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Hepatic Encephalopathy and Light and Electron Micrographic Changes of the Baboon Liver After Portal Diversion

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Six baboons of varying weights and estimated ages had complete portal diversion. All animals became emaciated and lost hair. Five of the 6 developed hepatic encephalopathy so serious that it either killed them or required their sacrifice after an average of 109 days. One exceptional animal which lived for 208 days without encephalopathy had markedly elevated blood ammonia levels. In one brain that was examined, greatly increased numbers of Alzeheimer's Type II astrocytes were diffusely distributed in the cerebral cortex. Changes in liver function tests were similar to those reported by many authors in dogs. The 6 baboons' livers underwent striking atrophy during the 49 to 208 days of postoperative observation. With some variations in degree, the same light and electron microscopic changes were observed that have now also been seen after completely diverting portacaval shunt in rats, dogs and humans. Thus the hepatic injury of Eck fistula is common to all species so far studied although most of the metabolic consequences of the procedure seem to selectively spare rats and man.

ALTHOUGH HUMANS with normal liver function seemingly tolerate portacaval shunts with no obvious adverse effects,[25,30] Eck's fistula in normal dogs regularly causes weight loss and hepatic encephalopathy, the latter complication having been termed "meat intoxication" by Hahn, Massen, Nencki and Pavlov,[8] who noted the harmful effect of meat powder ingestion in their animals. It has been widely believed that the devastating neurologic and other effects of Eck's fistula in dogs represent a species specific phenomenon. This belief has persisted in spite of descriptions of hepatic encephalopathy after portal diversion in monkeys[6,36] and of reports of inanition and inexplicable deaths in swine.[5,10]

In this study, the effects of complete portal diversion were studied in 6 baboons of varying ages. The clinical behavior of the animals and the morphologic changes in their livers were the main end points but, in addition, changes in serum lipids were examined. Within 7 months, 5 of the 6 baboons had died of hepatic encephalopathy and the sixth was well on the way to a similar fate. All had similar changes in their livers.

Methods

The features of the 6 male baboons are summarized in Table 1. They all had been retained in the University laboratories for about 1½ years and had been in perfect health during that time.

The portacaval shunts were performed under ketamine hydrochloride (Ketalar[R]) anesthesia. In brief, the technique consisted of side-to-side anastomosis after excision of an ellipse from both vessels. After completion of the anastomosis, the portal vein was ligated at its bifurcation into the right and left branches taking pains to eliminate any incoming venous tributaries to the portal branches above the site of their ligation. At the time of portal diversion, the liver was biopsied for comparison with a sample obtained at autopsy or at the time of sacrifice.

Submitted for publication February 7, 1976.

The work was supported by research grants MRIS 8118-01 and 7227-01 from the Veterans Administration; by Grant Numbers AM-17260 and AM-07772 from the National Institutes of Health; and by Grant Numbers RR-00051 and RR-00069 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.

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TABLE 1. *Clinical Features of Six Baboons Submitted to Eck's Fistula*

No.	Survival, (Days)	Body Weight (kg)		Weight Loss (%)	Liver Weight at Autopsy		Encephalopathy†
		Preop	Postop		Total (gm)	Per Cent Body Weight	
1	Died, 103	9.5	7.0	26%	—	—	Yes
2	Sacrificed*, 49	11.3	8.6	24%	—	—	Yes
3	Died, 202	10.9	7.7	29%	98	1.3%	Yes
4	Sacrificed, 208	11.0	7.4	33%	127	1.7%	No
5	Sacrificed*, 130	13.6	9.0	34%	110	1.2%	Yes
6	Sacrificed*, 164	8.6	6.3	27%	77	1.2%	Yes

* Sacrificed in a terminal state.
† *All* baboons had alopecia, muscle wasting, and ascites.

Postoperatively, the animals were given the same diet as that on which they had been maintained for the previous 18 months. It consisted of Purina[R] monkey chow (15% protein and 5% fat), supplemented daily with fresh fruit ad libitum. At approximately biweekly intervals before and after portacaval shunt, the animals were anesthetized with ketamine hydrochloride and peripheral venous blood samples obtained. In addition to standard liver chemistries, the serum cholesterol and other lipid concentrations were analyzed by previously described methods.[27]

Tissue specimens from biopsy or autopsy were fixed in 10% neutral buffered Formalin[R] (aqueous solution of formaldehyde). Frozen sections were cut and stained with Sudan 4 for fat, and then the remaining tissue was processed and the paraffin sections were stained with hematoxylin and eosin, Gordon and Sweet's trichrome for collagen and fibrin, periodic acid Schiff reaction for glycogen, and Pearse's method for ceroid and lipofuscin. Brain tissue was also stained with cresyl violet.

Additional small tissue samples were initially fixed in glutaraldehyde solution, then postfixed in osmic acid and embedded in Epon[R] (synthetic embedding medium). Half micron and ultrathin sections were cut. The former were stained with Azure II for examination in the light microscope, while the latter were stained with lead citrate and examined in the electron microscope.

The sizes of the midzonal hepatocytes before and after portacaval shunt were determined on hematoxylin and eosin stained sections by a method previously described.[26] In essence, the technique consists of tracing large numbers of hepatocytes on standard thickness paper, cutting out the silhouettes and weighting them. Midzonal hepatocytes identified in half micron Epon sections were also used for measuring the length of rough endoplasmic reticulum per area of cytoplasm by a morphometric method.[16]

Results

Clinical Observations

Postoperatively, all of the animals were initially healthy, but within a month they had lost weight and had developed alopecia, muscle wasting and variable ascites (Table 1). All but one of the baboons eventually developed disequilibrium and convulsive movements, which ranged from a kind of "liver flap" to generalized seizures and unconsciousness, unrelieved by the intravenous administration of dextrose. Two of the animals died overnight, three more were sacrificed in a terminal state and the sixth, which did not have overt encephalopathy after 208 days, was sacrificed electively. The average survival of

TABLE 2. *Changes in Serum Chemistries in Six Baboons Submitted to Eck's Fistula**

No.	SGOT (Sigma-Frankel Units) Normal <40		SGPT (Sigma-Frankel Units) Normal <25		Alkaline Phosphatase (Bodansky Units) Normal <10		Postoperative Blood Ammonia Levels (μg/100 ml) Normal <50	Serum Cholesterol (mg/100 ml) Normal 120 to 180	
	Preop	Postop†	Preop	Postop†	Preop	Postop†		Preop	Postop
1	7	320	—	385	—	14.2	63 (42 days)	134 →	38
2	—	216	16	260	4.4	14.7	66 (49 days)	171 →	51
3	22	123	10	123	4.5	18.6	—	130 →	40
4	21	66	25	92	7.2	13.5	443 (208 days)	148 →	63
5	21	630	6	400	3.4	15.5	—	148 →	49
6	7	92	4	71	4.7	14.6	500 (164 days)	152 →	29

* Serum bilirubin concentrations were always normal.
† The postoperative values given represent the maximum abnormalities recorded.

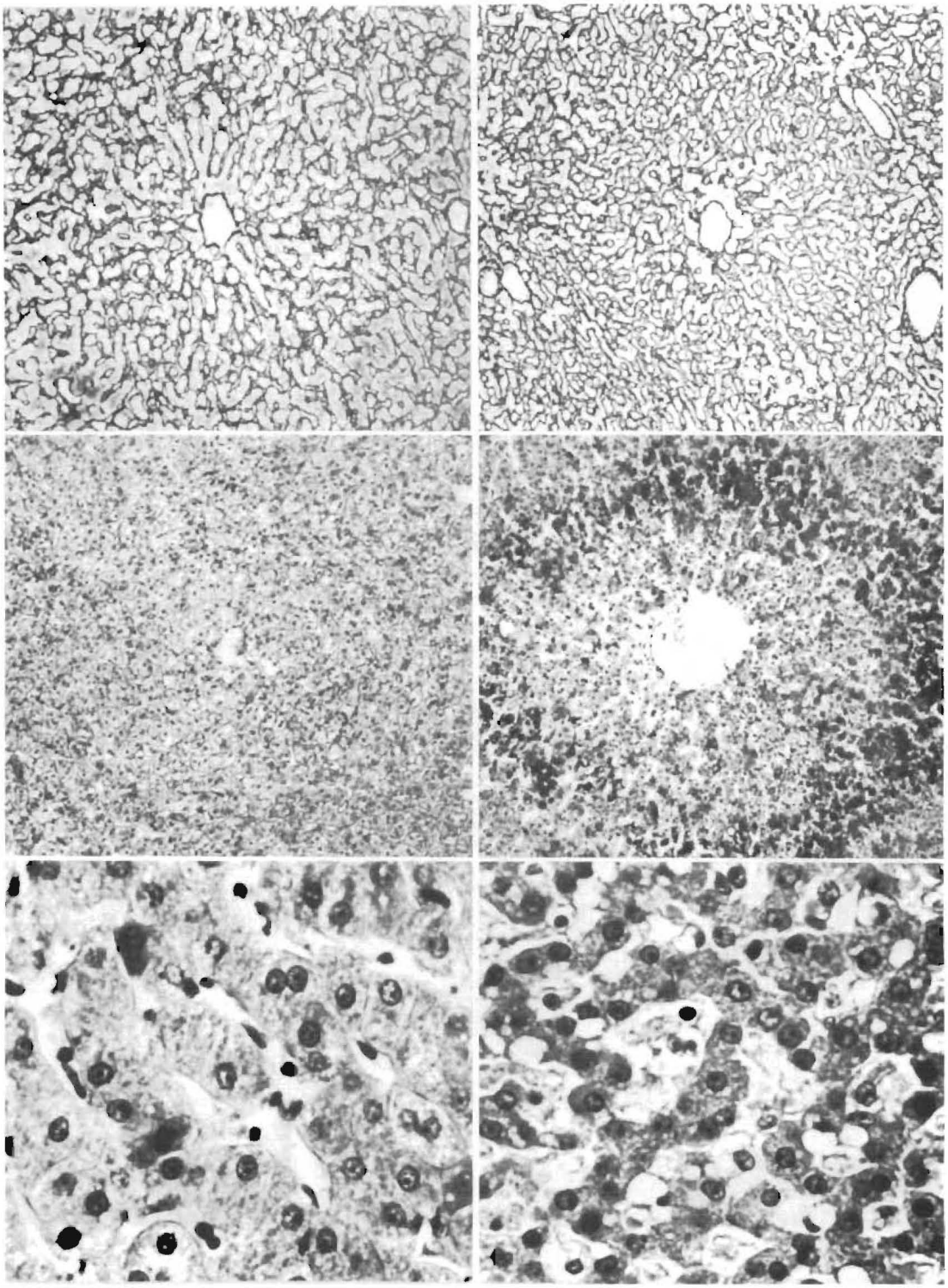

FIG. 1. Photomicrographs of sections from a baboon liver subjected to portal diversion. The panels on the left show the liver structure as seen in a biopsy immediately before portacaval anastomosis. The panels on the right show the condensation of lobular reticulin, accumulation of fat and atrophy of hepatocytes 208 days after operation. (Top) reticulin stain, ×20; (middle), frozen section stained with Sudan, 4, ×30; (Bottom), and E, ×175.

the 5 animals which had encephalopathy causing death or requiring sacrifice (Table 1) was 109.3 days.

Biochemical Studies

After portacaval shunt, the serum bilirubin concentrations remained normal throughout the period of observation in all 6 baboons. However, there were significant elevations of the SGOT, SGPT and alkaline phosphatase in all animals (Table 2).

In 4 baboons blood ammonia levels were obtained 42 to 208 days postoperatively. The results ranged from 63 to 500 μg/100 ml with a normal range of 20 to 50 μg/100 (Table

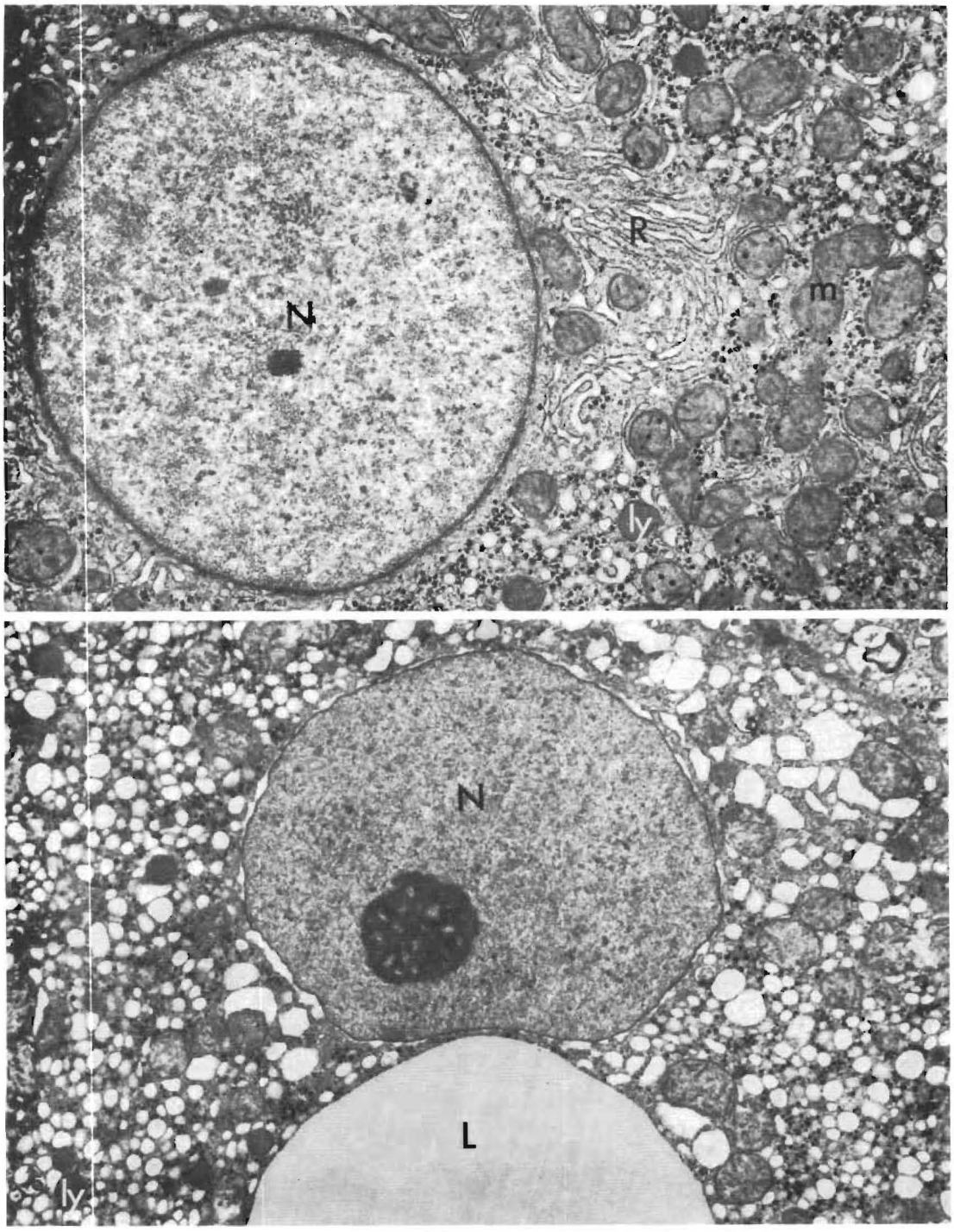

FIG. 2. (Top). Ultrastructure of a hepatocyte from the liver of baboon Number 4 immediately before portacaval shunt. The cytoplasm contains abundant rough endoplasmic reticulum (R) with normal cisternae and membranes studded with ribosomes. Glycogen granules are abundant. There are no fat droplets and few lysosomes (ly). m, mitochondrion; N, nucleus. Lead stain (×11,400). (bottom) Ultrastructure of the same liver 208 days after portacaval shunt. The cisternae of the rough endoplasmic reticulum are dilated and the membranes depleted of ribosomes. The amount of smooth endo plasmic reticulum is increased. There is a large lipid droplet (L) and lysosomes (ly) are numerous. N, nucleus. Lead stain (×11,400).

2). A serum ammonia concentration of 443 μg/100 ml was recorded on the day of sacrifice in the only animal which did not have obvious hepatic encephalopathy, indicating that even this baboon was near the end of survival.

The changes in serum cholesterol concentrations in these 6 animals are summarized in Table 2. In addition to substantial falls in the serum cholesterol values, the phospholipid concentrations were also depressed postoperatively, as previously reported by us[27] in dogs and baboons, and as originally recorded by Winter[35] in dogs. There were only minor changes in the triglycerides.

Histopathologic Studies

The biopsy specimens obtained before portacaval shunt were normal by light microscopy (Fig. 1). Ultrastructurally, hepatocytes sectioned through a central area showed many complexes of rough endoplasmic reticulum (Fig. 2,

TABLE 3. *The Effect of Eck Fistula on Liver Cell Size**

No.	Hepatocyte Size	
	Biopsy	Sacrifice or Death
1	0.29888	0.11716
2	0.34494	0.12352
3	0.34170	0.13707
4	0.55200	0.19705
5	0.30650	0.13489
6	0.43945	0.30683
Mean	0.381	0.169

* See text for definition of size units.

top). Each complex consisted of many parallel cisternae of ribosome-studded membranes. Free polyribosomes were also present in the cytoplasm. Glycogen was abundant.

After portacaval shunt the liver lobules and hepatocytes became smaller (Fig. 1). The mean hepatocyte size at operation was 0.381 size units, and only 0.169 size units when the animal was sacrificed or died. Thus, the average hepatocyte size had been more than halved (Table 3). The amount of stainable glycogen in the liver cell cytoplasm fell and stainable lipid appeared. The Kupffer cells, particularly in the central parts of the lobules, increased in size and number after the operation and their cytoplasm contained ceroid but not hemosiderin. There were more binucleate and trinucleate hepatocytes and hepatocyte mitoses after portacaval shunt, and in the half micron sections the hepatocytes showed variable staining properties, producing an irregular mixture of light and dark cells. The dark cells were smaller than the light ones and more numerous. The lobular reticulin framework was condensed and in some of the livers the sinusoids appeared collapsed.

Ultrastructurally, the cytoplasm of many of the hepatocytes after portacaval shunt contained lipid droplets of different sizes and glycogen granules were scanty (Fig. 2, Bottom, and Fig. 3). The amount of rough endoplasmic reticulum was greatly reduced and what remained was dilated and fragmented. Morphometric analysis showed that the area of rough endoplasmic reticulum per volume of cytoplasm was reduced to less than one third the quantity found in the preoperative biopsy. There were fewer ribosomes on the membranes of the endoplasmic reticulum than normal. The amount of smooth endoplasmic reticulum was increased. Free ribosomes were relatively abundant. The Golgi apparatus was poorly developed. Some of the mitochondria were enlarged and their cristae disrupted. Small bundles of collagen fibrils were present in the space of Disse and around central veins. The enlarged Kupffer cells contained masses of lipid, lipofuscin and ceroid in their cytoplasm. The bile canaliculi appeared normal.

In one animal (Number 4) brain tissue was studied. This showed greatly increased numbers of enlarged protoplasmic astrocytes diffusely distributed in the cerebral cortex. The majority of the abnormal astrocytes were Alzheimer's Type II cells. A few nerve cells were damaged or destroyed but most appeared normal.

Discussion

Portacaval shunts in four *Macaca mulatta* monkeys were reported by Zuidema et al.[36] as part of an experiment

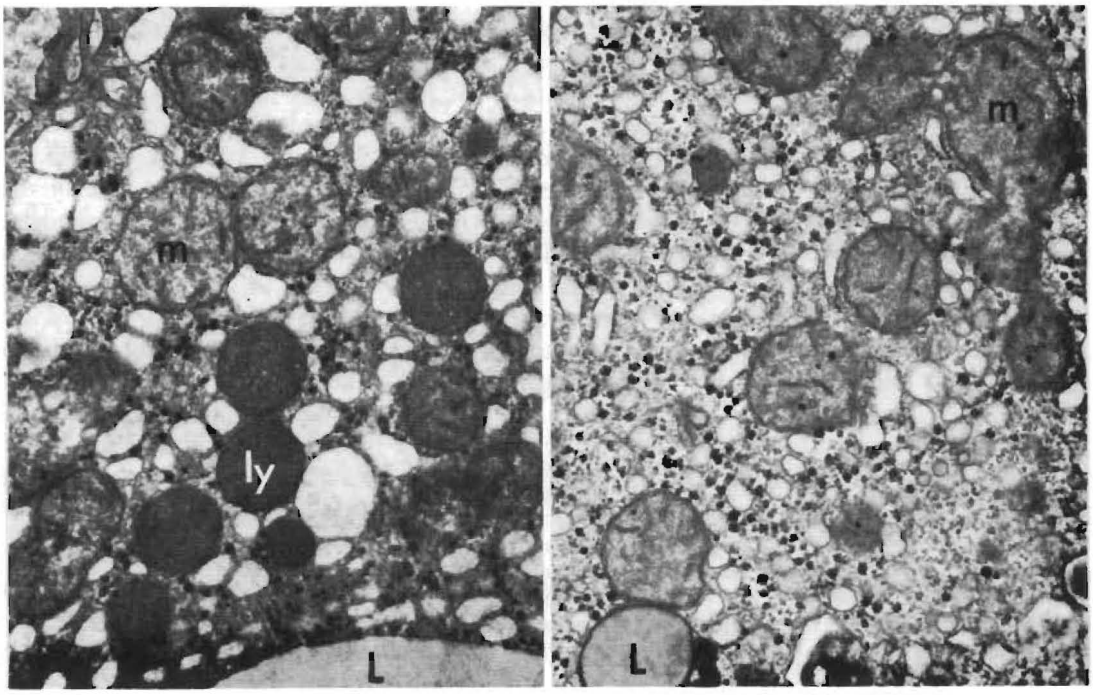

FIG. 3. Higher power views of part of hepatocyte shown in Fig. 2, (Bottom). The cisternae of rough endoplasmic reticulum are dilated and the membranes lack ribosomes. The smooth endoplasmic reticulum is increased in amount. Lipid droplets (L) of various sizes are present. Lysosomes (ly) are numerous. Some of the mitochondria (m) are enlarged and their internal structure disorganized. Lead stain (×17,100).

in which blood was administered by gavage a few days later. The observations concerning the response to enteral blood were short term, but in the Discussion, the authors remarked that the animals died 4 to 6 months later with loss of hair, weight loss and neurologic disability. Detrie[6] noted the same syndrome in Rhesus monkeys with some amelioration by total colectomy. Kline, Crook and Nance[11] followed 10 *Macaca malatta* monkeys from 332 to 345 days. Only one had evidence of neurologic findings, but elevations in serum blood ammonia were the rule. At autopsy, the atrocyte swelling and proliferation were present which Nance and Kline[19] have correlated with encephalopathy in other work. No studies of liver pathology were mentioned in any of these articles.

The baboons of our study developed an unequivocal portoprival syndrome, rivaling that in the dog for its swift and lethal qualities. The animals lost hair and became wasted, with an average weight loss of about 30% of their body weight. All but one were neurologically invalided or dead within 6 months.

Histopathologically, the livers showed severe hepatocyte atrophy with accumulation of fat in the liver cell cytoplasm and loss of glycogen. The number of hepatocytes in mitosis were increased. Severe ultrastructural changes including depletion of rough endoplasmic reticulum occurred that closely resembled those we reported earlier in two baboons that had total portal diversion in two stages[27]; the only difference was the presence of increased amounts of smooth endoplasmic reticulum in the present group of animals.

It may be useful to compare the pathological findings caused in baboons by Eck fistula with those observed in other species. Shrinkage of the liver occurs in all species that have been studied. The hepatocyte atrophy and tendency to fat accumulation has been seen by light microscopy in rats,[9,15] dogs[2,8,14,18,23,24]* and pigs,[5] although the changes are probably less marked in rats.[1,13] It is noteworthy that ultrastructural changes caused in rats[7,20,22,33] and in dogs[17,27] closely resemble those reported here in the baboon. Furthermore, both the light and electron microscopic findings in all the species are analogous to those in the human.[25,30] Thus the hepatic injury of Eck fistula afflicts all species so far examined.

Interpretation of the alterations that occur in the hepatocyte ultrastructure after portacaval shunt is hampered by the vulnerability of the various organelles, in particular the endoplasmic reticulum, to a variety of humoral and hormonal stimuli.[24,31] However, it is undoubtedly relevant that the liver changes after portacaval shunt are remarkably similar to those that occur in the liver of the rat made diabetic by the administration of alloxan.[21] The abnormalities in the diabetic rat liver are rapidly reversed when the animal is treated with insulin. Moreover, we have found that after Eck fistula in dogs constant insulin infusion into the tied off portal vein at the liver hilum can prevent much of the damage to the canine liver that occurs within a few days without this treatment.[29] This and other recent evidence from our laboratories suggest that the basic cause of the liver injury after portacaval shunt is its deprivation of endogenous hormones which ordinarily reach it in high physiologic concentrations from the splanchnic viscera.[26–28] The most important of these so-called hepatotrophic hormones in the splanchnic blood seems to be insulin.

The foregoing hormonal hypothesis would explain the uniformity of the hepatic changes caused by Eck fistula in different species. At the same time, it is evident that a spectrum of susceptibility to the adverse effects of Eck fistula does exist. The rat has been resistant to all of the lethal complications of Eck fistula and most of the minor ones except for a temporary failure to gain weight. Normal man has aligned himself, as he so often does, with the rat.[25,30] On the other hand, monkeys and baboons are placed at great risk by the procedure of portacaval shunt, and the same is probably also true for pigs.

The most frequently lethal complication of Eck fistula is encephalopathy. The manner in which these liver changes alter brain function is obscure. In the brains of patients and animals with portacaval shunts the protoplasmic astrocytes are enlarged and increased in numbers in the grey matter of the cerebrum and cerebellum and in the putamen and globus pallidus.[12,32] The morphological changes in the astrocytes are probably an expression of metabolic hyperactivity and their severity is in proportion to the duration of the post-operative rise in plasma ammonium levels.[3,4] It is possible that the changes in the astrocytes are directly contributed to by the prolonged high levels of circulating ammonia. The astrocytes may be the cells in which ammonia ions derived from neuronal metabolism are incorporated into alpha-ketoglutarate and glutamic acid to form glutamine, and the astrocyte changes after portacaval shunt could be the result of storage of the excess glutamine which is known to be present in the brain.[3] However, there is little reason to believe that complex neurologic disorders caused by portal diversion can be explained solely by hyperammonemia.

* The article by Hahn, Massen, Nencki and Pavlov[8] contains the first, albeit brief, description of the atrophy and fatty changes in the liver caused by Eck fistula. Whipple and Sperry published the first photomicrograph of these findings. Their animals with portacaval shunt were two control dogs that were part of a larger experiment.[34] In Fig. 6 of that article are to be found all the light microscopic abnormalities that continue to occupy us 67 years later. Dr. Whipple died on January 31, 1976, at the age of 97 years.

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