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Uptake of Radioiodine Labeled Atabrine by Enlarged Spleen In Leukemic Mice

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Ackerman and Shemesh (1,2) have investigated the possible use of radioiodinated atabrine in the clinical localisation and diagnosis of malignant tumors.

The accumulation and excretion of nonlabeled atabrine has been studied by Dearborn (3); and a similar study with the iodine-131 labeled atabrine, injected intravenously, has been performed by the author (4).

In this experimental work, the iodine-131 labeled atabrine was injected intraperitoneally in order to minimize the diffusion throughout the tissues and to give more reliable results, as noted by Ackerman (2).

EXPERIMENTAL PROCEDURES AND RESULTS

Ten mC of iodine-131 labeled atabrine (Specific activity 0.1 mC/mg), prepared according to the author's technique (4), were dissolved in 1 ml of distilled water and passed through a Dowex 1-X8 (200 mesh) ion exchange resin (acetate form) column of 20 cm X 0.8 cm^2. The elution was carried out with 10 ml of distilled water followed by 30 ml of 50% methanol and, finally, 30 ml of 100% methanol. The eluted material was collected in 1 ml fractions. By plotting the radioactivity of each fraction, measured by scintillation counting, a graph was obtained (Fig. 1). The pool of the fractions comprising each peak (A, B and C) was analyzed by ascending chromatography on paper using n-butanol saturated with 0.2 M phosphate buffer (pH 2.0) as a solvent (4). The radioactivity of the chromatogram was scanned and automatically recorded. The radioactivity corresponding to the peak A gave Rf=0.35, similar to that corresponding to the unlabeled atabrine. During the elution most of the yellow coloration was eluted with this peak of activity. The other peaks B and C, gave similar values, Rf=1.0.

Afterwards, the eluted solution corresponding to each peak was evaporated to dryness under vacuum, and the residue was dissolved in 2 ml of 0.1 N hydrochloric acid. The pH was 1.0 and was altered to 5.0-6.0 by addition of 1 N sodium hydroxide.

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Three series of 10 BALB mice injected days before intraperitoneally with leukemia L 370-III 86 cells and presenting a very enlarged spleen (10-20 times the normal size), have been injected intraperitoneally with the radioiodinated atabrine in the following way. One series with the material from peak A (compound A), a second series with the material from the peak B (compound B) and a third series with the original radioiodinated atabrine (compound M), without any processing. Another series of 10 animals, without a priori injection of leukemic cells, was injected with compound M. Each animal was injected with approximately 25 μC of radioactive compound.

The animals were sacrificed at different times and Table I gives the values corresponding to the radioactivity found in the spleen and liver as a percentage of the total activity present in the whole body at the moment the mice were sacrificed.

Spleen, liver and intestine were minced and extracted twice with methanol. The extracts were analyzed chromatographically in the way described above. Activity was found at Rf=0.60-0.65 in liver and spleen. Intestine showed activity in the iodide zone (Rf=0.16). Only the spleen presented activity at Rf=1.0.

In general, the mice with enlarged spleen presented a higher percentage of the total activity in the spleen and this activity remains localized there longer.

FIG. 1. ION EXCHANGE CHROMATOGRAPHY OF IODINE-131 LABELED ATABRINE.

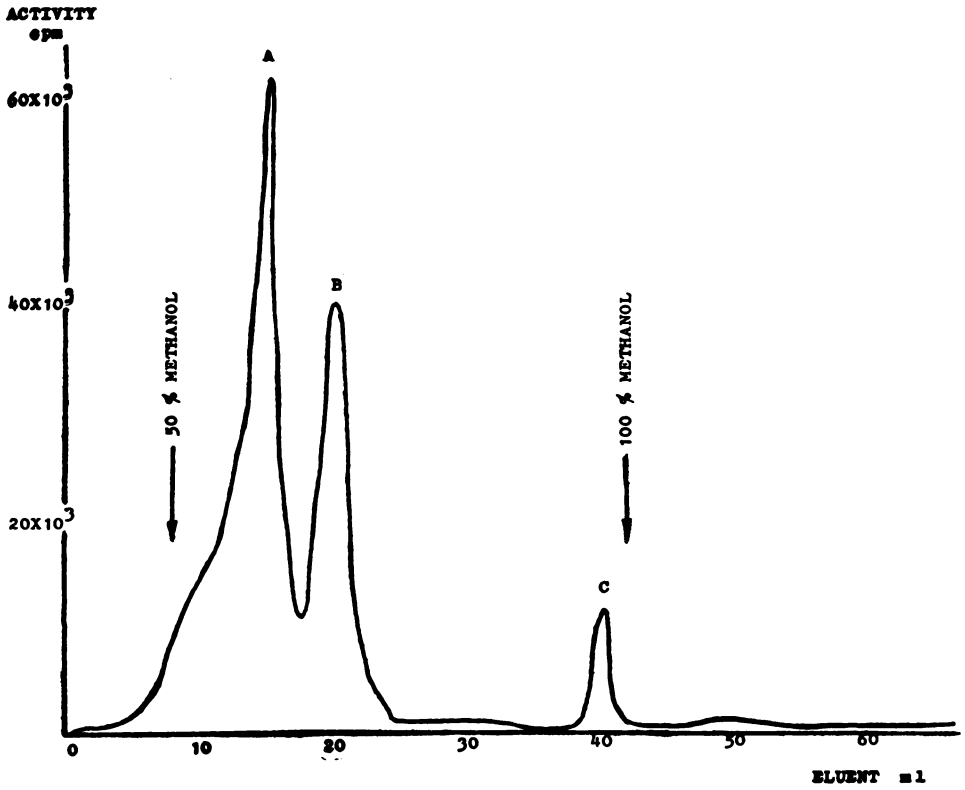

Fig. 1

Compound B presented a higher uptake during the first day and a higher remaining activity after six days. The compound M behaved as expected, as a mixture of both compounds A and B (The very low activity due to the peak C is considered as not important in this case).

Previous studies demonstrated experimentally that different batches of iodine 131 labeled atabrine give variable amounts of compounds A and B; depending on time, temperature and reactants' concentration during the iodination, as well as the storage time of the iodinated material.

TABLE I

DISTRIBUTION OF THE RADIOACTIVITY AFTER THE INJECTION OF RADIOIODINATED ATABRINE

		Spleen		*Liver*		
		Weight gr	*Activity*‡ %	*Weight* gr	*Activity*‡ %	*Ratio* $\frac{\text{S.A. Liver}‡‡}{\text{S.A. Spleen}}$
Group A	1 day*	0.152	5.9	1.300	54.6	9.0
	3 days**	0.165	6.7	1.420	66.3	10.8
	6 days*	0.172	2.7	1.250	93.1	5.0
Group B	1 day*	1.867	5.9	1.400	31.8	1.6
	3 days*	1.691	7.5	1.540	45.6	3.6
	6 days**	2.487	9.5	1.425	82.0	12.6
Group C	1 day*	1.698	2.2	1.390	13.6	5.2
	3 days*	1.476	1.5	1.450	11.6	6.4
	6 days**	1.901	6.1	1.375	46.6	12.9
Group D	1 day**	1.157	23.5	1.425	44.4	1.9
	3 days*	1.650	8.7	1.390	56.0	6.9
	6 days*	1.836	9.9	1.510	88.8	6.4

Group A: Nonleukemic injected with compound M (total).
Group B: Leukemic injected with compound M (total).
Group C: Leukemic injected with compound A.
Group D: Leukemic injected with compound B.

‡As percent of the whole body's activity.
*Average of 3 animals.
**Average of 4 animals.
‡‡Ratio—Specific Activity Liver/Specific Activity Spleen.

DISCUSSION

As demonstrated before (4), three radioactive compounds are present in the iodine 131 treated atabrine.

The higher uptake of compound B observed in enlarged leukemic spleen as compared to atabrine, indicates that this compound is selectively incorporated.

The compound with Rf=0.60-0.65 observed chromatographically in the liver is possibly a metabolic product.

The amount of radioactivity incorporated into the enlarged spleen is in direct relation with its size, and more significantly, the ratio specific activity of the liver/specific activity of the spleen is higher in the nonleukemic animals.

All these observations indicate a potential usefulness of the compound B separated from the radioiodinated atabrine in further studies of spleen scanning and local irradiation.

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