdivided into three subgroups, 1, 2 and 3.

Subgroup 1.—The preliminary experiments of the investigation fall under this heading. The object was to determine as quickly as possible whether the ingestion of large amounts of protein would produce demonstrable injury of the kidney in a relatively short time.

For this purpose six rabbits were each fed the whites of two hard boiled eggs daily for one week. During this time they received no other food. Thereafter rabbits Nos. 1 and 3 each ate the whites of four or five hard boiled eggs, and, in addition, were each allowed from 25 to 50 gm. of carrots daily. The feeding was continued in this way for thirty days in the case of rabbit No. 1; and for thirty-eight days in the case of rabbit No. 3. Rabbits Nos. 2, 4, 5 and 6, after the first week, ate the whites of from three to four eggs and received no other food. This second period for these four rabbits lasted, respectively, nine, ten, fourteen and twenty days.

The urine of none of these six rabbits contained either albumin or casts, the day preceding the experimental diet. A persistent albumin-

uria developed during the experimental period in the case of each rabbit. It was noted as early as the third day in the urines of rabbits Nos. 1 and 6; and on the fifth day in the urines of rabbits Nos. 2 and 3. Casts were seen in the urines of four of the rabbits, Nos. 1, 2, 3 and 6.

Rabbit No. 5 was found dead on the nineteenth day of the experiment. The remaining rabbits were killed as soon as they refused to eat the hard boiled egg white.

Microscopic examination of the kidneys was made. In every instance cloudy swelling and congestion was seen. Numerous casts were present in the kidneys of each of the rabbits with the exception of No. 6.

What value shall be placed on the data obtained from these rabbits? These data are albuminuria, casts in the urine and numerous casts seen in the microscopic sections of the kidneys, cloudy swelling and congestion. The last two, swelling and congestion, are features which all histologists recognize as variables. They are, in part, at least, related to the preparation of the tissues for microscopic examination. Taken by themselves, in the absence of other signs, they are feeble evidence of renal damage due to high protein diets.

What significance has the albuminuria? Is the albumin in the urine merely white of egg, absorbed as such from the intestine and filtered out of the blood as such by the kidney? It has been clearly demonstrated that this is not the cause of the albuminuria, but that the albumin found in the urine comes from the rabbit itself. The evidence appears in the following two experiments.

EXPERIMENT 1.—Rabbit No. 8, March 1, 1917. The diet has consisted entirely of hard boiled egg white for the past six days. The rabbit has a well marked albuminuria. Ten c.c. of blood are withdrawn from the heart. It is defibrinated by beating and the plasma is obtained by centrifuge. One c.c. of the whole plasma and 1 c.c. of each of the following dilutions, 1:10, 1:20, 1:50, 1:100, are placed in a series of tubes. To each tube 1 c.c. of a 1:1,000 solution of Kaulbaum's egg white is added. The tubes are kept at 38 C. over night. No precipitate is seen in any of the tubes the next morning.

A rabbit whose urine contains albumin consequent to the ingestion of egg white shows no detectable trace of the egg white in his blood.

EXPERIMENT 2.—Rabbit 13, Feb. 12, 1917. The diet has consisted entirely of hard boiled egg white for the preceding ten days. The urine shows a well marked albuminuria. One c.c. of this urine is injected into the peritoneal cavity of each of four guinea-pigs.

Blood is also withdrawn from the heart of this rabbit. It is allowed to clot and the serum is obtained by centrifuge. One c.c. of this serum is injected into the peritoneal cavity of each of four guinea-pigs.

February 24. The guinea-pigs which received the intraperitoneal injection of albuminous urine twelve days ago, are today each given intraperitoneally, 2 c.c. of a 1:100 solution of Kaulbaum's egg white. No reaction is observed.

One-half hour later, two of these same guinea-pigs are given an intravenous injection of normal rabbit's serum. Both showed typical anaphylactic shock.

The group of guinea-pigs which received the intraperitoneal injection of serum obtained from Rabbit 13, twelve days ago, today are each given intravenously 2 c.c. of a 1:100 solution of Kaulbaum's egg white. No reaction is observed. One-half hour later these same guinea-pigs are given an intraperitoneal injection of normal rabbit's serum. Typical anaphylactic shock resulted.

The intraperitoneal injection of the albuminous urine from a rabbit eating egg white, failed to sensitize guinea-pigs to egg white, but did sensitize them to rabbit's serum albumin. The intraperitoneal injection of serum obtained from a rabbit eating egg white failed to sensitize guinea-pigs to egg white, but did sensitize them to rabbit's serum albumin.

The albuminuria produced by feeding egg white may accordingly be accepted as satisfactory evidence of renal injury.

Casts are agglomerations of broken down renal epithelium. They are not found in the urines of normal rabbits. A very few casts may occasionally be found in the sections of supposedly normal rabbit kidneys. Their presence in large numbers in the urines and in the kidneys of the rabbits that received the egg white, is an unquestionable sign of kidney injury.

There is, then, clear evidence that a diet restricted to egg albumin with or without the addition of a small amount of carrot, is capable of quickly causing some renal damage.

Subgroup 2.—These experiments were planned with the object of showing whether recurring periods of excessive protein metabolism would injure the kidneys.

The animals were fed as much hard boiled egg white as they would eat for intervals of about nine days, alternating with periods of stock diet lasting about a week. This type of feeding was continued as long as the rabbits would eat the egg white.

There are four satisfactory experiments, lasting, respectively, four, four and one-half, five and one-half and six and one-half months. Three of the rabbits ate about three egg whites daily. One rabbit (No. 9) ate about five egg whites daily.

This rabbit was found dead at the end of six and one-half months. The microscopic examination of the kidneys revealed cloudy swelling, marked congestion, hemorrhage into the subcapsular space compressing the tufts and many casts. The kidneys of another one of these rabbits (No. 10) showed abnormalities of the same sort, but less well marked.

Both these animals were found dead. The necropsies revealed no important disturbances other than those found in the kidneys. Rabbit No. 9 had, however, lost 600 gm., and rabbit No. 10 lost 900 gm.

during the experimental period. Death may, accordingly, have been caused by inanition.

The two remaining animals of this group were killed. Their kidneys showed no changes which could certainly be attributed to the effect of the diet.

The urines of each of the four rabbits contained albumin during the experimental period.

The ingestion of large amounts of egg white as administered in this subgroup does not cause any serious chronic renal lesions, but evidence of some kidney disturbance has been obtained. No further conclusions are warranted.

Fig. 1.—Microphotograph of the kidney of a normal rabbit.

Subgroup 3.—All the animals thus far dealt with lost weight while living on the experimental diets. It might be contended that the malnutrition was at least partly responsible for the renal injury. In order to obviate this possible source of error, a third series of rabbits was fed the egg white combined with other food in such a way as to guard against nutritional disturbances.

Each animal received the following mixture daily: three egg whites; 30 gm. of corn meal; sufficient bran (about 25 gm.) to make a stiff paste when the three ingredients were stirred together. The mass was

then transferred to a buttered pan and baked in a hot oven for about twenty minutes. In addition to this, each animal received 25 gm. of bread daily, and some cabbage or a carrot several times a week. This diet furnishes about 230 calories daily. It contains about 17 gm. of protein, about 33 gm. of carbohydrate, and about 2 gm. of fat. One third of the dry weight of the diet is protein.

Three rabbits, Nos. 110, 111 and 113, ate this diet for six months, four months and seven months, respectively. No. 110 maintained its weight, No. 111 gained about 450 gm., and No. 113 gained about 225 gm. The urine of each of the animals showed a marked albuminuria, and many granular and hyaline casts on every examination.

Rabbit No. 110 was killed at the end of six months. It had shown no sign of disease other than the abnormal urine. At necropsy the organs revealed no gross abnormality. Microscopic examination of the kidneys did not disclose any abnormalities which could be attributed to the diet.

Rabbit No. 111, after four months of the diet, began to refuse the egg white mixture and was accordingly killed. The kidneys weighed 21 gm., which is about twice the normal average. On microscopic examination, severe parenchymatous injury was seen. This was especially marked in the convoluted tubules whose epithelium showed granulation, swelling, vacuolization and desquamation. Some nuclei were unstained, others were much swollen. There were many casts in the convoluted and collecting tubules. The glomeruli were somewhat swollen, but there was no exudate in the subcapsular space. Many tufts showed extreme congestion. There was a little increase of young connective tissue in the cortex. Edema and engorgement were marked throughout the section.

Rabbit No. 113 ate the diet containing the whites of three eggs for seven months. During this time he gained 225 gm. and showed no signs of disease other than albuminuria and cylindruria. At the end of this period, the diet was changed so that it contained the whites of five eggs daily. None of the other factors in the ration were altered. The animal ate this latter diet for three months. During this period he gained about 330 gm. and appeared to feel exceptionally well. At the end of these three months he was killed. For seven months he had ingested three egg whites daily and for the next three months he had eaten five egg whites daily.

At necropsy the kidneys were found to weigh 22 gm., which is about twice the normal. The microscopic examination of the kidneys showed a well marked injury of the tubular epithelium, especially severe in the convoluted tubules. Desquamation, vacuolization and irregular disappearance of the parenchymatous cells was widespread.

Casts were numerous. In places, dilatation of tubules was seen. The kidney as a whole showed engorgement and edema. The glomeruli showed no specific injury. There was no fibroblastic increase. The thoracic aorta from the aortic valves to the diaphragm showed very many raised yellow plaques and streaks.

A fourth rabbit, No. 112, was fed the hard boiled egg white as such instead of the mixture of egg white, corn meal and bran. He also ate 50 gm. of bread and a few greens daily. This diet furnished about 180 calories daily. The rabbit took this diet well for three months. During this time he lost 200 gm. Thereafter, for the next

Fig. 2.—Early stage of chronic nephritis produced by the soy bean diet. Note the beginning contraction of the cortex and the pinching off of a group of tubules by connective tissue. Casts are seen.

ten days he ate only the egg white. Two or three days before he was killed he began to show marked weakness. This increased until he became ataxic and unable to hold up his head. He lost 330 gm. during these ten days. He was then killed by a blow on the head. At necropsy a few small areas of pulmonary congestion were found. The kidneys were pale and cloudy. They weighed 15 gm. (average normal is 11.8 gm.). The urine obtained from the bladder contained a large amount of albumin, many granular and hyaline casts, and numerous red blood cells. The microscopic examination of the kidney gave clear

evidence of severe acute injury. Many glomeruli were crowded with red blood cells. Masses of red blood cells and desquamated epithelium were seen in a few subcapsular spaces. The tubular epithelium, and more especially that of the convoluted tubules, showed granulation, much desquamation and marked vacuolization. Red blood cells were seen in the lumen of a few tubules. Some nuclei were swollen, while others were unstained. There were many casts in all parts of the tubules. The collecting tubules were dilated. This experiment presents an example of acute Bright's disease caused by the ingestion of large amounts of protein in the form of egg white.

At first sight it would appear as if the egg white diet had had a greater effect on this rabbit than on any of the three preceding ones which had eaten the same amount of egg white for longer periods. But a consideration of the nitrogen metabolism² of these three rabbits shows that such a conclusion would be incorrect. The total urinary nitrogen eliminated during five days was determined for each of these rabbits. The average twenty-four hours' amount for the rabbits that ate the egg white cooked with corn meal and bran was as follows: Rabbit No. 110, 1.2 gm. total nitrogen; rabbit No. 111, 1.5 gm. total nitrogen; rabbit No. 113, 1.2 gm. total nitrogen while eating the mixture containing three egg whites, 2.3 gm. total nitrogen while eating the mixture containing five egg whites; rabbit 112, 2.2 gm. total nitrogen. Obviously, the mixture of egg white with corn meal and bran resulted in a very poor absorption of the digestion products of the egg white. But when plain egg white was fed the absorption was excellent, and adequately explains the much more acute injury to the kidney observed in rabbit No. 112.

These data for the nitrogen metabolism of the four rabbits in this subgroup have a further significance. It will be noted that rabbit No. 110, whose twenty-four hours urinary nitrogen was only 1.2 gm., showed no renal changes which could be recognized by the microscope. The absorption of nitrogen from the diet was so poor in this instance as to result in only a slight increase over the normal nitrogen metabolism. The average total twenty-four hours urinary nitrogen in one group of controls was 0.8 gm.; in a second group it was 1.0 gm. Rabbit No. 111, whose absorption was better, 1.5 gm. total nitrogen, showed a subacute nephritis, but was able to gain weight and appeared to feel well up to the time when he was killed four months from the beginning of the experiment. Rabbit No. 113, whose absorption was only 1.2 gm. while he was eating a mixture containing the egg whites, gave no evidence of disease except albumin and casts during the seven

2. A full discussion of the procedure used for determining the urinary nitrogen will be found in the discussion accompanying Table 2, page 375.

months of this diet. The change in his diet which caused him to eat five egg whites daily for the next three months, resulted in a nitrogen absorption of 2.3 gm. daily. He continued to gain weight and to give the outward appearance of health during this second period. But in spite of the steady gain in weight, the autopsy revealed a well marked subacute parenchymatous nephritis.

The data obtained from these four rabbits in Subgroup 3, demonstrate that the renal lesions observed were not a part of a nutritional

Fig. 3.—A later stage of the soy bean nephritis showing more marked contraction, atrophy of some glomeruli, slight thickening of Bowman's capsule and casts. A broad band of connective tissue occupies the central portion of the field.

disturbance, but were, in fact, the direct result of the injection of egg white.

The information obtained from the various types of experiments with egg white may be summed up by the following statements: (1) When rabbits eat several egg whites daily, evidence of renal injury is very quickly and constantly noted. (2) When rabbits absorb the digestion products of egg white in sufficient quantity for a considerable time, a well marked acute or subacute nephritis may result.

GROUP B.—CASEIN EXPERIMENTS

In order to decide whether the nephritis observed in the preceding group of experiments was the result of the prolonged secretion by the kidney of an excessive amount of one or more of the nitrogenous bodies which, in lesser amounts, are normal constituents of the blood; or whether it might possibly have been caused by some nephrotoxic substance peculiar to egg white, it was necessary to determine whether nephritis would follow the prolonged ingestion of some other protein in excessive amounts.

Casein was chosen for this purpose. The diet was prepared by placing 25 c.c. of milk, 20 c.c. of water and 20 gm. of scraped carrot together in a pan over a hot water bath. When the mixture was warm, the casein and about 1 gm. of sodium bicarbonate was added. The whole was stirred. The gas evolved produced a porous mass which, when cool, somewhat resembled a "baking powder biscuit." Four rabbits were fed 30 gm. of casein daily in this way. Two rabbits received fifteen grams daily. In addition to this the animals received from 25 to 50 gm. of bread daily and a handful of greens about once a week.

The four rabbits which ate 30 gm. of casein daily, were killed at the end of three weeks, ten weeks, four months and eleven months.

The rabbit which was allowed to live only three weeks on the diet, was found acutely ill one morning, and for this reason the experiment was ended. The kidneys weighed 22 gm., or 7.5 gm. per kilogram. (The average weight of the kidneys of eight normal rabbits was 11.8 gm., or 4.9 gm. per kilogram.) On microscopic examination the kidney showed very marked edema and engorgement. There were numerous red blood cells scattered through the subcapsular spaces and the lumina of the tubules. Very many casts were seen. The other organs were normal. The type of renal disturbance found strongly suggests that the symptoms had been caused by acute nephritis.

The second rabbit had seemed to be ill for some days prior to his death. He was killed after he had been eating 30 gm. of casein daily for ten weeks. The kidneys weighed 20 gm.; and, on microscopic examination, showed moderate parenchymatous injury, casts and congestion.

The third rabbit, killed after four months of the diet, showed changes similar to those found in the preceding one, but they were much more pronounced. The kidneys weighed 21 gm.; and, on microscopic examination, revealed marked congestion, severe parenchymatous injury, very many casts and beginning fibroblastic increase.

The fourth rabbit that ate 30 gm. of casein daily was killed at the end of eleven months. The urine contained no albumin before the

casein diet was started, but did give positive tests for albumin thereafter. The kidneys weighed 16 gm., but gave no other evidence of injury due to the diet. Evidently, the high protein diet had either had less effect on the kidneys of this rabbit than upon those of the three preceding animals, or else this fourth rabbit had recovered from a nephritis such as was observed in the others at a much earlier stage of the diet. No choice between these two alternatives is possible.

Two other rabbits ate 15 gm. of casein daily for one year. Their urines showed no albumin before the diet was started. During the course of the experiment the slightest trace of albumin was often noted,

Fig. 4.—A still later stage of the soy bean nephritis showing marked injury of the epithelium of the convoluted tubules. Many convoluted tubules are lined by a flat epithelium; others have entirely lost the epithelial lining. Many tubules are widely dilated. There are many casts. Bowman's capsule shows more thickening. A patchy increase of connective tissue involving most of the field is seen.

but only once were a few casts seen. The kidneys weighed 13.5 gm. and 14.5 gm., respectively. Microscopic examination did not show any abnormalities.

The outcome of the experiments with casein is this: When the nitrogenous metabolism is increased by means of casein, rabbits suffer no renal injury from eating 15 gm. of casein daily, even though this

amount of casein causes the nitrogen metabolism to be about double the normal. But when the daily intake of casein is 30 gm., and the nitrogen metabolism is about three times the normal, a well marked deleterious effect on the kidney was produced in three rabbits out of four.³

GROUP C.—SOY BEAN EXPERIMENT

Even though there can be little doubt that the kidney lesions thus far described bear a causal relation to the large amount of protein in the diet, nevertheless it was felt that the evidence would be strengthened if similar results should be obtained by feeding a third type of protein.

For this purpose soy beans were used as the sole food. The soy bean was chosen for a number of reasons: (1) Rabbits habitually eat vegetable protein. A renal injury occurring in herbivora as a result of the ingestion of a large amount of vegetable protein would have more significance than if it had been produced by means of an animal protein. (2) It was to be expected as the result of the studies of Osborne and Mendel,⁴ and of Daniels and Nichols,⁵ that animals could live on the soy bean⁶ for many months without acquiring nutri-

3. The necropsies of the three rabbits whose casein feeding had lasted for a year, revealed an unexpected lesion of great interest. The thoracic aorta in each case was the seat of a well marked arteriosclerotic process. The inner surface of the aorta presented many raised yellow plaques and streaks. The lesion was most extensive at the very beginning of the aorta and included the aortic valves. It gradually became less marked as one passed down the aorta and ended before the diaphragm was reached. None of the other large vessels were involved. The microscopic appearance of the lesion was described by Dr. C. V. Weller as follows: "Rabbit 58. Aorta. Hemalum and eosin and Van Gieson's stains. The intima is thickened irregularly through the presence of elevated plaques, many of which are three to four times as thick as the normal aortic wall. These elevated patches in most cases involve only the intima. In a few instances the innermost lamellae of the media are included. The elevated patches show a proliferation of the intima, increase in the elastic fibers and large areas of secondary fatty degeneration and liquefaction necrosis, accompanied by marked formation of cholesterol. The picture is that of an advanced intimal atherosclerosis in varying stages up to and including secondary atheromatous change with cholesterol formation. The greater part of the media and the adventitia are negative."

This disease of the aorta was found only in the three rabbits that had eaten the casein diet for a year and in the one rabbit (No. 113) of the egg white group that had eaten the egg albumin for ten months. Further study is required to determine whether arteriosclerosis may be regularly caused by the prolonged injection of high protein diet.

4. Osborne, T. B., and Mendel, L.: *J. Biol. Chem.* **32**:369 (Dec.) 1917.

5. Daniels, A. L., and Nichols, N. B.: *J. Biol. Chem.* **32**:91 (Oct.) 1917.

6. Bulletin No. 28, Office Experiment Stations, Washington, D. C., states the composition of the soy bean as follows: water, 5.5 per cent.; protein from 36 to 43 per cent.; fat, 17 per cent.; ash, ?; carbohydrate (by difference), 34.5 per cent.

tional disturbances. (3) The soy bean is very rich in protein. The beans used by us contained 37.8 per cent. of protein. If the rabbits ate enough of the beans to maintain their weight, they would of necessity also eat very much more protein than was their habit.

Twenty-six rabbits were placed in a large pen consisting of three compartments. The dividing walls were made of wire netting. Some of the rabbits were full grown. The remainder were about six months old and about three fourths grown. The adults were all put in the first compartment. Six of the partly grown rabbits were kept in the second compartment. The remainder of the young rabbits were placed

Fig. 5.—A higher magnification of the same field as Figure 4. Note especially the extreme atrophy and dilatation of the tubules.

in the third division of the pen. The six rabbits in the second compartment served as controls. They were fed hay, bread, corn cake, cabbage and other greens. The other two groups in the pen, consisting of twenty rabbits, lived on soy beans and water. All of the rabbits were exposed to the same conditions, except as regards diet. If at the end of the experiment any consistent difference between the kidneys of the control rabbits and of the soy bean groups should be found, this difference would have to be attributed to differences in diet.

The average individual weight of the control rabbits after seven months residence in the pen was 2,415 gm. The partly grown rabbits

averaged 2,179 gm. at the beginning of the soy bean diet. Three months later their average weight was 2,497 gm., an average individual gain of 318 gm. The average weight of the adults at the beginning of the soy bean diet was 2,382 gm. Three months later they weighed, on the average, 2,780 gm. At the end of another three months these animals averaged 2,892 gm. It is evident from a consideration of the above weights that the soy bean, when fed as the only source of food, is not alone adequate for maintenance, but also for growth.

The control rabbits were killed at the end of seven months. Neither the gross nor the microscopic examination of their kidneys revealed any abnormalities.

Of the twenty rabbits that were fed soy beans, five were killed at the end of two months. The kidneys of four of these animals showed no changes. But the kidneys of the fifth rabbit showed degeneration of the epithelium of the convoluted tubules and very many casts.

Fifteen rabbits lived on an exclusive diet of soy beans for from four and one-half months to twelve months. Eight of these fifteen rabbits died. Microscopic examination of the kidneys showed acute or chronic nephritis in every instance. In addition to this, two of the animals had pneumonia and one a bacterial endocarditis. In five of the eight animals that died while on the soy bean diet, no cause of death other than Bright's disease could be found. Seven of the fifteen rabbits were killed by a blow on the head after they had lived on the soy bean diet for from four and one half to twelve months. The microscopic examination of these kidneys showed, with one exception, subacute and chronic nephritis. The kidneys of one rabbit, killed at the end of five months, showed only slight injury of the tubular epithelium.

The following protocols and the microphotographs will illustrate the type of kidney lesion which develops in the rabbit as the result of a diet restricted to soy beans.

RABBIT 90.—Nov. 19, 1917: This is one of the young rabbits. He was about three-fourths grown at the beginning of the experiment. April 1, 1918: He was found dead this morning. He had eaten nothing but soy beans for four and one-half months. He weighs 1,590 gm. Necropsy: All organs except the kidneys appear to be normal. The bladder urine contains albumin and many hyaline casts. The heart weighs 7 gm. The kidneys weigh 12 gm. The capsule strips easily, leaving a smooth surface which presents a peppered appearance. Microscopic examination⁷ of the kidney: "Patchy degenerative nephritis, shown by cloudy swelling, atrophy, dilatation of tubules and obliteration of some convoluted tubules by connective tissue. Bowman's capsule is thickened in many instances. There is some thickening of the arterial walls. The glomeruli show varying stages of atrophy, congestion of capillaries and degeneration of epithelium. Van Gieson's stain shows marked increase in

7. I am grateful to Dr. A. S. Warthin for this and other descriptions of the microscopic anatomy of the kidneys.

connective tissue, especially marked in the labyrinths but extending into the medullary rays. There is very little cellular infiltration in these areas. Many granular and hyaline casts are seen."

RABBIT 98.—Nov. 5, 1917: Soy bien diet started.

June 25, 1918: After seven and one-half months of the diet the rabbit weighs 3,120 gm. Nov. 5, 1918: The rabbit has eaten nothing but soy beans for one year. It weighs 2,725 gm. Blood was withdrawn from the heart today. It contained 123 mg. of incoagulable nitrogen per 100 c.c., and 128 mg. of urea per 100 c.c. The urine shows a trace of albumin and a few coarsely granular casts.

The animal is now placed on a diet of greens, oats and bread. Nov. 17, 1918: It weighs 2,610 gm. November 18: The total incoagulable nitrogen of the blood is 89 mg. per 100 c.c.; the urea is 52 mg. per 100 c.c. December 2, 1918: The animal weighs 2,440 gm. The total incoagulable nitrogen of the blood is 123 mg. per 100 c.c.; the blood urea is 60 mg. per 100 c.c. The urine shows a trace of albumin but no casts.

A consideration of the figures for incoagulable nitrogen and blood urea shows that both were about twice the normal for rabbits, four weeks after this animal had been placed on a normal diet. The nitrogen retention is evidence of a serious disturbance in renal function. It is also worthy of special note that the animal lost 285 gm. during the last four weeks of his life in spite of the fact that he was receiving a normal diet. The continuous loss of weight under these conditions, indicates that the rabbit was seriously ill.

Dec. 3, 1918: Necropsy: All organs but the kidneys appear to be normal. They weigh 15 gm. The capsule strips easily, leaving a finely granular surface. Microscopic examination shows a well marked irregular increase in connective tissue. Just beneath the capsule, fairly large patches of connective tissue have caused contraction in these areas. The connective tissue extends in irregular bands through the cortex deep into the medulla. Many of Bowman's capsules are much thickened. Many glomeruli show atrophy, and a few, marked scarring. The convoluted tubules are dilated and lined with a flattened epithelium whose nuclei are picnotic. Desquamation of the tubular epithelium is seen in some places. There are many casts throughout the section.

The protocols and the photographs of the kidneys make it clear that the nephritis produced by the soy bean closely resembles the chronic nephritis commonly found in human subjects—the secondarily contracted kidney of the pathologists. The lesion is not the result of arteriosclerosis, for the arteries are not diseased. Nor are we dealing with a primary glomerulonephritis. Judging by the constancy and severity of the damage to the epithelium of the convoluted tubules, the firm impression is gained that the offending substance has its primary and chief deleterious effect upon the epithelium of these tubules and that this slowly progressing injury of the convoluted tubular epithelium is accompanied by an overgrowth of the connective tissue.

The soy bean nephritis causes a marked interference with renal function. There is an early and persistent retention of blood urea. Table 1 shows the blood urea readings in normal animals and in animals that had lived on soy beans for (1) seven weeks and for (2) six months. The normal animals ate hay, oats and bread. All the animals were fasted for twenty-four hours before drawing the blood.

This precaution was taken in order to avoid an error in favor of the nephritis rabbits. Since these animals were eating abnormally large amounts of protein, their blood urea might be abnormally high during the digestive period. But the undamaged kidney would eliminate any excess of blood urea from that source during the twenty-four hours' fast.

TABLE 1.—BLOOD UREA IN NORMAL AND IN SOY BEAN RABBITS

Normals		Rabbits Living on Soy Beans			
Hay, Oats, Bread		Seven Weeks		Six Months	
Number	Urea per 100 C.c.	Number	Urea per 100 C.c.	Number	Urea per 100 C.c.
A	42 mg.	33	83 mg.	—	—
B	33 mg.	34	90 mg.	—	—
C	17 mg.	35	84 mg.	35	176 mg.
D	36 mg.	36	96 mg.	36	118 mg.
E	29 mg.	37	98 mg.	37	78 mg.
F	53 mg.	38	98 mg.	38	87 mg.
Average	31 mg.	Average	100 mg.	Average	115 mg.

The experiments described above leave no doubt that a well marked chronic nephritis is produced in rabbits when the diet is restricted to soy beans. Evidence has been presented to show that the renal lesion is not the outcome of a lack of any essential food constituent. Nor can the lesion be attributed to an hypothetical nephrotoxic substance in the bean. For the habitual use of the soy bean by the Japanese and Chinese as food, and the excellent results obtained by Osborne and Mendel,⁴ and by Daniels and Nichols⁵ when the bean was the sole source of protein in the diet, make it highly improbable that the bean contains any poisonous substance which could so regularly and seriously damage the kidney of the rabbit. The only tenable explanation, in the light of the results obtained with egg white and casein, is one which relates the nephritis observed in each group of experiments, to the common factor, namely, the high protein content of the diet.

NITROGEN METABOLISM

Having now shown that nephritis may be produced in rabbits by feeding high protein diets, it is of interest to compare the nitrogen metabolism of such rabbits with that of rabbits eating a normal diet. To that end the following plan was used.

The rabbits used as normals were taken from a stock cage and placed in metabolism cages. They were fed oats and carrots, and allowed to eat as much of the weighed amount offered as they chose. The first twenty-four-hour specimen of urine was discarded. All the urine voided during the next five days was saved and measured.

Enough strong HCl was placed in the receptacles to make the urine acid. The urine was acidified to prevent loss of urea. It had been found previously that the urea in the alkaline rabbit urine gradually breaks down. The total amount of each food eaten during the five day period was determined, and the approximate amount of protein eaten was calculated from Atwater and Bryant's tables. The urines were analyzed for total nitrogen, urea nitrogen and ammonia nitrogen. All calculations were reduced to a twenty-four hour period. The normal data were obtained from six rabbits. Exactly the same procedure was followed with the three rabbits of Subgroup 3, eating egg white, three rabbits on casein diets and four of the rabbits living on soy beans.

TABLE 2.—PROTEIN EATEN, PROTEIN ABSORBED AND NITROGEN EXCRETED IN URINE

	Rabbit No.	Protein Eaten, Gm.	Protein* Absorbed, Gm.	Urine			
				Total N, Gm.	Urea N, Gm.	Urea, Gm.	Amount, C.c.
Controls	—	8.5	5.3	0.85	0.64	1.37	105
	—	11.2	5.7	0.91	0.50	1.07	109
	—	11.4	4.2	0.67	0.53	1.13	74
	—	8.0	4.5	0.72	0.59	1.26	107
	—	13.0	4.8	0.78	0.61	1.31	62
	—	9.7	5.7	0.91	0.89	1.90	90
	Average	10.3	5.0	0.81	0.63	1.34	91
Egg white	110	15.0	7.9	1.21	1.12	2.40	87
	111	15.0	9.2	1.48	1.44	3.08	120
	112	15.0	13.9	2.23	2.05	4.40	182
	113	15.0	7.4	1.19	1.17	2.50	155
	113	25.0	14.3	2.24	1.80	3.90	120
	Average	—	—	—	—	—	—
Casein	57	15.0	11.5	1.54	1.66	3.65	70
	58	15.0	11.9	1.91	1.87	4.0	93
	61	30.0	14.8	2.38	2.25	4.80	220
	Average	—	—	—	—	—	—
Soy Beans	38	16.7	8.2	1.32	1.08	2.31	114
	92	26.6	14.0	2.24	2.05	4.39	202
	96	19.0	12.9	2.07	1.83	3.92	111
	95	8.4	8.2	1.32	1.15	2.46	60
	Average	17.7	10.8	1.74	1.53	3.27	122

* This figure is obtained by multiplying the total urinary nitrogen by 0.25.

Table 2 shows that when rabbits are living on what may be considered a normal diet, they eat by choice about 10 gm. of protein daily; but that they absorb only about 5 gm., or half of the protein eaten. The same relation holds in regard to the mixture of egg white, corn meal and bran. Rabbits which ate this mixture containing about 15 gm. of protein absorbed about 8 gm. of protein. When, however, plain, hard boiled egg white was fed, nearly all of it was absorbed. A correspondingly greater effect on the kidney was noted. The absorption of the digestion products of casein was

very good in the case of the rabbits that received 15 gm. of casein daily. They had a nitrogen metabolism of more than twice the normal. It will be seen from the table that the ingestion of soy beans by the four rabbits tested was quite variable. In order to get a more reliable figure for the average daily consumption of the beans, the amount eaten by eight different rabbits during seven-day periods was determined. The consumption was measured for thirty such periods. The resulting average was 40 gm. of beans eaten daily by each rabbit, with a variation from 22 to 80 gm. The beans used by us contain 37.8 per cent. of protein, hence the animals were eating 15 gm. of protein daily. This is somewhat less than the ingestion by the four rabbits during the test period. However, the individual averages of these four rabbits determined in the case of the first one from seven periods, for the second, third and fourth ones from four periods each, gave the following average intakes: 13.9 gm., 18.1 gm., 17.0 gm., 12.0 gm. The average is 15 gm., which is the general average. The figure found in the table for average protein absorption is probably also a little high, but cannot be far from correct. Accordingly, it may be stated that the protein metabolism of rabbits whose sole food is the soy bean, is about twice the normal.

When these figures for protein absorption from the various diets are compared with each other, it is seen that a chronic nephritis is produced when soy beans are eaten in sufficient quantity to double the total nitrogen metabolism. But casein and egg white must be fed in sufficient amounts to treble the total nitrogen in order to injure the kidney seriously; and furthermore, the injury from both casein and egg white tends to be acute rather than chronic. Apparently, the kidney is not injured by the secretion of that portion of the total nitrogen which is formed in amounts corresponding to the quantity of protein absorbed, for if that were the case, the injury to the kidney would be expected to be proportionate to the amount of protein absorbed, regardless of the source; and urea, which is the quantitatively important end-product of all protein metabolism, would be expected to damage the kidney in proportion to the amount secreted by it. But a comparison of the urea figures for soy bean rabbits with the average urea output of three rabbits eating casein, shows that the soy bean produced nephritis in rabbits that were secreting 3.3 gm. of urea daily, whereas the casein food failed to produce nephritis in rabbits whose urea averaged 4.1 gm. daily. This question was investigated further by feeding 5 gm. of urea daily to three rabbits that were eating an otherwise normal diet. The rabbits ate the urea for two, three and nine months, respectively. Analysis of the urine for urea showed that they were eliminating a little more than twice the normal amount. The

urines occasionally contained the slightest trace of albumin, but never any casts; and microscopic examination of the kidneys failed to show any abnormalities which could be attributed to the urea. It may accordingly be definitely stated that the renal lesion produced by feeding high protein diets is not caused by the passage of too much urea through the kidney.

It would appear rather that the kidney injury was related to those digestion products of protein which vary both quantitatively and qualitatively with the type of protein eaten. In this connection, one thinks of the amino acids. They form a constant constituent of the urine, and the quantity of amino acid nitrogen found in the urine is proportionate to the protein metabolism. On the other hand, individual members of the group are found in large amounts in some proteins and are nearly absent from others. Whether the amino acids are responsible for the nephritis can only be determined by studying their effect on the kidney. At present, no data which will throw any light on this question are available.

SUMMARY

In the majority of cases of chronic Bright's disease the cause of the nephritis cannot be discovered. This investigation has been undertaken with the intention of determining whether the elimination of abnormally large amounts of nitrogenous material by the kidney will cause nephritis. To this end, high protein diets have been fed to rabbits. The following results were obtained:

1. Renal injury was very quickly and constantly noted in rabbits that ate several egg whites daily.
2. Prolonged egg white feeding caused acute and subacute nephritis.
3. When the nitrogenous metabolism was increased by means of casein, rabbits suffered no demonstrable renal injury from eating 15 gm. of casein daily; but when the daily intake of casein was 30 gm., and the nitrogen metabolism was about three times normal, a well marked deleterious effect upon the kidney was produced.
4. Rabbits that lived on soy beans for months regularly acquired chronic nephritis and frequently died of it. The nitrogen metabolism from this diet was about twice the normal.
5. The renal lesion produced by feeding high protein diets was not caused by the passage of too much urea through the kidney.

The data suggest that the kidney injury is related to those digestion products of protein which vary both quantitatively and qualitatively with the type of protein eaten.