Surgically induced hepatic failure in animals

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Cirrhosis of the liver accounts for approximately 30,000 deaths annually in the United States. In addition, many patients die of acute hepatic failure. Approximately one third of all deaths from liver disease are directly related to hepatic insufficiency. Hepatic coma is frequently the end stage of progressive hepatic insufficiency. Despite abundant reports on clinical and experimental findings, the cause of hepatic coma is still only partially understood. The reason for this can be traced back to a lack of knowledge of the total scope of liver function, leading to a limited understanding of hepatic failure.

To develop effective hepatic support systems, the establishment of good animal models of hepatic failure has been the goal of many investigators. Various methods have been conceived, tried, and evaluated. These models can be classified into two main groups: drug induced or surgically induced.

One factor of critical importance in establishing animal models is that the pathologic situations must be well controlled and comparable to clinical disease states. The models must be critically reproducible to eliminate possible false-negative or false-positive results. Drug-in-

duced hepatic necrosis by CCl_4,[1] anesthetic agents,[2] dimethylnitrosamine,[3,4] and yellow phosphorus[5] has many disadvantages, such as low reproducibility and the possible involvement of other organs. For this reason, drug-induced hepatic insufficiency has been abandoned.

Surgical procedures used to induce hepatic failure should be as simple as possible, reproducible, and should produce varying types of liver cell damage and failure. Three surgical procedures have been selected for use in our studies of hepatic failure: (1) total hepatectomy—complete loss of hepatic function, (2) hepatic blood deprivation—progressive hepatic cellular deterioration, and (3) biliary obstruction—loss of the excretory pathway.

The different types of pathophysiologic procedures performed in these animals with their consequent effects have provided us with additional information on hepatic failure and have led to a better understanding of the mechanisms involved.

Materials and methods

Twelve male mongrel dogs, each weighing 15 to 20 kg, were used. Before surgery they were fasted overnight. Four dogs were assigned to each of three groups. Anesthesia was induced with thiamylal sodium and maintained with gas mixtures of oxygen, nitrous oxide, and halothane (Fluothane). A total of 15 to 20 mg/kg of thiamylal sodium was administered intravenously. After intubation, using a 40F Lanz endotracheal tube, respiration was controlled with a respirator. A gas mixture of 30% to 50% nitrous oxide with 1% halothane was administered. These anesthetic gas mixtures were discontinued before the end of the surgical procedures; in operations to create anhepatic and ischemic models, halothane was discontinued before the start of the portacaval anastomosis. The endotracheal tube was removed after spontaneous respiration was complete, when the animal started chewing the tube. After the induction of anesthesia, an intravenous catheter for transfusion and blood sampling was inserted into the femoral vein. In the case of anhepatic animals, a catheter for monitoring systemic pressure was also placed in the femoral artery. The animal was placed in the supine position with a pillow under its back. The surgical area was prepared and draped. The abdominal cavity was entered by a midline incision from just under the xyphoid process.

Anhepatic model. A one-stage total hepatectomy, followed by an end-to-side portacaval anastomosis was performed. The inferior vena cava anastomotic site was isolated, and the portal vein was dissected from its junction with the splenic vein to the hepatic branches, cutting off the pancreatoduodenal vein. The inferior vena cava was partially occluded with a Satinsky vascular clamp, and an opening was made by removing a segment of its wall. The portal vein was divided between a tie and a clamp. An end-to-side portacaval anastomosis was performed by continuous sutures with 6-0 prolene. The liver was then mobilized downward by dissection of its phrenic attachment, cutting both phrenic arteries. The minor omentum was tied en masse, including the hepatic artery and the common bile duct, and sharply divided. Each lobe of the liver was individually removed by ap-

plying large clamps close to its junction with the inferior vena cava, sharp dissection, and mass ligation with silk. To avoid hypoglycemic shock, which develops approximately 6 hours postoperatively,[6] glucose, 0.25 g/kg of body weight/hr, was administered intravenously. No single electrolyte solution was given consistently. To suppress the growth of anaerobic organisms, 1 g cephalothin sodium was injected intravenously every 6 hours postoperatively. The patency of the portacaval anastomosis was confirmed at autopsy.

Ischemic model. Both triangular ligaments, including the phrenic arteries, were ligated and divided. An end-to-side portacaval anastomosis was then performed in the same manner as in the anhepatic models. Mean portal occlusion time was 11.3 ± 1.5 minutes. After completion of the portacaval anastomosis, the common hepatic artery was isolated from the hepatoduodenal ligament and surrounding nerves. A silk ligature was placed around the common hepatic artery and passed through a vinyl tube, 3 mm inside diameter and 15 cm in length. This tube was guided outside through the incision and fixed to the peritoneum. By pulling this suture from the outside of the body, the hepatic artery was occluded the same day, and 2 and 6 days after the portacaval anastomosis, without anesthesia. A 50% dextrose solution, 200 to 300 ml, was administered intravenously to avoid death due to hypoglycemia.[7] No electrolyte solutions were given after ligation of the common hepatic artery. Cephalothin sodium was injected, 2 g/day, after the portacaval anastomosis and 1 g/6 hr after ligation of the common hepatic artery to suppress the overgrowth of anaerobic organisms in the dearterialized liver.[8] Patency of the portacaval anastomosis and complete occlusion of the hepatic artery were confirmed at autopsy.

Jaundiced model. The gallbladder was removed from the fundic portion toward the cystic duct. The cystic duct was transfixed and ligated. The common bile duct was isolated from the hepatoduodenal ligament, ligated, and divided as near as possible to the duodenum. One gram of cephalothin sodium was administered 4 days postoperatively.

Laboratory studies included the following: blood gas analysis (pO_2, pCO_2, and pH measured on the IL-513 blood gas analyzer); hematology (RBC analyzed with automatic analyzer Model-S, Coulter Co.; WBC and platelets visually counted); blood chemistry (SMA-18 Autoanalyzer, Technicon Inst. Co.); ammonia nitrogen (Berthelot reaction); amino acids (multisample amino acid analyzer, Technicon Inst. Co.); free fatty acid (modification of Antonis' procedure); fibrinogen (monochloroacetic acid reaction).

Results

Anhepatic model. These four animals survived 26, 17, 17, and 11 hours respectively after completion of total hepatectomy. The average time of anesthesia and operation in the four dogs was 3.8 ± 0.4 and 2.6 ± 0.1 hours. All four dogs had similar postoperative courses. After recovery from anesthesia, they lifted their heads, moved their paws, and showed good response to sound and pain stimulations; their response to stimulation gradually became weak, and the animals went into coma.

Coma began about 9 hours after hepatectomy. They vomited gastric contents and later, coffee-groundlike material. The dogs had running movements with their front legs, spastic motions of the body, and clonic convulsions. Breathing was shallow and fast but became deep and slow before death. Systolic blood pressure was well maintained for the first 6 hours, then declined gradually (75 mm Hg) at 7 to 12 hours after hepatectomy. The pulse rate was initially high (140–170/min) but decreased (60–70/min) before death. Respiratory rate was also high, sometimes over 60/min but decreased to about 20/min. The animals became oliguric and then anuric 4 to 5 hours before death, although liquid transfusions were continued throughout. Average biochemical parameters of the anhepatic models after completion of the hepatectomy and before death are listed in *Table 1*. In the end stage of coma, severe acidosis and hypercapnea were suggested, although PO_2 levels were maintained consistently. During coma, respiratory alkalosis due to hyperventilation was remarkable, whereas arterial pH decreased continuously. All blood cell components decreased gradually, reaching low levels in the end stage. Serum sodium and chloride levels decreased; in contrast, potassium levels increased. Total protein, albumin and fibrinogen reduced gradually with similar tendencies. Liver function tests, such as total bilirubin, alkaline phosphatase, and serum glutamic oxaloacetic transaminase (SGOT) increased to maximum levels 8 hours after hepatectomy, then leveled off; increments were not remarkable. Cholesterol and blood urea nitrogen (BUN) levels declined rapidly after hepatectomy; the BUN became negligible 11 hours after surgery. Conversely, creatinine and uric acid levels increased. Levels of ammonia and free fatty acids increased remarkably. Analysis of amino acids indicated the slight decrease of branched-chain amino acids: leucine, isoleucine, and valine (*Fig. 1*). Phenylalanine and tyrosine in aromatic amino acids increased moderately. Among the nonessential amino acids, glutamic acid, glycine, and histidine increased remarkably, while methionine levels tended to decrease. Glutamine, lysine, alanine, aspartic acid, threonine, and serine levels indicated no great changes.

Ischemic model. Four dogs were

Table 1. Average biochemical changes of the anhepatic models

Laboratory values	Initial value*	Final value	Laboratory values	Initial value*	Final value
Arterial pH	7.37 ± 0.11	7.03 ± 0.06	Albumin, g/dl	3.0 ± 0.3	0.5 ± 0.2
PO^2, mmHg	97.8 ± 0.3	95.5 ± 9.0	Fibrinogen, mg/dl	270 ± 48	45 ± 15
PCO^2, mmHg	36.5 ± 4.7	54.3 ± 4.7	Total bilirubin, mg/dl	0.2 ± 0.0	0.4 ±0.2
RBC, × 10^6	5.95 ± 0.64	2.00 ± 0.85	Alkaline phosphatase, mU/ml	54 ± 7	102 ± 28
Hematocrit, %	39.7 ± 3.1	15.0 ± 4.2	SGOT, mU/ml	87 ± 11	772 ± 468
WBC, × 10^3	7.4 ± 0.9	4.0 ± 1.8	Cholesterol, mg/dl	166 ± 43	48 ± 3
Platelet, × 10^4	12.7 ± 0.6	8.3 ± 3.1	BUN, mg/dl	14 ± 1	0
Na, mEq/liter	143 ± 3	95 ± 22	Creatinine, mg/dl	0.9 ± 0.1	1.6 ± 0.5
K, mEq/liter	4.2 ± 0.4	5.1 ± 0.8	Uric acid, mg/dl	0.4 ± 0.1	4.9 ± 1.4
Cl, mEq/liter	109 ± 4	71 ± 20	Ammonia, μg/dl	132 ± 59	1012 ± 526
Total protein, g/dl	5.8 ± 0.5	1.7 ± 0.4	Free fatty acid, μmoles/liter	0.216 ± 0.054	0.910 ± 0.161

* Initial value: obtained after completion of the total hepatectomy.

operated on to create ischemic hepatic failure. The procedure consisted of two parts: first, portacaval anastomosis and second, occlusion of the common hepatic artery (*Table 2*).

All animals except Dog 3 subsequently went into hepatic coma and died. The common hepatic artery of Dog 3 was occluded 48 hours after portacaval anastomosis, but the animal did not go into coma. Therefore, a week after the second procedure, the proper hepatic artery was ligated, and the animal died in coma 18 hours after the last operation. Some relationship between time intervals of the portacaval anastomosis and hepatic artery ligation and survival time and liver weight at autopsy is apparent. The longer the duration of first and second operations, the longer the animal seemed to survive and the lighter the weight of the liver. The average biochemical data on these four dogs preoperatively, 48 hours after portacaval anastomosis, and before death, after the ligation of the hepatic artery, are shown in *Table 3*.

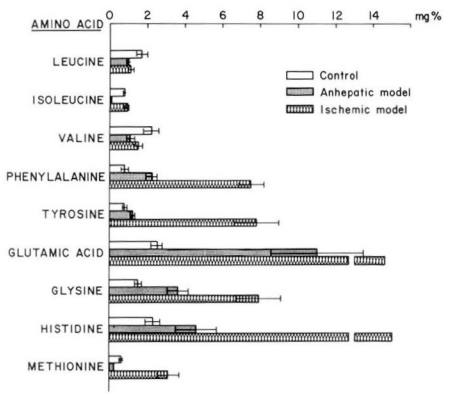

Fig. 1. Levels of representative amino acids in anhepatic dogs and in dogs with ischemic livers. Branched-chain amino acids (leucine, isoleucine and valine) decreased. The other amino acids increased in both anhepatic and ischemic models; the rate of increase was more remarkable in ischemic models.

Biochemical data 48 hours after the portacaval anastomosis showed no great changes compared to data in the preoperative period except for liver function tests. Changes of data after ligation of the hepatic artery indicated almost the same tendencies as those of the anhepatic model to a smaller degree, except for liver function tests. The amino acid analysis showed that changes of branched-chain amino acids were not significant, but those of other amino acids listed in *Figure 1* were remarkable. Compared to the anhepatic model, the ischemic model showed increas-

Table 2. Relationship between surgical procedures and survival time, onset time of coma and liver weight in ischemic dogs

Ischemic model	Duration between PCA and ligation of CHA	Survival time after last procedure	Onset time of coma after last procedure	Liver weight at autopsy, g
1	0 (same time)	16 hr	7 hr	1100
2	48 hr	18 hr	12 hr	800
3	48 hr	. . .	No coma	. . .
	(6 days)*	18 hr	14 hr	380
4	6 days	38 hr	35 hr	600

PCA = portacaval anastomosis; CHA = common hepatic artery.
* This animal had the third operation, ligation of the proper hepatic artery, 6 days after ligation of the common hepatic artery.

Table 3. Average biochemical changes of the ischemic liver failure models

Laboratory values	Preoperative value	48 hr after PCA	Before death, after ligation of common hepatic artery	Laboratory values	Preoperative value	48 hr after PCA	Before death, after ligation of common hepatic artery
RBC, $\times 10^6$	6.22 ± 0.11	6.85 ± 0.71	4.74 ± 0.19	Albumin, g/dl	2.6 ± 0.3	2.6 ± 0.3	1.7 ± 0.5
WBC, $\times 10^3$	6.3 ± 0.3	15.6 ± 1.9	27.1 ± 6.8	Fibrinogen, mg/dl	360 ± 45	380 ± 85	248 ± 88
Hb, g	15.1 ± 0.4	16.8 ± 2.2	13.5 ± 1.6	Total bilirubin, mg/dl	0.2 ± 0.0	0.8 ± 0.5	1.9 ± 0.9
Hematocrit, %	42.7 ± 1.2	47.6 ± 5.0	35.3 ± 3.7	Alkaline phosphatase, mU/ml	50 ± 8	423 ± 76	1063 ± 159
Platelet, $\times 10^3$	178 ± 31	164 ± 28	145 ± 36	SGOT, mU/ml	53 ± 22	>3500	>3500
Na, mEq/liter	143 ± 11	145 ± 8	129 ± 5	Cholesterol, mg/dl	173 ± 16	172 ± 21	88 ± 31
K, mEq/liter	4.0 ± 0.4	4.4 ± 0.4	3.6 ± 0.7	BUN, mg/dl	18 ± 1	12 ± 3	5 ± 2
Cl, mEq/liter	108 ± 8	103 ± 8	95 ± 9	Creatinine, mg/dl	1.1 ± 0.2	0.9 ± 0.1	1.2 ± 0.4
Ca, mg/dl	8.5 ± 0.3	7.0 ± 0.4	7.3 ± 1.0	Uric acid, mg/dl	0.5 ± 0.1	0.7 ± 0.5	2.9 ± 1.1
P, mg/dl	3.0 ± 0.1	3.8 ± 1.4	7.0 ± 2.7	Ammonia, μg/dl	79 ± 8	88 ± 14	360 ± 134
Total protein, g/dl	6.1 ± 0.7	6.0 ± 0.2	4.2 ± 1.0	Free fatty acids, μmole/liter	0.329 ± 0.079	0.590 ± 0.327	0.954 ± 0.248

ingly greater degrees of amino acid level.

Jaundiced model. Four dogs were operated on to induce obstructive jaundice. The animals survived for 5, 5, 7, and 17 weeks after surgery respectively (*Table 4*). Dog 1 died 37 days after surgery of severe liver abscesses combined with renal failure. At autopsy, complete necrosis of the liver and pus were found instead of normal liver tissue. Dog 2 died of pneumonia 39 days after surgery. Dog 3 died of gastric bleeding 7 weeks after the procedure. At autopsy multiple hemorrhagic erosions were found in the stomach. Dog 4 survived 17 weeks after the surgery; the cause of death was hepatic failure from biliary cirrhosis.

Figure 2 indicates by graph the principal biochemical alterations of the four dogs. Serum albumin levels decreased gradually, although total proteins were maintained.

Total bilirubin reached maximum levels at 1 week after the surgery, then leveled off. Alkaline phosphatase levels remained at a high level, over 3500 mU/ml for 6 weeks after surgery, then gradually decreased. SGOT and cholesterol values increased to maximum levels in 1 or 2 weeks, then decreased. Ammonia levels tended to increase, and all these levels before death were over 400 mg/dl. Free fatty acid levels showed some tendency to increase, but they indicated great variations. Amino acids were analyzed, but they showed great variations in each case; therefore, no constant trends could be obtained.

Discussion

Hepatic coma is frequently the end stage of progressive hepatic insuffi-

Table 4. Summary of the four jaundiced animal models

Dog	Survival time	Cause of death	Principal laboratory findings before death
No. 1	37 days	Liver abscess with renal failure	WBC 59,000, fibrinogen 720 mg/dl, total bilirubin 18.5 mg/dl, SGOT 290 mU/ml, alkaline phosphatase >3500 mU/ml, BUN 80 mg/dl, creatinine 720 mg/dl
No. 2	39 days	Pneumonia	WBC 28,000, fibrinogen 230 mg/dl, total bilirubin 9.2 mg/dl, SGOT 140 mU/ml, alkaline phosphatase >3500 mU/ml, ammonia 520 μg/dl
No. 3	7 weeks	Gastric bleeding	Hemoglobin 9.5 g, A/G ratio 0.4, fibrinogen 520 mg/dl, total bilirubin 13.8 mg/dl, SGOT 240 mU/ml, alkaline phosphatase 1790 mU/ml, BUN 58 mg/dl, ammonia 460 μg/dl
No. 4	17 weeks	Liver failure	A/G ratio 0.3, total bilirubin 3 mg/dl, SGOT 70 mU/ml, alkaline phosphatase 1370 mU/ml, ammonia 420 μg/dl, free fatty acid 1.07 μmole/liter

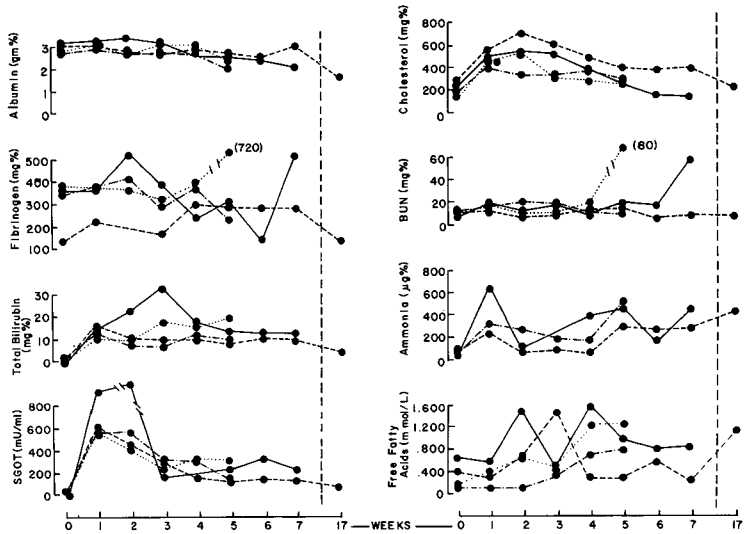

Fig. 2. Biochemical alterations of four jaundiced dogs. Changes varied in accordance with the cause of death. Dog 1 = ... ; dog 2 = _._._._; dog 3 = _____; dog 4 = — — — —.

ciency and is defined as a disorder of consciousness with increased or decreased psychomotor activity occurring in patients with severe liver disease.

Establishment of good animal models of hepatic failure has proved to be difficult for many investigators. The first model of hepatic failure was that of the anhepatic animal originated by Mann,[9] in which total hepatectomy was performed by a two-stage procedure. This technique was recently simplified by subsequent investigators to a one-stage procedure.[4] To study the metabolic and hemodynamic aspects of total hepatic failure, this animal model is highly significant.

The animal model of ischemic liver failure was first described by Rappaport et al,[1] and Giges et al.[8] These investigators produced hepatic coma in dogs by a two-stage procedure in

which permanent hepatic artery ligation was performed some days after a portacaval shunt. The significance of this animal model and its similarity to hepatic coma in man was proved subsequently by other investigators.[5, 10, 11]

These two types of surgically created liver failure in animal models, complete loss of hepatic function and progressive (ischemic) hepatocellular deterioration produced acute and complete hepatic failure. In our experiments, all dogs died of hepatic coma. Total hepatectomy was performed by a modification of the technique of Starzl et al.[4] Coma and death ensued in 11 to 26 hours. Differences in survival time seemed to depend upon the volume of intraabdominal bleeding caused by progression of a hemorrhagic diathesis after the hepatectomy.

In the ischemic liver failure group, the longer the duration between the first and second surgical procedures, the longer the animals seemed to survive and the lighter the weights of the livers. In our experiments hepatic coma developed in all animals except one. According to the reports of Mattson and Turcotte,[10] only 25% of dogs with 96-hour intervals between portacaval shunt and hepatic artery ligation became comatose; all dogs with 24- to 48-hour intervals died in coma. Giges et al[8] reported that only 8% of dogs became comatose if the interval was 4 days or longer. In Rappaport's[1] studies, the variation in survival was imposed by a varying arterial collateral flow. Holper et al[12] pointed out that after a certain ischemic period, survival or nonsurvival depended on the reticuloendothelial system in the liver.

Terminal biochemical changes of these two types of animal models indicated many similarities to hepatic coma in man. The degree of biochemical changes, except for liver function tests and amino acid analysis, were more extreme in the anhepatic model than in the ischemic model. Serum albumin is synthesized in the microsome of the liver; α_1, α_2, and β globulin are synthesized predominantly in the liver (90%, 75%, and 50% respectively).[2] In the terminal stages of hepatic failure, serum protein levels were sharply decreased; liver function tests (bilirubin, SGOT, and alkaline phosphatase levels) were increased. Royer et al[13] suggested that the spleen, intestine, and kidney may be the site of bilirubin formation and conjugation in the hepatectomized animal. Ammonia, which is produced from the deamination of amino acids, is metabolized to urea by the Krebs-Henseleit cycle in the liver. In hepatic coma, blood ammonia concentrations were elevated, but a good correlation with clinical status was not evident.[14] Ammonia is still considered to be the most important pathogenic factor inducing hepatic coma, but the mechanism that causes brain toxicity is still only partially understood. Zieve et al[15] studied the synergism between ammonia and fatty acids in the production of coma. For each of these substances, the blood level associated with coma is substantially lowered when both are present. In hepatic failure, free fatty acids are increased in the bloodstream; short chain fatty acids are considered important factors in inducing hepatic coma.

In animal models, changes in amino acids are similar to those of human hepatic failure.[16–18] Branched-chain amino acids decreased in the

anhepatic models; there were no significant changes in the ischemic models. Other amino acids, aromatic amino acids, nonessential amino acids, and essential amino acids were increased in both anhepatic and ischemic models of liver failure. In many theories for the pathogenesis of hepatic encephalopathy, false neurotransmitters, principally octopamine, have been considered important. Levels of phenylalanine and tyrosine are increased in both plasma and brain tissue of humans and animals.[7, 19, 20] Phenylalanine and tyrosine may accumulate and be synthesized into tyramine and octopamine;[21] serum and urine octopamine levels parallel the mental state.[22] Fischer and Baldessarini[23] suggested that octopamine competes with the normal central neurotransmitter, dopamine, for uptake and release, hence leading to encephalopathy.

Compared to the anhepatic and ischemic causes of acute hepatic failure, obstructive jaundice has different aspects. The causes of death in patients with obstructive jaundice include hepatic failure as well as renal failure and gastrointestinal bleeding. However, patients who die from hepatic failure usually do not show the massive hepatic cell necrosis frequently seen in patients with acute hepatic failure. In our studies and biochemical tests, hepatic cell necrosis was not observed; the reduction of serum proteins was not remarkable; the maximum SGOT levels were all under 600 units/ml; and there was no reduction of urea synthesis. Increases of bilirubin, alkaline phosphatase and cholesterol resulted from cholestasis. Blood ammonia levels were maintained with moderately elevated values 4 weeks after biliary obstruction. Free fatty acid levels showed variable tendencies up to 4 weeks after surgery, then the levels became uniformly high. From these studies, deterioration of the converting ability of ammonia and free fatty acid in the liver is suggested in the later stages of biliary obstruction when cirrhotic changes occur. The clinical and biochemical changes in the later stages of biliary obstruction were strongly influenced by the mode of death of the animals.

The procedures we have performed in these animals, total hepatectomy, hepatic blood vessel deprivation, and biliary obstruction, with their consequent effects, have provided additional information on understanding hepatic failure. The clinical course and biochemical changes associated with these animal models suggest that they closely resemble hepatic failure in humans. These animal models will, therefore, aid us in evaluating hepatic assist devices and in completing future studies, now planned, on extracorporeal metabolic hepatic assistance for support of the animal (or patient) with hepatic failure.

Summary

Three types of hepatic failure were surgically induced in dogs: anhepatic, ischemic, and jaundiced models. Postoperative survival, the clinical course, and biochemical abnormalities were studied.

Anhepatic dogs showed high reproducibility in clinical and biochemical changes. Models with ischemic livers showed different survival times related to the time interval between the portacaval anastomosis and hepatic artery ligation. However, clinical and biochemical changes were re-

producible. All dogs died of hepatic coma. Jaundiced animal models had differing causes of death, an observation frequently found in patients with obstructive jaundice. The clinical and biochemical changes in late stages were, therefore, different, according to the cause of death.

The clinical course and biochemical changes associated with these animal models of hepatic insufficiency suggest that they closely resemble hepatic insufficiency in humans.

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