Reprinted from the Archives of Surgery
August 1968, Volume 97

166

Specific Bacteriologic Problems After Orthotopic Liver Transplantation in Dogs and Pigs

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IN THE literature of experimental hepatic homotransplantation, it has not been emphasized that liver sepsis posed an unusual problem or occurred with exceptional frequency although reports from our laboratory[1] and by Stuart et al[2] and Fonkalsrud et al[3] mentioned the development of liver abscesses as one postoperative complication. In these dogs, heavy antibiotic therapy was given. Moreover, Alican and Hardy[4] observed hepatic abscesses in dogs which had received autotransplantation of the liver and biliary drainage by cholecystoduodenostomy. Their findings were particularly significant since neither an immunologic barrier for immunosuppression were involved in their experiments.

Since then, interest has been directed to the specific problem of liver homograft sepsis by the development of hepatic abscesses in four of six children who were treated at our institutions by orthotopic liver homotransplantation. The features and treatment of this complication in patients has been discussed elsewhere.[5] The present report is concerned with experimental studies designed to elucidate the pathogenesis of the posttransplantation liver sepsis and to establish guidelines for its prevention by evaluating the influence of ischemia, type of biliary drainage, rejection, immunosuppression, and antibiotics.

Submitted for publication March 13, 1968.

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Read before the 25th annual meeting of the Central Surgical Association, Cleveland, Feb 23, 1968.

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Methods

General Techniques.—The animals used were mongrel dogs and pigs of mixed breeds (predominantly Yorkshire and Hampshire) which weighed 13 to 17 kg (29 to 38 lb), and 15 to 27 kg (33 to 60 lb), respectively. The dogs were anesthetized with sodium pentobarbital and phencyclidine hydrochloride (Sernalyn) and a combination of sodium thiamylal (Surital) and succinylcholine chloride (Anectine) was used for the pigs. Postoperatively, liver function was followed with analyses of serum bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT). Antibiotics were not administered during the preoperative or postoperative period except in group 5. Fifteen animals surviving less than three postoperative days were eliminated from the study since these early fatalities were generally attributable either to technical or postanesthetic complications. Three animals that died later of intussusception were also excluded.

Further mention will be made only of the 51 completed experiments.

Experimental Groups

Sham Operations.—*Group 1A.*—Five dogs had the abdominal cavity left open for three to 3½ hours after liver biopsy, approximately the same time as that required for orthotopic transplantation. Cholecystotomy and duodenotomy were made initially and closed at the end of the waiting period. The vascular supply to the liver was not interrupted.

Group 1B.—Four pigs received the same operation described in group 1A with the addition of splenectomy.

Simulated Autotransplantation in Pigs.—*Group 2A (Intact Biliary Drainage).*—In five pigs the liver was isolated from the circulation for 40 to 46 minutes by crossclamping the suprahepatic and subhepatic vena cava, portal vein, and hepatic artery. The blocked splanchnic system was decompressed with a splenic-jugular venous bypass during the anhepatic phase. After isolation, the liver was quickly cooled by infusion through the portal vein of 1,000 to 2,000 ml of chilled (4 C) lactated Ringer's solution. The fluid was allowed egress from a venotomy in the suprahepatic vena cava. The common duct was temporarily crossclamped. Cholecystotomy and duodenotomy were made and closed separately, thereby maintaining a natural biliary drainage.

Group 2B (Cholecystoduodenostomy).—Five pigs underwent the simulated autotransplantation described in group 2A with liver isolation for 39 to 56 minutes. However, the common duct was ligated and divided and a cholecystoduodenostomy was performed.

Orthotopic Homotransplantation Without Immunosuppression.—*Group 3A.*—Five dogs were provided with orthotopic homografts from nonrelated donors using previously described techniques including a donor hepatic artery to recipient hepatic artery anastomosis and a cholecystoduodenostomy.[6,7] The donors were cooled to between 30 to 34 C and killed by removal of their livers at the same time as the organs were core-cooled by intraportal infusion of chilled lactated Ringer's solution.

Group 3B.—Five pigs received orthotopic homografts. Deviations from the techniques used for dogs included splenectomy and the employment of a splenojugular venous bypass during the anhepatic phase. A portacaval shunt or separate decompression of the inferior vena cava was not employed.

Orthotopic Homotransplantation with Immunosuppression.—*Group 4A.*—Five dogs were pretreated for two to three days with subcutaneously administered canine antilymphocyte globulin (ALG). Azathioprine therapy was started at the time of transplantation and continued in the maximum daily doses which did not cause leukopenia. A one-week course of prednisone was administered in progressively diminishing daily doses, which started at 3 mg/kg of body weight on the day of operation. As in the other animals of groups 1 to 3, antibiotics were not given.

Group 4B.—Five pigs were treated with azathioprine and prednisone as in group 4A. Antilymphocyte globulin was not employed.

Orthotopic Homotransplantation with Immunosuppression and Antibiotic Treatment.—*Group 5.*—Twelve dogs were treated with ALG, azathioprine, and prednisone as in group 4A. They were evaluated for survival and bacteriological studies were not obtained. On the day prior to transplantation, benzathine pencillin G therapy (1.2 million units) was started. In addition, a two-week course of chloramphenicol (250 mg to 500 mg daily) or tetracyline (500 mg daily) was begun three to four days prior to operation. Chronic survivors had their spectrum antibiotic changed every two weeks.

Bacteriologic Studies

At operation, both the donor and recipient were examined bacteriologically by culturing pieces of the livers and gallbladder walls, bile, duodenal contents, and portal venous blood. Daily blood cultures were obtained by femoral venipunctures, beginning one day before transplantation. With the development of two or three positive blood cultures, or after an arbitrary period of approximately 21 days, the animals were killed. Seven animals which died before they could be killed were autopsied within two hours. In either case, the cultures obtained at the original operation were repeated under sterile conditions.

Blood samples were placed in tryptic soy broth bottles, and if no growth was evident by 14 days, subcultures were made to blood agar. The other specimens of tissues or body fluids were inoculated on duplicate plates of 5% sheep blood trypticase soy agar and lactobacillus selection agar; one of each kind of plate was incubated aerobically at 35 C and the other anaerobically in BBL gas pack jars. MacConkeys agar plate was also used for some of the samples. In

addition, fungus cultures were made on bottles of plain and antibiotic-containing mycology agar and incubated at room temperature for 21 days before being discarded as negative.

Prior to the foregoing inoculations, the liver specimen was made into a brei by fragmenting it in a tissue grinder (Ten Broeck) which contained tryptic soy broth. By weighing the tissue introduced, adding a given volume of broth to the grinder, and inoculating the agar plates with a known quantity of the resulting brei supernatant, it was possible to estimate the bacterial count of different organisms for each gram of tissue.

Positive cultures from the blood, fluid, or tissue specimens were further studied by conventional staining and differential testing procedures. Anaerobic organisms were retested for inability to grow aerobically. Facultative organisms were counted as aerobes. Partial identification schemes based on methods used by Zubrzycki and Spaulding[8] were used on some organisms. An identification of *Bacteroides* was made if the colonies were strictly anaerobic and if they were sporeless, gram-negative pleomorphic bacilli, and had a pungent, fetid odor. Strictly anaerobic gram-positive bacilli, (with or without spores) were identified as clostridia. *Clostridium perfringens* was identified by gram-stain morphology, colony appearance on blood agar, and stormy fermentation of milk. Other clostridia were simply listed as *Clostridium* species.

Results

The results of approximately 1,500 cultures are summarized in Tables 1 to 5. Before and during operation, the bacterial flora were similar in both the 25 normal dogs and the 34 normal pigs (Table 1). In both species, organisms were occasionally found in the peripheral and portal venous blood, the liver, gallbladder wall, and bile. Bacteria, most commonly of the gram-negative variety, were found in more than half the duodenal cultures.

Postoperatively, this bacteriologic profile was altered more or less drastically according to the procedure carried out. The least profound changes followed the sham operations (group 1). However, even in these animals there was a high incidence of postoperative bacteremia as well as an increased number of positive cultures from the gallbladder, bile, and liver (Table 2).

After either simulated autotransplantation (group 2), or homotransplantation to untreated (group 3), or immunosuppressed (group 4) recipients, bacterial growth became ubiquitous inasmuch as sterile cultures were rare from any of the areas of sampling (Table 2). The bacteria were of all varieties with a strong representation of kinds normally found in the gastrointestinal tract (Table 3).

In the pigs with simulated autotransplantation, the enteric organisms appeared in the peripheral venous blood at a later time when the common duct was left intact than when cholecystoduodenostomy was performed. This resulted in the killing of the former animals (group 2A) after only eight days as compared to 16 days in group 2B.

In all instances in groups 2 to 4 in which the liver tissue contained bacteria, at least one of the other specimens had the same microorganisms. Nevertheless, there was wide variability in given animals in the bacterial strains isolated from the individual samples. Thus, the microorganisms ultimately isolated from the liver tissue often had not been previously found in the peripheral venous samples (Table 4). Similarly, bacteria grown from the portal vein, the gallbladder wall, bile, or duodenum were not necessarily represented in the infected liver tissue; this was particularly true after autotransplantation with an intact common duct (Table 4).

Although there were not significant qualitative differences in the livers of the animals with simulated autotransplantation (group 2) as opposed to those with true homotransplantation (group 3 band 4), the magnitude of sepsis seemed greater in the latter two series as quantitated by rough bacteriologic counting (Table 5). It was also in groups 3 and 4 that evidence of major hepatic necrosis was demonstrated as reflected primarily by increases in serum enzyme values and by other measures of liver function (Table 6).

The virulence of the septic complication was also highly variable in the four test

Table 1.—*Frequency of Positive Cultures and Types of Bacteria Found in Normal Dogs and Pigs*

Specimen		No. of Animals with Positive Cultures	Number of Positive Cultures*														
			Bacteroides	**Clostridium perfringens**	**Clostridium** species	Diphtheroids	Enterococcus	**Escherichia coli**	**Lactobacillus** (aerobic)	**Lactobacillus** (anaerobic)	**Pasteurella multocida**	**Proteus** species	**Staphylococcus** (coagulase-negative)	α-hemolytic **Streptococcus**	β-hemolytic **Streptococcus**	Nonhemolytic **Streptococcus**
Peripheral blood	Dog	1/25											1			
	Pig	6/34											4		2	
Liver	Dog	3/25	2					1				1	1	1		
	Pig	1/34						1								
Portal blood	Dog	1/25											1			
	Pig	5/34		1				1					2		1	
Gallbladder wall	Dog	1/25						1				1				
	Pig	4/34			1			2		1						
Bile	Dog	1/25						1				1				
	Pig	5/34		1				2	1	1	1			2		
Duodenal contents	Dog	14/25	4	4		2	2	9	3	4		1	1	2	1	1
	Pig	25/34	3	8		1	1	18	10	11				2		

* More than one organism was often grown from individual specimens. Consequently, the sum of the individual positive cultures could be greater than the number of animals with positive cultures.

Table 2.—*Animals That Developed Positive Cultures Postoperatively*

Group	Total No. of Animals	No. of Animals With Positive Cultures											
		Peripheral Blood		Liver		Portal Blood		Gallbladder		Bile		Duodenum	
		Preoperative	Postoperative	Operative	Death	Operative	Death	Operative	Death	Operative	Death	Operative	Death
1A	5	0	4	0	0	1	1	0	2	0	1	3	2
1B	4	0	4	0	1	2	2	0	4	0	3	3	4
2A	5	0	5	0	4	0	5	1	5	1	5	4	5
2B	5	0	5	0	4	0	4	1	5	0	5	2	5
3A	5	0	5	2	5	0	5	1	5	1	2	4	5
3B	5	1	5	1	5	1	5	0	5	0	5	4	5
4A	5	0	5	0	5	0	4	0	5	0	5	3	5
4B	5	2	5	0	4	0	5	1	5	2	5	4	5

Table 3.—*Bacteria Found in Postoperative Peripheral Blood Cultures and in Various Specimens Obtained at Termination of Experiment*

		No. of Positive Cultures																			
Group	Specimen	**Alcaligenes faecalis**	Bacteroides	**Clostridium perfringens**	**Clostridium** species	**Corynebacterium pyogenes**	Diphtheroids	Enterococcus	**Escherichia coli**	**Lactobacillus,** (aerobic)	**Lactobacillus,** (anaerobic)	**Pasteurella multocida**	**Proteus**	**Pseudomonas**	**Staphylococcus** (coagulase-negative)	**Staphylococcus** (coagulase-positive)	α-hemolytic **Streptococcus**	β-hemolytic **Streptococcus**	Nonhemolytic **Streptococcus**	**Neisseria** species	Total
1A	Peripheral blood			2								2			3	4			9		20
	Liver																				0
	Portal blood														1						1
	Gallbladder				1				1		1						1				4
	Bile										1						1				2
	Duodenum		1				1		1	1	1		1			1	1				8
1B	Peripheral blood		1	7			2		1						17	1	1	5		1	36
	Liver								1								1				2
	Portal blood														2			1			3
	Gallbladder			2					2	1	1	1			1		1				9
	Bile			1					2	1	1	1									6
	Duodenum						1		4	2	2		1				3				13
2A	Peripheral blood			3			1	1	1			1			3	5					15
	Liver		1	1		2			1				1			1					7
	Portal blood			1				1	1				1		1	1					6
	Gallbladder			2		1			1				1		2	2					9
	Bile			2	1				1							1	1				6
	Duodenum			3	1				5	3	4	1	2		1		1				21
2B	Peripheral blood	1		5			3		2						17	3		1			32
	Liver		1	1			2		4				1		1						10
	Portal blood			2			1		1				1			1					6
	Gallbladder			4					5		1										10
	Bile			4				1	5	1	3										14
	Duodenum			4			1		5		4		1				1				16
3A	Peripheral blood		1						2							2		12			17
	Liver		4	4					4				1			1		3			17
	Portal blood			1				1	3							1		2	1		9
	Gallbladder		1						5	1			2				1	2			12
	Bile								2												2
	Duodenum		1	1					5	1	1		2				1	1			13

Table 3.—*Bacteria Found in Postoperative Peripheral Blood Cultures and in Various Specimens Obtained at Termination of Experiment (Continued)*

Group	Specimen	No. of Positive Cultures																			
		Alcaligenes faecalis	Bacteroides	**Clostridium perfringens**	**Clostridium** species	**Corynebacterium pyogenes**	Diphtheroids	Enterococcus	**Escherichia coli**	**Lactobacillus,** (aerobic)	**Lactobacillus,** (anaerobic)	**Pasteurella multocida**	**Proteus**	**Pseudomonas**	**Staphylococcus** (coagulase-negative)	**Staphylococcus** (coagulase-positive)	α-hemolytic **Streptococcus**	β-hemolytic **Streptococcus**	Nonhemolytic **Streptococcus**	**Neisseria** species	Total
3B	Peripheral blood		5					2					1		4	4		4			20
	Liver		4	1				2	5						1		1	1			15
	Portal blood		2	1					3					1	1	1					9
	Gallbladder		4	4				1	5	1	2		2								19
	Bile		4	2				1	4	1	2		2			1		1			18
	Duodenum		1	4				1	5	3	3		2			1		1			21
4A	Peripheral blood		2						7					1	1	5		1			17
	Liver		3	3	1			1	4		1		1	1	1	2	1				19
	Portal blood		1	2					3			1				2					9
	Gallbladder		3	3					4				2	1			1				14
	Bile		2	2					5		2		1	1			1				14
	Duodenum		2	3	2				5		2		1	1			1				17
4B	Peripheral blood		1	8	1		3		4				1		4	1		4			27
	Liver			3			1		3	1	2		1		1			1			13
	Portal blood		1	1					2				1				1	1			7
	Gallbladder			5					5	2	3		2								17
	Bile			4				1	5	1	3		3				1				18
	Duodenum			5					4	3	4		2								18

Table 4.—*Frequency of Same Organisms Found Postoperatively in Individual Animals in Various Pairs of Specimens*

Group	Peripheral Blood and Liver	Liver and Gallbladder Wall or Bile	Liver and Portal Venous Blood	Liver and Duodenal Contents
1A	...	...	...	...
1B	1/2	2/4	0/1	2/2
2A	1/7	3/13	0/6	2/22
2B	5/10	5/15	4/5	6/16
3A	8/18	8/12	6/9	9/12
3B	4/15	10/19	5/9	8/18
4A	6/21	15/18	7/9	14/17
4B	8/13	10/20	1/7	10/20

Table 5.—*Bacterial Data on Liver Specimens Obtained at Termination of Experiments*

Group	Average Bacterial count/gram Liver Tissue
1A	—
1B	10×10^2
2A	135×10^2
2B	138×10^2
3A	$23{,}539 \times 10^2$
3B	$17{,}152 \times 10^2$
4A	$1{,}950 \times 10^2$
4B	$16{,}638 \times 10^2$

Table 6.—*Mean Values of Liver Function Tests

Group	Observation (days)	Bilirubin		SGOT†		SGPT†		Alkaline Phosphatase‡	
		1st POD	Death	1st POD	Death	1st POD	Death	1st POD	Death
1A	23.0	0.1	0.2	69	47	88	43	5.2	4.5
1B	24.3	0.2	0.6	134	53	33	31	5.7	2.4
2A	16.0	0.2	0.1	187	24	34	21	5.2	3.6
2B	8.0	0.2	0.3	203	56	56	21	7.3	5.4
3A	5.3	0.4	10.8	120	2855	376	3663	8.9	381.5
3B	6.0	1.2	4.3	116	594	177	15	9.0	12.8
4A	8.5	0.4	4.6	220	1371	476	1610	12.2	126.2
4B	4.5	0.3	3.1	218	927	33	109	7.3	19.6

* On the first postoperative day (POD) and the last day of life.
† Normal range, 20-60 Sigma Frankel units.
‡ Normal range, 2-6 Bodansky units.

groups as judged by the clinical courses. The animals subjected to sham operation or simulated autotransplantation usually seemed quite normal in spite of the well-documented postoperative bacteremia. All survived until they were killed. In contrast, both the untreated and immunosuppressed homograft recipients tended to follow a malignant course. Frequently, they passed from apparent well-being to a moribund state within a few hours. For example, the deterioration was so rapid in the immunosuppressed dogs (group 4A) that only one of five experiments could be electively terminated. The survival in the other four was three, seven, eight, and nine days, respectively.

The addition of intensive antibiotic therapy after canine homotransplantation to immunosuppressed recipients (group 5) radically changed the outlook. Of the latter 12 dogs, two died of hepatic artery occlusion after four and seven days, respectively, and two more of rejection at seven and eight days. The eight remaining animals lived for three weeks or more, and six survived for at least six weeks. Using a maximum credit of 70 days for any individual dog, the mean survival for the group was 38.7 ± 29.8 days (standard deviation).

Comment

The foregoing findings establish that highly characteristic infectious complications occur with orthotopic hepatic transplantation, and that sepsis selectively afflicts the liver homograft probably by virtue of its strategic location in relation to the rest of the bacteria-rich gastrointestinal tract. The multiple factors which could contribute to invasion of hepatic parenchyma by pathogenic microorganisms have been analyzed in the present study in which antibiotic therapy was for the most part avoided.

In both dogs and pigs, bacteria were cultured from both the liver and portal vein at the time of initial laparotomy with a much lower frequency than that noted by previous authors.[9-13] When the wounds were closed after a relatively nontraumatic sham operation, consisting only of duodenotomy and cholecystotomy, the incidence was only slightly increased with later sampling from the same location. However, postoperative systemic bacteremia was now demonstrable in eight of nine animals. The peripheral microorganisms were usually gram-positive cocci which in only one case were subsequently cultured from the liver.

When an ischemic injury was superimposed in pigs by performing simulated autotransplantation with or without division of the common duct, the bacteriologic pattern was markedly altered. Postoperative bacteremia again occurred, but there was a much higher representation of organisms which are indigenous to the gastrointestinal tract (Table 3). These same organisms were now also commonly although not invariably found in the liver, portal vein, gallbladder, bile, and duodenum; the presence in multiple sites of the same bacterial species was more pronounced after cholecystoduodenostomy than when the common duct had been left intact. Mixed flora were common in all the samples.

The foregoing sham procedures or simulated autotransplantations appeared to have provoked a potentially serious infectious

complication to a degree which was roughly proportional to the magnitude of the procedure. However, the majority of the animals did not appear ill and all 19 survived until the experiments were electively ended in order to obtain specimens; presumably many could have lived indefinitely. The introduction of two additional factors into this dangerous environment resulted in explosive infections when antibiotic therapy was not used.

The first was the replacement of the animals' own livers with homografts. The transplanted organs seemed to have little capacity to resist the bacterial invasion when subjected to the added duress of rejection. All the nonimmunosuppressed dogs and pigs developed overwhelming sepsis before or at the same time as there was biochemical evidence of liver necrosis. Often, there was an early bacteremia with gram-positive organisms which was succeeded within a few days by gram-negative septicemia. When the latter finding appeared, death was usually imminent. At sacrifice or autopsy, the same gram-negative organisms were very often found as part of a mixed and variable flora in the liver, gallbladder, upper gastrointestinal tract, and portal vein.

In animals which did not receive antibiotic therapy, attempts with host immunosuppression to prevent the liver necrosis caused by rejection did not improve the situation in terms of the promptness and the virulence of the sepsis; the postoperative course in these dogs and pigs was not significantly improved over that in animals in which rejection was allowed to run its natural course. Only when antibiotics were used in conjunction with immunosuppression was there significant prolongation of life.

Appreciation that septic complications after homotransplantation of the liver often center around the homograft should make it possible to avoid them. That this can be done is demonstrated by the fact that survival of as long as four years has been achieved in our laboratory after orthotopic transplantation of the dog liver. Two general approaches seem feasible.

First, incisive antibiotic therapy is mandatory as has been shown after other kinds of severe liver injury.[14,15] Since bacterial seeding is probably through the biliary tract and portal vein, prophylactic treatment should be primarily based upon culture data on the flora in the recipient gastrointestinal tract. Mechanically, it may be possible to reduce the extent of contamination by providing biliary tract drainage with choledochocholedochostomy.

Of far greater importance, however, is the prevention of either immediate or delayed ischemic injury since, once extensive liver necrosis has started, the chance of preventing or controlling growth in this ideal culture medium has probably been lost. This was pointed out in a recent more complete analysis of four orthotopic liver recipients who died from two to six months after transplantation. Death was the direct or delayed consequence of septic hepatic infarction of the right liver lobe. In all four children, there was selective thrombosis of the right hepatic artery.[16] Evidence was presented that distortion of the hepatic arterial anatomy could have been an underlying mechanical factor, to which low-grade rejection could conceivably have contributed.

Summary

A bacteriologic study was performed in dogs and pigs after sham operation, simulated hepatic autotransplantation, and orthotopic hepatic homotransplantation to unaltered and immunosuppressed recipients. When antibiotics were not given, each of the procedures was followed by bacteremia. With either autotransplantation or homotransplantation, bacterial invasion of the liver apparently occupied a central role in the complex septic course that followed. Ischemic injury, rejection, and immunosuppression without antibiotic coverage all aggravated the infections. After simulated autotransplantation, sepsis developed earlier after cholecystoduodenostomy than when the common duct was left intact. Long-term survival could be obtained in the recipients of homografts only if intensive antibiotic therapy was added to effective immunosuppression. These findings have direct implications for clinical liver transplantation.

This study was supported by Public Health Service grants AM-06344, HE-07735, AM-07772, AI-04152, FR-00051, FR-00069, and FO5-TW-1154.

Generic and Trade Names of Drugs

Pentobarbital—*Nembutal.*
Phencyclidine hydrochloride—*Sernylan.*
Sodium thiamylal—*Surital.*
Succinylcholine chloride—*Anectine.*
Azathioprine—*Imuran.*
Prednisone—*Deltasone, Deltra, Meticorten, Paracort.*
Benzathine penicillin G—*Bicillin.*
Chloramphenicol—*Chloromycetin.*
Tetracycline—*Achromycin, Bristocycline, Panmycin, Steclin.*

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Discussion

ROBERT HERMANN, MD, Cleveland: I am sure every one here recognizes the enormous contributions which have been made by the University of Colorado group to the development of transplantation surgery, especially in the field of liver transplants. Under the direction of Dr. Tom Starzl, with the inspired help of his active team and Dr. Brettschneider, this group has continued to point the way in this extraordinarily complicated field.

Dr. Brettschneider has taken the complication of liver abscesses that developed in their clinical transplants to the laboratory to study this problem under controlled circumstances. He has defined at least four factors which appear to play a role in the development of hepatic infections. The first, of course, is ischemic injury to the liver. The second is rejection injury to the liver. The third is the alternation and the attenuation of the host's capacity to resist infection, and, finally, he has identified the sources of entry of organisms over the biliary system and the portal vein from the duodenum and the gastrointestinal tract.

The prevention of ischemia of the transplanted liver and of providing adequate protection against rejection is evident to all. In addition, Dr. Brettschneider has concluded that it is essential to protect the homograft by the liberal use of parenteral antibiotics. He has postulated that a common bile duct-to-common duct anastomosis might be preferable to a gallbladder-duodenal anastomosis and may reduce one potential entry of bacteria, that over the biliary system.

I would like to ask Dr. Brettschneider and his group two questions. How long does he believe the survivors will have to be covered by antibiotics after liver transplants; and second, has he considered the use of oral or nonabsorbable antibiotics to sterilize the upper intestinal tract?

FRASER N. GURD, MD, Montreal: I might just make one comment, if I may. The contamination of a dog liver is a natural thing. The contamination with clostridia is light, so light that biopsy of a gram will not give a positive culture, whereas a larger fragment, say 5 gm or so, will be positive almost invariably. However, that is not really the problem facing these authors because the light normal contamination with clostridia is readily controlled with antibiotics.

I wonder if the problem here is perhaps not the permeability of the intestine to a variety of bacteria resulting from the stressful situation to which the animal is exposed in connection with the operation and the subsequent treatment. Dr. Fred Cross mentioned yesterday morning that he has been able to overcome the tendency to develop positive portal blood cultures in dogs after extracorporeal cardiopulmonary circulation by the use of Dr. Bounous' elemental diet as described last September in the *Annals of Surgery* (**166**:312, 1967). When the diet was employed, Dr. Cross found that antibiotics became effective in protecting the dogs. Now that it is an easy thing to do, the diet should be applied in any field where it makes any sense to apply it. This might be one.

FREDERICK CROSS, MD, Cleveland: I think that those in the session yesterday morning heard me comment on our valve work in dogs. Dr. Richard Jones and I feel quite strongly that sterilization of the dog's gastrointestinal tract and rigorous postoperative antibiotic therapy will greatly improve the results following prosthetic valve-replacement surgery. Prior to our use of this technique, the incidence of clotting on valve implants was very high and survival over one month was less than 20%. A regimen of preoperative elemental diet with oral neomycin and polymixin for two days has resulted in complete sterilization of the bowel in over half the dogs. When this is followed by postoperative cephaloridine, 1 gm three times daily for a week, survival for one month or more has increased to 93%, and the incidence of thrombus formation on the valves is very low.

We have discussed extending the use of this technique to transplantation procedures in the dog, where I am certain a reduction of the incidence of infection would be of great importance.

HENRY GANS, MD, Minneapolis: I rise to comment on one parameter which was not mentioned by Dr. Brettschneider in his otherwise superb report. It concerns the clearance function of the liver which, as you know, exerts a very crucial role in clearing microorganisms and their products from portal vein blood during its passage through the liver.

My question is whether you or Dr. Starzl have any information concerning the clearance function of the liver after liver transplantation? Could the difficulties you described result from an impairment of this function?

LAWRENCE BRETTSCHNEIDER, MD, Denver: The answer to Dr. Hermann's question concerning the length of time we carry these patients on antibiotics remains unclear. The patients who had drainage of their livers probably will be treated indefinitely.

In the past, intestinal sterilization has not improved the results with orthotopic liver transplantation in dogs. However, in one of our patients, we used intestinal antibiotics and will consider it in the future.

We are especially thankful for Dr. Gurd's comments, since he did a great deal of interesting work in 1951 in reference to the production of hepatic sepsis in normal dogs, and also because of the information he presented at the American Surgical Association meeting last year on the elemental diet. We very seriously are considering the latter approach as a means of preventing contamination from the gastrointestinal tract. As you well know, crossclamping of the splanchnic and portal systems in the transplantation procedure may lead to an injury to the gut and a subsequent outpouring of organisms.

Printed and Published in the United States of America