Eck Fistula Encephalopathy:

Long-Term Studies in Primates

DAVID G. KLINE, M.D., JOHN N. CROOK, M.D.,
FRANCIS C. NANCE, M.D.

From the Department of Surgery/Neurosurgery, Louisiana State University School of Medicine, New Orleans, Louisiana, and Delta Regional Primate Center, Covington, Louisiana

PATIENTS with encephalopathy as a result of severe hepatic disease or following portacaval shunt for cirrhosis and esophageal varices have characteristic neurologic findings including episodic stupor, "liver flap," asterixis, and extrapyramidal findings such as tremors, rigidity, and dystonic movements.[1, 3, 7] Neurologic symptoms in man have been associated with cerebral morphologic changes. These changes consist of a proliferation of abnormal large astrocytes called Altzheimer cells. Adams and others feel that the presence of these cells is the hallmark of cerebral dysfunction due to hepatic disease.[1, 8] Many neurologic findings in patients with hepatic encephalopathy have also been observed in dogs with portacaval shunts.[2] Indeed, the majority of dogs that have large portacaval shunts die from encephalopathy or intercurrent infection. Cerebral morphologic changes in the dog are also similar, in most respects, to those observed in the human with hepatic encephalopathy and the severity of the changes can be related to either the completeness of the shunt or the gastrointestinal protein load or both.[6]

There have been no reports of neurologic changes or cerebral histologic changes following portacaval shunts in primates. As a result, biochemical and neuropathologic studies were undertaken to see if symptoms and morphologic findings of encephalopathy could be reproduced in the monkey which phylogenetically is more closely related to man than is the dog.

Several short-term studies in monkeys with portacaval shunts have been previously reported. In one, monkeys given blood by gavage several days after a shunt responded with hyperammonemia in a fashion similar to that of dogs.[10] A companion study compared end-to-side with side-to-side shunts in a small group of primates after blood gavage. Serum ammonia was found to clear more rapidly in animals with side-to-side shunts than in those with end-to-side shunts.[11] Colectomy in primates with portacaval shunts has been reported to decrease the rise in blood ammonia after blood gavage.[5] Experiments were short-term and no report of survival or of neurologic findings was given. Despite the apparent similarities in biochemical behavior between dog and primate suggested by these reports, there have been no long-term studies tracing the course of primates with portacaval shunt.

Method

Seventeen maccaca mulatta monkeys were given a normal protein diet (22%) and followed for 3 weeks to insure good health and normal neurologic function. Baseline serum ammonia and other biochemical determinations including serum

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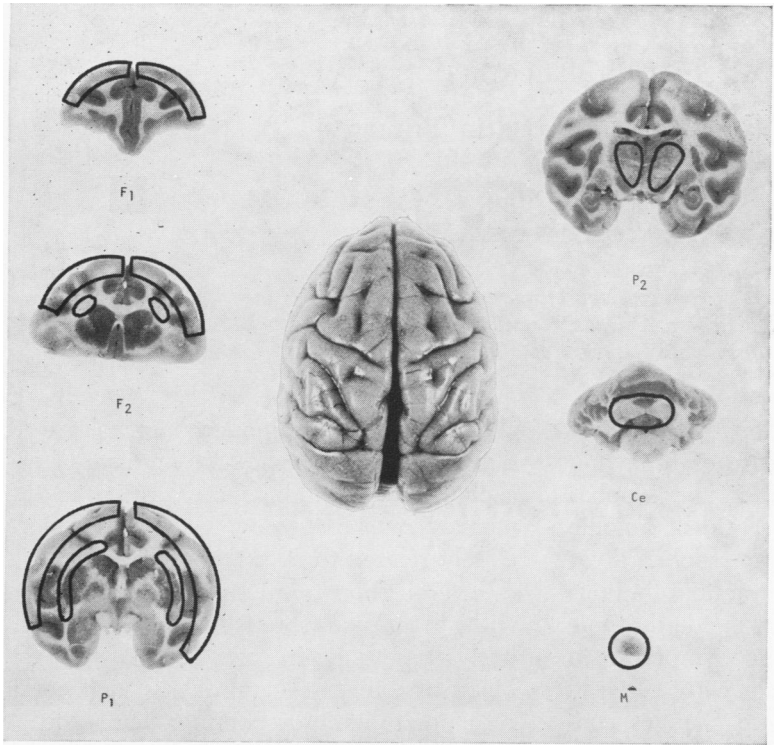

FIG. 1. Perfused and fixed macacca mulatta brain in the center flanked by serial slices. Circled areas are those usually counted for abnormal astrocytes or Altzheimer cells. Fl = frontal (cortical), F2 = posterior frontal (cortical and medullary), Pl = parietal (cortical and medullary), P2 = posterior parietal (basal ganglia), Ce = cerebral and M = medulla.

proteins, bilirubin, alkaline phosphatase, and SGOT were determined. End-to-side portacaval shunts were constructed in 14 monkeys while three monkeys were not operated upon and served as controls. Operations were performed under intravenous pentobarbital anesthesia. Every attempt was made to keep blood loss minimal and even small losses were replaced by lactated Ringer's solution. The entire portal venous return was shunted into the vena cava by constructing the shunt after the entrance of the last tributary into the portal vein. Special care was taken to make the stoma of the shunt as generous as possible. Animals did not receive antibiotic drugs postoperatively. Four animals succumbed within 48 hours of operation due either to blood loss or to technical errors in construction of the shunt or both. The ten animals surviving portacaval shunt were continued on a normal protein diet (22%) for 3 months. Five animals were then given a high-protein diet (42%), while the remaining five animals were maintained on the normal diet. Periodic neurologic observations for lethargy, irritability, tremors, abnormal posturing, and convulsive behavior were made. Animals were weighed and periodic biochemical determinations were also obtained. Animals operated upon were observed for approximately a year after shunt and sacrificed. Control animals not operated upon were also sacrificed after a year. After making terminal observations, all monkeys were anesthetized using intravenous Nembutal and sacrificed by perfusion with 10% buffered formalin. Each portacaval shunt was examined for patency and stomal size was measured. The porta hepatis was examined carefully to rule out collateral circulation. The brain was then removed and suspended in formalin by means of a sling suture placed beneath the basilar artery. The liver, spleen, and kidney were biopsied.

After a 10-day period of fixation, the brain was sectioned at multiple levels in-

TABLE 1. *Primate Portacaval Shunts*

Animal	Survival	Symptoms	% Wgt. Change	Average Astrocytes (per 350 μ field)	PreOp. NH3	Interim Peak NH3	Terminal NH3	Shunt Status
				Normal Protein Diet				
K 10	424 days	0	−21	7.1 ± 3.3	300 μg./100 ml.	983 μg./100 ml.	432 μg./100 ml.	open
K 20	336	0	−40	8.8 ± 1.5	343	864	455	open
K 17	322	*	−18	4.4 ± 1.0	311	1,150	406	open
K 13	384	0	+10	4.1 ± 2.1	240	1,060 to 300	337	closed
K 14	383	0	+15	2.5 ± 0.8	210	711 to 300	216	closed
				High Protein Diet				
K 2	435 days	0	−17	12.8 ± 2.6	207 μg./100 ml.	894 μg./100 ml.	595 μg./100 ml.	open
K 6	408	0	−28	12.4 ± 4.2	154	592	343	open
K 12	385	0	−30	6.1 ± 1.0	294	975	412	open
K 3	338	**	−15	9.3 ± 1.4	300	1,080	200	open
K 9	332	0	−21	10.1 ± 1.4	300	1,104	304	open
				Control				
K 19	—	0	+6	1.2 ± 0.4	—	200 μg.%	242 μg./100 ml.	no shunt
K 21	—	0	+10	1.4 ± 0.4	—	250	226	no shunt
K 23	—	0	+10	1.0 ± 0.3	—	196	200	no shunt

* — ? had convulsions.
** = Lethargy, irritability, convulsions.
Liver function studies remained normal in all primates.

cluding frontal, parietal, basal ganglia, cerebellar, medullary, and cervical spinal cord levels. After paraffin embedding, multiple sections at each level were stained with H and E and Nissl technics. Each of the eight brain levels were counted for abnormal astrocytes or Altzheimer cells using a randomized technic which has been described previously.[6] Ten random fields of 350 microns in diameter were counted in each section making a total of 80 fields in each animal (Fig. 1). Altzheimer cells are larger than normal astrocytes and have pale cytoplasm, fine granular chromatin, and sometimes an irregular form. Such cells are frequently grouped together suggesting recent division.

Liver, spleen, and kidney biopsies were embedded in paraffin, sectioned and stained by the H and E technic. Neurologic findings, weight change, and biochemical determinations were then correlated with the cerebral pathologic changes.

The 42% protein diet, which was fed to five of the shunted monkeys, was composed of blood meal (60% protein) with normal monkey chow (22% protein). Water was added and the mixture was frozen so that it could be fed to the monkeys in the form of pellets.

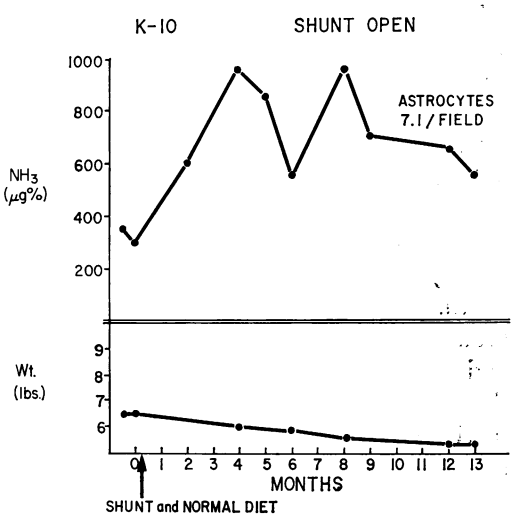

FIG. 2. Course of a primate with portacaval shunt fed a normal diet. Shunt was open at the time of sacrifice. Although animal lost 21% of preoperative weight, there were no neurologic findings, and animal remained alive until sacrificed 424 days postoperatively. Postoperative serum ammonia values remained elevated and the averaged abnormal cerebral astrocyte count was 7.1 per high powered field.

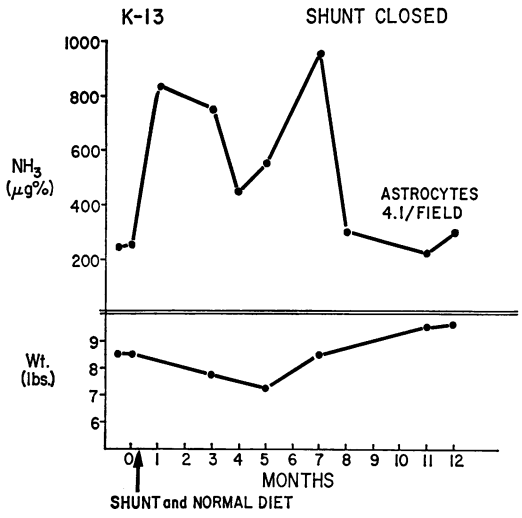

FIG. 3. Course of a primate with a portacaval shunt fed a normal protein diet. Shunt was found to be closed at the time of sacrifice. Animal lost weight for several months postoperatively and then began to gain weight. Serum ammonia values subsequently decreased and this change probably corresponded with complete closure of the shunt. A relatively low but not normal astrocyte count was found.

Results

Table 1 summarizes the long-term course of monkeys on normal protein diets, monkeys on high-protein diets, and control monkeys. Although monkeys with portacaval shunts lost weight, they survived until sacrifice 332 to 435 days following operation. One monkey (K-20) which died prior to sacrifice had a large weight loss (−40%). No other cause was found for death. One animal in the high protein fed group (K-3) had some lethargy, irritability, and one questionable convulsion. Despite these symptoms, this animal survived until sacrifice 338 days postoperatively. The remainder of the animals behaved in a normal fashion and did not develop neurologic symptoms.

Abnormal astrocyte or Altzheimer cell counts averaged 6.8 ± 1.2 cells in three monkeys fed normal diets in which shunts remained open. Serum ammonia values remained extremely elevated throughout the course of these animals (Fig. 2) but serum bilirubin, alkaline phosphatase, and SGOT remained normal. Two monkeys maintained on normal diets were found to have closed shunts a year after operation. Astrocyte counts in these animals were relatively low (3.3 ± 0.8). These two animals had weight losses for 6 months and then, began to gain weight (Fig. 3). Serial serum ammonia values changed also midway through the courses of these animals. Both biochemical and weight changes corresponded with spontaneous closure of the portacaval shunts. Thus, serum ammonia levels of monkey K-13 dropped from an interim peak value of 1,000 μg.% to 300 μg./100 ml. while monkey K-14's values dropped from 771 to 300. Abnormal cerebral astrocyte counts averaged 4.1 and 2.5, respectively, and these values were much lower than those in animals in which shunts remained open. The low cerebral astrocyte counts correlated well with the clinical and biochemical courses of these animals. These findings were in contrast to those in primates in which shunts stayed open

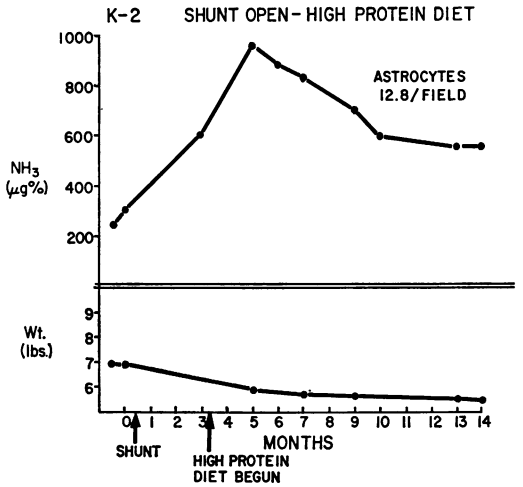

FIG. 4. Course of a primate with portacaval shunt fed a high protein diet. Shunt remained open, the animal lost weight, serum ammonia values remained high, and the abnormal astrocyte count was high at sacrifice.

where ammonia values tended to remain elevated, animals continued to lose weight, and cerebral astrocyte counts were elevated.

The five shunted monkeys on high-protein diets lost weight but had minimal or no symptoms despite consistently elevated serum ammonia values and elevated abnormal astrocyte counts at the time of sacrifice (10.2 ± 1.9). Figure 4 portrays the usual course of such a shunted monkey.

Liver function studies including bilirubin, SGOT, and alkaline phosphatase remained normal in all animals. Serum protein values and A/G ratios did not alter and liver, spleen, and kidney biopsies showed no abnormalities. The splenic veins of three animals in which shunts stayed open were injected with contrast material at the time of sacrifice. Venous collaterals led into the vena cava both above and below the liver but not into the liver. Control nonshunted animals had normal liver function and serum ammonia values and low cerebral astrocyte counts (1.2 ± 0.4) at the time of sacrifice.

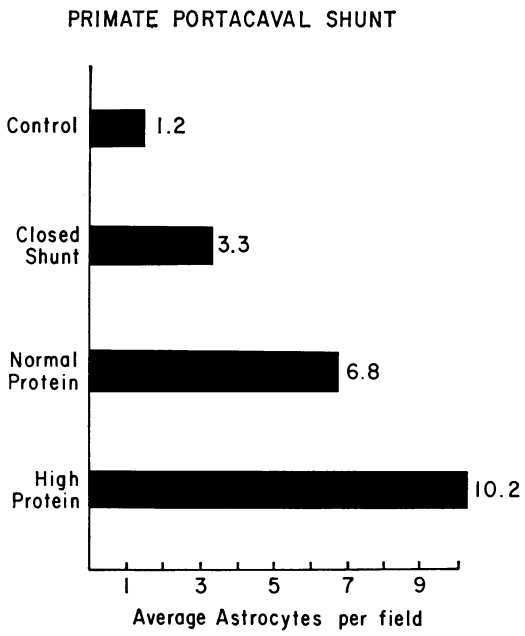

FIG. 5. Summary of averaged abnormal astrocyte counts in three control primates, in two primates fed a normal protein diet whose shunts closed midway through their course, in three primates fed a normal protein diet whose shunt remained open, and in five primates fed a high protein diet whose shunt remained open.

Discussion

These studies demonstrate, for the first time, that a monkey with a portacaval shunt can survive for a long period. With one exception, ten animals with shunts remained alive until sacrifice. Shunted monkeys survived even when placed on high-protein diets. Such prolonged survival, especially in the face of a high-protein gastrointestinal load, represents a distinct species difference between dog and monkey. The majority of dogs with portacaval shunts succumb either with signs of encephalopathy or intercurrent infection some weeks to months following shunt. A high-protein diet increases the severity of the encephalopathy and accelerates the dog's death.

All monkeys in which shunts remained open had elevated serum ammonia values throughout their courses. Despite hyperammonemia, primates had no neurologic findings and only suffered from weight loss. Altzheimer cells were present in the brains of monkeys on normal protein diets as well as on high-protein diets but average astrocyte counts were relatively low in monkeys fed normal protein diets. Only when shunted monkeys were fed high-protein diets did astrocyte counts approach those reported in dogs with Eck fistulas.

Species differences between the dog and primate hepatic circulation have been known for years.[8] The hepatic veins of dogs have smooth muscle bundles which can contract, constricting the venous outflow from the liver, whereas primates do not have this anatomic arrangement. In addition, operative attempts to produce esophageal varices in the dog produce lesions which lie in the outer esophageal wall rather than in the submucosal layer as in humans with hepatic disease and as can be

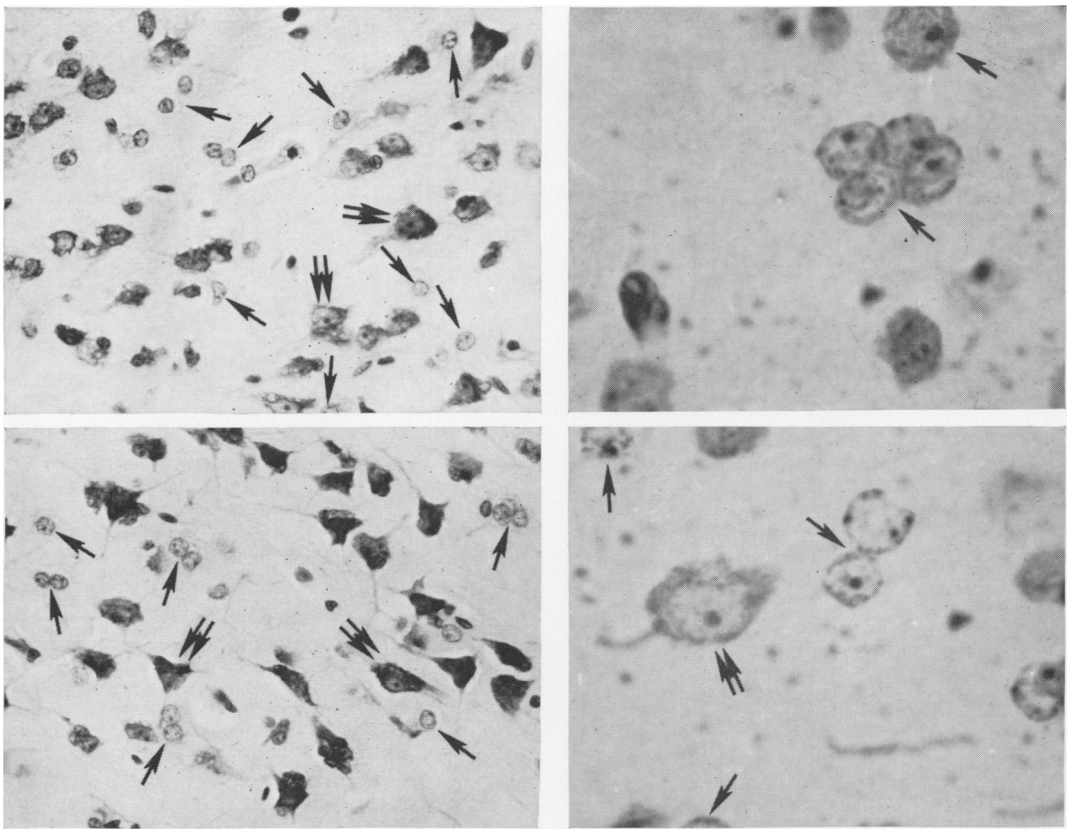

FIG. 6. Abnormal astrocytes or Altzheimer cells in primate brain. Astrocytes are marked by single arrows while several examples of neurons are marked by double arrows. Composites to the left are Nissl ×240 while those to the right are Nissl ×970 under oil. Abnormal astrocytes are often grouped together as if they had recently divided.

reproduced in the monkey. Ligation of the portal vein in the dog is almost uniformly fatal while ligation of this vessel in the monkey or the human is not.[4] These anatomic differences do not seem sufficient to explain the absence of neurologic findings in the monkey with a portacaval shunt.

Orloff *et al.*, showed that the incidence of encephalopathy following Eck fistula in dogs can be related to stomal size of the shunt.[9] In our study, stomal size was gauged, however, to be generous in the eight primates in which shunts remained open. Of importance, hepatic function remained normal in these monkeys. There was no decrease in serum proteins or reversal of the A/G ratio as is frequently seen in the dog with a portacaval shunt. If

TABLE 2. *Summary*

	Number of Animals	Weight Changes	S(X)'s	Abnormal Astrocytes
Normal protein (shunt open)	3	−29%	0	6.8 ± 1.2
Normal protein (shunt closed)	2	+12%	0	3.3 ± 0.8
High protein (shunt open)	5	−23%	occ.	10.2 ± 1.9
Control (non-operated)	3	+ 9%	0	1.2 ± 0.4

cirrhosis or similar liver damage were produced in primates with portacaval shunts, more severe clinical and histologic signs of encephalopathy might occur.

Cerebral pathologic changes were not as impressive in monkeys as in dogs. On the other hand, the significance of elevated abnormal astrocyte counts in primates in which shunts remained open was emphasized by animals in which shunts closed approximately 6 months postoperatively. These animals gained weight, serum ammonia values decreased, and there were low abnormal cerebral astrocyte counts by the time of sacrifice.

Despite marked hyperammonemia, in some cases, for more than a year, there were few neurologic symptoms in monkeys. By way of contrast, ammonia levels in dogs subjected to portacaval shunts have been reported lower than those found in the shunted primates of this study. Despite relatively low ammonia levels, the dog with a portacaval shunt has neurologic symptoms and more marked cerebral histologic changes than the primate. These differences in response to Eck fistula suggest that hyperammonemia is not the only biochemical abnormality responsible for encephalopathy in the primate.

Summary

Five of ten primates surviving portacaval shunts were given normal protein diets (22%) while five were given high-protein diets (42%). All shunted animals lost weight but only one had neurologic findings. Shunted animals, with one exception, survived until sacrificed from 332 to 435 days following shunt. Hyperammonemia was uniformly observed but primates did not develop encephalopathy as has been observed in dogs. Abnormal astrocyte counts in monkeys on normal protein diets did not approach values reported in the dog while counts in primates fed high-protein diets did. These species differences suggest that hyperammonemia may not be the only biochemical abnormality responsible for encephalopathy.

Two monkeys on normal protein diets had elevated ammonia values and lost weight for 6 months. Each of these animals had an abrupt decrease in ammonia levels and a gradual weight gain. Closed shunts were found at autopsy and this correlated well with low abnormal astrocyte counts.

The monkey seems to be a better experimental animal than the dog for studies involving the portal circulation.

Acknowledgments

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