By preventing the acidotic shifts characteristic of hypoxia and leading to disturbance of the activity of certain enzyme systems, including glutamine synthetase, sodium hydroxybutyrate may speed up the binding of ammonia accumulating under hypoxic conditions. The experimental data given above suggest that sodium hydroxybutyrate may prevent the development of the accumulation of toxic end products of nitrogen metabolism, one of the undesirable manifestations of excessive physical work.

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INTRAHEPATIC CIRCULATION OF ^{14}C-PHENAZEPAM AND ITS METABOLITES IN ALBINO RATS

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Excretion of phenazepam and its metabolites in the bile and its intrahepatic circulation were studied in two groups of rats: bile donors receiving an intravenous injection of ^{14}C-phenazepam (14 mg/kg) previously, and recipients into whose duodenum the donors' bile was introduced. Phenazepam (compound I), its free-hydroxy metabolites (compound II), and a metabolite with a hydroxyl group in the aromatic ring (compound III) were shown to be excreted in the bile of these animals. Hydroxyl derivatives are excreted in the bile in the form of glucuronides also. Compounds I and III and the glucuronide of compound III undergo intrahepatic circulation.

KEY WORDS: phenazepam; metabolism; intrahepatic circulation.

With the introduction of phenazepam, a tranquilizer of the 1,4-benzdiazepine series, into medical practice the study of its metabolic pathways in experimental animals and in man and the rate of its excretion is interesting.

The investigation described below was carried out to study the ratio of phenazepam metabolites, both free and conjugated with glucuronic acid, entering the bile of albino rats following direct administration of ^{14}C-phenazepam. The composition of metabolites involved in intrahepatic circulation in the experimental animals also was studied.

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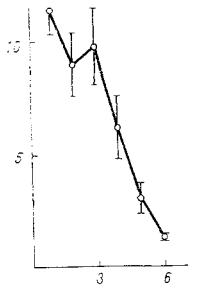


Fig. 1

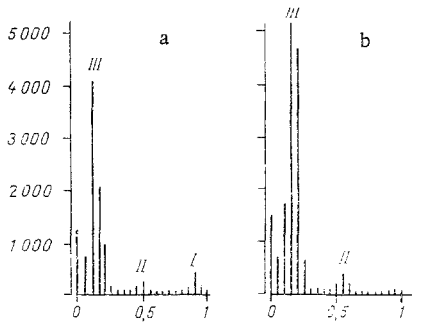


Fig. 2

Fig. 1. Excretion of total radioactivity (^{14}C-phenazepam and its metabolites) with bile in rats. Abscissa, time of investigation (in h); ordinate, excreted radioactivity (in % of injected dose of original compound).

Fig. 2. Radiochromatogram of chloroform extract of bile (a) and of chloroform extract of aqueous phase of bile treated with β-glucuronidase (b). Abscissa, R_f values; ordinate, radioactivity of sample (in cpm). Roman numerals denote compounds.

TABLE 1. Content of ^{14}C-Phenazepam and Its Metabolites (in % of injected dose) in Bile of Albino Rats after Intravenous Injection of Original Compound in Dose of 14 mg/kg (M ± m)

Compound	0—1 h	1—2 h	2—3 h	3—4 h	4—5 h	5—6 h
I (Phenazepam)	0,02±0,003	0,02±0,004	0,015±0,004	0,01±0,002	0,008±0,001	—
II	0,03±0,006	0,03±0,001	0,01±0,004	0,01±0,003	0,01±0,003	—
III	0,27±0,07	0,27±0,05	0,14±0,02	0,12±0,03	0,07±0,009	—
IV	0,04±0,009	0,03±0,008	0,08±0,004	0,10±0,02	0,07±0,01	0,03±0,007
V	0,31±0,09	0,21±0,02	1,48±0,27	1,22±0,15	0,80±0,09	0,44±0,01
Residual radioactivity	10,44±1,10	8,16±1,37	7,89±1,72	4,74±1,39	2,02±0,7	0,70±0,1

EXPERIMENTAL METHOD

Phenazepam was first synthesized in the writers' laboratory [1, 2]. The radioactivity of its labeled analog was 1 Ci/mole.

Experiments were carried out on male albino rats weighing 210-230 g. In the experiments of series I, in order to determine the relative quantitative proportions of phenazepam metabolites in the bile, the rats were anesthetized by intraperitoneal injection of barbital sodium (200 mg/kg). ^{14}C-Phenazepam (14 mg/kg) in Tween emulsion with isotonic NaCl solution was injected into the caudal vein. Exteriorization of the bile duct and collection of bile were carried out as described in [3]. The bile was collected hourly for 6 h. Samples of bile were made up with water to 2.5 ml and extracted twice with 5 volumes of chloroform. After extraction of the chloroform-soluble metabolites the aqueous phase was acidified to pH 4.7 and treated with β-glucuronidase (3000 units). The mixture was incubated for 24 h at 37°C. The aglycones thus set free were extracted, just as in the case of the free metabolites. The metabolites were fractionated on plates with an attached layer of "Silufol UV-254" silica-gel in a solvent system of acetone—chloroform—30% ammonia (30 : 70 : 1). Radioactivity was determined on the chromatograms at R_f intervals of 0.05, beginning from the starting line, on an SL-30 liquid photometer (Intertechnique, France).

The metabolites were identified by comparing their R_f values and staining in UV-light (λ = 253.7 nm) with corresponding values for synthesized substances. The mass-spectra of metabolites eluted from the chromatograms were obtained on the MKh-1303 instrument with an ionization energy of 50 eV and an emission current of 1.5 mA.

In the experiments of series II the animals were divided into two groups. The rats of group 1 received ^{14}C-phenazepam by the method described above; these rats served as donors of bile in which the original compound and its metabolites were found. The animals of group 2 were recipients; they were given bile from the donors by cannula into the duodenum after intervals of 60, 120, 180, and 240 min. The bile duct of these animals

TABLE 2. Quantity of ^{14}C-Phenazepam and Its Metabolites (in cpm × 10^3) Excreted with Bile of Donor and Recipient Rats (M ± m)

Time, h	Donors	Recipients			
		compound I	compound III	compound V	residual radioactivity
0—1	9959±840	2,4±0,8	4,9±1,7	4,3±0,8	84,8±26,0
1—2	7535±1151	1,8±0,7	2,3±0,8	20,0±6,6	72,7±31,5
2—3	8215±1462	1,5±0,4	3,9±1,3	35,8±5,5	128,4±30,6
3—4	5208±1176	1,5±0,1	5,5±1,5	—	129,4±33,6

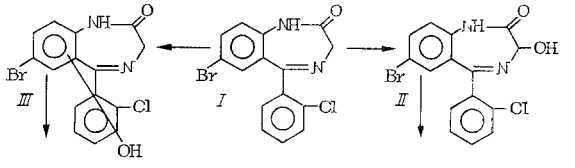
was exteriorized and their bile collected hourly for 4 h. Just as in the experiments of series I the phenazepam metabolites in the bile were identified and estimated quantitatively. A similar blood analysis also was carried out on the recipient animals in order to determine the nature of the phenazepam metabolites absorbed from the gastrointestinal tract.

EXPERIMENTAL RESULTS AND DISCUSSION

Analysis of the radioactive material showed that in the course of 6 h 40.5 ± 2.76% of the injected dose of phenazepam and its metabolites was excreted with the bile. Excretion of the total quantity of substances was distributed unevenly in time and was most rapid between 60 and 180 min (Fig. 1). By 360 min the excretion of radioactivity was negligible.

During chromatographic fractionation of the chloroform extracts of bile three compounds were found (Fig. 2a). Identification of the metabolites showed that one of them was the original compound (I) and the second was its 3-hydroxy derivative (II). The mass spectrum of metabolite III contained peaks of a molecular ion with m/e 366, (M – H) with m/e 365, (M – H_2CN) with m/e 338, (M – H, CO) with m/e 337, and (M – Cl) with m/e 321. The difference between this metabolite and the 3-hydroxy derivative according to the mass spectra and R_f values and also the fact that it formed large quantities of a glucuronide in vivo point to the presence of a hydroxyl group in its molecule. The writers suggest that metabolite III may be a phenazepam molecule with oxidized aromatic ring.

Besides free metabolites of phenazepam excreted into the bile, glucuronides of compounds II and III also were found (Fig. 2b). The above facts suggest that the phenazepam metabolites excreted into the bile can be represented by the following formulas:



Glucuronide (V) Glucuronide (IV)

Among the free and conjugated (glucuronide) metabolites, most radioactivity belonged to compound III and its glucuronides (Table 1). Incidentally, besides the metabolites already mentioned, the bile also contained water-soluble compounds, not extractable with chloroform, in amounts much greater than those of substances I-V (Table 1).

Together with the bile, substances I-V and residual water-soluble metabolites were thus excreted into the intestine of the recipient rats. Analysis of the blood of these animals showed that it contains phenazepam and metabolites III and V.

Data on the quantity of phenazepam and its metabolites excreted with the bile after reabsorption are given in Table 2. Most of the radioactivity belonged to the water-soluble metabolites, excretion of which increased with time. Among the metabolites identified, there were large quantities of compounds III and, in particular, V.

The results are evidence that metabolite III is particularly actively involved in the intrahepatic circulation. This compound is excreted into the bile in large quantities, both in the free form and as the glucuronide (compound V). They are then reabsorbed into the blood stream, where they were present in the ratio of 1:1. Later, via the portal vein they reenter the liver, where the glucuronide is synthesized and the ratio between metabolites III and V in the animals' bile was 1:20 between the 1st and 3rd hour of the investigation.

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