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Research Article

STUDIES ON NEOPLASMS WITH THE AID OF RADIOACTIVE PHOSPHORUS.[1] III. THE PHOSPHORUS METABOLISM OF THE PHOSPHOLIPID, ACID SOLUBLE AND NUCLEOPROTEIN FRACTIONS OF VARIOUS TISSUES OF NORMAL AND LEUKEMIC MICE FOLLOWING THE ADMINISTRATION OF "TRACER" AND "THERAPEUTIC" DOSES OF RADIO-PHOSPHORUS

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"Tracer" doses of radio-phosphorus (P^{32}) are small amounts which are conceivably insufficient to cause significant changes in the metabolism of the animal's cells in which they are retained, while "therapeutic" doses are large amounts of P^{32} which significantly alter the metabolism of cells because of the quantity of beta-radiation spontaneously emitted. A previous paper (1) presented the retention of radio-phosphorus, which had been administered intraperitoneally, in "phospholipid", "acid soluble" and "nucleoprotein" fractions of various tissues of normal mice and mice with generalized lymphomata. This paper presents the retention of P^{32} in the same fractions of similar groups of mice following the administration of both "tracer" and "therapeutic" doses of radio-phosphorus.

MATERIALS AND METHODS

The same materials, methods and techniques were used in this experiment as in the experiment mentioned above. The radio-phosphorus was produced by the Berkeley cyclotron (2) and converted into a sodium phosphate (15 mgm. per cc.) solution which was sterilized and assayed for radioactivity. It has been determined (unpublished data) that a dose of radio-phosphorus emitting 70 microcuries of beta radiation given intraperitoneally (as a sodium phosphate solution containing P^{32}) is lethal for mice weighing 20 grams in one to two weeks. The "tracer" dose of P^{32} used in this experiment consisted of ½ cc. of a sodium phosphate solution that contained 7.5 mgm. of Na_2HPO_4 at a pH of 7.4, and emitted 8 microcuries on the day of administration. The "therapeutic" dose used in this experiment was of the same volume, the same concentration of Na_2HPO_4 and the same pH, but emitted 80 microcuries of beta radiation on the same day. Five-tenths per cent, 1 per cent, 2 per cent, 5 per cent and 10 per cent quantities of both solutions, accurately measured, were kept as reference samples and their radioactivities were compared with those of the fractions studied, thus obviating calculations for decay of radio-phosphorus. All measurements of radioactivity were made by the use of a DuBridge type of ion chamber electrometer.

One hundred and seventy-six highly inbred Strong A strain mice (each weighing between 18 and 22 grams) were housed in thirty-two clean wire-bottomed cages, were fed almost identical amounts of Purina dog chow and oats ten days before and during the experiment, and were divided into 16 groups. Eight groups of 10 mice each (5 males and 5 females) served as the control or normal series. Eight groups of 12 mice each (6 males and 6 females), which served as the leukemic series, were injected intraperitoneally with 15 million cells of a lymphoma (3) fourteen days before being sacrificed. This lymphoma "takes" 100 per cent in the Strong A strain, produces uniform generalized leukemic infiltrations of the liver, spleen and lymph nodes and, in addition, grows in the peritoneal cavity as a very cellular, non-necrotic localized tumor mass. Six of the 176 died before completion of the experiment and were discarded. The remaining 170 were sacrificed twelve, twenty-four, thirty-six and forty-eight hours after intraperitoneal injections of "tracer" (8 microcuries per mouse) and "therapeutic" (80 microcuries per mouse) doses of radio-phosphorus.

The spleens, livers, lymph nodes (central and peripheral) both of the normal and the leukemic animals and the intraperitoneal tumor masses of the leukemic animals of the 16 groups respectively were pooled, weighed and prepared for analysis. The respective carcasses were pooled and ashed at 400 degrees Centigrade. The "phospholipid" fraction of the four tissues was extracted by means of ether, alcohol and reflux condensers, the "acid soluble" by cold 5 per cent trichloracetic acid, and the residue was considered the "nucleoprotein" fraction (1).

The amounts of P^{32} excreted by these animals were unfortunately not determined.

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TABLE I

Retention of radio-phosphorus in "phospholipid", "acid soluble" and "nucleoprotein" fractions of various tissues and remainder of bodies of normal mice and mice with lymphoma twelve, twenty-four and forty-eight hours following its intraperitoneal administration in small and large doses

			μc. of P^{32} administered intraperitoneally	Hours after administration of P^{32} (*expressed in per cent of dose per gram wet weight*)			
				12	24	36	48
Spleen	Normal	Phospholipid	8	0.834		1.19	0.592
			80	0.854	0.689		0.545
		Acid soluble	8	2.0	1.61	1.27	0.888
			80	1.64	1.10	1.01	0.872
		Nucleoprotein	8		2.32	2.08	1.76
			80	2.02	1.64		1.25
	Leukemic	Phospholipid	8	0.877	0.898	0.932	
			80	0.702	0.791	0.864	0.748
		Acid soluble	8	2.49	1.64	1.64	
			80	2.13	1.53	1.49	1.32
		Nucleoprotein	8	3.26	3.43	3.85	
			80	2.81	2.97	3.12	2.87
Liver	Normal	Phospholipid	8	2.16	1.63	1.36	0.985
			80	1.76	1.32	1.20	0.947
		Acid soluble	8	1.47	1.08	1.02	0.672
			80	0.920	0.802	0.745	0.532
		Nucleoprotein	8	0.368	0.571	0.683	0.640
			80	0.460		0.558	0.550
	Leukemic	Phospholipid	8	1.74	1.44		1.17
			80	1.31	1.30	1.28	1.01
		Acid soluble	8	2.02	1.37	1.12	1.18
			80	1.36	0.954	1.10	0.887
		Nucleoprotein	8		1.62	1.99	1.87
			80	1.27	1.34	1.37	1.62
Lymph nodes	Normal	Phospholipid	8	0.431	0.563	0.824	0.794
			80	0.292	0.470		0.474
		Acid soluble	8	1.54	0.988		0.775
			80	1.12	0.760	0.706	
		Nucleoprotein	8	1.14	1.03	1.16	1.13
			80	0.820	0.850	0.858	0.882
	Leukemic	Phospholipid	8	0.786		0.777	0.776
			80	0.447	0.434	0.592	0.621
		Acid soluble	8	1.87		1.62	
			80	1.57	1.01	0.635	0.632
		Nucleoprotein	8	2.18	2.41	2.44	2.52
			80	2.04		2.42	2.42

μc = microcurie. P^{32} = radio-phosphorus.

TABLE I—*Continued*

		μc. of P^{32} administered intraperitoneally	Hours after administration of P^{32} (*expressed in per cent of dose per gram wet weight*)			
			12	24	36	48
Lymphoma	Phospholipid	8	0.604	0.711	0.803	0.791
		80	0.458	0.618	0.683	0.666
	Acid soluble	8	2.59	1.24	1.54	1.35
		80	1.63	1.36	1.21	0.845
	Nucleoprotein	8		2.38	3.45	3.34
		80	1.19	2.53	2.53	2.11
Carcass	Normal		*	*	*	*
		8	2.06 (9)	1.52 (10)	1.79 (9)	1.17 (10)
		80	1.52 (10)	1.40 (10)	1.24 (10)	0.900 (10)
	Leukemic	8	2.31 (10)	2.04 (12)	2.31 (12)	1.78 (12)
		80	1.74 (12)	1.79 (11)	1.67 (11)	1.74 (12)

* Number of mice on which above data were obtained.

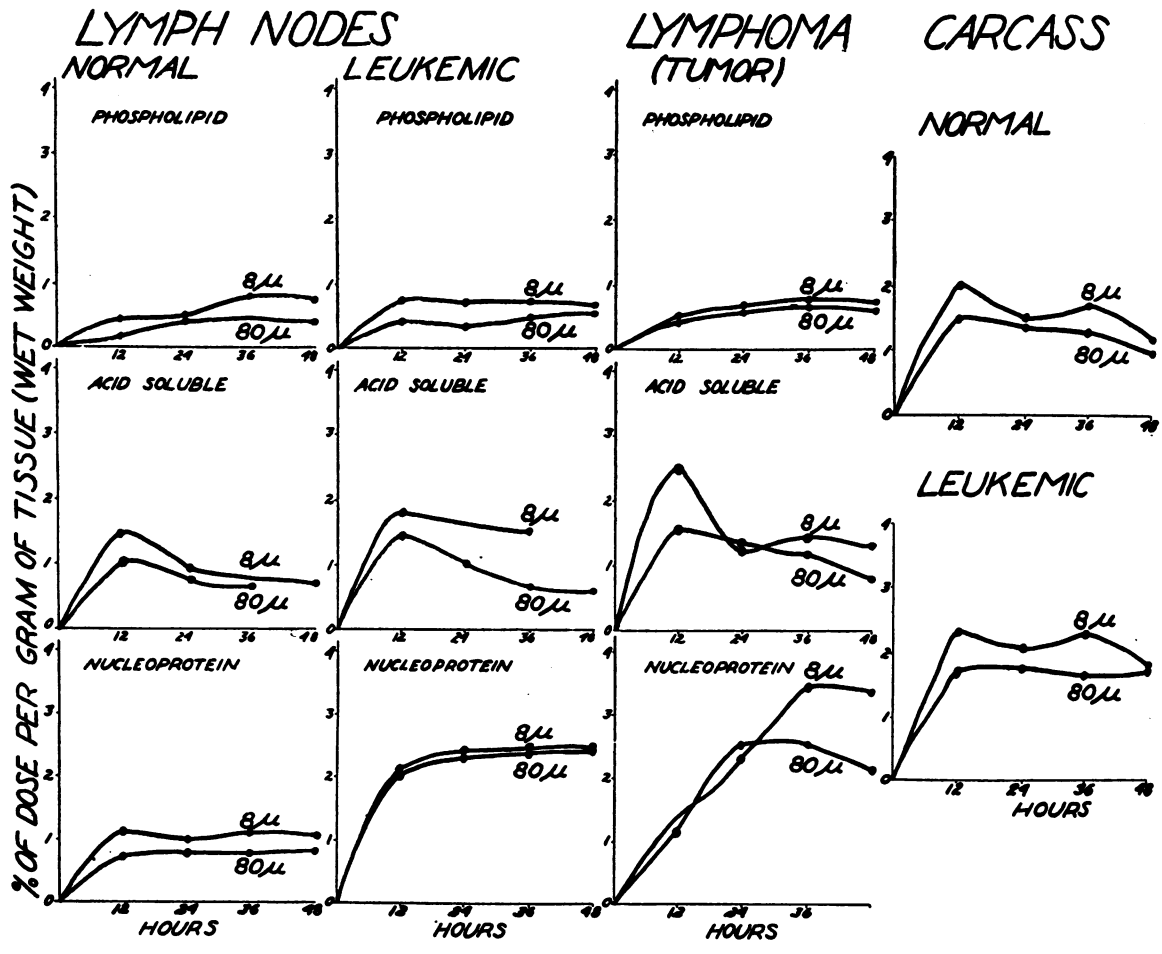

FIG. 1. THE COMPARATIVE RETENTION OF RADIO-PHOSPHORUS IN FRACTIONS OF TISSUES AND CARCASSES OF NORMAL AND LEUKEMIC ANIMALS 12, 24, 36 AND 48 HOURS FOLLOWING THE INTRAPERITONEAL ADMINISTRATION OF P^{32} EMITTING BOTH 8 AND 80 MICROCURIES OF BETA RADIATION PER MOUSE

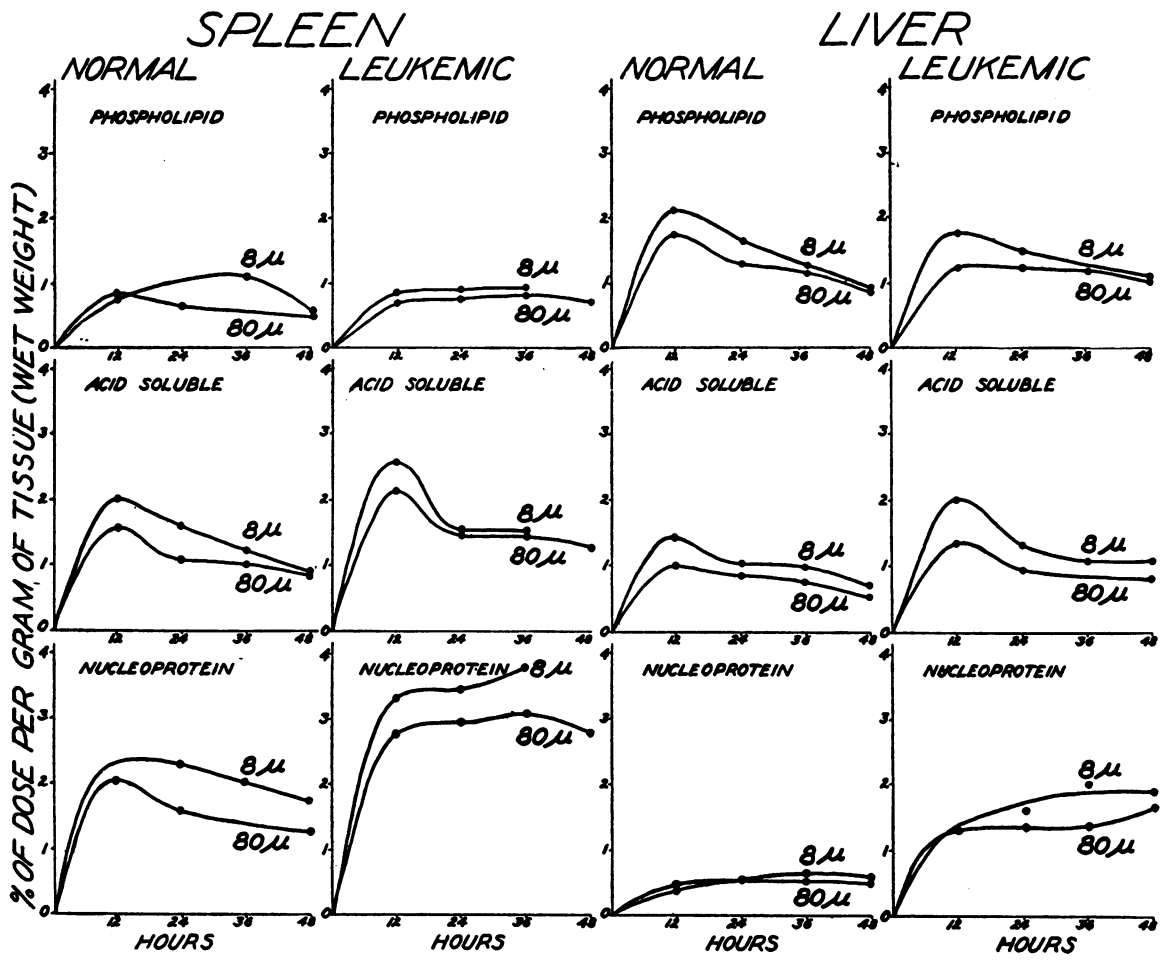

FIG. 2. THE COMPARATIVE RETENTION OF RADIO-PHOSPHORUS IN FRACTIONS OF TISSUES AND CARCASSES OF NORMAL AND LEUKEMIC ANIMALS 12, 24, 36 AND 48 HOURS FOLLOWING THE INTRAPERITONEAL ADMINISTRATION OF P^{32} EMITTING BOTH 8 AND 80 MICROCURIES OF BETA RADIATION PER MOUSE

RESULTS

The results are listed in Table I and are illustrated in Figures 1 and 2.

The amounts (as per cent of the dose of P^{32} administered per gram of tissue-fresh wet weight) of radio-phosphorus retained in the various fractions of the four tissues and in the carcasses were consistently less following the "therapeutic" dose (80 microcuries per mouse) than those following the "tracer" dose (8 microcuries per mouse). In the majority of instances this difference was noted twelve hours after administration and persisted at the forty-eight-hour period also. The difference was perhaps slightly greater in the "nucleoprotein" fractions than in the "phospholipid" and "acid soluble" fractions. Although the leukemic animals retained more P^{32} than the corresponding normal animals, the differences between levels of retention of P^{32} were quite similar in the corresponding fractions of the tissues of the 2 groups following "tracer" and "therapeutic" doses, respectively.

DISCUSSION

These results indicate that, when large doses of radio-phosphorus are used, the effects of the irradiation can be measured. Therefore, in interpreting the results of metabolic investigations, it must be known whether large or small doses were used if radioactive agents were employed in obtaining these results. Since the results reported here, when 8 microcuries per animal were used,

are identical at the forty-eight-hour period with those when 5.5 microcuries were used (1) and almost identical with those when 30 microcuries and 50 microcuries per animal were used,[3] it would seem safe to conclude that 8 microcuries per animal is a safe "tracer" dose for mice on which such studies are to be made.

The results also indicate that there is no difference in radiosensitivity of the metabolic processes studied in the normal animals when compared with those of the leukemic animals.

This technique could well be used as a method to compare the radiosensitivity of various types of cellular metabolism both in normal and neoplastic tissues, and it may prove to be a valuable method of comparing the effects on these tissues of different types of radiation such as x-radiation or neutron radiation.

[3] See footnote, page 60, reference 1.

SUMMARY

Less P^{32} was retained in the "phospholpid", "acid soluble" and "nucleoprotein" fractions of spleen, liver and lymph nodes, and the carcasses both of normal mice and mice with lymphoma after intraperitoneal administration of large or "therapeutic" doses of radio-phosphorus than when small or "tracer" doses were given.

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