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Absorption and Excretion of ^{131} Iodine Labeled Atabrine

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Recently, Ackerman (1,2) used ^{131}iodine labeled Atabrine as a tagged material which was selectively absorbed by tumor tissues.

Direct iodination of Atabrine [2-methoxy-6-chloro-9-(1-methyl-4-diethylamine-butylamine)acridine] gives a very poor yield (between 0.5 and 1.5 %). The method worked out in our laboratory increases that yield to 10-30 per cent. It is based on the technique of Terentyev *et al* (3) for the halogenation of amines. A cold preparation of the iodinated compound permitted labeling by a simple isotopic exchange.

The absorption and excretion, as well as chromatographic identification of the labeled material, was also studied.

Experimental procedures and results:

One gram of Atabrine dissolved in 20 ml of water was made alkaline with concentrated ammonium hydroxide (to pH 10.0) and the acridine base was extracted with ethyl ether. The ether extract was dehydrated with anhydrous sodium sulfate. After this, 0.2 ml of iodine monochloride, dissolved in 20 ml of dioxane, was added to the extract. By gentle heating (50-60°C) the ether was evaporated and the remaining liquid was heated during a half hour at the same temperature. After the return to room temperature, it was acidified to pH 1.5 with 2 N HCl. After adding ⅓ of its volume of methanol, it was extracted with ethyl ether, until there was no more pink coloration in the ether phase. At this

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point, the aqueous layer was made alkaline with concentrated ammonium hydroxide and again extracted with ethyl ether. Under these conditions, the Atabrine and the cold iodinated Atabrine were in the ether extract. After evaporation of the ether in a water bath, they were dissolved in methanol and finally kept under refrigeration.

Four hundred mg of the cold iodinated Atabrine, prepared according to the preceding technique, dissolved in 20 ml of methanol, was mixed in a glass stopper flask, with 30 mc of carrier-free ^{131}NaI in a volume of 6 ml of distilled water, 5 ml of choloroform, and one drop of 30% hydrogen peroxide. This was shaken one hour. Under these conditions, if the cold material preparation was correct, no pink coloration in the choloroform layer was seen. Ten to twenty minutes after the addition of sufficient methanol, to obtain one homogenous phase, one drop of sodium sulfite solution (saturated) was added. By gentle heating and under a nitrogen stream, the liquid was evaporated to 5 ml. After addition of an equal volume of methanol, the solution was passed through a Dowex 1-X8 (200 mesh) ion exchange resin (acetate form) column of 10 cm $\times$ 1 cm^2. The elution was carried out with 50 per cent methanol and the eluate was brought to pH 1.5 with 2 N HCl. The free radioiodide remains on the column. The yield of radioactive iodinated Atabrine, by this exchange was between 10-30 per cent.

The determination of the radioactive purity was carried out by ascending paper chromatography, using n-butanol saturated with 0.2 M phosphate buffer (pH 2.0). With this solvent the iodide Rf value is 0.16-0.19. The labeled Atabrine presents two components: A minor component (approximately 30%) with the Rf value 0.3-0.4 (the same Rf as that for the unlabeled Atabrine) and a major component (approximately 70 %) with an Rf value of 1.0. This major component could be other form of iodinated Acridine base.

The methanol solution from the elution was evaporated by gentle heating using a carbon dioxide stream. The residue, dissolved in water, was injected into adult rats through the tail vein, 0.2 ml and approximately 90 μc each. A group of five animals was counted (whole body counting) throughout the whole experiment at different intervals. Groups of five (three male and two female) were sacrificed at different intervals. The total activity in organs and tissues was determined with a scintillation counter (Table I). After this, the organs with the highest activity were homogenized in saline, alkalinized with concentrated ammonium hydroxide and extracted with ethyl ether. The tendency of the homogenized material to form a stable emulsion was avoided by saturating the aqueous phase with ammonium acetate. The ether layer was washed three times with ammonium acetate solution (saturated), alkalinized with ammonium hydroxide, containing 0.1 percent of potassium iodide. After this, the extracts were counted and this activity (due to the Atabrine fraction) was compared with the total (Table II). In the same way, the excretion products at diverse intervals were analyzed (Table III).

Table I shows the specific activities in different organs and tissues. From the first moment, the activity in organs with a high blood perfusion is high, in-

TABLE I

VALUES OF RADIOACTIVITY IN DIFFERENT ORGANS AND TISSUES*

		30 min.	*1 hr.*	*3 hr.*	*6 hr.*	*12 hr.*	*24 hr.*	*144 hr.*
Thyroid	a	139	199	1115	1736	5582	10031	2531
	b	0.190	0.279	1.566	2.437	7.841	14.105	3.552
Stomach	a	2.9	3.4	5.19	20.9	45.9	15.32	0.41
	b	0.900	0.996	10.357	5.590	9.352	2.197	0.071
Lung	a	0.82	0.75	2.39	2.01	3.30	1.73	0.06
	b	0.160	0.150	0.369	0.471	0.533	0.153	0.005
Heart	a	0.71	0.75	1.81	2.26	2.86	1.53	0.14
	b	0.006	0.063	0.127	0.216	0.232	0.090	0.007
Kidney	a	0.57	0.56	1.74	1.39	2.58	3.79	0.49
	b	0.150	0.148	0.294	0.401	0.442	0.495	0.065
Spleen	a	0.48	0.51	1.92	2.51	8.31	0.99	0.11
	b	0.040	0.029	0.134	0.187	0.385	0.044	0.004
Liver	a	0.21	0.23	0.90	0.66	1.96	0.99	0.11
	b	0.271	0.300	0.728	0.781	1.344	0.698	0.077
Intestine	a	0.15	0.17	0.43	0.76	1.47	4.48	0.09
	b	0.243	0.401	0.568	1.185	1.514	4.004	0.107
Brain	a	0.17	0.18	0.28	0.20	1.01	0.33	0.02
	b	0.010	0.010	0.031	0.048	0.069	0.025	0.0015
Testicle	a	0.34	0.44	0.56	1.47	1.22	0.61	0.10
	b	0.062	0.068	0.081	0.136	0.111	0.108	0.0080
Ovary	a	0.32	0.39	0.79	1.38	0.75	0.64	0.46
	b	0.081	0.088	0.089	0.069	0.203	0.004	0.0032
Muscle	a	0.04	0.04	0.09	0.17	0.36	0.35	0.002
	b	—	—	—	—	—	—	—
Bone	a	0.11	0.14	0.31	0.40	1.57	0.23	0.12
	b	—	—	—	—	—	—	—

a: Specific activity cpm/mg.
b: Percent of injected dose. (per organ)
*All these values are without correction by decay and related to the initial injected dose. They are the average of five animals.

TABLE II

VALUES OF ACTIVITY CORRESPONDING TO LABELED ATABRINE FRACTION (AS % OF TOTAL RADIOACTIVITY IN HOMOGENIZED MATERIAL).

	30 min.	*½ hour*	*3 hours*	*6 hours*	*12 hours*
Stomach	—	—	0.0003	0.0006	0.0009
Kidney	0.06	0.19	1.05	0.12	0.08
Liver	0.08	0.12	0.18	0.09	0.25
Intestine	0.59	0.67	0.20	0.21	1.02
Urine					0.52*
Feces					0.41*

*Determined only at 12 hours.

creasing progressively in spleen and kidneys. The values for liver are relatively lower and in muscle and bone are almost constant.

The fraction of the injected dose found in the various organs (Table I and Fig. 1) shows that in the first 12 hours the stomach has a maximum, then it decreases and there is a corresponding increase in the intestine and thyroid.

The average of the whole body counting is given by Fig. 2. After 15 days

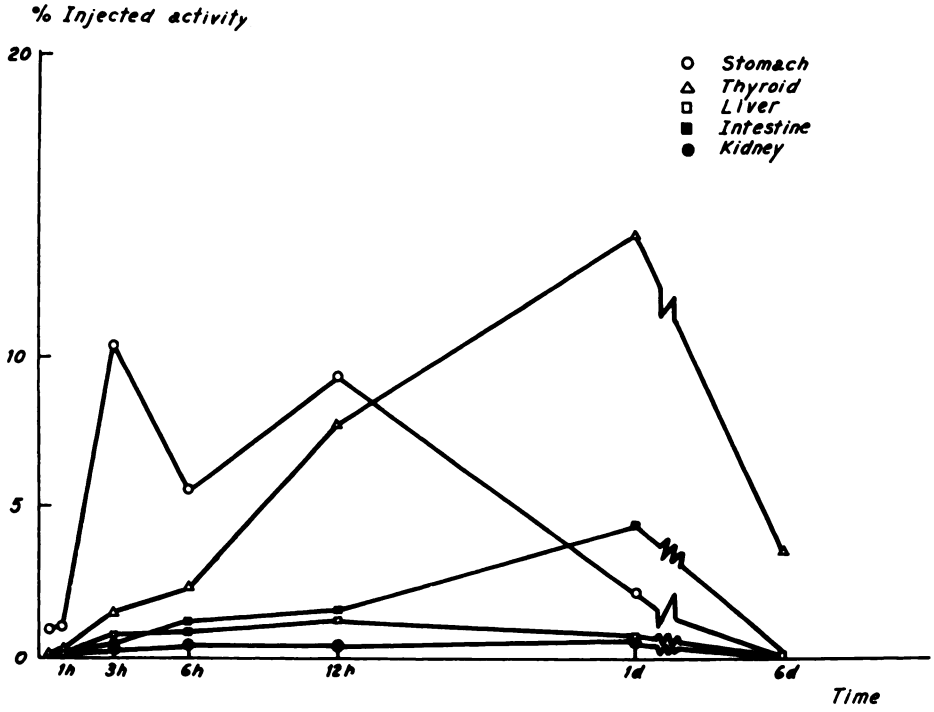

Fig. 1

TABLE III

ELIMINATION OF RADIOACTIVITY THROUGH THE EXCRETAS (%)

	12 hs.	*24 hs.*	*3 days*	*6 days*	*15 days*
Urine	3.03	21.3	29.6	35.6	50.8
Feces	0.03	1.03	5.6	9.8	12.

these animals were sacrificed, and the activity found in the thyroid was 23.3 percent of the original dose. The whole body activity was 27.5 percent of the original dose.

The analysis of homogenized tissues indicates that the activity due to labeled Atabrine was very low. The kidneys present a maximum of labeled Atabrine activity at three hours. The excretion in urine is higher than in feces; in the first 24 hours the ratio is approximately 20:1.

Discussion:

An analysis of the radioiodine labeled Atabrine indicated the presence of two compounds, a minor one with Rf value 0.3-0.4 and a major with Rf value 1.0. The minor component is excreted rapidly and the major component is excreted

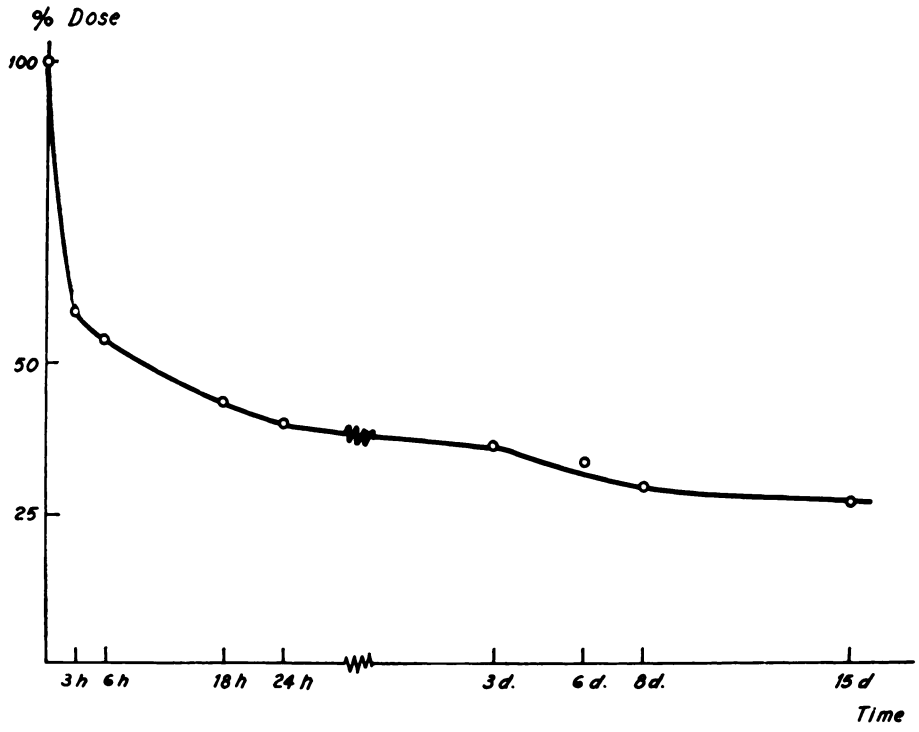

Fig. 2

more slowly. In agreement with this conclusion is the high activity found in the stomach, the normal pathway of iodide elimination (4)(5)(6), and the maximum of labeled Atabrine found in the kidney at three hours. The minor compound could be identified with the fraction having an Rf 0.3-0.4, using n-butanol saturated with phosphate butanol saturated with phosphate buffer, pH 2.0. The major compound is distributed throughout the whole body and its excretion occurs slowly through the liver.

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