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I hereby recommend that the thesis prepared under my supervision by Martin H. Kuizenga *entitled* Blood changes after Nephrectomy as Affected by Cortin.

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Approved by: Albert P. Mathews

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BLOOD CHANGES AFTER NEPHRECTOMY AS AFFECTED BY CORTIN

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by

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Table of Contents

Table of Contents (continued)

General Introduction.

The first part of this thesis is concerned with answering the question "Is there an internal secretion of the kidney?" The literature concerning this subject is reviewed and the experiments to determine prolongation of life after nephrectomy by administration of certain concentrated kidney extract are given.

In the second part there is taken up the results of total and partial nephrectomy. The determination, of the greatest possible reduction of kidney substance in the rabbit, is given. There is presented the character and course of the renal insufficiency produced by surgical removal of sixty to seventy per cent of the kidneys.

In part III the effects of administration of cortin to animals with renal insufficiency are reported and the changes in blood non protein nitrogen and sugar after cortin injection into nephrectomized rabbits are recorded. From the results obtained there is presented the proposition that cortin influences kidney function.

In 1889 Brown Sequard published his theory of internal secretion, and included the kidney as an organ of internal secretion. In 1893 he stated that, while nephrectomized animals lived only thirty hours on the average, similar animals to which kidney extracts were given lived sixty hours (1). The dog was the experimental animal used by Brown Sequard. He concluded from his experiments that the accumulation of the nitrogenous waste products was not the only factor responsible for the appearance of uremic symptoms; the disturbance of the internal secretion of the kidney being a more powerful and active factor.

Vitzon in 1902 (2), also investigating the internal secretion of the kidney, claimed to have kept animals alive following a two stage double nephrectomy for as long as from 146 - 164 hrs., by no other measure than the injection of blood serum from the renal veins of healthy animals of the same species. This, he interpreted, as due to the internal secretion of the kidneys.

The Englishman, J. R. Bradford (3), shortly before the work of Vitzon made some interesting studies on dogs in which he removed not all the kidney tissue, but merely about two-thirds. The manner in which this was accomplished is as follows. The animals were submitted to two operations. The first operation consisted in the removal of a V shaped piece of kidney on the left side. After the animal, - inthis case again the dog, - had survived this operation, the whole right kidney was removed. Removal of two-thirds of the kidney tissue in this manner "causes" says Bradford, "emaciation, if animals do not die." "Hemorrhage from the gum has been seen; ulcerated sores on the lips and mucous membranes of cheek are common and circular superficial ulcers have been observed on legs just above the ankles. These ulcers are symmetrical and probably arise from injury. They do not heal, but discharge and become offensive. Doubtless in the normal dog these same causes of injury are at work, but no ulceration and loss of substance occurs, such as seen in these dogs after extensive ablation of kidney substance." When two-thirds to three-fourths of the total kidney tissue was removed Bradford found an increase in the amount of urine, and also an increase in the non-protein-nitrogen of the blood accompanied by an increase of urea excretion. These observations of Bradford have been corroborated by the findings of many later workers in the field. Because of the great emaciation, the increase in urinary secretion, and amount of blood non-protein-nitrogen as well as urea excretion, Bradford postulated the existance of an internal secretion of the kidney which controlled or inhibited protein metabolism.

The work of Ajello and Parasiendalo, as quoted by Pearce (4), consisted in the determination of the effect of glycerine kidney extracts on the length of life of bilaterally nephrectomized dogs. Twelve control dogs died in from 4 - 48 hrs. after double nephrectomy with symptoms of dyspnea, convulsions and gastro intestinal disturbances considered to be uremic; while of ten nephrectomized dogs treated with glycerine extracts of the kidney substance, one lived four days, six lived three days and three died in 48 - 52 hrs. "Many other investigations", says Pierce, "gave similar results."

The older work thus far reviewed indicates that the life of bilaterally nephrectomized animals can be prolonged by the administration of kidney extracts, and the results, including those of Bradford, may be adduced to explain the pressure of an internal secretion of the kidney. The length of life of the treated nephrectomized animals, however, wasonly little greater than of the untreated. More recent work has shown that the earlier observations are inconclusive.

Andrews (5) in 1927 reported several cases of dogs surviving from 4 - 10 days following total nephrectomy.

Ivy (6) in 1929 found that subcutaneous injection of Ringer's solution prolonged the life of totally nephrectomized dogs and brought about a condition of edema. Andrews observed that his animals showed no evidence of acid intoxication even in terminal stages. Swingle (7) also observed that dogs do not develop acid intoxication after nephrectomy and survived 80 to 95 hours. In some cases a slight fall in alkali reserve was observed, but the change was in striking contrast to the acidosis that followed adrenal removal. The suggestion is made by Swingle that the acid intoxication of adrenal insufficiency and probably also of uremia is extra renal in origin, since it is not present in nephrectomized dogs with total suppression of kidney function.

The results of these recent investigations show that the length of life after total nephrectomy is much longer than the work of earlier investigators indicates. The perfection of operative technique is, no doubt, the explanation of the great increase in length of life recently obtained. The observations on acid intoxication in adrenal insufficiency, which was not made in cases of total absence of kidney is merely mentioned at this time, since the relationship between the adrenal glands and kidney function will be considered in a following section of the report of this investigation.

Although the length of life of nephrectomized animals reported by Brown Sequard, Vitzon, and Ajello and Parosciendalo is shorter than that reported by Andrews, Ivy and Swingle, the fact still remains that the earlier observers found an increase in prolongation of life after extract treatment. This, assuredly, may have been due to the saline containing the kidney extract, in the light of the experiment of Ivy in which injection of Ringers solution was followed by longer life after nephrectomy.

It therefore becomes quite necessary to determine whether the injection of a concentrated kidney extract could prolong the life of bilaterally nephrectomized animals. The animal used in these experiments was the rabbit. These animals were all healthy rabbits of very nearly the same weight ranging from two to two and one half kilos. Both kidneys were removed at one operation and by as aseptic technique as possible. An incision was made on the linea alba about five centimeters below the end of the sternum. The kidneys were each in turn, beginning with the left one, located, decapsulated, the blood vessels and ureter ligated in one operation, and removed. The animals were kept under ether anaesthesia and the length of operation was usually forty-five minutes from the beginning of anaesthesia till the completion of the skin suture.

The first extract used was an alcohol benzene soluble portion of ground sheep kidney. Six sheep kidneys were finely ground and allowed to macerate in 95% alcohol for three days at room temperature. The alcohol was removed and extraction continued for 3 days with 80% alcohol. The combined alcoholic extractionswere concentrated in vacuo at a temperature of 40 - 45° C to 1/20 of the original volume. This acqueous residue was mixed with an equal volume of benzene and the benzene washing repeated several times in a separatory funnel. The benzene was removed in vacuo and the lipid residue suspended in water and used.

Four rabbits were bilaterally nephrectomized as above described and two of them injected twice daily, in the morning and in the evening with 2 c.c. of the extract. All injections were made intra-peritoneally. The two control rabbits Nos. 90 and 1290 died one in 68 hrs., the other in 65 hrs. The rabbit No. 592 and 1300 that received the injection died in 62 and 80 hrs. respectively. The difference in length of life between the control and those receiving the injection, is therefore not great. It was observed however that the acqueous suspension of the lipid material was not easily absorbed. It was therefore decided to try to obtain a water soluble extract that could easily be absorbed. The method of extraction employed was that used by Swingle and Pfiffner (8) in obtaining cortin from the adrenal glands.

2000 gms. of beef kidney were finely ground in a meat grinder and the extraction begun as above. The benzene residue was taken up in acetone by repeated washing and rubbing of the lipid residue in acetone. The acetone dry residue was taken up in petrolic ether. The 30 c.c. of petrolic ether solution was washed in a separatory funnel with 70% alcohol. Several portions of alcohol were used. These were combined and alcohol removed and residue made up to 50 c.c. with water. NaCl was added to make the preparation isotonic, and was then filtered through a Berkfeld filter and kept in a sterile flask.

Ten animals were used in this experiment, of which five were injected with the preparation from beef kidneys and five were used as controls. The protocols of these animals are given below. The average length of life of the control animals was seventy-nine hours, while that of those receiving extract was ninety-three hours. The extract was administered again twice daily in the morning and in the evening.

Although the animals receiving the injection did live, on an average, a little longer than the controls, the greatest length of life in any one case was not longer than the greatest length of life of the controls. Two rabbits, numbers 10 and 640, were treated with a saline extract of the beef kidney after they had been bilaterally nephrectomized. Two beef kidneys were passed through a meat grinder into 1% saline. One liter of saline was used and after allowing to macerate at room temperature with occasional stirring for five hours, the mixture was placed in the ice box for three days. At the end of this time the liquid portion was separated from the tissue by straining through a muslin cloth. The extract thus obtained was concentrated to 200 c.c. in vacuo and this filtered through coarse filter paper and used for injections. The length of life of the animals, numbers 10 and 640, given the extract was, respectively, 101 and 80 hrs. Again the length of life of the treated animals was not greater than that of the control group. Although it is true that this last salt extract was not sterile, there was at autopsy no bad sepsis at the site of injection.

Those animals which at autopsy were found to have had peritonitis or tubercles in the lungs or liver were excluded. All the nephrectomized animals looked very well 24 - 48 hrs. after the operation, and symptoms began by a drooping of the head. The weakness gradually increased, but at no time were the animals comatose and death always occurred quite suddenly.

Protocols of rabbits used in determination of effect of the alcohol benzene soluble kidney extract on prolongation of life after total nephrectomy.

Protocol of Rabbit No. 90.

Bilaterally nephrectomized at 11 a.m. January 19, 1932.

Death occurred between 1 and 7 a.m. Friday Jan. 22.

Length of life after nephrectomy was about 65 hrs.

At autopsy there could be found no sepsis or adhesions. The lungs looked very good. The liver, except for a dark color, was also of very healthy appearance.

Protocol of Rabbit No. 1200.

Bilaterally nephrectomized at 3:30 o'clock. January 28, 1932.

Death occurred at 11:45 a.m. January 31.

Length of life afternephrectomy was 68 hrs.

Autopsy:-

1. Slight adhesion of subcutaneous tissue at place of incision.

2. No peritonitis and no adhesion or infection at site of operation.

3. Liver, lungs and other organs were very healthy and normal except that the liver was quite dark.

Protocol of Rabbit No. 592.

1. Bilaterally nephrectomized at 10 p.m. Feb. 2, 1932.
2. Injected with 2 c.c. of extract twice daily in the morning and the evening, beginning 9 a.m. Feb. 3.
3. Death occurred at 12 o'clock, Feb. 5. Length of life = 62 hrs.
4. Autopsy showed presence of slight sepsis.

Protocol of Rabbit No. 1300.

1. Bilaterally nephrectomized at 4 p.m., Feb. 3.
2. Injection of 2 c.c. of extract twice daily beginning at 9 p.m., Feb. 3 to 10 a.m., Feb. 7.
3. Death occurred at 12:15 p.m., Sunday, Feb. 7. Length of life = 80 hrs.
4. Autopsy:-
    1. Slight subcutaneous adhesion.
    2. No peritonitis, no adhesion at site of operation.
    3. Liver and lung normal, except for a dark color of the former.

Protocols of Rabbits used in determination of effect of kidney extract prepared according to Swingle-Pfiffner technique for preparation of cortin.

Protocol of Rabbit No. 12.

1. Bilaterally nephrectomized at 12 m, Friday, Feb. 19, 1932.
2. Death occurred on Monday morning, Feb. 22.
3. Length of life = 65 hrs.
4. Autopsy:-
    1. No sepsis.
    2. Slight subcutaneous adhesion.
    3. Lungs and liver normal.

Protocol of Rabbit No. 1252.

1. Bilateral nephrectomy 10 p.m., Friday, Feb. 19.
2. Died morning of Feb. 24.
3. Length of life = about 102 hours.
4. Autopsy:-
    1. No sepsis or adhesions at site of operation.
    2. Slight subcutaneous adhesion.
    3. Lungs and liver were normal in appearance.

Protocol of Rabbit No. 108.

1. Bilaterally nephrectomized at 12 p.m., Feb. 19.
2. Died immediately after 12 p.m., Feb. 23.
3. Length of life = 96 hrs.

4. Autopsy:-

    1. Slight subcutaneous adhesion.

    2. No sepsis.

    3. Lungs and liver normal.

Protocol of Rabbit No. 1250.

1. Bilaterally nephrectomized 2:30 p.m., Friday, Feb. 12, 1932.

2. Received injection of 2 c.c. kidney extract twice daily beginning Saturday a.m., Feb. 13 to Feb. 16.

3. Died at 10:30 a.m., Feb. 16. Length of life was 92 hrs.

4. Autopsy:-

    1. No adhesions and no peritonitis.

    2. Normal liver and normal lungs.

Protocol of Rabbit No. 104.

1. Bilaterally nephrectomized Saturday 3 p.m., Feb. 13, 1932.

2. Injection of extract from Sunday Feb. 14 to a.m. Feb. 17.

3. Death at 2 a.m., Feb. 18.

4. Length of life after nephrectomy = 107 hrs.

5. Autopsy:-

    1. Subcutaneous infection.

2. No peritonitis, nor adhesions at site of operation.
3. Lungs and liver were normal.

Protocol of Rabbit No. 3.

1. Bilateral nephrectomy Monday, Feb. 15, 1932, at 4 p.m.
2. Injection of 2 c.c. twice daily from Monday 9:00 p.m. to Wednesday 10 a.m., Feb. 18.
3. Death occurred at 10 p.m., February 18. Length of life = 84 hrs.
4. Autopsy:-
   1. No peritonitis and no adhesion.
   2. Liver and lungs normal.

Protocol of Rabbit No. 1251.

1. Bilaterally nephrectomized Feb. 16 at 12 m.
2. Injection of extract
   (a) Feb. 16, 11 p.m. 2 c.c.
   (b) Feb. 17, 10 a.m. 2 c.c.
   (c) Feb. 17, 10 p.m. 2 c.c.

   This animal and No. 64 were given the last of the extract and only three injections each.
3. Death occurred at 4 p.m., Friday, Feb. 19. Length of life = 76 hrs.

4. Autopsy:-

    1. No adhesion at site of operation and no sepsis.

    2. Slight subcutaneous adhesions.

    3. Normal lungs and liver.

Protocol of Rabbit No. 64.

1. Bilaterally nephrectomized at 10:30 p.m. Feb. 16.

2. Injection of extract

    (a) Feb. 16, 11 p.m. 2 c.c.

    (b) Feb. 17, 10 a.m. 2 c.c.

    (c) Feb. 17, 10 p.m. 2 c.c.

3. Death occurred Sunday, 9 a.m., Feb. 21. Length of life = 108 hrs.

4. Autopsy:-

    1. No peritonitis or sepsis.

    2. No adhesion.

    3. Lungs and liver were normal.

Summary of Experiment to Determine Effect of Kidney Extracts on Prolongation of Life of Nephrectomized Rabbits.

I. Control Group.

No. 90 lived after nephrectomy 65 hrs.

No. 1200 lived after nephrectomy 68 hrs.

No. 12 lived after nephrectomy 65 hrs.

No. 1252 lived after nephrectomy 102 hrs.

No. 108 lived after nephrectomy 96 hrs.

Average length of life after bilateral nephrectomy = 79 hrs.

II. Group that received alcohol benzene soluble extract.

No. 592 lived after nephrectomy 62 hrs.

No. 1300 lived after nephrectomy 80 hrs.

III. Group that received extract prepared according to Swingle Pfeffner technique for cortin.

No. 1250 lived after nephrectomy 92 hrs.

No. 104 lived after nephrectomy 107 hrs.

No. 3 lived after nephrectomy 84 hrs.

No. 1251 lived after nephrectomy 76 hrs.
(only 3 injections)

No. 64 lived after nephrectomy 108 hrs.
(only 3 injections)

Average length of life after bilateral nephrectomy = 93 hrs.

Protocols of nephrectomized rabbits that received injections of salt extract of the kidney.

Protocol of Rabbit No. 640 ^{o}

1. Bilaterally nephrectomized at 11 a.m. March 19, 1932.
2. Injection of 5 c.c. of extract tissue daily beginning March 19 and ending March 22, 11a.m.
3. Death occurred March 22, 7 p.m. Length of life = 80 hrs.
4. Autopsy:-
    1. No infection or sepsis at site of operation or injection and no peritonitis.
    2. Lungs and liver were normal in appearance.

Protocol of Rabbit No. 10 ^{o}

1. Bilaterally nephrectomized at 12 m. March 19, 1932.
2. Injection of extract daily in 5 c.c. amounts beginning 4 p.m., March 19. On the 22nd two injections, one at 11 a.m. and one at 9 p.m. of 10 c.c. each were made.
3. Death occurred at 5 p.m. March 23. Length of life after nephrectomy was 101 hrs.

4. Autopsy:-

    1. No peritonitis or sepsis at site of operation.
    2. Slight subcutaneous adhesion.
    3. Lungs and liver were normal in appearance.

From the results of these experiments, there can be deduced no good evidence for the existence of an internal secretion of the kidney. On the other hand, the absence of such a secretion has not been proven. It can at present not be definitely stated whether the accumulation of metabolic end products is the sole cause of death after bilateral nephrectomy or whether there is absent also in nephrectomized animals a vital principle normally elaborated by the kidney as Brown Sequard postulated in 1893.

Part II.

The Results of Partial Nephrectomy.

To what extent can the kidney tissue of the rabbit be removed without causing immediate renal insufficiency? What will be the effects of such partial nephrectomy on nitrogen retention, and how will the blood sugar concentration be changed by partial reduction of kidney tissue? Is the hyperglycaemia caused by lack of kidney tissue, or is this increase in blood sugar only present when kidney substance has been removed sufficiently to cause rapid increase of metabolic end products? An attempt to answer these questions is given in the following pages.

That extirpation of the kidneys results in an accumulation of the end products of metabolism, especially protein metabolism, was observed by von Schroeder (9) fifty years ago. He determined the effect on the accumulation of urea of the extirpation of the kidneys and demonstrated that there was always an accumulation and that the longer the animal lived the greater became the concentration of urea in the blood. He and other experimenters observed that carnivores survive the loss of the kidneys for about three days. The cause of death which ensues is not yet certain. Whether death is caused solely by the accumulation of metabolic end products, or by the absence also of a vital principle normally elaborated by the kidneys is difficult to prove, as shown in the preceding chapter.

It is our purpose in this chapter to determine the effect in the rabbit of the partial removal of kidney substance. The experiments of Bradford (3), to which reference has already been made, comprise the first work on the subject of partial extirpation of the kidneys. He observed the changes brought about by such procedure in dogs and his observations and conclusions have already been given.

Hilding Anderson (10) adopted Bradford's technique in his "investigation undertaken to determine the effect of prolonged renal insufficiency on the blood pressure, and the influence of high or low protein diet on the course of chronic renal disease". He found in rabbits that the blood chemistry changes were characterized in general by a progressive accumulation of metabolites. There was a slight rise in the concentration of creatinine after removal of the wedge from the left kidney but was apparent only after averaging many cases. Beginning almost immediately after removal of the right kidney there was a definite gradual increase in the concentration of creatinine and urea nitrogen, which was progressive until death. "The rapidity of the increase bore an inverse ratio to the length of time the animals lived." "The health of the animals," says Anderson, "continued apparently good until a decided retention had developed." "When the concentration of urea rose as high as from 80 - 100 mgms. and creatinine to 3 - 5 mgms., the animals began to lose weight and became less lively. Weakness began to be apparent and the animals failed to eat well. As the concentration rose still higher a day or two before death, they quite eating entirely. The more severe symptoms developed only a few days before death. No convulsions were observed in any." These symptoms of renal insufficiency observed by Anderson are much the same that Bradford (3) observed in his dogs.

In those animals in which total nephrectomy was followed by blood analysis for non-protein nitrogen and sugar, it was my own observation that an hyperglycemia invariably existed when the non-protein nitrogen concentration rose to something over 200 mgms. per 100 c.c. Although the blood sugar value was not always extremely high an hyperglycemia invaraibly existed in the last stages of uraemia. The experiments will be given later. Hyperglycemia in nephritis is very often observed clinically and also after nephritis artificially induced by uranium salts. Hendrix and Bodansky (12) observed hyperglycemia in uranium nephritis in dogs and sought a relation between the pH and sugar of the blood in such nephritis. They state that, in confirmation of much earlier work, acidosis, as shown by the CO_2 combining power and pH of the blood, hyperglycemia and glucosuria have been observed after the administration of uranium acetate to dogs. They state also; however, that although some relationship between degree of acidosis and hyperglycemia is indicated, it did not appear that the relationship was a quantitative one. That this relationship between acidosis and hyperglycemia does not exist is indicated by the observation of Swingle (7) that after complete nephrectomy no acidosis is observed, while after complete adrenalectomy there is a very severe acidosis. And this acidosis after adrenalectomy is accompanied, not by an hyperglycemia, but an hypoglycemia.

It was considered very interesting, therefore, to determine the effect of sixty to seventy-five per cent removal of kidney substance, both on the retention of total non-protein-nitrogen in the blood, the relationship between the extent of such removal and the amount of retention, and also the degree of hyperglycemia if any is produced after such reduction of kidney substance.

First of all, then, there is given in the following table the summary of experiments showing the extent of hyperglycemia after total nephrectomy.

Insert Table I.

The normal blood sugars were 95 to 105 mgms. per 100 c.c. It is very evident therefore that a definite hyperglycemia is present after nephrectomy when the non-protein-nitrogen of the blood has reached great concentration. Is this hyperglycemia also present after reducing the kidney substance to a minimum compatible with life?

The rabbit, again, was the animal used in these experiments. Healthy rabbits ranging from two to two and one half kilos were used. The animals were kept on a diet of oats and hay, with greens once a week. The greens consisted of lettuce leaves, carrots and cabbage. The animals did very well on this diet. Not one unoperated animal that was healthy when received into the laboratory died during the entire year.

The kidney substance was removed by a two stage

TABLE I

Rabbit	Non-protein-nitrogen	Blood Sugar	Length of life after nephrectomy	Remarks
386	330	200	112 hrs.	Bilateral nephrectomy
118	480	165	77 hrs.	Two unilateral nephrectomies
130	326	170	52 hrs.	Two unilateral nephrectomies
39	230	150	80 hrs.	Two unilateral nephrectomies
125	262	209	60 hrs.	Two unilateral nephrectomies

operation, very much after the technique of Bradford. A wedge shaped piece was first removed from the left kidney, and after the animal had completely recovered from this operation, the right kidney was removed. Both the wedge and the whole right kidney were weighed immediately after extirpation. The percentage of kidney tissue then remaining was calculated on the basis that the total kidney weight was twice that of the right kidney.

The animals were prepared for operation by shaving a sufficient area at the proposed site of operation. The clean skin was then washed with 80% alcohol followed by an alcoholic solution of iodine. The operators hands were merely washed in 80% alcohol and the instruments boiled in water containing a little sodium carbonate. A sterile cloth was placed over the animal and an opening made of sufficient size to allow the operative procedure necessary. Lateral incisions were made for each nephrectomy. The incisions were made parallel to the direction of the lowest rib and about five centimeters lower than the lowest rib on the left side and only about two centimeters lower than the lowest rib on the right side.

Ether anaesthesia was maintained during the time of the operation. The first operation, consisting of the removal of the wedge from the left kidney, required about forty-five minutes from the time anaesthesia was begun till the completion of the skin suture. The second operation, consisting of the removal of the whole right kidney, required only about thirty minutes.

The blood samples for analysis were obtained from the ear veins by means of a needle and syringe, but where only occasionally, samples were obtained, cardiac puncture was done. Samples were obtained immediately before the first operation and afterward to determine the effect on the non-protein-nitrogen of the blood. From time to time after the second operation samples of blood were obtained to determine the non-protein-nitrogen and sugar. Both determinations were made according to the Folin-Wu method of blood analysis (13).

These partially nephrectomized animals consist of two groups. The first group comprises those whose kidney substance was reduced to an extent that was incompatible with long life after removal of the right kidney. The second group comprises those that lived eighty days or longer after removal of the right kidney. In the last group a smaller wedge had been removed from the left kidney.

The blood non-protein-nitrogen of the first group began increasing immediately after the second operation and there was a continuous gradual increase till death. The length of life, the increase in non-protein-nitrogen and the hyperglycaemia are very much the same as after complete nephrectomy. A summary of the changes in blood non-protein-nitrogen and sugar after such partial nephrectomy is given in the following table. The order in which they are given is that of the order of length of life after removal of right kidney, those living the shortest length of time, appearing first. It is apparent from the results summarized in this table that more than seventy per cen reduction of kidney substance results in death in a few days and that blood sugar and non-protein-nitrogen are as high just before death, as they are just before death after total nephrectomy. In the group of animals that suffered less than seventy per cent reduction of kidney the non-protein-nitrogen of the blood increased after the last operation. The high concentration was not maintained however if the animal survived the first few days, but decreased again until just before death when the high values observed before death after total nephrectomy were again obtained. This is best shown in rabbits 432 and 436. Coincident again with the high non-protein-nitrogen was a high blood sugar. These changes are represented graphically in figure one which was

26a

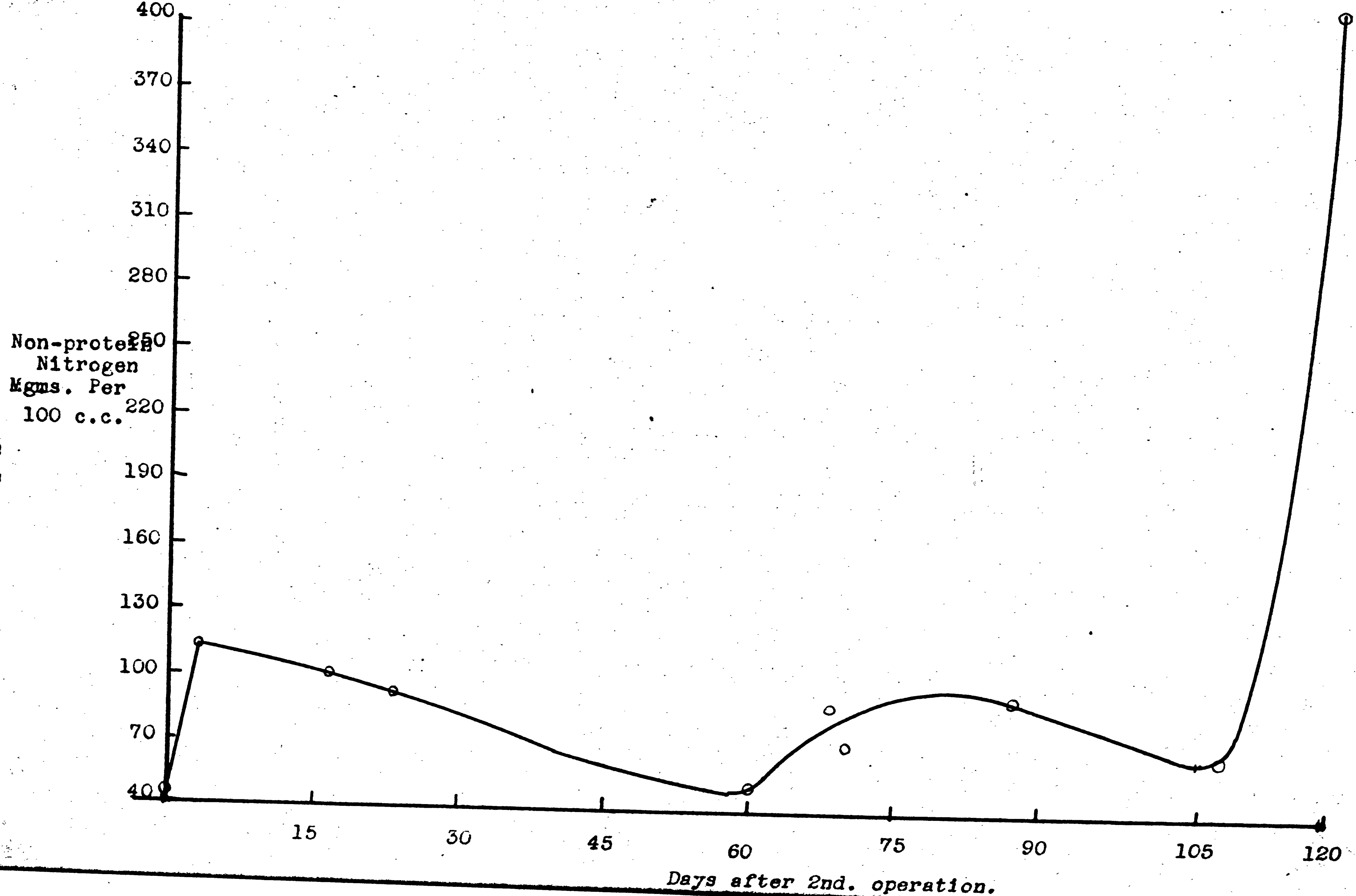
Non-protein Nitrogen Mgms. Per 100 c.c.
400
370
340
310
280
250
220
190
160
130
100
70
40
15
30
45
60
75
90
105
120
Days after 2nd. operation.

TABLE II.

Summary of Change in Blood non-protein-nitrogen and Sugar after Nephrectomy.

Rabbit	Days survived after 2nd operation	% of kidney substance remaining	Lowest non-protein-nitrogen value after 2nd operation	Highest non-protein-nitrogen value after 2nd operation	Highest Blood Sugar
593	2	27	140 (1)	* 149 (2)	285 (2)
439	3	21	53 (1)	* 307 (3)	442 (3)
753	3	33	122 (1)	* 315 (3)	180
764	4	28	39 (1)	280 (4)	135 (4)
433	4	32	100 (2)	264 (4)	160 (4)
751	5	20	46 (1)	302 (5)	244 (5)
758	6	32	120 (1)	374 (6)	151 (6)
434	7	34	98 (2)	* 176 (7)	320 (7)
445	8	21	70 (1)	122 (8)	126 (8)
767	7	27.5	50 (1)	450 (7)	190 (7)
443	19	32	99 (3)	620 (19)	165 (19)
432	80	30	60 (22)	116 (14)	
444	102	37	59 (24)	102 (102)	125
436	120	33	51 (60)	450 (120)	150 (120)
446	129	33	52 (92)	81 (10)	134
415	202	35	40 (7)	132 (173)	140 (173)
6000	250	35	33 (15)	92 (148)	164 (6)

TABLE II. (continued)

Rabbit	Days survived after 2nd operation		% of kidney substance remaining	Lowest non-protein-nitrogen value after 2nd operation	Highest non-protein-nitrogen value after 2nd operation	Highest Blood Sugar
757	25		30	* 59 (4)	* 114 (14)	
437	110		32	61 (72)	89 (2)	154 (3)
447	136	Still Living	36	52 (23)	97 (80)	119
775	164		28.9	52 (100)	103 (116)	Normal
429	175		36	44 (60)	55 (7)	Normal

The small figures in brackets indicate the day after 2nd operation the analysis was made.

* The values given for these animals were obtained after the injection of cortin.

TABLE III.

Summary of Operative Procedures and Necropsies.

Rabbit	Days survived after 2nd operation	Weight of piece of left kidney excised	Weight of right kidney	% of kidney substance remaining	Weight of remainder of left kidney
593	2	4.5 gms.	9.7 gms.	27	5.2 gms.
753	3	3.5 gms.	10.5 gms.	33.3	3.2 gms.
439	3	4. gms.	6.9 gms.	21.	4.4 gms.
764	4	4.7 gms.	10.9 gms.	28.	6. gms.
433	4	3.3 gms.	9.4 gms.	32.	3.1 gms.
751	5	4.7 gms.	7.8 gms.	20	3.2 gms.
758	6	3.5 gms.	10. gms.	32.5	3.7 gms.
767	7	4.1 gms.	9.1 gms.	27.5	5.4 gms.
434	7	4.2 gms.	13. gms.	34.	5. gms.
445	8	5.2 gms.	9. gms.	21.	3.7 gms.
443	19	3.5 gms.	10.1 gms.	32.6	3.7 gms.
432	80	3.2 gms.	8.5 gms.	30.	6.0 gms.
436	120	3.3 gms.	9.9 gms.	33.3	8.5 gms.
446	129	3. gms.	9.2 gms.	33.3	13.0 gms.
415	202	3. gms.	10. gms.	35.	9.6 gms.
6000	250			35.	9.2 gms.
444	102	3.0 gms.	11.4 gms.	36.	12.6 gms.
429	175	2.5 gms.	9. gms.	36.	
757	25	3.9 gms.	9.9 gms.	30.	
437	110	3.8 gms.	10.6 gms.	32.	
447	136	2.2 gms.	8.3 gms.	36.7	
775	164	3.7 gms.	8.8 gms.	28.9	

constructed with the data obtained from rabbit 436. This is merely typical of the results obtained with the entire group, 432, 436, 415, 6000, 757, 437, 447, 775 and 429, as is seen from the protocol of these animals given in the following pages.

In table 3 are given the summary of operative procedures and necropsies. The average per cent of kidney substance remaining in those animals that died in eight or less days after the second operation was twenty-seven. The average per cent remaining in those that lived eighty days and longer was 33.3 . Rabbit number 775 is an exception to the general rule that no more than seventy per cent of the kidney can be removed without ending in death in a few days. This animal, however, received several injections of cortin and will be discussed in the next section. It can be observed also from table 3 that marked hypertrophy of the kidney remnant occurred in those animals that lived long after the second operation. The average weight of the remnant in five animals was 10.3 gms. Microscopic sections were made of the pieces removed, of right kidney, and of the remnant obtained at autopsy. The kidney remnants presented in all cases marked enlargement of the glomeruli.

Summary of Results.

1. Total nephrectomy is followed by an hyperglycaemia. This hyperglycaemia is present just before death, when the non-protein-nitrogen is extremely high.

2. Reduction of kidney substance by more than seventy per cent is followed by a gradual increase in non-protein-nitrogen, and again by an hyperglycaemia just before death when the non-protein-nitrogen is high.

3. Reduction of kidney substance by more than sixty per cent and less than seventy per cent is followed by a maintained high level of non-protein-nitrogen in the blood. The blood sugar values are normal, until a condition of renal insufficiency is developed where the non-protein-nitrogen is high. At this time again there is an hyperglycaemia.

4. There is great hypertrophy of the kidney remnant after sixty to seventy per cent removal of kidney substance. This hypertrophy is so great that the weight of the remnant is equal to or greater than the weight of a normal functioning kidney.

Discussion of Results.

It is evident that the hyperglycaemia after total nephrectomy is not brought about by a lack of kidney substance, since reduction of kidney by seventy per cent does not disturb the sugar concentration of the blood. It is only when the non-protein-nitrogen has reached values greater than 200 mgms. per 100 c.c. that an hyperglycaemia is observed, both after total nephrectomy and partial nephrectomy. Bodansky (12) has sought to explain the hyperglycaemia by disturbance of acid base equilibrium; the acidosis and hyperglycaemia having been thought to occur together. This, however, does not consistantly happen, even in nephritis as Bodansky himself mentions. Swingle and Andrews, indeed, have observed no acidosis after total nephrectomy, but high blood sugar values are obtained. What then can be the cause of the hyperglycaemia observed? An attempt to answer this question will be given in the next section.

Anderson ( ) has observed after limitation of the kidney to from thirty to forty per cent that the urea nitrogen gradually increased till death, even in those animals that lived a hundred days or more, but finally died of renal insufficiency. Such a gradual increase in the total non-protein-nitrogen has not been observed in the experiments here reported. There was a higher concentration of non-protein-nitrogen the first few days after nephrectomy, than there was one to two months afterward. Before death occurred of renal insufficiency this concentration did again gradually increase till just before death, when it was highest. The hypertrophy, reported by Anderson, of the remnant of the left kidney in four of his rabbits is confirmed by the results here reported.

According to Anderson's results (11) it is impossible to remove more than 63 - 65% of the kidney without having the rabbit die in a few days. The results here reported indicate that 68 - 70% of the kidney may be removed and the animal live eighty to two hundred days before death of renal insufficiency. The average weight of the wedge removed from the left kidney of the five rabbits that died in 46 to 168 days reported by Anderson was 2.1 gms. The average weight of the wedge removed from the left kidney of those five animals already dead and here reported is 3.1 gms. These animals survived eighty to two hundred and fifty days after the second operation. The average weight of the wedge removed from the left kidney of four others still living at the time of this writing, 110 to 175 days after the second operation, is also 3.1 gms. Anderson used rabbits of approximately the same weight as those here studied and the difference in extent of limitation of kidney substance, before death from renal insufficiency in approximately the same number of days cannot be explained by us. It may, perhaps, be due to the fact that the animals used here were in a better state of recovery from the first operation when the second was done. The general state of health of the two groups of animals may have been different.

A very marked arteriosclerosis was observed in the aorta of rabbit No. 415, the report of which is given in the protocol of this animal. It was thought that there might be a relationship between the development of arteriolesclerosis in the kidney and sclerosis of the aorta. Consequently the aorta of all animals dead of renal insufficiency was examined. In no other case was sclerosis observed. Again we would refer to the report of Anderson. He also has observed the occurrence of arteriosclerosis in his animals. He states that the aorta of rabbits on a low protein diet and with renal insufficiency showed no sclerosis. Those on a high protein diet did show sclerosis, and this sclerosis did not extend to the small arteries or arterioles. "The sclerotic changes", says Anderson, "did not seem to bear any relation to the amount of kidney substance functioning."

"Two of the normal aortas were found in those having 50% removed, and one in those having 12% removed. Both of the controls not operated on presented sclerosis." "In one of them the disease was far advanced; just as far advanced as in rabbit 43, which had undergone both operations." The renally deficient controls on a low protein diet developed no aortic lesions, but those on a high protein diet, both the group that had submitted to partial kidney removal and those that were not nephrectomized, did develop sclerosis. These observations of Anderson indicate that the severe arteriosclerosis observed, occurs just as often when renal insufficiency is not present and that a high protein diet predisposes to the production of the lesions.

Insert Protocol of Rabbit 439.

This case is very interesting. When the last kidney was removed, there was left only 20%. At autopsy this small amount of kidney was apparently very hydro-nephrotic.

The nitrogen immediately and greatly increased in the blood. The injection of cortin in this animal is very interesting. The sugar is greatly increased. The non-protein-nitrogen decreased.

Protocol of Rabbit No. 764 °

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10-8-32	2500		30	98
10-10-32		4.7 gm. of left kidney excised		
10-11-32			56	
10-18-32	2525		46	105
11- 7-32	2800	Right kidney removed. wt. = 10.9 gm.	39	98
11- 8-32			106	93
11- 9-32		72% of kidney removed	195	121
11-10-32			280	135
11-11-32		Found dead in the morning		

Autopsy:-

1. Normal heart. No ill effect from cardiac puncture.
2. Lungs and liver appeared normal.
3. Portion of left kidney remaining appeared normal.

Microscopic:-

Slide 764A - right kidney - normal.

Slide 764B - left kidney at autopsy. No marked changes seen.

It is quite evident that this animal died of renal insufficiency caused by too great limitation of renal tissue.

Protocol of Rabbit No. 433.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10- 6-32	2400		38	
10- 8-32		3.3 gms of left kidney excised		
10-11-32			47	
10-16-32			34	135
11-17-32		Right kidney removed. wt.= 9.4 gms.		
11-19-32	2050		100	156
11-20-32			264	160
11-21-32	2050	Found dead at 8:30 a.m. Wt. of remaining kidney was 3.1 gms.		

Autopsy:-

1. Portion of left kidney remaining was functioning since it appeared normal.

2. Lungs were somewhat congested.

3. Peritoneum looked good. Incisions not infected.

3.1 gms. = wt. of kidney remaining, which is 20% of total kidney weight. 3.1/15.8 = .20

Microscopic:-

Slide 433A - Right kidney - congestion of kidney.

Slide 433B - Left kidney at autopsy:
1. Scarring.
2. Slight atrophy and inflamation around suture. p.m. changes

Although the right kidney, when removed weighed 9.4 gms., the total weight of the left kidney, the piece removed and the piece remaining was only 6.4 gms. There was functioning, therefore, after removal of the right kidney no more than 20% of the total kidney tissue originally present. It is therefore quite certain that the animal died of uremic poisoning. The microscopic study of the left kidney revealed that there was scarring, slight atrophy and inflamation so that kidney function was limited almost to a minimum.

Protocol of Rabbit No. 445.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10-11-32	3100	5.2 gms. of left kidney excised	47	
10-13-32			78	
10-18-32	2825		57	89
11-11-32	3100		40	97
11-12-32		Right kidney removed. wt.= 9 gms.		
11-13-32			70	108
11-14-32	2700	80% of kidney removed.	89	129
11-15-32			119	139
11-16-32			94	141
11-17-32			94	129
11-19-32	2625		93	109
11-20-32		Rabbit died of cardiac hemorrhage.	122	126

Autopsy:-

1. Lungs and liver normal.
2. Heart greatly hypertrophied.
3. Remaining portion of left kidney weighed 3.7 gms.

Microscopic study:-

Slide 445A - Right kidney - slight congestion.

Slide 445B - Left kidney at autopsy:
1. Slight atrophy of the tubules.
2. Necrosis of the pelvis of the kidney.
3. Incomplete granulation.

In this animal also, the removal of 80% of the kidney tissue caused a severe condition of renal insufficiency. The blood non-protein-nitrogen kept increasing. The weight of the rabbit steadily declined. The blood sugar also slightly increased. Although the non-protein-nitrogen was not extraordinarily great at time of death, the great decline in weight is a good indication that the condition would have ended fatally in a few days.

Protocol of Rabbit No. 443. o

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10-13-32			36	
10-15-32		3.5 gms. of left kidney excised		
10-18-32	2400		65	112
10-20-32			60	
11-15-32			46	117
11-16-32		Right kdiney removed. Wt.= 10.1 gms.	62	109
11-19-32	2700		99	138
11-20-32			177	147
11-21-32			187	206
11-22-32			273	187
11-23-32			233	213
11-23-32		2:30 p.m. Injection of cortin 2 c.c. 2 x		
		4:00 p.m.	188	173
		5:00 p.m. Injection of cortin 2 c.c.		
		7:30 p.m. Sample		
		10:00 a.m.	162	195
11-24-32			203	128
11-29-32			174	160
11-30-32			214	161
12- 1-32			232	158
12- 3-32	2025		400	210
12- 4-32	1900		540	
12- 5 -32	1900		620	

Protocol of Rabbit No. 443 o (continued)

Rabbit died.

Autopsy:-

1. Clot in pericardium.
2. Kidney remaining weighed 3.7 gms.
   79% of kidney removed.

The kidney was reduced in this animal to the extent of 79%. This caused an increase of blood non-protein-nitrogen to 820 mg. before death. The blood sugar was already high when the non-protein-nitrogen was 180. Even when the nitrogen was 820, the blood sugar had not increased above what it was when the nitrogen was 180.

The increase of nitrogen was quite gradual. The operation was done on November 16th, and the animal died on December 15th.

Protocol of Rabbit No. 432.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10- 6-32	2200	3.2 gms. of left kidney excised	38	
10 -8-32			42	105
11- 8-32			39	121
11- 9-32		Right kidney removed. Wt.= 8.5 gms.		
11-10-32			80	105
11-11-32	2225	70% of kidney removed	104	116
11-12-32			65	116
11-14-32	2125		86	130
11-22-32			116	160
11-23-32		Injection of cortin (2 x) 2 c.c. at 2:30 p.m.	88	138
		Sample at 4 p.m.	97	285
		Injection at 5 p.m. Sample at 7:30	85	254
11-24-32 10 a.m.			70	148
12- 1-32			60	125
12- 3-32			74	124
12- 4-32			65	125
1- 5-32			65	110
1-14-32			83	139
1-18-32		10:30 a.m. injected 6 c.c. cortin Prep. 3 x	73	119

Protocol of Rabbit No. 432 (continued)

Date	Weight grams	Operation		Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
1-18-32		12 m.	2 months old	76	156
		2 p.m.		78	152
		4 p.m.		69	153
		7 p.m.		75	125
		9 p.m.		73	129
		11 p.m.		74	120

Protocol of Rabbit No. 444.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10-11-32	2600	3 gms. of left kidney excised	50	
10-13-32			46	
11-22-32			53	140 greatly excited
12-12-32	3300	Right kidney removed. Wt.= 11.4 gms.		
1- 5-33			59	100
		63% of kidney removed		
2-3-33			71	125
2-23-33				129
2-24-33			67	106
2-25-33			82	122
2-26-33			72	111
3-21-33			102	
3-23-33		Found dead.		

It is evident from the results obtained with this animal that removal of kidney tissue to the extent of 63% causes no appreciable increase in non-protein-nitrogen over a period of two months.

There was an increase, however, after the first two months which resulted in death at the end of the third month.

At autopsy the remaining portion of kidney was seen to be greatly hypertrophied. There was no fluid present and the kidney tissue weighed 12.6 gms.

Protocol of Rabbit No. 436 °.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
9-28-32	2200		25	
10- 3-32			34	
10- 5-32		3.3 gms. of left kidney excised		
10- 8-32			30	88
11- 4-32			46	103
11- 5-32	2350	Right kidney removed. Wt.= 9.9gms.	50 (8 1/2 hrs. P.O.)	92
11- 6-32		66 2/3% of kidney removed	74	105
11- 7-32			84	116
11- 8-32			113	115
11- 9-32			104	108
11-11-32			106	97.5
11-14-32			88	122
11-23-32			103	120
12- 1-32			94	115
12- 3-32			63	115
1- 5-33			51	100
1-14-33			89	119
1-16-33	9 a.m.		69	120
1-16-33	1 p.m.		70	118
2- 3-33			80	123
2-16-33			56	107
2-23-33			66	133
3- 3-33		Rabbit is weak. Soon prostrate. Died at 11 p.m.	450	

The change in blood non-protein-nitrogen and blood sugar is the same here as in No. 429 except that the increase is much greater. A greater amount of kidney tissue was removed. 66 2/3% as compared with 64%.

The blood sugar increased after removal of right kidney. Although the increase is not great, it is quite evident that a definite higher blood sugar was maintained than is normal.

After more than three months the non-protein-nitrogen is still twice the normal concentration.

After four months the blood non-protein-nitrogen increased and was 450 mgm. % before death.

The autopsy revealed that the kidney was greatly hypertrophied, but was also full of viscid milky fluid. Very advanced pyonephrosis was, evidently, present.

Protocol of Rabbit No. 446.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10-13-32	2100		32	
10-15-32		3 grams. of left kidney excised		
10-18-32	2050		38	103
11-17-32			31	145 Excitement very great
11-21-32	2535	Right kidney removed. Wt.= 9.2 gms.	73 (8 hrs.P.O.)	136
12- 1-32			81	134
1- 7-32		67% of kidney removed	62	
2- 3-32			72	
2-23-33			52	125
3-19-33		Found dead		

Autopsy:-

The remaining portion of kidney was greatly enlarged. Wt. = 13 gms. after removal of a little viscid fluid that was present in the pelvis. Capsule was not adhered to kidney cortex except at site of suture. On gross examination, the kidney did not present a bad appearance.

The left lung was very greatly infected so that it filled the entire left side of the thorax. The cause of death, therefore cannot be ascribed solely to renal insufficiency.

Protocol of Rabbit No. 415.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
4-23-32	2875	1/3 of left kidney removed. Wt. = 3 gms.	31	108
5 -9-32	2685		32	116
5-10-32		Right kidney removed. Wt.=11gms.	37	107
5-13-32	2675		42	141
5-14-32		63.6% of kidney removed.	46	125
5-15-32	2600		55	140
5-16-32			40	110
5-17-32			49	112
5-19-32			56	111
5-20-32			49	113
5-21-32			47	117
9-26-32			62	120
9-28-32			51	102
10- 4-32			93	
10-18-32			96	107
10-20-32			91	
10-26-32			123	109
10-27-32			132	116
11-28-32		Found dead and autopsied. Wt. of remaining kidney was 9.6 gms.		

It is evident from the results obtained with this animal that reduction of the kidney tissue to nearly 64% caused a maintained increase of non-protein-nitrogen in the blood. This, however, did not become dangerously high till five months after removal of the last kidney. After the fifth month the non-protein-nitrogen of the blood kept increasing. The animal died sooner than was expected since he looked very well until found dead November 28th.

Autopsy showed normal lungs, large heart. The piece of left kidney remaining weighed 9.6 gms. and looked swollen.

There was very severe arteriosclerosis of the ascending aorta. The sclerosis was so bad that death due to aortic insufficiency was very possible.

The kidney and aorta were preserved for microscopic study.

Protocol of Rabbit No. 6000

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
3-20-32	2500	Left kidney removed. Wt.=10.5gms.		
5- 4-32	2575	1/3 of right kidney excised. Wt.=2.5 gms.	29	113
5- 5-32	2465			
5- 6-32	2460	62% of kidney removed.	70	112
5- 7-32	2400		50	141
5- 8-32	2350			
5- 9-32	2400			
5-10-32			52	164
5-13-32	2385		40	122
5-14-32	2450		40	114
5-15-32	2500		41	112
5-17-32	2475		38	114
5-19-32	2450		33	96
5-20-32	2485		39	106
9-26-32			50	100
9-28-32			48	130
10- 3-32	2700		92	
10-18-32	2850		83	117
10-20-32			75	
10-26-32			76	127
12- 1-32			69	
12- 3-32			63	115
1-10-33		Died of cardiac puncture.	62	133

The reduction of kidney tissue to the extent of sixty-two per cent does not immediately bring about abnormally high non-protein-nitrogen levle. After four months the non-protein-nitrogen began to increase and did not return to normal.

Autopsy showed:-

1. Lungs somewhat discolored.
2. Liver looked very dark.
3. Kidney remaining weighed 9.2 gms. This kidney tissue looked edematous and swollen. The kidney was incised but no free water found. There were several renal calculi present.
4. Clot in the pericardium.
5. No arteriosclerosis was found. The presence of arteriosclerosis in the grey and not in the white may be of much interest.

Protocol of Rabbit No. 437 o

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10-15-32	2425	3.8 gms. of left kidney excised	35	108
1- 7-33			42	
1- 9-33	2425	Right kidney removed. Wt.= 10.6 gms.		
1-10-33			89	97
1-11-33		68% of kidney removed.	84	149
1-12-33			66	154
1-14-33			69	
2- 3-33			65	127
2-23-33			66	129
3-21-33			61	
4-22-33	10 a.m.		68	108
4-22-33	1:30 p.m.		65	105
4-22-33	4 p.m.		64	
4-22-33	8 p.m.		63	117
4-23-33	10:30 a.m.		63	119
4-24-33	9 a.m.		65	116

Protocol of Rabbit No. 447.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
9-28-32	1800		35	128
10- 3-32			36	
10- 5-32		2.2 gms. of left kidney excised.		
10- 8-32			38	98
12-13-32		Right kidney excised. Wt.= 8.3 gms.		
1- 5-33			52	107
		63% of kidney removed		
2- 3-33			62	119
2-23-33			84	106
3-22-33			97	
3-25-33			71	
3-29-33			77	
4-19-33			64	95

Protocol of Rabbit No. 775.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
10- 8-32		3.7 gms. of left kidney excised.	37	107
10-11-32	2625		74	104
10-18-32			48	
11-14-32	3100		46	138
11-15-32		Right kidney removed. Wt.=8.8 gms.		
11-16-32			69	141
11-17-32			65	138
11-19-32	2975		80	127
11-20-32			91	117
11-21-32			86	152 Blood from the ear. Excited.
11-22-32			95	157
12- 1-32			79	121
1- 5-33			82	118
1-10-33			65	110
1-21-33		2 p.m. Injection 6 c.c. cortin 3 x 2 months old	73	121
1-21-33		4 p.m.	79	146
1-21-33		5:30 p.m.	64	138
1-21-33		8 p.m.	64	125
1-21-33		10 p.m.	59	114

Here again it is seen that reduction of kidney tissue to about 70%, (here 71%), causes the non-protein-nitrogen of the blood to remain high over a long period of time. When 66% or less is removed, after an increase, the blood non-protein-nitrogen drops to nearly normal and then slowly increases until after a period of five or six months, the animal dies.

The injection of cortin caused its characteristic action on blood sugar. There was also a decided decrease in non-protein-nitrogen after cortin . 59 mgs. per 100 c.c. was the lowest since removal of the right kidney. It may be that cortin reduces the non-protein-nitrogen of the blood in nephrectomy and animals. More data are necessary to prove this point.

Changes in blood non-protein-nitrogen and blood sugar by reducing the total kidney tissue by more than 60% and less than 70%.

Protocol of Rabbit No. 429 o

Work was begun with this animal on September 28th, when a sample of blood was taken to determine normal non-protein nitrogen.

<u>On October 5th</u> 2.5 gms. of left kidney were excised.

<u>On November 4th</u> the right kidney was removed.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
9-28-32	2400		29	
10- 3-32			31	
10- 5-32		2.5 gms. of left kidney excised.		
10- 8-32			42	98
11- 4-32		Right kidney excised. Wt.= 9 gms.	30	125
11- 5-32			42	105
11- 6-32			46	121
11- 7-32		64% of kidney removed.	47	131
11- 8-32			52	117
11- 9-32			53	108
11-11-32	2650		55	103
11-12-32			50	105
12- 1-32			48	108
1- 5-33			44	120
2- 3-33			50	127
2-23-33			54	134
3-21-33			57	
4-19-33			45	112

Protocol of Rabbit No. 434.

Date	Weight grams	Operation	Non-protein-nitrogen mgms. per 100 c.c.	Blood Sugar mgms. per 100 c.c.
12-14-32	3500	4.2 gms. of left kidney excised.	45	
1- 7-33			51	
1- 9-33	3400	Right kidney excised. Wt.= 13 gms.		
1-10-33			98	96
1-11-33			117	143
1-12-33			131	160
1-12-33		2:30 p.m. Injection of cortin(3x) 1 c.c. intravenously	131	139
1-12-33		1 c.c. intraperitoneally		
1-12-33		4:30 p.m. Injection of 2 c.c. intraperitoneally.	123	210
1-12-33		7:45 p.m. Injection 2c.c. intraperitoneally.	130	175
1-12-33			111	107
1-13-33		10 a.m.	124	139
1-13-33			142	150
1-14-33			186	153
1-15-33			172	
1-16-33		9 a.m. Injection 2 c.c. cortin(3x) intraperitoneally.	177	145
1-16-33		11 a.m. Injection 2 c.c. cortin.	168	140
1-16-33		1 p.m. Injection 2 c.c. cortin.	176	320
1-16-33		3 p.m. Rabbit dead.		

Blood from ear with needle and syringe.

Autopsy:-

1. Clot in pericardium.
2. Kidney looked good macroscopically.

Here, again, too much kidney was removed and the non-protein nitrogen of the blood kept on increasing.

The injections of cortin into this animal are interesting. When the non-protein-nitrogen was at a level of 131 per 100, the injection of 2 c.c. cortin caused a very decided increase in blood sugar. When the non-protein-nitrogen was at 177, cortin caused a still greater increase, when injected in the same amount. In the former instance it increased the blood sugar 51% and in the latter 134%. The effect on the non-protein-nitrogen in the latter instance could not be learned, since the rabbit died six hours after the first injection. There was no change during this period. It seems quite evident, however, that there was a decrease in nitrogen after the first injection of cortin.

Part III.

Is there any relationship between the adrenal glands and the normal functioning of the kidney? Indeed, the occurrence of urea retention after adrenalectomy is now an accepted fact. It is not known what is the cause of this urea retention. Is it merely the result of a circulatory disturbance as Swingle (14) supposes, or the secondary result of a primary disturbance of carbohydrate metabolism as Britton (15) believes, or the result of the lack of the influence of the cortical hormone on the kidney itself? This last explanation will be considered here by observing the affect of the injection of cortin, the hormone obtained from the cortex of the adrenals, on the high blood non-protein-nitrogen concentration of experimental renal insufficiency, produced in the manner described in the last section. There will also be here reported the changes in blood sugar if any is observed after cortin injection. Let us first, however, review the literature concerning the affect of adrenalectomy on the kidney.

The affects of double adrenalectomy have been observed by several investigators. Marshal and Davis (16) in 1916 found that, after double adrenalectomy, in cats, blood urea was increased to twice the normal concentration.

It remained at this higher level and just before death rose still higher. They state that the cause of this increase appeared to be reduction of kidney function, since urea and creatinine injected into the blood were excreted very slowly. Bierry and L. Malloigel (17) observed reduction in blood sugar. Lucas (18) in 1926 found a concentration of blood solids and an increase in blood phosphorus and urea. Banting and Gairns (1926) (19) described hyperemia of the kidney with swelling of the tubules. They also found albuminous casts and blood cells in the tubules. Rogoff and Stewart (20) found that the non-protein-nitrogen of the blood rose markedly when the characteristic symptoms, especially anorexia, developed. The increase in urea nitrogen was the great factor. These authors also described an increase in blood solids. Swingle (7) found an increase in blood phosphorus and a lowering of the blood sugar. He also found albumin in the urine of animals showing marked symptoms and found congestion and hemorrhage in the kidneys. Hartman (21) and his co-workers also report that besides the increase in calcium, creatinine, and uric acid to slightly above normal values the blood urea increased, and that this increase ran parallel with the loss of appetite and other symptoms.

The blood sugar fell in the later stages reaching as low as 50% of normal at death in some individuals. These workers also report an increase in blood solids. In the later stages the acid soluble phosphates were considerably higher than in the normal blood. The anatomical changes which appeared rather constantly, as reported by Hartman, were purplish discoloration of the gums, hemorrhages in the thymus, general hyperemia of the internal organs and, what is of special interest to us here, an accumulation of large quantities of lipoid substance in the tubuli contorti of the kidney.

In the work of Swingle (7) on blood CO_2 capacity after adrenalectomy as compared with CO_2 capacity after nephrectomy, to which reference has already been made, it was found also that sulfur, phosphorus, urea, and sugar increased after nephrectomy. After adrenalectomy the same increases were observed, except that the blood sugar fell markedly after adrenal removal. It is also suggested by Swingle "that possibly one reason why bilaterally nephrectomized dogs maintain an approximately normal acid base equilibrium up to the time of death is that an extra renal factor is responsible for the maintenance of the normal equilibrium and that the mechanism may consist of an active principle or hormone elaborated by the suprarenal cortex. In nephrectomized dogs the kidney function is eliminated, but the suprarenal cortex remains intact and assists in maintaining normal equilibrium. In uremia, on the other hand, this extra renal factor, i.e, the suprarenal cortex, breaks down along with the kidney function. In adrenalectomy the conditions would be essentially similar to those of uremia."

Although it is maintained, therefore, that suprarenalcortical hormone controls acid base equilibrium, Swingle explains the increased concentration of blood urea after adrenalectomy, as due to the decrease in blood volume and concentration of blood solids. Swingle himself, however, observed a decrease in blood sugar after adrenalectomy. If this decrease of sugar is due to the passage of it, in the plasma, through the capillary walls, urea should also decrease in concentration. It, however, increases greatly in amount. Britton and Silvette (15) have shown that extirpation of the adrenals causes a marked decrease in liver and muscle glycogen as well as blood sugar. The administration of cortin, the extract of the adrenal glands according to Swingle's method, to animals suffering from adrenal insufficiency caused blood sugar, liver and muscle glycogen to return to normal. It seems impossible therefore to explain the changes of concentration of blood constituents after adrenal removal, as secondary to the increased concentration of blood solids. Blood sugar on the one hand is decreased, while blood urea nitrogen is increased.

Britton (14) has also observed that the injection of cortin into normal animals is followed by an increase in blood sugar, but large amounts were necessary to bring about an appreciable increase.

Can the high blood non-protein-nitrogen of renal insufficiency be decreased by cortin injection? This determination is of great importance in view of the facts presented above. If blood sugar concentration is also affected after cortin injection into renally deficient animals would the increase be of the same magnitude as in normal animals? It is the purpose of the work to be here given to give an answer to these very important and very interesting questions.

The method of Swingle and Pfiffner (8),(22),(23), was used in the preparation of the cortin. Beef adrenals were obtained that had been removed from the animals shortly after they had been killed and were still warm. The glands were packed in ice, taken to the laboratory and extraction begun immediately. The fat and connective tissue was removed and the whole glands passed through a meat grinder into 95% alcohol. Ten pound quantities of glands were usually employed. This ground gland substance was allowed to macerate in 2.5 volumes of the 95% alcohol at room temperature with occasional stirring for 3 days. At the end of this time the mixture was strained through muslin and the tissue residue thoroughly expressed in a tincture press. The strained fluid was filtered through soft filter paper.

The gland residue was reground and extracted in a similar manner for three days with two volumes (based on fresh gland weight) of 80% alcohol. The alcoholic extracts were concentrated separately in partial vacuo at an external temperature of 50 - 60° C. to 1/15 to 1/20 the original volume. Each aqueous residue was transferred to a cylinder with an equal quantity of benzene. After thorough mixing the mixture was set aside to settle. From this stage on all material was kept in the refrigerator except when under manipulation.

The benzene solution was removed and washing repeated until the last benzene washing was colorless or only slightly yellow. Three or four washings were usually sufficient. The benzene solutions were all combined and the benzene completely removed in partial vacuo at an external temperature of 45° - 50° C.

To the residue in the flask were added 500 c.c. of acetone. The material was thoroughly rubbed and set aside in the ice box for 24 hrs., with occasional rubbing. The acetone solution was then decanted and the residue re-extracted in the same manner. After decanting the second acetone solution, the residue was transferred to a mortar and rubbed with five one hundred c.c. portions of cold acetone. The acetone was removed at an external temperature of 45° - 50° C.

The residue from the acetone was transferred to a separatory funnel with 30 c.c. petroleum ether (20° - 40° B.P.) and 74 c.c. of 95% alcohol. 26 c.c. of distilled water were added and the contents of the funnel gently mixed. The 70% alcohol layer was washed five times with thirty c.c. portions of petroleum ether. The fifth washing gave a negative Lieberman-Burchard color reaction for cholesterol.

The petroleum ether solution and washings were returned to the original flask and the petroleum ether removed in partial vacuo at an external temperature of 20° - 40° C. The distribution procedure was repeated and the two 70% alcohol solutions combined and alcohol removed in partial vacuo at an external temperature of 45° - 50° C. Toward the end of the distillation small quantities (20 - 40 c.c.) of absolute alcohol were added to facilitate the removal of water. The residue was taken up in 100 c.c. of 95% ethyl alcohol, in which it was completely soluble, and the solution filtered through two 30 gm. portions of permutit at rate of 1 - 2 drops per second to remove the adrenaline.

The apparatus used for this purpose consisted of two cm. glass tubing of convenient lengths. Each tube was fitted at one end with an one hole rubber stopper, through which was inserted a short piece of 6 mm. glass tubing. Rate of flow was controlled with a short piece of gum rubber tubing and pinch clamp. Two tubes were placed one above the other.

A small pledget of cotton was placed in the tube immediately above the stopper. The permutit was poured in, lightly tamped and washed by draining 100 c.c. of

95% alcohol slowly through the filter. Excess alcohol was removed by suction.

After the alcohol solution of the active fraction had entered the filter, the permutit was washed with one 100 c.c. and one 300 c.c. portion of 95% alcohol, which was allowed to pass through the filter at the same rate as the alcoholic solution of the active fraction.

Following concentration of filtrate to 100 c.c. the filtration procedure was repeated. Swingle advises to use 15 gm. portions of permutit for this second filtration, but since the whole glands were used instead of just the cortex, thirty gram portions were again used.

The alcoholic solution was then concentrated to about 100 c.c. and 70 c.c. distilled water added. The alcohol was removed and the extract diluted with distilled water. Enough water was added so that one c.c. represented 50 gms. of whole adrenal gland. The milky suspension obtained was rapidly clarified and sterilized by passing through a Berkfeld filter. The filtered extract was crystal clear and pale yellow in color. Before the Berkfeld filtration, the addition of .9% NaCl rendered the solution isotonic. This procedure for the removal of adrenaline was used in every preparation except one.

To insure complete removal of adrenaline in one of the preparations for the purpose of comparing effect on blood sugar of the injection of this solution, with the effect of the injection of the others, an ether solution of the active principle was made and washed with .1 N. NaOH. The solvents were removed as before from the 70% alcohol solution. The residue was transferred to a separatory funnel with 100 c.c. of ether. The ether solution was washed with five 50 c.c. portions of .1 N. NaOH and then with three 50 c.c. portions of distilled water. The alkali and water washings were washed in order with one 100 c.c. portion of fresh ether. The ether solution and ether washings were combined, the ether removed by distillation in vacuo and the residue taken up in 95% alcohol. 80 c.c. H_2O were added, the alcohol removed by distillation as usual, the volume brought to desired quantity with H_2O and extract clarified and sterilized by Berkfeld filtration.

In all of the above procedures, as much of the solvents as possible were recovered and used over again.

Adrenalectomized rats were used to test the final product for the presence of cortin. Four healthy white rats of the same litter and ranging in weight from 112 to 137 gms. were bilaterally adrenalectomized. Two of these, receiving no injection, died in seven and nine days. The third rat on the eighth day after adrenalectomy showed symptoms of adrenal insufficiency. It remained in a corner of its cage and its hair was ruffled and back arched and took only very little food. Two cubic centimeters of the cortin preparation were injected intraperitoneally. The next day the rat appeared much better and two c.c. more were given. After this no more cortin was administered and the rat died on the twenty-second day.

The fourth rat, on the seventh day after adrenalectomy received also 2 c.c. of cortin preparation intraperitoneally, since on this day evident symptoms of adrenal insufficiency were present. 2 c.c. were injected daily for four days. 1/2 c.c. was then given twice daily for eight more days and 1 c.c. daily for seventeen days following. After loss of weight and evident symptoms of insufficiency on the seventh day this rat gained weight and looked well, while the injections were being given. After the thirty-sixth day no more injections were given and soon loss of weight was observed, and death occurred on the fiftieth day. The protocol of these rats will be given here.

Protocol of Rat No. 3.

Oct. 24, 1932. Left adrenal gland removed.
Wt. of rat = 125 gms.

Nov. 1, 1932. Right adrenal gland removed.
Wt. = 132 gms.

Nov. 2, 1932. Wt. = 128 gms.

Nov. 5, 1932. Wt. = 122 gms.

Nov. 10, 1932. Rat found dead in the morning.
Wt. = 110 gms.

Length of life after removal of last adrenal gland was 9 days.

Protocol of Rat No. 7.

Nov. 2, 1932. Bilaterally adrenalectomized. Wt. = 132 gms.

Nov. 7, 1932. Wt. = 125 gms.

Nov. 9, 1932. Evident symptoms of adrenal insufficiency observed in the morning. Died in the evening. Wt. = 120 gms.

Length of life after bilateral adrenalectomy was 7 days.

Protocol of Rat No. 11.

Nov. 4, 1932. Wt. = 130 gms.
Bilaterally adrenalectomized.

Nov. 12, 1932. Wt. = 120 gms.
Symptoms of adrenal insufficiency apparent.
Injection of 2 c.c. of cortin preparation intraperitoneally.

Nov. 13, 1932. Rat looks better.
Injection of 2 c.c. cortin preparation.

Nov. 15, 1932. Wt. = 132 gms.

Nov. 26, 1932. Dead of adrenal insufficiency.
Wt. = 112 gms.

Protocol of Rat No. 12.

Nov. 4, 1932. Wt. = 112 gms. Bilaterally adrenalectomized.

Nov. 11, 1932. Wt. = 102 gms. Adrenal insufficiency apparent.
2 c.c. of cortin preparation administered.

Nov. 12, 1932. 2 c.c. of cortin preparation administered.

Nov. 14, 1932. 2 c.c. of cortin preparation administered.

Nov. 15 to Nov. 23. 1/2 c.c. twice daily in the morning and in the evening.

Nov. 23 to Dec. 10. 1 c.c. given daily in the morning.

Nov. 30. Wt. = 118 gms.

Dec. 10. Dead of adrenal insufficiency.
Wt. = 102 gms.

Four months after the experiments outlined above, two more rats, also litter mates belonging to the same colony as the above four, but of a different litter, were used to test the seventh cortin preparation that was made. Observations were here made on the ability of cortin to maintain body temperature of adrenally insufficient rats. Rat No. 27 was the control for Rat No. 29, which was injected with cortin.

The protocols of these rats are here given.

Protocol of Rat No. 27.

March 7, 1933. Bilaterally adrenalectomized.

		Rectal Temperature	Wt.
March 7, 1933.	before operation	101.2	198 gms.
March 8, 1933.	9 a.m.	100.3	
March 9, 1933.	9 a.m.	100.8	
March 10, 1933.	11 a.m.	99.	
March 10, 1933.	5 p.m.	98.6	
March 11, 1933.	9 a.m.	97.2	170 gms.
March 11, 1933.	6 p.m.	98.4	
March 12, 1933.	12 m.	100.	168 gms.
March 13, 1933.	9 a.m.	98.6	162 gms.
March 13, 1933.	4:30 p.m.	98.4	167 gms.
March 14, 1933.	10 a.m.	100.3	167 gms.
March 16, 1933.	10 a.m.	99.	
March 17, 1933.	10 a.m.	98.6	
March 17, 1933.	9 p.m.	98.2	
March 18, 1933.	9 a.m.	97.6	
March 18, 1933.	9 p.m.	94.6	160 gms.
March 19, 1933.	Found dead in the morning		158 gms.

Length of life after adrenalectomy was 12 days.

Loss of body weight and inability to maintain body temperature were very marked.

Protocol of Rat No. 29.

		Rectal Temperature	Wt.
March 30, 1933. Bilaterally adrenalectomized		101.5	120 gms.
March 31, 1933.	4 p.m.	99.5	115 gms.
April 1, 1933.	9 a.m.	100.6	110 gms.
April 2, 1933.	9 a.m.	99.6	99 gms.
April 3, 1933.	9 a.m.	97.4	94 gms.
April 3, 1933.	5 p.m.	99.	99 gms.
April 4, 1933.	9 a.m.	100.6	102 gms.
April 5, 1933.	9 a.m.	100.	106 gms.
April 9, 1933.	1 p.m.	100.	108 gms.
April 10, 1933.	7 p.m.	98.8	103 gms.
April 11, 1933.	9 a.m.	97.8	103 gms.
April 12, 1933.	10 p.m.	94.1	100 gms.
Injection of 1 c.c. preparation 7 x			
April 12, 1933.	12 p.m.	96.2	100 gms.
April 13, 1933.	10 a.m.	97.5	
April 13, 1933.	9 p.m.	92.8	98 gms.
April 14, 1933.	Found dead in the morning.		97 gms.

It is seen that injection of the cortin preparation was immediately followed by increase in body temperature. This gives very good evidence that the preparations contained the active principle called cortin as shown first by Hartman (24) and his coworkers.

It is very obvious from the experiments just given that the preparations made from the adrenals contained the active principle, cortin. The experiments to determine whether the high blood non-protein-nitrogen of renal insufficiency can be decreased by administration of cortin will be given. The renal insufficiency was produced in the manner described in Part II. A number of animals in which enough kidney had been removed to cause a steady increase of blood non-protein-nitrogen, with death in a few days, were used. Injections of the cortin were made and blood samples taken every two or three hours for 12 - 16 hrs., and the non-protein-nitrogen and sugar values compared with those found in blood taken immediately before the injections. Also a number of animals, whose kidney substance had not been reduced sufficiently to cause death in a few days, but in which there was a maintained high non-protein-nitrogen level, were used. Blood samples were also obtained from these animals immediately before injection and at two hour intervals for the remainder of the day the injection had been made. Samples of blood were then taken daily in a few cases, to determine whether the changes observed after cortin injection were maintained over longer periods of time. All injections were made intraperitoneally and the blood samples obtained from the ear vein with a needle and syringe.

There is given in table IV the summary of experiments to determine the affect after nephrectomy of the injection of cortin, both on the blood non-protein-nitrogen and sugar. The small figures in parentheses in the last two columns indicates the hour after injection that the value reported, existed. The changes are shown much better in figures two to eleven, in which a graphic representation of the results is given.

In figure two there is shown the steady increase in non-protein-nitrogen of the blood after double ureter ligation. The gradual increase that occurred in rabbit No. 751 after removal of 80% kidney substance is also shown in this figure. It is seen that the injection of 10 c.c. .9% saline did not alter the increase. Since several blood analyses were made in each animal before and after the injection of cortin, each one is its own control. In every rabbit there was gradual increase in blood non-protein-nitrogen after nephrectomy, and cortin injection was followed by a slowing of the increase, or as was evident in most cases, by an actual decrease from the amount present at the time of injection. In figures three to nine are shown the effects

Table IV.

Summary of experiments to determine changes in blood non-protein-nitrogen and sugar after injection of cortin into renally deficient rabbits.

Rabbit No.	Amount of Injection	Blood Non-protein-nitrogen per 100 c.c. at time of injection mgms.	Sugar per 100 c.c. at time of injection mgms.	Lowest Non-protein-nitrogen within 24 hrs. after injection	Highest Blood Sugar within 12 hrs. after injection
439	5 c.c. of 3x twice 4:30 5 c.c. 5:30 5 c.c. Total 10 c.c.	266	174	250 (5)	442 (5)
443	3 c.c. of 2 x twice 2:30 3 c.c. 4:00 3 c.c. Total 6 c.c.	233	-	162 (5)	-
434	2 c.c. of 3 x three times 2:30 2 c.c. 4:30 2 c.c. 6:30 2 c.c. Total 6 c.c.	131	139	111 (8)	210 (2)
432	3 c.c. of 2 x twice 2:30 3 c.c. 4:30 3 c.c. Total 6 c.c.	88	138	70 (17)	285 (2)
593	6 c.c. of 5 x	163	189	149 (3)	285 (3)
775	6 c.c. of 3 x	74	121	59 (6)	146 (2)
767	10 c.c. of 6 x twice 2:00 10 c.c. 4:00 10 c.c. Total 20 c.c.	336	143	248 (8)	191 (4)

Table IV (continued)

Rabbit No.	Amount of Injection	Blood Non-protein-nitrogen per 100 c.c. at time of injection	Sugar per 100 c.c. at time of injection	Lowest Non-protein-nitrogen within 24 hrs. after injection	Highest Blood Sugar within 24 hrs. after injection
758	10 c.c. of 7x	138	106	126 (2)	147 (4)
757	10:30 10 c.c. 7x 1:30 5 c.c. 7x Total 15 c.c.	98	117	59 (22)	196 (5 1/2)
753	10 c.c. of 7x	216	111	188 (4)	153 (4)
753	10 c.c. of 7x	260	123	183 (4)	155 (4)
775	5 c.c. of 5x	70	122	55 (18)	155 (2 1/2)
775	10 a.m. 10c.c. 7x 1 p.m. 5c.c. Total 15 c.c.	75	100	54 (13)	133 (3)

of cortin administration in cases of severe renal insufficiency.

In figures three and four are shown the results obtained in two cases, where injection was followed in a few hours by death of the animal. It is obvious, however, that, whereas in all other cases blood non-protein-nitrogen kept increasing to the time of death, where it was highest, cortin injection was followed by a decrease before death.

As is shown in figures seven and eight repeated administrations of cortin to the same animal, were followed by a decrease in non-protein-nitrogen concentration of the blood. When rabbit No. 767 was in the last stages of insufficiency, a large dose (20 c.c.) of the cortin preparation was followed by a great decrease in the non-protein-nitrogen. It is obvious, as seen from the figures, that such great decreases in non-protein-nitrogen concentrations as were observed after administration of cortin, were never observed, when no injections were made, or when only .9% saline was injected.

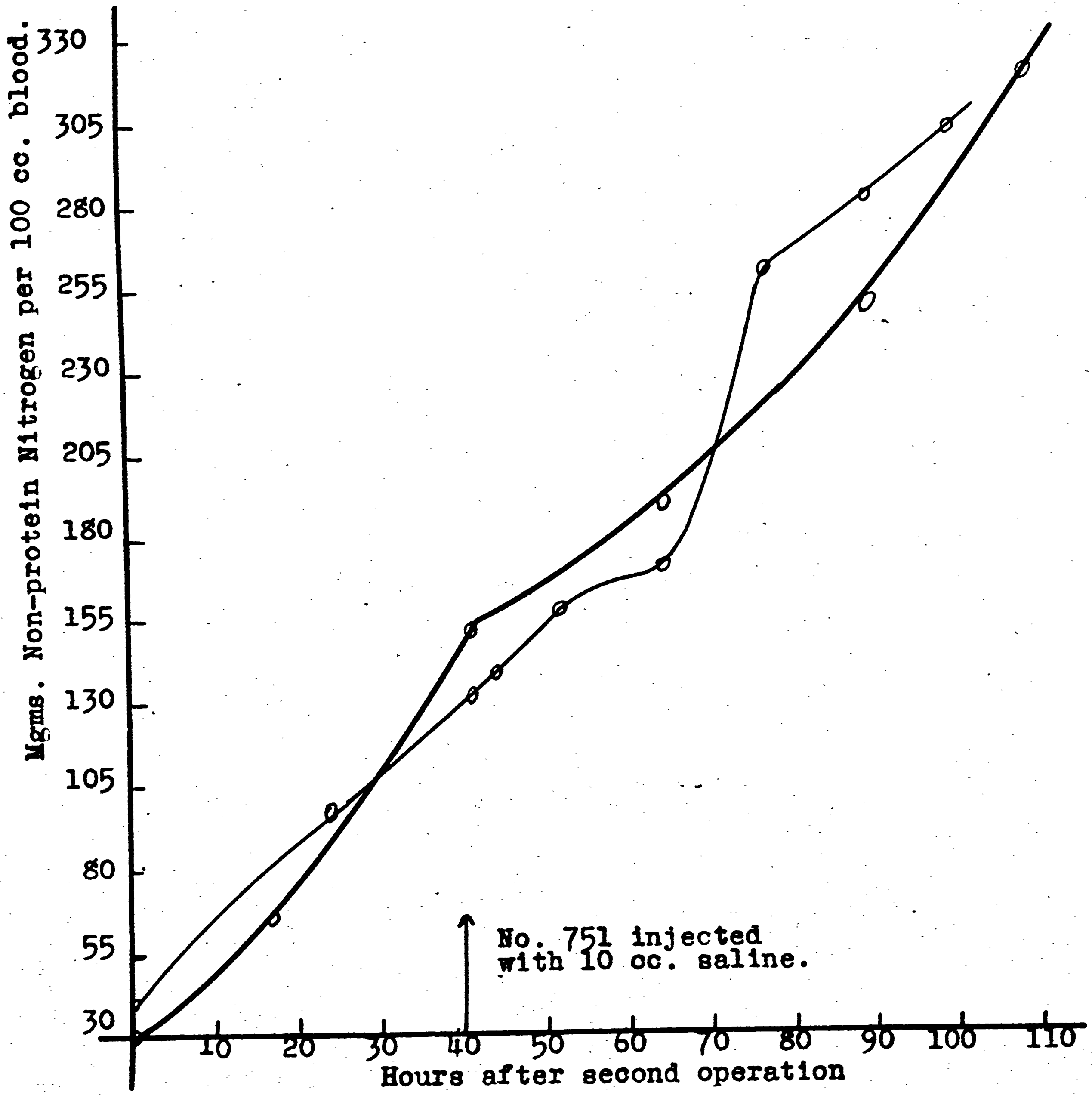

Fig. 2. Controls.

Rabbit No. 751, 80 per cent kidney removed.

Rabbit No. 386, double ureter ligation.

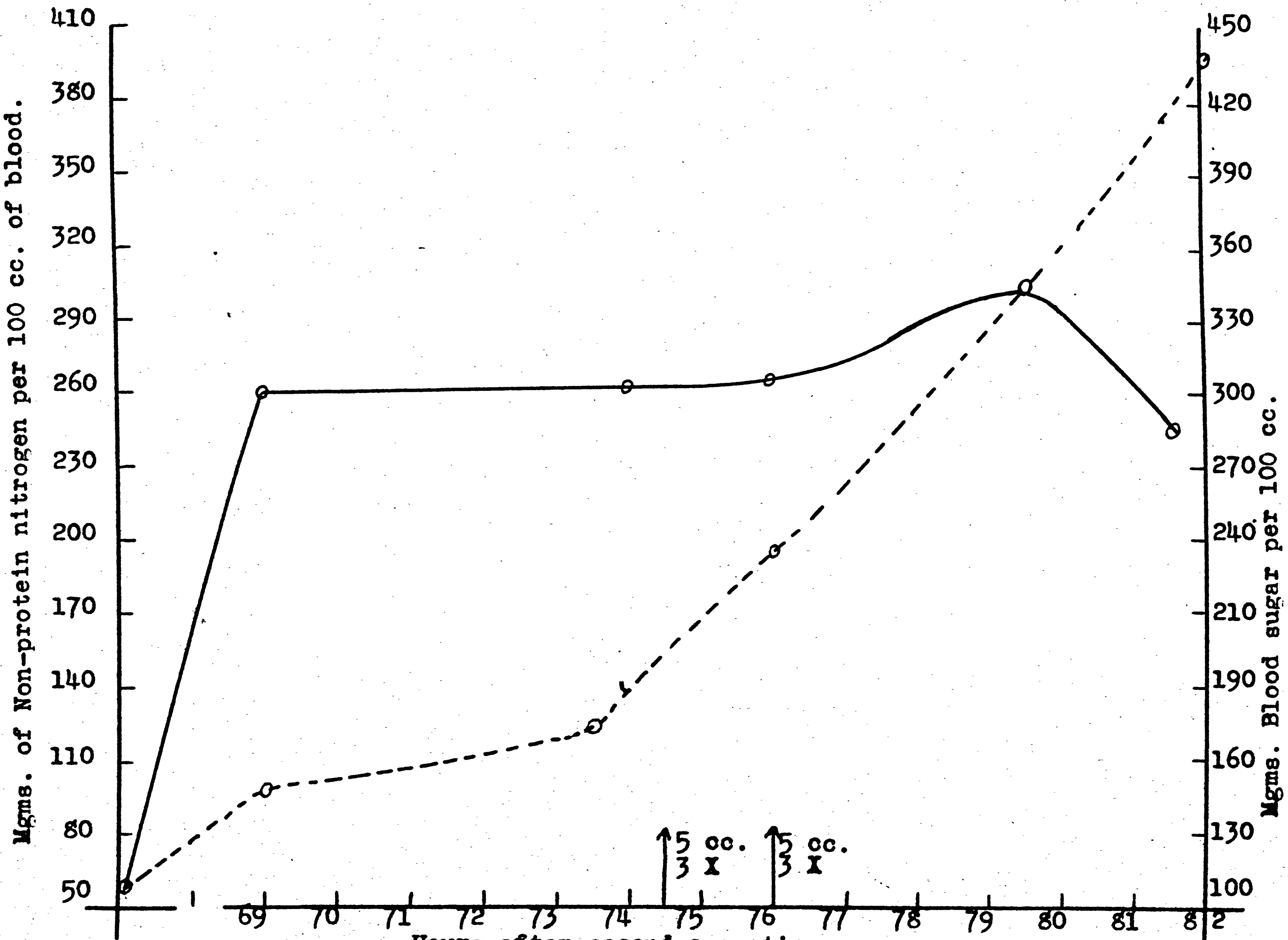

Fig. 3. Rabbit No. 439

———— Non-protein nitrogen.

- - - - - - - - Blood sugar.

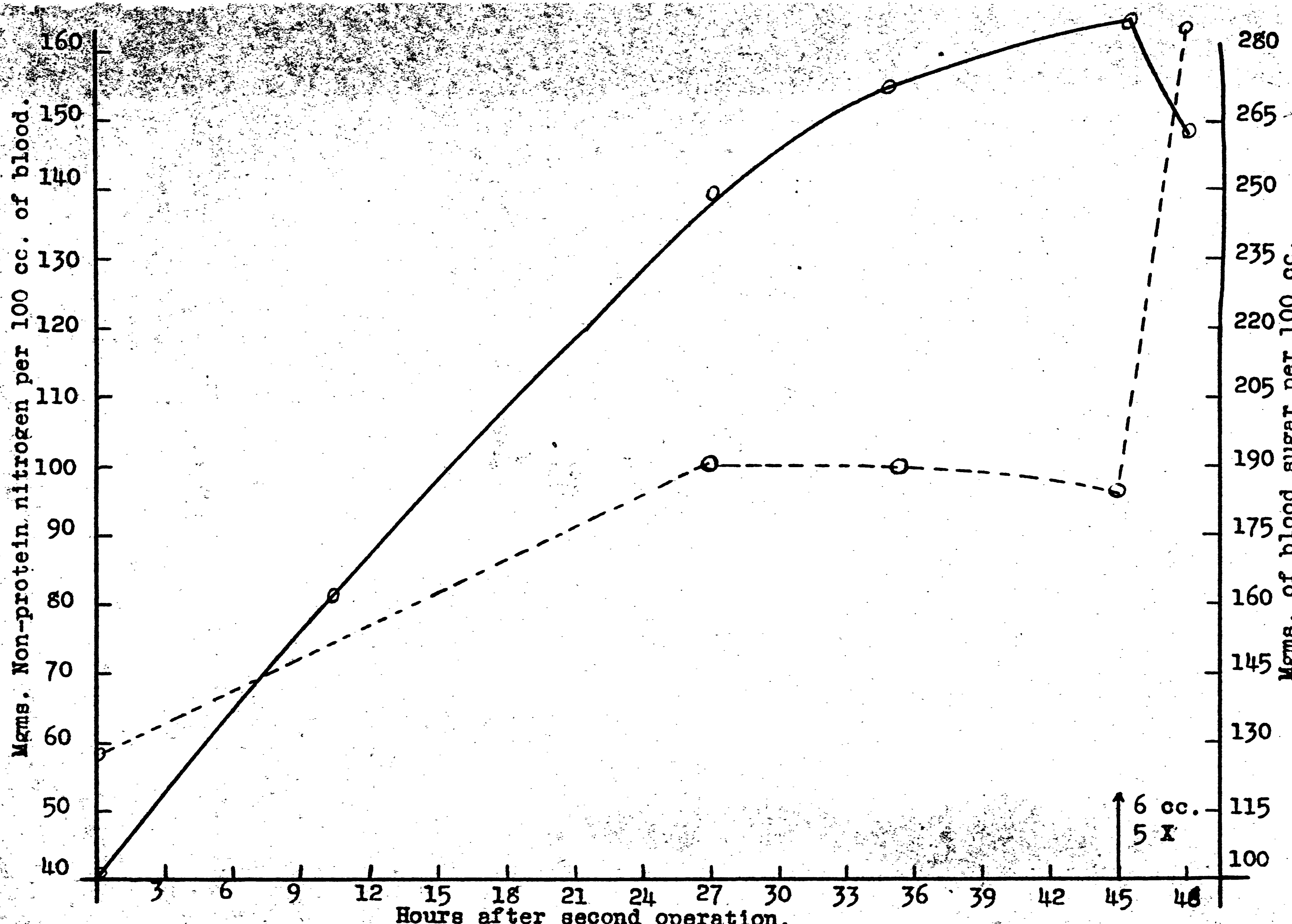

Fig. 4. Rabbit No. 593.

——— Non-protein nitrogen.

- - - - - Blood sugar.

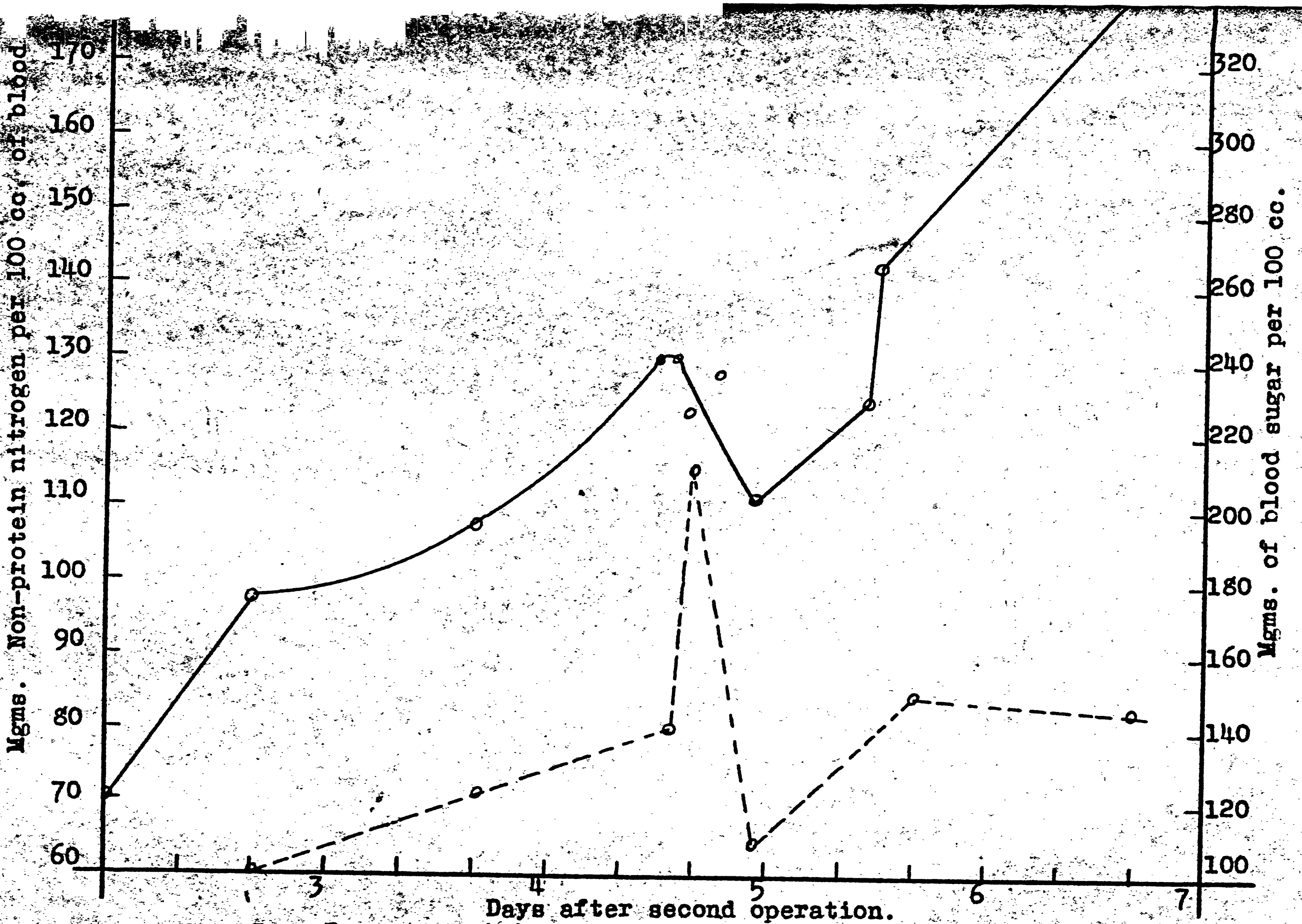

Fig. 5. Rabbit No. 434.

——————Non-protein nitrogen.

- - - - - - -Blood sugar.

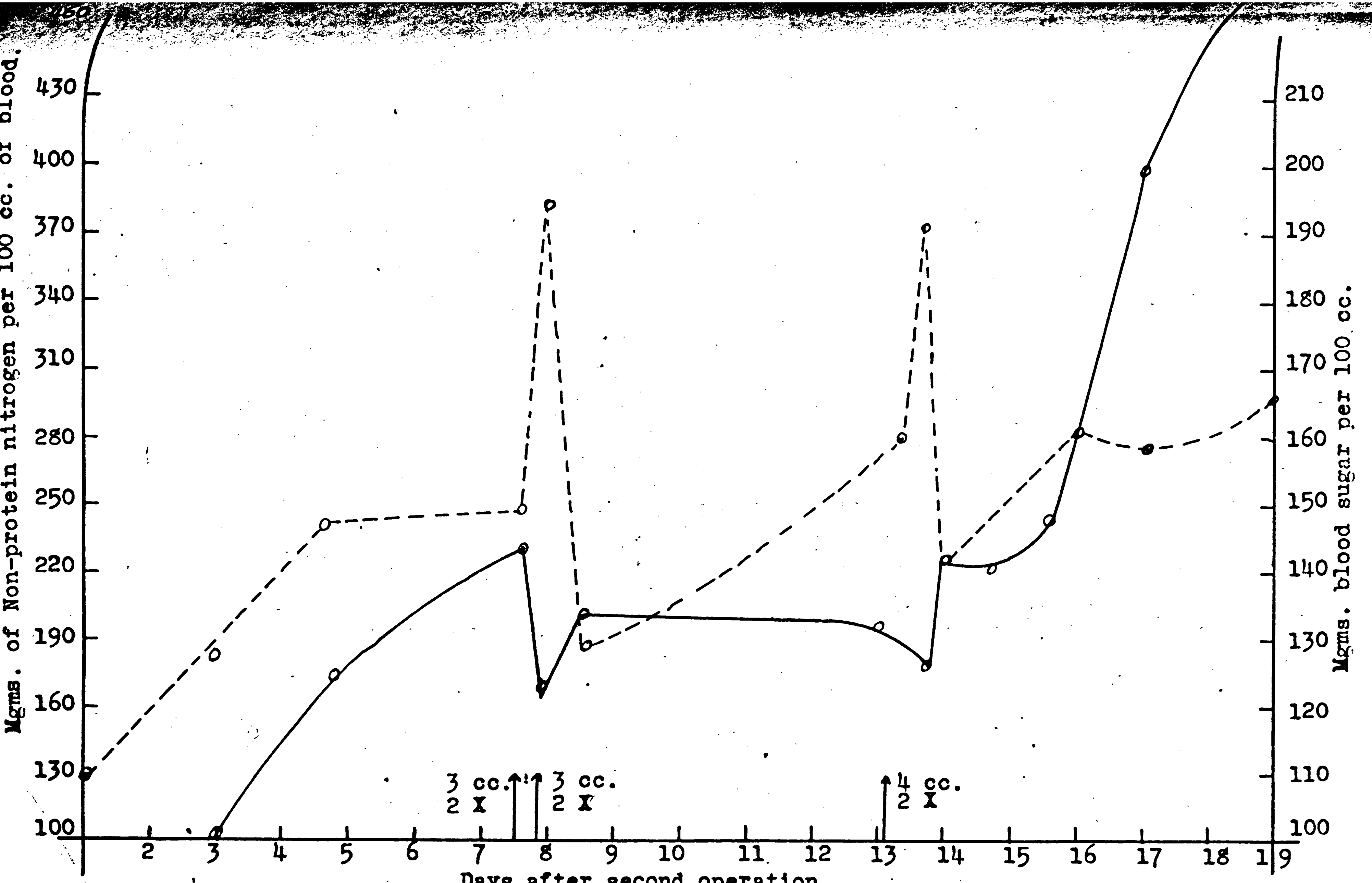

Fig. 6. Rabbit No. 443

——— Non-protein nitrogen.
- - - - Blood Sugar.

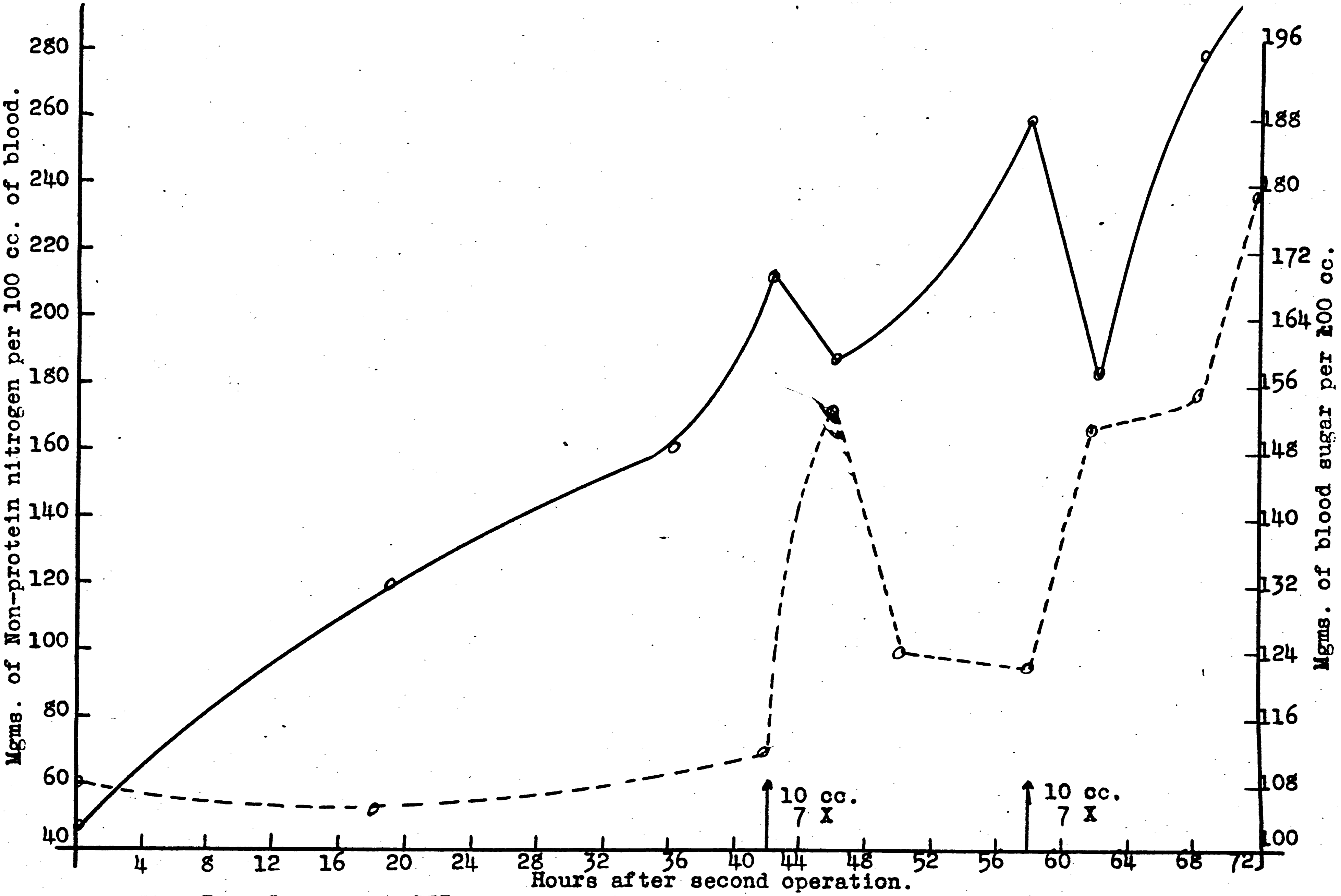

Fig. 7. Rabbit No. 753.

———— Non-protein nitrogen.

- - - - - - Blood sugar.

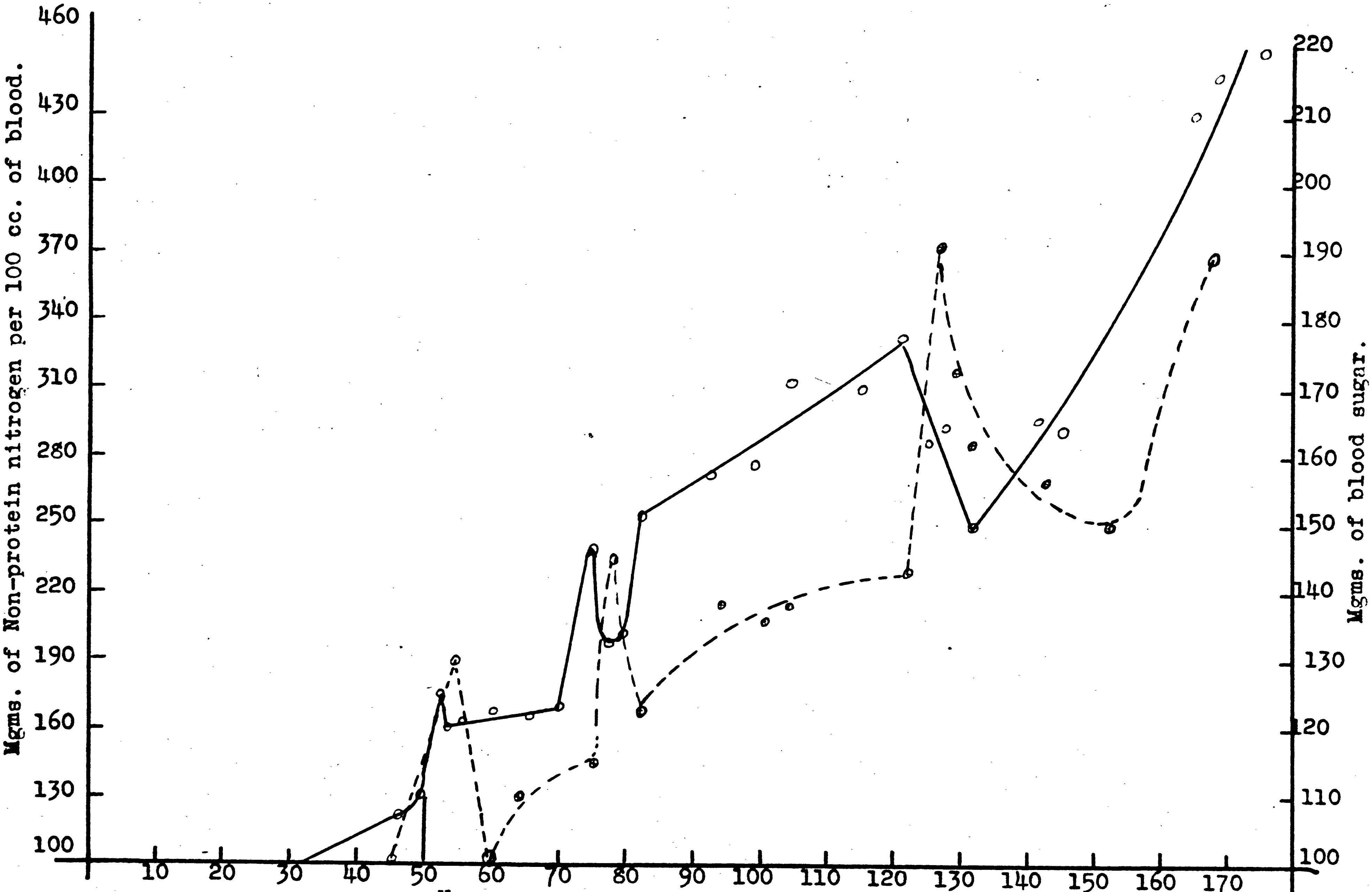

Fig. 8. Rabbit No. 767.

——— Non-protein nitrogen.

– – – – Blood sugar.

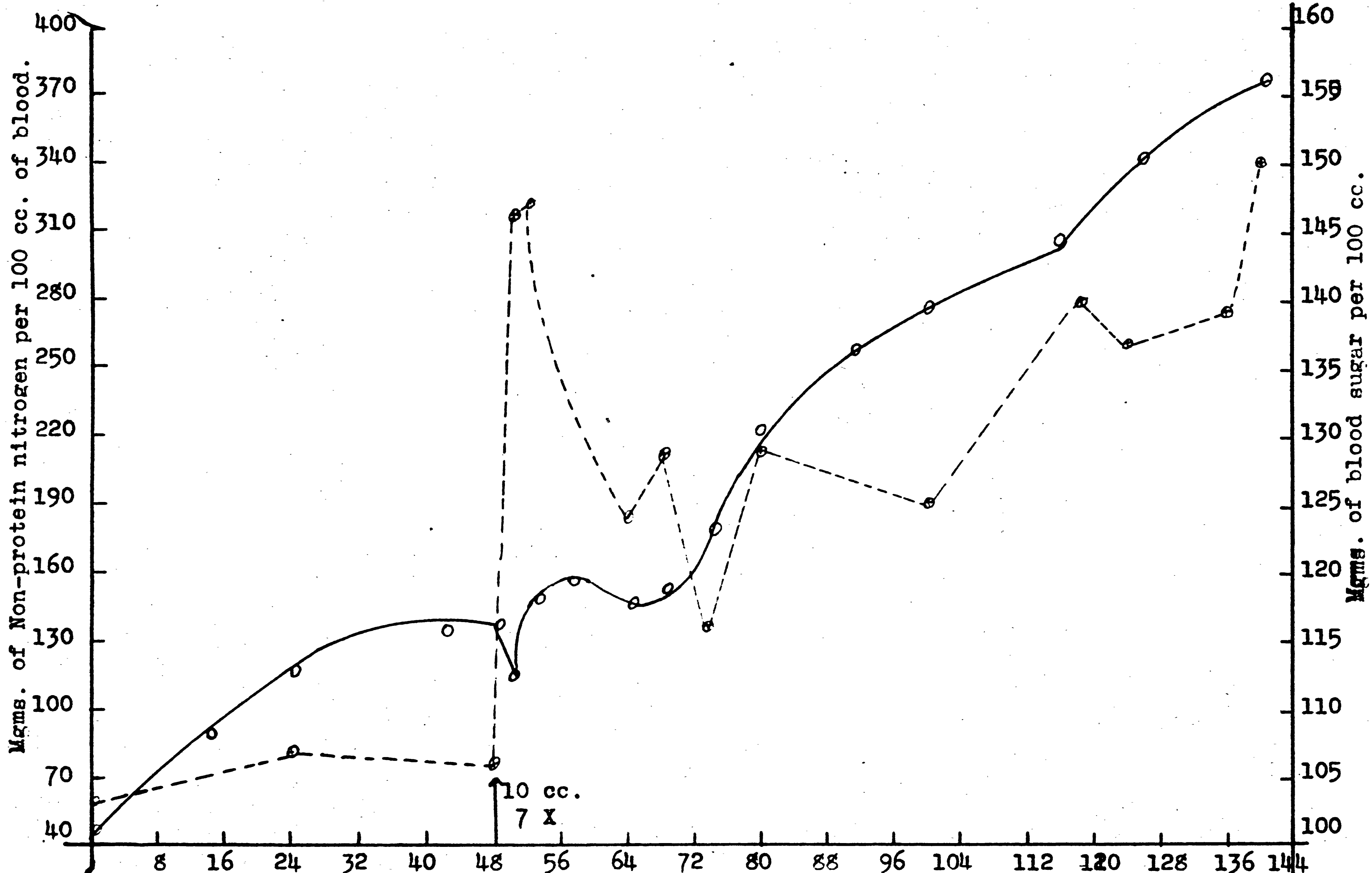

Fig. 9. Rabbit No. 758.

——— Non-protein nitrogen.

- - - - - - - Blood sugar.

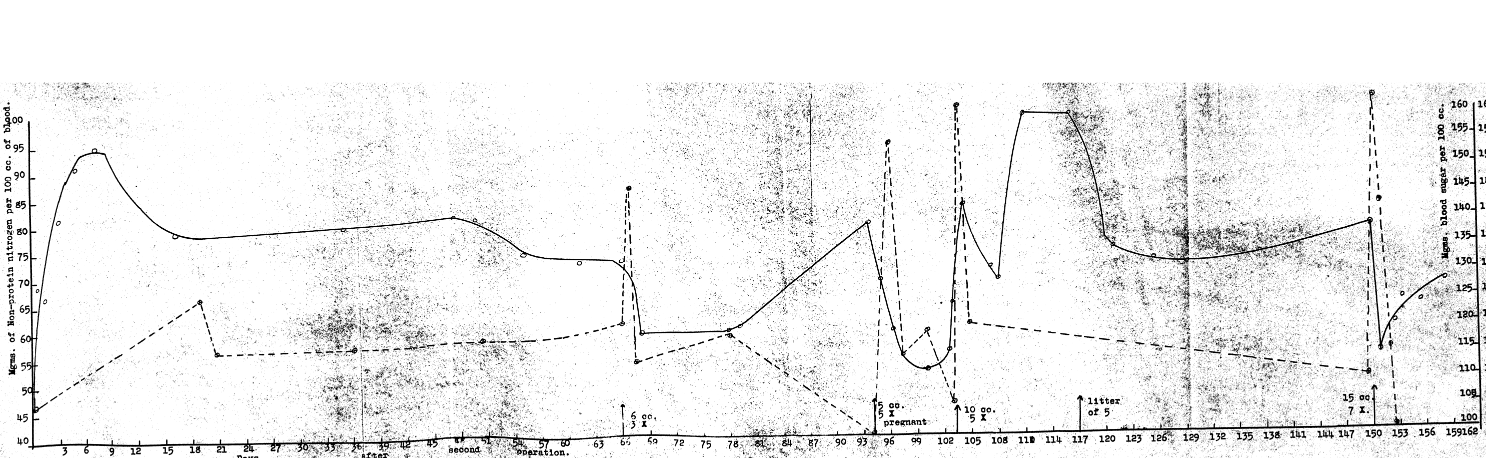

Fig. 10. Rabbit No. 775.

Non-protein nitrogen.

Blood sugar.

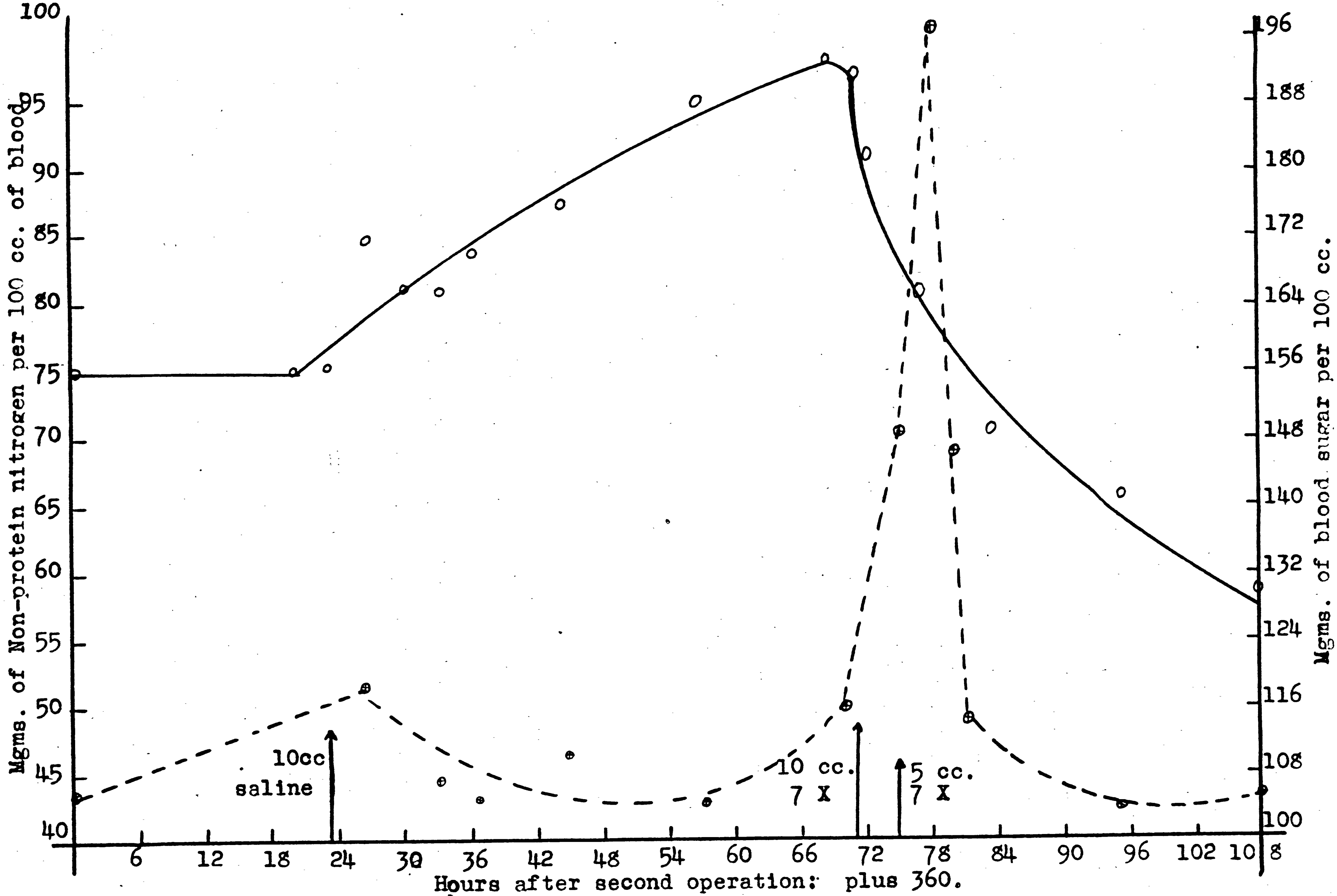

Fig. 11. Rabbit No. 757.

——— Non-protein nitrogen.
- - - - - Blood sugar.

In figures ten and eleven are given the graphic representation of the effects of cortin administration to animals that lived a long period of time after nephrectomy. It is plainly seen in figure ten, that cortin administrations were followed by lower blood non-protein-nitrogens than were observed at any other time during the life of the rabbit. These observations were confirmed with rabbit No. 757, as shown in figure 11.

To be certain that the error of the nitrogen determinations was not great, a number of controls are given in table V. These determinations were made either after injection of .9% saline, or of preparation 4 x, which was apparently inactive.

Insert Table V.

In every instance of cortin administration there was observed an increase in blood sugar. To rule out the possibility that traces of adrenaline caused this increase in sugar, four of the preparations were tested for adrenaline by the Folin uric acid reagent (25). Preparation 6 x which had been washed with .1 N. NaOH to remove adrenalin gave no color at all with this reagent. Preparations 5 x, 4 x, and 7 x gave a definite pale blue color, which, when compared with the Folin

Table V.

Rabbit No.	Injection	Non-protein-nitrogen: Before injection	2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs.	Blood Sugar: Before injection	2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs. after injection
757	10 c.c. saline	75	86	81	84	81	86	106	115	119	109	105	104
437	10 c.c. saline	68	-	65	64	63	65	108	105	117	119	116	-
775	6 c.c. saline	52	-	52	-	-	53	123	-	120	-	-	114
436	5 c.c. prep. 4x	56	53	53	-	-	55	107	120	118	-	-	110
444	5 c.c. prep. 4x	67	65	69	-	-	72	106	100	117	104	122	111
775	10 c.c. prep. 4x	80	76	80	76	-	76	85	85	100	98	-	110

uric acid standard amounted to seven parts per million Preparation 4 x, however, was made from glands that had been stored for 18 months in the cooler at the abatoir. The injection of this preparation into the animals caused no increase in sugar as shown in table V. But it gave with the uric acid reagent as strong a color as did 5 x or 7 x. Preparation 6 x, although it gave no color at all with the reagent, markedly affected the blood sugar, as is shown very clearly in figure 8. It is certain, therefore, that cortin does increase blood sugar, and that the effects observed were not due to adrenalin, since preparation 4 x, which evidently contained no cortin, but did contain 7 pts. per million of adrenalin, did not affect the blood sugar, and since preparation 6 x did increase blood sugar, although it contained no adrenalin.

That the blood changes observed were real and not merely due to changes in blood volume was proven by counting the red blood cells after cortin injection. The results are given in table VI. Although the non-protein-nitrogen decreased and the sugar increased, the red blood cell count remained the same. Rabbit No. 437 was used as control and the data obtained with this animal are given also in table VI along with data

Table VI.

Rabbit No.	Injection	Time after injection	Red blood corpuscles per c.mm.	Non-protein-nitrogen mgms.	Blood Sugar mgms.
775	none	same	3,072,000	66	107
775		4 hrs.	2,969,600	64	114
757	none	-	3,072,000	98	117
	15 c.c. of cortin	3 hrs.	3,276,800	83	149
		5 1/2 hrs.	3,174,400	82	196
		9 1/2 hrs.	3,328,000	68	114
		24 hrs.	3,277,000	66	103
		46 1/2 hrs.	2,969,000	59	105
437	10 c.c. of saline	-	4,900,000	68	108
		3 1/2 hrs.	4,600,000	65	105
		6 hrs.	-	64	-
		10 hrs.	4,659,000	63	117
		48 hrs.	4,812,000	63	119

obtained with 757, in which the cortin injection was made.

Discussion of Results.

In rabbit No. 775, in which 72% of the kidney had been removed pregnancy was accompanied, as shown in figure 10, by an increase in the blood non-protein-nitrogen. In spite of a small decrease after cortin injection, the value reached 103 mgms. per cent just before delivery. A litter of five was born and well attended by the mother. The non-protein-nitrogen of the blood then again decreased. Four of the litter of five have survived and are very healthy. In one of Anderson's rabbits in which merely 63% of kidney had been removed, pregnancy in the sixth week after nephrectomy was followed by death with urea nitrogen of 400 mgms. per cent. Anderson reports that none of his nephrectomized rabbits were successful in raising their litters, even though the reduction of kidney was in no case more than 65 per cent. Is it not significant, therefore, that rabbit No. 775 with kidney substance reduced 72 per cent was able to successfully deliver a litter of five and care for them, and have its non-protein-nitrogen increase merely to 103 mgms. per cent?

Indeed figure 10 clearly shows that every injection of cortin was followed by a decrease in non-protein-nitrogen, and it was, no doubt, on account of this action of cortin that the pregnancy was successfully brought to term.

The sharp drop in blood non-protein-nitrogen, when this value was high, and the slower drop when the value was lower, indicates that stimulation of the kidney had been effected by the cortin injection. When only little functioning kidney was present and the accumulation of metabolic end products in the blood was very great, the action of the injected cortin on the kidney was not apparent for more than a few hours. When only twice the normal concentration of non-protein-nitrogen existed in the blood, there was present, obviously, a greater amount of functioning kidney. Cortin injection was then followed by longer maintenance of lower non-protein-nitrogen values.

A greater amount of functioning kidney would, after added hormonal stimulation, be able to secrete more efficiently for a longer period of time, than would a very much smaller amount of kidney substance.

From the literature on the effects of adrenalectomy, it is learned that kidney disfunction follows total removal of adrenals. From the results here given, it is apparent that the hormone from the cortex of the adrenals decreases the accumulation of protein metabolic end products, occurring in renal insufficiency. There is good evidence, therefore, for the existance of an adrenal hormonal control of kidney secretion.

The administration of cortin was followed by increases of blood sugar. The amount of the increase was greatest when blood non-protein-nitrogen retention was highest. The very high blood sugars observed in the first few cases, figures 2, 3 and 4, may have been due to the presence of some adrenalin, since the preparations used here were not tested. The same technique, however, was used in removing adrenalin as was used in 4 x, 5 x and 7 x. That the increase in blood sugar after injection of these preparations was not due to adrenalin, has already been proven. It may be that the removal of adrenalin was not as complete in the first preparation and that the very high blood sugars were due to both cortin and adrenalin. It cannot be doubted, however, that cortin alone caused an increase of blood sugar, which was greatest when the renal insufficiency was most marked.

In severe renal insufficiency alone there is a definite hyperglycaemia. No good explanation has been given for this phenomenon. If cortin is necessary for stimulating the secretion of an overloaded kidney, it is quite certain that there is more cortin in the blood when accumulation of metabolic end products occurs. This presence of cortin also causes, then, the increase in sugar observed. There is thus afforded a good explanation of the hyperglycaemia occurring in the last stages of renal insufficiency, which, heretofore, has had no good explanation.

The results here reported support the theory of Britton and Silvette that cortin plays a role in carbohydrate metabolism. That the cortin injections were not followed by changes in blood volume is indicated by the fact that blood non-protein-nitrogen decreased, and blood sugar increased, and that the red blood cell count remained the same.

Summary.

1. The kidney tissue of the rabbit can be limited to the extent of 70 per cent.

2. Such limitation of kidney tissue is followed by an initial increase in the non-protein-nitrogen of the blood, followed by several weeks of lower values still higher than normal, and finally by increases up to 400 mgms. per cent before death.

3. An hyperglycaemia exists when the non-protein-nitrogen is very high, (over 200 mgms.) both after total and partial nephrectomy.

4. Great hypertrophy of the kidney remnants occurred, when life continued long after removal of 65 to 70% of the kidney.

5. Cortin administration caused a decrease in non-protein-nitrogen of the blood in renally deficient animals. There is good evidence presented, therefore, that cortin has a direct action upon the kidneys.

6. The blood sugar values were increased after cortin injection.

7. There is put foward an explanation for the hyperglycaemia in renal insufficiency.

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