Investigation of Hepatic Drug Metabolism by Isolated Perfused Liver *In Situ*

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The metabolizing activity of the hepatic tissues in an isolated *in situ* liver preparation has been examined. In this experiment the organ remained connected to the other parts of the body, but its blood circulation was supplied by a pump–oxygenator system. By this method some hepatic metabolic activities, such as demethylation, acetylation, and glucuronide-conjugation, have been evaluated.

THE TECHNIQUE of local perfusion of organs was developed with the specific purpose of minimizing the toxicity and increasing the chemotherapeutic activity of carcinolytic agents. Klopp *et al.* (1) maintained in 1950 that the therapeutic effect of drugs could be enhanced and the toxicity greatly reduced by intravascular injections into regions of the body containing tumors. In 1958 Creech *et al.* (2) and Ryan *et al.* (3) began the use of local therapy by isolating the tumor-bearing area from the remainder of the circulation and perfusing it with the aid of a pump–oxygenator system. According to these authors, the technique of regional perfusion is based on the concept that various anatomic regions can be isolated from the general circulation, supplied with a separate extraneous system for pumping and oxygenating the blood, and maintained for varying periods of time under these conditions without significant systemic effects.

Subsequently, these or related techniques have been utilized by many investigators, and Stehlin *et al.* (4), Creech *et al.* (5), Shingleton *et al.* (6), Ausman and Aust (7), Clarkson and Lawrence (8), and others have published excellent descriptions.

In the course of systematic investigations of drug metabolism (9–11), we developed a method to evaluate the liver metabolic activity *in situ*, utilizing the concepts of the authors quoted above. To test the method itself, we have also examined some metabolic transformations of aminopyrine and oxazepam.

EXPERIMENTAL

The abdominal technique of liver perfusion was carried out on 16 dogs (14.5 to 29.0 Kg.) and on 4 monkeys (*C. hamadryas;* 11.4 to 16.2 Kg.) preanesthetized with urethan (0.4 Gm./Kg. i.p.). Anesthesia was induced and maintained in closed circuit by nitrous oxide, cyclopropane, or ethyl ether. The animals were given artificial ventilation after tracheal intubation by Warne tube during succinylcholine chloride action (1 mg./Kg. i.v.). The operative procedures are indicated in Fig. 1 and are briefly summarized here.

Before laparotomy, a large outflow tube is inserted into the right external iliac vein (*via* femoral vein, 10) and connected with a large inflow tube inserted into the right external jugular vein, 9, in the dog or into the right internal jugular vein in the monkey; the connection is made by a large silicone coated para-rubber cannula joined to a Sigmamotor pump, 12.

After laparotomy, the inferior cava vein, 6, is ligated above the renal veins, 17. The phrenico-abdominal veins are also ligated or excluded. Into the inferior cava vein, clamped below the liver, a special three-way outflow cannula is inserted immediately above the ligature; this tube, 5, contains a movable rubber balloon (connected with a syringe, 14), which after clamp removal, is passed into the cava, 7, over the diaphragmatic foramen. The rubber balloon, 18, remains deflated, and the outflow tube, 5′, is clamped. It is necessary to tie the accessory portal veins, and particularly the gastro-hepatic, paraumbilical, diaphragmatic, and cardial groups.

A T-shaped outflow tube, 13, inserted into the porta or the mesenteric vein, is connected, by a silicone coated para-rubber cannula, with the Sigmamotor apparatus, 12.

An inflow cannula, 3, is inserted into the portal vein and ligated below the liver but immediately before the portal vein divides into two or more branches. Both the portal inflow cannula, 3, and the cava outflow cannula, 5, are connected with the pump–oxygenator system, 4.

The rubber balloon, 18, is inflated by the syringe, 14, and drawn back (under X-ray examination) to

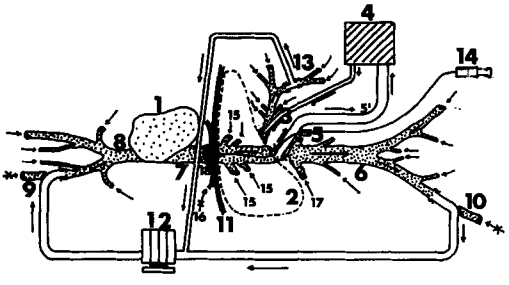

Fig. 1—Diagram of the experimental arrangement in dog. Key: 1, *heart;* 2, *liver;* 3, *portal inflow cannula;* 4, *pump–oxygenator system;* 5, *three-way cannula with outflow tube,* 5′; 6, *under-diaphragmatic inferior cava vein;* 7, *over-diaphragmatic inferior cava vein;* 8, *superior cava vein;* 9, *right external jugular vein;* 10, *right femoral vein;* 11, *diaphragm;* 12, *Sigmamotor pump;* 13, *T outflow cannula;* 14, *syringe connected with the rubber balloon,* 18; 15, *hepatic veins;* 16, *phrenic veins;* 17, *renal veins;* 18, *movable rubber balloon.*

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block, in the diaphragmatic foramen, both the cava vein and the blood flow from the phrenic tributaries, 16. At the end, the hepatic artery is ligated and the perfusion begins. It is necessary to make several ligatures to block the lymphatic ducts of the hepatic hilum and the pancreas.

The pump–oxygenator system consists of: a venous reservoir, an oxygenator with gasmeter, a roller-type pump with flowmeter, a blood filter, an apparatus to eliminate the blood foam, a perfusion pressure regulator, and a blood heat exchanger with telethermometer. Before the extracorporeal liver perfusion, the pump–oxygenator system is filled with 300–400 ml. of heparinized blood previously obtained from the animal itself that was infused with 300–400 ml. of blood from donor animals. The priming blood is circulated through the extracorporeal system, fully oxygenated, and warmed. A flow of oxygen into the oxygenator is maintained at the rate of 4–8 L./min. During the extracorporeal liver perfusion the blood flow rate was 4–10 ml./min./Kg.

The evaluation of any leakage from the extracorporeal circuit can be determined: (*a*) during the metabolic research, by taking samples from the general circulation of the blood and the lymph to verify the absence of the studied substance or its metabolites; (*b*) at the end of the metabolic research, by adding to the blood of the extracorporeal circuit either a dye (which can be examined, for its eventual leakage, by autopsy) or a radiopaque substance (which can be traced by radiography).

To confirm the proper function of the liver, the concentration of serum glutamate-oxalacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), sorbit dehydrogenase (SDH); leucine aminopeptidase (LAP) and seric hemoglobin was determined in blood samples from the extracorporeal circuit. From bile samples, the time of bromosulphonthalein (BSP) elimination from the blood of the extracorporeal circuit was determined.

To evaluate the metabolizing activity of the liver, the following were examined: (*a*) the demethylation by studying the transformation of aminopyrine to 4-aminoantipyrine, using the method of Brodie and Axelrod (12), after the addition of 150–300 mcg./ml. of aminopyrine into the extracorporeal circuit; (*b*) the acetylation, by studying the transformation of 4-aminoantipyrine to *N*-acetyl-4-aminoantipyrine under the above-mentioned conditions; (*c*) the glucurono-conjugation, by studying the transformation of oxazepam to glucuronide, using the method of Walkenstein *et al.* (13), after addition of 25–50 mcg./ml. of oxazepam (7-chloro-1,3-dihydro - 3 - hydroxy - 5 - phenyl - 2*H* - 1,4 - benzodiazepine-2-one) into the extracorporeal circuit.

RESULTS

Following these preparations, the venous blood from the hepatic veins (Fig. 1, 15) passes through the inferior cava into the pump–oxygenator system which is connected with the portal inflow tube. The abdominal venous blood by-passes through an artificial cava into the right side of the heart.

During the extracorporeal liver perfusion (from 2 to 3 hr.) no difference was observed between initial and terminal values of the following parameters: SGPT, SDH, LAP, and the time of BSP elimination. In contrast, there was a slight linear increase of SGOT during the perfusion depending on the increase of serum hemoglobin content. In fact, it has been shown that even a low-grade hemolysis leads to false positive values for SGOT (14).

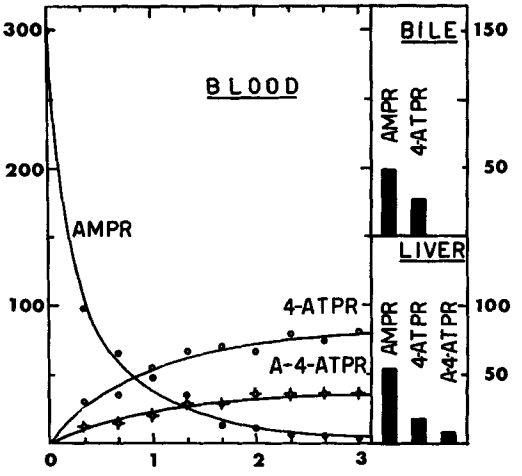

Fig. 2—Aminopyrine metabolism studied by the isolated liver perfusion in situ *in a dog weighing 25.8 Kg. Blood in extracorporeal circuit = 600 ml.; blood flow rate of the liver extracorporeal circuit = 130 ml./min.; initial concentration of aminopyrine in the plasma of the extracorporeal circuit = 300 mcg./ml. At left, the plasmatic concentration curves (mcg./ml., in ordinate) of aminopyrine (AMPR), 4-aminoantipyrine (4-ATPR), and* N*-acetyl-4-aminoantipyrine (A-4-ATPR) during 3 hr. (in abscissae) after aminopyrine addition. At right, bile and liver concentrations (mcg./ml. or mcg./Gm., in ordinate) of aminopyrine and its two metabolites, 3 hr. after adding the aminopyrine into the extracorporeal liver perfusion.*

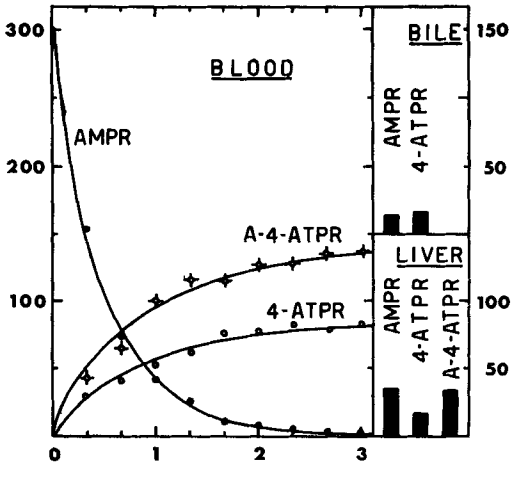

Fig. 3—Aminopyrine metabolism studied by the isolated liver perfusion in situ *in a monkey* (C. hamadryas) *weighing 13.8 Kg. Blood in liver extracorporeal circuit = 480 ml.; blood flow rate of the extracorporeal circuit = 70 ml./min.; initial concentration of aminopyrine in the plasma of extracorporeal circuit = 300 mcg./ml. Left and right same as Fig. 2.*

Concerning the metabolizing activity of *in situ* isolated liver preparation, aminopyrine and oxazepam disappear from the extracorporeal blood of dog and monkey at a very rapid rate: the half-life for aminopyrine ranges from 10 to 14 min. in dog and from 16 to 21 min. in monkey, and for oxazepam from 7 to 10 min. in both animals.

Figures 2 and 3 show two typical examples of the rate of transformation of aminopyrine in two metabolities: the demethylation is quite similar in dog and monkey, whereas the acetylation is higher in monkey than in dog. Likewise, at the end of the liver perfusion, the hepatic concentration of 4-aminoantipyrine is similar in both species, while the hepatic concentration of *N*-acetyl-4-aminoantipyrine is higher in the monkey than in the dog.

Figure 4 shows a typical example of the high glucurono-conjugation produced by the liver which eliminates the glucuronide into the bile.

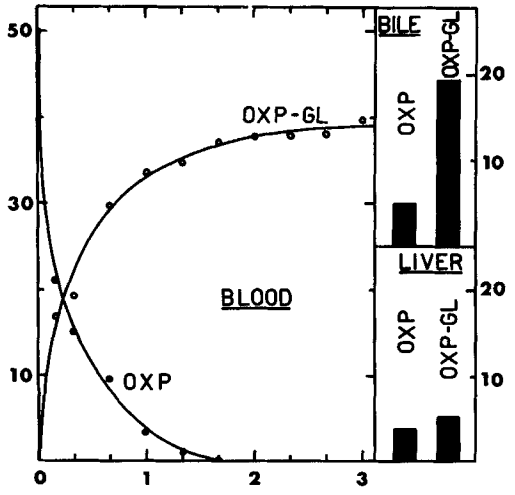

Fig. 4—Oxazepam metabolism studied by the isolated liver perfusion in situ *in a dog weighing 23.5 Kg. Blood in liver extracorporeal circuit = 600 ml.; blood flow rate of extracorporeal circuit = 120 ml./min.; initial concentration of oxazepam in the plasma of the extracorporeal circuit = 50 mcg./ml. At left, the plasmatic concentration curves (mcg./ml., in ordinate) of oxazepam (OXP) and its glucuronide (OXP-GL), assayed for 3 hr. (in abscissae) after oxazepam addition. At right, the bile and liver concentrations (mcg./ml. or mcg./Gm., in ordinate) of oxazepam and its glucuronide, assayed after 3 hr. of the extracorporeal liver perfusion with the addition of oxazepam.*

CONCLUSIONS

The described method permits us to investigate *in situ* the metabolizing activity of the liver normally connected with the body except that its blood circulation is supplied by a pump–oxygenator system. The liver functional tests remained within normal limits during the experiment (2 to 3 hr.). In such a way, the hepatic metabolic activity can be examined under experimental arrangements more similar to the normal conditions than traditional *in vitro* methods.

SUMMARY

The technique of the local perfusion of the organs *in situ* has been applied to evaluate the metabolizing hepatic activity. The liver can be isolated from general circulation, supplied with a separate extraneous system for pumping and oxygenating the blood, and maintained from 2 to 3 hr. under these conditions without significant local damage.

To test the method, the authors studied certain hepatic metabolic activities such as demethylation, acetylation, and glucurono-conjugation. The results suggest that the method could be useful in evaluating the metabolizing activity of the liver *in situ* without interference from other tissues.

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