Uterine–Placental–Fetal Preparation *In Situ* on the Dog. Investigation of Metabolizing Activity and Tissue Distribution

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The extracorporeal perfusion technique *in situ* has been used to evaluate the metabolizing activity of the uterine–placental–fetal preparation on the dog. The pregnant uterus isolated from general circulation was supplied with a pump-oxygenator system. The method was tested studying the metabolic transformation of aminopyrine to 4-aminoantipyrine and *N*-acetyl-4-aminoantipyrine, and evaluating their distribution in fetal tissues.

TOXICOLOGICAL and pharmacological importance of drug metabolism in fetal and uterine tissues is well known. Nevertheless, the studies on pregnant animals *in toto* present complications related to maternal metabolism. The evaluation of the fetal physiological activities *in situ* can be performed while maintaining the fetus detached from its placental connection by an extracorporeal supporting system reproducing the placental circulation (1–7). Likewise, the uterine or placental influence on the transfer and utilization of the substances can be studied by the perfused placenta or pregnant uterus (8–14). Nevertheless, the lack of data prompted the study of drug metabolic activity of the fetus–uterus complex isolated *in situ*, so as to eliminate any interference of extrauterine tissues.

In a systematic investigation of the metabolism and tissue distribution during the pregnancy (15–17) the metabolic fate of aminopyrine was detected in the uterine–placental–fetal preparation *in situ* using a method of extracorporeal circulation. In the experimental arrangement, the pregnant uterus remained connected with the other parts of the body, except its blood circulation was supplied by a pump-oxygenator system.

EXPERIMENTAL

The experiments were carried out on 11 pregnant dogs (16.8 to 27.5 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 by a tracheal Warne tube after succinylcholine chloride (1 mg./Kg. i.v.) administration. Arterial blood pressure was measured from a cannula inserted into a femoral artery.

The abdomen was incised along the midline from xiphoid process to pubis; the uterine artery and vein on both sides of the uterus were isolated at the neck of the uterus. The vaginal, ovaric, and peritoneal vascular connections with the extrauterine tissues were occluded by ligature or compression. Both isolated uterine veins were ligated, cannulated, and connected with the venous reservoir of the pump-oxygenator system through the gravitational flow. Both isolated uterine arteries were cannulated and connected with the pump-oxygenator system; at this point the perfusion started.

Hematocrit, clotting time, pH, and arteriovenous blood oxygen were measured from samples of both extracorporeal and systemic circulation. The perfusion apparatus consists of: a venous reservoir, an oxygenator with gas meter, a roller-type pump with flow meter, a blood filter, an apparatus to eliminate the blood foam, a perfusion pressure regulator with manometer, and a blood exchanger with telethermometer (18).

Before the extracorporeal perfusion, the pump-oxygenator system was filled with 400–600 ml. of heparinized and defibrinated blood, previously obtained from the animal itself, filtered through cloth, preserved by ampicillin (1:10,000), and stored in a refrigerator. Before the perfusion the blood was filtered, diluted with Tyrode solution (to O_2 capacity = 13 ± 1 vol.%), and added to glucose (5%). 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 5–10 L./min. During the extracorporeal circulation, the blood-flow rate was 10–12 ml./min./Kg.; the blood pressure was maintained at constant values of 80–110 mm. Hg; the time of perfusion was limited to 60 min.

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

To investigate the metabolizing activity, the authors studied: (*a*) *demethylation*, by evaluating the transformation of aminopyrine to 4-aminoantipyrine using the method of Brodie and Axelrod (19) after addition of 100–200 mcg./ml. of aminopyrine into the extracorporeal circuit; (*b*) *acetylation*, by evaluating the transformation of 4-aminoantipyrine to *N*-acetyl-4-aminoantipyrine under the above-mentioned conditions and method.

At the end of the perfusion, the tissue distribution of aminopyrine and its two metabolites in some fetal organs (plasma, liver, kidney, lung, heart, brain, thymus, muscle, amniotic fluid, placenta) was determined, for every pregnant dog, pooling together the organs of its fetuses.

RESULTS

In the dog at eighth–ninth week of pregnancy, the uterine–placental–fetal preparation *in situ* shows a metabolizing activity. In fact, 29% (25 to 38%) of aminopyrine disappears in 60 min. and is partially replaced by the appearance of the 4-aminoantipyrine and *N*-acetyl-4-aminoantipyrine; an example is indicated in Fig. 1.

Figure 2 is a typical example of the fetal tissue concentration of aminopyrine and its two metabolites assayed after 60 min. of extracorporeal perfusion of an uterine–placental–fetal preparation. The following observations were made: (*a*) the diffusibility of aminopyrine across the placenta to the

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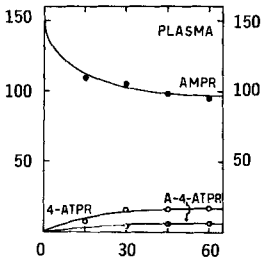

Fig. 1—Aminopyrine metabolism studied by the isolated uterine–placental–fetal preparation in situ *in a pregnant dog weighing 22.9 Kg. at ninth week of pregnancy; number of its fetuses = 6. Circulating blood in extracorporeal circuit = 680 ml.; blood flow rate of the extracorporeal circuit = 270 ml./min.; initial concentration of aminopyrine in the plasma of the extracorporeal circuit = 153 mcg./ml. On ordinate, the plasmatic concentrations (mcg./ml.) of aminopyrine* (AMPR), *4-aminoantipyrine* (4-ATPR), and N-*acetyl-4-aminoantipyrine* (A-4-ATPR). *On abscissa, time (in minutes) after aminopyrine addition.*

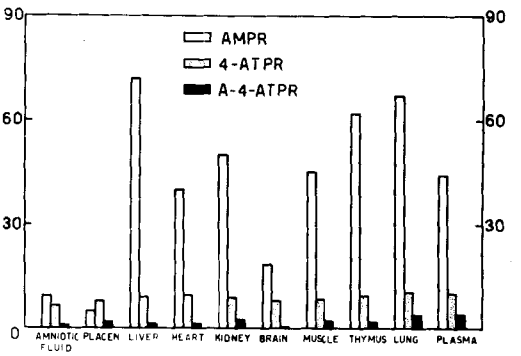

Fig. 2—Aminopyrine (AMPR), *4-aminoantipyrine* (4-ATPR), *and* N-*acetyl-4-aminoantipyrine* (A-4-ATPR) *concentrations (mcg./Gm. on ordinate) in the placental–fetal tissues 60 min. after adding aminopyrine into the extracorporeal circuit of the isolated uterine–placental–fetal preparation* in situ *of the pregnant dog as in Fig. 1. The concentrations are obtained by pooling together the organs from its six fetuses.*

fetuses; (*b*) the different concentrations of aminopyrine in the fetal organs studied; (*c*) the various proportions of aminopyrine to 4-aminoantipyrine and *N*-acetyl-4-aminoantipyrine in the fetal organs. Additional information will be necessary to state if these various proportions depend on a different ability in taking up the circulating metabolites.

In conclusion, the uterine–placental–fetal complex seems to be a useful method to investigate *in situ* both a detailed metabolizing activity and the drug-tissue distribution in the pregnant uterus normally connected with the body, except that its blood circulation is supplied by a pump-oxygenator system.

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Keyphrases

Uterine–placental–fetal preparation—*in situ*
Perfusion technique—drug administration
Metabolic activity
Fetal tissue—drug distribution