hydroxypropyl methylcellulose 90 HG, 15,000 cps. matrix would release 82% of drug through diffusion in approximately 9.5 hr. Attrition, based on the slope obtained in Fig. 12, would contribute 18% drug at 9.5 hr. Similarly, the hydroxypropyl methylcellulose 90 HG, 25 cps. matrix would be totally depleted of drug after 2.8 hr. Attrition accounts for 36% of drug release in this case while diffusion contributes the remainder. It appears from these data that the lower molecular weight polymer is approximately two times more susceptible to attrition than the higher molecular weight species.

The area of the planar surface of the tablet is 0.98 cm.^{2}. The area for the standard whole tablet is 3.06 cm.^{2}. The ratio of experimental drug-release rates between the whole tablet and the planar surface was 4:1, while the ratio for the dry, unhydrated surfaces was 3:1. These results are not unexpected if the hydrated surface is considered in both cases. Hydration of the planar surface results in essentially a projection of the same area. Hydration of the whole tablet, however, changes the tablet diameter resulting in an increase in the area of the planar surface in proportion to the square of the radius. In addition, the increase in the circumference provides more area to the rim of the tablet in proportion to the radius. The added diffusional area resulting from hydration would account for the increased drug release observed over that expected from the unhydrated surface.

Chlorpheniramine maleate release patterns obtained from methylcellulose MC, 4000 cps. tablets appear to be completely dependent on attrition since a straight-line relationship holds when drug release is plotted against time.

It would appear that since the surface of the tablet was diminishing, a nonlinear release rate would be expected. However, erosion was not uniform, and fragmented particles continued to add to the drug concentration.

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Keyphrases

Matrices—compressed, hydrophilic
Polymers—compressed, hydrophilic matrices
Release rates—compressed hydrophilic matrices
Diffusion—drug from matrices
Diluent effect—drug release rates, matrices
Temperature effect—drug release rates, matrices
UV spectrophotometry—analysis
Hydroxypropyl methylcellulose tablets—hydration layer

Renal Metabolizing Activity Studied in Dog and Monkey by the Isolated Perfused Kidney *In Situ*

By G. BENZI, F. BERTÉ, A. CREMA, and E. ARRIGONI

The drug metabolizing power of the renal tissues in an isolated *in situ* kidney preparation has been evaluated in the dog and monkey. Under these experimental conditions, the kidney remained connected to the other parts of the living animal, except for blood circulation which was supplied by a pump-oxygenator system. The disappearance of the tested substances (oxazepam and aminopyrine) from extracorporeal blood was partially replaced by their metabolites. In fact, both the drugs and the metabolites were present at different concentrations in the excreted urine and in the cortical and medullar renal tissues.

In previous papers (1–3) the techniques were described for the perfusion of isolated areas of the body (liver, brain, uterine–fetal complex) to study some of their drug-metabolizing activities, such as demethylation, acetylation, and glucuronide conjugation.

In a systematic investigation (4, 5) on drug

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metabolism and tissue distribution, the present research was extended to the metabolizing activity of the kidney isolated *in situ* in living animals.

The techniques of the perfusion *in vitro* of isolated kidney suspended in a special box and placed in an extracorporeal circuit have been utilized for about a century (6, 7).

The present research, instead, utilizes the concept of Creech *et al.* (8) that various anatomic regions of the living animal 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 damages.

To test the metabolizing activity of the kidney isolated *in situ*, some transformations of oxazepam (glucuronide conjugation) and of aminopyrine (demethylation and acetylation) have been examined.

METHOD

The simple abdominal technique of kidney perfusion *in situ* was carried out on 39 dogs (9.8–18.8 kg.) and six monkeys (*C. hamadryas:* 7.8–13.8 kg.) preanesthetized with urethan (0.4 g./kg. i.p.). Anesthesia was induced and maintained in closed circuit by nitrous oxide, cyclopropane, or ethyl ether.

The animals were given artificial ventilation by a tracheal Warne tube placed during succinylcholine chloride action (1 mg./kg. i.v.). Arterial blood pressure was measured from a cannula inserted into a femoral artery and connected with a Polygraph-Schwarzer recorder.

After laparatomy, the left kidney and the upper part of the left ureter were exposed; the peritoneal sheath around the left ureter was opened 3–6 cm. from the renal pelvis to expose the ureter and the vessels of the surrounding fat. All the vessels were ligated and a polythene catheter was gently inserted in the ureter, pointing towards the renal pelvis.

The left adrenal gland was removed and the adrenal vessels ligated. The left renal artery and vein were isolated from the surrounding fat and a chain of loose ligatures placed around the vasal connection of the renal area. Both isolated left renal artery and vein were ligated, cannulated, and connected with a pump-oxygenator system; the ligatures around the vasal renal area were tightened to block the communications between the left kidney and the remainder of the body. The time from clamping of the renal artery until the start of perfusion was between 3 and 6 min.

The renal perfusion apparatus employed (9) consists of a venous reservoir, an oxygenator with gas meter, a roller-type pump with flow meter, a glass-wool or a polyester staple blood filter, an apparatus to eliminate blood foam, a blood exchanger with telethermometer, and a perfusion pressure regulator connected with a Polygraph-Schwarzer recorder.

Before the extracorporeal kidney perfusion, the pump-oxygenator system was filled with 400–600 ml. of heparinized blood drawn from donors. The collected blood was filtered through cloth, preserved by ampicillin (1:10,000), and stored in a refrigerator. The perfusions were made with (*a*) whole blood, (*b*) blood diluted with Tyrode solution (3:1), (*c*) blood diluted with Tyrode solution (3:1) and added to glucose (10%), (*d*) blood diluted with Tyrode solution (3:1) and added to both glucose (10%) and cyproheptadine (5–10 mcg./ml.) plus dibenamine hydrochloride (2.5–5 mcg./ml.). When whole blood was used, this was obtained from a donor 20 min. prior to use, to prevent the accumulation of lactic acid which would require a subsequent large neutralization. Before the perfusion, the heparinized blood, compatible with that of the subject, was filtered through glass wool or polyester staple, and adjusted to pH 7.35 using 1 *M* sodium bicarbonate. The priming blood was circulated through the pump-oxygenator system fully oxygenated and warmed. A flow of O_2 or O_2–CO_2 mixture (95:5) into the oxygenator was maintained at the rate of 4–6 l./min.; at the beginning of the extracorporeal renal perfusion, the blood flow was kept at the rate of 5–7 ml./kg./min. related to a pressure equal to the initial systemic pressure of the animal (90–145 mm. Hg). The time of kidney perfusion was limited from 60 to 120 min. Blood samples from extracorporeal circuit were collected every 15 min. for determination of hematocrit, clotting time, arterovenous blood oxygen, pH, and plasma electrolytes (Na, Cl, K). The urine from isolated and perfused kidney was collected and measured every 15 min. for determination of flow, pH, and electrolytes excretion (Na, K, Cl).

The metabolizing activity of the kidney isolated *in situ* was investigated by (*a*) the glucuronide conjugation, by evaluating the transformation of oxazepam to glucuronide (10) after addition of 10–20 mcg./ml. of oxazepam into the extracorporeal circuit, (*b*) the demethylation, by evaluating the transformation of aminopyrine to 4-aminoantipyrine (11) after addition of 40–80 mcg./ml. of aminopyrine into the extracorporeal circuit, (*c*) the acetylation, by evaluating the transformation of 4-aminoantipyrine to *N*-acetyl-4-aminoantipyrine (11) under the conditions mentioned in *b*.

The eventual leakage of perfusate into the systemic circulation was evaluated by taking samples of blood and lymph, to verify the absence of the tested substance or of its metabolites.

RESULTS AND DISCUSSION

The most frequent observation during an extracorporeal kidney perfusion isolated *in situ*, was the steady increase of blood perfusion pressure at constant rates of flow.

In the experimental conditions, the authors preliminarly minimized one of the various responsible factors by filtration of the blood through glass wool or polyester staple to remove both platelet aggregates and fibrin clots that tend to block the microvasculature of the organs (12–14).

Table I shows occurrence and degree of the increase of the renal vascular resistance, in different experimental conditions of dog's extracorporeal kidney perfusion *in situ*. A mixture of 5% carbon dioxide and 95% oxygen, used instead of pure oxygen in the aeration of the extracorporeal blood, reduced the degree of the perfusion pressure increase, in agreement with the results of Bachrack *et al.* (15),

TABLE I—OCCURRENCE IN DOG OF INCREASE OF THE RENAL PERFUSION PRESSURE AT CONSTANT RATE OF BLOOD FLOW (5–7 ml./kg./min.) IN DIFFERENT EXPERIMENTAL CONDITIONS

Aeration in Oxygenator		Medium of Perfusion		With Addition of			Occurrence After 2 Hr. of the Increase of Perfusion Pressure in Comparison with the Initial Values		
O_2(100%)	O_2(95%) + CO_2(5%)	Whole Blood	Diluted Blood (3:1)	Glucose (10%)	Cyproheptadine + Dibenamine	No. of Dogs	<10%	10–30%	>30%
yes	—	yes	—	—	—	8	1	2	5
—	yes	yes	—	—	—	8	1	4	3
—	yes	—	yes	yes	—	8	2	5	1
—	yes	—	yes	yes	yes	8	2	3	3

Malette *et al.* (16), and Steyn *et al.* (17). In comparison with the whole blood, the use of the blood diluted with Tyrode solution (3:1) and added to glucose (10%) reduced occurrence and degree of the increase of vascular resistance, in agreement with the data of Dottori *et al.* (18), Harvey (19), and Steyn *et al.* (17). The use of antihistaminic, antiserotoninic, and adrenergic-blocking agents was without any effect in reducing the increase of renal perfusion pressure, in partial agreement with Steyn *et al.* (17) and in contrast with Hollenberg *et al.* (20) and Belzer *et al.* (14). During the extracorporeal renal circulation *in situ*, no significant difference was observed between initial and terminal values of both the plasma electrolytes concentration of the perfusing blood, and the urine pH and electrolytes excretion, as indicated in Fig. 1.

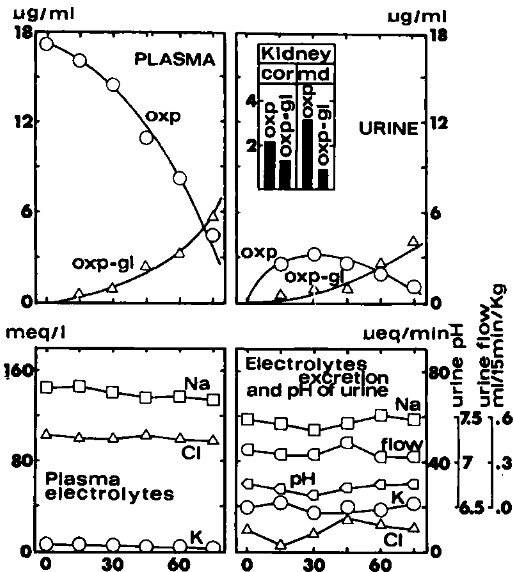

Fig. 1—Oxazepam metabolism studied by the isolated kidney perfusion in situ *in a dog weighing 18.2 kg. Blood in kidney extracorporeal circuit = 520 ml.; blood flow rate of extracorporeal circuit = 110 ml./min.; initial concentration of oxazepam in the plasma of the extracorporeal circuit = 17.5 mcg./ml. Top, at left the plasma concentration curves (mcg./ml., in ordinate) of oxazepam (oxp) and its glucuronide (oxp-gl), assayed every 15 min., for 75 min. At right, the urine concentration curves (mcg./ml., in ordinate) of oxazepam and its glucuronide; insert shows the cortical (cor) and medullar (md) renal concentration (mcg./g., in ordinate) of oxazepam and its glucuronide, evaluated 75 min. after the extracorporeal kidney perfusion with addition of oxazepam. Bottom, at left the plasma electrolytes concentrations (meq./l.) of the extracorporeal circulating blood assayed, every 15 min., for 75 min. At right, the urine pH, flow (ml./15 min./kg., in ordinate) and electrolytes excretion (meq./min., in ordinate) assayed as above indicated.*

In the extracorporeal circuit of both dog and monkey, the decrease of oxazepam plasma levels was 52% (42–58%) in 60 min.; the drug disappearance was partially replaced by its glucuronide. Both oxazepam and its studied metabolite appeared in the urine, as shown in Fig. 1; the oxazepam urine concentration decreased with the decrease of plasma concentration as the glucuronide urine concentration increased with the increase of its plasmatic concentration.

In the extracorporeal circuit of both dog and monkey, the decrease of aminopyrine plasma levels was 35% (28–41%) in 60 min.; the drug disappearance was partially replaced by its metabolites. Aminopyrine and its demethylated and acetylated metabolites appeared in the urine as indicated in Fig. 2. Figures 1 and 2 show the cortical and medullar concentrations of the drugs and their studied metabolites after the perfusion of the kidney: the affinity for aminopyrine, or instead for oxazepam, seems similar in both animal species.

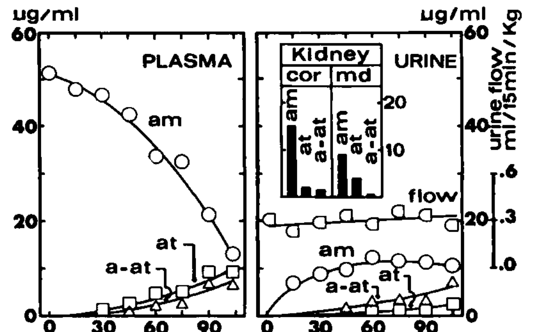

Fig. 2—Aminopyrine metabolism studied by the isolated kidney perfusion in situ *in a monkey* (C. hamadryas) *weighing 12.3 kg. Blood in kidney extracorporeal circuit = 480 ml.; blood flow rate of extracorporeal circuit = 74 ml./min.; initial concentration of aminopyrine in the plasma of the extracorporeal circuit = 51.9 mcg./ml. At left, the plasma concentration curves (mcg./ml., in ordinate) of aminopyrine (am), 4-aminoantipyrine (at), and* N-*acetyl-4-aminoantipyrine (a-at) assayed every 15 min. for 105 min. At right, the urine flow (ml./15 min./kg., in ordinate) and urine concentration curves (mcg./ml., in ordinate) of aminopyrine and its mentioned metabolites. Insert shows the cortical (cor) and medullar (md) renal concentrations (mcg./g., in ordinate) of aminopyrine and its two metabolites, assayed 105 min. after the extracorporeal kidney perfusion with addition of aminopyrine.*

CONCLUSIONS

The kidney isolated *in situ* shows a good metabolizing power evaluated by studying the glucuronide conjugation of oxazepam and the demethylation and acetylation of aminopyrine, both in the dog and the monkey. The tested substances and their metabolites were present in the excreted urine and in the renal tissues. During the extracorporeal renal perfusion (from 1 to 2 hr.) no significant differences were observed between the initial and final values of plasma electrolytes concentrations and urine pH, flow, and electrolytes excretion.

In comparison with the metabolizing power of other organs isolated *in situ* the kidney shows a lower activity than the liver (1) and a higher activity than the brain (2).

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Keyphrases

Renal metabolism—*in situ* determination
Kidney, perfused—dog, monkey
Oxazepam—renal metabolism
Aminopyrine—renal metabolism

Influence of Calcium and Antiarrhythmic Drugs on Palmitate Uptake by Rabbit Heart Slices

By C. M. MOKLER* and P. P. MATHUR

Nonesterified fatty acids diffuse into myocardial cells at a rate which is proportional to the concentration of unbound acid, and are used in intracellular oxidative and synthetic reactions. Quinidine is known to inhibit cellular oxidative processes. The effects of quinidine, disopyramide, and pronethalol on oxygen consumption and palmitate uptake by rabbit ventricle slices were determined by standard Warburg manometric techniques. In the absence of Ca^{++}, all three drugs in concentrations of 10^{-5}–10^{-4} moles/l. induced marked increases in the rate of palmitate uptake from its albumin-bound form in incubation media without significant changes in tissue Q_{O_2}. This effect was not seen at drug concentrations of 10^{-6} or 10^{-3} moles/l., or when 2 m*M* Ca^{++} was present. A relationship is suggested between palmitate, Ca^{++}, antiarrhythmic drugs, and extracellular protein, which may involve the binding of fatty acid to the protein moiety.

Plasma nonesterified fatty acids (NEFA) serve an important function in mammalian myocardial nutrition (1). They apparently diffuse across cell membranes and become available to cytoplasmic and mitochondrial enzyme systems in quantities which are related to the molar ratio of NEFA–plasma albumin (2) because of the reversible manner in which NEFA are bound to plasma albumin. Quinidine is also bound to plasma albumin (3), as is ionic calcium (Ca^{++}) (4), which is known to play an important role in cardiac function (5). Nayler (6) has shown an additional relationship between Ca^{++}, quinidine, and cell lipids. Quinidine will inhibit the movement of Ca^{++} from an aqueous phase into a chloroform phase which contains lipids extracted from myocardial membranes and

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