AMMONIA TOLERANCE STUDIES IN ECK-FISTULA DOGS

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The importance of determination of patency of Eck-fistuala in post-operative period is well recognsied (1), but so far methods of its evaluation are not standardised. One of the methods to find out the state of the shunt in the living post-operated individuals, is by radiological studies in which the shunt can be visualised by splenovenography and portovenography; unfortunately, these carry a certain risk and the spleen is not uncommonly surgically absent in this group of patients. Measurements of the portal circulation time have also been employed but these are difficult to perform and as well inaccurate in interpretation.

The purpose of this paper is to assess the chemical method of ammonia tolerance studies to find out the patency or otherwise, of Eck-fistula in post-operative period.

In ammonia tolerance test, the blood ammonia is determined and a standard dose of an ammonium salt is given by mouth followed by a series of determinations of blood ammonia at different time intervals. Normally, ammonia concentration in systemic blood rises for about half to one hour after the administration of ammonium compound; after that the ammonia concentration begins to fall down sharply and within another hour it comes down to the normal fasting level. This is due to the fact that ammonium salt, when taken by mouth, is absorbed from the gastro-intestinal tract and is transported via the portal vein to the liver where the ammonia radical is converted to urea. When however the liver is by passed by means of a patent shunt as in the case of Eck-fistula, systemic blood ammonia level should remain elevated for a long time, and thus to know as to whether or not the shunt remains patent, the ammonia tolerance test would appear to be useful.

METHODS AND MATERIALS

Blood Ammonia Determination. This estimation is particularly difficult for two main reasons. First, the quantities of ammonia involved are so small that extreme precautions have to be taken in analytical procedure. Second, there is spontaneous evaluation of ammonia after blood is shed; this reaction

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is so explosive that the original concentration of blood ammonia is multiplied several folds in a short time. Parnas and Hellar (2) and Conway (3) observed that there were two factors which depressed this spontaneous production of ammonia from shed blood, viz. by keeping the blood cool and by surrounding it with carbon dioxide atmosphere. Taking advantage of these observations Singh, Barclay and Cooke (4, 5) collected blood in syringes which were previously filled with carbon dioxide gas and were kept cold at the temperature of 0°–4°C; this quite efficiently depressed evolution of ammonia for a reasonable time. In the present studies blood was collected according to the modification of Singh et al. (4, and the analyses were made by Conway's micro-diffusion method (6).

Collection of Blood.–A 20 ml. glass syringe was well cleansed with distilled water and dried by rinsing with absolute alcohol and ether. A little amount of finely powdered ammonia-free crystalline potassium oxalate was introduced and the syringe was filled upto 5 ml. mark with carbon dioxide under pressure. The nozzle of the syringe was quickly closed with a hub (amputed and soldered needle) and the syringe was then stored in crushed ice contained in a thermos flask. About 10 ml. of blood from the femoral artery of the dog was collected in this cold syringe. The hub was replaced and the blood was well mixed with the oxalate and carbon dixoide, and the syringe immediately put back into the flask. The analyses in triplicate, were done immediately with 1 ml. portions of this blood; the results were found to be reasonably concordant.

Proeedure of Ammonia Tolerance Test. The normal or the operated Eck-fistula dogs on which these tests were to be performed, were starved on the day of study. These were anaesthetised with I/V Nembutal. Normal dogs were given 25 mg./kgm. body weight of the anaesthetic but Eck-fistulae dogs were given only 15-20 mg./kgm. body weight as they have got low power to detoxicate barbiturates. Femoral artery was then exposed in femoral triangle and a thread loop was passed to surround it. About 10 ml. of blood was collected in the cooled syringe and its ammonia concentration estimated.

To administer ammonium salt, the stomach tube was passed and through it the dog was given 1% solution of ammonium chloride, the dose of which was 100 mg./kgm. of the body weight. Blood samples were collected every half hour for a period of two hours and the curves of these results were plotted.

Thc dogs used were of stray breeds, of both sexes and 6-14 kgms. body weight, kept under observation in the laboratory for a period of one week before actual studies were made. Ammonia tolerance studies were carried out in six unoperated dogs to serve as a control. In five other dogs Eck-

fistulae were constructed by different techniques and after variable periods, the studies were made; in two of these, ammonia tolerance studies were also carried out in the preoperative phase to serve as an extra control.

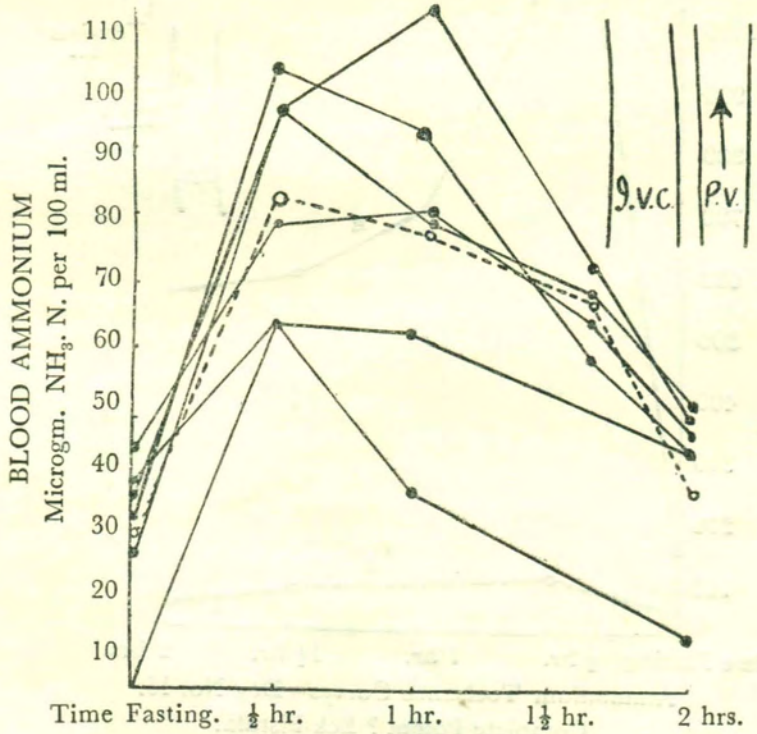
Eck-fistulae were accomplished in these animals through right subcostal incision. In two dogs end to side (end of portal vein to side of inferior vena cava) anastomoses, and in three side to side anastomoses were carried out. Two of these latter three were left as incompleted Eck-fistulae, and the third was converted into a complete Eck-fistula by ligaturing the portal vein proximal to the site of shunt thereby completely by-passing the liver.

The post-mortem examinations were made in all the five cases to assess the patency or otherwise of the shunt and to compare the results of chemical studies.

RESULTS AND DITCUSSION

Normal Dogs The results of the ammonia tolerance studies made with six normal dogs are shown in figure 1; the dotted curve is the average of

Fig. 1

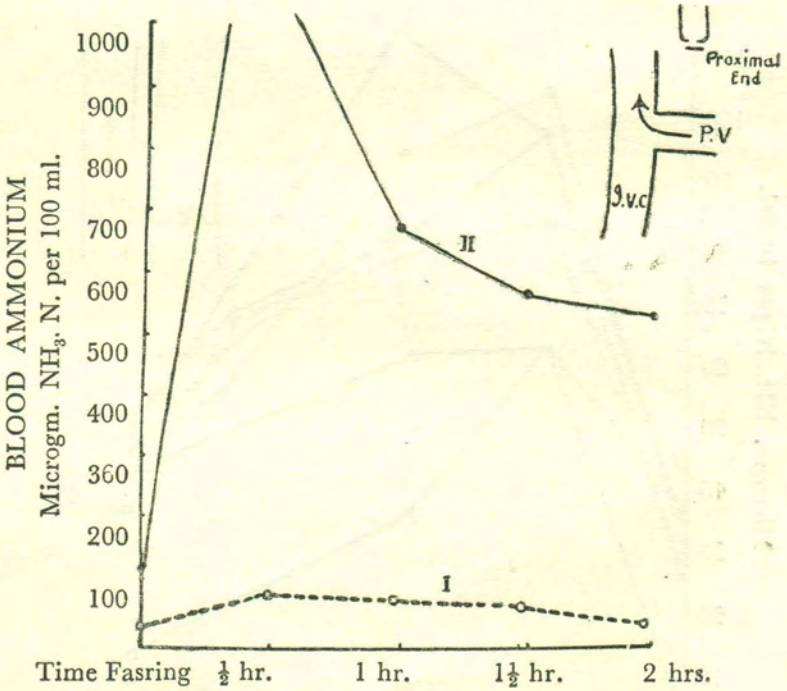


Ammonium Tolerance Curve.
(Normal Dog.)

the six and this was taken as normal to serve as the control. Fasting blood ammonia level ranged between 10–45 μg. NH_3—N per 100 ml. with an average of 33.3 microgm. After the administration of ammonium chloride, peak was obtained in half to one hour and the maximum blood ammonia level did not exceed 110 μg, %. This was followed by a sharp fall in blood ammonia level and in two hours it was approaching the fasting level.

Eck-Fistulae Dogs. DOG No. 16–In it complete Eck-fistula was accomplished by end to side anastomosis. Chemical study done on the eighth day of operation revealed that the fasting blood ammonia level was higher than the normal and that on administration of ammonium chloride an abrupt steep rise of blood ammonia level was obtained in the first half hour period (over 1000 μg. %). At the end of two hours the blood ammonia concentration was well above the fasting level (see figure 2). The abrupt steep rise and the sustained elevated level were suggestive of patent Eck-fistula;

Fig. 2

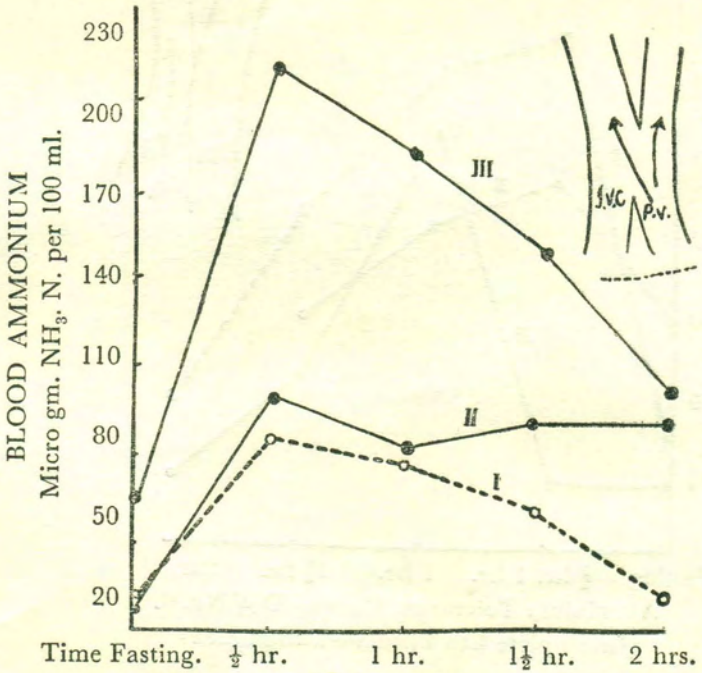


Ammonium Toeliance Curves—Dog No. 16.
Complete Potent ? Eck Fistula.
No. I Average normal curve.
No. II Curve 8 days after operation.

this was confirmed on postmortem examination. In the complete and patent Eck-fistula the peripheral blood ammonia concentration mirrors the amount being absorbed by the gastro-intestinal tract as the liver is totally by-passed.

DOG No. 2–(see figure 3) In it an incomplete Eck-fistula was constructed by side to side anastomosis. The studies were made on the fifth and the twelfth post-operative days. First study revealed a curve closely resembling the normal upto one hour of the administration of ammonium chloride but after two hours, the blood ammonia level was about 90 μg. %, unlike in in the normal. Second study on the twelfth day showed fasting blood ammonia level which was within normal limits but an immediate steep rise was obtained (220 μg. %) half an hour after the administration of ammonium chloride; however, this was much lower than in the cases of dog No. 16. After this there was a rapid fall, the concentration remaining a little higher than the fasting level at the end of two hours.

Fig. 3.



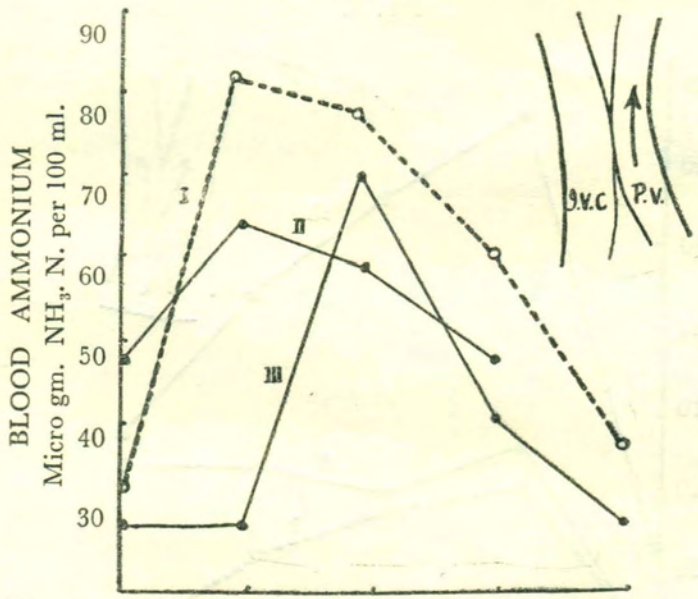
Ammonium Tolerance Curves—Dog No. 2,
(Incomplete Patent Fistula)

No. I. Average normal curve.
No. II. Curve 5 days after the operation.
No. III. Curve 12 days do. do.

This pattern in both these studies suggested an incomplete patent Eck-fistula which was confirmed on postmortem. In the first study the curve was rather flat suggesting that perhaps more blood was passing through the liver at that occasion ; however, even in this case, the curve was different from the normal in as much as that the blood ammonia concentration at the end of two hours remained elevated suggesting some by-pass of the liver. This study in addition, suggests the use of serial tests to arrive at a definite conclusion.

DOG No. 6—(see figure 4). In it an incomplete Eck-fistula was constructed by side to side anastomosis. Repeated chemical studies on the seventh and forty second post-operative days showed findings within normal limits suggestive of complete thrombosis which was later confirmed on postmortem examination,

Fig 4



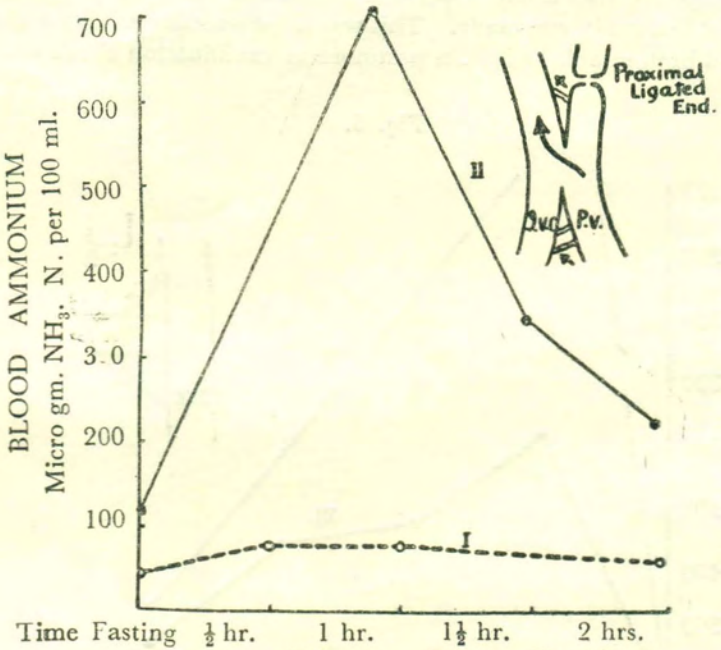
Amminium Tolerance Curve—Dog No. 6.
(Incomplete Eck Fistula—thrombosed)

No. I. Average normal Curve.
No. II. Curve 7 days after operation.
No. III. Curve 42 days do. do.

DOG No. 3—In it a side to side anastomosis was done but was converted into a complete fistula by ligaturing the portal vein proximal to the shunt.

The results are shown in figure 5, where curve I shows the normal findings before the operation. Curve II(5th day of operation) revealed a fasting blood ammonia level higher than the normal but the rise was gradual to a peak of 700 μg% in one hour period. At the end of two hours the blood ammonia concentration remained elevated and did not touch the fasting level. This pattern of curve in a complete fistula can only be due to (i) complete thrombosis of shunt or (ii) complete thrombosis with establishment of collaterals. Post-mortem examination showed that the shunt was incompletely thrombosed.

Fig. 5.



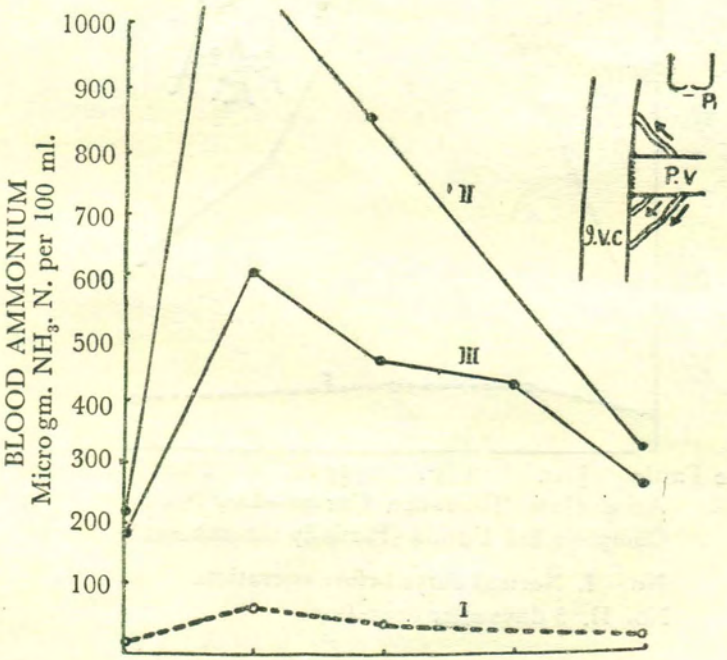
Ammonium Tolerance Curves—Dog No. 3.
Complete Eck Fistula (Partially thrombosed)

No. I. Normal curve before operation.
No. II. 5 days after operation.

It appears that in this dog though the portal blood was getting shunted into systemic blood the process was not as abrupt as in the patent fistula (dog. no. 16). It is likely that some time was taken by the ammonia saturated blood to reach the systemic circulation and therefore the magnitude of peak in this is lower.

DOG No. 13—In it a complete fistula, by end to side anastomosis was constructed. The results are given in figure 6, where curve I shows the findings of the ammonia tolerance study done before the operation. Curves II and III gave respectively the findings of studies done on the fifth and twelfth days of the operation. Curve II is identical with the curve obtained in the case of dog No. 16 suggesting complete and patent shunt. But on repeating the study on the twelfth day, there was a flattening of the curve which was more or less similar to that in the case of dog No. 3, attaining a peak of about 600 μg. %. This suggested (i) a partially occluded shunt or (ii) completely occluded shunt with drainage by collaterals. The post-operative record of this dog showed that it had developed ascites and bleeding per rectum by the time the last study was made. This was a presumtive evidence that collaterals had been established. On postmortem examination shunt was found

Fig. 6.



Ammonium Tolerance Curves—Dog No. 13.
(Complett Eck Fistula 1st. Patent, later thrombosed).

No. I. Before operation.
No. II. 5 days after operation.
No. III. 12 days after do.

to be completely occluded, thus confirming the suggestion no. (ii) above of the chemical study.

These experimental studies show clearly that the most reliable method of interpretting the ammonia tolerance test is in the comparison of the studies performed pre-operatively, and serially during the post-operative period when shunt patency is in doubt. An abrupt change in the test curve provides the most important indication of the shunt.

While this test indicates promise as a simple and an inexpensive test in assessing the patency of a shunt, it needs to be emphasized here that it is not without danger to apply this test in patients with severe uncompensated liver disease. It has been observed by Eiseman et al. (7) and by one of the authors (I. D. S.) that severe hepatic coma may result even with a small amount of an ammonium salt given to such patients.

SUMMARY

Ammonium tolerance studies were carried out in 11 dogs in order to determine the patency or otherwise of Eck-fistula constructions. A complete Eck-fistula was constructed by end to side or side to side anastomosis of the portal vein with inferior cava with the proximal end of the portal vein tied, while an incomplete preparation was possible by a side to side anastomosis with the proximal end of portal vein untied. The ammonium tolerance studies were undertaken after oral administration of 100 mgms. of NH_4Cl/kgm. of body weight and determination of blood ammonia concentration every half an hour upto 2 hours. The results of the tolerance studies in Eck-fistula dogs were compared with 6 normal control dogs. In a patent complete Eck-fistula the fasting blood ammonium level was much higher and it showed a steeply rising curve, whereas in a patent incomplete Eck-fistula the level was only slightly higher than normal. Thrombosis of the fistula was suspected in one dog when the steep curve was suddenly replaced by a lower and flattened curve on the twelfth day of the operation. This was subsequently confirmed at autopsy. In 2 other dogs also, the suspicion of thrombosis was duly confirmed. It is concluded that serial ammonium tolerance studies are of value in assessing the patency of Eck-fistule constructions.

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