

cluded that factors in addition to ACTH are concerned in the growth of the adrenal in renal hypertension.

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## Hepatic Artery Ligation in Rabbits Followed by Thoracic Caval Constriction.\* (18881)

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Acute ligation of the hepatic artery, as shown by Markowitz(1), is compatible with life in dogs if an antibiotic such as penicillin is given in the postoperative period. This discovery has led to new approaches to the problem of ascites in animals and in man.

It has been indicated by Reinhoff(2), in cases of portal hypertension, that hepatic artery ligation might prevent the formation of ascites. Perfusion experiments have shown that blood flow through the hepatic artery in cirrhosis encounters less resistance than in the normal liver. The higher pressure of blood flow through the hepatic artery, with less resistance, tends to increase the resistance to the flow through the liver from the portal vein. The rationale of ligation of the hepatic artery in these cases was based upon the fact that lowering the intra-arterial pressure also lowers that in the portal vein. In a previously reported experiment(3), it was found that the ascites formed in rabbits by thoracic caval constriction was not affected by previous 2-stage portal ligation. The present experiment, as a continued study, was carried out to determine what effect hepatic artery ligation

would have on the type of ascites produced by experimental constriction of the thoracic inferior vena cava. The fluid formed by such a procedure is believed to be the result of venous congestion in the liver. McKee(4,5) produced ascites in dogs within a few days by means of an adjustable aluminum band placed on the thoracic inferior vena cava midway between the heart and diaphragm. Volwiller(6) and Volwiller and co-workers(7), also working with dogs, used a cellophane band placed around the thoracic segment of the inferior vena cava. The fibrous reaction of the cellophane would cause a progressive constriction of the vessel lumen to approximately one-quarter of its original diameter within three to four weeks. This resulted in marked ascites.

Rabbits weighing 3 to 4 kg were used in this experiment. The diet consisted of stock rabbit pellets containing 17% protein, oats and water. All surgical procedures were executed under aseptic operating conditions. In the cases in which thoracotomy was performed, local anesthesia and positive endotracheal oxygen pressure were utilized. Postoperative intake and output were not measured.

**Method and results.** Ten control rabbits were subjected to hepatic artery division and ligation just distal to the gastroduodenal

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1. Markowitz, J., Rappaport, A., and Scott, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, v70, 305.

2. Reinhoff, W. F., *Bull. Johns Hopkins Hosp.*, 1951, v88, 368.

3. Milnes, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1951, v76, 147.

4. McKee, F. W., *Surg., Gyn. and Obst.*, 1950, v89, 529.

5. McKee, F. W., *J. Exp. Med.*, 1948, v87, 457.

6. Volwiller, W., *Proc. Staff Meetings, Mayo Clinic*, 1950, v25, 31.

7. Volwiller, W., Grindley, Bollman, *Gastroenterology*, 1950, v14, 40.

TABLE I. Comparison of Survival Rate in Rabbits With and Without Penicillin Following Hepatic Artery Ligation.

|          | No. Rabbits | Survived | Died | Avg days survived |
|----------|-------------|----------|------|-------------------|
| Controls | 8           | 0        | 8    | 2                 |
| Treated  | 18          | 12       | 6    | 5                 |

branch. In every case, careful search was made for aberrant or extra hepatic branches of the hepatic artery. No antibiotics were given following surgery. On the 10 rabbits, 9 died within a period of 2 days, and 1 died after 5 days. Immediate post mortem examination revealed large amounts of gas present in the peritoneal cavity and gross necrosis of the liver. Culture of peritoneal exudate taken just before death and immediately afterward showed gas forming *Clostridium welchii* to be predominant. This is in accord with the experience of others (8-10) that the mechanism of death in liver autolysis is bacterial. It is thought that hepatic artery ligation allows activation of anaerobic bacilli in the liver by diminishing the oxygen supply to that organ. Table I gives results in this group.

In another group of 18 rabbits, the hepatic artery was ligated and divided in the same manner as in the control group. Postoperatively, however, 300,000 units of procaine penicillin were given twice daily for a period of 7 days. Twelve animals survived without visible effect. The 6 that died showed massive liver necrosis, gas in the peritoneal cavity, and presented a picture similar to that shown by the animals which received no penicillin. It was interesting to note that those animals that did not survive died in 4 to 6 days rather than in 2 days, as in the control group. (See Table I for comparison with the control group). The penicillin apparently contained anaerobic organisms for a time, at least. In none of the rabbits was ascitic fluid demonstrable in frequent paracentesis.

A second control series of 8 rabbits was

8. Mann, F. C., as cited by Mason and Davidson.
9. Mason, E. C., and Davidson, E. C., *J. Lab. and Clin. Med.*, 1925, v10, 622.

10. Lewis, F. J., and Wangenstein, O. H., *Proc. Soc. Exp. Biol. and Med.*, 1950, v73, 533.

operated upon. Each animal was subjected to constriction of the thoracic inferior vena cava midway between the heart and diaphragm, with a polyethylene band measuring .5 cm in width. The vein was measured carefully with calipers so that the vessel could be reduced as nearly as possible to 60% of its normal diameter. Closure of the chest was accomplished with interrupted silk, and the lungs re-expanded by means of positive endotracheal pressure. Following operation, the presence of ascites was determined by daily paracentesis. Ascites was found to occur in 3 to 4 days. Samples of fluid were removed every 7 days for a period of 2 months and analyzed for total protein and A/G ratio. The volume that could be removed at each sampling varied from 20 to 40 cc, with a specific gravity of 1.012 to 1.019. The values for total protein averaged 4.2 g per 100 cc, and the A/G ratio averaged 1.3. Table II outlines the 8 control experiments.

The 12 rabbits that survived hepatic artery ligation were reoperated upon again in 10

TABLE II. Ascitic Fluid in Rabbits with Thoracic Caval Constriction.

| Exp. | Onset of ascites (days) | S. G. | T.P. (g/100 cc) | A/G |
|------|-------------------------|-------|-----------------|-----|
| 1    | 4                       | 1.017 | 3.9             | 1   |
| 2    | 3                       | 1.019 | 4.3             | .9  |
| 3    | 4                       | 1.012 | 4.5             | 1.5 |
| 4    | 4                       | 1.018 | 4.6             | 1.3 |
| 5    | 3                       | 1.012 | 3.9             | 1.7 |
| 6    | 3                       | 1.014 | 3.8             | 1.6 |
| 7    | 4                       | 1.018 | 4.3             | 1.4 |
| 8    | 4                       | 1.016 | 4.2             | 1.4 |
| Avg  |                         |       | 4.2             | 1.3 |

TABLE III. Hepatic Artery Ligation Followed by Thoracic Caval Constriction.

| Exp. | Onset of ascites (days) | S. G. | T.P. (g/100 cc) | A/G |
|------|-------------------------|-------|-----------------|-----|
| 1    | 3                       | 1.013 | 3.6             | .9  |
| 2    | 4                       | 1.016 | 3.8             | 1.4 |
| 3    | 4                       | 1.014 | 4.4             | 2.1 |
| 4    | 3                       | 1.014 | 4.3             | 1.2 |
| 5    | 4                       | 1.016 | 4.2             | 1.7 |
| 6    | 3                       | 1.017 | 4               | 1   |
| 7    | 3                       | 1.015 | 3.6             | 1.3 |
| 8    | 3                       | 1.017 | 4.3             | 1.4 |
| 9    | 2                       | 1.016 | 3.8             | 1.5 |
| 10   | 3                       |       | 4.3             | 1.6 |
| Avg  |                         |       | 4               | 1.4 |

days. The same technic of thoracotomy and thoracic inferior vena caval constriction, as employed in the second control group, was applied to this series. Positive endotracheal oxygen pressure was given throughout. No antibiotics were utilized in the postoperative period. Following operation, daily paracenteses were performed, and the formation of ascites was usually demonstrated in 3 to 4 days. This fluid was examined for total protein and A/G ratio every 7 days for a period of 2 months. No appreciable change in average total protein or A/G ratio was noted when compared with the fluid produced in the ani-

mals with thoracic inferior caval constriction alone. Total protein averaged 4.0 g per 100 cc, A/G ratio 1.4 and specific gravity 1.013 to 1.017. Table III outlines the results obtained in this group of animals.

**Conclusion.** 1. Acute hepatic artery ligation in the rabbit resulted in 100% mortality in this experiment. 2. Massive penicillin therapy following hepatic artery ligation in rabbits resulted in a 66% survival rate. 3. Ascites produced by thoracic caval constriction in the rabbit is not prevented or appreciably affected by previous hepatic artery ligation.

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### Influence of Vitamin B<sub>12</sub> and Folacin on the Synthesis of Choline and Methionine by the Rat.\* (18882)

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It was reported(1,2) that vit. B<sub>12</sub> exerted a sparing action on the choline requirement of rats and chicks. It was later noted(3) that both folacin and vit. B<sub>12</sub> were involved in the

methylation process. Schaefer *et al.*(4) reported that chicks required vit. B<sub>12</sub> for the synthesis of choline from methylaminoethanol and methionine or betaine. Jukes *et al.*(5) noted that vit. B<sub>12</sub> was essential for the methylation of homocystine for growth in chicks fed a methionine-deficient diet. Oginsky(6) observed that livers of vit. B<sub>12</sub>-deficient rats exhibit a lower ability to form methionine from homocystine and either betaine or choline as compared with those from animals dosed with vit. B<sub>12</sub>. The role of vit. B<sub>12</sub> and folacin in the methylation of guanidoacetic acid was reported by Dinning *et al.*(7). Stekol and Weiss(8) concluded that rats are able to grow on a diet free of all the known labile methyl group donors but

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4. Schaefer, A. E., Salmon, W. D., and Strength, D. R., *Fed. Proc.*, 1950, v9, 369.

5. Jukes, T. H., Stokstad, E. L. R., Broquist, H. P., *Arch. Biochem.*, 1950, v25, 453.

6. Oginsky, E. L., *Arch. Biochem.*, 1950, v26, 327.

7. Dinning, J. S., and Day, P. L., *J. Biol. Chem.*, 1949, v181, 897.

8. Stekol, J. H., and Weiss, K., *J. Biol. Chem.*, 1950, v186, 343.