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SOME EFFECTS OF PORTO-CAVAL ANASTOMOSIS IN THE MALE RAT

MA HTA KYU AND J. B. CAVANAGH

*From the M.R.C. Research Group in Applied Neurobiology, Institute of Neurology, Queen Square, London, W.C.*1

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SUMMARY.—Porto-caval anastomoses have been made on male rats which survived for up to 38 weeks. The acute phase of the consequences of this operation was associated with rapid fall in liver and body weight and a slow fall in testicular weight. Appetite also was considerably reduced and the plasma ammonium nitrogen became set at a new level of 400–500 μg./100 ml. After 12 weeks all animals were tending to gain weight, and their appetite was returning towards normal. At this phase there was an increase in both liver and testicular weight and a slow fall in plasma ammonium nitrogen. It is considered that all these features were linked and probably dependent upon the return of portal blood to the liver through adhesions around the operation site. While the plasma ammonium nitrogen levels were lowered by antibiotic treatment, they could not be further raised by dietary means.

THE Eck fistula has been used by a number of authors (Bollman, 1961) for the study of the cerebral complications of experimental liver disease since Hahn, Massen, Nencki and Pawlow (1893) first used this method. While several studies have been made in dogs by this technique the rat has been used less frequently, although its small size is no bar to successfully performing this operation (Lee and Fisher, 1961; Fisher, Lee, Fisher and Saffer, 1962; Flynn and Kennan, 1968; Doyle, 1967).

Use has been made of this experimental preparation by us (Cavanagh and Kyu, 1969, 1970*a* and *b*; Kyu, 1970) in an attempt to understand how the anomaly in astrocyte nuclei, known as Alzheimer's change, comes about. Since, at the same time, general changes in weight and food consumption as well as in plasma ammonium levels were also studied, and since also the histological alterations in certain organs other than the brain were followed in these animals, these findings were considered worth reporting separately from the specific brain changes.

MATERIALS AND METHODS

Animals.—Male rats either 300–420 g. or 180–250 g. were used. The strains employed were Porton obtained from M.R.C. Animal Centre, Carshalton, and specific-pathogen-free Wistar (C.F.H.B.) and Sprague-Dawley (C.F.E.) obtained from Carworth (Europe) Ltd. They were housed in plastic boxes and fed Chardex and 41B. This was supplemented in certain cases with high protein foods. They were weighed weekly. In certain groups the food was weighed daily to assess the daily consumption.

The operation.—The technique of porto-caval anastomosis was shown to us by Professor K. Weinbren of Nottingham University. It was essentially the same as that of Lee and Fisher (1961) and of Bismuth, Benhamou and Lataste (1963). Under ether anaesthesia, the coronary vein was ligated and the portal vein was then clamped, tied and cut, and subsequently stitched to an incision in the anterior wall of the inferior vena cava just above the

junction with the right renal vein. Continuous sutures using a 7/0 Ethicon silk thread were used. The wound was closed and the animals usually recovered within a short time. Patency of the anastomosis was tested at post-mortem examination and found to be satisfactory in every case.

A sham operation was done on 5 animals in which the procedure was taken as far as clamping the portal vein for 10 min. The clamp was then removed, the abdominal contents replaced in the peritoneal cavity and the wound closed with clips as in the other animals.

Plasma ammonium nitrogen (NH_4-N) estimations.—The method of Fenton and Williams (1968) was used and an estimation was made in every animal immediately before killing. Under light ether anaesthesia a needle was thrust into the heart through the chest wall and 1·5–2·0 ml. of blood removed into a heparinized syringe. It was rapidly centrifuged and the plasma removed, care being taken not to take it from too close to the buffy coat. Estimates were reliable if these two precautions were taken and if the blood was not allowed to clot. Unexpectedly high figures were invariably found if clotting occurred or if centrifugation was too long.

The histological methods.—When the animals were killed by an overdose of chloroform the aorta was canulated and formol-acetic acid (10 per cent/1 per cent) fixative was perfused into the aorta through the left ventricle to fix the brain rapidly. The descending aorta was clamped and the liver removed after inespecting the anastomosis. The liver, kidneys, testes, suprarenals and spleen were weighed and then fixed by immersion in formol-acetic acid. In a few instances fragments of liver were fixed in 70 per cent alcohol for glycogen.

Sections were routinely stained with haematoxylin and eosin, and stains for reticulin (Gordon and Sweet), for iron (Perl) and for glycogen (Best) were also done.

RESULTS

The general health of all animals remained good after the immediate post-operative recovery. Older rats (Porton strain) tended to become unkempt and infested with fleas, but the younger rats (C.F.E. and C.F.H.B.) groomed themselves better and kept healthier.

Food consumption (Table I)

This was tested in 11 post-operative rats and 2 unoperated controls by weighing the food before and after each day for 16 post-operative weeks. For the first 5

TABLE I.—*Daily Food Consumption of Experimental and Control (weight matched) Rats at Various Times after Operation*

Number of animals	Period of assessment (weeks after operation)	Average daily food consumption (g.)
2	1–4	8
2	5–8	12
3	8–12	17·5
2	13–14	25
2	15–16	17
2 (Controls)	16	27·5

post-operative weeks these rats ate about 1/3 the amounts of the control rats. From 5–8 weeks after operation they ate about half the normal amounts while from 8 weeks onwards they ate 2/3 or more of the control amounts.

Changes in body weight (Fig. 1)

All 86 operated rats, 5 sham operated rats and 6 normal controls were weighed weekly. The post-operative weight changes differed in the younger and the

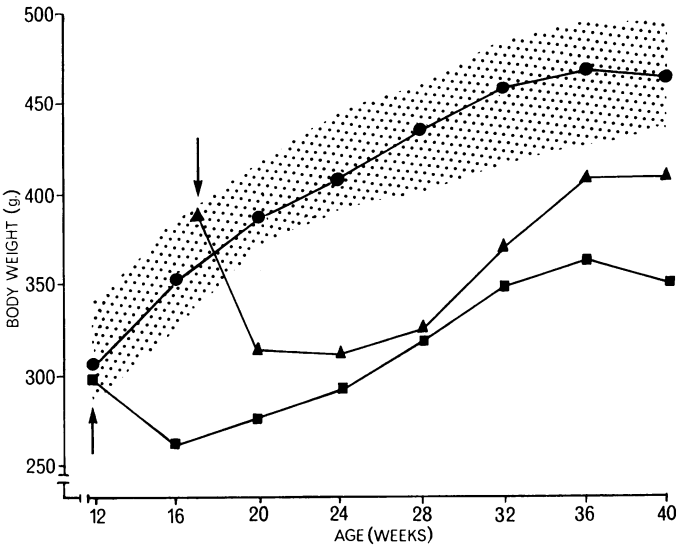


FIG. 1.—Mean weekly body weight of control rats (●—●. Hatched area is range of weights) and 4 representative rats in the greater (▲—▲) and lesser (■—■) body weight range. Arrows indicate time of operation.

older rats. Younger rats lost 10–20 per cent of their original body weight but recovered faster so that by the 4th week they were beginning to gain weight again. They reached their pre-operative weights by 10–14 weeks and continued to gain weight in parallel with the controls. On the other hand, older rats lost 20–30 per cent of their initial body weight soon after the operation and did not begin to gain weight again until 7 weeks after operation. Although they were thus more affected and slower to recover than the younger group, they gained weight finally in parallel with the controls. In parallel with the controls also, they reached a peak weight at about 36 weeks of age and thereafter declined in weight. The 5 sham operated controls lost up to 5 g. of body weight during the first two post-operative days, but thereafter gained weight at the same rate as unoperated controls.

Those rats (4 animals) in the older (Porton strain) group at operation that lost more than 35 per cent of their body weight in the post-operative weeks died, and were found to have a lung infection at post-mortem examination.

State of the anastomosis at death

All 86 animals were examined at death by gently running a finger along the portal vein to test for patency of the anastomosis. It was patent in every instance and no thickening or other evidence of recanalization of a thrombosis was found.

Adhesions around the operation site were present in every instance. Even by the end of the first post-operative week light adhesions were present between the omentum and the small intestine. Usually after about 10 weeks the duodenum was adherent to the anterior part of the right lateral lobe of the liver, and the omentum tended to be adherent to its under surface in the region of the stump of the portal vein. Although these older adhesions were firm it was not possible,

by the methods of inspection used, to identify effective collateral connections between the portal system and the liver. However, it was noted that in the longer survivors, more than 16 weeks, in which plasma NH_4-N levels approached normal figures, these adhesions were more dense and firm than in animals whose NH_4-N levels remained high.

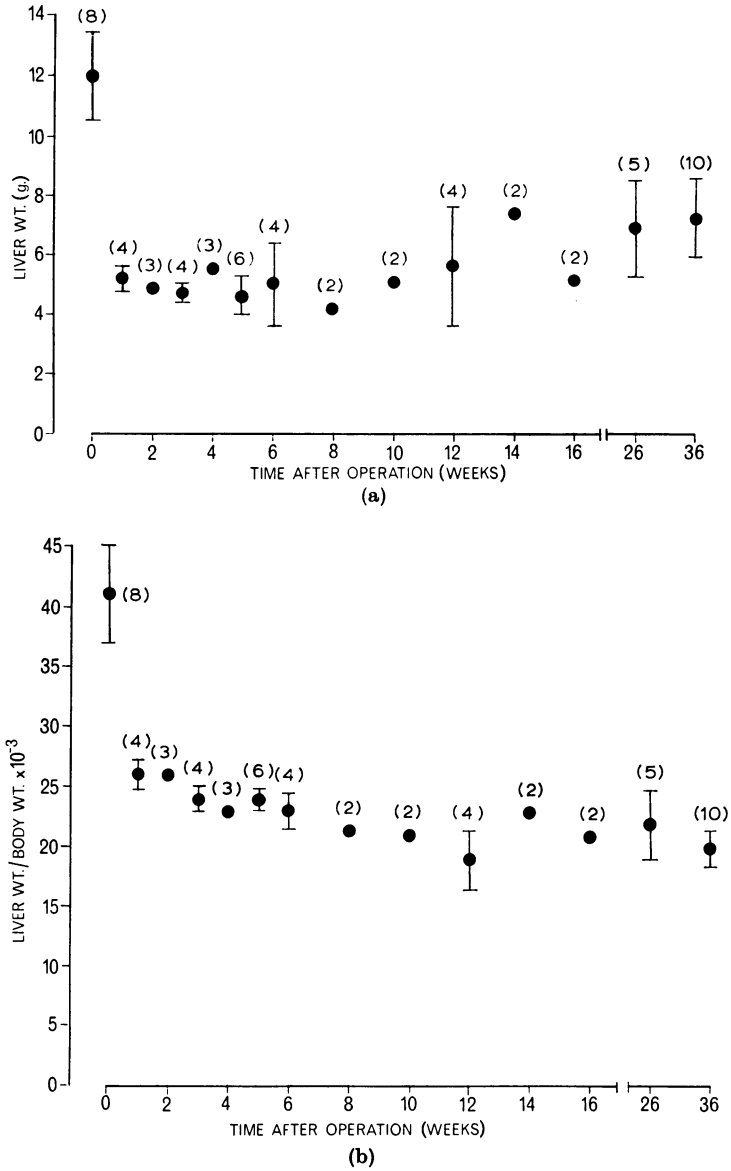


FIG. 2.—(a) Mean liver weight and (b) Mean liver/body weight ratios from 1–36 weeks after porto-caval anastomosis. Standard deviations are shown as bars and the number of animals in each group in brackets.

Effects of porto-caval anastomosis on the liver

One week after operation the liver looked shrunken and wrinkled, and was flabby with sharp edges. By 10 weeks it tended to look more rounded and smooth, and this was more evident when adhesions were more dense.

Liver weights and liver/body weight ratios.—In Fig. 2(a) it will be seen that the mean liver weight fell to less than half the mean normal within a week of operation. There was a slight subsequent fall which was probably not significant, but after 8 weeks there was a steady increase in mean liver weights, and the variation between animals was more marked. This weight increase closely paralleled the increase in body weight at this period. The curve of liver/body weight ratios (Fig. 2(b)) shows an abrupt change immediately following the operation, a further slight decline and then a level trend after 8 weeks which reflected the closely parallel changes in these two parameters.

Histological changes.—The lobular architecture was preserved throughout. There was marked collapse of the sinusoids due to the diminished blood flow but no demonstrable reduction in the size of the liver cells was found. Cell density as measured by nuclear counts per unit area more than doubled (Table II) by the end of the first week.

TABLE II.—*Mean Nuclear Density per Unit Area in Sections of Liver*

Time after operation (weeks)	Number of animals	Mean nuclear density per unit area (±S.D.)	Plasma NH_4-N range (μg./100 ml.)
Normal	2	24·0	—
1–2	5	44·8 (±6·8)	375–544
12–14	4	28·2 (±4·1)	268–527

Ten fields counted per animal. No correlation between individual estimates and plasma NH_4-N level was evident.

Although the cell size did not appreciably change, the cells no longer showed the normal vacuolated pale appearance of the cytoplasm, and there was marked loss of glycogen from all liver cells (Fig. 3). In livers examined 3–4 weeks or more after operation, glycogen was apparent in cells around veins beneath the capsule. These areas became obvious in routinely stained preparations since the cells in these regions returned to the normal pale and vacuolated appearances. Those in the centre of the liver, however, remained homogeneous and eosinophilic throughout.

Fatty changes were seen in frozen section stained with Oil red 0 from the 11th week onwards, which were always centrilobular. Usually the fat was finely granular, but occasionally larger vacuoles appeared. Perl's iron stain was invariably positive in Kupffer cells at all stages, but in longer term animals the liver cells also showed a fine granular positive reaction.

Effects of porto-caval anastomosis on the testes

Mean testicular weights showed a slow decline during the first 5 weeks to less than half the mean normal values. From then onwards there was a slow upward tendency so that by 30–38 weeks the mean weights were approaching normal (Fig. 4(a), (b)).

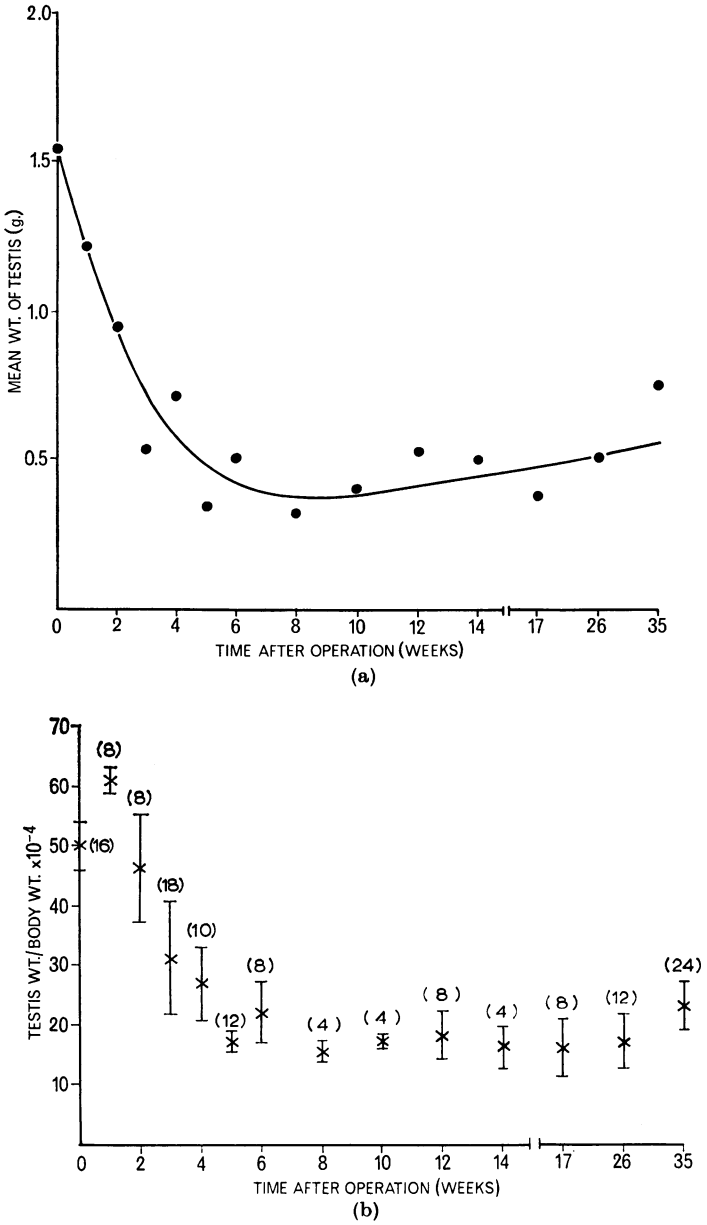


FIG. 4.—Mean weights of tests (a) and mean testis/body weight ratios, (b) at various times after operation.

The right testis was consistently equal in weight to the left testis, which is unusual since the left is normally consistently smaller. Moreover, the right testicular vein was consistently more prominent in operated animals than the left. The right renal vein runs directly into the inferior vena cava, which was

always noticeably dilated, sometimes sufficiently to form an impression on the under surface of the liver, so that it would tend to be subjected to a slightly greater hydrostatic pressure than the left testicular vein.

Atrophy began to be patchily evident in the seminiferous tubules from the 2nd week onwards. By the 3rd week all tubules showed some degree of atrophy which was maximal at 5–8 weeks (Fig. 5(a), (b)). The tubules at this stage contained no spermatozoa or spermatids. Spermatogonia and spermatocytes were the chief surviving cells, the latter showing major abnormalities, such as pyknosis, nuclear swelling and multinucleate forms. Sertoli cells were not conspicuous. Because the testes had been carefully weighed unfixed and fixation was therefore delayed, the conditions were not suitable for studies on mitotic activity in the spermatogonia or spermatocytes, nor were Leydig cells well seen.

By the 10th week after operation normal spermatogenesis began to be seen in some tubules, while others were still severely atrophied. No spermatozoa were seen, however, until after the 12th week (Fig. 6(a), (b)). Although at 28 weeks many tubules were normal in appearance many others still showed atrophic linings and contained only cell debris and clear fluid.

Effects of porto-caval anastomosis on the kidney

Five out of 86 animals had hydronephrosis of the right kidney. Since however, these were all in the younger group of animals (32) operated upon when between 180–265 g. it occurred in 15·6 per cent of this group. The anastomosis, because of the smaller size of the animals, tended to be closer to the opening of the right renal vein, and is likely that the right renal vein had a substantially higher pressure than the left for this reason.

The renal pelvis of the hydronephrotic kidneys contained brown urate crystals, but the cortex and medulla were not obviously narrowed. The mean kidney weights of those without hydronephrosis did not differ significantly from normal (Table III).

Effects of porto-caval anastomosis on the spleen

Mean splenic weights were slightly but not significantly less than normal (Table III). Excess stainable iron pigment was constantly present in the medulla from the 3rd week onwards.

TABLE III.—*Mean Weights (± S.D.) of Spleen and Kidney and Ratios with Body Weights in P–C Shunted Animals whose Body Weights Lay within the Range of that of Control Animals*

	Mean spleen wt (g.)	Mean $\frac{\text{spleen wt}}{\text{body wt}}$ ratio ($\times 10^{-4}$)	Mean kidney wt (g.)	Mean $\frac{\text{kidney wt}}{\text{body wt}}$ ratio ($\times 10^{-4}$)
P–C shunt (7–24 weeks)				
	0·67 (10)*	22·0	1·4 (10)	41·1
	±0·15	±2·4	±0·17	±5·6
Controls				
	0·70 (7)	22·7	1·3 (8)	40·7
	±0·12	±2·9	±0·15	±5·1

* Number of observations in brackets.

Effects of porto-caval anastomosis on the stomach

All were carefully inspected for ulceration and other changes but none were found. The observation of ulceration by Doyle (1967) was therefore not confirmed in this series.

Effects of porto-caval anastomosis on the plasma ammonium nitrogen levels (Table IV)

The mean plasma NH_4-N level of blood taken by cardiac puncture from 25 normal rats (180–340 g.) was 67·8 μg./100 ml. (S.D. ± 19·4). The range of readings was 37·0–102·0 μg./100 ml. Figures higher than 110 μg./100 ml. (more than two standard deviation from the mean) were therefore taken as being abnormally high.

TABLE IV.—*Plasma NH_4-N Levels after Porto-caval Anastomosis Taken by Cardiac Puncture just before Killing*

Time after porto-caval shunt (weeks)	Number of animals	Mean Plasma NH_4-N (μg./100 ml.)	S.D.	Range
1	4	400	±113	270–544
2	2	468	—	442–493
3	4	372	±559	315–447
4	2	572	—	433–711†
5	5	463	±59·6	392–538
8	2*	649	—	603–695†
10	2	539	—	527–552
12	4*	269	±133·2	125–446
14	2*	465	—	450–480
16	2*	390	—	307–474
23–28	5	291	±46·4	241–341
33–38	10	223	±89·9	84–357
Sham operated controls	5	66	±27·8	40–101
Normal controls	25	67·8	±19·4	37–102

* Fed blood daily 7–14 post-operative weeks.
† Samples centrifuged for too long accounting for high estimation.

After sham porto-caval anastomosis (5 animals) when measured 1–5 weeks later the mean level was 66·0 μg./100 ml. (S.D. ± 27·8).

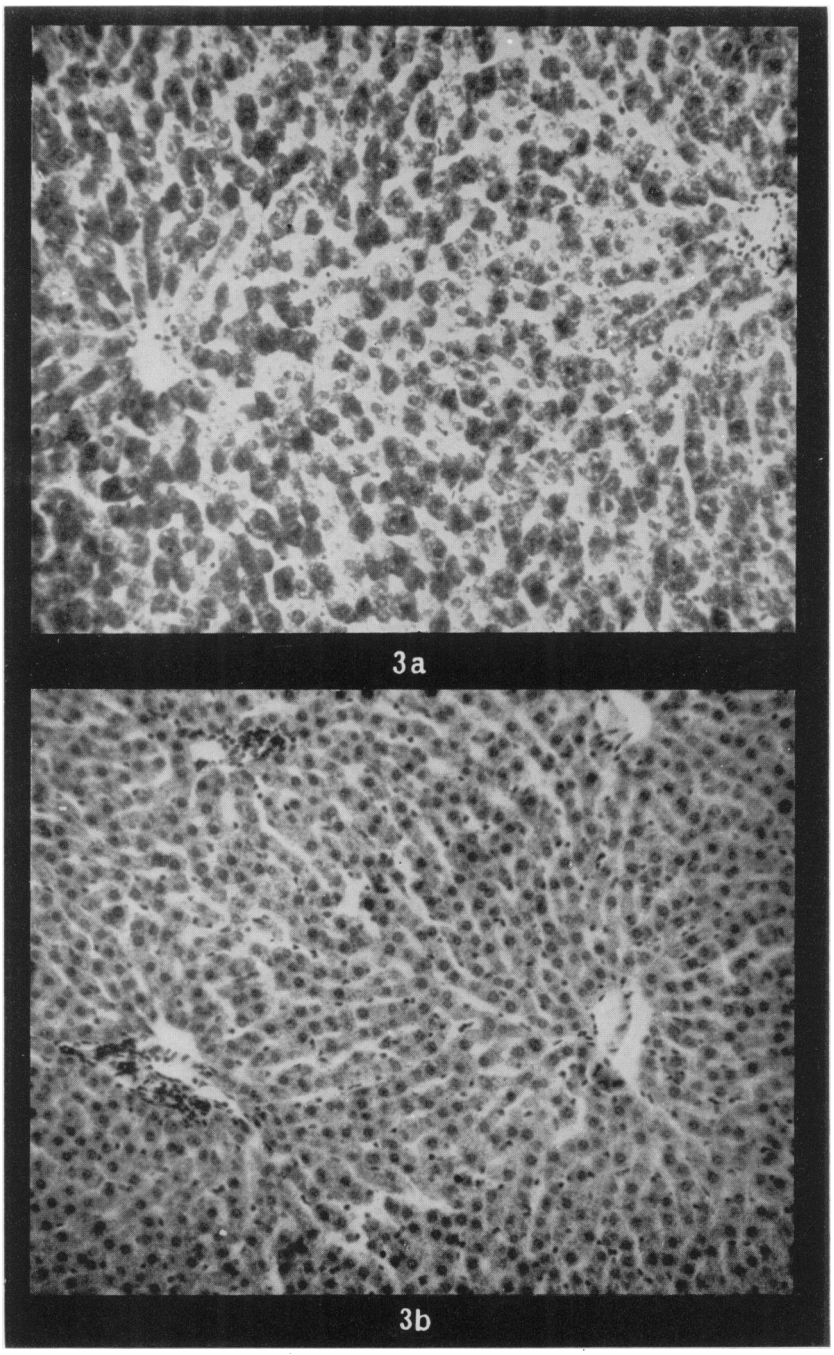
From the first week after operation the figures for porto-caval operated animals was markedly raised. This level of 4–8 times normal remained substantially unchanged until 10 weeks post operation after which time occasional animals showed lower figures. Lower figures occurred progressively from 23–28 weeks after operation. These changes tended to correlate with increasing body weight and the development of firm adhesions to the liver.

EXPLANATION OF PLATES

FIG. 3.—Liver from normal animal (a) and experimental animal (b) 4 weeks after operation stained for glycogen after fixation in 70 per cent alcohol. Best's Carmine. ×150.

FIG. 5.—Testicular tubules 5 weeks after operation showing absence of spermatozoa and spermatids. Spermatocytes show regressive changes. H. and E. (a) ×135. (b) ×335.

FIG. 6.—Testicular tubules 16 weeks after operation showing return of cellularity and spermatozoa. H. and E. (a) ×135. (b) ×335.



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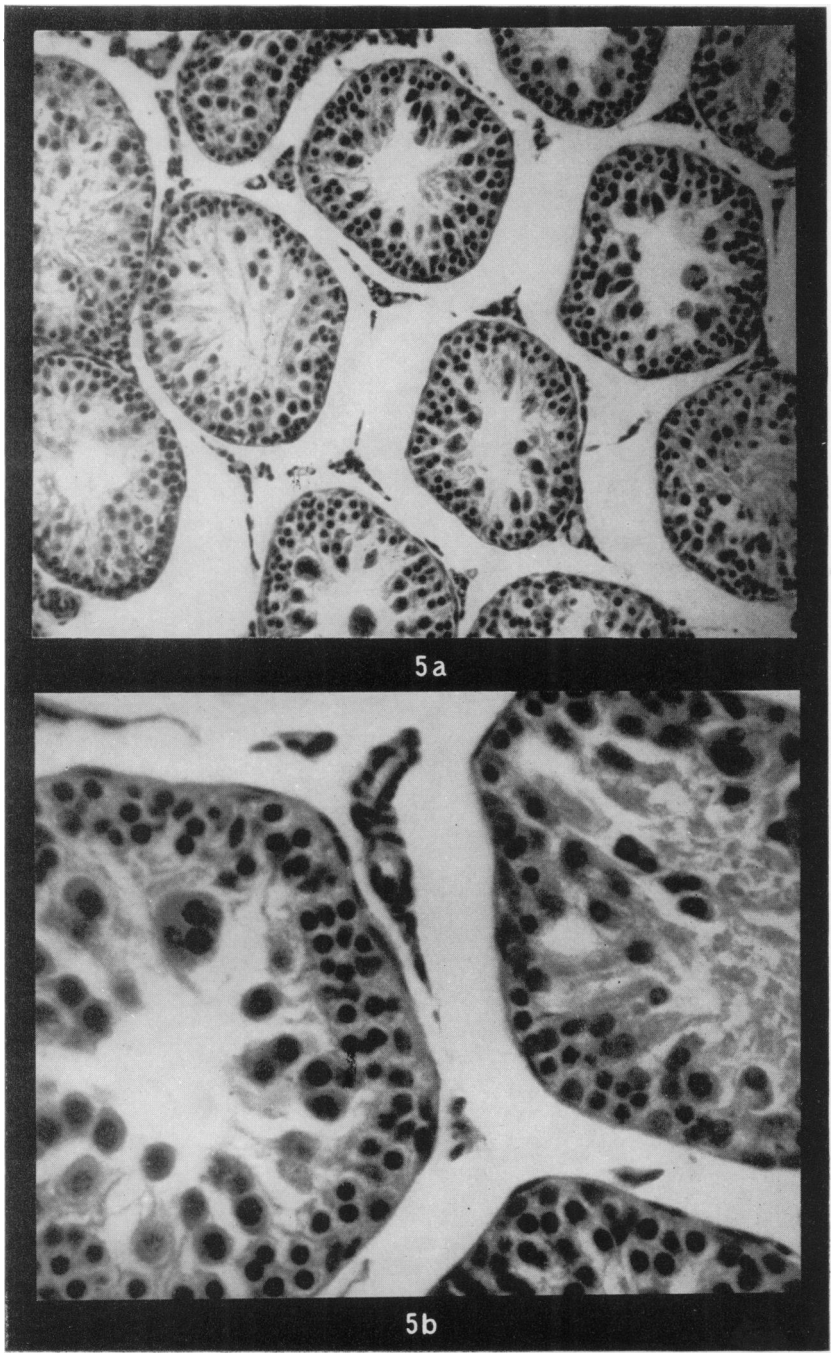

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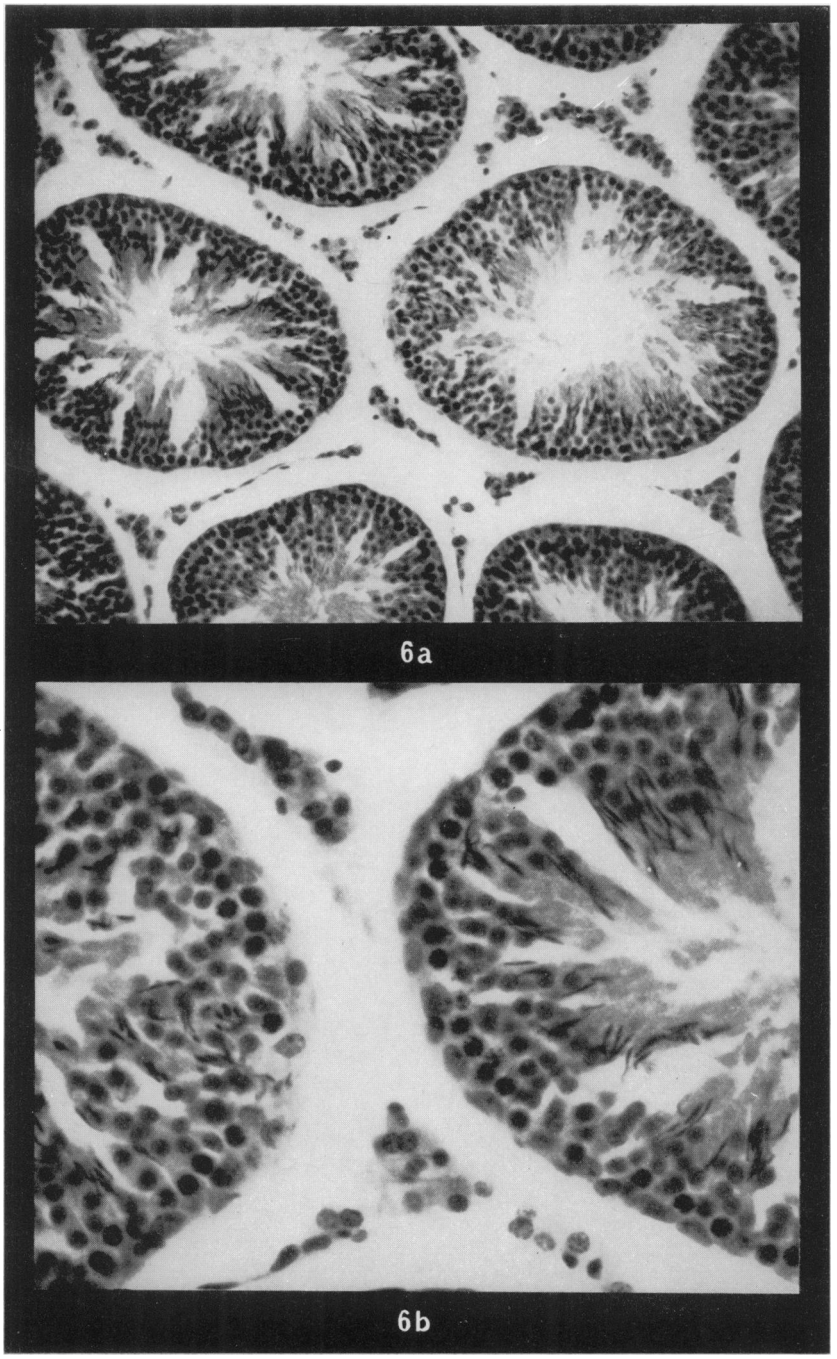

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Attempts to influence the plasma NH_4-N levels

With a high protein diet.—A number of animals (Table V) were fed, in addition to the normal diet, a commercial baby food " Farlene " that contains 25 per cent protein of animal and vegetable origin. They ate 4–8 g./day for 5 weeks after the operation. These were compared with controls fed 41B diet. No significant difference in the plasma NH_4-N levels between the 2 groups was found.

" Farlene " was also fed to animals from 23 weeks onwards after operation (Table V). There was a slight, but probably not significant, difference between those fed protein and those on a normal diet ($P < 0{\cdot}02 > 0{\cdot}01$).

TABLE V.—*Effects on Plasma Ammonium Nitrogen of Addition of " Farlene " Containing 25 per cent Protein*

Diet	Number of animals	Time to death (duration of diet) (weeks)	Mean plasma NH_4-N level (μg./100 ml.)	±S.D.
41B	3	5 (whole)	462·3	—
" Farlene " + 41B	4	1 (whole)	399·7	±113·0
" Farlene " + 41B	4	2–3 (whole)	439·2	±48·6
" Farlene " + 41B	11	4–5 (whole)	518·5	±142·3
" Farlene " + 41B	9	23–24 (last 5–10 weeks)	276·0	±58·3
41B	6	25–38 (whole)	168·5	±74·5

For tests of significance see text.

TABLE VI.—*To Show Lack of Effect of Feeding Blood on Plasma NH_4-N Level*

Diet	Number of animals	Time of killing after operation (weeks)	Mean plasma NH_4-N levels (μg./100 ml.)	±S.D.
Normal	7	5–14	485·1	±45·8
Normal + 5 ml. blood daily	9	8–16	375·8	±189·9

t Test $P < 0{\cdot}2 > 0{\cdot}1$.

TABLE VII.—*To Show Lowering Effect of Neomycin on the Plasma NH_4-N Level and its Reversal with Ammonium Acetate by Mouth*

Regimen	Number of animals	Time after operation (weeks)	Mean plasma NH_4-N level (μg./100 ml.) ±S.D.	t test
Neomycin (40 mg./rat/day)	3	3	220·3 ±56·6	($P < 0{\cdot}01 > 0{\cdot}001$)
Neomycin (40 mg./rat/day) + Ammonium acetate (40 mg./rat/day)	4	2–4	394·0 ±42·4	($P < 0{\cdot}01 > 0{\cdot}001$)

By feeding blood.—Discarded human blood was fed 5 ml. per animal each day from 0–6 weeks after operation for 7–14 weeks (Table VI). Again no significant effect was obtained ($P < 0{\cdot}2 > 0{\cdot}1$).

By antibiotic treatment.—The antibiotic Neomycin (Squibb) was given daily in

water to 3 animals by stomach tube (40 mg./day) for 4 weeks, beginning 1 week before the operation. The mean plasma NH_4-N levels (Table VII) after this regime was 220·3 μg./100 ml. which was about half the mean level in control animals. The difference is statistically significant ($P < 0{\cdot}01 > 0{\cdot}001$). This effect was successfully nullified by feeding another group of animals at the same time ammonium acetate (40 mg./rat/day) in their drinking water. The mean level of 4 animals was 394·0 μg./100 ml. which was not substantially different from animals without either antibiotic or ammonium acetate (Table VII).

DISCUSSION

Lee and Fisher's (1961) initial study of the effects of porto-caval anastomosis on the rat showed many features that we have confirmed in this present study. The initial weight loss occurs constantly, as was also found by Bismuth, Benhamou and Lataste (1963), but the rate of recovery from this is clearly from the present findings to some extent age dependent. It would appear also to be dependent probably upon the rate of formation of adhesions between the portal circulation and the liver. Bismuth *et al.* (1963) in one animal showed that a collateral circulation can be demonstrated by radiography, but whether this is true of every instance when the various parameters return towards normality is not certain. In general, in this series, where the plasma NH_4-N was low, the weight gain was satisfactory and the liver weights were increasing. In such cases the adhesions tended to be more dense and firm than where these features were less apparent. Indeed, we would like to believe that the level of the plasma NH_4-N is a good indirect evidence of the degree to which portal blood is finding its way back to the liver. The histological evidence tends to support this also. Histochemically, virtually all stainable glycogen disappears from the liver after porto-caval anastomosis. It can be found in later animals, however, but only in cells beneath the capsule, and around the superficial veins, where adhesions are present.

The importance of food consumption in the loss of body weight was evident in the present series. Lee and Fisher (1961) reported that at 14 weeks after operation, although their rats ate 18 g. of food per day (normal 17–22 g.) they were not at this stage gaining weight. Our sequential study shows that there is a marked loss of appetite during the early weeks (Table I) which returns towards normal at a time when the blood ammonia is tending to fall and the other parameters are also tending to pass towards normality, although usually not achieving it. Lee and Fisher's estimation of food consumption was done at a time when our animals were beginning to gain weight and the plasma NH_4-N was beginning to return to normal. Increased appetite may also therefore reflect return of portal blood to the liver through adhesions.

Of considerable importance to using this experimental model for studying the mechanism of cerebral complications was the difficulty which we experienced in raising the plasma NH_4-N higher than 400–500 μg./100 ml. By using the antibiotic, neomycin, we were able to reduce the plasma ammonium concentration to about half the expected level, and this could be reversed by additionally giving ammonium acetate in the drinking water. By feeding a high protein diet, or blood, daily, however, we were not able to raise the ammonium level still further, as might be expected from the work on human liver disease (Sherlock, Summerskill, White and Phear, 1954). Only in one animal, that appeared to be suffering from an incidental liver infection (? viral), was a figure of 1389 μg./100 ml. obtained

for the plasma NH_4-N, and this animal was in a stuporose state from which it could only briefly be roused by stimulation. It is possible that these differences in response between rat and man might be a reflection of the different bacterial flora in the colon of rats. Whatever the reason, we were not able intentionally to raise the circulating ammonium levels by the methods used, nor could we produce functional encephalopathy as was clearly achieved by Kline, Doberneck, Chun and Rutherford (1966) in dogs by porto-caval shunts. Changes in astrocyte nuclei, none the less, of both Type I and Type II (Alzheimer) were regularly produced in the brains of these rats (Cavanagh and Kyu, 1969; 1970*a* and *b*; Kyu, 1970).

The changes in the testis are of interest for they too show variations that parallel the other features discussed. The atrophy was much slower than the reduction in liver weight and the testicular weights continued to decline so long as reduction in cellular activity in the tubules was evident. The final pictures closely resembled those reported by Dordal, Glagov and Debarros (1967), but with the increase in body and liver weight there was a corresponding increase in testicular weight. This was accompanied by a return in spermatogenesis in some but not all tubules, and even 38 weeks after operation normal appearances had not been fully achieved. The lower the plasma ammonium level, in such long duration animals, the more complete the degree of tubular recovery suggesting, since it is generally understood that oestrogen inactivation is principally at fault in such circumstances, that recovery of all these parameters is linked to returning activity of liver cells.

We wish to thank Professor K. Weinbren of the Department of Pathology, Nottingham University, for teaching one of us (M.H.K.) the operative technique and Dr. J. C. B. Fenton of St. Bartholomew's Hospital, for his advice over the plasma ammonia techniques. Ma Hta Kyu gratefully acknowledges a scholarship from the Ministry of Education, Union of Burma, in association with the Colombo Plan. We wish to thank Mrs. Christine Fry for the histological preparations.

REFERENCES

Bismuth, H., Benhamou, J. P. and Lataste, J.—(1963) *Presse Med.*, **71**, 1859.
Bollman, J. L.—(1961) *Physiol. Rev.*, **41**, 607.
Cavanagh, J. B. and Kyu, Ma Hta—(1969) *Lancet*, ii, 620.—(1970*a*) *J. Neurol. Sci.*, in press.—(1970*b*) *J. Neurol. Sci.*, in press.
Dordal, E., Glagov, S. and Debarros, T. S.—(1967) *Archs Path.*, **83**, 49.
Doyle, D.—(1967) M.D. Thesis 'Portocaval Shunt in the Rat'. University of Edinburgh.
Fenton, J. C. B. and Williams, A. H.—(1968) *J. clin. Path.*, **21**, 14.
Fisher, B., Lee, S. H., Fisher, E. R. and Saffer, E.—(1962) *Surgery*, **52**, 88.
Flynn, P. J. and Kennan, A. L.—(1968) *Archs Path.*, **85**, 138.
Hahn, M., Massen, O., Nencki, M. and Pawlow, J.—(1893) *Arch. exp. Pathol. Pharmak.*, **32**, 161.
Kline, D. G., Doberneck, R. C., Chun, B. K. and Rutherford, R. B.—(1966) *Ann. Surg.*, **164**, 1003.
Kyu, Ma Hta—(1970) Ph.D. Thesis 'Reactions of Neuroglial Cells to Experimental Liver Disease'. University of London.
Lee, S. H. and Fisher, B.—(1961) *Surgery*, **50**, 668.
Sherlock, S., Summerskill, W. H. J., White, L. P. and Phear, E. A.—(1954) *Lancet*, ii 453.