3. A. I. Maiskii and S. V. Shoshina, The Pharmacology of Experimental Alcoholism [in Russian], Moscow (1982), p. 28.
4. A. I. Maiskii, N. A. Khodorova, and V. N. Vlasova, Byull. Éksp. Biol. Med., No. 9, 284 (1984).
5. G. V. Morozov, Abstracts of Proceedings of the First Congress of Neuropathologists and Psychiatrists of Moldavia [in Russian], Vol. 2, Kishinev (1981), pp. 3-9.
6. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1980).
7. D. Yu. Rusakov and A. V. Val'dman, Farmakol. Toksikol., No. 5, 107 (1983).
8. V. N. Solov'ev, A. A. Firsov, and V. A. Filov, Pharmacokinetics [in Russian], Moscow (1980), pp. 35-40.
9. K. Eriksson, Scand. J. Clin. Lab. Invest., Suppl. 113, 61 (1970).
10. P. Eriksson and H. Sippel, Anal. Biochem., 80, 35 (1977).
11. A. K. S. Ho and J. H. Tsai, J. Neurochem. Behav., 3, 1073 (1975).
12. T. Koivula, M. Koivusalo, and K. O. Lindros, Biochem. Pharmacol., 24, 1807 (1975).
13. G. McClearn and R. Kakihana, Behav. Genet., 3, 409 (1973).
14. R. D. Porsolt, G. Auton, et al., Eur. J. Pharmacol., 47, 379 (1978).

BIOTRANSFORMATION OF A ^{14}C-HYDROGENATED PHENAZEPAM ANALOG IN INBRED MICE *IN VIVO*

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It was shown previously that the pharmacological activation of some psychotropic drugs depends on some genetically controlled features of their metabolism [4, 5].

The aim of this investigation was to study excretion of a ^{14}C-hydrogenated analog of phenazepam (5-o-chlorophenyl-7-bromo-1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-one) (I), synthesized by ourselves [6], and which, compared with phenazepam itself, induces a much weaker degree of muscle relaxation and ataxia [5], and excretion of its metabolites, from inbred lines of mice with different oxidation phenotypes [4, 5].

TABLE 1. Content (in % of injected dose) of ^{14}C-I and Its Metabolites (total quantities) in Excreta of Inbred Lines of Mice, Excreted over a Period of 1-4 Days (M ± m)

Interval, days	Line of mice	Urine			Feces			
		total radioactive material	free metabolites lites	residual radioactivity (aqueous)	total radioactive material	free metabolites	glucuronides	residual radioactivity (aqueous)
1	B6	7,75±1,86	3,91±0,74	3,38±0,59	35,71±3,68	8,62±0,25	4,60±0,55	7,65±0,78
	C	3,95±0,63	0,96±0,01	2,09±0,09	43,14±4,35	9,56±0,94	5,92±0,57	5,12±0,14
	CBA	4,17±0,62	1,28±0,10	2,00±0,34	46,19±3,99	8,95±0,62	7,40±0,62	5,25±0,34
2	B6	2,06±0,33	0,75±0,11	1,15±0,09	11,39±1,04	2,46±0,09	2,09±0,19	2,17±0,23
	C	1,42±0,62	0,31±0,03	0,92±0,09	13,39±1,72	3,35±0,47	2,19±0,31	1,49±0,16
	CBA	1,29±0,10	0,25±0,05	0,90±0,08	13,29±1,97	2,67±0,19	2,30±0,14	1,74±0,21
3	B6	0,92±0,15	0,29±0,07	0,36±0,06	7,59±0,55	1,54±0,31	1,12±0,13	1,34±0,17
	C	0,71±0,08	0,18±0,02	0,43±0,05	7,10±0,74	1,48±0,07	0,94±0,08	0,75±0,09
	CBA	0,68±0,09	0,20±0,03	0,39±0,04	8,01±0,97	1,39±0,09	1,10±0,10	0,80±0,06
4	B6	0,39±0,08	0,12±0,03	0,14±0,02	4,64±0,82	0,97±0,14	0,65±0,12	0,70±0,13
	C	0,35±0,05	0,09±0,01	0,19±0,03	4,11±0,39	0,91±0,01	0,36±0,04	0,36±0,05
	CBA	0,32±0,05	0,11±0,01	0,19±0,02	5,25±0,44	0,50±0,04	0,42±0,04	0,36±0,04

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TABLE 2. Content (in % of injected dose) of ^{14}C-I and Its Free Metabolites in Excreta of Inbred Lines of Mice Excreted in the Course of 1-4 Days (M ± m)

Interval, days	Line of mice	Urine				Feces			
		I	II	III	V+VI	I	II	III	V+VI
1	B6	0,600±0,130	0,225±0,090	0,298±0,053	0,296±0,037	1,119±0,091	0,657±0,080	0,968±0,075	0,568±0,069
	C	0,208±0,015	0,058±0,009	0,060±0,009	0,108±0,009	0,973±0,099	0,918±0,088	0,926±0,096	1,079±0,166
	CBA	0,132±0,012	0,062±0,008	0,081±0,002	0,110±0,014	1,272±0,112	0,878±0,057	0,921±0,054	1,036±0,084
2	B6	0,095±0,021	0,100±0,011	0,067±0,006	0,096±0,015	0,272±0,030	0,356±0,076	0,399±0,026	0,164±0,019
	C	0,049±0,004	0,027±0,003	0,027±0,005	0,048±0,003	0,255±0,031	0,385±0,054	0,357±0,051	0,405±0,042
	CBA	0,034±0,004	0,035±0,007	0,024±0,005	0,047±0,005	0,300±0,025	0,272±0,043	0,281±0,019	0,366±0,021
3	B6	0,048±0,022	0,032±0,005	0,039±0,005	0,060±0,009	0,135±0,044	0,221±0,015	0,226±0,012	0,114±0,018
	C	0,034±0,004	0,016±0,002	0,020±0,003	0,030±0,003	0,090±0,010	0,119±0,009	0,176±0,019	0,139±0,013
	CBA	0,024±0,003	0,019±0,002	0,016±0,003	0,034±0,008	0,112±0,005	0,092±0,007	0,150±0,015	0,127±0,012
4	B6	0,022±0,006	0,018±0,004	0,015±0,002	0,035±0,001	0,107±0,013	0,100±0,015	0,124±0,006	0,045±0,001
	C	0,023±0,003	0,007±0,001	0,015±0,001	0,020±0,001	0,040±0,003	0,049±0,005	0,099±0,009	0,047±0,004
	CBA	0,017±0,002	0,011±0,001	0,012±0,001	0,023±0,002	0,048±0,005	0,030±0,010	0,080±0,011	0,050±0,005

EXPERIMENTAL METHOD

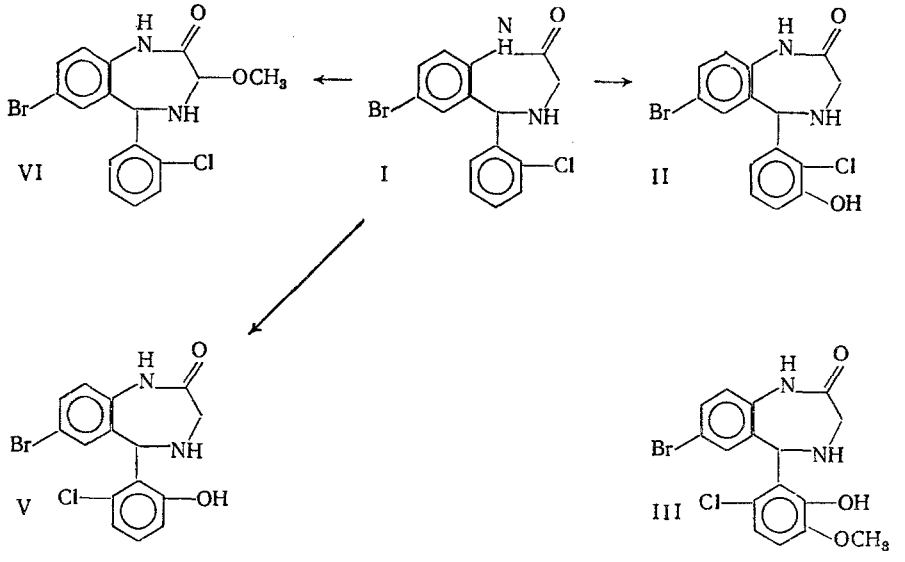
Experiments were carried out on male mice of lines C56BL/6 (B6), BALB/c (C), and CBA weighing 18-20 g. ^{14}C-I ($4.1 \cdot 10^{10}$ Bq/mole) was injected intraperitoneally with Tween-40 in a dose of 10 mg/kg. The urine and feces were collected every 24 h for 4 days and processed [2]. Chloroform extracts of the excreta were chromatographed on Silufol-254 plates (Czechoslovakia) in a system of hexane—ethyl ether—acetone—NH_4OH in the ratio 15:15:20:0.5. The arrangement of the ^{14}C-compounds was recorded on a Berthold KB 2832 linear analyzer (West Germany) and determined quantitatively on an Intertechnique SL-4000 liquid scintillation phtometer (France).

The structure of the metabolites found were determined mass-spectrometrically on an MS-1302 photoionization mass spectrometer (Special Design Office for Analytical Instruments, Academy of Sciences of the USSR).

EXPERIMENTAL RESULTS

Radiochromatography revealed at least five labeled compounds in the chloroform-extracted fraction of the excreta from the mice. Much of the radioactive material was fixed to the starting line and it was not determined quantitatively.

The process of biotransformation of ^{14}C-I can be represented as follows:



Characteristically, dehydrogenation of its molecule with the formation of a 3-oxy-metabolite, characteristic of other tranquilizers of the benzodiazepine series [1, 7], was not observed for I.

TABLE 3. Content (in % of injected dose) of Glucuronides of I and Its Metabolites in Feces of Inbred Lines of Mice Excreted in the Course of 1-4 Days (M ± m)

Interval, days	Line of mice	I	II	III	V+VI
1	B6	0,363±0,031	0,387±0,041	0,342±0,041	0,323±0,032
	C	0,693±0,098	0,314±0,059	0,361±0,046	0,560±0,090
	CBA	0,735±0,049	0,407±0,066	0,534±0,049	0,734±0,110
2	B6	0,139±0,017	0,113±0,011	0,152±0,016	0,164±0,020
	C	0,134±0,027	0,098±0,014	0,086±0,010	0,232±0,025
	CBA	0,215±0,026	0,086±0,012	0,156±0,033	0,300±0,035
3	B6	0,081±0,015	0,045±0,003	0,101±0,012	0,100±0,019
	C	0,060±0,004	0,045±0,008	0,053±0,001	0,125±0,009
	CBA	0,090±0,001	0,041±0,005	0,109±0,007	0,094±0,011
4	B6	0,042±0,003	0,026±0,003	0,032±0,006	0,050±0,010
	C	0,029±0,002	0,028±0,003	0,027±0,003	0,049±0,006
	CBA	0,040±0,004	0,021±0,004	0,043±0,005	0,046±0,005

Analysis of excretion of the total radioactive material with the urine and feces showed that the excretion process is biphasic, and its kinetic parameters were very similar in the animals of the various lines studied. The content of metabolites in the feces was greater than in the urine: by 5-6 times in B6 mice, by 10-11 times in C and CBA mice (Table 1).

In the course of 96 h, 70-80% of the injected dose of the substance was excreted by the animals. Comparison of the kinetic and quantitative parameters of elimination of the ^{14}C-compounds showed the absence of deposition of any of them in the mice after a single injection of I.

Total content of free metabolites in the urine of the B6 mice was higher than in that of the C and CBA mice. However, comparison of the concentrations of individual products reveals that in the animals of these lines their concentrations were in fact in the following order: I > V + VI > III > II, and the quantities recorded were proportional (Table 2). Considering also that the kinetic curves characterizing excretion came close together toward the end of the experiment, it can be postulated that despite the absence of interlinear differences in the total fraction of glucuronides, ^{14}C-I and its derivatives were bound to a lesser degree in the B6 mice than in C and CBA mice with the plasma proteins. Under these circumstances, all the ^{14}C-compounds were present in the form of glucuronides in the mice, but they were excreted with the feces (Table 3).

In the course of 96 h, significantly more unchanged I was excreted from the B6 and CBA mice than from the C mice. Interlinear differences for their content in the feces also were observed for the derivatives of I (Table 2).

It can thus be concluded from these results that biotransformation of I is qualitatively similar in animals with different oxidation phenotypes. Under these circumstances no metabolite was found whose concentration significantly determined the direction of metabolism of I in one line of mice. The quantitative differences in excretion of the original compound and its products which were found suggest that the pharmacological action of this preparation may be characterized by quantitative differences, due to genetically determined features of the organization of the metabolic systems.

LITERATURE CITED

1. A. V. Bogatskii, S. A. Andronati, and N. Ya. Golovenko, Tranquilizers [in Russian], Kiev (1980).
2. N. Ya. Golovenko, V. G. Zin'kovskii, and S. B. Seredenin, Khim.-farm. Zh., No. 12, 14 (1980).
3. N. Ya. Golovenko, O. P. Rudenko, T. L. Karaseva, et al., Abstract lodged in the All-Union Institute of Scientific and Technical Information, No. 1805-83, April 6, 1983.
4. S. G. Seredenin, V. G. Zin'kovskii, B. A. Badyshtov, et al., Byull. Éksp. Biol. Med., No. 10, 450 (1981).
5. S. B. Seredenin and I. V. Rybina, Farmakol. Toksikol., No. 4, 79 (1985).
6. V. A. Sozinov, O. P. Rudenko, V. G. Zin'kovskii, et al., Khim.-farm. Zh., No. 10, 1232 (1984).
7. L. Otvos, Zs. Tegyey, L. Vereczkey, et al., Drug. Metab. Dispos. Biol. Fate Chem., 6, 213 (1978).