There are contradictory opinions on the role of processes of binding in the prolongation of the action of sulfanilamides. Some authors indicate a direct dependence of the prolongation of the action of preparations in the organism on the degree of binding [3, 12]; others do not find this dependence [13].

Our investigations showed that the binding of sulfamonomethoxine and its N-methylglucamine salt by blood serum proteins and erythrocytes does not differ. The interaction of the preparations with blood proteins depended on the sulfanilamide concentration, while with erythrocytes at concentrations from 10 to 500 μg/ml, the binding indices were the same. Complexes of sulfamonomethoxine with serum proteins and erythrocytes dissociated practically completely.

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SYNTHESIS OF [^{14}C]PIPERAZINE AND FEATURES OF ITS DISTRIBUTION IN ANIMALS

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We have previously reported on the preparation and distribution in the organism of [^{32}P]dipin [1] and [^{14}C]dipin with the label in the ethyleneimine groups [2], and of [^{14}C]dipin with the label in the piperazine ring. Considering the important role of piperazine in the dipin molecule (the bearer of the phosphoramide groups), and also the fact that piperazine and its salts are widely used in medical and veterinary practice for treating ascariasis and other helminthiases [3, 4], it was of interest to investigate its distribution in the organism of animals.

In the present communication we give data on the synthesis and distribution of [^{14}C]piperazine, and we have also made a comparative analysis of the behavior of [^{14}C]piperazine and of [^{14}C]dipin having a label in the piperazine ring in the animal organism.

The basis for the synthesis of [^{14}C]piperazine was made a procedure which made it possible to use [1, 2-^{14}C]dibromoethane as the starting radioactive compound; we have previously used this in the synthesis of labeled ethylenimine [2].

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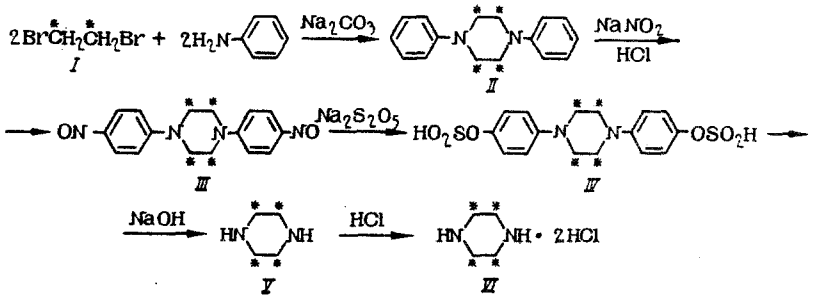

By reaction of [1, 2-^{14}C]dibromoethane (I) with aniline in the presence of anhydrous sodium carbon we prepared N,N'-diphenylpiperazine[^{14}C] (II), which, by nitrosation with sodium nitrite, was converted into p-dinitrosodiphenyl-N,N'-piperazine [^{14}C] (III), and further, by sulfonation with sodium bisulfite, into p-disulfodiphenyl-N,N'-piperazine [^{14}C] (IV). On basification with caustic soda, the latter gave [^{14}C]piperazine (V), isolation of which was performed by distillation with steam into a receiver containing hydrochloric acid.

The yield of [^{14}C]piperazine was 56.6% (calculated on [^{14}C]dibromoethane), with a specific activity of 11.75 μCi/mg. Confirmation of the radiochemical and chemical purity of the preparation was carried out by the thin-layer chromatography method and by autoradiography with subsequent densitometry of the prints obtained. The chromatographing was carried out on Silufol plates in the systems propanol–ammonia (7:3) and butanol–water–acetic acid (4:5:1); development was carried out in iodine vapor. The R_f values of the [^{14}C]-piperazine and of the "indicator" coincide in these systems and are equal to 0.25 and 0.21, respectively.

In a study of the distribution of [^{14}C]piperazine (VI) in rats having sarcoma 45, a rapid resorption of it from the blood was observed after injection. Thus, after 5 min, the absorption coefficient of the blood was 0.82; and after 30 min, it was only 0.15 in all. The exponential dependence for radioactivity content of the blood corresponded to the following equation:

$$C_t = 0.67 \cdot e^{-4.16t} + 0.15 \cdot e^{-0.12t},$$

where C_t is the absorption coefficient in a definite time, expressed in hours (t).

Hence it follows that a radioactivity corresponding to an absorption coefficient of 0.67 was removed from the blood with a half-removal period of 10 min, but a part of it, equal to 0.15, left the blood with a $T_{1/2}$ of 6 h. The reduction of radioactivity which characterized the content of [^{14}C]piperazine in the blood took place in the tumor-bearing rats more rapidly than that of [^{14}C]dipin which was labeled in the piperazine ring. The [^{14}C]-piperazine rapidly accumulated in the organs and tissues. The maximum radioactivity content was observed 5-15 min after injection of the preparation. The highest absorption coefficient of the radioactivity (10.9) was in the kidneys, as is evident from Table 1. In the lungs it was 4.67; in the adrenals, 5.16; in the lymph nodes, 3.11; in the pituitary gland, 3.77; in the stomach, 3.91; in the small intestine, 3.03; in the thymus, 2.46; in the thyroid gland, 2.37; in the spleen, 2.83; in the heart, 2.34; and in the liver, 2.01. In the remaining organs and tissues, the largest value of the radioactivity absorption coefficient was in the range 1.94-0.76. In the tumor the absorption coefficient reached 0.61.

On comparison of the absorption coefficients of radioactivity in the tissues and blood, it was discovered that this ratio in the experimental animals was higher than upon injection of [^{14}C]dipin. The absorption coefficient (AC) tissue: blood ratio for the kidneys was 24.8 (15 min after injection of the [^{14}C]piperazine); for the liver, 6.4; for the spleen, 12.7; for the lungs, 14.3; for the tumor, 2.5 (30 min). It was also high for the other organs (2.1-15 after 15 min). All this indicates that, in addition to its content in the blood, the radioactivity accumulates as a result of penetration of the [^{14}C]piperazine into the structural formations of the cells. The faster entry of [^{14}C]piperazine into the tissues as compared with [^{14}C]dipin also caused the high tissue AC : blood AC ratio. The comparatively low tissue AC : blood AC ratio in the organs of animals to which we had injected dipin labeled with ^{14}C in the piperazine ring characterizes the somewhat retarded penetration of this preparation into the cell structure.

The [^{14}C]piperazine accumulated in the organs in 5-15 min; thereupon the total content in the muscles was 27.5%; in the liver, 8.5%; in the kidneys, 7.8%; in the skeleton, 7.1%; in the small intestine, 12%; in the stomach, 3.9%; in the large intestine, 3.28%; and in the tumor, 2.76%. In the remaining organs and tissues the total amount of it was 1.0-0.015% of that introduced. The change in radioactivity content can be represented by an exponential function. Then

TABLE 1. Absorption Coefficient of [^{14}C]Piperazine in Rats with Sarcoma 45 on Intravenous Injection

	Time, min						
	5	15	30	60	120	240	24 h
Blood	0,82	0,35	0.15	0.12	0,156	0,10	0,012
Liver	1.88	2,01	0,96	0,39	0,52	0,39	0,17
Kidneys	10.92	8.69	3,04	2.21	2,07	1,83	0,22
Spleen	1,67	2,83	1,90	1,11	0,82	0,63	0,24
Lungs	2.19	4,76	2.14	0,79	0,88	0.56	0.12
Heart	1.58	2,34	0,89	0,60	0,47	0,14	0,06
Muscles	0,62	0,87	0,57	0,39	0,34	0,26	0,03
Skeleton	0,36	0,72	0,35	0,27	0,16	0,11	0,038
Brain	0,076	0,02	0,045	0,015	0,025	0,026	0,018
Lymphatic glands	1,24	3,11	1,29	1,32	0,42	0,36	0,14
Thymus	1,11	2,46	1,62	1,09	0,82	0,63	0,076
Bone marrow	0,13	0,24	0,021	0,03	0,013	0,03	—
Pituitary gland	3.19	3,77	2,33	2,16	1,48	1,35	1,24
Thyroid	1.28	2,37	1,15	1,34	0,7	—	—
Adrenals	1,14	5,16	2,03	1,91	0,44	0,38	0,17
Pancreas	1,16	1,75	1,48	0,97	0,35	0,23	0,052
Stomach	0,89	3,91	2,27	1,66	0,83	0,62	0,56
Small intestine	1,08	3,03	1,39	0,86	0,76	0,52	0,24
Large intestine	0,76	1,94	0,61	0,26	0,97	1,54	0,35
Tumor	0,26	0,61	0,37	0,30	0,26	0,16	0,026
Tail (as site of injection)	3,68	2,15	0,76	0,35	0,54	0,39	0,29

$$A_t = 63\% \cdot e^{-2{,}08\,t} + 37\% \cdot e^{-0{,}09\,t},$$

where A_t is the total amount of radioactivity (in percent of that introduced to the time t, in h).

From the equation given it follows that 63% of the radioactivity injected was removed from the organism with a half-removal time of 20 min, and 37% with a period of 7 h. The [^{14}C]piperazine is metabolized in the organism of rats more rapidly ($T_{1/2}$ = 7 h) than is [^{14}C]dipin ($T_{1/2}$ = 10 h).

On study of the distribution of [^{14}C]piperazine in the organism of intact rats, it was found that the radioactive preparation is removed from the blood more slowly than in the case of the tumor-bearers. Thus, after 5 min the absorption coefficient of the blood was 0.91; after 30 min, 0.27; and after 1 h, 0.18.

The radioactivity level in the organs and tissues in intact rats rose rather rapidly, and after 5-15 min it reached the maximum. The AC in the kidneys was 6.72; in the liver, 1.34; in the spleen, 1.74; in the lungs, 1.94; in the bone marrow, 0.17; in the brain, 0.041; in the lymph nodes, 2.07; in the pituitary, 2.04; in the thymus, 1.3; in the pancreas, 1.04; in the heart, 1.12; in the stomach, 2.12; in the small intestine, 1.72; and in the thyroid, 1.14. In the remaining organs and tissues, the AC was equal to 1.12-0.43. The high radioactivity content of the gastrointestinal tract is a very important fact in estimating the accumulation of the preparation under study in treatment of helminthiases.

In intact rats, the ratio of the AC of most of the organs to the blood AC during the period of maximum radioactivity level (after 5-15 min), in distinction from the tumor-bearers, was less than unity. This indicates a rather rapid binding of the [^{14}C]piperazine by structural elements of the cells.

Results of chromatographic analysis of the urine of intact rats (Silufol plates; system, propanol – ammonia, 7 : 3) showed that even 2 h after intravenous injection of [^{14}C]piperazine hydrochloride, along with the unchanged preparation (R_f 0.25) four metabolites containing the radioactive label were detected in the urine. Their R_f values were 0.37 (30.3%); 0.46 (8.8%); 0.52 (2.8%); and 0.62 (traces). In the following periods (3-6 h), the number of metabolites did not change. One day after the moment of injection, these metabolites were not recorded, even by autoradiography. The data obtained indicated that [^{14}C]piperazine is rather rapidly converted into its metabolism products. In this connection, the observed radioactivity in the organs and tissues in a 2-h period is equivalent to the content of [^{14}C]piperazine itself, but in the farther removed periods it is caused by the presence of labeled metabolites.

EXPERIMENTAL

Chemistry

[^{14}C]Piperazine Hydrochloride. A mixture of 10.965 g (0.06 mole) of [1,2-^{14}C]dibromoethane having a specific activity of 4.9 μCi/mg, 6.05 g (0.06 mole) of aniline, and 6.52 g (0.07 mole) of dry sodium carbonate was boiled for 5-6 h with thorough stirring. The melt was extracted with hot water until the sodium bromide had been removed. There was obtained 6.4 (92%) of [^{14}C]diphenylpiperazine (II). To a suspension of II in 25.6 ml of concentrated hydrochloric acid, with cooling (−4 to −8°) and stirring, was slowly added, dropwise, a

saturated solution of 5.12 g of sodium nitrite. At the end of the reaction the precipitate was filtered off and it was washed with cold water. The moist p-dinitrosodiphenyl-N,N'-piperazine[^{14}C] (III) was added to a 40% solution of sodium metabisulfite (22 g of $Na_2S_2O_5$) and the suspension was heated to 80° with stirring. Thereupon a reddish-orange solution of p-disulfodiphenyl-N,N'-piperazine[^{14}C] was formed (IV), plus a considerable amount of suspended matter, which was filtered off and discarded. The product IV was made basic with a sodium hydroxide solution and was concentrated by distillation. The distillation was continued with superheated steam until the distillate had a neutral pH. The [^{14}C]piperazine was caught in hydrochloric acid. The solution was evaporated to dryness, the residue was recrystallized from dilute alcohol, and there was obtained 2.6 g (56.6%) of [^{14}C]piperazine hydrochloride, based on the starting [1,2-^{14}C]dibromoethane, having a specific activity of 11.75 μCi/mg.

RADIOMETRY OF INTERNAL ORGANS OF RATS AND RADIOCHROMATOGRAPHY OF URINE

The distribution of the [^{14}C]piperazine was studied in experiments on 102 mongrel male rats having sarcoma 45 and on healthy rats having a weight of 130-160 g. The animals with tumors were brought to the experiment 7 to 10 days after transplanting. The weights of the tumors reached 7-10 g. The [^{14}C]piperazine was injected into the tail vein in the form of an aqueous solution. After injection of the [^{14}C]piperazine, the rats were decapitated after 5, 15, or 30 min, or 1, 2, 4, or 24 h; the blood was collected in test tubes to which a 5% sodium citrate solution had been added preliminarily, and the organs were removed for radiometry. Specimens of the organs and tissues were dissolved in 2 ml of 1 N alkali on a water bath at 80-90°. The alkaline hydrolysate of the tissues was neutralized with acetic acid to pH 7.0-8.0. Eleven ml of scintillation liquid was poured into flasks, to which 0.1 ml of the tissue hydrolysate was added. The scintillation liquid was prepared as indicated in [5]. Radiometry of the specimens was carried out in an Ansitron liquid scintillation counter. The efficiency of the count was determined by use of [^{14}C]toluene as an internal standard. The count efficiency was 80-90%.

For estimation of the radioactivity content in the organs and tissues, the data obtained by radiometry were expressed in two indices. One index was the absorption coefficient, which characterized the ratio of the radioactivity concentrations in pulses present per gram of tissue to that introduced per gram weight of rat. The second index characterized the detected radioactivity in organs and tissues in percent referred to that introduced, which was taken as 100%. Removal of the label from the rat organism was estimated by the exponential equation.

For the chromatographic study of the urine, we introduced 1.5 mg each (17.62 μCi) of [^{14}C]piperazine intravenously to intact rats. After definite periods from the moment of injection of the radioactive preparation (2, 3, 4, 6, 24, and 48 h) urine samples were collected and they were chromatographed in the systems propanol–ammonia (7 : 3) and butanol–water–acetic acid (4 : 5 : 1) on Silufol plates, from which autoradiograms were then prepared. Density scanning of the darkening in the autoradiograms was carried out on a Uvifot densitometer.

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