Isoniazid Distribution in Body Fluids of the Dog and the Rabbit.* (20766)

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Since the advent of isonicotinic acid hydrazide isoniazid as an agent in the treatment of tuberculosis(1,2), efforts have been directed at determining the absorption and distribution of the drug in body tissues and fluids(3-8). A series of determinations are reported here which further elucidate the distribution of isoniazid in body fluids.

Experimental. Five pregnant rabbits were anesthetized with 40 mg per kg of 'Amytal Sodium' (Amobarbital Sodium, Lilly) intravenously, about 2 hours after intravenous administration of 10 mg per kg of isoniazid. The body cavity was then opened and 2 or 3 intact loculi removed. After one hour, the remaining loculi were detached. Amniotic fluid free from blood contamination was withdrawn with a hypodermic needle and syringe from the intact placentae. Fetal blood was secured by cardiac puncture, and pooled. Maternal blood was taken from the medial artery of the ear or by cardiac puncture. To secure sufficient volume for accurate isoniazid determination amniotic fluid was obtained from 20-22-day pregnant animals, and the fetal blood from 28-30-day-old fetuses. In the dog experiments isoniazid was given orally by stomach tube in a dose of 20 mg per kg. Blood samples were taken from the jugular vein. Three lactating dogs were milked dry manually before, and at intervals after, drug administration. Isoniazid was given to 5 other dogs before phenobarbital anesthesia. The pancreatic and left submaxillary ducts were cannulated. The samples were collected for 15 minutes at the beginning of each hour. Prior to each collection period secretions were stimulated by the intravenous administration of secretin and pilocarpine. The common bile duct and the ureters of 3 of the dogs were also cannulated. Bile and urine were collected continuously throughout the assay period. Two unanesthetized female dogs with ligated cystic ducts and duodenal fistulas(9) so arranged that plastic tubing could be inserted into the common bile duct were used for an excretion study. Bile was collected continuously for six hours with the dog resting on its side. Urine was obtained by catheterization.

TABLE I. Isoniazid Concentrations in Maternal and Fetal Plasma, and Amniotic Fluid of 5 Rabbits, All Expressed in μg per ml.

Hr after i.v. admin.	Maternal plasma	Fetal plasma	Amniotic fluid
2	4.9	5.2	
3	2.3	5.2	
2	6.2	6.8	
3	5.4	5.9	
2	4.8		5.5
3	3.0		5.5
2	5.2		3.5
3	5.0		5.2
2	4.6		5.5
3	4.4		5.1

TABLE II. Isoniazid Concentrations in Plasma and Milk of the 3 Lactating Dogs, All Expressed in μg per ml.

Hr after oral admin.	Plasma	Milk
1	15.7	16.5
2	17.8	31.2
4	8.4	16.4
2	18.2	17.6
4	10.6	15.6

The analysis of the isoniazid content of the various body fluids was performed according to the technic of Kelly and Poet(10). This procedure relies on extraction of isoniazid from biological material with an ether-isoamyl alcohol mixture, redistribution in 0.1 N HCl and subsequent color formation with *p*-dimethylaminobenzyaldehyde. Photometric readings were made with a Beckman model DU spectrophotometer at 450 mμ. All blood was heparinized. Plasma samples exhibiting hemolysis gave unreliable results and were discarded. Bile, when analyzed by this procedure, produced a cloudiness in the color reaction. This was avoided by using small

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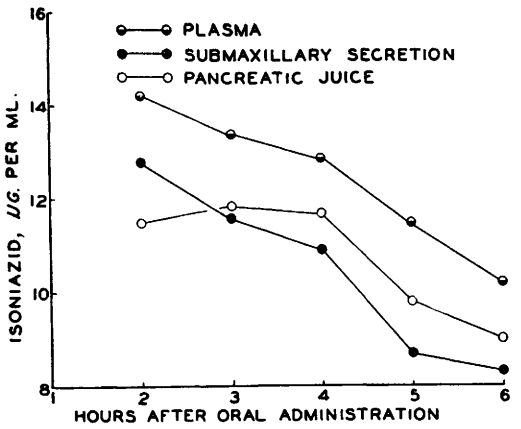

FIG. 1. Average isoniazid concentrations in the plasma, submaxillary secretion, and pancreatic juice of 5 dogs receiving 20 mg/kg orally.

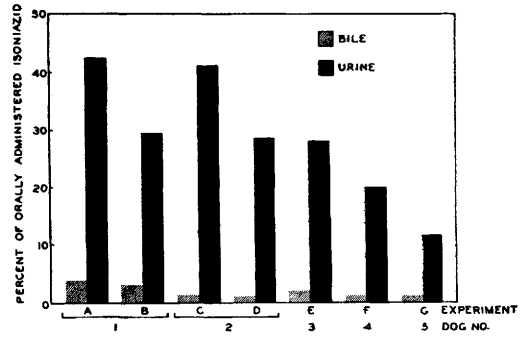

FIG. 2. Isoniazid excretion for the first 6 hr following oral administration of 20 mg/kg in the dog. Numbers 1 and 2 were unanesthetized while 3, 4 and 5 were anesthetized with phenobarbital.

volumes (.02-.04 ml). The method yielded reliable results only when bile isoniazid concentrations exceeded 25 µg per ml. No difficulty was encountered with the other fluids analyzed.

Results. A study was performed in the pregnant rabbit to detect whether isoniazid is transferred from the maternal circulation to the fetus and the amniotic fluid. Results as shown in Table I demonstrate that isoniazid readily passes through the placental barrier.

In 3 of the 5 milk samples taken from 3 dogs, the isoniazid concentration exceeded that of the circulating plasma and was equivalent in the other 2 (Table II).

The isoniazid levels in submaxillary secretion and pancreatic juice approximated the plasma levels throughout the period of 2 to 6 hours after oral administration as shown in Fig. 1. The disappearance rate also closely followed that of the plasma in both instances.

Biliary and urinary excretion was followed in 3 anesthetized and 2 unanesthetized dogs for 6 hours subsequent to oral administration of isoniazid. Total recovery in the bile ranged from 0.9 to 3.6% of the dose during the 6 hours. Urine excretion varied from 12.0 to 42.5% in the same dogs (Fig. 2). In 4 experiments with the unanesthetized dogs, the bile recovery varied from 3.5 to 9.2% of the total combined urinary and biliary excretion. In the 3 anesthetized dogs the bile recovery varied from 5.8 to 8.5% of the total. Since it appeared that anesthesia produced no preferential effect on either the biliary or urinary excretion of the drug, the mean rates of excretion were computed. As is shown in Fig. 3, the mean maximal excretion rate for both urine and bile was reached simultaneously at the second hour. Diminution of the rates occurred nearly proportionately.

Discussion. The dose levels of isoniazid employed in our experimental animals were higher than those in clinical usage. High concentrations in body fluids enhanced the accuracy of the chemical determinations, particularly in the case of the bile. No toxic symptoms were noted in any of these acute experiments. The small number of animals used make conclusions valid only in so far as

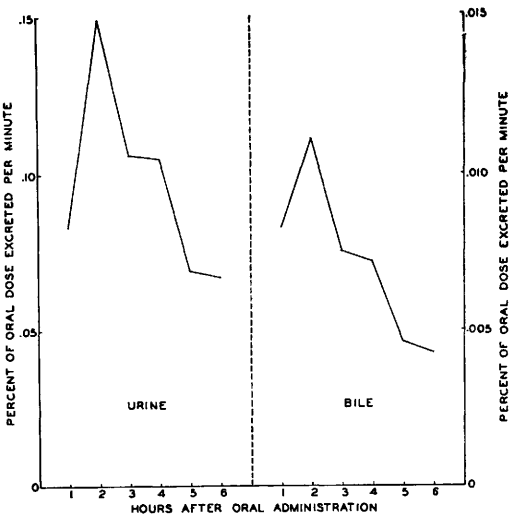

FIG. 3. Average urinary and biliary excretion rate of isoniazid in 7 experiments with 5 dogs receiving 20 mg/kg orally.

trends of isoniazid distribution and excretion are concerned.

Rubin *et al.*(6) found that in the dog the spinal fluid and most tissue concentrations follow plasma levels closely. Our data are in general agreement with theirs in that the isoniazid concentration in the pancreatic juice and the submaxillary secretion of the dog, as well as the fetal plasma and the amniotic fluid of the rabbit, also correspond to plasma levels. Camurri(7) reported a similar finding in the fetal plasma of the rabbit, but found the amniotic fluid to have a greater isoniazid concentration than the maternal plasma.

We found the isoniazid levels in the milk of the dog to be higher than in the plasma in 3 out of 5 instances. Camurri(8), on the other hand, reported isoniazid levels in human milk as significantly lower than plasma. This might be due to a species difference. Certainly further work is necessary to establish the role of the mammary gland in isoniazid excretion.

The peak urinary excretion of isoniazid in the dog was reported by Rubin *et al.*(6) to occur between the second and fourth hour after oral administration. This was confirmed by us in both anesthetized and unanesthetized dogs. The biliary excretion reached its peak at the same time as the urinary excretion, but at less than one-tenth the magnitude.

Summary. 1. In the rabbit isoniazid was found to pass freely through the placental barrier. 2. In the unanesthetized dog isoniazid appeared in the milk at concentrations equal to or greater than those of the plasma. 3. In the phenobarbitalized dog the concentrations of isoniazid in the submaxillary secretion and the pancreatic juice were found to be at near plasma levels. 4. In both anesthetized and unanesthetized dogs, total isoniazid recovered from urine and bile at the sixth hour following oral administration varied from 12.9 to 46.0%. Biliary recovery was less than one-tenth of the total in the 5 dogs studied.

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