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Application of Perfused Rat Liver Preparation to Isotopic Study. (19866)

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It seems a reasonable assumption that an isolated liver forming bile is a living liver. Such a preparation lends itself to many useful studies. Many apparatus and methods for perfusing the isolated liver of the rat have been used. A particularly good method emphasizing bile production has been developed by Brauer and coworkers(1). The authors have presented evidence, in addition to bile production, that the liver is viable during perfusion. This recent paper appeared subsequent to the completion of the present study. A perfusion apparatus that performs reasonably satisfactory has been made in this laboratory and adapted to the investigation of liver physiology in relation to radioactive materials. Gallium^{72} citrate was used in preliminary experiments.

General perfusion technic. The perfusion model, designed by one of the authors (M.B.), was fashioned in the shop of this laboratory from lucite. The essential parts comprising the apparatus are shown Fig. 1. They consist essentially of an oxygenation chamber, an artificial heart, a windkessel, which acts to convert a pulsatile into a constant flow, and an adjustable side reservoir. Polyethylene tubing is used for connections. About 90 ml of blood are required for operation of the present model. The artificial heart is driven by a variable stroke pump† set to deliver about 110 ml of air per stroke at a stroke rate of about 190 per minute. Blood flow from the portal vein cannula, when attached separately to the apparatus, has been measured as about 7.5 ml per minute. However, blood flow from the inferior vena cava during perfusion has been measured as about 2.5 ml per minute. The flow from the portal vein cannula is delivered under a head of constant

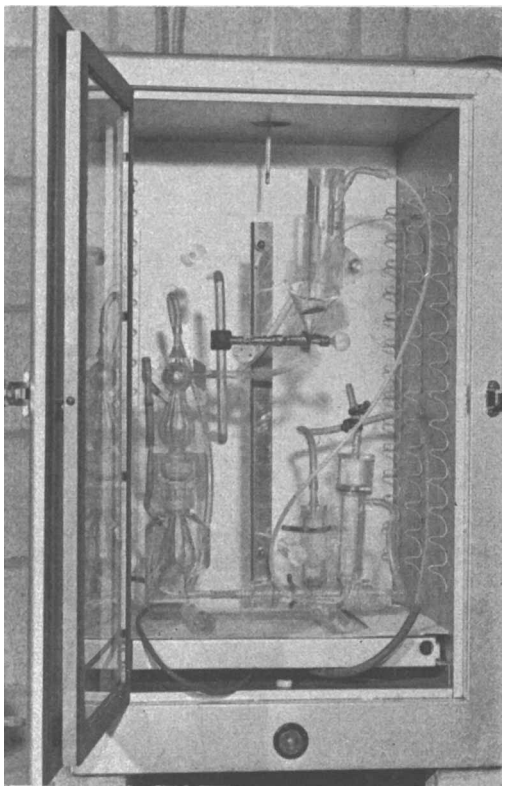

FIG. 1.

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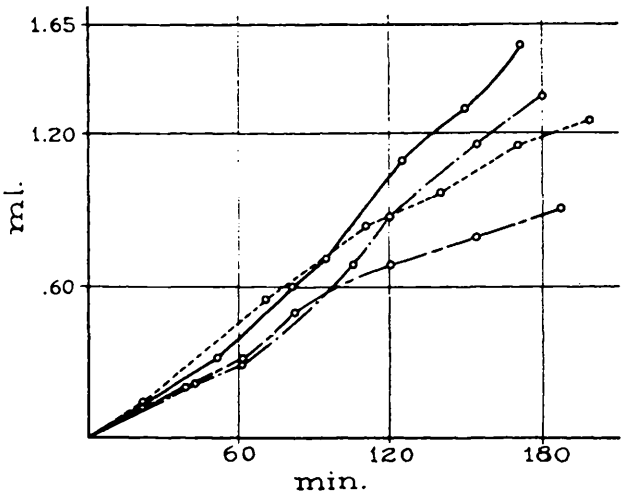

FIG. 2. Bile flow as evidence of viability in liver perfused with solution containing .26 millicurie of Gallium^{72}.

pressure of approximately 10 in. of water. A mixture of 95% oxygen and 5% carbon dioxide is supplied the oxygenation chamber at the rate of about 2 liters a minute. The gas mixture is passed through water before entering the oxygenation chamber. Temperature of the heating cabinet in which the perfusion machine is placed is kept at 37° to 38°C. The blood used is heparinized fresh whole rat blood. A typical perfusion experiment is carried out as follows. A large adult rat (Wistar), preferably male, is anesthetized with sodium pentobarbital, the abdomen opened, and the blood heparinized. The bile duct is cannulated with polyethylene tubing (i.d. .019") by approaching it from the duodenum. The inferior vena cava is then cannulated with a short polyethylene cannula, preferably slightly larger than that used in the portal vein. This cannula is stoppered. Lateral vessels (usually 2) of the portal vein, just before entrance into the liver, are tied. The portal vein is then cannulated with tubing (i.d. .062") which has been carefully filled with blood. It is very important that no air be in this cannula. This cannula is also stoppered. The perfusion apparatus is removed from the heating cabinet while cannulation of the portal vein is proceeding. Circulation of the blood in the apparatus is begun, and the portal vein cannula is then unstoppered and attached as quickly as possible. The stopper is removed from the inferior vena cava. The liver is cut out of the animal and placed in the organ receptacle with the inferior vena cava cannula pointing downward. The interval elapsing from the time the portal vein is cannulated to the time the liver is finally installed is about 2½ minutes. The apparatus is then placed back in the heating cabinet and bile flow begins almost immediately.

Gallium^{72} citrate. The present study was conducted primarily to demonstrate the feasibility of using a radioactive material with the isolated rat liver perfusion system with bile production. Gallium^{72} citrate has been used. In Fig. 2 are presented the curves of bile production plotted from the data of 4 experiments, in each of which a separate rat liver was used. Bile and dehydrocholic acid were added not primarily to measure volume of bile flow as affected by these agents but rather to enhance bile flow which furnished evidence for viability of the liver.

Four curves of bile flow are shown in Fig. 2. They are as follows: 1) Nothing was added to the perfusing blood (control); 2) 0.75 ml of perfused bile and 1.14 ml of bile

TABLE I. Activity of Blood, Liver, and Bile in Perfused Rat Liver Preparation.

Sample	Counts/min/ml or g ($\times 10^4$)
Blood (30 min intervals)	
1	8.5
2	8.4
3	7.7
4	8.5
5	8.5
6	8
Liver	2.2
Bile	1.8

obtained from another rat *in vivo* were added; 3) 0.60 ml of perfused bile plus 0.9 mg of dehydrocholic acid were added; and 4) 0.5 ml of gallium citrate solution, containing Gallium^{72},‡ and 0.5 mg of dehydrocholic acid were added. The latter solution contained 0.26 millicurie of Gallium^{72} activity. The quantity of gallium metal in the gallium citrate solution usually prepared was 8 to 10 mg per ml. All substances were added by way of the side reservoir.

Each perfusion was carried out about 3 hours, as shown in Fig. 2. Brauer and coworkers have obtained maximum rate of bile flow in this early phase(1). The bile and dehydrocholic acid were added at various times throughout the course of the perfusion.

The gallium citrate solution, containing Gallium^{72} was introduced into the perfusing blood 20 minutes after perfusion had begun. In this experiment samples of blood (0.1 ml) were taken every half hour for radioactivity measurement. The liver was perfused with isotonic saline several times and then digested with concentrated nitric acid. A diluted sample was placed in a small Petri dish for counting. The diluted samples of blood and bile were similarly counted. A lead-walled, horizontal counting chamber with a thin-walled Geiger-Mueller tube was used. The activity is expressed as counts per minute, corrected to a zero time, per ml, or g (Table I).

Results. It is evident that the introduction of the 0.26 millicurie of Gallium^{72} and stable gallium citrate into the perfusing blood did not prevent the isolated liver from producing bile for a period of at least about 3 hours. On the assumption that the isolated liver producing bile is a living liver, it is concluded that the liver was viable throughout this time. It seems the curves presented in Fig. 2 indicate strongly that the volume of bile produced was not, at least, drastically modified for that length of time after the 0.26 millicurie of activity had been introduced.

The radioactivity measurements are presented in Table I. The level of activity in the blood throughout the 3 hours remained rather constant. The liver had considerably less activity than the blood and the bile slightly less than the liver. Thus, it seems evident the bile per unit volume contained nearly as much Gallium^{72} as the liver. These data indicate that the particulate state of the gallium or gallium compound was not of sufficient size to be very effectively retained by the reticuloendothelial system of the liver. Both the higher level of activity in the blood and the nearly equal levels of activity of the liver and bile seem to support this.

Conclusion. The technic of isolated rat liver perfusion with bile production seems to be quite applicable to the study of the effects of radioactive isotopes on certain aspects of liver function; 0.26 millicurie of Gallium^{72} citrate solution with stable gallium has been used in the present study. The liver remained viable within the interval specified in the experiment. Blood activity levels remained constant. Both liver and bile contained Gallium^{72} although in lesser quantities than the blood.

‡ Prepared in the laboratory of the Medical Division from irradiated $Ga(NO_3)_3$ by Dr. J. D. Perkinson.

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