Heterologous Liver Perfusion in Treatment of Hepatic Failure *

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THE UNIQUE regenerative capacity of the liver provides the rationale for temporary support of the patient in reversible hepatic failure. The metabolic complexity of the liver makes it unlikely that attempts to eradicate only a single "toxic" product of liver failure would meet with much lasting clinical success. In all likelihood, only another normal liver can adequately substitute for one that has failed.

The present study is a laboratory and clinical evaluation of the isolated perfused *ex-vivo* heterologous liver as it may provide temporary support for patients in reversible hepatic failure.

The perfused liver has been a well recognized experimental preparation for the study of hepatic metabolism since its use by Claude Bernard [1] in 1855. Increasing familiarity with pump-oxygenators has, during the past decade, permitted study of this preparation utilizing heparinized homologous or autologous blood as the perfusate.[7] We, as well as others,[16] have utilized an extracorporeal homologous liver or a cross perfusion [8, 11] to support a hepatectomized animal.

The clinical utilization of such a preparation requires the simultaneous availability of a patient with reversible yet severe liver failure and a fresh, undiseased liver from a patient with the proper blood type whose family is available and willing to authorize quick postmortem harvest of the cadaver liver. Such a fortuitous set of circumstances is obviously unlikely.

In an effort to resolve this clinical dilemma, it was decided to investigate the feasibility of utilizing a heterologous liver washed free of its own blood for such temporary hepatic support.

Part I. Experimental Studies

A variety of liver function tests were performed on the excised liver of 22 pigs perfused with heparinized human blood in a sterile chamber.

Technic

Pigs weighing between 20 and 45 Kg. were placed on nothing but sugar water by mouth 12 hours prior to operation. One gram of streptomycin and one million units of penicillin were administered at that time.

Hepatectomy was performed under sterile conditions with the animal anesthetized with 20 mg./Kg. body weight of an intravenous 4 per cent Pentothal solution. Subsequent intravenous Pentothal was administered as required through the cannulated jugular vein while the animal was respired via a cuffed endotracheal tube placed through a tracheostomy. With the animal on his back, a long right subcostal incision was made, extending well into the flank. The vena cavae above and below the liver were suitably isolated and the liver freed of its peritoneal attachments. The cystic duct was ligated to avoid gall bladder bile

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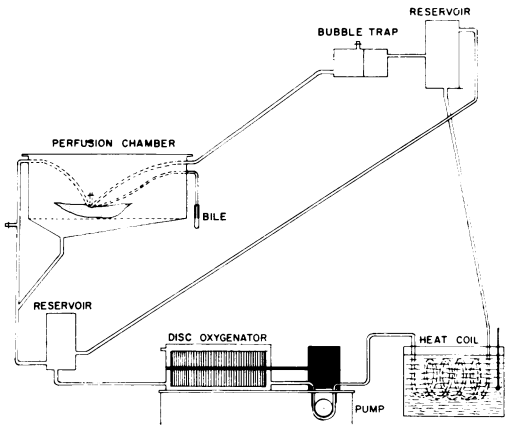

FIG. 1. Diagram of laboratory perfusion system.

from mixing with that subsequently produced during perfusion. The common duct was severed and cannulated. The portal vein and hepatic arteries were isolated at a distance from the liver to avoid damage to branches leading to the left lobe of the liver. When only the vessels connected the liver, 3 mg./Kg. of Heparin were administered intravenously.

A size 10–14 Bardic arterial cannula was inserted into the hepatic artery. The infrahepatic vena cava was severed at the level of the renal veins. A size 24–28 French Bardic cannula was inserted into the portal vein. As a final step, a size 30–36 Bardic venous cannula was placed in the suprahepatic vena cava which was severed at least 2 cm. above the diaphragm. In all cannulations care was taken to avoid air emboli.

The excised liver was washed free of blood by 8 to 10 L. of iced, sterile, heparinized (50 mg./L.) Ringers lactate solution perfused through both the hepatic artery and portal venous lines. Pressures were by 3 feet of gravity into the portal vein and at 200 mm. Hg into the hepatic artery. This ordinarily required 6 to 10 min. The effluent soon became clear and the liver blanched. Intermittent occlusion and release of the outflow catheter alternately distended the distal reaches of the liver with the heparinized solution and provided more complete wash-out.

The asanguinous liver was immediately transferred to a sterile perfusion chamber and the afferent lines attached to the portal vein and hepatic artery. A recording temperature probe was inserted into the liver substance. The common duct cannula was brought out through an aperture in the perfusion chamber. Characteristically, no longer than 15 to 30 min. of hepatic anoxia elapsed between clamping of the portal vein in the animal and resumption of perfusion in the chamber with oxygenated blood. This period of anoxia even at room temperature did not appreciably alter subsequent liver function.[5]

In 12 animals perfusion of the portal vein only was employed.

The vena cavae were variously left open (14 experiments) or cannulated with a 30–36 Fr. catheter (8 experiments) with the effluent leading to a reservoir. Ascitic fluid from the capsule also drained to this reservoir. Fig. 1 is a diagram of this perfusion circuit.

Physiologic conditions of temperature (37° C.), perfusion pressure (200 mm. saline) and pH (7.4) and strict asepsis were maintained. Perfusion from the raised reservoir was non-pulsatile at a rate of 800 to 1,200 cc./min. to the 1-Kg. liver.

Acidosis was asiduously avoided by suitable addition of a 7.5 per cent solution of sodium bicarbonate, since we have shown [6] that acidosis increases vascular resistance.

It was determined that the asanguinous pig liver functioned when perfused with heparinized human blood of any type. All but type B human plasma agglutinates pig erythrocytes, but all human cells are agglutinated by pig plasma. In actuality the prime consisted of 2 L. of heparinized human blood regardless of type utilized the day previously in a pump oxygenator cardiotomy. The blood was reheparinized (2 cc./500 cc.) and one million units of peni-

cillin was added prior to use in the liver perfusion system.

In all 22 experiments blood flow, bile flow and pH were monitored. Clearance of a 7.5-Gm. load of galactose was studied in five experiments quantitating galactose concentration by the enzymatic technic.[10] Acute loads of 0.5 Gm. of ammonium citrate were administered in 10 experiments. Blood ammonium concentrations were measured by the Seligson technic.[17]

Twenty-five microcuries of radioactive Rose Bengal were administered to the perfusion system in 13 experiments, with the subsequent measurement of its disappearance from the blood and its appearance in the bile.

Two hundred fifty mg. of unconjugated bilirubin were added to the system in three experiments and its disappearance from the blood and its appearance in the bile similarly quantitated, utilizing the Mallon technic.[14]

Oxygen utilization was determined by measuring afferent and efferent oxygen content and was correlated with other measurements of liver function. In three experiments response of the perfused liver to 1 to 2 cc. of the choleretic agent Decholin (dehydrochloric acid) was noted by measurement of bile production hourly six times following addition of the drug.

Liver function with perfusion solely via the portal vein was compared to function with additional perfusion through the hepatic artery. Total flow was maintained at 1,000 cc./min. A single line from the reservoir led via a Y-connector to both perfusion cannulae, with no attempt made to control the comparative flows through either portal vein or hepatic artery.

Control studies were performed for each liver function by excluding the liver from the perfusion circuit in order to confirm that neither the mechanical system nor contaminating organisms affected the blood concentrations of the substances under study. Only experiments in which sterility was proved by culture of the perfusate were included.

Three livers were left unperfused in the chamber overnight and perfusion restarted 24 hours thereafter to determine whether such an obviously dead organ could excrete bile by purely mechanical filtration.

To determine hepatic fatigue of the system Rose Bengal, ammonia and galactose loading experiments were performed serially at 6-hour intervals. The longest perfusion, lasting 30 hours, was terminated only for reasons of practicality. Three others were continued over 24 hours. During these and all other perfusions lasting over 3 hours, 75 cc./hour of 10 per cent glucose in water was added to the perfusate.

Results

It was immediately evident that the pig liver washed free of its own blood functioned satisfactorily when perfused with heparinized human blood.[13] There has been no evidence of "outflow block."[6] Flow rates have varied from 800 to 1,200 cc./min. Bile flow, which has proved to be an accurate gross guide of liver function, average 6 to 10 cc./hr. Both blood and bile flow decrease about 30 per cent each 5 hours of perfusion for reasons that are not entirely clear. This occurs despite reheparinization and is not due to major vascular thrombosis.

Perfusion Solely via Portal Vein

Table 1 compares the results of liver function tests when only the portal vein was perfused with similar values when perfusion was through both the portal vein and the hepatic artery. It is important to appreciate that these function tests are made in a high flow system with perfusion of 100 per cent oxygenated blood at a minimum rate of 0.9 cc./Gm. of liver/min. At these high flows perfusion via the portal vein alone fulfills the metabolic demands of the liver since liver function is not al-

TABLE 1. *Liver Function with Perfusion through Portal Vein Compared to Additional Use of Hepatic Artery**

		No. Exp.	Hours 1	2	3	4	5	6	7
Blood flow (cc./min.)	HA ± PV	14	1,050	1,160	1,110	1,110	1,110	1,020	910
	PV	4	1,150	1,125	900	890	960	855	885
Bile flow (cc./hr.)	HA + PV	11	5.9	9.9	9.8	9.1	5.8	5.8	
	PV	2	8.4	10.2	9.2	10.9	5.3		
			Base	5′	10′	15′	30′	45′	60′
Ammonia (49%)	HA ± PV	6	136	1,447	548	265	170	99	84
	PV	4	172	1,474	495	287	194	140	124
RARB % 5 min. cts.	HA + PV	8	7	100		55.3	40.5	37.7	34.3
	PV	5	5.7	100		51.7	41.3	36.9	28.8

* Total non-pulsatile flow 1 cc./Gm. liver/min.

All values are mean of total experiments shown in third column. HA + PV = both vessels perfused. PV = portal vein alone employed. Ammonia = concentration following 0.5 Gm. ammonium citrate. RARB = radioactive Rose Bengal.

TABLE 2. *Clearance of Ammonia Load by Pig Liver Perfused with Human Blood*

Exp. No.	Control	5′	10′	15′	30′	45′	60′
7 a	85	1,390	1,200	1,199	437	223	174
b	112	763	391	225	104	187	
16 a	200	2,121	988	825	591	400	340
b	345	1,525	417	153	130	105	97
19 a	209	1,610	268	74	63	57	56
b	76	1,740	696	222	52	52	52
Average	167	1,525	660	449	229	170	154
Control (without liver)	80	912	1,074	1,085	1,200	1,016	1,018

Ammonium citrate, 0.5 Gm., added to 2 L. perfusate at 0-time.
Annomia expressed as μGm./100 ml.

TABLE 3. *Bilirubin Clearance by Pig Liver Perfused with Human Blood*

		Minutes				Hours				
Exp. No.	Control	5	15	30	60	2	3	4	5	6
12	0.7	11.8	11.8	11.1	10.3	7.9	7.2			
13	0.3	13.3	12.2	11.2	6.0	3.5	3.2			
14	0.6	9.8	10.0	9.3	8.2	6.9	5.7	4.7	4.3	3.7
Average (3 exp.)	0.5	11.6	11.3	10.5	8.1	6.1	5.3	4.7	4.3	3.7
Control (without liver)	0.4	8.7	10.0	10.3	10.3	10.1	10.1	10.3	10.1	10.1

Bilirubin load of 250 mg. at 0-time.

TABLE 4. *I^{131} Rose Bengal Clearance by Pig Liver Perfused with Human Blood*

Exp. No.	Control	5′	15′	30′	45′	60′
1	3.4	100	47.9	42.1	42.3	40.7
2	5.2	100	59.8	33.7	29.5	27.8
5	1.0	100	59.2	31.4	24.6	23.5
	19.0	100	72.5	58.0	50.5	47.5
6	3.0	100	52.0	39.8	34.4	33.2
	5.6	100	56.7	49.5	51.5	40.7
7	14.7	100	49.3	42.6	41.1	39.3
	29.3	100	58.1	52.9	51.6	50.0
13	4.6	100	28.1	13.3	12.3	13.3
14	0.6	100	71.1	54.4	55.2	42.2
15	10.8	100	56.2	36.7	31.2	30.5
Average (11 exp.)	8.8	100	55.5	41.3	38.5	35.3

I^{131} Rose Bengal 25 microcurie added at 0-time. Values are % counts at 5 minutes.

TABLE 5. *I^{131} Rose Bengal Excretion in Bile During Pig Liver-Human Blood Perfusion*

Exp. No.	1 Hr.	2 Hr.	3 Hr.	4 Hr.	Total % of Load
16	6.3	5.3	5.3	5.1	22.0
14	7.1	13.5	10.1	7.0	37.7
19		15.7	16.2		31.9
20	12.0	13.9	9.5	8.9	45.3
Average	6.6	10.1	8.3	6.2	34.2
Dead Liver	0.05	0.06	0.06	0.03	0.2

25 microcurie I^{131} Rose Bengal. Values are % total injected I^{131} appearing in bile.

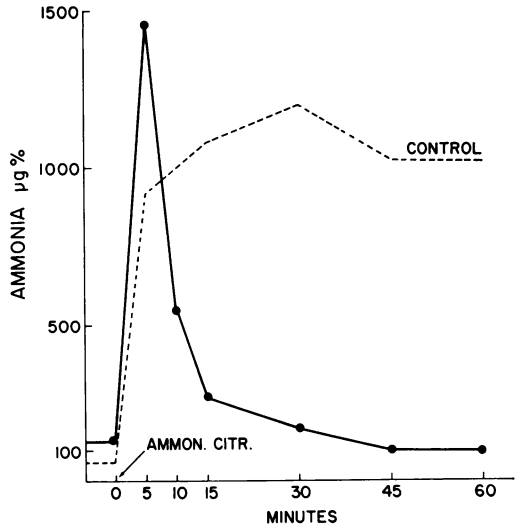

FIG. 2. Graph of mean ammonia clearance (6 experiments) compared to control without liver in the circuit.

tered by omitting hepatic artery perfusion. Since a flow of 1.0 cc./Gm. of liver/min. is achieved easily in this system, all subsequent laboratory perfusions were solely via the portal vein at approximately 1,000 cc./min. with the reservoir 200 mm. above the hepatic hilum.

Ammonia Clearance. Table 2 records the ammonia clearance in six ammonium citrate loading experiments performed on three pig livers. Figure 2 graphically illustrates the mean ammonia concentrations of six such experiments, compared to controls in which the liver was excluded from the circuit. It is evident that even the unphysiologic concentrations of ammonia are cleared by the pig liver within 15 to 30 min.

Bilirubin Clearance. Pertinent data on the three bilirubin clearance studies is shown in Table 3. The pig liver efficiently clears bilirubin into the bile in high concentrations even during prolonged periods of perfusion.

Rose Bengal, Galactose, and BSP Excretion. Table 4 records the data of the I^{131} Rose Bengal clearance and Table 5

TABLE 6. *BSP Retention During Pig Liver-Human Blood Perfusion*

Exp. No.	Control	2′	5′	10′	15′	30′	45′	60′
16	1	50	38	18.2	18.0	19.0	15.7	11.2
19	0	85	57	25	22	22	27	30
20	0	85	16	11	11	13	7	7
Average (3)	0.3	73.3	37.0	18.0	17.0	18.0	16.6	16.0

Values are in percentage of administered load of BSP.

TABLE 7. *Galactose Clearance During Pig Liver-Human Blood Perfusion*

Exp. No.	Control	15′	30′	45′	60′	75′	90′	2 Hr.	3 Hr.
11 a	0.05	1.16	0.78	0.43	0.09	0.09	0.09	0.09	0.09
b	0.03	1.57	1.05	0.57	0.13	0.13	0.09	0.09	0.09
12	0.02	1.00	0.65	0.35	0.39	0.05	0.05	0.05	0.05
13	0.05	2.73	1.84	1.62	1.20	0.90	0.20	0.09	0.05
16	0.06	1.82	1.60	1.36	1.16	0.92	0.70	0.44	0.18
Average (5 exp.)	0.04	1.63	1.18	0.87	0.59	0.42	0.22	0.14	0.09
Control (without liver)	0.04	1.46	1.40	1.40	1.40	1.40	1.40	1.40	1.40

Galactose load of 7.5 Gm. Values are mg./cc.

analogous data quantitating its appearance in the bile. Tables 8–10 document analogous metabolism of BSP, galactose, and oxygen utilization. BSP clearance is graphically shown in Figure 3. The choloretic activity of Decholin® (dehydrocholic acid) routinely evoked a response in the perfused pig livers manifested by an increased volume of bile excretion. Analysis of the bilirubin and I^{131} Rose Bengal content of the bile following addition of Decholin demonstrated that this merely represented dilution, not an increase in solute excretion.[4]

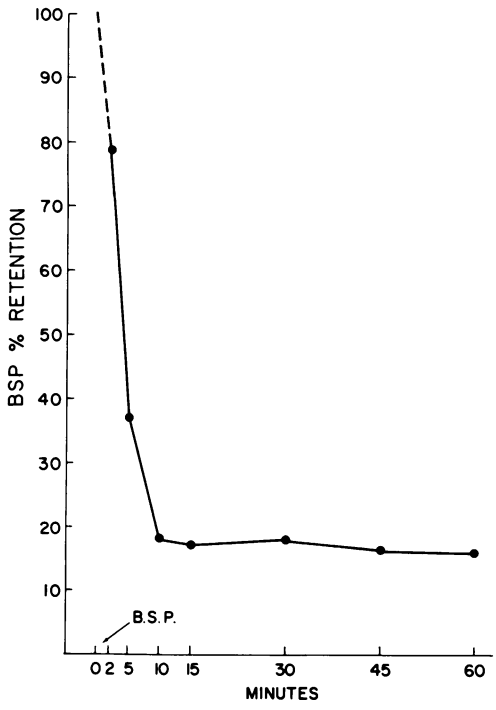

FIG. 3. Graphic illustration BSP clearance, pig liver with human blood (3 experiments).

Part II. Clinical Studies

These laboratory studies provided sufficient evidence that the pig liver would function when perfused with human blood and led to clinical trial of the technic for the temporary support of patients in hepatic failure. Operative technics were perfected for attachment of both dogs and pigs to an extracorporeal homologous liver in a series of 18 experiments prior to clinical use.

To date, eight patients in terminal liver coma have had temporary hepatic assistance by perfusion of their blood through an extracorporeal pig liver.

TABLE 8. *Oxygen Consumption* During Pig Liver-Human Blood Perfusion*

Exp. No.	1st Hr.	3rd Hr.	6th Hr.
9	0.020	0.009	
10	0.027		
11	0.022	0.019	
15	0.016	0.007	0.005
16	0.029	0.014	0.009
Average (5 exp.)	0.023	0.012	0.007

* cc./Gm. liver/min.

TABLE 9. *Clinical Experience: Bile and Bilirubin Excretion from Pig Liver*

Patient No.	15′	30′	45′	60′	75′	90′	105′	120′	135′	3 Hr.	4 Hr.	5 Hr.	6 Hr.	Bile Total	Bile Mean/Hr.	Total Bilirubin (mg.)
1. Bile cc.	3	2	2	2										9	8	
Conc. mg.%																
Bilirubin mg.																
2. Bile cc.		8		9		7								24	16	
Conc. mg.%		281		370		256										
Bilirubin mg.		22		33		18										73
3. Bile cc.	3	4	5.5	5.3	5.3	4.3	4.2	3.9						35.5	17.7	
Conc. mg %	28	80	94	66	59	80	94	101								27.2
Bilirubin mg.	0.84	3.2	5.2	3.5	3.1	3.4	4.0	4.0								
4. Bile cc.		6.5		8.5					29	15.5				59.5	19.8	
Conc. mg.%		456		352					352	360						
Bilirubin mg.		29.6		29.9					102	55.8						217.3
5. Bile cc.				15				16		14.5	21.5	15	16	98	16.3	
Conc. mg.%																
Bilirubin mg.																
6a. Bile cc.				20				22.5		23.5	27.5	24.5	22	140	23.3	
Conc. mg.%				1,080				920		880	910	1,000	1,000			
Bilirubin mg.				216				207		206.8	250	245	220			1,344.2
6b. Bile cc.				5				6		5	4	3	3	26	4.3	
Conc. mg %				640				1,080		560	800	640	800			
Bilirubin mg.				32				64.8		28	32	19.2	24			200
7a. Bile cc.		1.3		1.3				1.4		3.1				7.1	2	
Conc. mg.%				40				18		38						
Bilirubin mg.				1.04				0.252		1.178						2.47
7b. Bile cc.		0.75		0.4				0.35		1.25	1.1			3.75	1	
Conc. mg.%				50				48		19	29					
Bilirubin mg.				5.7				0.168		0.237	0.264					6.36
7c. Bile cc.				1.2				1.6						2.8	1	
Conc. mg.%				18				28								
Bilirubin mg.				0.216				0.320								0.536
8. Bile cc.	0.5			1.0		2.0		2.0	3.5	3.5	6.5	9.5	1.5	30.0	5.4	
Conc. mg.%	11.2			11.2		112.8		76.8	74.4	76.8	112.8	107.2	57.6			
Bilirubin mg.	0.05			0.11		2.25		1.5	2.6	2.6	7.2	10.1	0.85			27.26

TABLE 10. *Blood Ammonia Concentration* Prior to, During and Following Clinical Perfusion*

Patient No.	Preop.	Perfusion											Postop. Day							
		5′	10′	15′	30′	45′	60′	75′	90′	120′	3 Hr.	4 Hr.	5 Hr.	6 Hr.	1	2	3	4	5	6	7	8
1	1,590	1,292	180		239	209	584								642							
2		78		88	120	104	88	1,200	104													
3	280	120		196	168	100	100			100					196	190						
4	400			30			30			20												
5	320			180	170	70	60								230				100		3,640	1,100
6a	390			240	220		100								250	300						
6b							308															
7a	163			99.9	84.2	99.9	105			105	68.4				93.75	153.5	113	134				
7b	166			120						46	66.7	113			169	155.8	75	120	138			
7c	199				170		105			135	140				189	138						
8	151				109		0			0	0	0	0	0								

* In μGm.

Selection of Patients

Because of the obvious unpredictable outcome of these studies only patients totally resistant to other therapy and judged to have a hopless prognosis have thus far been admitted to this study. The experimental nature of the procedure was fully explained to a responsible relative. All but one patient has been in terminal coma following esophogeal bleeding secondary to alcoholic cirrhosis. One boy of 17, partially comatose for 14 days and totally unresponsive for 10 days with viral hepatitis, also has been perfused through a pig liver.

These pioneer efforts have been timid and arbitrarily limited in length as we have carefully evaluated the technic. Progressive confidence has developed both in the safety and potentiality of such perfusions.

Technic

As clinical experience has accumulated, there has been a progressive change in the perfusion technic. The first patient was placed in parallel to a perfusion system duplicating the laboratory experiment as shown in Fig. 4a. Cannulae led from the common femoral artery to a reservoir and thence to the portal vein and hepatic artery.

With the second patient (Fig. 4b), the pump oxygenator was entirely excluded once the patient was in the system.

In the third patient (Fig. 4c) the common femoral artery cannula led directly to the portal vein of the extracorporeal liver, thus omitting both the oxygenator and the reservoir. The hepatic artery was left unperfused in the next four cases.

In the fourth patient, the superficial femoral artery and the saphenous vein were used for cannulation in order to allow the patient unhindered movement as he began to respond during perfusion.

The fifth perfusion was the first in which a cannula was placed as an effluent in the suprahepatic vena cava in order to mini-

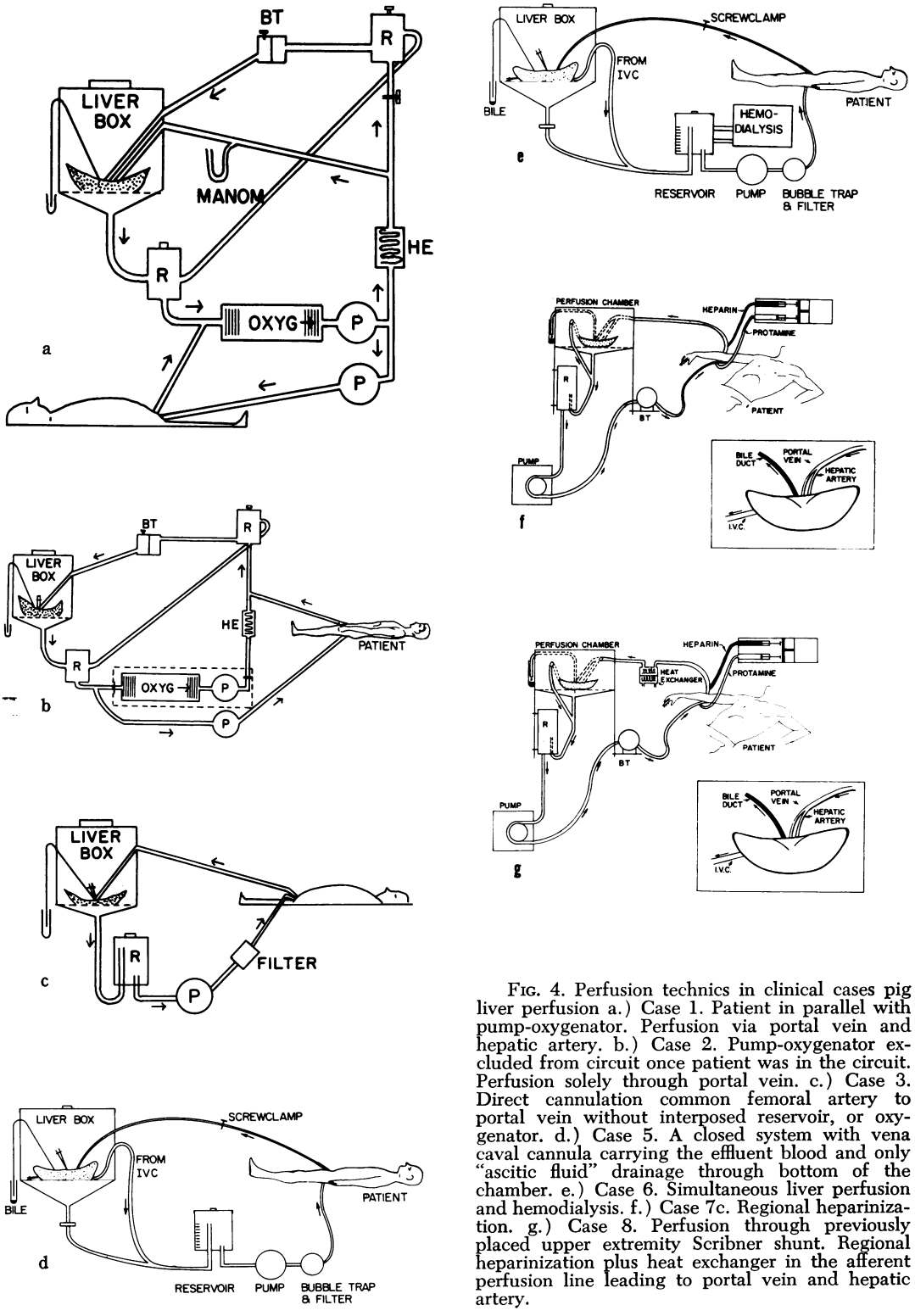

FIG. 4. Perfusion technics in clinical cases pig liver perfusion a.) Case 1. Patient in parallel with pump-oxygenator. Perfusion via portal vein and hepatic artery. b.) Case 2. Pump-oxygenator excluded from circuit once patient was in the circuit. Perfusion solely through portal vein. c.) Case 3. Direct cannulation common femoral artery to portal vein without interposed reservoir, or oxygenator. d.) Case 5. A closed system with vena caval cannula carrying the effluent blood and only "ascitic fluid" drainage through bottom of the chamber. e.) Case 6. Simultaneous liver perfusion and hemodialysis. f.) Case 7c. Regional heparinization. g.) Case 8. Perfusion through previously placed upper extremity Scribner shunt. Regional heparinization plus heat exchanger in the afferent perfusion line leading to portal vein and hepatic artery.

mize the air-blood interface and damage to elements in the blood (Fig. 4d).

The sixth patient was perfused twice. During the second 6-hour perfusion, an artificial Kol kidney was placed in parallel to the liver perfusion system to assist in the treatment of anasarca and the pre-existing renal shutdown (Fig. 4e).

The seventh patient underwent three separate perfusions. In the first, both the hepatic artery and the portal vein were again utilized for perfusion since laboratory studies had shown better hepatic function at low flow rates if both vessels were thus employed. In the second perfusion on this patient, regional heparinization was employed, thus keeping the liver heparinized but neutralizing the perfusate with protamine at the point of its reintroduction into the patient's saphenous vein. This was also the first perfusion in which low flow were employed (0.5 cc./Gm. liver/min.). In the third perfusion on this patient, cannulation was via a previously placed Scribner shunt [9] fixed in the brachial artery and vein (Fig. 4f). A very low flow (0.2 cc./Gm. liver/min.) was purposely employed.

In the eighth patient, a disposable heat exchanger was placed proximal to the liver and low flow employed (0.3 cc./Gm. of liver/min.) from the upper arm Scribner cannula (Fig. 4g).

Site of Cannulation. Unconscious and unresponsive prior to perfusion, the majority of the patients became restless, variously responsive and uncomfortable during perfusion. Restlessness was minimized by placing pillows beneath the patient on the table and by avoiding indwelling esophageal or rectal thermometers. Patients totally comatose prior to perfusion reacted to the discomfort of the open cut-down wound. Moving the cannula to the saphenous vein and superficial femoral artery allowed greater mobility, but with prolonged and repeated perfusion it became necessary to employ an indwelling upper extremity Scribner shunt. A flow of 600 cc./min. from a forearm artery has been achieved without the use of a pump in such a cirrhotic patient.

Temperature. Temperature of the pig liver washed free of its cells with cold ringers lactate was 12° C. when transferred to the perfusion chamber. Characteristically, it required 15 to 30 minutes of perfusion for the liver to reach 37° C. During this time, function of the hypothermic liver was invariably impaired.[2] Even when perfused at high flows the isolated livers tended to cool. To achieve optimum function, therefore, a heat exchanger in the afferent line was employed in Patient 8 to maintain a constant temperature of 37° C.

Occasionally a transient hypotension and chill accompanied the re-entry to the patient of blood traversing the chilled liver during the first few minutes of perfusion.

Hypotension. The asanguinous liver as it is transferred to the perfusion chamber is like an empty sponge and requires blood for its engorgement. To avoid oligemia in the patient as he bleeds into the liver, a transfusion of 500 cc. is rapidly performed from the reservoir, thus avoiding hypotension that follows the sudden establishment of such a negative gradient.

Flow. The perfusion system imposes an extracorporeal burden on the heart equal to the total flow through the liver. The minimum flow achieving maximum hepatic function is obviously best suited to the clinical needs. Under conditions of low flow (less than 0.5 cc./Gm. liver/min.) perfusion through both the portal vein and the hepatic artery achieves more efficient function than attainable at similar low flows through the portal vein alone.[18] For this reason, both the hepatic artery and portal vein were utilized during the latter part of this clinical experience.

Preparation of Animal Donor. For the first six patients the liver was excised from the pig in an operating room adjacent to

the one utilized for the patient. This has obvious administrative disadvantages! Subsequent laboratory studies have shown that the liver left within a cadaver will maintain its efficiency for 1 hour [19] and if excised and left at room temperature will function normally after 2½ hours of vascular interruption.[5] In these preparations the liver is cooled by the blood wash out as soon as it is excised, so that there is no immediacy for its transferance to the perfusion chamber. Hepatectomy from the donor animal thus can be performed in a nearby animal laboratory and the excised sterile liver carried at leisure to the hospital operating room for attachment to the patient.

Bleeding and Heparinization. The greatest problem in the first four patients was the onset of bleeding when the patient was reheparinized. The problem is complex in these patients who have advanced liver disease: a low prothrombin activity, diminished platelets, increased fibrinolysins, the necessity for anticoagulation and exposure to prolonged perfusion. Reheparinization was diminished from 4 mg./Kg./hr. to 2 mg./Kg. every four hours. An entire spectrum of coagulants were variously employed, including fresh blood, platelet transfusions, fibrinogen, epsilon amino caproic acid, calcium and a commercially available trypsin inhibitor (Trasylol®). The problem was not solved satisfactorily until regional heparinization was employed in the seventh case. One mg. of heparin was added per 100 cc./min. flow of arterial blood as it emerged from the patient. Protamine, 1.5 mg./100 cc./min., was used to neutralize the anticoagulant as the effluent from the liver was pumped into the patient's vein. The efficiency of this technic in achieving anticoagulation in the perfused liver and a normal clotting time in the patient is illustrated in Figure 5.

Liver Function Tests During Perfusion. Rose Bengal clearance, BSP clearance, plasma bilirubin and ammonia blood concentration were measured within a few

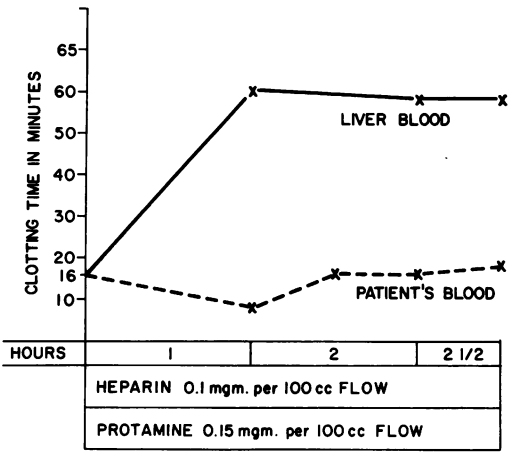

FIG. 5. Effectiveness of regional heparinization in achieving anticoagulation in the liver perfusion system and a normal clotting time in the patient.

hours prior to, and again serially while, the patient was on the pig liver perfusion.

Blood and bile flow were recorded every 15 to 30 min., with quantitation of bilirubin, Rose Bengal or BSP in the bile excreted by the pig liver. Serum electrolytes and a series of hematologic studies were made during the early clinical perfusions but gradually were omitted as they proved to be unrewarding. Blood pH was measured hourly and sodium bicarbonate added, when necessary, to avoid acidosis.

Blood Flow was limited only by the size of the cannulated artery, the arterial pressure and the height of the liver above the patient. There was never any evidence of outflow block in the liver. Regulated flows ranged from 1,200 to 200 cc./min. The latter (in Patient 7c) was inadequate for proper function of the 1-Kg. liver.

It was observed that the best bile flow was attained in those perfusions in whom a short line transmitted a pulsatile flow from the patient's artery to the liver. The value of pulsatile flow to the hepatic artery is currently being examined experimentally.

With the vena cava cannulated, the only fluid that escaped from the liver capsule represented "ascites" which was drained into the reservoir. The volume of such

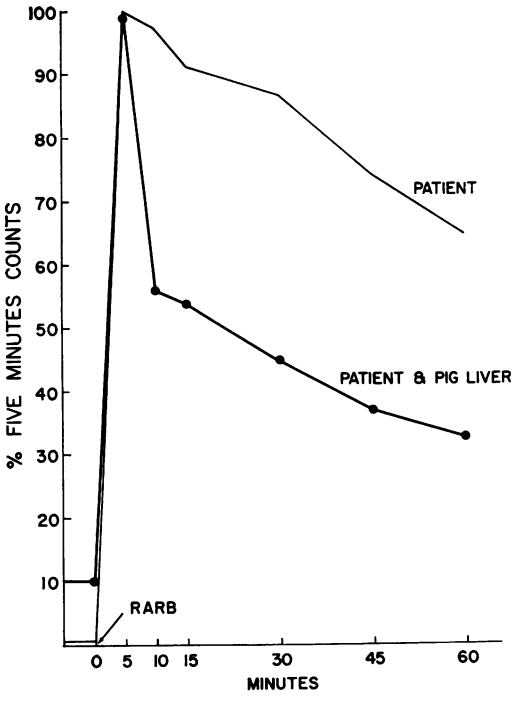

FIG. 6. Rose Bengal I^{131} clearance in Patient 2 prior to and during pig liver perfusion.

"ascites" formation varied directly with the hepatic venous pressure. With no resistance, such fluid collection characteristically was 4 to 8 cc./min. but trebled when hepatic venous outflow was obstructed.

Bile Flow has varied from 1 to 27 cc./hr. as shown in Table 9. Both in clinical and laboratory studies bile flow has proved to be the single most reliable index of hepatic function. It is perhaps significant that the greatest flows were achieved where there was a large bore, short cannula leading from the pulsating femoral artery to the pig liver placed immediately over the thigh. Under such circumstances there was minimal dampening of arterial pulse or cooling.

Bilirubin. The patients were all jaundiced at the time of perfusion. Data concerning bile and bilirubin output is shown in Table 9. Bile output was 27 cc./hr. in one patient (6A) who excreted 1,344 mg. of bilirubin and 140 cc. of bile during the 6-hour perfusion. Bilirubin concentration in the bile ranged from 3 to 1,080 mg./100 ml., indicating efficient metabolic activity of these heterologous livers.

Rose Bengal and BSP. Both of these function tests demonstrated the supplementary activity of the extracorporeal liver compared to control measurements made immediately prior to perfusion. Figure 6 illustrates that Rose Bengal was cleared at almost twice the control rate during perfusion in Patient 2.

Ammonia Clearance. Table 10 records the values of circulating blood ammonia prior to, during and after the various perfusions. Although the data have a wide variation, it is evident that these extracorporeal livers promptly clear enormous amounts of ammonia from the patient's blood. The exceedingly high ammonia concentrations of the priming blood occasionally distort the measurements immediately prior to perfusion. During the latter part of this experience, the prime was carefully circulated through the patient for 10 to 15 min. prior to putting the liver in the system. Figure 7 illustrates one of the more effective perfusions in reducing blood ammonia concentration. As in other clinical experience, the correlation was not exact between blood ammonia concentration and liver flap or neurologic status.[8]

Appearance of the Liver. Although the liver often appeared irregularly cyanotic after 3 or more hours of perfusion, its color

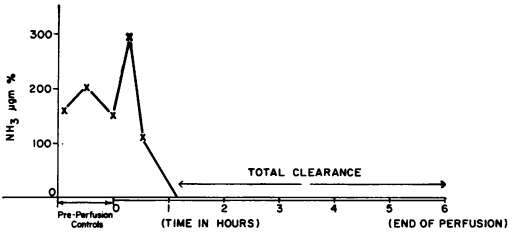

FIG. 7. Case 8, illustrating effectiveness of the pig liver in lowering blood ammonia concentrations.

did not necessarily reflect its functional efficiency. Even after 6 hours of such clinical perfusion there was no evidence of thrombosis either by post-perfusion arteriography or upon histologic examination.

Oxygen Utilization. The mean oxygen utilization during the clinical perfusions was 1.8 cc./100 Gm. liver/min. During the periods of lowest flow the nadir of oxygen saturation, 69 per cent, was reached. As flow decreased, the effluent blood from the pig liver became visibly more cyanotic.

Clinical Response. Every patient thus far perfused with a heterologous liver has been comatose and unresponsive prior to connection to the extracorporeal liver. When cut-downs were needed for cannulation, local anesthesia characteristically was not even required. There has been a wide variation in the clinical response to perfusion, which is summarized in Table 11. In every instance there has been some evidence of neurologic improvement during the perfusion. The least response (7c) was in an inefficient low-flow (200 cc./min.) run in which there was little bile flow from the hypothermic liver and little alteration in blood ammonia.

The most dramatic improvement came in Cases 3, 5, 6a, 7a and 8, in which the patients awoke sufficiently to respond to spoken commands. Patient 5 (the only case of viral hepatitis) had been totally unreactive for 10 days, yet began to respond within 15 minutes after the start of perfusion and was speaking coherently at the end of the 6-hour perfusion.

In Case 2 and Case 4, the patient began to react to painful stimuli within 9 and 11 minutes, respectively, after the onset of perfusion. In neither had there been either a preperfusion acidosis or hypoglycemia.

The neurologic improvement occasioned by perfusion lasted a variable period of time. At best (in Case 5), the patient was coherent for 4 days after perfusion and then began a gradual deterioration that ended in death on the eighth post-perfusion day.

The most remarkable relapse occurred within minutes following the termination of the first perfusion in Case 7. Awake, talking and asking for a cigarette at the end of the 3 hour and 40 minutes of perfusion, he became somnolent as he was moved off the operating table. A blood sugar determination performed 30 minutes later was 57 mg./100 ml. and when repeated 10 minutes later was 30 mg./100 ml. With the administration of 50 per cent glucose solution, the patient again awoke but required enormous amounts of intravenous glucose to avoid hypoglycemic coma. Despite 20 per cent and even 50 per cent intravenous glucose solutions, the patient never spilled sugar in his urine. None of the other patients demonstrated hypoglycemia.

Perfusion by a heterologous liver has neither impaired nor helped renal function. The four patients anuric prior to pig liver perfusion continued thereafter without urinary output. On the contrary the other four patients excreting urine prior to perfusion continued to do so at an unaltered rate.

The seventh patient was eating and talking and appeared to be recovering from his liver failure 5 days after the first pig liver perfusion when he suddenly had a torrential hemorrhage from his esophageal varices. These were ligated through a gastrotomy, at which time a Scribner shunt was placed in his arm. Six hours later he was comatose and was placed for the second time on a liver perfusion. During the 4-hour perfusion he began to respond and showed marked improvement for 3 days when he again became comatose. He did not respond to the third perfusion which was performed with a flow of 200 cc./min.

The survival of these patients following heterologous perfusion is shown in Table 11. None has lived more than 12 days. They were chosen for this study only when they were moribund and their prognosis was

TABLE 11. *Clinical Summary of Response to Pig Liver Perfusion During Hepatic Failure*

Patient No.	Age Sex	Etiology	Lgth. Perf.	Ammonia a. Pre b. Post	Bilirubin a. Pre b. Post	Bilirubin Bile mg. total	Bile Flow 1 Hr.	Perf. Flow cc./min.	React. Perf.	Clin. Change	Complications	Survival
1.	42 M	Alcohol cirrhosis	70″	1,590 584	31 11		9.3	600 1,200	0	+	Bleeding foll. rehepar.	48 hr.
2.	M	Alcohol	1½ hr.	104	27.4	73.4	16	500 900	0	+	Bleeding following rehep.	8 hr.
3.	47 M	Alcohol cirrhosis	2 hr.	2,800 1,000	20.5 15.3	27.2	16.2	500	0	++	Bleeding 6 hrs. after rehep.	48 hr.
4.	40 M	Alcohol	3 hr.	400 200	18.4 7.8	216.5	19.8	500 750	0	+++	Bleeding after rehep.	12 hr.
5.	17 M	Post necr.	6 hr.	320 60	34.3 21.4		16.3	960	0	++++	0	8 days
6a.	54 M	Alcohol	6′10″	390 220	62.9 38.5	1,344	23.3	600 750	0	+++	0	2nd perfusion (6b) 48 hr. after the first
6b.			6 hr.	308 308	48.6 44.4	199	4.3	200 375	0	+	0	12 hr.
7a.	48 M	Alcohol cirrhosis	3½ hr.	99.9 68.4	2.73 2.9	2.47	2	400 1,000	0	+++	Hypoglycemia bleeding foll. rehep.	2nd perfusion 5 da. after 7a
7b.			3½ hr.	134 113	20 18	6.36	1	600 1,000	0	++	Bleeding from tracheostomy + aspiration	3rd perfusion (7c) 6 da. after 7b
7c.			3 hr.	103.6 140	20 23	0.536	1	150 200	0	0	Inadequate perfusion flow	2 da.
8.	49 M	Hemochrom-atosis	5 hr. 45 min.	151 0	10.2 7.9	27.26	5.2	300 800	0	++	0	1 da.

otherwise judged hopeless. None of the patients has appeared to be detrimentally affected by the perfusions. Neither in the original perfusions nor in the two patients in whom perfusions were repeated has there been any evidence of an allergic or antigenic reaction.

Discussion

The pig liver was chosen for these heterologous perfusions because of our long experience with perfusing this species and because of the sterility of pig liver compared to that of the dog. Pig livers are remarkably free of the increased vascular resistance which so plagues perfusion of the liver of some other species.[6] Following the initial pig liver human blood studies, we have confirmed that a calf liver will function equally well when perfused with human blood. A dog liver will not. It is our intention to utilize a calf liver should a patient prove to be sensitive to a pig liver perfusion.

Carbon [14] tagged glycine and lysine both appear in the albumin fraction of protein emerging from the perfused liver. It is assumed that such a protein carries the pig protein genetic code. Although bovine plasma has been extensively evaluated as a plasma substitute, there is little data as to the comparative antigenicity of porcine plasma fractions.[12] The liver does not synthesize gamma globulin.[15]

Neither on the original, nor on the repeat, perfusion was there any evidence of a sensitivity reaction to the perfusion. The multiple perfusions employed so far have been on desperately ill patients in whom the immune mechanisms are probably depressed, and the total period between first and last perfusion has been no more than 10 days.

These are only preliminary clinical trials of this new technic by which a patient suffering from hepatic failure might receive temporary metabolic assistance from an extracorporeal liver. The ultimate value of the procedure remains to be defined. It may be of benefit to three types of patients: 1) the cirrhotic with marginal liver reserve who has suffered an acute overpowering metabolic insult, but who might recover if he could survive the initial episode of coma; 2) a patient comatose with acute viral hepatitis or acute toxic hepatitis with a potentially reversible hepatic lesion; and 3) a patient immediately prior to or following homograft replacement of an irreversibly damaged or disease liver. In the latter circumstance, the heterologous extracorporeal liver could function as a temporary support much as the artificial kidney or peritoneal dialysis supports patients undergoing kidney homotransplantation.

When infection and tumor can be excluded, cross circulation has been suggested by Joyeuse [11] and practiced by Volwiler.[3] Such a technic implies an inevitable risk to the donor which is obviated when a temporary extracorporeal heterograft liver is employed.

Summary and Conclusions

The excised pig liver washed free of its own blood will function in a normal manner when perfused with heparinized oxygenated human blood.

Eight patients otherwise moribund from hepatic coma have been perfused a total of eleven times for from 1 to 6½ hours through an extracorporeal pig liver. Each has evidenced the metabolic activity of the perfused liver. Clinical improvement has varied from dramatic return of consciousness during perfusion to only slight neurologic improvement. None of these moribund patients with far advanced hepatic failure thus far selected for perfusion has been a long term survivor.

One patient has undergone three separate perfusions without incident. In another patient, simultaneous hemodialysis for renal shutdown was employed while the patient was being perfused through a pig liver.

The progressive changes made in the perfusion technic have been described and their physiologic rationale outlined.

It is believed that this new technic for supporting patients with potentially reversible hepatic insufficiency is worthy of further careful clinical trial.

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Discussion

Dr. Lloyd M. Nyhus (Seattle): There is no question that these animal livers, used in perfusion technics, will clear by-products of metabolic processes. The many problems which relate to the new approach, of course, remain to be studied in depth.

Drs. Bibler, Condon and I have undertaken a study to determine if heterologous protein is present in human blood and canine blood after perfusion through a heterologous (bovine) liver, and to determine if the presence of such protein would lead to sensitization. In four or five calves we flushed the liver completely with Ringers solution until we were quite certain that all or most of the calf blood had been removed; we then perfused human blood through these livers for 6½ hours.

(Slide) This is a slide of immuno-electrophoretic plate showing rabbit antibovine serum which has been placed at this spot. The human blood was put in here (Indicating). The top portion of the slide shows the normal preperfusion antigen-antibody precipitation reaction. After perfusing this line of precipitation indicates new protein—new calf protein—in this human blood as represented by these new curves.

(Slide) We were interested to see if this would happen with porcine or pig liver. We see here the preliminary precipitation which all of us have within our body in cross-reactivity with porcine protein, but again, we see the new protein in the human blood, which is porcine protein.

(Slide) Does this antigen-antibody reaction result in sensitization? We studied this matter further by producing hepatic insufficiency in dogs.