TABLE III—RANKING AND COMPARISON OF MEAN LOCOMOTOR ACTIVITY OF SIX STRAINS OF MICE COMBINED ACROSS NONINJECTION AND SALINE TREATMENTS

	Mice Tested Singly[a]					
Strain	CBA	C3H/An	DBA/2	NLW	BALB/c	C57BL/6
Activity	2.733	2.821	2.894	2.900	2.908	2.926

	Mice Tested in Groups[a]					
Strain	DBA/2	BALB/c	CBA	C3H/An	C57BL/6	NLW
Activity	3.820	3.311	3.344	3.356	3.406	3.465

[a] Values not sharing underline differ significantly ($p < 0.05$).

TABLE IV—COMPARATIVE EFFECTS OF GROUPING ON ACTIVITY IN SIX STRAINS MEASURED AS MEAN DIFFERENCES FROM SINGLY TESTED MICE[a]

Strain	DBA/2	BALB/c	C57BL/6	C3H/An	NLW	CBA
Difference	0.386	0.403	0.480	0.535	0.565	0.611

[a] Values not sharing underline differ significantly ($p < 0.05$).

phenomenon indicated by these data is its occurrence in mice tested in groups, as well as those tested singly. Although the present observations include four of the same inbred strains utilized in the study by Meier *et al.* (4), the data do not confirm their implication that the effect of saline treatment varies between strains. While there was a superficial suggestion in the untransformed data that the degree of response to saline might differ between strains, the nonsignificant injection x strain interaction term failed to support such an inference. Any such trend surely was not consistent enough to be detected by the analysis.

The pharmacological basis for the phenomenon of saline inhibition of activity remains quite unknown. The authors have termed it a placebo effect following the definition of Wolf (9), "... any effect attributable to a pill, potion or procedure, but not to its pharmacodynamic or specific properties." It surely cannot have the same explanation as that given for a placebo effect of saline injection upon locomotor activity of rats (10), *i.e.*, simple Pavlovian conditioning of the depressant effect of a previously administered pharmacologically active agent, scopolamine.

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Cerebral Drug Metabolism Investigated by Isolated Perfused Brain *In Situ*

By G. BENZI, F. BERTÉ, A. CREMA, and G. M. FRIGO

The *in situ* isolated brain, supplied by an extracorporeal pump-oxygenator system, shows a drug metabolizing activity. The tested substances (aminopyrine and oxazepam) show a disappearance from the extracorporeal blood of dog and monkey related to the metabolic transformations (demethylation, acetylation, or glucurono-conjugation) and to the fixation at the cerebral tissues.

IN A PREVIOUS PAPER (1) the authors described a method to investigate *in situ* the metabolizing activity of the liver connected normally with the body, except for blood circulation, which was supplied by a pump-oxygenator system. In such conditions, the liver functional tests remained within normal limits throughout the experiment, and it was possible to study the rate of some hepatic metabolizing activities, such as demethylation, acetylation, and glucurono-conjugation. In the course of systematic investigations on drug metabolism and tissue distribution (2–4) the research was extended to the metabolic activity of the brain, isolated *in situ* in the

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living animal. A cat's brain perfusion method *in vivo* was described in 1947 by Geiger and Magnes (5) and modified by Geiger and associates (6, 7). Experiments of brain perfusion were performed either on the severed head (8–10) or with incomplete separation of the brain circulation from the head circulation (11) and from the systemic circulation *in situ* (12–17).

According to Geiger (6), the choice of experimental animals depends on the vascular anatomy of the head and the reasonable availability. The monkey is more suitable for the isolation of the arterial side of the circulation, since no major communication exists between the brain and the rest of the body, except *via* the internal carotid and the vertebral arteries; nevertheless, the blockage of the vertebral sinuses is difficult. The dog is suitable for availability and size, but it shows a *rete mirabile* between the brain and the extracerebral circulation, and a rather large anterior spinal artery. It is impossible to separate the arterial side of the cerebral circulation from that of the extracranial circulation in the cat, because the blood from the carotid arteries reaches the Willis' circle through a *rete mirabile*. Thus, the Geiger procedure (18) consists mainly of the isolation and the occlusion of all the venous outlets of the cat's brain and of opening a new outlet by tapping the cerebral sinuses. In view of the possibility of extending the research to the isolated fetus (19), the dog and monkey were chosen because of their size.

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

METHOD

The experiments were carried out on 18 dogs (10.3 to 19.7 Kg.) and 6 monkeys (*C. hamadryas*, 8.2 to 12.8 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, following succinylcholine chloride (1 mg./Kg. i.v.) administration. Arterial blood pressure was measured from a cannula inserted into a femoral artery. Extradural electrodes were set in place some days before.

The operative procedure consists mainly of the isolation of the jugular external veins in the dog or the jugular internal veins in the monkey, and the isolation of the common carotid arteries and ligature of all their branches except the internal carotid arteries. The vertebral vessels are ligated before their entrance into the transverse foramen of C2 or C3. The numerous muscular branches arising from the vertebral vessels, the anastomosis between vertebral and carotid arteries, the anastomosis between vertebral and jugular veins, the internal jugular veins in the dog or the external jugular veins in the monkey, the vascular muscular branches of the neck, the vessels running under the carotid arteries and vagus nerves, the zygomatic, maxillary, auricular, and supra-orbital vessels are occluded by ligature or compression. The occlusions of the sinus columnae vertebralis and of the anterior spinal artery were made (*a*) by opening the rachis in C2, ligating the dog's spinal artery according to the method of Greeley and Greeley (20), and compressing the venous vessels; (*b*) by opening the rachis between C2 and C3, and compressing all the vessels around the spinal cord.

Both isolated jugular veins were ligated, cannulated, and connected with the venous reservoir of the pump-oxygenator system through the gravitational flow. Both the isolated carotid arteries were cannulated and connected with the pump-oxygenator system; at this point the perfusion started.

Hematocrit, clotting-time, and arterio-venous blood oxygen were measured from samples of both extracorporeal and systemic circulation.

The brain perfusion apparatus employed consists of a venous reservoir, an oxygenator with gasmeter, a roller-type pump with flowmeter, a blood filter, an apparatus to eliminate blood foam, a perfusion pressure regulator with manometer, and a blood exchanger with telethermometer.

Before the extracorporeal brain perfusion, the pump-oxygenator system was filled with 400–500 ml. of heparinized, defibrinated blood. This blood was previously obtained from the animal infused with blood from donor animals. The collected blood was filtered through cloth, preserved by ampicillin (1:10,000), and stored in a refrigerator. Before the perfusion, the blood was filtered, diluted with Tyrode's solution (to O_2 capacity = 13 ± 1 vol. %), and added to glucose (10%). The priming blood was circulated through the pump-oxygenator system fully oxygenated and warmed. A flow of O_2–CO_2 mixture (95:5) into the oxygenator was maintained at the rate of 4–8 L./min.; during the extracorporeal brain perfusion, the blood flow rate was kept very high (8–10 ml./min./Kg.). In most experiments, it was possible to maintain the blood pressure constantly equal to the initial systemic pressure of the animal (100 to 140 mm. Hg).

The time of brain perfusion was limited to 60 or 90 min. and was related to the presence of cerebral electric activity.

The eventual leakage of perfusate into the systemic circulation was evaluated (*a*) during the metabolic research, by taking samples from the general circulation of the blood and of the lymph to verify the absence of the tested substance or its metabolites; (*b*) at the end of the metabolic research by adding either a dye or a radiopaque substance to the blood of the extracorporeal circuit.

The metabolizing activity of the brain was investigated *in situ* by (*a*) the demethylation, by evaluating the transformation of aminopyrine to 4-aminoantipyrine using the method of Brodie and Axelrod (21) after addition of 50–100 mcg./ml. of aminopyrine into the extracorporeal circuit; (*b*) the acetylation, by evaluating the transformation of 4-aminoantipyrine to *N*-acetyl-4-aminoantipyrine under the above-mentioned conditions; (*c*) the glucuronoconjugation, by evaluating the transformation of oxazepam to glucuronide, using the method of Walkenstein *et al.* (22) after addition of 10–20 mcg./ml. of oxazepam into the extracorporeal circuit.

RESULTS

The *in situ* isolated brain shows a metabolizing activity. In the extracorporeal circuit of both the dog and monkey, the decrease of aminopyrine plasma levels is 16% (14 to 21%) in 60 min., and 21% (17 to 23%) in 90 min.; the decrease of oxazepam plasma

levels is 22% (20 to 25%) in 60 min., and 25% (23 to 30%) in 90 min. The drugs' disappearance is partially replaced by their metabolites.

The affinity of aminopyrine and oxazepam for the brain tissues seems similar. Furthermore, no significant difference is noted on the brain metabolizing activity in the two different ways of occlusion of the sinus columnae vertebralis.

Figure 1 is a typical example of the rate of transformation of aminopyrine in two metabolites. The demethylation and the acetylation are quite similar in the dog and monkey.

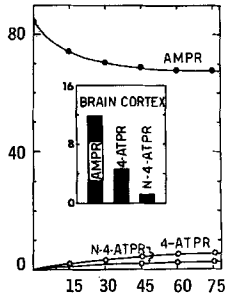

Fig. 1—Aminopyrine metabolism studied by the isolated brain perfusion in situ *in a monkey* (C. hamadryas) *weighing 12.8 Kg. Blood in extracorporeal circuit = 500 ml.; blood flow rate of the extracorporeal circuit = 115 ml./min.; initial concentration of aminopyrine in the plasma of the extracorporeal circuit = 86.5 mcg./ml. An ordinate, the plasmatic concentration (mcg./ml.) of aminopyrine* (AMPR), *4-aminoantipyrine* (4-ATPR), *and* N-*acetyl-4-aminoantipyrine* (N-4-ATPR), *assayed for 75 min. (in abscissae) after aminopyrine addition. Insert shows the brain cortex concentration (mcg./Gm. in ordinate) of aminopyrine and its two metabolites, assayed after 75 min. of the extracorporeal brain perfusion with addition of aminopyrine.*

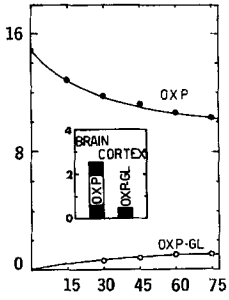

Fig. 2—Oxazepam metabolism studied by the isolated brain perfusion in situ *in a monkey* (C. hamadryas) *weighing 10.7 Kg. Blood in extracorporeal circuit = 480 ml.; blood flow rate of extracorporeal circuit = 95 ml./min.; initial concentration of oxazepam in the plasma of the extracorporeal circuit = 15.3 mcg./ml. An ordinate, the plasmatic concentrations (mcg./ml.) of oxazepam* (OXP) *and its glucuronide* (OXP-GL) *assayed for 75 min. (in abscissae) after oxazepam addition. Insert shows the brain cortex concentration (mcg./Gm. in ordinate) of oxazepam and its glucuronide, assayed after 75 min. of the extracorporeal brain perfusion with addition of oxazepam.*

Figure 2 is a typical example of the glucurono-conjugation of the isolated brain; also the glucurono-conjugation is quite similar in both species.

CONCLUSIONS

The brain isolated *in situ* shows a drug metabolism evaluated by studying the demethylation and the acetylation of aminopyrine and the glucurono-conjugation of oxazepam. In dogs and monkeys, the decrease of aminopyrine plasma levels is 16% and of oxazepam is 22% in 60 min. In studies (1) on the isolated liver *in situ* it was found that the half-time for aminopyrine ranged from 10 to 14 min. in the dog, and from 16 to 21 min. in the monkey, and for oxazepam from 7 to 10 min. in both animals.

These great differences on drug metabolism between brain and other tissues *in situ* seem to be interesting, particularly when the action on the nervous system is not induced by the drug itself, but by its metabolic products.

SUMMARY

The cerebral drug metabolism of dog and monkey has been investigated by the *in situ* isolated brain supplied by an extracorporeal pump-oxygenator system.

To test the brain metabolizing activity, some transformation of aminopyrine (demethylation and acetylation) and oxazepam (glucuronide-conjugation) was examined.

The drugs disappeared from the extracorporeal blood at the rate of 16% for aminopyrine and of 22% for oxazepam in 1 hr.; at the same time their metabolic products appeared. The concentration in the brain cortex of the substances has been evaluated also.

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