

PROTEIN METABOLISM IN ECK'S-FISTULA DOGS.

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Among the first investigators to undertake any very extensive metabolic experiments upon animals with the blood supply to the liver more or less interfered with were, Hahn, Massen, Nenki, and Pawlow (I). In addition to cutting off the portal blood to the liver by means of an Eck's-fistula, they ligated the hepatic artery and even went so far as to try to extirpate the liver altogether so as to totally remove its influence. The latter they only accomplished in part for they always left behind about fifteen to twenty per cent of the total mass of the organ. However, the partial extirpation of the liver in no way changed the results obtained by ligating the hepatic artery after an Eck's-fistula ; in either case the animal only survived the operation from ten to twenty hours. It is quite evident from their experiments, that the condition of the animal during the short lease of life left it after the operation, rendered it impossible to obtain any trust-worthy information concerning what might have been the changes in metabolism had the conditions imposed by the operation permitted the animal to live.

During the course of their experiments they found that the portal blood might contain, especially during protein digestion, three to five times as much ammonia

as the blood of the general circulation; but after the establishment of an Eck's-fistula, while they were unable to find any appreciable increase in the ammonia content of the blood of the general circulation, the proportion of ammonia to total nitrogen and urea in the urine was increased. They also found in their experiments, when in addition to an Eck's-fistula they ligated the hepatic artery and cut away a large part (85 %) of the liver tissue, the ammonia not only increased in the urine at the expense of the urea, but the ammonia content of the systemic blood actually increased, even to a point equal to that present in the portal blood, and gave rise to symptoms similar to those of ammonia poisoning, which proved fatal.

Perhaps the most interesting observation made by these investigators as well as a most stimulating one, was that in some of the Eck's-fistula dogs, the ingestion of meat was followed sooner or later by a train of symptoms of a nervous character, similar in some respects at least, to the symptoms of ammonia poisoning. These observations led to a number of subsequent investigations by these same authors as well as by others; viz.: Biedl and Winterberger (2), Rothberger and Winterberger (3), Salaskin (4), Nenki and Zaleski (5),

and later by Macleod and Haskins (6), Fischler (7), Grafe and Fischler (8), all of whom dealt more or less with the relationship of ammonia to these toxic symptoms. While all agreed as to the symptoms and increase in the ammonia content of the blood, yet all, except Salaskin, were unconvinced that ammonium carbonate or ammonium carbamate, or in fact any other ammonia compound or NH_2 compound upon which the liver is capable of acting, was the sole cause of the intoxication. Salaskin, however, was fully convinced that the symptoms were entirely due to ammonia.

The question to which attention was first directed in the investigations was the fate of the products of intestinal digestion and absorption which under normal conditions pass into the portal blood to be carried to the liver before entering the general circulation. Of the nitrogenous compounds formed in the intestines, ammonia was the one to which special attention was directed, because it was always present and was about the only one known to possess any very marked toxic properties. Also the action of the liver upon ammonia formed in the intestines was quite well known.

Macleod (9) confirmed in general the findings of Hahn, Massen, Nenki, and Pawlow and also noted that

there was present in the urine a large amount of albumin the day following the feeding of Liebig's meat extract. He further noted that glycosuria followed carbohydrate feeding to Eck's-fistula dogs.

Hawk (10) in a series of feeding and injecting experiments on Eck's-fistula dogs found that the animals showed the toxic symptoms described by Biedl and Winterberger (2), Rothberger and Winterberger (3), Salaskin (4), Nenki and Zaleski (5), Macleod and Haskins (6), Grafe and Fischler (8), as well as Hahn, Massen, Nenki, and Pawlow (1), when fed on Liebig's meat extract for several days. He failed, however, to corroborate Macleod's observation that albuminuria followed Liebig's meat extract feeding and glycosuria followed carbohydrate feeding. He also noted that the animals invariably lost weight, the loss in weight for the different animals ranging from fourteen per cent to forty-two per cent of the body weight. He further noted that meat was refused by the animal after recovery from the toxic symptoms induced by the meat feeding. The toxic symptoms appeared in from eight to ten days when Liebig's meat extract was fed to the animals in connection with a meat diet but did not appear during a period of thirty-one days when the extract alone was

fed to the animal. He concluded from this that the toxic symptoms were due to some constituent of the extract but these constituents are toxic only in the presence of a meat diet.

S. A. Matthews and E. M. Miller (II) found that on feeding Eck's-fistula dogs on a heavy meat diet for several days that a train of symptoms usually developed, at first having the character of mild cocaine poisoning., i.e. restlessness and increased irritability. In some animals these were the only symptoms noticed, but in others these symptoms increased to the proportions of intense clonic convulsions which when left alone proved fatal. They noticed that during the meat feeding the ammonia content of the blood was increased and that when the diet was changed to bread and milk, all toxic symptoms disappeared as well as the excess of ammonia in the blood. They noted further that these toxic symptoms, no matter how severe, could be allayed in from twelve to forty-eight hours by feeding the animal warm milk even if the animal was unable to drink the milk and had to be fed by means of a stomach tube. They further noted that ligation of the hepatic artery alone produced the same effects on the excretion of urea and ammonia as did an Eck's-fistula operation,

i.e., the urea was diminished and the ammonia compounds increased in the urine, but no toxic symptoms resulted from meat feeding. If the hepatic artery was tied in Eck's-fistula dogs, either at the time of the operation or some weeks after, the animal only survived the operation for from twelve to forty-eight hours. Also in Eck's-fistula dogs of from six months to a year standing the nitrogen metabolism tended to approach the normal. They concluded from this that the liver plays an important role in the formation of urea from the ammonia compounds absorbed from the intestines, but after it had been shunted out from the circulation for some time the other tissues of the body assume this power.

S. A. Matthews and C. F. Nelson (12) found that by the intravenous injection of a diuretic salt solution of the following composition:-

Sodium chloride.....	4	Grams.
Sodium sulfate.....	80	"
Sodium citrate.....	27	"
Calcium chloride.....	0.5	"
Water to make a	1000 cc.	

at the rate of three cc per minute into the femoral vein of an Eck's-fistula dog after ligation of the hepatic, superior and inferior mesenteric arteries and with about eighty-five per cent of the total mass of

the liver removed, that after the injection had been kept up for one hour, the nitrogen content was found to be very small, but more ammonia than urea. At this point of the experiment the intramuscular injection of ten grams of Ereptone, dissolved in fifty cc of water, in an hours time showed a marked increase in the ammonia and urea content of the urine, the ammonia being in excess of the urea. But two hours after the injection of the Ereptone, there was a marked decrease in both the ammonia and urea content of the urine. They concluded from this that the first action of the tissues upon Ereptone (amino-acids) was the formation of ammonia and later the production of urea.

The evidence introduced in this paper depends upon the determination of the Total non-protein nitrogen and Urea nitrogen in the blood. The blood was always drawn from the external saphenous vein of the dog unless otherwise stated.

In as much as the technique of the operation is so well known a description is unnecessary.

The method of total non-protein nitrogen and urea nitrogen determination is essentially that devised by Folin (13) with a slight modification which is worthy of note, i.e., the digestion mixture is transferred to a U-shaped tube made out of a fifty cc. pipette with a second fifty cc. bulb and a small reflux condenser placed between the digestion mixture and the acid solution used to collect the ammonia liberated. Furthermore the digestion mixture is placed in a water bath which is heated to eighty or ninety degrees Fahrenheit during the passage of the air current. The air current was drawn through the mixture by means of a filter pump and was allowed to run for at least thirty minutes. Preliminary determinations on known amounts of nitrogen as ammonium-sulfate and urea nitrogen as urea showed that at least ninety-eight per cent and in many instances one-hundred per cent of the known amounts of nitrogen or urea could be recovered.

One rather interesting phenomena was noticed on several Eck's-fistula dogs and that was, if the animals had been on a meat diet for several days before the operation, they seldom lived more than twelve to forty-eight hours after the operation, even if apomorphine was given the day before to empty the stomach of all its contents. However, if the animals had been on a mixed diet of bread and meat or a bread and milk diet ~~it~~^{they} survived the operation much better and recovered from the shock much sooner, in some cases in twelve hours. On other dogs the day following the operation they appeared restless, they drank heartily and ate their food but would vomit in a few minutes after drinking or eating and only lived from two to four days after the operation. Autopsy showed that death was due to intusseption of the bowel, and in one instance the intusseption was six inches long. When animals exhibited these signs of intestinal disturbances an attempt was made to remove the disturbing factors by the subcutaneous injection of from two to five milligrams of atropine depending on the size of the animal, and in one instance successful results were obtained .

In all the operations the dogs were prepared as dog No. I unless otherwise stated.

Dog No. I. This was a large male dog weighing fifteen kilos. It had been kept on a mixed diet, bread and meat scraps from the table, the week before the operation and had nothing to eat two days before the operation except a few pieces of bread. The operation September 24/ 15 was successful. The following day it drank heartily and ate its food, a mixed diet consisting of bread and meat scraps, but vomited immediately after drinking or eating, showing those signs of intestinal disturbances described above. A subcutaneous injection of three milligrams of atropine was given in hopes that it would break up the intusseption provided this was the cause of the trouble. The next day the animal was apparently no better, a second injection of five milligrams of atropine was given and on the third day the animal was found dead in its cage. Autopsy showed that it had died of intusseption, the fistula was wide open and the portal was tied off in good shape. There were no adhesions.

Dog No. 2. Operation October 1/15.

Date	Total Non-P.N. per 100 cc blood.	Urea Nitrogen per 100 cc blood.	Remarks.
Nov. 5/15....	18.64 mg....	3.94 mg....	Bread and meat diet.
" 8/15....	20.00 ".....	3.65 "....	Bread and Meat diet.
" 10/15....	14.57 ".....	4.16 "....	Bread and Milk.
" 11/15....	11.42 ".....	4.34 "....	Bread and Milk.
" 15/15....	17.28 ".....	3.96 "....	Meat four days
" 16/15....	18.88 ".....	3.89 "....	Meat diet.
" 26/15....	22.22 ".....	3.67 "....	Meat diet.
" 27/15....	25.80 ".....	3.05 "....	Meat diet.
Dec. 10/15...	25.80 ".....	3.44 "....	Meat diet.
Jan. 5/16...	22.85 ".....	4.51 "....	Bread and Meat diet.
" 11/16...	26.47 ".....	3.95 "....	Meat for six days.

This was a large male dog weighing fourteen kilos. The operation was successful and completely healed in three weeks. Following the operation it was kept on a mixed diet, (bread and meat), and thirty-four days after the operation the total non-protein nitrogen content of the blood was 18.64 milligrams per 100 cc of blood, and a urea nitrogen content of 3.94 milligrams per 100 cc of blood, while that of a normal dog on the same diet showed a total non-protein nitrogen content

of 13.18 milligrams per 100 cc. of blood and 3.20 mgs. of urea nitrogen per 100 cc of blood. The Eck's-fistula dog showed a decided increase in both total non-protein nitrogen and urea nitrogen. The animal was somewhat restless and irritable, it was given a small amount of meat in addition to its regular meal and the following day showed more pronounced toxic symptoms. The total non-protein nitrogen content had increased to 20.00 mgs. per 100 cc of blood while the urea nitrogen had decreased. Its diet was changed to a generous allowance of bread and milk and two days later the total non-protein nitrogen showed a decided drop while the urea nitrogen had increased. All toxic symptoms were gone and the animal was apparently in a normal condition. The bread and milk diet was continued and the total non-protein nitrogen content steadily dropped and the urea nitrogen likewise increased. The dog was now subjected to a meat diet alone for four days and as indicated by the table the total non-protein nitrogen increased and the urea nitrogen decreased to about what it was at the time of the first examination when it had been on a bread and meat diet for thirty-four days. The toxic symptoms were returning and the animal was removed from its cage

to the dog pen with the other dogs and the meat diet was continued. While in the pen it was extremely restless and irritable, it was continually running around the pen barking and fighting the other dogs. One night it escaped from the pen but returned two days later when it was caught and put in its cage where it had previously been. It remained in the cage quiet and apparently not in a good condition. A total non-protein nitrogen determination showed a decided increase over what it had ever been while the urea nitrogen was lower. It was given a mixed diet of bread and meat and the next day the total non-protein nitrogen content had increased somewhat while the urea nitrogen had decreased. The next day the nitrogen and urea content had not changed to any very marked degree, but the animal laid in the cage in a comatose condition, when removed from the cage and placed on its feet it would lie down without attempting to walk about the room. It was given a quart of warm milk and in six hours showed signs of returning to normal, it would stand up in the cage and walk around the room. It was kept on a bread and meat diet for thirteen days and seemed to be somewhat relieved from the comatose condition but not normal. The total non-protein

nitrogen had decreased some and the urea nitrogen had increased as indicated by the table. It was now again subjected to a meat diet for six days, the toxic symptoms returned, the total non-protein nitrogen increased more than it had ever been before and the urea nitrogen was lower than at any other time during the experiment. For the next ten days it was kept on a mixed diet, but only a small amount of meat was given the animal. An attempt to completely extirpate the liver proved fatal. About ninety-five per cent of the liver was removed, it survived the anaesthetic but died five hours later from loss of blood caused by one of the ligatures of a small vein breaking. The fistula was wide open, there were no adhesions. The liver was small and necrosed, and a histological examination showed many fat cells scattered throughout the tissue. The bile was thick and viscous and of a light yellow color. During a period of a III days, the animal had lost about twenty-five per cent of its body weight. Photograph No. I.

Dog No. 3. This was a large male dog weighing 15 kilos, operated on October 2, 1915, and died two days later of intusseption, atropine failing to remove the disturbing factor. The fistula was wide open.

Dog No. 4. Operation November 6, 1915.

Date.	Total non-protein nitrogen per 100 cc Blood.	Urea nitrogen per 100 cc Blood.	Remarks.
Nov.8/15...	16.74 mgs....	4.13 mgs..	Bread and Meat diet.
" 11/15...	17.69 "	3.91 " ..	Bread and Meat diet.
" 16/15...	22.67 "	3.42 " ..	Meat alone.
" 22/15...	9.42 "	4.95 " ..	Milk for six days.

This was a large female bulldog weighing seventeen kilos and was normal after the operation. It was kept on a mixed diet of bread and meat following the operation and the total non-protein nitrogen showed a gradual increase while the urea nitrogen had gradually decreased. The animal was becoming quite noisy and restless and exhibited those toxic symptoms which are characteristic of meat poisoning. Its diet was changed to milk and the toxic symptoms completely disappeared within forty-eight hours. The milk diet was continued for six days when the animal showed a surprisingly large drop in total non-protein nitrogen and an increase in the urea nitrogen content of the blood. The dog was taken to the pen where it was given a meat diet along with the other dogs, the toxic

symptoms returned rapidly and the dog was soon fighting the other dogs and four days later it was found dead in the pen. It had lost about twenty per cent of its body weight during the fifty days of the experiment. Autopsy showed the fistula wide open, no adhesions, the liver was small and necrosed. A histological examination showed that the liver tissue had almost all undergone fatty degeneration. Photograph No. 2.

Dog No. 5. Operation Februrary 8, 1916.

Date.	Total non-P.N. per 100 cc of Blood.	Urea Nitrogen per 100 cc of Blood.	Remarks.
2/8/16...	32.12 mgs.....	2.96 mgs....	Vena Cava Blood.
2/18/16..	16.05 mgs.....	1.38 mgs....	Bread and Meat diet.

This was a small male dog weighing twelve kilos and was in good condition following the operation, but lost weight rapidly. Blood drawn from the portal vein at the time of the operation showed a total non-protein nitrogen content of 32.12 milligrams per 100 cc of blood and a urea nitrogen content of 2.96 milligrams per 100 cc of blood. The animal was kept on a mixed

diet for ten days when the total non-protein nitrogen content was 16.05 milligrams per 100 cc of blood and the urea nitrogen was 1.38 milligrams per 100 cc of blood. The dog was given a mixed diet for the next seventeen days when the pancreas was completely removed for class experiment. The following day the urine, which was the first voided after the operation, showed a sugar content of 4.76 per cent. A subcutaneous injection of pituitrin was given to see if the sugar content dropped but negative results were obtained even after a second injection. This animal was sacrificed for class experiment.

Dog No. 6. Operation February 11, 1916.

Date.	Total non-P.N. per 100 cc of Blood.	Urea Nitrogen per 100 cc of Blood.	Remarks.
2/11/16..	40.98 mgs...	5.22 mgs...	Bread and Meat diet. Portal blood.
2/17/16..	29.66 mgs...	2.85 mgs..	Bread and Meat diet.
3/22/16..	32.65 mgs...	4.68 mgs.	Bread and Meat diet. Blood from Heart.
4/6/16...	20.52 mgs...	3.75 mgs..	Bread and Milk diet.
4/14/16..	36.36 mgs...	2.31 mgs..	Meat for 7 days.
4/21/16..	25.80 mgs...	2.06 mgs.	Bread and Meat diet.

This was a small female dog weighing twelve kilos, and was normal after the operation. Blood drawn from the portal vein at the time of the operation showed a total non-protein nitrogen content of 40.98 milligrams per 100 cc of blood and 5.22 milligrams of urea nitrogen per 100 cc of blood. After the operation it was kept on a bread and meat diet for six days when the total non-protein nitrogen content was 29.66 milligrams per 100 cc of blood while the urea content was 2.85 milligrams per 100 cc of blood. To get blood from the vena cava would necessitate opening the abdominal cavity, so in order to obtain some idea of the non-protein nitrogen content of the vena cava without opening the abdomen, blood was drawn from the heart and it gave a total non-protein nitrogen content of 32.65 milligrams per 100 cc of blood and 4.68 milligrams of urea nitrogen per 100 cc of blood. There were no apparent signs of meat poisoning even though the animal had been on a bread and meat diet. It was kept on a bread and milk diet for fifteen days and the total non-protein nitrogen content of the systemic blood decreased while the urea nitrogen increased as was the case in all the other dogs which had been kept on a meat free diet. It was now placed on a purely meat

diet for seven days. The last two days of the meat feeding the toxic symptoms of restlessness, irritability and ataxia were quite apparent and the total non-protein nitrogen had remarkably increased while the urea nitrogen had decreased. It was now placed on a mixed diet but it continued to become weak and emaciated, it had lost control of its hind legs. The dog had lost about twenty-five per cent of its body weight. An attempt to completely extirpate the liver April 21, 1916, proved fatal. Blood drawn from the portal vein at the time of its death showed a marked decrease in both total non-protein nitrogen and urea nitrogen. The fistula was wide open, the site of the operation was adhered to the neighboring bowel and the pancreas. The liver was small, yellowish in appearance and quite friable. It was adhered to the right kidney but all adhesions were easily broken up. A histological examination showed evidence of fatty degeneration. Photograph No.3.

Dog No. 7. Operation March 10, 1916.

Date.	Total non*P.N. per 100 cc Blood.	Urea Nitrogen per 100 cc Blood.	Remarks.
3/22/16.....	25.00 mgs....	2.87 mgs,.....	Bread and Meat diet
4/6/16.....	21.68 mgs....	3.06 mgs.....	Bread and Meat diet.

This was a large male Grey-hound weighing sixteen kilos. It had been on a bread and meat diet and was used the day before the operation for class experiment in which the animal was bled dry from the carotid artery and the blood from another dog was transfused into its jugular vein, so that it possessed practically none of its own blood. After the operation the animal was kept on a bread and meat diet for eleven days, when the blood showed a total non-protein nitrogen content of 25.00 milligrams per 100 cc, and 2.87 milligrams of urea nitrogen per 100 cc. The bread and meat diet was continued, but the animal became weak and emaciated, however, it did not exhibit any toxic symptoms, except that when it was permitted to run loose in the room where there was another dog it began to fight at once, even when the other dog tried to keep out of its way. It developed such an ataxic condition that it was sacrificed for tissue examination. The autopsy showed no adhesions, the fistula was wide open, the bile was thick and viscous and of a light yellowish color. The liver was small and necrosed. Histological examination showed many fat cells. Photograph, No. 4.

Normal Dogs.

Date.	Total non-P.N. per 100 cc. Blood.	Urea nitrogen per 100 cc. Blood.	Remarks.
II/5/I5..	13.18 mgs....	3.20 mgs....	Bread and Meat diet.
II/19/I5.	15.09 mgs....	2.35 mgs....	Bread and Meat diet.
I2/I0/I5.	18.74 mgs....	3.44 mgs....	Meat diet alone.
2/8/I6...	29.62 mgs....	2.18 mgs....	Bread and Meat diet. Portal vein Blood.
3/20/I6..	38.04 mgs....	2.65 mgs....	Bread and Meat diet. Portal vein Blood.
4/6/I6...	44.44 mgs....	2.85 mgs....	Bread and Meat diet. Portal vein Blood.

The total non-protein nitrogen content of normal dogs blood showed a gradual increase while the animal was kept on a bread and meat diet, but exhibited a much larger increase when placed on a purely meat diet. While on a bread and meat diet and the total non-protein nitrogen was increasing the urea nitrogen content fell somewhat, but when placed on a purely meat diet the urea nitrogen as well as the total non-protein nitrogen increased, however, at no time did any toxic symptoms develop in normal dogs.

The total non-protein nitrogen content of the portal blood was taken from different dogs in each case in order to get some idea of the nitrogen content of the portal blood compared with the systemic, and in every instance the portal blood was much higher.

The changes most apparent in nitrogen metabolism observed in this paper is the gradual increase in total non-protein nitrogen of the general circulation after the establishment of an Eck's-fistula, and a decrease in the urea nitrogen. Also, that when the animals are kept on a meat diet alone or a diet consisting largely of meat, there is a gradual increase in the total non-protein nitrogen and a decrease in the urea nitrogen content of the general circulation. Furthermore, that after about fifteen days of meat feeding there always appear toxic symptoms, at first, restlessness and increased irritability, which later develop into clonic convulsions. However, if meat is excluded from the diet, the total non-protein nitrogen decreases and the urea nitrogen increases, and all toxic symptoms disappear. The increase in total non-protein nitrogen always exceeds the decrease in the urea nitrogen, and a decrease in total non-protein nitrogen always exceeds the increase in urea nitrogen.

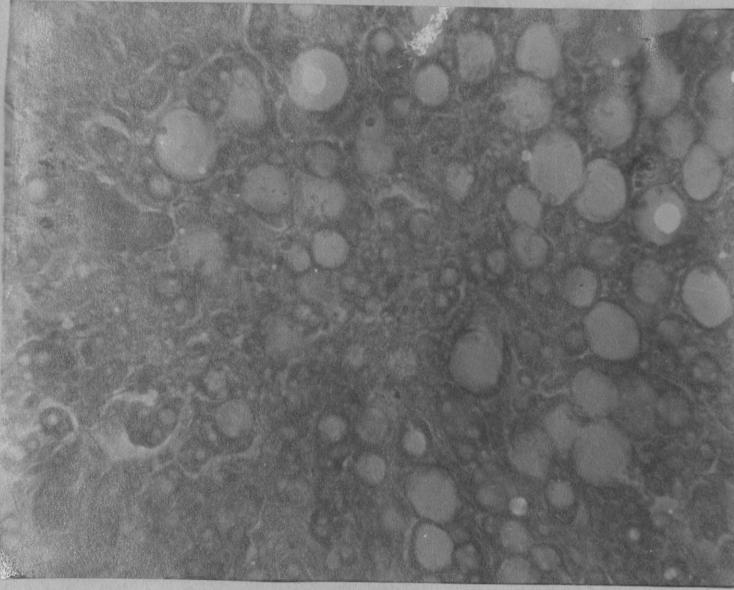
I wish to thank Dr. Matthews, under whom this work was done, for his valuable instructions and assistance, and to express my appreciation for his kindness in allowing me to use his translation of some of the Bibliographs which I was unable to obtain.

I also want to thank Mr. Ray West for his assistance in all the operations.

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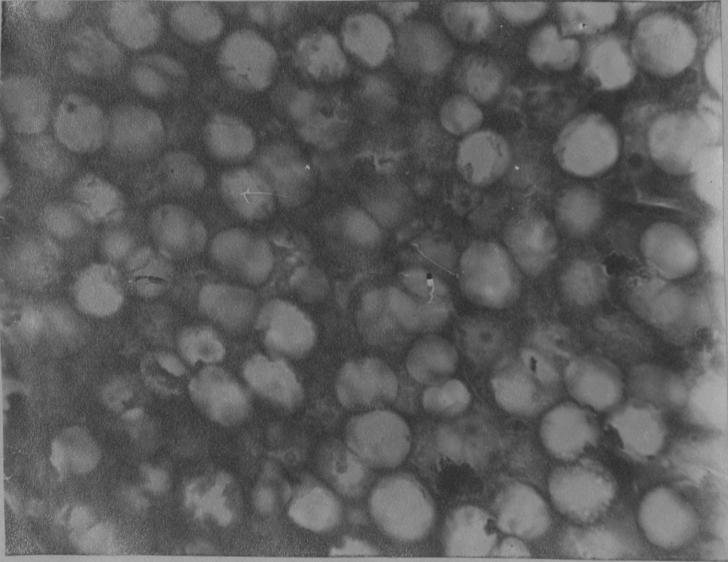
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Photograph No. I.



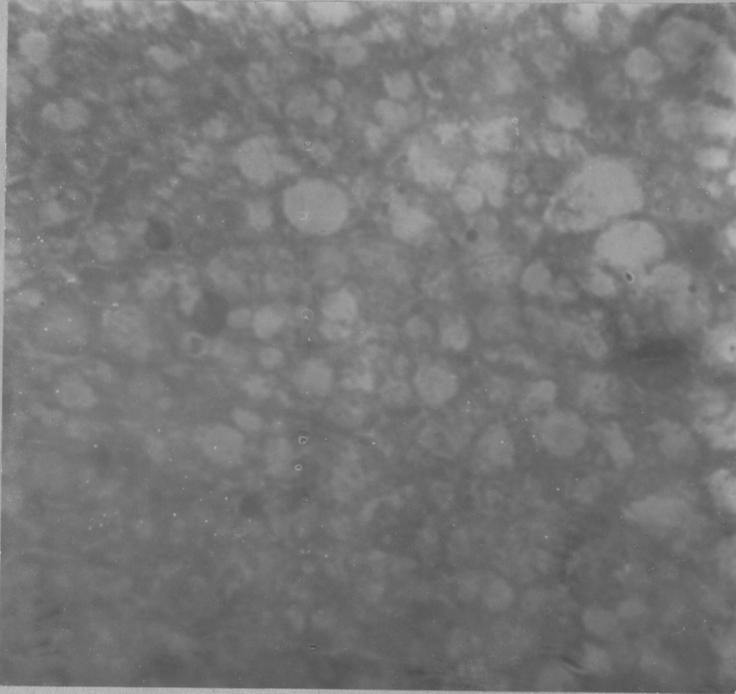
Photograph of the liver tissue of dog No. 2 showing the presence of numerous fat cells.

Photograph No. 2.



Photograph of the liver tissue of dog number 4 showing almost complete fatty degeneration fifty days after the operation.

Photograph No. 3.



Photograph of the liver tissue of dog number 6 showing the presence of many fat cells sixty-nine days after the operation.

Photograph No. 4



Photograph of the liver tissue of dog number 7 showing the presence of many fat cells forty-five days after the operation.