Hydrocholeresis in the Isolated Perfused Rat Liver

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The construction and operation of a simplified isolated rat liver perfusion apparatus consisting of readily available components are described. In 30 determinations this procedure successfully maintained isolated rat liver function to a degree equivalent to that obtained by other investigators.

PIONEER STUDIES with perfused livers as reported by Bauer, *et al.* (1), were made by Mautner and Pick in 1915. These early perfusions, which employed saline perfusates, lead to the more recent and refined investigations of Brauer, *et al.* (2), who used expanded blood perfusates. This latter procedure required considerable glass manipulation, for the apparatus was tailored to the morphology of the liver. Young, *et al.* (3), later developed a rabbit liver perfusion apparatus which had the advantage of being composed largely of standard glassware, facilitating initial construction and replacement. An adaptation of this sytem was used in the work herein reported.

Isolated liver perfusion as a means of studying liver function and metabolism is useful because it permits hepatic activity to be studied in the absence of extrinsic nerve supply and lymphatic plexus. Brauer, *et al.* (2), reported that maintenance of a liver in near physiologic state was excellent if a blood-containing perfusate were used.

METHODS AND MATERIAL

Perfusion Apparatus.—The perfusion apparatus (Fig. 1) consisted of a perfusate reservoir, oxygenating column, organ chamber, circulating pump and filter system, hydrostatic perfusate reservoir, and a thermostatically (37.5°) controlled thermal plywood cabinet with a glass door. Heat was supplied by four 100-watt shielded light bulbs.

The reservoir [1] was a 100-ml. three-neck distilling flask with a standard taper 24/40 center-neck. All other ground-glass joints in this apparatus were standard taper 24/40. The oxygenation system consisted of an oxygenating column [2] made by fusing an outer and inner Pyrex joint to give a column of 20 cm. total length. The connecting tubes [3] on either end of this column were Pyrex with 105° side arms. The Pyrex inner joint [4], above the upper connecting tube, was cut to fit the lower neck of the organ chamber [5] with the aid of a bored-rubber stopper gasket. The organ chamber consisted of two 150-mm. desiccator covers with greased, ground-glass surfaces to insure an air-tight seal for good oxygen circulation through the apparatus. A small hole in the lower cover allowed for egress of the bile duct cannula. The organ mounting cradle [6] was made of No. 50-mesh stainless steel screen. Special attention is directed to the cannula attachment [7, 8] which was fashioned by fitting a rubber stopper in the upper desiccator neck with a short length of 9-mm. Pyrex glass tubing which extended above the stopper $^1/_4$ inch. An 11.5-cm. length of 7-mm. Pyrex tubing was slipped into the 9-mm. length, and the upper juncture of these sealed with a short length of rubber tubing. This allowed for sliding the inner glass tubing leading to the portal cannula up or down for positioning the excised liver. The double perfusion valve [11] was available from the Macalister-Bicknell Co., Cambridge, Mass. The blood filters [10] were Baxter Filterdrip assemblies which were modified to reduce their volume by use of 7-mm. Pyrex glass tubing extensions [13]. These extensions were inserted into the top of the assemblies and terminated a few millimeters above the filter elements. The double filter system enabled transfer of filters should one become clogged during a perfusion. The pulsating tube [12] was made from a 2-inch length of 22-mm. Pyrex glass tubing joined to a $1^1/_2$-inch length of 7-mm. Pyrex tubing. The diaphragm of this pulsating pump was a large rubber finger cot which was folded over the large end of the pulsating tube and held in place by rubber cement and closed by the rubber adapter cap of a disposable Baxter blood administration set. A pipetting machine [21] with valves removed was fitted with a lubricated 30-ml. syringe to provide the reciprocating action necessary to expand and collapse the finger cot diaphragm of the pulsating chamber. The hydrostatic reservoir [15] was made from a $2^1/_2$-inch length of 22-mm. Pyrex tubing attached at the bottom to a short length of 7-mm. Pyrex tubing. The overflow arm was made from 9-mm. Pyrex tubing with its lower end reduced to 7 mm. This side arm was attached to the reservoir 1 inch from the top. The increase in tube size in this arm permitted a noncontinuous overflow of the perfusate which bipassed the liver. This reservoir was elevated to give 21.5 cm. of hydrostatic pressure from overflow arm to portal vein of the liver. The infusion system [17] consisted of a Phipps and Bird syringe driver adapter fitted to a kymograph and adjusted to deliver 0.012 ml./min. of infusate into the system via the latex tubing above the organ chamber. A few drops of Dow Corning antifoam B were added to the saline humidifier [16] to prevent excessive foaming. The rubber connecting tubing used throughout this system was amber latex rubber tubing with 5 mm. i.d. and 1.5 mm. wall thickness. Especially constructed glass cannulas were used for the portal and vena cava cannulations, as adapted from the work of Werthessen (4).

The entire system except for the pulsating tube

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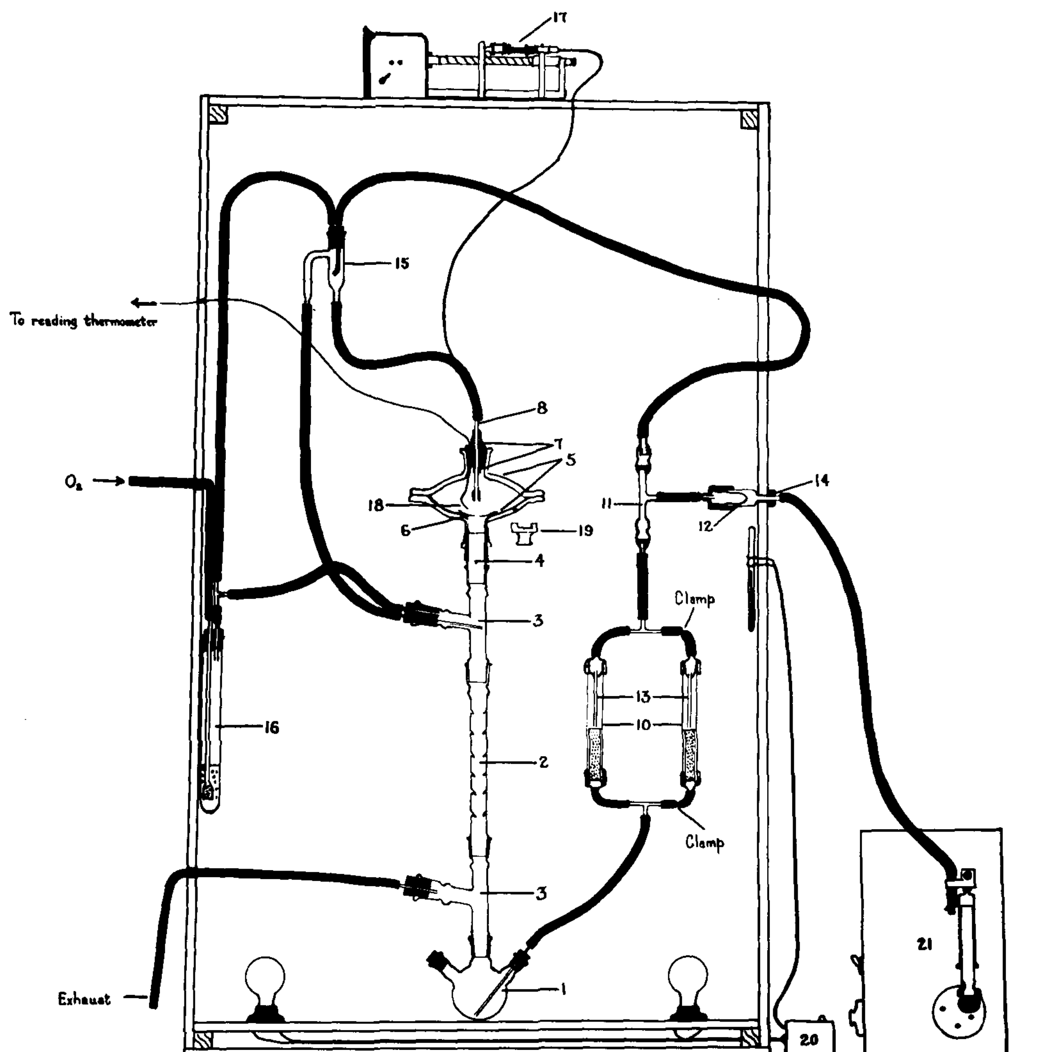

Fig. 1.—Liver perfusion apparatus. 1, Reservoir; 2, oxygenating column; 3, connecting tubes; 4, inner joint; 5, organ chamber; 6, organ mounting cradle; 7 and 8, adjustable portal cannula attachment; 10, dual blood filters; 11, double perfusion valve; 12, pulsating tube; 13, tubular extensions; 14, rubber friction washer; 15, hydrostatic reservoir; 16, gas saline humidifier; 17, infusion system; 18, thermometer thermocouple; 19, planchet magazine; 20, thermostat; 21, pipetting machine.

was steam sterilized prior to use, and the finger cot diaphragm of the pulsating tube was soaked with benzalkonium chloride 1:500 for 3 hours prior to assembly.

Perfusate.—The perfusate and infusate mixtures were adopted from Brauer, *et al.* (2), to give a basis for performance comparisons with his biliary flow rates. Perfusate solutions A (expanded physiologic saline)[1] and B (phosphate buffer system) were mixed, added to the reservoir and equilibrated with oxygen for 2 minutes, and then solution C (rat blood) was added. The infusate mixture consisted of an antibiotic and nutrient in an expanded saline medium.

Operative Technique.—Under ether anesthesia the bile duct of Sprague-Dawley rats was cannulated with P.E. 50 tubing. The inferior vena cava of the heparinized animal was ligated just superior to the right renal vein and divided. The inferior vena cava was cannulated just superior to the diaphragm and divided. The portal vein was cannulated last and severed. The organ was then removed by excising the diaphragm and dividing the suspensatory ligaments attached to the organ. The liver was mounted, diaphragm down, on the stainless steel cradle and attached to the filled perfusion system making sure all lobes were free for circulation. The bile duct cannula was placed through the hole in the organ chamber provided for it and positioned over the planchet magazine. Then the covers of the organ chamber were sealed. The thermocouple to the thermometer was then positioned to rest lightly on a lobe of the liver. Surface temperature varied from 38.5 to 39.5° which was within the temperature range of maximal bile production as reported by Brauer, *et al.* (5).

[1] The authors are indebted to the Armour Laboratories for the bovine plasma albumin used in the perfusate.

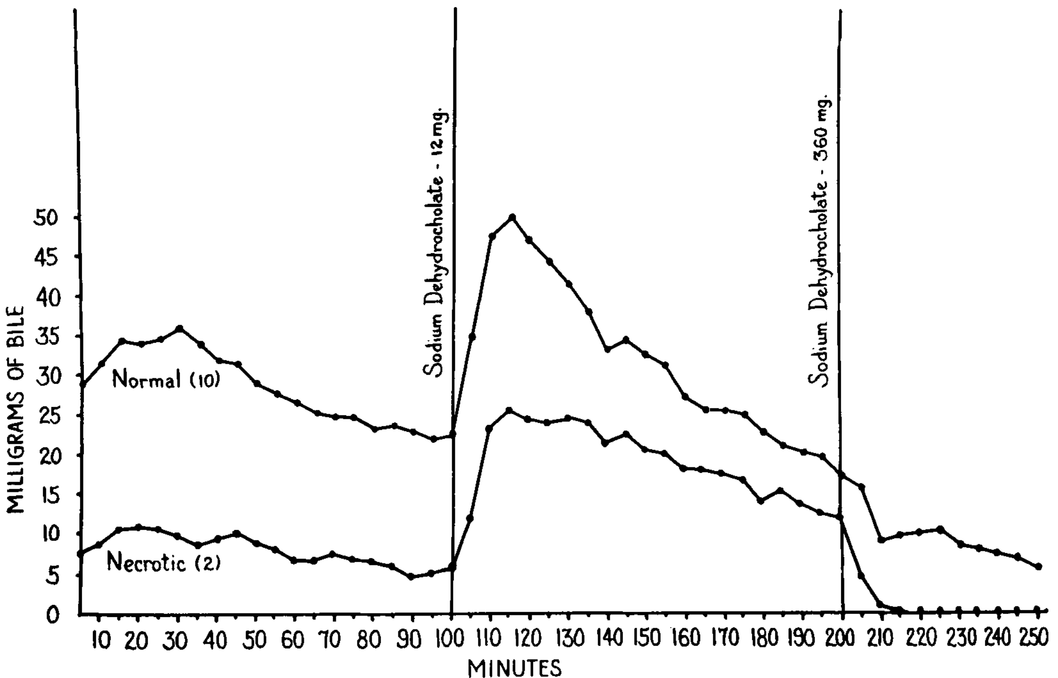

Fig. 2.—Bile flow of normal and necrotic isolated perfused rat livers.

Operation of Apparatus.—Oxygen flow was maintained at 2 L./min. The perfusate pumping rate was regulated to pass about one-half as much perfusate over the hydrostatic reservoir overflow as that which passed throuhg the organ. Perfusate flow varied somewhat in each individual organ, but the usual rate was 100 to 150 ml. per minute.

A properly functioning organ was evidenced by the gradual change in lobe color from dark to pink, as well as by the rate of bile flow. A nonperfusing lobe remained dark and required repositioning to initiate perfusion.

After an equilibration period of 15 minutes, bile was collected at 5-minute intervals in tared cup planchets and quickly weighed.

EXPERIMENTAL DATA

Fifteen Sprague-Dawley albino rats, ranging in weight from 350 to 400 Gm., served as liver donors in the early phases of the experiment where adjustment of the oxygenation rate was necessary, as well as the perfusion design and technique. In addition, 30 animals were used for varying periods of time. Of these, 12 were complete runs of 250 minutes and are included to illustrate the operation of the equipment. The high rate of failures indicates the technical complexities of isolated rat liver perfusion. Mean perfusion rates of 10 normal livers and two necrotic livers and their response to sodium dehydrocholate[2] are reported. The data are preliminary and are to be extended.

Twenty-four-hour liver necrosis was induced by an oral dose of 3 ml./Kg. of a 40% mixture of carbon tetrachloride in olive oil (6). Special care in handling these organs was needed because the carbon tetrachloride induced considerable fragility.

A rapid increase in bile flow occurred during the first 45 minutes of perfusion followed by a gradual decline for about the same time-interval, finally reaching a steady phase of flow of approximately 23 mg. per 5-minute interval (Fig. 2).

During the steady phase the initial 12-mg. dose of sodium dehydrocholate was given into the latex tubing of the hydrostatic reservoir overflow which allowed for maximal mixing with the perfusate before reaching the liver. The average bile flow (by weight) at the time of the dose was 22.2 mg., while the average peak bile flow 15 minutes later was 49.9 mg. per 5-minute interval, an increase of 125 $\pm$ 14.7%. During hydrocholeresis the perfusate flow was unchanged.

The initial high rate of bile flow was not as pronounced in the necrotic preparations but had the same general pattern as the normal. The necrotic livers exhibited considerably less biliary flow than their normal counterparts. The relative percentage response of the necrotic livers to the 12-mg. dose of sodium dehydrocholate was greater than in the normal livers. The average bile flow (by weight) of the necrotic livers at the time of the dose was 5.5 mg.; 15 minutes later the average peak bile flow was 25.3 mg. per 5-minute interval, a 360 $\pm$ 68.7% increase. Again the perfusate rate remained constant. The stimulated response was considerably more prolonged in the necrotic livers as compared to the normal.

After allowing 100 minutes for the initial response to sodium dehydrocholate (12 mg.) to subside, the 360-mg. dose was given as before. This dose was equivalent to the 200 mg./Kg. dose used routinely in this laboratory with *in situ* work.

[2] Appreciation is expressed to George A. Breon and Co. for the Dilabil-Sodium Injection.

The response from this massive dose resulted in an abrupt decrease in normal liver biliary flow from an average weight at the time of administration of 17.2 mg. to 5.9 mg. of bile per 5-minute interval at depth of depression, a 67.5 ± 11.8% decrease. Again the perfusate flow remained constant.

Although necrotic livers did not return to their stable biliary flow rate in 100 minutes, the 360-mg. dose of sodium dehydrocholate was given for experimental continuity. Bile flow decreased from an average flow at time of administration from 12.0 to 0.00 mg. of bile per 5-minute interval, a 100 ± 0.00% decrease. The perfusate flow remained constant as before. Obviously, the latter data are suggestive only.

DISCUSSION

The described liver perfusion unit differs from others in that standard glassware and simple cabinet construction were used. Using identical perfusate and infusate mixtures and the same hydrostatic pressure as Brauer, *et al.* (2), a normal bile flow pattern identical with his was found. However, the hydrocholeretic response was not proportionally as great as that from the 2 mg. of sodium dehydrocholate used by Brauer. Perhaps this was due to the addition of the drug by Brauer into the system just above the liver, which resulted in a greater initial hepatic concentration of drug.

The decrease in biliary flow due to the 360-mg. dose was apparently not a vascular response because the perfusate flow remained constant. During preliminary runs, large doses (360 and 840 mg.) of sodium dehydrocholate were found to elicit a depression of bile flow during the first hour of perfusion. Also, 2-mg. doses of sodium dehydrocholate given at 40, 95, 135, and 160 minutes resulted in equivalent increments in biliary flow indicating that the depressive response is a true reaction and not just the malfunction of a fatigued or subnormal organ. Goodman and Gilman (7) and Clark (8) noted that the toxic effects of bile salts resulted in inhibition of cholinesterase. Perhaps endogenously released acetylcholine caused a decrease in bile flow through biliary tract constriction.

The perfusion rate of the isolated perfused rat liver was considerably above the *in situ* rate of blood flow. Brauer, *et al.* (9), attributed this fivefold increase in flow rate to the inactivation of a hepatic vasoconstrictor factor resulting in full vasodilatation of the hepatic vascular tree.

The initial rise in bile flow may be due to a recovery from liver hypothermia following the surgical procedure. Brauer, *et al.* (10), noted that bile elaboration was depressed when liver temperature was reduced from its optimal value of about 40°. Upon rewarming, an excessive flow with increased bilirubin content resulted for a brief period. This surge was perhaps due to an accumulation of intermediate metabolites leading to excessive flow once metabolic balance was restored upon rewarming. The initial collections of bile in this work were noticeably darker, which quite possibly indicated an increment of bile salts.

It is quite apparent that necrotic liver data are insufficient for evaluation, but were included to indicate a trend.

SUMMARY

The assembly and operation of a functional isolated rat liver perfusion apparatus is described using simple materials and standard glassware. Preliminary data are included to illustrate the operation of the unit.

The mean per cent response of normal isolated perfused rat livers to 12 mg. of sodium dehydrocholate from point of administration to peak of hydrocholeretic rise was 125 ± 14.7% (22.2 to 49.9 mg. bile per 5-minute interval). The comparable response of necrotic livers was 360 ± 68.7% (5.5 mg. to 25.3 mg. bile).

The bile flow response to 360 mg. sodium dehydrocholate resulted in a decrease from point of administration to depth of depression of 67.5 ± 11.8% (17.2 to 5.9 mg. bile per 5-minute interval) for normal livers, and 100 ± 0.00% (12.0 to 0.00 mg. bile) for necrotic livers.

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