Encephalopathy in Graded Portacaval Shunts

DAVID G. KLINE,* M.D., RAYMOND C. DOBERNECK, M.D., PH.D., BYUNGKYU K. CHUN, M.D., ROBERT B. RUTHERFORD, M.D.

From the Department of Experimental Surgery, Walter Reed Army Institute of Research, Washington 12, D. C.

DESPITE universal use of the Eck fistula to study hepatic circulation and physiology, descriptions of neuropathologic changes in brains of animals with post-shunt encephalopathy are few. Nor does unanimity concerning the neuropathologic changes reported in the literature exist. Changes consistent with encephalitis are stressed by some authors [3, 10, 11, 20, 22] while others describe degenerated neurons, demyelination, or glial proliferation.[8, 10, 14, 19] Furthermore, it is generally accepted that unless the portacaval shunt is constructed proximal to the highest branch of the portal vein, symptoms of encephalopathy do not ensue.[5, 12] This observation has not been documented by neuropathologic studies in animals with shunts at various levels of the portal vein.

Thus, the original intent of this work was to delineate the changes seen in the brains of completely shunted animals, using quantitative histologic methods. It became apparent, however, that such data might provide more information if an attempt was made to vary the gastro-intestinal origin and volume of blood shunted away from the liver.

Method

Principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. Animals were anesthetized with Pentobarbital, sodium 25 mg./Kg. of body weight.

Four groups of adult mongrel dogs of both sexes were used. The first group of 12 dogs had a complete portacaval shunt with an end-to-side anastomosis made proximal to the entry of the gastroduodenal vein into the portal vein (Grp. I). The second group of eight dogs had an end-to-side portacaval shunt carried out below the insertion of the gastroduodenal vein into the portal vein (Grp. II). The third group of four dogs had a gastroduodenal vein-to-vena cava shunt (Grp. III). Branches of the gastroduodenal vein draining structures other than the duodenum and head of the pancreas were divided in the incompletely shunted animals (Grp. II and Grp. III). Tributaries in the region of the ligament of Treitz, pylorus and pancreaticoduodenal mesentery were divided in an attempt to restrict gastroduodenal venous return to that from the duodenum.[9, 17] A fourth group of four dogs served as controls and were not operated upon (Grp. IV).

With the exception of four dogs in Grp. II which were fed canned meat rations, all dogs were fed commercial dog chow. Preoperative and presacrifice bromsulphalein retention was determined in the Grp. I and Grp. II meat fed animals. Animals were observed neurologically and sacrificed when severe encephalopathy ensued or a suitable period of time had elapsed to insure that encephalopathy would not develop.

At the time of sacrifice animals were

* Department of Neurosurgery, University of Michigan Medical Center, Ann Arbor, Michigan.

Submitted for publication February 11, 1966.

Presented in part to the 51st Meeting of the American College of Surgeons, Atlantic City, New Jersey, October 20, 1965.

TABLE 1. *Abnormal Astrocyte Counts*—Complete Shunt (Grp. I)*

Animal	Survival (Days)	Symptoms	F1C	F2C	F2M	P1C	P1M	P2C	P2M	C	M	Average (excluding M)
X54	13	LC_1C_2	13.9	9.7	8.2	18.6	10.2	20.9	14.1	6.5	5.2	12.7
7Y3	26	LC_1C_2	19.1	19.4	16.3	18.6	15.2	17.1	12.2	21.9	4.2	17.4
4X8	27	LC_1D	14.2	14.7	16.3	16.7	19.2	16.5	12.8	19.6	14.2	16.2
Y55	30	LC_1	10.7	17.0	12.0	12.2	10.1	9.0	10.2	3.0	1.7	10.5
49X	30	C_1C_2	20.6	12.5	6.1	19.4	8.9	17.6	6.7	10.6	10.9	12.8
2Y1	41	L	12.0	17.9	8.0	13.1	9.2	12.4	7.3	10.4	6.0	11.4
X03	43	LC_1	5.4	3.8	4.8	4.2	0.7	1.5	0.3	5.8	0.4	3.3
X64	43	L	17.3	7.6	1.8	2.6	2.5	5.4	1.7	3.9	3.6	5.4
6Y0	49	LC_1	10.4	16.8	9.6	18.3	8.9	13.5	11.9	4.4	1.8	11.7
1Y9	53	LC_1C_2	21.8	18.8	6.8	13.4	5.3	13.1	6.3	11.8	9.0	12.2
88X	56	LC_1	14.3	13.3	11.6	15.6	14.5	9.0	5.3	15.9	—	12.4
0W2	78	L	18.5	16.0	6.5	15.1	19.8	9.8	12.9	20.3	7.9	14.8
Average	48	—	14.8	14.0	9.0	14.0	11.3	11.6	8.2	11.2	5.9	11.7

* Each count represents the average of ten random fields, 350U in diameter of each section.

F1C—Frontal pole cortex; F2C and F2M—midfrontal cortex and medullary layers; P1C and P1M—parietal cortex and medullary layers; P2C and P2M—basal ganglia and adjacent white matter; C—cerebellar nuclear regions; M—medulla or cervical spinal cord; L—lethargy; C_1—coma; C_2—convulsions; D—decerebrate.

anesthetized and perfused with formalin. Shunts were inspected grossly to insure patency. Retrograde injections of hypaque were carried out in some of the incompletely shunted animals to determine the presence of collateral flow. Perfused brains were cut, embedded in paraffin, and microtomed in serial sections approximately eight microns in thickness. Hematoxylin and eosin, Nissl, and Morgan's myelin staining methods were used.

A survey of the histologic findings in each group was made. Using a method similar to that described by Adams [1] for human material, counts of abnormal astrocytes (Altzheimer cells) were made. Sections studied included frontal pole cortex (F1C), midfrontal cortex (F2C) and medullary layer (F2M), parietal cortex (P1C) and medullary layers (P1M), basal ganglia (P2C) and adjacent white matter (P2M), cerebellar nuclei (C), and medulla (M). Ten random fields 350 microns in diameter were counted in each section making a total of ninety fields counted in each animal.

Separate groups of control, complete portacaval (Grp. I), and incomplete portacaval (Grp. II) shunted animals were given 40 ml./Kg. of bank blood per nasogastric tube. Serial determinations of serum ammonia and blood urea nitrogen were made every two hours over an eight to twelve hour period. Serum ammonia values were determined by a modified Conway technic.[21] Astrocyte counts were analyzed by a number of one factor analyses of variance to determine differences between animals with various types of shunts. Analyses were carried out for each of the nine brain regions. Duncan's test was then carried out to ascertain which group or groups of animals were significantly different from the rest.

Results

Completely shunted animals (Grp. I) sustained an average weight loss of 30% and the gradual onset of lethargy. Crossed leg catatonic posturing, hyperreflexia, and drooling of saliva were observed in several animals. Many completely shunted animals became stuporous and four had generalized convulsions prior to sacrifice (Table 1). Postoperative bromsulphalein determinations were elevated in eight of the twelve completely shunted animals. Although animals shunted below the gastroduodenal vein (Grp. II) did not lose weight, two dogs in the meat fed group developed

TABLE 2. *Incomplete Portacaval Shunts below Gastroduodenal and Fed Chow-(Grp. IIa)*

Animal	Survival	Symptoms	F1C	F2C	F2M	P1C	P1M	P2C	P2M	C	M	Average (excluding M)
7S2	160	0	1.3	11.4	7.1	9.0	4.6	3.4	1.6	3.8	2.2	5.2
8S5	165	0	7.1	5.8	2.2	5.4	2.4	0.7	0.3	0.4	0.3	3.0
2HI	173	0	5.0	7.3	2.9	7.0	2.2	3.1	2.0	1.1	0.7	3.8
13R	181	0	8.9	8.0	5.0	12.8	6.4	7.0	4.5	1.5	0.4	6.7
Average	169	—	5.6	8.1	4.3	8.6	3.9	3.6	2.1	1.7	0.9	4.7

Incomplete Portacaval Shunts below Gastroduodenal and Fed Meat (Grp. IIb)

Animal	Survival	Symptoms	F1C	F2C	F2M	P1C	P1M	P2C	P2M	C	M	Average (excluding M)
A992	34	0	7.0	9.5	6.6	7.9	4.4	6.0	5.3	5.8	2.6	6.6
B816	47	L	8.8	12.9	5.7	8.9	5.9	5.2	4.1	4.0	1.2	6.9
C267	57	L	5.0	7.3	3.0	8.1	2.4	4.8	3.5	4.5	1.2	4.8
C110	58	0	6.2	8.0	7.2	6.5	7.0	6.8	5.2	4.6	2.0	6.4
Average	49	—	6.7	9.4	5.6	7.9	4.9	5.7	4.5	4.7	1.8	6.2

See Table #1 for key.

lethargy without specific neurologic findings. Dogs with gastroduodenal-to-vena cava shunts gained weight and had no neurologic disturbances (Tables 2, 3).

On gross examination brains had no significant abnormalities. Meninges were not thickened and there were no areas of gross destruction, infarction, or hemorrhage. The predominant histologic finding in completely shunted animals was an increased number of abnormal astrocytes. Such cells resembled Altzheimer cells described by Hösslin and Altzheimer in 1912.[13] These astrocytes had enlarged ovoid nuclei with lightly stained basophilic nucleoplasm by the Nissl technic (Fig. 1–6). Cytoplasm was not discernible with Nissl stain but an eccentric nucleolus was frequently seen. As-

TABLE 3. *Gastroduodenal-to-Vena Cava Shunts (Grp. III)*

Animal	Survival	Symptoms	F1C	F2C	F2M	P1C	P1M	P2C	P2M	C	M	Average (excluding M)
0X7	323	0	2.4	2.6	1.6	5.4	1.9	1.9	0.4	3.9	1.7	2.5
8X3	324	0	3.0	6.5	5.2	4.3	2.5	0.9	1.4	1.3	1.4	3.1
21X	325	0	1.5	3.9	2.4	2.6	1.6	0.7	1.3	2.4	1.4	2.1
43W	330	0	4.6	2.3	2.6	3.6	1.8	1.1	1.7	2.1	1.5	2.5
Average	326	—	2.9	3.8	2.9	3.9	1.9	1.2	1.2	2.4	1.5	2.6

Control Non-operated (Grp. IV)

Animal	Survival	Symptoms	F1C	F2C	F2M	P1C	P1M	P2C	P2M	C	M	Average (excluding M)
B573	42	0	0.5	6.7	1.0	3.9	1.0	1.4	0.5	2.3	0.4	2.2
B286	42	0	2.6	8.0	2.4	6.1	3.5	3.3	1.1	0.9	1.2	3.5
D701	42	0	1.5	2.7	2.1	2.7	0.5	0.9	0.5	0.5	0.3	1.4
D713	46	0	2.3	2.9	0.5	3.1	1.3	2.6	0.7	0.6	1.2	1.7
Average	43	—	1.7	5.1	1.5	3.9	1.6	2.1	0.7	1.1	0.8	2.2

See Table #1 for key.

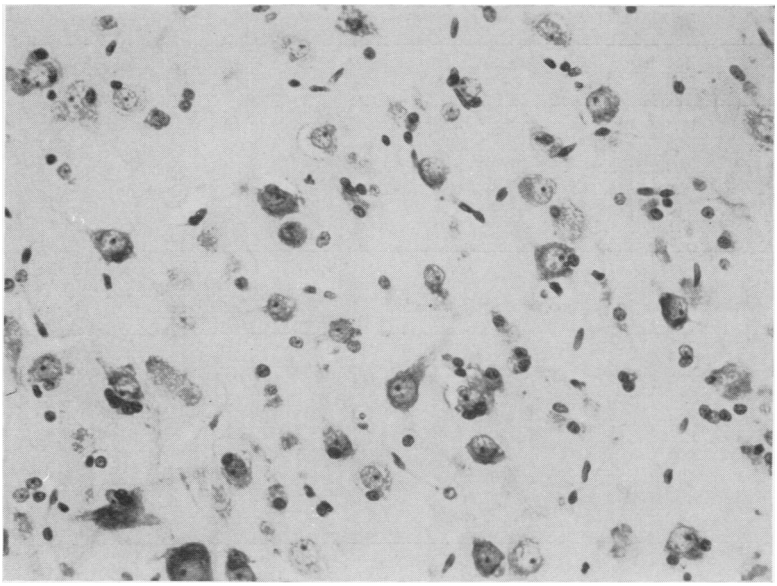

FIG. 1. Cortical region of control dog #B573. Neurons and neuroglia including astrocytes are present. Abnormal astrocytes or Altzheimer cells are absent (Nissl ×240).

trocytes were often grouped together suggesting recent division.

Astrocyte counts were markedly increased in 10 of the 12 completely shunted animals (Table 1). As may be seen in Table 2, some increase in abnormal astrocytes was noted in animals shunted below the gastroduodenal vein especially in those fed a meat diet (Grp. IIb). Animals with a gastroduodenal-to-vena cava shunt (Grp. III) had no increased counts (Table 3). Figure 7 correlates the average astrocyte count per section with the number of sections in which such a count was observed.

Analysis of variance for each brain region indicated that the five groups were

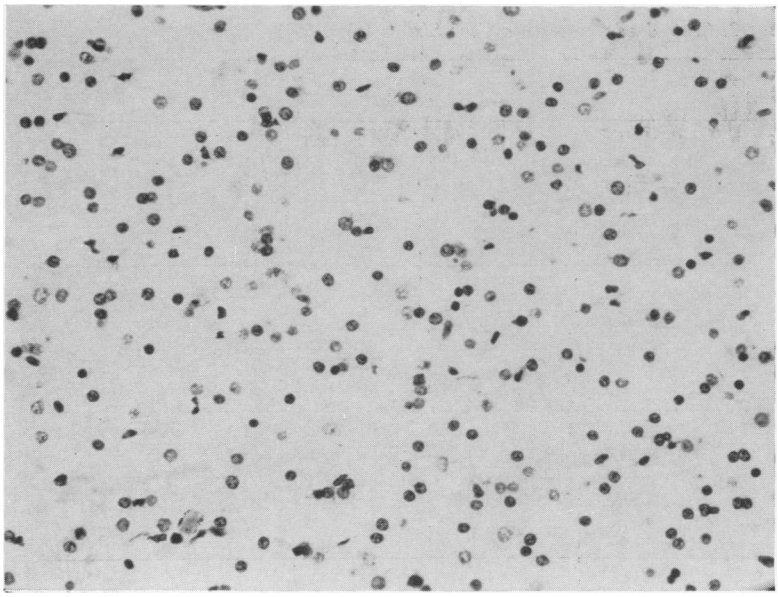

FIG. 2. Medullary region (white matter) of control dog #B573. Oligodendroglia, microcytes, and astrocytes are seen. Abnormal astrocytes are absent (Nissl ×240).

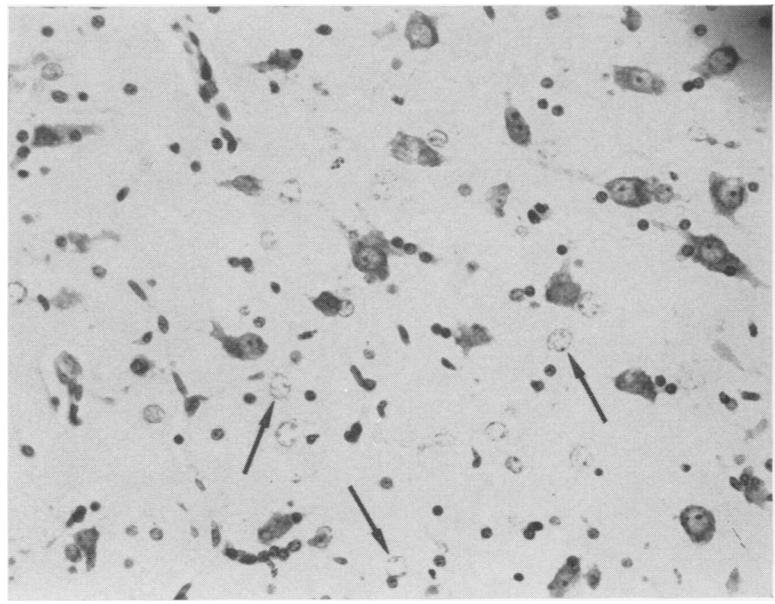

FIG. 3. Parietal cortical section of dog #4XB sacrificed 27 days following complete portacaval shunt. Animal was comatose and decerebrate prior to sacrifice. Average abnormal astrocyte count was 16.2 per 350U field (Ssee Table #1). Some of the astrocytes are marked by arrows (Nissl ×240).

statistically different (p less than 0.01 except in medulla where p was less than 0.05). By Duncan's tests, astrocyte counts of completely shunted animals were statistically different from all others except those animals incompletely shunted and fed meat (p less than 0.05). Completely shunted dogs were also significantly different from incompletely shunted and meat fed animals except for brain regions F2C, F2M, and P2M.

Thus, completely shunted animals had an overall mean count of 11.7 abnormal astrocytes per 350 micron field (SD ± 2.2) whereas those shunted below the gastroduodenal vein and fed commercial chow

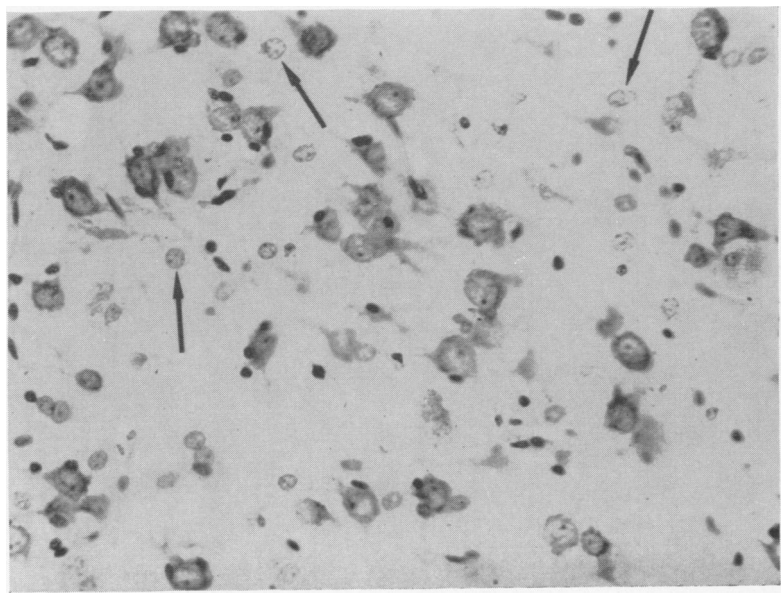

FIG. 4. Cortical section of dog #88X sacrificed 56 days after complete portacaval shunt. Average astrocyte count was 12.4 per 350U field. The animal was comatose prior to sacrifice (Nissl ×240).

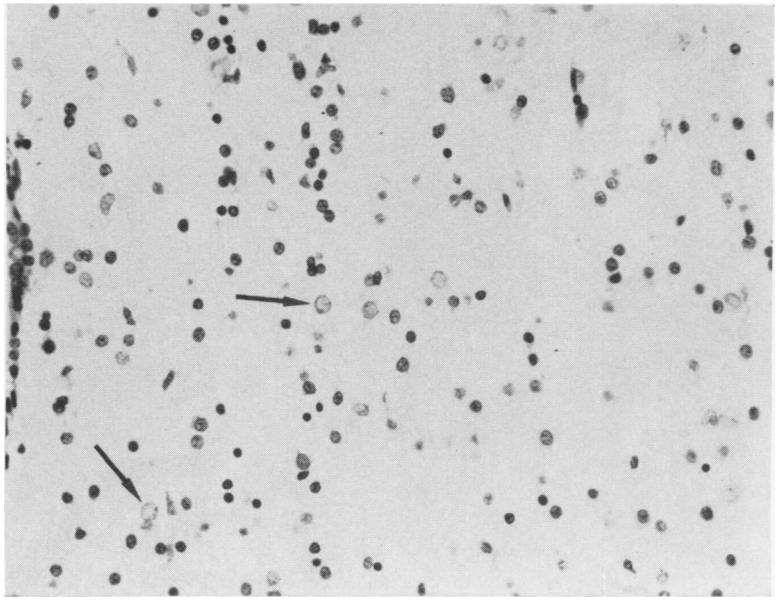

FIG. 5. Medullary region from dog #88X. Abnormal astrocytes are present although not seen as frequently in the white matter as in the cortical sections. Compare with Figure 2 (Nissl ×240).

had a count of 4.7 (SD ± 1.6). Animals with a shunt below the vein and fed meat had an average count of 6.2 (SD ± 1.5). Dogs with a gastroduodenal-to-vena cava shunt had a count of 2.6 (SD ± 1.7). Control animals had a count of 2.2 (SD ± 1.7). As seen in Table 4, counts could be correlated in a general fashion with weight change, bromsulphalein values, and neurologic findings.

Partially degenerated neurons were found in shunted as well as control animals but appeared more ubiquitous in those completely shunted than in those incompletely shunted. Parenchymal changes such as vacuolation and partial demyelination

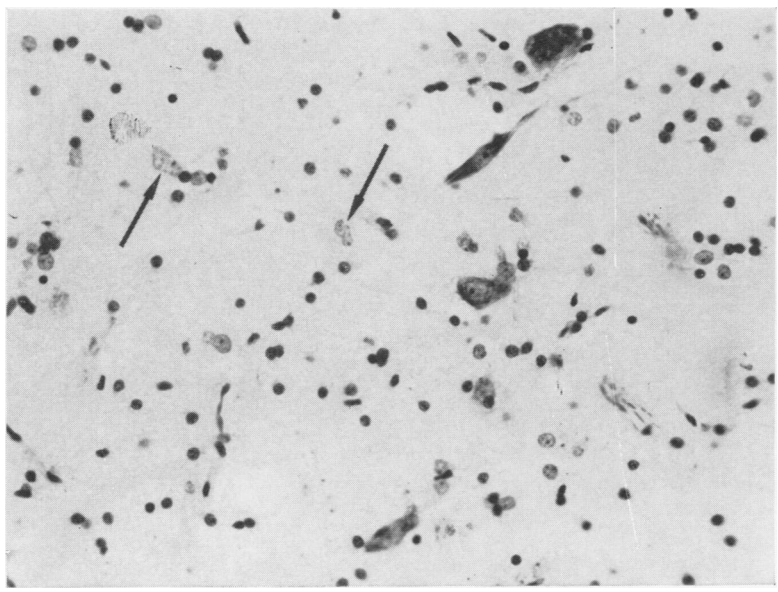

FIG. 6. Cerebellar nuclear region of dog #49X sacrificed 30 days after complete portacaval shunt. Several large abnormal astrocytes are seen (Nissl ×240).

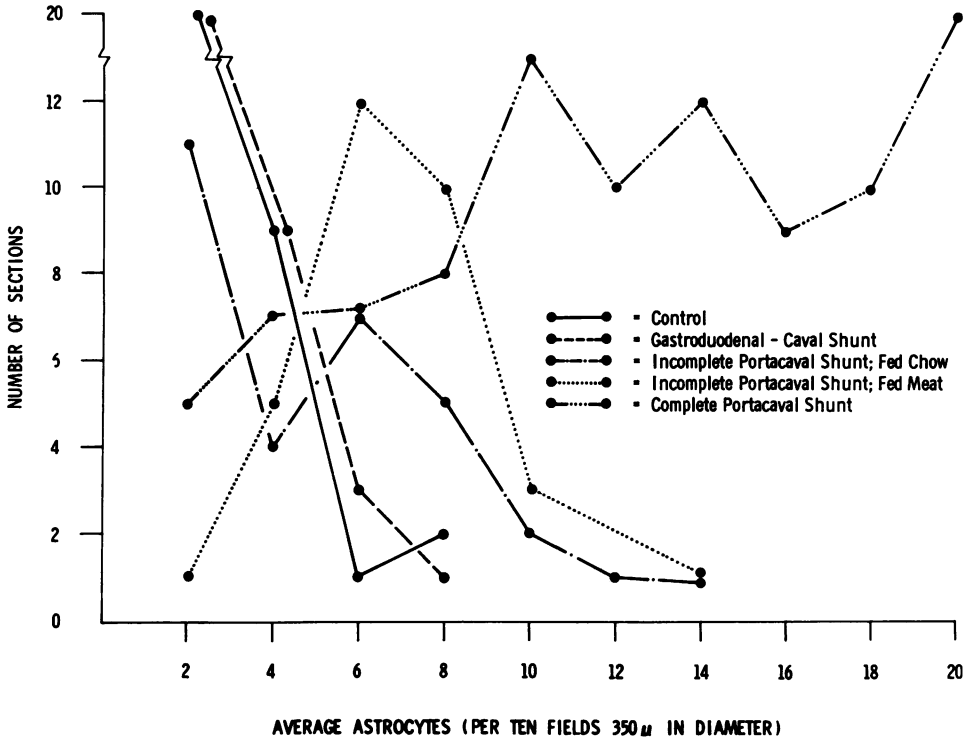

FIG. 7. Average astrocyte count per section correlated with the number of sections in which such a count was observed. Note similarity in gastroduodenal-caval shunt and control groups. Counts were elevated in the incompletely shunted animals, especially those fed meat, but did not approach those seen in the completely shunted animal (See Table 4).

of white matter were observed in both completely and incompletely shunted animals. These changes could be seen in many sections of the control specimens however and were difficult to quantitate.

The number of shunted animals given blood by gavage was small. Furthermore, curves were not as sharply peaked as reported in the past [25, 26] (Fig. 8). Mean ammonia peaks in animals given blood by gavage were 95 mcg.% in controls, 264 mcg.% in incompletely shunted dogs, and 373 mcg.% in completely shunted dogs. No attempt was made to correlate these values with the size of each shunt [18] although there was obvious gradation of stomal size in the types of shunt used.

Discussion

These studies demonstrate that animals with an Eck fistula have a significant increase in abnormal cerebral astrocytes. The findings correspond with those reported in patients with liver disease and with neurologic disorders.[1, 6, 16, 20, 24] Partially degenerated neurons appeared frequently in the completely shunted animals but were difficult to quantitate. Changes described by earlier investigators were not consistent and were not considered significant. Vascular congestion and perivascular cellular infiltrates suggestive of encephalitis [3, 10, 11, 22] were not seen. Parenchymal changes such as vacuolation and demyelination of white matter have been described in brains of patients dying of hepatic coma or portacaval shunt encephalopathy.[2] Such changes, although occasionally seen, were not consistent in shunted animals and were seen in the control animals as well. Instead, a generalized increase in abnormal astrocytes was the hallmark of cerebral morphologic

TABLE 4. *Summary Table*

	Control	I	IIa	IIb	III
Animals in group	4	12	4	4	4
Average days postop.	43	48	169	49	325
Weight change (%)	+0.5	−28	+5	+6	+11
Neurologic findings	none	multiple	none	occ. lethargy	none
Postoperative BSP (mg. %)	—	7.7	—	3.2	—
Mean peak ammonia (mcg. %)	95	373	—	264	—
Average astrocyte count per section	2.2	12.1	4.6	6.2	2.6
Average astrocyte count for all brain sections sampled	17.7	94.0	37.8	49.5	20.4
Standard deviation	±1.7	±2.2	±1.6	±1.5	±1.7
Significance (p value)	—	<0.05	>0.05	>0.05	>0.05

Type I—Complete portacaval shunt; Type IIa—Incomplete portacaval shunt below gastroduodenal fed commercial chow; Type IIb—Incomplete portacaval shunt below gastroduodenal fed canned meat; Type III—Gastroduodenal-to-cava shunt.

change in Eck fistula animals. Although astrocyte counts were elevated in incompletely shunted animals, and especially in those fed meat, the counts were not statistically convincing.

Astrocytosis or gliosis is seen in many diseases of the central nervous system. Brown [6] examined brains from patients with neurologic diseases such as tuberous sclerosis, Marie's cerebellar ataxia, paralysis agitans, Huntington's chorea, multiple sclerosis, cerebral contusion, and tumors. In this material, Altzheimer cells were found, but not in the numbers reported in brains of

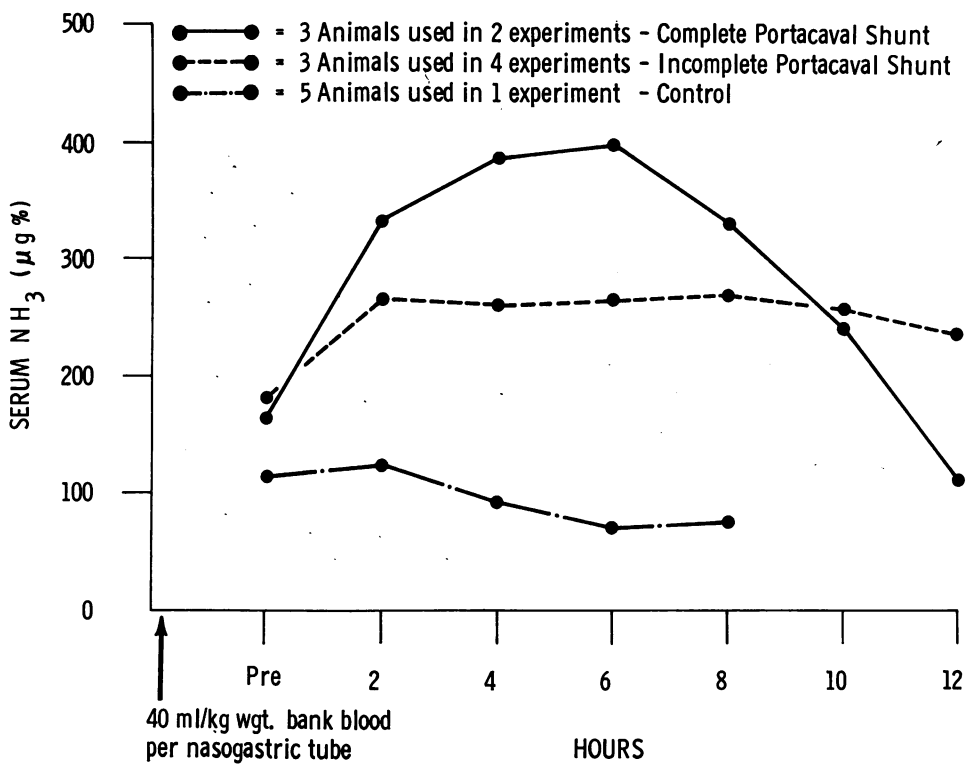

FIG. 8. Serum ammonia curves of animals fed blood by nasogastric tube.

patients with severe liver disease. Generalized astrocytosis, such as seen in this study, is believed by Adams [1] to be specific in altered liver-brain relationship. Disease entities responsible for changes in the morphologic appearance of the central nervous system are myriad, but ways in which the neurons and glia can react to these diseases are few. Thus, as pointed out by Wilson [24] and Brown [6] it is difficult to attribute specific effects to the astrocytic changes seen in these studies. Undoubtedly, proliferation and alteration in the character of astrocytes in shunted animals is a reflection of basic metabolic disturbances. Possible metabolic aberrations have been well reviewed and will not be discussed here.[15, 16, 23]

It is of interest that preservation of a relatively small vessel, the gastroduodenal, prevents not only clinical encephalopathy, but also the histologic changes seen in such encephalopathy in shunted animals. Despite variations in the origin of shunted blood from the gastro-intestinal tract, significantly elevated astrocyte counts were seen only in completely shunted animals, although a trend towards elevation was noted in incompletely shunted animals fed meat for a short period of time. These findings were not unexpected for Bollman [4, 5] emphasized the importance of collateral venous return to the liver in preventing encephalopathy. Such observations seem inconsistent with those made in patients with a side-to-side portacaval shunt. The side-to-side shunt should preserve some venous return to the liver. Post shunt encephalopathy can occur, however, in these patients. This apparent paradox has been explained in that shunts are constructed in patients with severely diseased livers and partial inflow block.[7] Thus, little, if any, of the blood intended for the liver in a side-to-side shunt actually perfuses the liver and blood flow may even be reversed in this portion of the shunt. These circumstances would, of course, not obtain in the experimental animal where the side-to-side or incomplete portacaval shunt is in an animal with a normal liver.

In these experiments, liver function, as measured by bromsulphalein retention, was normal in incompletely shunted animals. Serum ammonia could be elevated, however, by giving these animals a high protein load. One wonders if higher astrocyte counts would have resulted in incompletely shunted animals if a larger protein load had been given over a longer period of time. Available information suggests that prevention of encephalopathy, at least experimentally, depends on the functional integrity of the liver, the protein load presented to the gastro-intestinal tract, and preservation of some venous return to the liver. Astrocyte counts would be of interest in investigating other types of shunts and may be of value in further evaluation of changes in hepatic circulation.

Summary

Quantitative studies of the astrocyte population in multiple areas of the brain were made in control, completely portacaval shunted, and incompletely shunted dogs. Data were correlated with weight loss, neurologic findings, and mean peak ammonia levels after blood gavage. Despite variations in the origin of shunted blood from the gastro-intestinal tract, significantly elevated abnormal astrocytes were seen only in completely shunted animals. Astrocyte changes corresponded to those seen clinically in hepatic coma. Incompletely shunted animals fed meat had increased numbers of astrocytes, but not in statistically convincing numbers. Such animals had elevated mean peak serum ammonia values with blood gavage, despite normal liver function as measured by bromsulphalein. Experimentally, prevention of encephalopathy seems to depend upon the functional integrity of the liver,

the protein load presented to the gastrointestinal tract, and preservation of some portal venous return to the liver.

Acknowledgments

The authors would like to extend appreciation to Arthur A. Morse, M.D. for technical help and to Anthony Schork, Ph.D., Department of Biostatistics, School of Public Health, University of Michigan Medical Center for statistical analyses of the data.

References

1. Adams, R. D. and Foley, J. M.: The Neurological Disorder Associated with Liver Disease in Metabolic and Toxic Diseases of the Nervous System. Baltimore, Williams and Wilkins Co., 1953, 1198 pp.
2. Baker, A. B.: The Central Nervous System in Hepatic Disease. J. Neuropath. and Exper. Neurol., **8**:283, 1949.
3. Baló, J. and Korpóssy, B.: The Encephalitis of Dogs with Eck's Fistula Fed on Meat. Arch. Path., **13**:80, 1932.
4. Bollman, J. L., Flock, E. V., Grindlay, J. H., Bickford, R. G. and Lichtenheld, F. R.: Coma with Increased Amino-acids of Brain and Cerebrospinal Fluid in Dogs with Eck's Fistula: Prevention by Porta-systemic Collateral Circulation. Arch. Surg., **75**:405, 1957.
5. Bollman, J. L.: The Animal with an Eck Fistula. Phys. Rev., **41**:607, 1961.
6. Brown, I. A.: Liver-Brain Relationships. Springfield, Ill., Charles C Thomas, 1957.
7. Child, C. G.: The Hepatic Circulation and Portal Hypertension. Philadelphia, W. B. Saunders, 1954.
8. Crandall, L. A. and Weil, A.: Pathology of Central Nervous System in Diseases of the Liver. Arch. Neurol. and Psych., **29**:1066, 1933.
9. Doberneck, R. C., Kline, D. G., Morse, A. A. and Berman, A.: Relationship of Hemosiderosis to Portacaval Shunting. Surgery, **54**:912, 1963.
10. Fiessinger, N., Cornil, L., Poursines, Y. and Paillas, J.: Les lésions encéphaliques consécutives a la fistule d'Eck. Annales D'Anat. Pathol. et D'Anat. Normale Méd. Chir., **14**: 553, 1937.
11. Fuchs, A.: Experimentelle Encephalitis. Wien. Med. Wchnschr., **71**:709, 1921.
12. Hahn, M., Massen, O., Nencki, M. and Pavlov, J.: Die Eck'sche fistel zwischen der unteren hohlvene und der pfortader und ihre folgen für der organismus. Arch. F. Exptl. Pathol. u. Pharmakal., **32**:161, 1893.
13. Hösslin, C. and von Altzheimer, A.: Erin betrag. zur klinik und pathologoschen anatomie der Westphal—Strumpellschen pseudoklerose. Ztschr. Neurol. u. Psychiat., **8**: 183, 1912.
14. Krauspe, C. and Gebhardt, F.: Untersuchungen am Eck-Fistel-Hund: Uber gehirnveranderungen bei Eck-Fistel-Hunden nach Fleischkast. Z. ges. Exptl. Med., **93**:378, 1934.
15. McDermott, W. V., Adams, R. D. and Riddell, A. G.: Ammonia Metabolism in Man. Ann. Surg., **140**:539, 1954.
16. McDermott, W. V. and Adams, R. D.: Episodic Stupor Associated with an Eck Fistula in Man with Particular Reference to Ammonia Metabolism. J. Clin. Invest., **33**:1, 1954.
17. Miller, M. E., Christensen, G. C. and Evans, H. E.: Anatomy of the Dog. Philadelphia, W. B. Saunders, 1964.
18. Orloff, M. J., Wall, M. H., Hickman, E. B. and Neesby, T.: Influence of Stomal Size of Portacaval Shunts on Peripheral Blood Ammonia Levels. Ann. Surg., **158**:172, 1953.
19. Riddell, A. G.: Changes in Man and Animals with Portacaval Anastomosis. Proc. Roy. Soc. Med., **48**:48, 1955.
20. Scherer, H. J.: Zur frage der beziehungen zwischen leber und gehirvenkranbungen. Virchows Arch. F. Path. Anat., **288**:333, 1933.
21. Seligson, D. and Seligson, H.: A Microdiffusion Method for the Determination of Nitrogen Liberated as Ammonia. J. Lab. and Clin. Med., **38**:324, 1951.
22. Silberstein, F.: Experimentelle Encephalitis. Wien. Klin. Wchnschr., **37**:30, 1924.
23. Walshe, J. M.: Biochemical Studies in Hepatic Coma. Lectures on the Scientific Basis of Medicine, **8**:407, 1958.
24. Wilson, S. A. K.: Progressive Lenticular Degeneration: A Familial Nervous Disease Associated with Cirrhosis of the Liver. Brain, **34**:295, 1912.
25. Zuidema, G. D., Gaisford, W. D., Rakolta, G., Fletcher, M., Burton, W. and Child, C. G.: Comparative Blood Ammonia Studies in Dogs and Monkies. Arch. Surg., **85**:776, 1962.
26. Zuidema, G. D., Fletcher, M., Burton, W., Gaisford, W. and Child, C. G.: Blood Ammonia Studies in Monkies before and after Portacaval Anastomosis. Arch. Surg., **85**:152, 1962.