Study of the Cerebral Metabolizing Activity in the Newborn Dog Utilizing the Isolated Perfused Brain *In Situ* Technique

G. BENZI, F. BERTÉ, E. ARRIGONI, and L. MANZO

Abstract ☐ The *in situ* isolated brain of the newborn dog showed a drug metabolizing activity related to age. The tested substances were oxazepam and aminopyrine to show some metabolic transformations, as glucuronoconjugation, demethylation, and acetylation. Before 4 days of age no metabolizing ability is present; at 8–12 days the drug metabolism is evident, but only at 24 days of age does it begin to become quantitatively similar to that of the adult.

Keyphrases ☐ Cerebral metabolism—newborn dog ☐ Brain, *in situ* isolated—drug perfusion ☐ Metabolism, brain—age effect ☐ Perfusion technique—*in situ* isolated brain

According to Dawkins (1) the enzyme activity of the liver increases to adult levels in the neonatal period at varying rates for different functions and there exists a transient period after birth when homeostasis may be unstable perhaps because of low activity of enzymes concerned with special functions. Conjugation with glucuronic acid is the main metabolizing pathway for some drugs, such as chloramphenicol, morphine, steroids, and salicylates (2). The susceptibility of premature newborns to poisoning with chloramphenicol (3) and the high half-life of plasma hydrocortisone in newborns (4) can be related to low UDP-glucuronyltransferase activity which in many species (mouse, rabbit, guinea pig, and man) increases rapidly immediately after birth and then more slowly to reach adult levels in the first month of life (5–8).

Some researchers suggest that mammalian liver UDP-glucuronyltransferase does not develop during perinatal time at identical rates among species, nor among substrates in any one species (9, 10).

This behavior appears also in mammalian extrahepatic tissues; thus in the kidney of the guinea pig the conjugation of salicylate developed as in liver, but in the kidney of the rabbit neonatal conjugation of salicylate was higher than that in adult tissue (11).

Similar observations may also be related to other metabolizing activities. The poor acetylation of the human infant is indicated by the appearance of only small amounts of acetylated sulfonamides in the urine (12). Thus in the newborn rat the hepatic conjugation of bromosulfophthalein with glutathione is of low activity and increases to adult levels by 9 weeks of age (13). No data are known on the metabolizing activity of the brain *in situ* of the newborn. In a previous paper (14) the authors investigated in adult dog and monkey the glucuronoconjugation of oxazepam and the demethylation and the acetylation of aminopyrine, by the isolated perfused brain *in situ*.

In both animal species, the decrease of oxazepam cerebral plasma levels was 22% and of aminopyrine 16% in 60 min.; the drugs' disappearance was partially replaced by their metabolites.

In a systematic investigation on drug metabolism and tissue distribution in adult, newborn, and fetus (15–17) the authors extended the research to the cerebral metabolizing activity of the newborn, using the method of the isolated brain *in situ*, supplied by an extracorporeal pump-oxygenator system. To test the drugs' metabolism, some transformations of oxazepam (glucuronoconjugation) and of aminopyrine (demethylation and acetylation) have been examined.

METHOD

The experiments were carried out on 51 newborn dogs from eight natural births; the pups of every litter were tested, respectively, at 1, 2, 4, 8, 12, and, depending on the number of the pups, also at 24 and 180 days of age.

In three litters it was possible to test at the same days of age (8 and 12) both male and female newborns. Before the experiments, the pups were maintained with the mother under the same environmental conditions (22 ± 1°, RH = 60 ± 5%, lighting cycle, 14 hr. light and 10 hr. darkness). The surgical procedure of isolating the cerebral blood from that of the rest of the body was carried out on animals preanesthetized with urethan (0.6 g./kg. i.p.) and anesthetized by nitrous oxide, fluothane, or cyclopropane in closed circuit. The animals were given artificial ventilation after cannulation of the trachea; extradural electrodes were set in place to record EEG in frontal, parietal, and occipital leads monitored continuously, with both systemic and cerebral pressures, on a 12-channel polygraph.[1]

The operative procedure consisted mainly of the isolation of the common carotid arteries and the ligature of all their branches except the internal carotid arteries; the vertebral vessels were ligated before their entrance into the transverse foramen between the fourth and the fifth vertebras. The external jugular veins were bared and one of them was ligated and a thread was passed underneath the other. The internal jugular veins, the communicating branches of external jugular veins, the numerous muscular branches arising from the vertebral vessels, the anastomosis between vertebral and carotid arteries, the anastomosis between vertebral and both jugular and facial veins, all the vascular muscular branches of the neck, the vessels running under the carotid arteries, the vagus nerves, the trachea and the esophagus, the zygomatic, maxillary, auricular, and supraorbital vessels were occluded by ligature or compression. The occlusions of the sinus columnae vertebralis and of the spinal artery were made by opening the rachis in C4 or in C5 and compressing all the vessels around the spinal cord.

Occipital holes were drilled in ventrocaudal direction, deep enough to reach the confluence of the transversal sinuses with the sigmoid sinus; these holes closed until the perfusion starts, permitted a gravitational outflow of venous blood to a reservoir, when the animals were turned onto their back. Both the isolated carotid arteries were cannulated and connected with a pump-oxygenator system; the occipital holes were opened and the previously placed thread on one external jugular vein was now tied; at this point the perfusion started.

The brain perfusion apparatus employed consists of a venous reservoir placed under the occipital holes, an oxygenator with gasmeter, a blood filter, a blood exchanger with telethermometer, a

[1] Physioscript EE 12 Schwarzer.

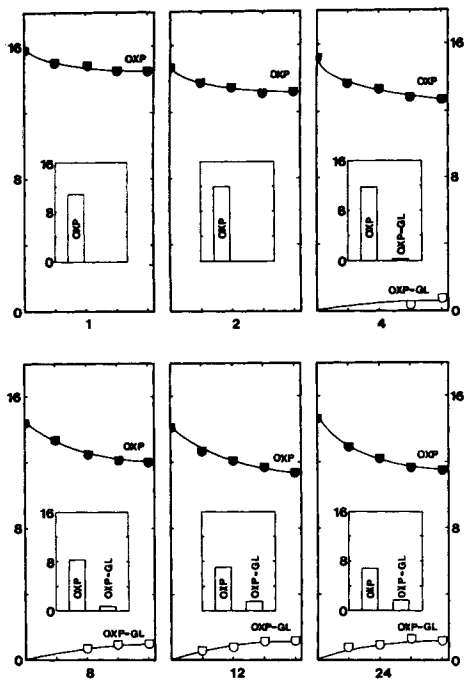

Figure 1—*Oxazepam metabolism studied by the dog's isolated brain perfusion* in situ *in six new-borns, from the same litter, at 1, 2, 4, 8, 12, and 24 days of age. Blood in extracorporeal circuit = 120 ml.; blood flow rate of the extracorporeal circuit = 10–12 ml./min./kg. The ordinates, the plasma concentrations (mcg./ml.) of oxazepam (OXP) and its glucuronide (OXP-GL) assayed for 60 min., every 15 min. (in abscissae) after oxazepam addition into the extracorporeal cerebral circuit. Inserts show the brain concentrations (mcg./g., in ordinate) of oxazepam (OXP) and its glucuronide (OXP-GL), assayed after 60 min. of the extracorporeal brain perfusion with addition of oxazepam.*

roller-type pump with flowmeter to pump both the venous blood from the reservoir to the oxygenator and the oxygenated blood from the oxygenator to the brain.

Before the extracorporeal perfusion, the pump-oxygenator system was filled with 80–180 ml. of heparinized blood diluted with Tyrode solution (3:1) and added to glucose (10%). The blood was obtained from the mother 20 min. prior to use, to prevent the accumulation of lactic acid which would require a subsequent large neutralization. Before the perfusion, the diluted blood was filtered through glass wool or polyester staple, and adjusted to pH = 7.35 using sodium bicarbonate 1 *M*. 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 0.8–1.8 l./min.; during the extracorporeal brain perfusion, the blood flow rate was kept from 8 to 12 ml./min./kg., at a pressure equal to the initial systemic pressure of the animal. The time of brain perfusion was limited to 60 or 90 min. and was related to the presence of a considerable cerebral electric activity.

Blood samples from extracorporeal circuit were collected every 15 min. for determination of hematocrit, clotting-time, arterovenous blood oxygen, pH, glucose, lactic acid, pyruvic acid, and LDH.

The brain of the newborn dog isolated *in situ* was investigated by (*a*) the glucuronoconjugation, by evaluating the transformation of oxazepam to glucuronide (18) 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 (19) after addition of 30–50 mcg./ml. of aminopyrine into the extracorporeal circuit; (*c*) the acetylation, by evaluating the transformation of 4-aminoantipyrine to *N*-acetyl-4-aminoantipyrine (19) under the conditions mentioned in *b*.

The brain concentrations of both oxazepam or aminopyrine and their metabolites were evaluated at the end of the perfusion. The eventual leakage of perfusate into the systemic circulation was evaluated by taking samples of blood, to verify the absence of the tested substance or of its metabolites.

The experiments in the 180-day-old dogs were carried out using the method described by Benzi *et al.* (14).

RESULTS AND DISCUSSION

The *in situ* isolated brain of the newborn dog initially shows a metabolizing activity only after 4 days of age. Before this age little or no concentrations of metabolites were found in both extracorporeal circuit and cerebral tissues. At 8–12 days of age the metabolizing activity is present, but only at 24 days does the activity begin to become quantitatively similar to that of the adult (14).

Figure 1 is a typical example of the glucuronoconjugation of the isolated *in situ* brain of six newborns from the same litter: it is possible to observe that at 1 and 2 days of age no glucuronide is present both in blood of the cerebral extracorporeal circuit and in brain, after 1 hr. of perfusion with oxazepam. At 4 days of age, glucuronide is significantly present in the samples of blood of the extracorporeal circuit only after 45 min. of perfusion: traces of glucuronide are present in the cerebral tissues at the end of the perfusion. At 8, 12, and 24 days of age, in the extracorporeal circuit the decrease of oxazepam plasma levels is always more evident simultaneously with an increase of the glucuronide concentrations: also the concentrations of glucuronide in brain tissues are always higher from the eighth to the twenty-fourth day. Figure 2 is a typical example of the rate of transformation of aminopyrine in demethylated and acetylated metabolites in the isolated brain *in situ* of six dogs from the same litter. At 1 and 2 days of age, no metabolites are evident both in blood of the cerebral extracorporeal circuit and in brain; traces of 4-aminoantipyrine are present in the plasma after 1 hr. of perfusion at second day of age, but these data are not constant in the various experiments. At 4 and 8 days of age the demethylated metabolite is present both in extracorporeal plasma and in brain and at 12 days appears also the acetylated metabolite in considerable concentrations. In Fig. 2 it is also possible to compare the rate of amino-

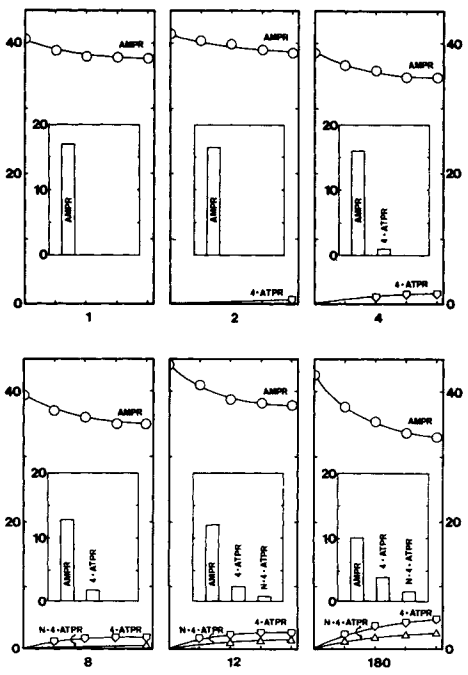

Figure 2—*Aminopyrine metabolism studied by the isolated brain perfusion* in situ *in six dogs, from the same litter, at 1, 2, 4, 8, 12, and 180 days of age. Blood in extracorporeal circuit = 120 ml. at 1, 2, 4, 8, 12 days, and 480 ml. at 180 days of age; blood flow rate of the extracorporeal circuit = 9–12 ml./min./kg. The ordinates, the plasma concentrations (mcg./ml.) of aminopyrine (AMPR), 4-aminoantipyrine (4-ATPR), and N-acetyl-4-aminoantipyrine (N-4-ATPR) assayed for 60 min., every 15 min. (in abscissae) after aminopyrine addition into the extracorporeal cerebral circuit. Inserts show the brain concentrations (mcg./g., in ordinate) of aminopyrine (AMPR) and its two metabolites (4-ATPR and N-4-ATPR), assayed after 60 min. of the extracorporeal brain perfusion with addition of aminopyrine.*

pyrine transformation at 12 days of age with that of a 6 months of age brother. Figures 1 and 2 show the ability of the brain, at every day of age, to take up the administered drugs, both oxazepam and aminopyrine.

In some experiments it was possible to test the metabolizing power of the isolated brain *in situ* of both male and female newborns from the same litter and at the same day of age: at the eighth day (glucuronoconjugation in two male and female newborns) and at the twelfth (glucuronoconjugation, demethylation, and acetylation in four male and female newborns). In these experimental arrangements no significant differences were noted between male and female newborn animals from the same litter.

CONCLUSIONS

Related to the days of age, the brain isolated *in situ* of newborn dogs from the same litter showed a drug metabolism which was evaluated by studying the glucuronoconjugation of oxazepam and the demethylation and acetylation of aminopyrine. No metabolizing activity was present before 4 days of age; at 8–12 days the drug metabolism was evident, but only after 12–24 days of age did it begin to become quantitatively similar to that of the adult.

These great differences in cerebral drugs metabolism induced by the age of the newborns, seem to be interesting particularly in view of both the ability of the brain to take up the administered drugs and the detoxication mechanisms of the brain itself. In the authors' experimental conditions no significant differences were evident between the metabolizing activity of male and female newborns from the same litter and of the same age.

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Detection and Separation of Aliphatic Polycarboxylic Acids by Reversed-Phase Partition Thin-Layer Chromatography

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Abstract □ The application of reversed-phase partition TLC, employing Silica Gel HF chromatoplates impregnated with silicone and developed in glacial acetic acid–dioxane–water–formic acid (4:1:1:6), proved suitable for the separation of agaricic acid (α-hexadecylcitric acid), norcaperatic acid (α-tetradecylcitric acid), and citric acid.

Keyphrases □ Polycarboxylic acids, aliphatic—detection, separation □ Phosphomolybdic acid solution—spot visualization □ Potassium permanganate solution—spot visualization □ TLC, reversed phase partition—detection, separation

Volatile compounds of a homologous series can be fractionated usually by adsorption TLC but compounds of longer chain lengths are resolved, in most cases, by reversed-phase partition chromatography. Kaufmann and Makus (1) employed this technique for the separation of homologs of fatty acids, alcohols, and triglycerides. Malins and Mangold (2) successfully separated saturated and unsaturated fatty acids by using siliconized chromatoplates. Other investigators have utilized reversed-phase partition procedures for the separation of fatty acids and their methyl esters (1–3), cholesteryl esters of higher fatty acids (4), keto acids, lactones, and hydroxy fatty acids (5).

A number of hydrophobic agents have been employed as the stationary phase in reversed-phase partition chromatography. Silicone appears to be the preferred hydrophobic agent but undecane, tetradecane, squalane, polyethylene, and paraffin have also been used. Numerous adsorbants have been utilized in this procedure but Silica Gel G and kieselguhr G are usually employed.

The recent isolation and identification of norcaperatic acid (α-tetradecylcitric acid) from the mushrooms *Gomphus floccosus* (Schw.) Singer [syn: *Cantharellus floccosus* Schw.] (6) and *Polyporus fibrillosus* Karst. (7) have accentuated the need for a convenient method for the differentiation and detection of members of this aliphatic polycarboxylic acid group. Agaricic acid