THE ISOLATED PERFUSED BOVINE LIVER*

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The technique of isolated liver perfusion has several unique features as an investigative tool for the study of hepatic physiology and biochemistry. These include the exclusion of non-hepatic tissues, the maintenance of intact cellular membranes, and the ability to control and reproduce more precisely the experimental hepatic environment than is possible in an entire animal. This investigative team has developed an isolated bovine liver perfusion preparation that is reproducible, bacterially sterile, and capable of continuous functional performance for extended periods of time.

The use of a large mammal, such as the bovine calf, as the experimental animal has other useful characteristics; the large liver permits serial tissue sampling, yielding specimens of generous size. The large volume of perfusate, with little intrahepatic accumulation, allows frequent generous sampling of this medium. The use of autogenous blood as the perfusate avoids interaction between foreign proteins which could occur if blood were pooled from several homologous donors.

The technique of perfusion reported herein permits simultaneous and independent perfusion through the hepatic artery and portal vein, and repetitive sampling of liver tissue and the circulating perfusate without interruption of the perfusion.

This report describes the characterstics of this preparation as experienced in over 90 separate perfusion experiments.

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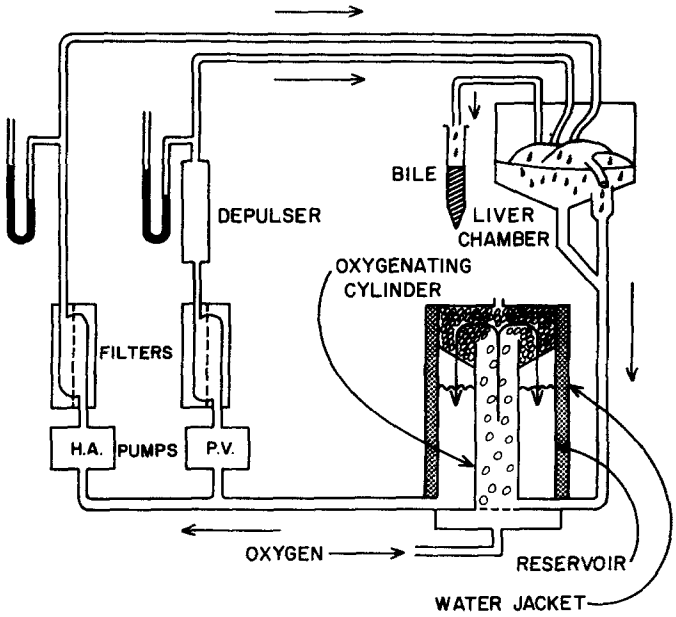
Methods

Experimental Animals.—Healthy bovine calves, 2 to 7 weeks of age, weighing 35 to 50 kg., were used. These were fasted for 24 hours just preceding hepatectomy; water was withdrawn 12 hours before operation.

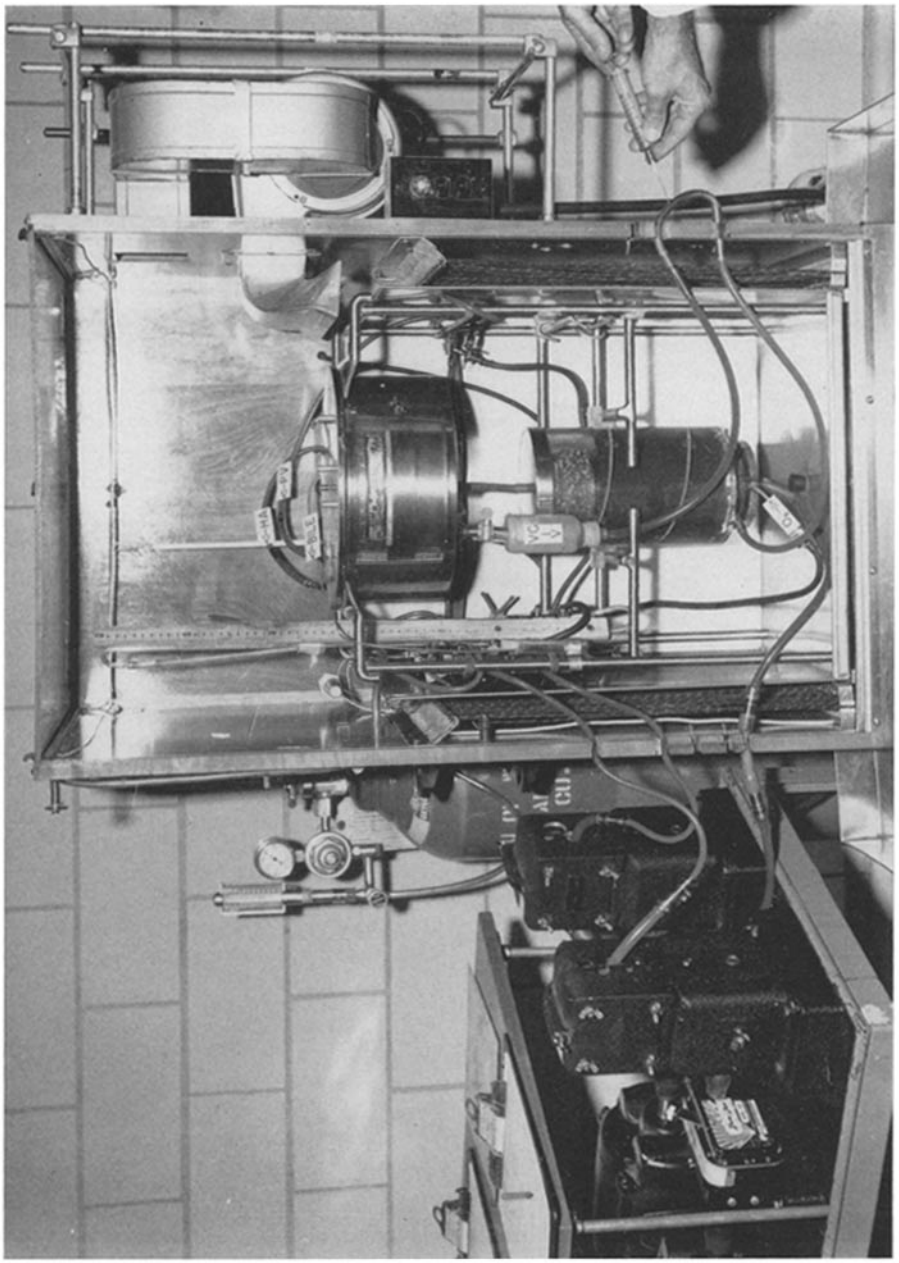
Calves younger than 2 weeks, although more readily available, were found to be unsatisfactory; these very young animals apparently cannot adapt to the nutritional or environmental changes of the laboratory as well as older animals. Poorly understood vascular and metabolic phenomena occur that frequently make perfusion of the liver of the very young animal unsatisfactory.



TEXT-FIG. 1. Isolated liver perfusion apparatus.

Apparatus.—The perfusion apparatus (Text-figs. 1 and 2), while of an established conventional design, requires comment; constant body temperature (37°C. ± 0.5°) is maintained by confining all parts except the two perfusion pumps within a thermostatically controlled cabinet. Supplementary control of the perfusate temperature is provided by a heated water jacket surrounding the reservoir. Drying of the liver surface is prevented and humidity is assured by suspending gauze, saturated with saline, on the inner walls of the stainless steel liver chamber. The liver is supported upon taut, loose-weave nylon mesh suspended just above the bottom of the chamber.

Perfusate from the supradiaphragmatic portion of the inferior vena cava is collected in the small funnel in the bottom of the liver chamber and flows by gravity into the bottom of the oxygenating cylinder at a position just above the bubble plate. Lymph, exudate from the liver surface, and perfusate flowing from tissue biopsy sites pass through the nylon liver support and flow out from the center of the funneled floor of the liver chamber to mix with the unoxygenated perfusate from the vena cava.



TEXT-FIG. 2. Isolated liver perfusion apparatus.

Oxygenation of the venous blood is accomplished by aeration with small gas bubbles (100 per cent O_2, or mixture of 97 per cent O_2 + 3 per cent CO_2) rising from holes in the plastic bubble plate (15). The foam formed during this process is broken by silicone[1]-coated stainless steel mesh placed over the top of the oxygenating cylinder. The perfusate then flows smoothly down the outside walls of the oxygenator, rather than being allowed to flow or splash upon the heated walls of the reservoir where drying and hemolysis of the erythrocytes would occur.

From the bottom of the reservoir the blood is passed through a section of rubber tubing used for arterial sampling and then is divided, going via two independently operated, occlusive, peristaltic Sigmamotor finger pumps, one for the portal venous (PV) and the other for the hepatic arterial (HA) circulation. The perfusate is pumped through fine mesh nylon[2] filters, through a depulser of Penrose rubber tubing (in portal vein circulation only), past manometer connections, and thence to the sites of cannulation of the portal vein or hepatic artery. These two cannulas, as well as the common bile duct cannula, enter the liver chamber through a lid constructed with radial slots which permits optimal orientation of these vessels.

The perfusate is in contact with tygon, plastic, and stainless steel surfaces only except for (*a*) dialysis membranes used to transfer pressures from the sterile circulation to the non-sterile manometers, (*b*) short sections of gum rubber tubing where injections are made and samples of perfusate are withdrawn with needle and syringe, (*c*) a short section of thin-walled Penrose rubber tubing used to damp the pulsations of the pump in the portal venous circulation, and (*d*) rubber gaskets in the filter block and oxygenator. Excluding the hepatic vasculature, the total circulatory volume is 1700 ml., of which 1200 ml. is in the reservoir.

The apparatus is prepared for use by successively: (*a*) cleaning the circulation vessels and chamber with alcoholic KOH, (*b*) rinsing with tap water, (*c*) coating with silicone[3], (*d*) cold-sterilizing with 0.5 per cent formalin for 24 hours immediately preceding the experiment, and (*e*) finally rinsing with 2 liters of sterile Krebs-Ringer-bicarbonate solution just prior to filling with perfusate. Rubber and metal parts such as the liver chamber are sterilized by autoclaving and connected to the formalin-sterilized portions of the apparatus shortly before perfusion. The short sections of rubber tubing through which perfusate samples are withdrawn are replaced after each experiment.

Anesthesia.—A mixture of 20 per cent nitrous oxide and 80 per cent oxygen is delivered by an anesthetic gas machine through a cuffed endotracheal tube inserted through a tracheotomy. This gas anesthesia is supplemented with intravenous succinyl choline to provide adequate muscular relaxation. An initial 100 mg. dose of succinylcholine is injected into a foreleg vein. This large dose relaxes the animal for 15 to 20 minutes, allowing sufficient time for skin preparation, sterile draping, and operative entry into the abdominal cavity. Thereafter, additional small doses of succinylcholine are injected as needed. Ventilation is maintained with a Palmer mechanical respirator.

Prevention of Bacterial Contamination.—Before being taken into the operating room, the animal is thoroughly washed and scrubbed. Following the induction of anesthesia, the animal is placed upon its back in a wooden frame (to stabilize it) on a standard operating table. The entire ventral surface from the top of the sternum to the pubis is clipped and shaved. This is followed by a 5 minute scrub with soap and water; the skin is then painted with 10 per cent tincture of iodine. Sterile surgical packs are not opened until the skin preparation has been completed and the clipped hair removed from the floor. The hepatectomy is conducted under standard aseptic surgical conditions.

[1] Antifoam A spray, Dow Corning Corporation, Midland, Michigan.

[2] American Standards for Testing Materials No. 200-74 nitex, nylon monofilament screen cloth, Tobler, Ernst and Traber, New York.

[3] Siliclad, Clay-Adams Inc., New York.

Hepatectomy.—A long midline, sternum-splitting, thoracoabdominal incision is made from the top of the sternum to the pubis. Blood loss must be controlled at all times. Poor hemostasis leads to a depleted blood volume which in turn produces an inadequate perfusate volume and a liver that may tolerate perfusion poorly.

Wide retraction of the incision is achieved by placing towel clips on each edge of the incision: (*a*) at the inferior end of the sternum, and (*b*) midway between the ensiform cartilage and the pubis. Heavy, sterile web tapes are tied to one towel clip, passed under the table (care being taken to avoid contamination), and attached to the towel clip on the opposite edge of the wound. In this manner, wide, self-retaining retraction is consistently achieved.

Anterior mediastinal structures must be divided to permit proper orientation of the heart and to avoid kinking of the pulmonary veins. Occasionally, to achieve this, a suture must be passed through the pericardium to elevate the heart. Unless attention is directed to these points, the animal may suddenly become hypotensive because of obstruction to the venous return to the heart.

Mechanical trauma to the liver during the course of the hepatectomy appears to influence significantly the success of this preparation. Manipulation of the liver must be minimized and utmost gentleness is necessary when handling the organ. Several fine sutures are appropriately placed through the free edge of the liver and the adjacent diaphragm; this elevates the anterior portion of the liver to provide sufficient room for dissection, thereby making manual retraction of the liver unnecessary.

The large stomach of the calf is retracted to the left and secured there with retention sutures. The remaining viscera are retracted from the operative site by an assistant.

The hepatectomy is begun by isolating and cannulating the common duct, after which the cystic duct is ligated. In the young bovine, the pancreas extends to the hilum of the liver, lying closely interposed between the common duct and the portal vein. By elevating the cannulated common duct, the pancreas can be easily dissected free of these structures. The hepatic artery is a branch of a large artery supplying the second portion of the stomach (reticulum) and can be seen after dividing the reticulo-hepatic ligament. The hepatic artery usually branches into 4 to 6 small vessels which enter the liver immediately beneath the portal vein.

After the hepatic artery and portal vein have been isolated and cleared for short distances adjacent to the liver, the surrounding diaphragmatic and visceral attachments are severed. This dissection is best begun on the left side of the inferior vena cava adjacent to the esophageal hiatus. By a combination of sharp and blunt dissection, a finger is passed behind the vena cava onto the bare surface area of the liver. This tissue plane is developed inferiorly and posteriorly, severing the peritoneal attachments of the liver and inferior vena cava. At this point, the stay sutures in the free liver edge are removed and the liver is retracted to the left by using the gall bladder as a handle. The right triangular ligament is transected and the space about the bare surface of the liver is now entered from the right. By a combination of sharp and blunt dissection, the remaining hepatic peritoneal attachments are severed and the vena cava is freed circumferentially just above the entrance of the right adrenal vein. The diaphragmatic veins are divided between clamps and ligated.

After completing this dissection, the animal is heparinized (3 to 4 mg./kg.). Partial exsanguination of the animal is accomplished through a large bore cannula which is inserted into the aorta and advanced to the level of the renal arteries. The blood is collected in sterile commercial intravenous bottles. An intravenous infusion of Krebs-Ringer-bicarbonate solution is begun simultaneously with the withdrawal of blood. The infusion rate is adjusted to replace approximately the volume of blood removed. The final hematocrit of this blood (perfusate) is in the range of 25 to 35.

A volume of approximately 1000 ml. of this blood is transferred to the perfusion apparatus, where it is circulated to remove all air from the tubing system. The exsanguination is stopped

until the portal vein is cannulated. Immediately thereafter, the flow of oxygenated perfusate through the portal vein cannula and further exsanguination are commenced simultaneously. The hepatic artery is rapidly cannulated and perfusion is begun through this vessel. Total hepatic inflow is then stopped. The vena cava above and below the liver is divided between clamps and the liver quickly transferred to the perfusion apparatus. The blood flow is resumed after removal of the clamps on the vena cava. During this transfer, total hepatic inflow is stopped to prevent engorgement of the liver.

During cannulation of the vessels, the liver is always supplied with oxygenated blood through either the hepatic artery or the portal vein. The time of cessation of total blood flow, occurring at the time of transferring the liver to the apparatus, is *less than 1 minute.* The average time from the beginning of surgery to completion of cannulation and initiation of perfusion is approximately 2 hours.

Sampling.—Samples of the circulating perfusate are withdrawn with a sterile syringe and needle from the appropriate sector of gum rubber tubing after sterilizing its surface with tincture of iodine. Samplings of hepatic tissue are accomplished with sterile scissors and forceps through the opened side port of the metal liver chamber. Tissue samples of generous size may be obtained. Hemostasis is unnecessary because the perfusate flows freely from the cut liver surface, drains quickly through the supporting nylon mesh, is efficiently collected in the bottom of the liver chamber, and is immediately returned to the oxygenator.

Bacterial Monitoring.—The sterility of each preparation is monitored during the course of the perfusion by frequent aerobic and anaerobic cultures of the perfusate. Aerobic cultures are made with pour plates containing tryptose blood agar base[4]; anaerobic cultures are made with pour plates containing Brewer anaerobic agar[4], and are incubated in oxygen-free Brul jars. All cultures are incubated at 37°C.

pH Regulation.—The pH of the perfusion medium is maintained between 7.2 and 7.5 by varying the proportions of CO_2 and O_2 entering the oxygenator. By diluting the blood at the time of the exsanguination with an isotonic solution of high bicarbonate content (Krebs-Ringer-bicarbonate solution), a mild metabolic alkalosis (to pH 7.5) is created which is compensated to a pH of 7.4 by aerating the blood in the oxygenator with 3 per cent CO_2 to 97 per cent O_2. Subsequently, during the perfusion the blood pH is monitored and the CO_2 content of the gas entering the oxygenator is gradually lowered, eventually to zero, to compensate for the slow replacement of the metabolic alkalosis by a metabolic acidosis (Text-fig. 3).

Analytical Methods.—Blood ammonia determinations were conducted by the method of Seligson and Hirahara (19); lactic acid determinations by the method of Barker and Summerson (5), and pyruvic acid determinations by the method of Segal, Blair, and Wyngaarden (18). Blood pH was measured with a Beckman blood electrode in a constant temperature water bath (37.5°C.) and read with a Beckman expanded scale pH meter. Blood oxygen and carbon dioxide concentrations were determined by the method of Van Slyke and Neill (23).

Morphologic Techniques[5].—For light microscopy study, tissue blocks were taken from the same liver before and at 2, 4, 6, 12, and 24 hours after the start of perfusion and fixed in Bouin's fluid, or 10 per cent formalin. Sections, 4 to 6 micra in thickness, of control and perfused tissue, were mounted upon the same slide and stained simultaneously with hematoxylin and eosin.

For electron microscopy, tissue blocks were taken from the same liver before and at the end of 8 hours of perfusion, fixed in buffered osmium, and embedded in either epon epoxy or araldite epoxy resin.

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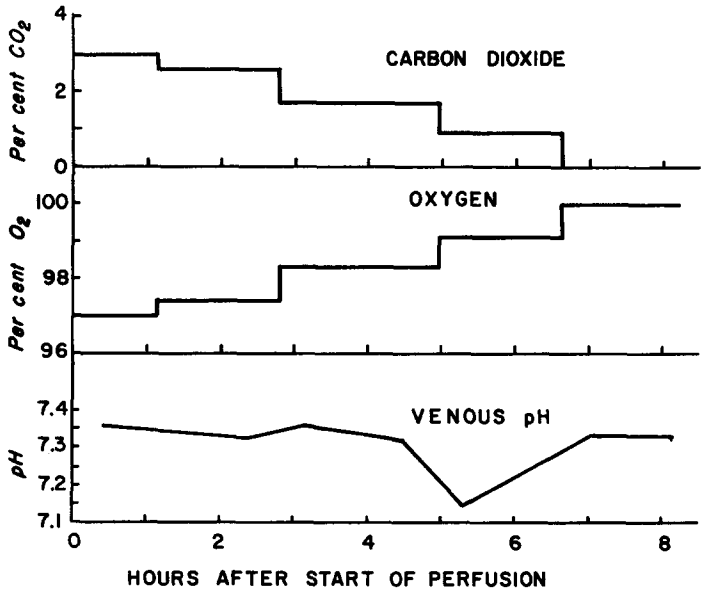
[4] Difco Laboratories Inc., Detroit, Michigan.

[5] The authors are indebted to Dr. Paul C. Griffith, Department of Pathology, for preparation and interpretation of the light microscopy material, and to Dr. Richard L. Wood, Department of Anatomy, for the electron microscopy studies.

RESULTS

Blood Flows and Pressures.—The flow of perfusate was set at the beginning of the experiment to adjust hepatic inflow pressures between desired limits; usually this was not varied during the remainder of the perfusion. At representative flow rates of 102 ml. per minute for the hepatic artery and 88 ml. per minute for the portal vein, the predominant pressures were 50 mm. of mercury and 12 cm. of water, respectively (Table I).

The total flow of perfusate through the liver varied between experiments



TEXT-FIG. 3. Effect of variation of CO_2 content of oxygenating gas upon the pH of blood in isolated bovine liver perfusion.

from 0.1 to 0.5 ml. per minute per gm. of wet liver. While minor variations occurred during the course of a perfusion, the pressures seldom exceeded the desirable maximum limits of 200 mm. Hg for the hepatic artery and 25 cm. water for the portal vein, and if this occurred it usually appeared only after 12 or more hours of perfusion.

Bile Flow.—The rate of bile flow in the isolated preparation was always found to be less than that in the intact animal. This decrease in the rate of flow occurred at the time the liver was transferred from the animal to the perfusion apparatus (shown by arrows in Text-fig. 4).

In 90 experiments, bile flows before hepatectomy averaged 20 ml. per hour (range of 2 to 50 ml. per hour) and after start of perfusion remained constant at average rates between 2 and 4 ml. per hour (range of 0.4 to 10 ml. per hour).

TABLE I

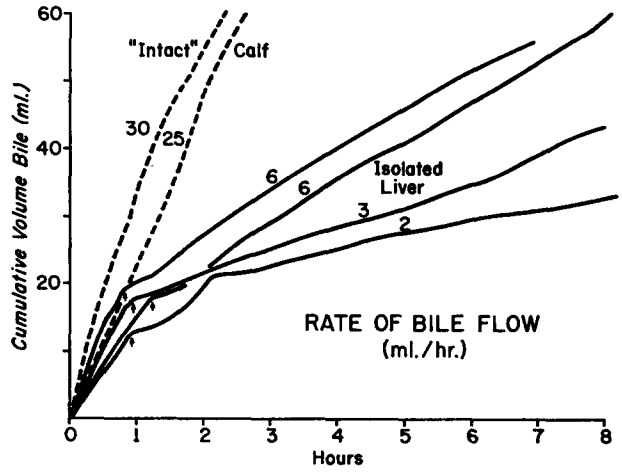
Representative Measurements of Perfusion Pressures and Flow Rates

Wet liver weight	Total blood flow		HA pressure* (mm. Hg)			HA flow	PV pressure‡ (cm. water)			Portal vein flow	Period of observation
			Max.	Min.	Predominant		Max.	Min.	Predominant		
gm.	*ml./min.*	*ml./min./gm. wet L*				*ml./min.*				*ml./min.*	*hrs*
1643	190	0.116	100§	40	45	102	21.5§	14.0	14.5	88	0 to 9¼
684	222	0.325	64‖	38	40	102	19.5§	14.2	16.5	120	0 to 24¼
1028	190	0.185	110§	40	55	102	11.5¶	8.0	8.5	88	0 to 13½
1882	190	0.101	100§	45	50	102	18.0	11.0	none**	88	0 to 15¼
1275	190	0.149	210	60	none**	102	15.5§	12.5	14.0	88	0 to 16
995	204	0.205	130	40	none‡‡	102	14.0	11.8	13.0	102	0 to 9½
773	245	0.317	44	32	40	102	24.0‖	20.0	24.0	143	0 to 8¾
1160	190	0.164	110§	20	25	102	24.0§‖	8.0	12.0	88	0 to 24
853	190	0.226	60	25	none¶	102	15.4	14.2	15.0	88	0 to 6½
944	190	0.202	60	45	50	102	15.0§	12.0	12.5	88	0 to 8¼
1326	190	0.143	60‖	30	50	102	7.5	6.0	6.5	88	0 to 6½
1805	88	0.049	0	0	0	0	14.0	12.0	13.0	88	0 to 5
813	88	0.108	0	0	0	0	11.0	10.0	10.5	88	0 to 4½
623	102	0.164	0	0	0	0	14.5§	9.0	9.5	102	0 to 23¾
547	115	0.210	0	0	0	0	15.0	12.5	12.5	115	0 to 6
456	147	0.252	0	0	0	0	14.0	13.5	13.5	147	0 to 12½
862	102	0.118	0	0	0	0	19.0	18.0	18.5	102	2 to 3
862	102	0.118	40	40	40	102	0	0	0	0	3 to 4
1290	190	0.147	125§§	85	85	102	4.5	4.0	4.5	88	0 to 7¼
1290	125	0.096	0	0	0	0	13.0	12.5	13.0	125	8 to 11¼
884	90	0.102	0	0	0	0	5.5	5.5	5.5	88	1 to 3
884	90	0.102	40	25	30	88	0	0	0	0	3 to 4½
712	100	0.141	0	0	0	0	15.0	12.5	14.0	100	1½ to 2½
712	100	0.141	130	115	125	100	0	0	0	0	2½ to 4½

* Expressed as mean values of the systolic and diastolic extremes (average range mm. Hg).
‡ Expressed as mean values of the systolic and diastolic extremes (average range cm. H_2O).
§ Terminal rise.
‖ Pressure change due to an initial rise
¶ Gradual drop throughout.
** Large fluctuations.
‡‡ Gradual rise throughout.
§§ Initial drop.

Intrahepatic Trapping of Perfusate.—During the course of a successful perfusion, the bovine liver became minimally congested. The degree of such congestion was quantitated by noting the decrease in the reservoir volume during the perfusion (corrected for samples withdrawn). The average accumulation of perfusate within the isolated calf liver organ at the end of 12 hours was 123 ml. (Table II).

Bacterial Growth.—The consistent failure to produce bacterial growth in aerobic and anaerobic cultures of liver tissue removed prior to perfusion was an indication that the normal bovine calf liver does not appear to possess a



TEXT-FIG. 4. Rate of bile flow from bovine liver, *in situ* and isolated.

bacterial flora. In spite of intensive efforts to prevent contamination, bacteria sterility of the perfusate was regularly maintained for only 12 to 14 hours The necessity for preventing bacterial contamination, if metabolic studies are to be made, may be seen in the effect of bacterial growth upon the concentrations of lactate, pyruvate, and ammonia (Text-fig. 5); a marked progressive rise in the content of these substances in the perfusion medium coincided with the appearance and growth of bacterial contaminants.

Oxygen Consumption.—Although the range of oxygen consumption for the different isolated liver preparations varied from 0.78 to 2.47 ml. O_2 per minute per 100 gm. of wet liver, the oxygen consumption did not change appreciably during a given experiment (Table III). These findings suggest that there may be differences in total oxidative metabolism between livers, but that for a given liver oxidative metabolic processes continue at a constant rate during its perfusion.

Morphologic Observations.—Histologic sections from livers which had been perfused for a number of hours without the occurrence of bacterial contamina-

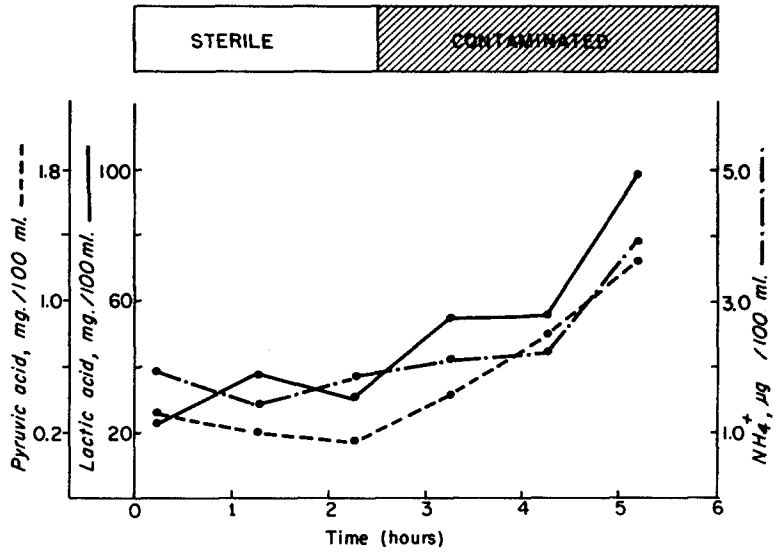
tion were indistinguishable from control tissue specimens taken from the same livers prior to perfusion (Fig. 1). Nuclear and cell membranes were intact; there was no clumping or dissolution of nuclear chromatin, nor changes in general staining characteristics. In many of the livers perfused for 12 hours, areas of mild sinusoidal congestion were seen occasionally, and all livers perfused for this length of time showed signs suggestive of glycogen depletion.

TABLE II

Intrahepatic Accumulation of Perfusate

Time from start of perfusion, *hrs.*	6	8	12	18	24
Average decrease in reservoir volume, *ml.**	100	100	123	248	355
No. of experiments	29	13	6	3	3

* Corrected for sampling.



TEXT-FIG. 5. Effect of bacterial contamination upon perfusate concentration of pyruvic acid, lactic acid, and ammonia in the isolated bovine liver perfusion.

Electron micrographs of perfused liver revealed remarkably intact cellular structures (Fig. 2). Cell membranes were sharp, and gave no indication of impending dissolution; likewise, the nuclear membrane was also intact. The mitochondrial particles were present in normal numbers and appeared to be intact. Certain differences between perfused and control tissue samples from the same liver were noted, however; in the perfused liver there were indications of glycogen depletion, a tendency toward more perisinusoidal spaces, and a

reduction in the number and length of the microvilli of the bile canaliculi. The perfused livers tended to have an increased number of elongate mitochondria and a looser, more spongy appearing endoplasmic reticulum. The significance of these changes[6] is unknown, but conceivably might relate to a lack of addition of nutrients during the course of the perfusion.

TABLE III

Oxygen Consumption of the Perfused Bovine Liver

Wet liver weight	Sample No.	Time of sample from beginning of perfusion	A-V O_2 Diff.	Total liver blood flow	Ml. O_2/min. 100 gm. wet liver
gm.		*hrs.*	*vol. per cent*	*ml./min.*	
684	1	½	5.8	252	1.9
	2	2	8.5	"	2.8
	3	4	6.6	"	2.1
	4	6	7.9	"	2.6
	5	8	6.7	"	2.2
	6	10	6.0	"	1.9
	7	12	6.0	"	1.9
995	1	½	3.5	204	0.7
	2	2½	5.7	"	1.2
	3	4½	3.5	"	0.7
	4	6½	3.2	"	0.7
	5	8½	3.3	"	0.7
1315	1	¼	8.5	190	1.2
	2	1½	4.3	388	1.3
	3	3	7.9	190	1.1
	4	4½	8.4	"	1.2
	5	6	7.9	"	1.1
1326	1	¼	4.7	190	0.7
	2	1½	6.9	"	1.0
	3	3	7.4	"	1.1
	4	4½	7.6	"	1.1
	5	6	7.9	"	1.1

Bromsulphalein Clearance.—The clearance of bromsulphalein dye from the plasma by the perfused liver and its appearance in the bile has been studied and reported in detail in a separate communication (9). The rate of clearance of this dye in the isolated liver is most efficient and parallels the clearance in the intact animal.

[6] Dr. Richard L. Wood, Department of Anatomy, has in press additional information and discussion of these observations.

DISCUSSION

The general technique of isolated liver perfusion has been described previously. Investigators have successfully perfused rat (8, 10, 16), rabbit (17), and frog (11) livers. Use of these species for isolated liver perfusion has been advantageous because of their small size, modest cost, reproducibility, and the simple, inexpensive apparatus required. For these reasons, perfusion of the isolated liver of these small animals has become a useful and practical method for the investigation of many biochemical and physiological phenomena.

Pooled heparinized blood supplied by several homologous donor animals is used as the perfusion medium for these small animals, thereby introducing potential problems associated with interaction of proteins from donors of only partial compatibility. Another feature of these preparations is that cannulation of the hepatic artery has not been possible; therefore, all blood must be supplied to the isolated liver through the portal vein.

In contrast, the large size of the bovine calf permits the use of autogenous blood as the principal perfusion medium. Simultaneous and independent perfusion through the hepatic artery and portal vein is possible, and repetitive generous tissue sampling of the liver itself can be accomplished without interrupting the flow of perfusate.

The pH of the perfusate is maintained within physiological limits. The data on oxygen consumption in the isolated liver suggest that during the useful life of the preparation, oxidative metabolic processes within the same liver remain stable.

The factors preventing maintenance of bacterial sterility for periods longer than 12 to 14 hours have not been fully explored. Probably the small number of bacteria introduced from time to time are held in check by defense mechanisms; when these are exhausted, bacterial growth appears unchecked. The utter essentiality of maintaining a bacterial-free perfusate within a physiological pH range cannot be overemphasized for such preparations made with intent to study physiologic and biochemical mechanisms.

The repetitive, normally efficient clearance of bromsulphalein and steady state of oxygen consumption in the bovine livers herein described attested to their metabolic "intactness." Negligible hemolysis of circulating erythrocytes occurred, plasma hemoglobin values never exceeding 20 mg. per cent. Especial reassurance as to the satisfactory state of the preparation was provided by the electron microscopic observations demonstrating intactness of the important submicroscopic cellular structures.

In our previous experience with the canine,[7] it did not appear possible to prepare consistently a satisfactory isolated perfused liver preparation; progressive and severe congestion was regularly observed, due to outflow obstruc-

[7] 48 separate canine liver perfusions were conducted in this laboratory in collaboration with Dr. T. W. Jones and Dr. R. V. DeVito.

tion from the occlusive spasm of the hepatic vein system. Identical problems of the isolated canine liver were described by Bauer (6). Acting as the principal anaphylactoid end organ of the dog, even the very small hepatic venules have been demonstrated to contribute to this "throttle" mechanism by virtue of their well developed, spirally arranged, smooth musculature (22). Arey (3, 4), after studying more than 30 mammalian species, reported that only 3, the domestic dog, raccoon, and seal had peculiar sphincter-like arrangements of smooth muscle in the walls of the hepatic veins; and that the dog's hepatic veins contained a relatively enormous amount of smooth muscle in comparison with the hepatic veins of the other mammals. Thomas and Essex (22) have demonstrated a diffuse occlusive spasm of the entire canine hepatic venous vasculature that was even more pronounced in the smaller vessels than in the larger ones. In spite of this difficulty, certain other investigators have claimed success with canine liver perfusions (1, 2, 13, 20).

In contrast, the bovine calf does not appear to possess an active hepatic vein throttle mechanism. This fortunate occurrence has permitted lengthy perfusions in this species without the intra-hepatic sequestration of large volumes of perfusate.

The intermediary metabolic pathways of the very young bovine are similar to those of monogastric mammals; however, after cessation of suckling and the development of rumenal metabolism, the intermediary metabolic pathways become somewhat different from those of the monogastric mammal (7, 12, 14, 21).

SUMMARY

1. A procedure is described for surgically isolating and artificially perfusing the liver of the young bovine. Heparinized autogenous blood from the hepatectomized animal diluted with Krebs-Ringer-bicarbonate solution is employed as the perfusate.

2. This preparation has been satisfactorily reproducible in this laboratory in more than 90 separate sequential perfusions. Absence of a sensitive hepatic venous smooth musculature contributes to the ability to maintain satisfactory perfusion for as long as 24 hours.

3. The perfusate was usually maintained bacterially free for at least the first 12 hours of perfusion without the use of antibiotics. The perfusate was maintained at normal body temperature and, by varying the CO_2 content of the oxygenating gas, within a physiological pH range. The importance of these features when studying problems of intermediary biochemistry and ultrastructure is emphasized.

4. The liver of the bovine calf is sufficiently large to permit (*a*) simultaneous and independent perfusion through the hepatic artery and portal vein, and (*b*) repeated sampling of heaptic tissue without interruption of the circulation.

5. Excellent, viable condition of the isolated liver, throughout many hours

of perfusion, was demonstrated by steady state of oxygen consumption, efficient clearance of bromsulphalein dye, continuous secretion of bile, constancy of blood flows and pressures, and very minimal alterations from normal in histologic and ultrastructural detail.

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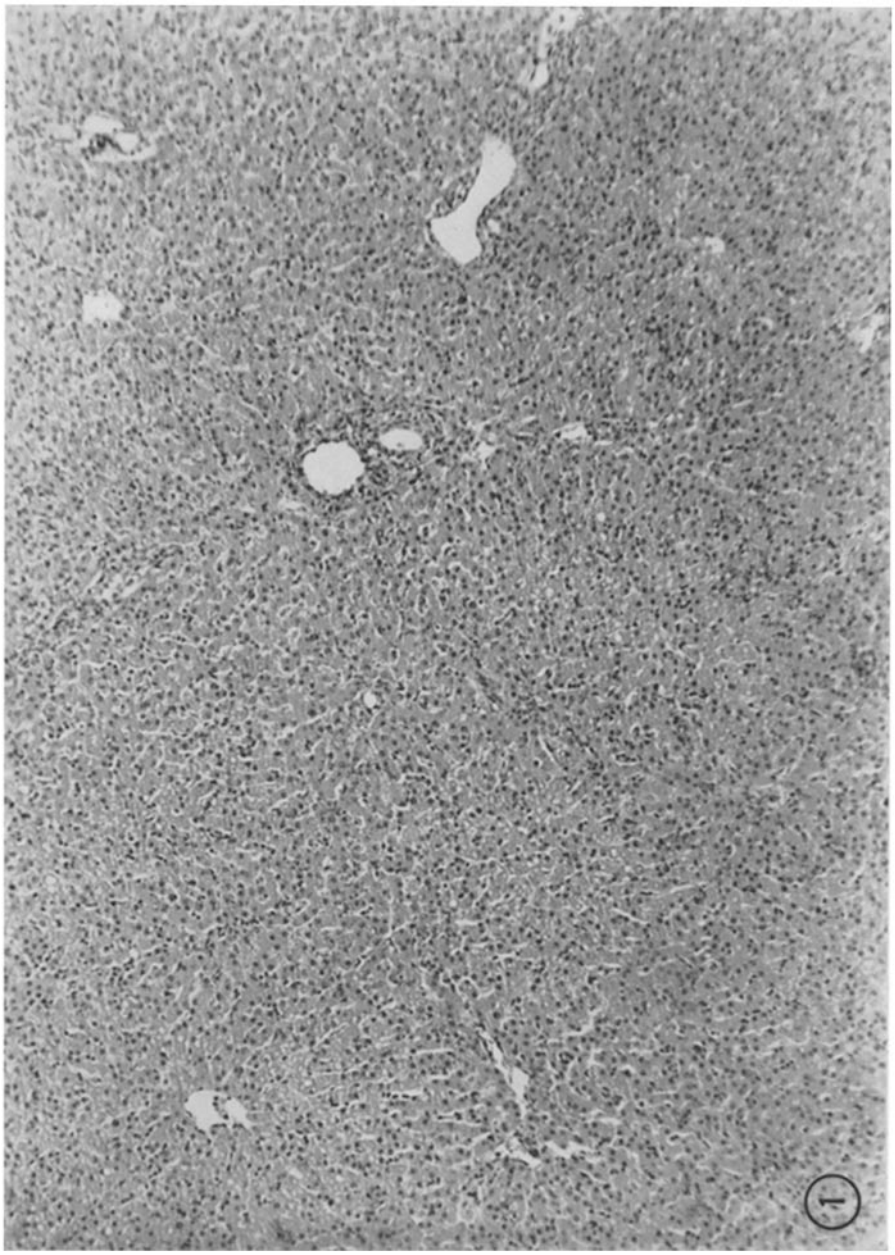
EXPLANATION OF PLATES

PLATE 101

FIG. 1. Histologic section from an isolated bovine liver 8 hours after start of perfusion. × 100.



(Chapman *et al.*: Bovine liver)

PLATE 102

FIG. 2. Electron micrographs of bovine liver. (*a*) Before perfusion, (*b*) 8 hours after start of perfusion. × 5000.

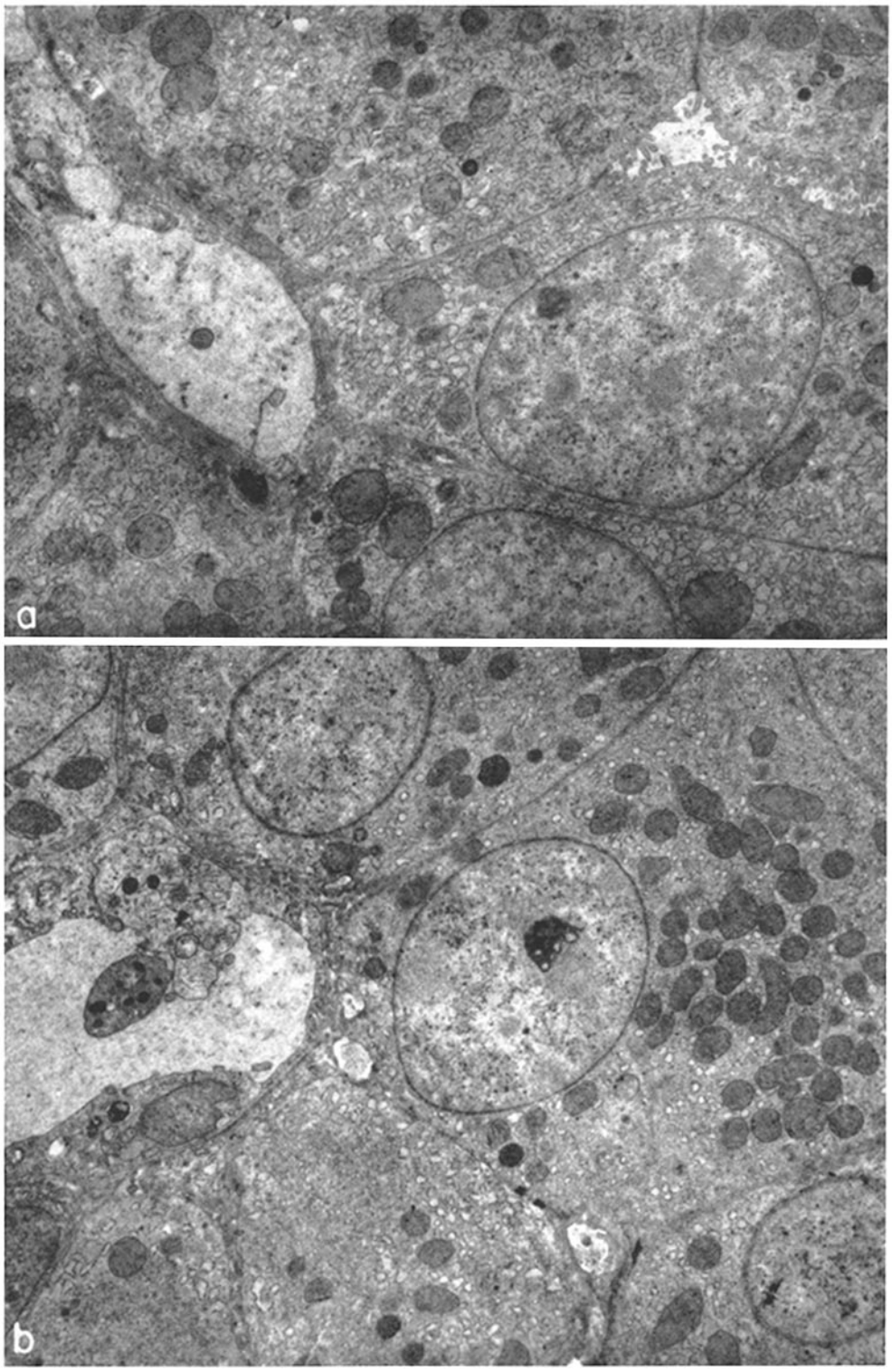


FIG. 2

(Chapman *et al.*: Bovine liver)