CLINICAL STUDIES WITH THE AID OF RADIOACTIVE PHOSPHORUS. I. THE ABSORPTION AND DISTRIBUTION OF RADIO-PHOSPHORUS IN THE BLOOD AND ITS EXCRETION BY NORMAL INDIVIDUALS AND PATIENTS WITH LEUKEMIA

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J Clin Invest. 1941;20(5):567-575. https://doi.org/10.1172/JCI101249.

Research Article

CLINICAL STUDIES WITH THE AID OF RADIOACTIVE PHOSPHORUS. I. THE ABSORPTION AND DISTRIBUTION OF RADIO-PHOSPHORUS IN THE BLOOD AND ITS EXCRETION BY NORMAL INDIVIDUALS AND PATIENTS WITH LEUKEMIA[1]

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(Received for publication April 9, 1941)

The purpose of this paper is (*a*) to indicate the amount of radio-phosphorus (P^{32}) retained by various fractions of the blood of 4 normal individuals, 12 patients with myeloid, and 15 with lymphoid leukemia, and the variations in retention following the administration of radio-phosphorus when given orally and/or intravenously and when accompanied by varying amounts of non-radioactive phosphorus (P^{31}); (*b*) to indicate the distribution of P^{32} in the bone marrow and in various fractions of white blood cells; and (*c*) to indicate the amount of radio-phosphorus excreted in urine and feces in these cases.

MATERIALS AND METHODS

The radioactive phosphorus was produced by the Berkeley cyclotron (1). The 4 normal individuals were robust, ambulatory workmen with recently healed fractures, all of whom had received the same type and quantity of food over a period of from 1 to 8 weeks, and each of whom had a single regular bowel movement daily during the same period previous to the administration of P^{32}. It was impossible to control the diets or the time of excretion of the patients, all of whom were ambulatory. The blood withdrawn from veins was heparinized, cooled and centrifuged exactly 20 minutes at 1450 times gravity to insure constant volume. The buffy coat was aspirated, suspended in equal amounts of heparinized Ringer's solution and centrifuged exactly 20 minutes at 1450 times gravity. The plasma was then removed from the original tube and finally the red blood cells were extracted. Bone marrow obtained by sternal aspiration was heparinized and centrifuged, as described previously. In some instances, the nuclei were separated from the cytoplasm of the peripheral white blood cells by violent agitation for 20 minutes in cold 5 per cent citric acid (2). The phospholipids were extracted from the white blood cells by the use of ether, alcohol and reflux condensers, and the acid-soluble substances by use of ice-cold 5 per cent trichloracetic acid, thereby leaving the nucleoprotein-like substances as residue (3). The assays of radioactivity were made by use of an electrometer.

[1] These investigations have been aided by a grant from the John and Mary R. Markle Foundation.

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RESULTS

The average percentages of the dose of administered P^{32} retained per 100 cc. of red blood cells, white blood cells and plasma of the normal individuals and of the patients are listed in Table I and illustrated in Figure 1.[3] The amounts and the method of administration of P^{32}, the number of cases studied, and the intervals in time are also noted. No attempts were made to correct for variations in the metabolic rate, blood volume, kidney and hepatic functions, diet, age, weight, etc. of the cases studied. In computing the averages (Table I), the determinations obtained from both acute and chronic cases of leukemia were used. The acute cases are indicated.

(*a*) *Retention of P^{32} in whole blood.* It can be observed that more P^{32} was retained, during

[3] All determinations in figures and tables were corrected for decay of radio-phosphorus (the half-life of which is 14.3 days) to date of administration. 1 microcurie (μc) or 1/1000 millicurie (Mc) is equal to 37,000 beta particles per second.

It must also be pointed out that all the doses of P^{32} administered to the patients discussed in this paper were "therapeutic" and not "tracer" in nature, for even the smallest dose (540 microcuries) administered caused a decrease in the white blood cell levels of the patient. Therefore, the values obtained in the various metabolic studies must be considered as having been "conditioned" by the biological effects of the beta radiation emitted by the P^{32} and the information about cellular metabolism, which was obtained incidental to therapeutic attempts, must be interpreted with reservation and caution.

Although the average doses of radio-phosphorus administered orally to the various groups (normal, myeloid and lymphoid) are not similar, those administered intravenously are comparable (Table I).

TABLE I

Average per cent of dose of P^{32} administered orally and intravenously which was retained per 100 cc. of various fractions of blood of normal individuals and patients with leukemia*

Oral: 2 Hours	Oral: 4 Hours	Oral: 6 Hours	Oral: 24 Hours	Oral: 48 Hours	Oral: 72 Hours	Oral: 96 Hours	Oral: 5 Days	Oral: 8 Days	Oral: 10 Days	Number of cases studied*		Number of cases studied	IV: 2 Hours	IV: 4 Hours	IV: 6 Hours	IV: 24 Hours	IV: 48 Hours	IV: 72 Hours	IV: 96 Hours	IV: 5 Days	IV: 8 Days	IV: 9 Days
											Whole blood											
0.093		0.1026	0.0736	0.0626		0.0419		0.02		2	Normal	2	0.170		0.1686	0.1726	0.08		0.0308		0.0263	
	0.131		0.108	0.100	0.116	0.084				9	Myeloid	10	0.177		0.13	0.131	0.129		0.103	0.082		0.089
0.096	0.103		0.099	0.0465	0.0858	0.0608	0.037			10	Lymphoid	10	0.277	0.222	0.18	0.182	0.145	0.1440	0.118	0.112	0.044	
											Red blood cells											
0.0981		0.1313	0.1286	0.1039		0.0426		0.030			Normal		0.2303		0.2336	0.1993	0.132		0.0693		0.0393	
0.029	0.098		0.151	0.1068		0.0853			0.045		Myeloid		0.195		0.23	0.218	0.135		0.103	0.087		0.072
0.15	0.121	0.0899	0.1098	0.0607	0.0733	0.069	0.056	0.0112			Lymphoid		0.332	0.396	0.342	0.308	0.224	0.169	0.1223	0.144	0.0932	
											White blood cells											
		0.0343	0.0554	0.1396		0.1410		0.1433			Normal				0.074	0.0946	0.107		0.112		0.1800	
0.021		0.088	0.210	0.258		0.248		0.216			Myeloid		0.368			0.493	0.291	0.225	0.385	0.247	0.267	
0.118	0.117		0.122	0.073	0.1529	0.208	0.162	0.083			Lymphoid		0.276	0.448	0.451	0.452	0.591	0.450			0.64	
											Plasma											
0.0716		0.021	0.0115	0.016		0.015		0.008			Normal		0.075		0.0219	0.018	0.020		0.0149		0.007	
	0.069	0.027	0.0212	0.0279	0.026	0.016		0.003			Myeloid		0.133	0.03	0.03	0.027	0.024	0.022	0.021	0.025	0.019	
0.080	0.054	0.018	0.0166		0.014	0.010	0.018	0.012			Lymphoid		0.14	0.1	0.063	0.040	0.038	0.032	0.020	0.033	0.032	

* See table of Administrations.

* *Administrations* †

Oral				Intravenous		
Mgm. of Na_2PO_4	Microcuries of P^{32}	Cases		Cases	Microcuries of P^{32}	Mgm. of Na_2PO_4
	Average				Average	
600	1500	1	*Normal*	1	1500	600
600	1500	2		2	1500	600
	1500				1500	
50	850	1	*Myeloid*	1	765	100
830	1000	2		2	765	3000
150	2300	3		3	850	50
2000	2300	4		4	1140	150
1250	4700	5		5	1260	150
3000	4700	6		6	1950	300
2800	5960	7		7	2000	143
2400	7200	8	→acute	8	2000	2000
20	12600	9		9	2350	300
				10	2350	2000
	4623					
					1543	
			Lymphoid			
5180	2440	1		1	540	150
450	3000	2		2	1360	150
3000	3000	3	→acute acute←	3	1900	150
3000	3050	4		4	1998	180
1500	3300	5	acute←	5	2160	180
750	4000	6		6	2250	300
142	4270	7		7	2550	2000
300	5000	8		8	2600	300
750	5000	9		9	2600	2000
3480	11680	10		10	5000	450
	4474				2295	

† The cases recorded here will be described individually and in detail elsewhere.

the period studied, in the whole blood of the *patients* than in that of the *normal individuals,* and that the concentration reached higher levels in both groups when the P^{32} was administered intravenously. This last feature was found to be true in all of the fractions of the blood.

(*b*) *Retention of* P^{32} *in red blood cells.* The same features were found as discussed under (*a*).

(*c*) *Retention of* P^{32} *in white blood cells.* The concentration of P^{32} in the white blood cells of the patients was considerably higher than that in the white blood cells of the normal individuals. The metabolic rates of the white blood cells were not determined. The concentration of P^{32} in the white blood cells of the leukemic patients (particularly the lymphoid cells) was higher following the administration of P^{32} by the intravenous than by the oral route.

(*d*) *Retention of* P^{32} *in plasma.* The concentration of P^{32} was higher in the plasma after intravenous than after oral administration for a short period of time only—approximately 2 to 4 hours. This finding was present in the normal individuals and in the patients.

Figure 2 also shows that there is more retention of radio-phosphorus in blood fractions following intravenous than following oral administration in individual patients receiving P^{32} by both routes, and that when relatively large amounts of P^{31} accompany the radioactive phosphorus, regardless of route of administration, less of the latter is retained by the fractions. One patient (Case 34) retained about as much P^{32} in the blood fractions after its oral administration, when accompanied with a small amount of P^{31}, as when the same quantity of radio-phosphorus was administered intravenously in the presence of more than 10 times more P^{31}.

Figure 3 shows that with one exception (R.B.C. of Case 23) the retention of P^{32} was less in the

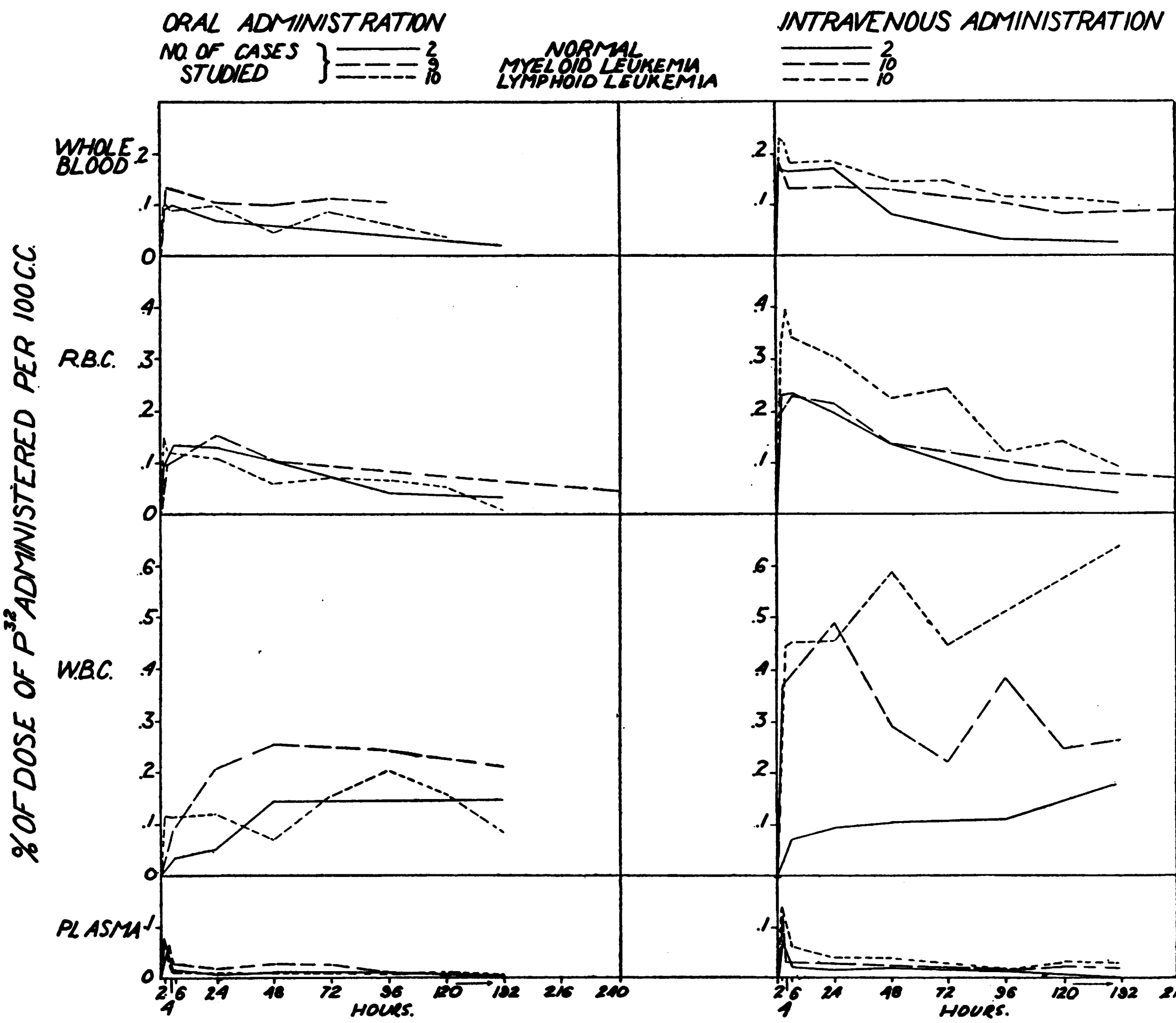

FIG. 1. AVERAGE RETENTION, IN BLOOD FRACTIONS, OF P^{32} ADMINISTERED ORALLY AND INTRAVENOUSLY IN NORMAL INDIVIDUALS AND IN PATIENTS WITH LEUKEMIA

blood fractions when large amounts of P^{31} accompanied the P^{32} than when small amounts of nonradioactive phosphorus were introduced at the same time.

Table II indicates that P^{32} is retained in the marrow in slightly higher concentrations than corresponding fractions in the peripheral blood at the same time period. The nucleated cells of the marrow include the nucleated red cells.

Table III indicates that the volumes of nuclei and cytoplasm of lymphocytes are about equal, while in myelocytes the nuclei comprise but about ⅕ to ⅓ of the volume of the cell. The ratio of the amounts of P^{32} retained in the lymphocyte nuclei to the cytoplasm is about 1 to 1, while in the myelocytes the ratio is 4 to 1 in the cases studied. Constant volumes were obtained by centrifuging samples for exactly 20 minutes at 1450 times gravity.

Table IV indicates that up to 48 hours after administration of P^{32} the greatest concentration occurs in the acid-soluble fraction of leukemic white blood cells. In 2 patients (Cases 28 and 67), it was noted that the concentration of P^{32} in the phospholipid and nucleoprotein fractions gradually increased following the administration of radio-phosphorus, while that in the acid-soluble fraction decreased. At the end of a period of 96

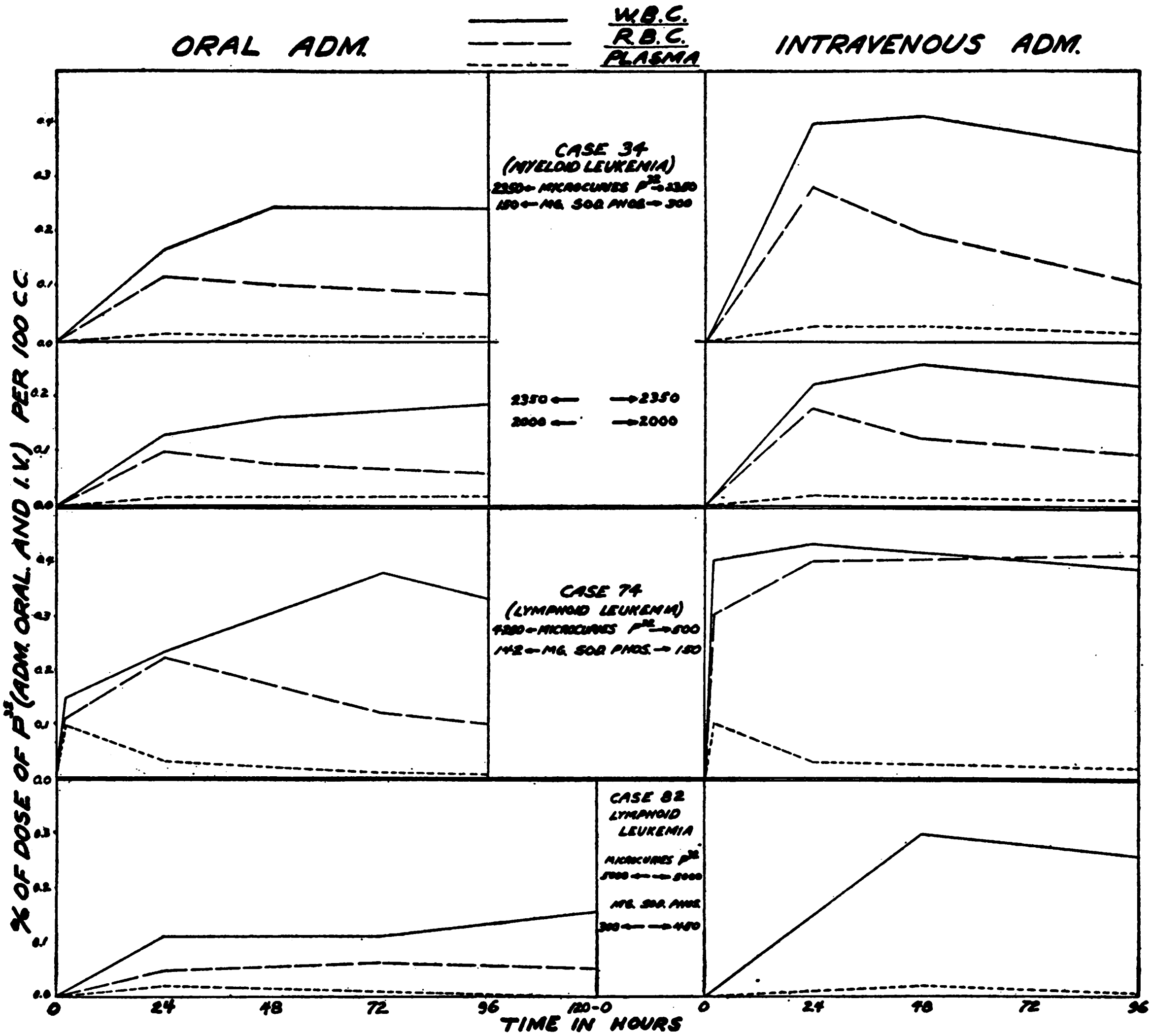

FIG. 2. VARIATIONS IN RETENTION, IN BLOOD FRACTIONS, OF P^{32} ADMINISTERED INTRAVENOUSLY AND ORALLY WHEN ACCOMPANIED BY LARGE AND SMALL AMOUNTS OF SODIUM PHOSPHATE

hours after the administration, the concentration of P^{32} in the nucleoprotein fraction was equal to or greater than the acid-soluble fraction in 4 of the 5 cases studied.

Figure 4 and Table V indicate the average per cent of the dose of administered P^{32} excreted in the urine and feces. When P^{32} is administered orally, from 15 to 50 per cent is excreted in the urine and feces in both normal individuals and patients during a 4- to 6-day period. In normal individuals the same percentages are excreted when P^{32} is administered intravenously, but in the patients from 5 to 25 per cent is excreted. When administered orally, the greater part of the P^{32} is excreted in the feces; when intravenously, a very small but definite amount is excreted in the feces. Normal individuals excrete large quantities in the urine following intravenous administration. In leukemic patients radio-phosphorus is probably more quickly fixed in the pathological tissues and cells (3, 4).

DISCUSSION

It has been observed (3, 5) that leukemic mice and their tissues retain more radio-phosphorus than normal mice and their tissues, that the con-

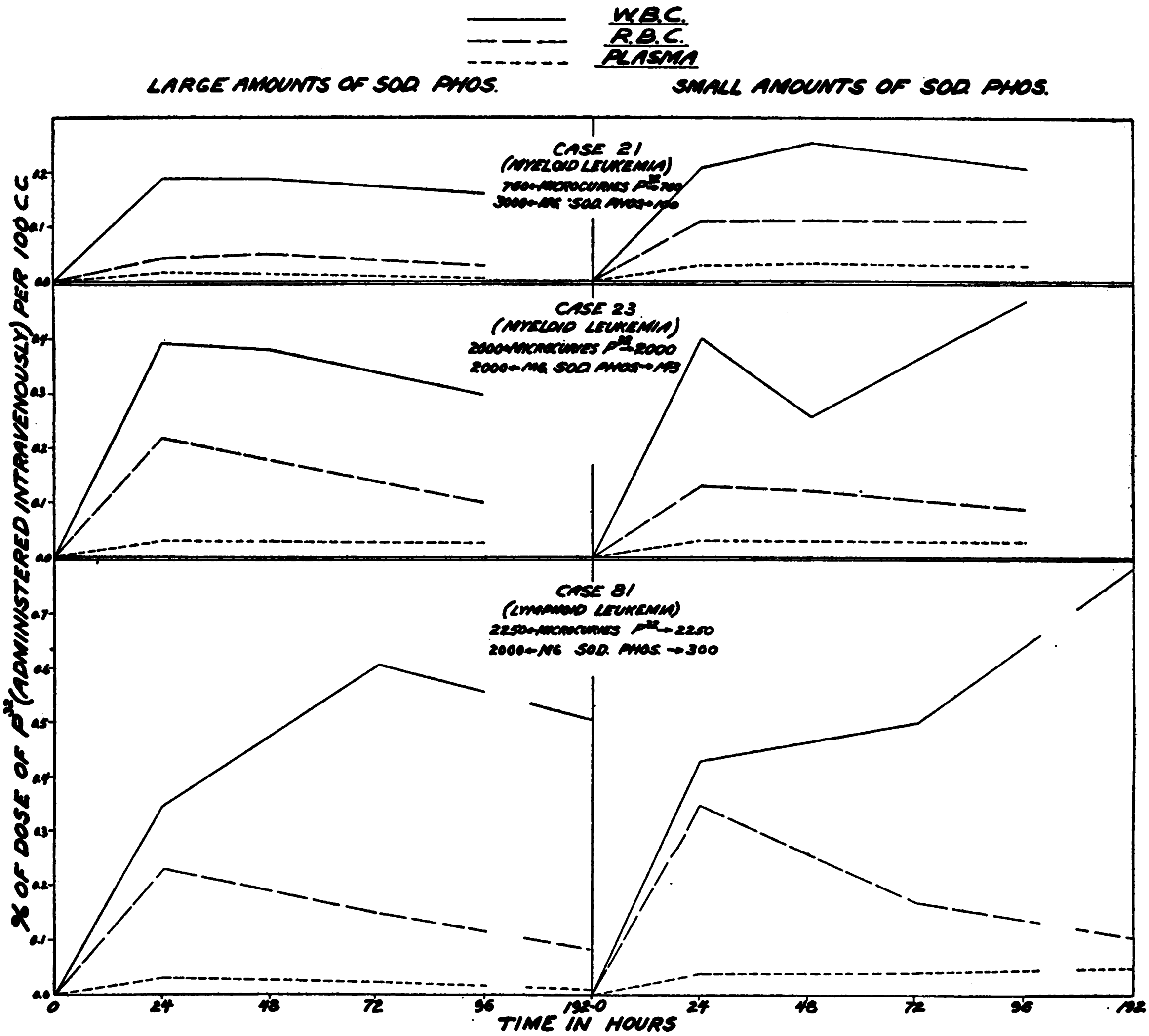

FIG. 3. VARIATIONS IN RETENTION, IN BLOOD FRACTIONS, OF P^{32} ADMINISTERED INTRAVENOUSLY WHEN ACCOMPANIED BY LARGE AND SMALL AMOUNTS OF SODIUM PHOSPHATE

TABLE II

Retention of radio-phosphorus in marrow and peripheral blood of patients with leukemia

Case number	Type of leukemia	Millicuries of P^{32} administered	Route of administration	Hours after administration		Red blood cells	*Nucleated cells* in marrow. *White blood cells* in peripheral blood	Plasma
						µc. per cc.	*µc. per cc.*	*µc. per cc.*
91	Lymphoid	2.16	Intravenous	4	Marrow:	0.073	0.079	0.020
					Peripheral blood:	0.063	0.064	0.021
67	Lymphoid	5.0	Oral	8	Marrow:	0.056	0.068	0.020
					Peripheral blood:	0.055	0.064	0.010
28	Myeloid	1.95	Intravenous	48	Marrow:	0.0260	0.0925	
					Peripheral blood:	0.0170	0.034	

TABLE III

Retention of radio-phosphorus in nuclei and cytoplasm of leukemic white blood cells

Case number	Type of leukemia	Millicuries of P^{32} administered	Route of administration	Days after administration	Nuclei	Cytoplasm	Nuclei	Cytoplasm
					volumes per cc.		*μc. per cc.*	
61	Lymphoid	2.0	Intravenous	1	0.50	0.50	0.0970	0.121
				4	0.50	0.50	0.0706	0.0763
77	Lymphoid	2.6	Intravenous	3	0.44	0.56	0.0708	0.0670
21	Myeloid	0.7	Intravenous	2	0.22	0.78	0.0258	0.0087
				5	0.20	0.80	0.0297	0.0078
21A	Myeloid	0.7	Intravenous	8	0.32	0.68	0.0244	0.0081
22	Myeloid	3.9	Oral	17	0.17	0.83	0.0436	0.0127

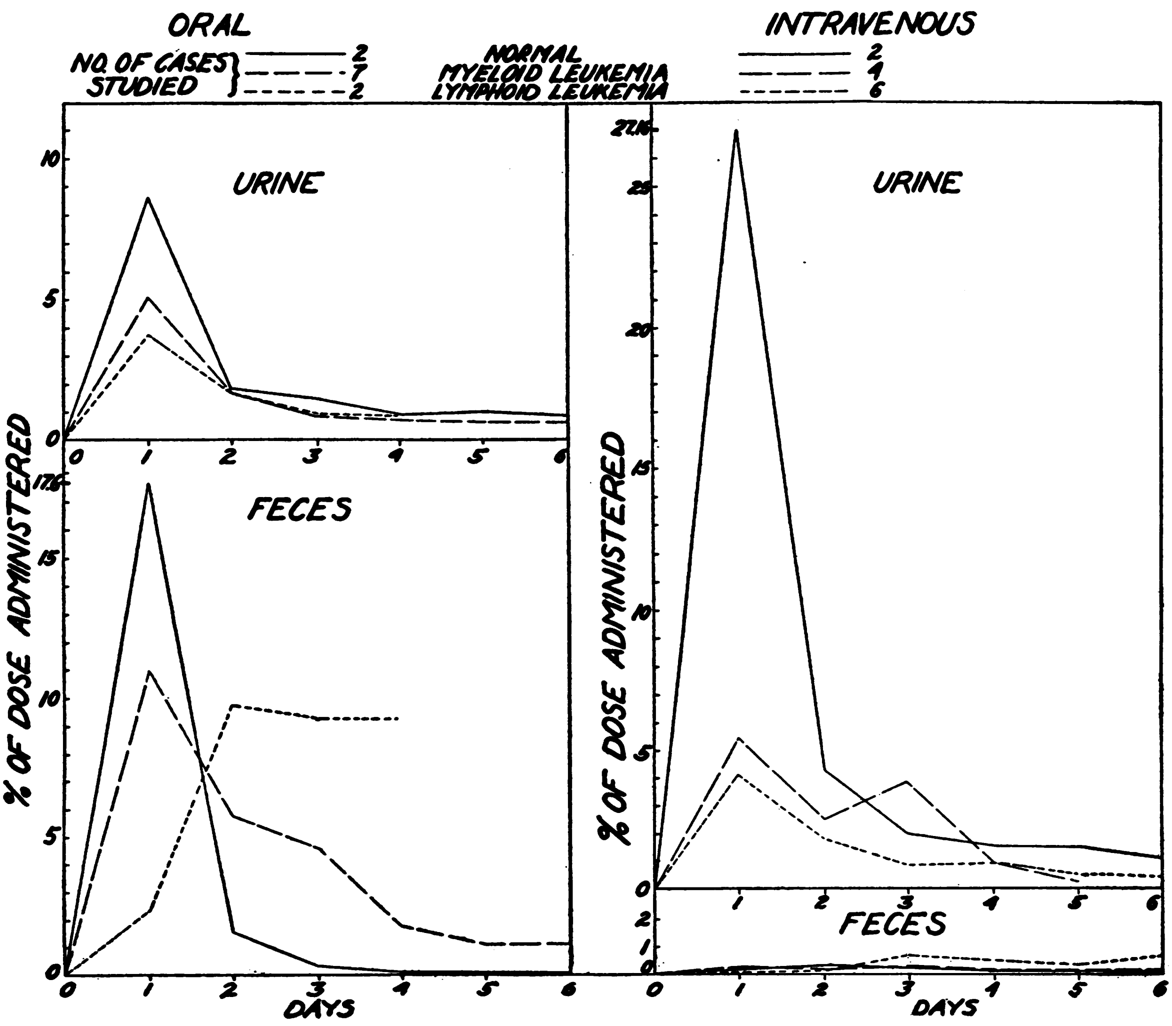

FIG. 4. AVERAGE EXCRETION OF P^{32} IN URINE AND FECES

TABLE IV

Retention of radio-phosphorus in the phospholipid, acid-soluble and nucleoprotein fractions of leukemic cells

Case number	Type of leukemia	Millicuries of P^{32} administered	Route of administration	Hours after administration	Fractionation of white blood cells: Phospholipids	Acid-soluble	Nucleoprotein
						μc. per cc.	
67	Lymphoid	5	Oral	12	0.0048	0.0465	0.0081
				24	0.0099	0.0435	0.0111
				48	0.0162	0.0417	0.0147
				96	0.0228	0.0187	0.0393
61	Lymphoid	2	Intravenous	96	0.0068	0.0079	0.0248
28	Myeloid	1.95	Intravenous	4	0.0018	0.0195	0.0027
				24	0.0039	0.0190	0.0058
				48	0.0037	0.0160	0.0077
				96	0.0057	0.0117	0.0105
14	Myeloid	0.87	Intravenous	12	0.0055	0.0064	0.0036
				120	0.0018	0.0016	0.0018
21	Myeloid	0.76	Intravenous	48	0.0031	0.0042	0.0065
				120	0.0036	0.0035	0.0059

TABLE V

*Average per cent of dose of P^{32} administered orally and intravenously (see below *) which was excreted in the urine and feces of normal individuals and patients with leukemia*

Days	1	2	3	4	5	6	Number of cases studied*		Number of cases studied*	1	2	3	4	5	6
								Urine							
	8.66	1.83	1.50	0.93	1.07	0.83	2	Normal	2	27.16	4.26	1.99	1.58	1.50	1.11
	5.11	1.71	0.909	0.707	0.634	0.634	7	Myeloid	4	5.50	2.55	3.88	0.97	0.23	
	3.86	1.63	0.98	0.87			2	Lymphoid	6	4.19	1.81	0.86	0.96	0.56	0.4
								Feces							
	17.76	1.55	0.30	0.06		0.03		Normal		0.089	0.35	0.24	0.16	0.02	0.03
	11.05	5.79	4.64	1.746	1.08	1.09		Myeloid		0.25	0.16	0.27	0.14		0.03
	2.33	9.75	9.3	9.28				Lymphoid		0.022	0.177	0.638	0.484	0.35	0.68

* *Administrations*

Oral: Mgm. of Na_2PO_4	Microcuries of P^{32}	Average	Cases		Intravenous: Cases	Microcuries of P^{32}	Average	Mgm. of Na_2PO_4
600	1500		1	*Normal*	1	1500		600
600	1500	1500	2		2	1500	1500	600
930	3850		1	*Myeloid*	1	870		150
1000	4000		2		2	1140		150
3000	4700		3		3	1260		150
3000	4700		4		4	1950	1305	300
	4700		5					
2800	5960		6					
20	12600	5787	7					
750	4000		1	*Lymphoid*	1	540		150
142	4270	4135	2		2	1360		150
					3	1900		150
					4	1998		180
					5	2160		180
					6	5000	2159	450

centration of radio-phosphorus in the nucleoprotein fraction of leukemic cells of mice increased, while that of the acid-soluble fraction decreased during a 4-day period, that large quantities of non-radioactive phosphorus, when accompanying radio-phosphorus on administration, tend to reduce the amount of P^{32} retained in the bodies of mice. Similar findings are apparent in humans, as reported in this paper.

The following features were also noted: (*a*) more radio-phosphorus is retained by patients with leukemia when it is administered intravenously than when given orally; (*b*) marrow retains radio-phosphorus in higher concentrations than blood per unit volume and at the same time period; and (*c*) relatively greater concentrations of P^{32} occur in the nuclei than in the cytoplasm of myeloid leukemic cells, while no differences in retention of P^{32} were noted in the nuclei and cytoplasm of the lymphoid cells studied. From the findings presented above phosphorus apparently passes from the acid-soluble substances of leukemic white blood cells (presumably through enzymatic carrier systems) to substances of nucleoprotein and phospholipid character. The practical point to be emphasized is, that if high concentrations of radio-phosphorus are to be obtained in circulating white blood cells, P^{32} should be introduced intravenously and it should be accompanied by the smallest amount of non-radioactive phosphorus possible. Reducing the phosphorus intake in the diet should also be considered.

SUMMARY

1. The variations in the retention of administered radio-phosphorus in blood of normal individuals and leukemic patients due to routes of administration and to amounts of accompanying non-radioactive phosphorus are presented.

2. The amounts of radio-phosphorus excreted in the urine and feces, after its administration both orally and intravenously, are given.

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