P_{32} UPTAKE BY NUCLEI

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As early as 1856, Francis Gurney Smith described and illustrated free nuclei obtained from skin and tumor tissue by the action of acetic acid (1), but curiously enough no use was made of this observation in subsequent biochemical studies of nuclei. From pus cells treated with artificial gastric juice, Miescher (2) isolated "nucleins," substances presumably characteristic of nuclei. Ploz (3) later showed that the insoluble residues of hemolyzed avian red blood cells gave characteristic "nuclein" reactions, and in 1904 Ackermann (4) determined the phosphorus and nitrogen content of nuclei obtained by this method. Eighty years after Smith's description of free nuclei, Crossmon (5) showed that small quantities of nuclei, sufficient for microscopic examination, could be isolated by teasing cardiac muscle in 5 per cent citric acid. Stoneburg (6) studied the lipid content of nuclei isolated from muscle and tumors by the combined action of citric acid and pepsin. He found it impossible to isolate nuclei from liver by this technique. It has been found possible to isolate nuclei from the liver and tumors of mice and rats by the action of 5 per cent citric acid alone. Studies of the rates at which P_{32} is bound by nuclei and cytoplasm, using this technique for isolating nuclei are described in the following experiments.

Materials and Methods

Mice (20–25 gm.) of the Strong A strain were used as hosts for the Lawrence-Gardner lymphoma (7), and mice of the Swiss strain for sarcoma 180. Inbred rats of the Slonaker strain were used for the carcinoma 256. Injections of P_{32} were timed so that the mouse tumors were 14–15 days old when removed. The rat tumors were 3–4 weeks old when used. The mice were given 0.1 cc. of isotonic Na_2HPO_4 containing 5–11 microcuries of P_{32} by intravenous injection. In the experiments with the mice 10 animals were used in each run.

Animals were anesthetized with nembutal and the livers perfused with saline through the hepatic vein until cleared of blood. Livers and tumors were then rinsed in ice cold saline, transferred to 5 volumes of cold 5 per cent citric acid in which the tissues were cut to small pieces with scissors, and allowed to stand for 30 minutes at

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2–5°C. They were then ground with mortar and pestle and passed through 2 layers of cheese-cloth. The suspension so obtained was centrifuged at low speed for 2 minutes to remove tissue fragments, and then at 2400 R.P.M. for 10 minutes. The nuclei were found in the bottom layer, the cytoplasmic fragments in the supernatant and upper solid layers. Nuclei with cytoplasmic fragments still attached were in the zone between the clean nuclei and the cytoplasm. In the liver, the latter was easily identified macroscopically since it had a brown color. The supernatant and upper solid layers were removed, the nuclei resuspended in 5 per cent citric acid and again centrifuged at 2,400 R.P.M. for 1–2 minutes. This process was repeated, usually 4–6 times, until very few cytoplasmic fragments were found in the upper solid layer, and the supernatant was no longer cloudy. The mass of washed nuclei so obtained was white. The same procedure was used with tumor tissue. In this case the cytoplasm had a grayish translucent appearance and since it was not as easily identified macroscopically each separation was examined with the microscope. The suspension of nuclei was then brought to a fixed volume with 5 per cent citric acid, 1 or 2 cc. pipetted into an ashing capsule for determination of radioactivity, and another fraction set aside for extraction. In each run a small portion of the suspension was used to determine the number of nuclei and the number and size of any non-nuclear fragments per cubic centimeter of suspension by hemocytometer count and measurement with an ocular micrometer. The volume of contaminating cytoplasmic fragments was found to be less than 0.5 per cent of the nuclear volume in all experiments. Another portion was centrifuged for exactly 30 minutes at 2400 R.P.M. in Bauer and Shenck (8) tubes to determine the relative volume of solid and fluid. If the centrifugation was prolonged beyond 30 minutes the nuclei became angular (usually hexagonal) in outline.

Small portions of tissues of all animals from which nuclei were to be isolated were put in weighing bottles and weighed as quickly as possible after removal. They were then transferred to ashing capsules and dried. The suspension of nuclei and an aliquot of the injected solution were also evaporated to dryness in ashing capsules. All activity measurements were made with an ionization chamber using a General Electric Co. FP-54 electrometer tube in a DuBridge circuit (9) and checked against a uranium standard.

To determine whether the liberation of nuclei was due to some specific action of the citrate ion or to the hydrogen ion concentration the following experiments were performed. Tissues were treated as previously described, substituting 5 per cent acetic acid and 5 per cent boric acid in place of citric. Nuclei could not be separated after treatment with boric acid but after treatment with acetic acid the nuclei could be isolated by centrifugation as readily as after citric. Liver pulped in 0.9 per cent NaCl showed on microscopic examination that the nuclei were completely free from the cytoplasm but were homogeneous rather than granular in appearance; *i.e.*, they apparently were not coagulated. When treated the same way in isotonic MacIlvaine buffer at pH 7.0, the nuclei did not separate from the cytoplasm. When pulped in buffer at pH 5.0, the nuclei were free with occasional small tabs of adherent cytoplasm, and had a granular appearance. At pH 6.0 the nuclei were free but did not appear granular until the tissue was immersed in the buffer for 1½ hours. Evidently the separation of the nucleus from the cytoplasm and its coagulation are due to an increase in hydrogen ion concentration and not to the action of a particular anion. In the case

of tissue ground in 0.9 per cent NaCl there is apparently sufficient acid liberated (the acid of injury of the microdissectionists) to free the nucleus from the cytoplasm, but not enough to produce coagulation.

RESULTS

Chemical Exchange vs. Metabolism

To determine whether the P_{32} was taken up by the nuclei by simple chemical exchange, the following experiments were performed: Nuclei were isolated from the livers of 10 mice and suspended in 3 cc. of a solution containing 15 mg. Na_2HPO_4/cc. with a total activity of 157.2 μc. After shaking for 1 hour the nuclei were washed 4 times with 5 per cent citric acid. With each washing, the activities recovered were 145.2, 8.60, 0.900, and 0.215 μc respectively. The nuclei contained 0.429 μc and since the volume of nuclei used was 0.695 cc., the activity per cubic centimeter of nuclei was 0.617 μc or 0.39 per cent of the activity originally contained in the solution. In a similar experiment the original solution contained 62.8 μc in 4 cc. solution. The nuclei were washed 7 times. The washings contained 57.0, 5.41, 0.290, 0.044, 0.009, 0.006, 0.004 μc respectively. The washed nuclei had an activity of 0.248 μc/cc. or 0.395 per cent of the total added activity. In a third experiment with 87.0 μc the nuclei retained 0.20 per cent of the activity in the solution after 7 washings. Isolated nuclei, therefore, will not bind more than 0.2-0.4 per cent of the phosphorus of an isotonic solution of Na_2HPO_4 in which they are immersed. Since clumping of nuclei occurs when the phosphate solution is added to the nuclei suspended in citric acid, a considerable portion of the P_{32} found associated with the nuclei may simply be adsorbed. If this were the case, presenting other surfaces on which the phosphate could be adsorbed should decrease the amount apparently bound by the nuclei. This is shown to be the case in the following experiment.

The livers of 10 mice were cleared of blood by perfusion, cut into thin slices, and shaken for one-half hour at room temperature in a 10 cc. solution containing 8 cc. of 0.9 per cent NaCl and 2 cc. of 1.5 per cent Na_2HPO_4 containing 206 μc. When the nuclei were removed and washed 7 times in citric acid they were found to have an activity of 0.023 μc/cc.; *i.e.*, 0.011 per cent of the total added activity. In the *in vivo* experiments where 5–10 μc of P_{32} was injected into the circulatory system the liver nuclei were found to contain 3 per cent of the added activity or about 300 times as much as in the case of the liver slices shaken with P_{32}. It follows from these and the previous experiments that the uptake of P_{32} from the blood stream cannot be accounted for by exchange but must be attributed to the metabolic activities of the cells involved.

Rate of P_{32} Uptake

The P_{32} retained by the liver and lymphoma tissue at various times after injection is shown in Fig. 1 and Table I. In the liver the maximum P_{32} content (9.5 per cent) is observed at 1 hour after injection as early as determinations

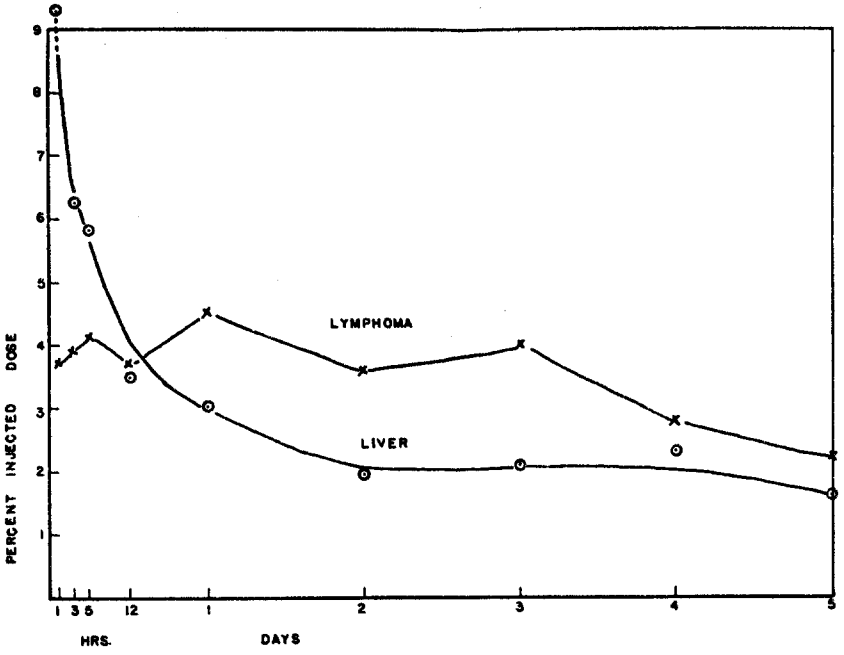

FIG. 1. P_{32} uptake by tissues

TABLE I

Lymphoma

Activity As Per Cent Injected Dose

Time after injection	Nuclei		Tissue		Nuclei/tissue	
	Liver	Tumor	Liver	Tumor	Liver	Tumor
1 hr.	2.995	1.255	9.57	3.72	0.311	0.337
3 "	1.76	2.57	6.24	3.90	0.286	0.689
5 "	1.66	4.85	5.825	4.155	0.284	1.13
12 "	1.25	6.41	3.45	3.70	0.371	1.37
1 day	1.23	6.395	3.035	4.11	0.412	1.59
2 "	0.945	7.17	1.96	4.06	0.398	1.78
3 "	0.97	7.02	2.12	3.68	0.461	1.91
4 "	1.03	6.09	2.34	2.80	0.449	2.19
5 "	0.71	5.47	1.64	2.27	0.438	2.35

The value at each time interval is the mean of 2–4 determinations with 10 mice. The activity of the nuclei is given as per cent/cm.^{3}, and that of the tissue as per cent/gm.

could be made, and is followed by a very rapid decrease to an asymptotic level at about 2 per cent. The lymphoma on the other hand shows a practically constant level at 4 per cent from 1 hour to 3 days after injection, followed by a

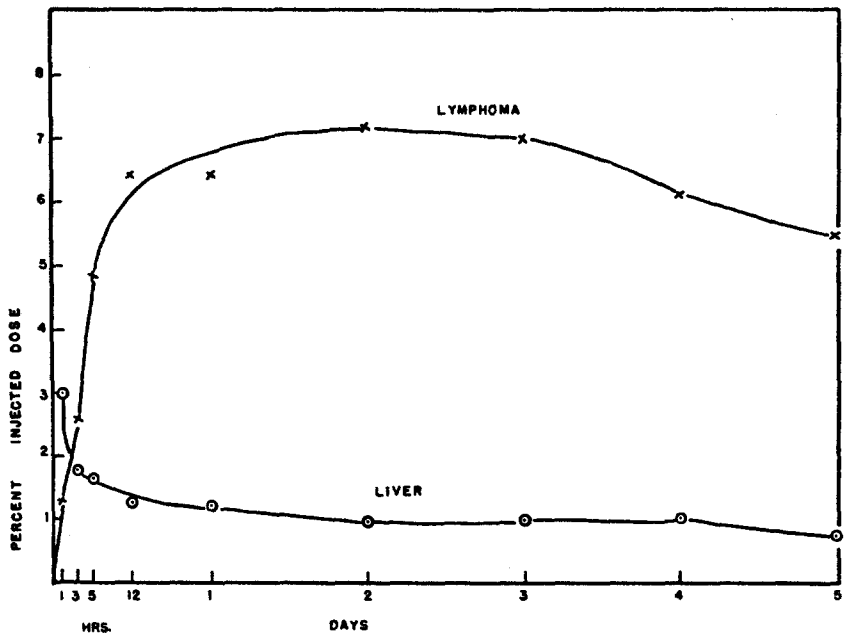

FIG. 2. P_{32} uptake by nuclei

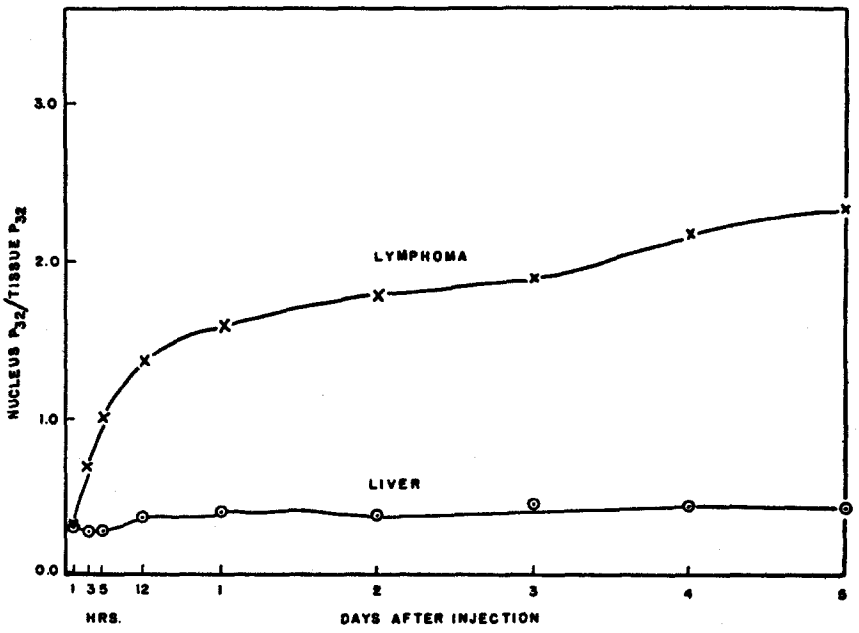

FIG. 3. Nuclear activity as a fraction of tissue activity

slow decrease. The P_{32} curve for liver nuclei (Fig. 2) parallels that of the whole tissue, with a maximum at 3 per cent 1 hour after injection, falling off rapidly to 1 per cent. The uptake by the lymphoma nuclei is strikingly different from that of the whole lymphoma tissue or of the liver nuclei. There is a very rapid rise in nuclear P_{32} concentration from 1 per cent at 1 hour after injection to 6.5 per cent at 12 hours. It continues to rise slowly till the 2nd day and then falls off gradually.

When the activity per cubic centimeter of packed nuclei as a fraction of the total activity per gram tissue (Fig. 3) is plotted as a function of time after injection, a striking difference in the behavior of tumor and liver nuclei becomes apparent. The relative concentration of P_{32} in the liver nuclei remains practically constant between 1 hour and 5 days after injection, while the concentration in the lymphoma nuclei rises rapidly from 1 to 5 hours after injection and continues to rise as late as 5 days afterward. The P_{32} concentration in the lymphoma nuclei is doubled in 2 hours and tripled in 5 hours. Evidently in the liver cell phosphorus not only enters the nucleus rapidly but also leaves it rapidly maintaining a steady state in the interchange of phosphorus between nucleus and cytoplasm. In the lymphoma cell the phosphorus enters the nucleus rapidly and there is no direct evidence from the curves of P_{32} uptake of a rapid loss of phosphorus by the nuclei.

Distribution of P_{31} and P_{32} in Nucleus and Cytoplasm

Table II shows the phosphorus content of liver and tumor tissues and the corresponding nuclei, determined by the Pregl (10) method. The number of determinations on different samples is given by the columns n.

The volume of the packed mass of nuclei in each suspension was obtained by centrifugation in Bauer and Shenck tubes as previously described and corrected to the true volume of nuclei by multiplying by the maximum packing fraction for spheres (0.74). By dividing these values by the number of nuclei per cubic centimeter of suspension the average volume of an individual nucleus was obtained. The average volume of a liver nucleus so obtained was $3.26 \pm 0.128 \times 10^{-10}$ cm.^{3} ($n = 31$) and of a lymphoma nucleus, $1.673 \pm 0.060 \times 10^{-10}$ cm.^{3} ($n = 30$). The densities of the nuclei determined by means of a pycnometer were 1.148 for the liver and 1.146 for the lymphoma nuclei. From these values the P_{31}/gm. nuclei was calculated.

The phosphorus content of lymphoma cells was calculated as follows: The diameters of 30 lymphoma cells which had been immersed in 5 per cent citric acid for half an hour were measured with an ocular micrometer. The mean diameter was $9.75 \pm 0.24 \times 10^{-4}$ cm. from which, assuming a spherical shape for the cell, a volume of 4.85×10^{-10} cm.^{3} was obtained. With no phosphorus in the cytoplasm, the P_{31}/gm. tissue would be:

$$P_{31}\text{/gm. nuclei} \times \frac{1.67}{4.85} = 2.30 \text{ mg.}$$

The tissue was found to have 2.995 mg. P/gm. and therefore the cytoplasm in 1 gram of tissue must have contained 0.695 mg. P. The P_{32} in the cytoplasm may be calculated in the same way.

The ratio $\frac{\text{Nuclear } P_{32}}{\text{Tissue } P_{32}}$ tumor reaches a value of 2.35 by the 5th day after injection of P_{32}, which is approximately the value (2.24) found for the ratio $\frac{\text{Nuclear } P_{31}}{\text{Tissue } P_{31}}$. Thus after a relatively long period of time the distribution of P_{32} in the cell becomes the same as that of P_{31} already present.

The complexity of the structure of the liver makes it more difficult to obtain an accurate measurement of the relative volume of nuclei and cytoplasm. However, a rough estimate has been obtained. When the suspension of pulped liver in 5 per cent citric acid is centrifuged and the clumps of cells are removed, the remaining nuclei are of three principal types easily distinguishable, liver, lymph, and endothelial; the latter two being more frequent in the upper layers

TABLE II

P_{31} Content in Milligrams

Liver	n	Tumor	n
Tissue, 1 gm. 2.882 ± 0.069	26	2.995 ± 0.106	19
Nuclei, 1 cc. 4.53 ± 0.248	25	7.66 ± 0.234	18
Nuclei, 1 gm. 3.95 ± 0.216		6.69 ± 0.204	

of the centrifuged mass of nuclei. Counts of suspensions of nuclei from normal mouse livers showed the relative frequencies of the three types of nuclei to be 35, 39, and 16 respectively. The mean diameter of the liver nuclei in 5 per cent citric acid was found to be $11.05 \pm 0.27 \times 10^{-4}$ cm.^{3} and in 0.9 per cent NaCl $12.05 \pm 0.33 \times 10^{-4}$ cm.^{3}. Liver cells are approximately parallelopipeds, the lengths of two sides of which were found to be $26.1 \pm 0.5 \times 10^{-4}$ cm. and $17.7 \pm 0.5 \times 10^{-4}$ cm. in citric acid and $26.6 \pm 0.7 \times 10^{-4}$ and $21.5 \pm 0.6 \times 10^{-4}$ cm. in saline. (Evidently the citric acid produces little or no change in size of either the nucleus or the whole cell.) From these measurements the volumes of the liver cell and nucleus were found to be 12.1×10^{-9} cm.^{3} and 7.26×10^{-10} cm.^{3} respectively. The endothelial nuclei are approximate prolate spheroids and have a volume of about 1.45×10^{-10} cm.^{3}. The volume of the lymph cell has already been given and the endothelial cell assumed to have approximately the same volume. It then becomes apparent from the relative frequencies and the volumes of the different types of cells, that the lymph cells represent only about 4.5 per cent and the endothelial about 2 per cent of the total volume of liver tissue. Therefore as a first approximation we may assume that practically all of the P_{32} found in the liver tissue is in the liver cells. The distribution of P_{31} in the cell and the specific activities of nucleus and

cytoplasm may be calculated in the same way as for the lymphoma. The calculation given in Table III is based on the volume of the liver cell nucleus as determined by measurement with the microscope (7.26×10^{-10} cm.^{3}), and not the mean nuclear volume of the suspensions of nuclei from the liver which

TABLE III

Activities As Per Cent of Injected Dose

	Time after injection	P_{32}/gm.			P_{32}/mg.P_{31}/gm.			
		Tissue	Nuclei	Cytoplasm	Tissue	Nuclei	Cytoplasm	Nuclei/cytoplasm
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Lymphoma	1 hr.	3.72	1.48	3.21	1.24	0.22	4.63	0.0475
	3 "	3.90	3.03	2.86	1.30	0.45	4.12	0.109
	5 "	4.16	5.72	2.19	1.39	0.86	3.15	0.273
	12 "	3.70	7.58	1.10	1.23	1.13	1.58	0.715
	1 "	4.11	7.55	1.52	1.37	1.12	2.19	0.511
	2 "	4.06	8.45	1.15	1.36	1.26	1.66	0.760
	3 "	3.68	8.29	1.83	1.23	1.24	1.20	1.03
	4 "	2.80	7.17	0.34	0.94	1.07	0.49	2.18
	5 "	2.27	6.45	0.05	0.76	0.97	0.07	13.9
Liver	1 hr.	9.57	3.53	9.36	3.32	0.90	3.52	0.256
	3 "	6.24	2.08	6.11	2.16	0.53	2.31	0.230
	5 "	5.83	1.96	5.67	2.02	0.50	2.16	0.232
	12 "	3.45	1.47	3.36	1.20	0.37	1.27	0.291
	1 day	3.04	1.45	2.94	1.05	0.37	1.11	0.334
	2 "	1.96	1.11	1.89	0.68	0.28	0.71	0.394
	3 "	2.12	1.14	2.05	0.74	0.29	0.78	0.372
	4 "	2.34	1.21	2.27	0.81	0.31	0.86	0.360
	5 "	1.61	0.84	1.56	0.56	0.21	0.59	0.356

	mg. P_{31}/gm.		
	Tissue	*Nuclei*	*Cytoplasm*
Lymphoma	2.995	6.69	0.695
Liver	2.882	3.95	2.644

were used (3.26×10^{-10} cm.^{3}). Thus with no phosphorus in the cytoplasm the P_{31}/gm. liver tissue would be:

$$P_{31}\text{/gm.} \times \frac{0.726}{12.05} = 3.95 \times 0.0604 = 0.238 \text{ mg.}$$

The cytoplasm therefore contains 2.64 mg. P_{31}/gm. tissue. If the mean volume of the different types of nuclei from the liver is used the P_{31}/gm. tissue due to nuclei is 0.108 and the cytoplasm has 2.77 mg. P_{31}/gm.

From Table III it is apparent that the concentration of P_{32} in the nuclei of the

lymphoma is already about 50 per cent of that in the cytoplasm at 1 hour after injection. 2 hours later the nuclear P_{32} content has doubled and is greater than that of the cytoplasm. By 5 days after injection practically all the lymphoma P_{32} is in the nuclei. In the liver the P_{32} content of the nuclei is about 35 per cent of the cytoplasmic P_{32} shortly after injection and is 50–60 per cent at 2–5 days after injection. In the lymphoma there is a rapid concentration of P_{32} in the nuclei accompanied by a depletion of the cytoplasmic P_{32}. The rate of decrease of cytoplasmic P_{32} is less than the rate of increase of nuclear P_{32}. This would be expected if (1) phosphorus moved out of the nucleus into the cytoplasm or if (2) phosphorus enters the cytoplasm more rapidly than it is bound by the nucleus or if both conditions obtain.

Specific Activities

Although the lymphoma nuclei show a rapid accumulation of P_{32}, the cytoplasm of the lymphoma has a greater specific per cent activity (per cent P_{32}/mg. P_{31}).[1] The lymphoma cytoplasm has a greater s.p.a. than the liver cytoplasm at all the intervals after injection except the last 2 days. If phosphorus enters the cytoplasm more rapidly than it is bound by the nucleus we would expect not only a greater initial concentration of P_{32} in the cytoplasm, but also a greater dilution of the cytoplasmic P_{32} by P_{31} in the later intervals after injection. This expectation is fulfilled since on the 5th day when the specific activity of the lymphoma nuclei is approximately 1, that of the cytoplasm is only 0.07. While in the lymphoma there is a rise in the s.p.a. of the nuclei with a corresponding decrease for the cytoplasm, in the liver both nuclei and cytoplasm decrease in specific activity after the 1st hour. Nevertheless there is a gradual increase in the relative s.p.a. of the liver nuclei by a factor of about 1.4 in 5 days (Table III, column 8) which indicates that the nuclei of the liver are synthesizing relatively non-labile phosphorus compounds even though no cell division takes place. In the lymphoma the concentration of P_{32} in the nucleus is much more striking, the relative specific activities of nucleus and cytoplasm increasing from 0.048 to 13.9 in 5 days, that is, by a factor of about 290.

P_{32} Uptake in Relation to Mitosis

To determine whether the rapid concentration of P_{32} in the nuclei is peculiar to the lymphoma, similar experiments were performed with mice of the Swiss strain carrying bilateral sarcoma 180 tumors. The results given in Table IV show that P_{32} is rapidly accumulated by the sarcoma nuclei in much the same way as was observed for lymphoma nuclei.

[1] Since specific activity has been defined as microcuries of activity per milligram we will use the term specific per cent activity (s.p.a.) as the activity in per cent of the injected dose per mg. P_{31}.

In order to determine whether the rapid phosphorus accumulation by nuclei is peculiar to tumor cells or is characteristic of mitotic activity of cells, the following experiment was performed. The median and left lateral lobes of the livers of three 150 gm. rats were removed and P_{32} as isotonic Na_2HPO_4 injected by way of the femoral vein 36 hours later. 3 normal rats and 3 rats carrying bilateral carcinoma 256 implants were injected at the same time. 2 days later the livers were cleared of blood by perfusion with saline and the nuclei isolated from the livers and tumors. For the normal liver the ratio of activity per cubic centimeter of nuclei to activity per gram tissue was 0.345, while for regenerating liver and tumor the ratios were 1.02 and 1.08 respectively. When the P_{32} was injected 4 days after partial hepatectomy and the nuclei removed 3 days later the ratios for normal and for hepatectomized animals were 0.28 and 0.32. At this time very few nuclei were found in mitosis, while at 36 hours after partial

TABLE IV

Sarcoma 180

Activity As Per Cent of Injected Dose

Time after injection	Activity/gm. tissue		Activity/cc. nuclei		Activity nuclei/activity tissue	
	Liver	Tumor	Liver	Tumor	Liver	Tumor
hrs.						
3	7.47	1.93	2.84	1.01	0.381	0.527
5	6.24	3.12	5.32	4.20	0.856	1.35
12	6.16	4.94	3.98	5.97	0.645	1.21

hepatectomy 3.7 per cent were in anaphase or metaphase and a much larger per cent in prophase. The rapid accumulation of P_{32} by nuclei may therefore be attributed to mitotic activity and not to metabolism peculiar to certain types of cells.

Distribution of P_{32} in Nuclei

The nuclei from tissues of 10 mice were extracted with 10 cc. of 95 per cent alcohol at 55°C. for 1 hour. They were then placed in 10 cc. of a mixture of 2 parts alcohol and 1 of ether and left at 55°C. overnight and then extracted 3 times with 10 cc. of alcohol-ether allowing 1 hour for each extraction. The extract was then evaporated almost to dryness, taken up in water, and shaken 5 times with petroleum ether. The alcohol extract was also evaporated until the alcohol was removed and extracted with petroleum ether. The nuclear residue was then extracted 3 times with 10 cc. 5 per cent trichloracetic acid at 5°C.

Table V gives the distribution of P_{32} in the different fractions as per cent of the activity in the untreated nuclei. There is considerable variation in the

P_{32} found in the three extracts of the liver nuclei. Only about one-half cc. of packed nuclei were used in each experiment, an amount apparently too small for the limits of error of the procedures used. However, the per cent activity in the residues is consistently about 60-70 per cent of the total nuclear activity.

TABLE V

Distribution of P_{32} in Nuclei

P_{32} as Per Cent of Nuclear P_{32}

	Time after injection	Water soluble		Lipid	Acid soluble	Residue	Total
Liver	1 hr.		21.4*		6.0	67.2	94.6
	1 day	15.2		6.1	3.3	68.3	92.9
	2 "		20.8*		13.9	61.7	96.4
	3 "	26.1		4.7	0.8	66.0	97.6
	4 "					68.0	
	5 "	2.7		8.6	1.9	66.9	80.1
	7 "	12.6		7.7	7.7	71.6	99.6
Lymphoma	1 day	2.5		0.6	0.6	94.8	98.5
	2 "	1.5		2.3	0.7	95.4	99.9†
	3 "	3.6		0.8	2.5	94.5	101.4
	4 "	2.0		1.4	5.1	91.6	100.1
	5 "	2.1		0.3	3.4	88.1	93.9
	7 "‡	8.4		1.4	26.4	70.4	106.6

* In these experiments the water-soluble and lipid fractions were assayed together.

† P_{32} in each fraction is given as per cent of the sum of all fractions.

‡ High P_{32} in water- and acid-soluble fractions is paralleled by comparably high P_{31} values for the same nuclei. P_{31} and P_{32} determinations on liver nuclei of the same animals show no increase in these fractions. The rise must therefore be associated with mitotic activity. Ionization by beta particles from P_{32} produces chromosome fragments which are not included in the daughter nuclei after anaphase but form micronuclei which at the onset of the next cell division become disorganized, stain diffusely, and eventually disappear. A tumor with 8μc/gm. will have 30 per cent of its anaphases with chromosome fragments at 24 hrs. after the P_{32} is injected and with 17μc will have 50 per cent such abnormal anaphases. (11) If the dissolved material (nucleic acid?) becomes incorporated in the new nuclei or adsorbed on their surfaces the rise in the acid- and water-soluble fractions may be accounted for. See Table VI.

Evidently even in the liver nuclei which do not undergo mitosis the greater portion of the P_{32} taken up is in the nucleoprotein fraction. Furthermore as early as 1 hour after the administration of the P_{32} the per cent in the nucleoprotein is as great as at several days after injection so that conversion of cytoplasmic phosphorus to nucleoprotein must be quite rapid.

The residues of the tumor nuclei contain 90–95 per cent of the total nuclear activity at all times until the 7th day after injection of the P_{32}. Since the tumor

is growing rapidly and therefore synthesizing new nuclei, it is not surprising to find practically all of the P_{32} in the nucleoprotein fraction. But in a so called resting nucleus, as in the liver, the phosphorus is also being replaced and since it has been demonstrated that the P_{32} is not held in the liver nucleus as the phosphate ion, it follows that nucleic acid or portions of it are continually being broken down and reformed from phosphorus compounds furnished by the cytoplasm.

TABLE VI

Distribution of P_{31} in Nuclei

Mg./Cc. Packed Nuclei

Nuclei		Water soluble	Acid soluble	Lipid	Residue	Sum of fractions
Liver	3.85	0.27	0.30	0.32	2.26	3.15
	4.50	0.25	0.13	0.20	3.75	4.33
m		0.26	0.22	0.26	3.01	3.74
Lymphoma	5.68	0.20	Trace	0.11	5.25	5.56
	7.06	0.18	0.28	0.15	5.25	5.86
m		0.19	0.25*	0.13	5.25	5.71
7 days after injection of P_{32}						
Liver	3.59	0.19	0.40	0.28	2.82	3.69
Lymphoma	6.26	0.96	1.30	0.13	4.15	6.54

* Analysis of a third batch of nuclei showed 0.23 mg. P/cc. packed nuclei. The mean is therefore taken at 0.25. The deductions remain unchanged whether 0.14 or 0.25 is used.

TABLE VII

	Fractions as per cent of Total						S. P. A.		
	P_{31}			P_{32}			P_{32}/P_{31}		
	H_2O and acid	Lipid	Residue	H_2O and acid	Lipid	Residue	H_2O and acid	Lipid	Residue
Liver	12.8	7.0	80.5	18.4	7.5	71.2	1.44	1.07	0.89
Lymphoma	7.7	2.3	92.0	4.9	1.1	94.0	0.64	0.48	1.02

Table VI gives the distribution of phosphorus in the nuclear fractions as determined by the Pregl method. Approximately 0.2–0.5 gm. nuclei were used in each of the lots extracted.

In Table VII the P_{31} content of the nuclear fractions is given as per cent of the sum of the fractions. The P_{32} is given as the mean per cent of the sum of the P_{32} fractions from 1 hour to 5 days after administration of the phosphorus. This procedure is not justifiable in the case of the acid-soluble fraction in the lymphoma nuclei which shows a very definite increase with time.

The quantity of nuclei used was too small for an accurate determination of the phosphorus distribution, but is adequate for a rough preliminary estimate. In the liver nuclei the acid-soluble fraction has the greatest specific activity and the nucleoprotein the least. In the lymphoma, as might be expected, the specific activity of the nucleoprotein is about twice as great as that of the other two fractions. If the specific activity of the trichloracetic acid-soluble fraction is calculated separately from the water-soluble fraction we find that it rises from 0.14 on the 1st day after P_{32} injection to 1.17 on the 4th day and 5.9 on the 7th day. The water-soluble fraction has a specific activity of 0.57 and shows no comparable increase with time until the 7th day.

Rate of P Replacement in Nuclei

The time required by the nuclei of the lymphoma to bind a quantity of phosphorus equal to that which they initially contained is the time required for the synthesis of an equal quantity of nuclei and should correspond to the time it takes the tumor to double its size. The time for complete replacement of the nuclear P_{31} may be calculated. Let:-

$U_{P_{32}}$ = P_{32} uptake by nuclei as per cent/gm. of the injected dose.

$C_{P_{32}}$ = P_{32} uptake by cytoplasm as per cent/gm. of the injected dose.

$C_{P_{31}}$ = Total P_{31} in the cytoplasm in milligrams.

$U_{P_{31}}$ = P_{31} uptake by nuclei in milligrams.

Using the value for $C_{P_{32}}/C_{P_{31}}$ observed at 1 hour after P_{32} injection, if all the cytoplasmic P is available to the nucleus:

$$U_{P_{31}} = C_{P_{31}} \times \frac{U_{P_{32}}}{C_{P_{32}}} = \frac{C_{P_{31}}}{C_{P_{32}}} \times U_{P_{32}} = 0.24 \text{ mg. } P_{31}/\text{hr.}$$

Since the nucleus contains 6.7 mg. P/gm. the time for complete replacement is 27 hours. The growth curve of the lymphoma (12) shows that a 14 day old tumor (0.6 gm.) will double its size in about 24 hours. The agreement is surprisingly good for an approximation but may be accidental.

Rate of Synthesis of Nucleotides

From the size of the lymphoma nucleus 1.67×10^{-10} cm.^{3} and the 7.66 mg. P/cc. nuclei, a single tumor nucleus is estimated as having 12.8×10^{-10} mg. A lymphoma nucleus will thus contain 129×10^{-10} mg. or 62.5×10^8 molecules of tetranucleotide, and if it takes 24 hours for a new nucleus to be formed, 7×10^4 molecules of tetranucleotide must be synthesized per second.

Effect of X-Ray on P_{32} Uptake

The decrease in P_{32} content of liver nuclei shortly after administration of the P_{32} suggests a movement of phosphorus out of the nucleus as well as into it. A

similar process may occur in the lymphoma but be obscured by the rapid accumulation of P_{32} due to the synthesis of new nuclear materials. If the outward movement could be interrupted an increase in the P_{32} concentration in the nuclei is expected. Mice carrying 13 day old lymphomas were given 200 roentgens, P_{32} injected intravenously one-half hour later, and tissues and nuclei isolated in the usual way at various intervals afterward. The results are given in Table VIII. At 2 hours after the x-ray treatment the P_{32} concentration in the lymphoma nuclei is 1.6 times that of the controls and then gradually approaches the control value. When the x-ray dose is increased to 260 r. the ratio of P_{32} concentration in the lymphoma nuclei is increased from 1.7 to 2.1 at 3 hours.

TABLE VIII

P_{32} Uptake Following X-Ray

207 r.—13 Day Tumors

Time after irradiation	Tissue per cent injected dose				Nuclei per cent injected dose				Nuclei/tissue			
	Liver	Control	Tumor	Control	Liver	Control	Tumor	Control	Liver	Control	Tumor	Control
hrs.												
2	6.16	7.2	3.54	3.9	1.52	2.0	2.56	1.60	0.246	0.30	0.72	0.46
3	5.75	6.5	3.54	3.9	1.66	1.75	3.66	2.50	0.288	0.28	1.03	0.60
6	5.65	5.8	3.48	3.9	1.71	1.65	5.38	4.9	0.274	0.30	1.558	1.02
12	4.05	4.0	3.82	3.9	1.54	1.40	7.45	6.1	0.381	0.36	1.95	1.36
24	2.92	3.0	3.30	3.9	0.815	1.20	7.19	6.8	0.280	0.40	2.18	1.60
					260 r.—15 day tumors							
3	5.15	6.5	3.79	3.9	2.12	1.75	5.17	2.50	0.412	0.28	1.36	0.60

After irradiation with x-rays a considerable proportion of the nuclei becomes pycnotic. To determine whether the increased accumulation of P_{32} in the nuclei might be a consequence of the pycnotic condition, mice were given 200 r. and the tumors removed and examined microscopically at 1/2, 1, 2, 3, 6, and 8 hours after irradiation. There is a small percentage of pycnotic nuclei in the non-irradiated control tumors. After irradiation there is no noticeable increase in pycnosis the first 2 hours after irradiation, at 3 hours there is a slight increase, which becomes very marked by the 6th and 8th hours. Since the maximum increase in P_{32} concentration is observed at 2 hours after irradiation this effect cannot be a consequence of pycnosis.

Table IX shows that the P_{32} concentration of the irradiated tumor tissue remains consistently about the same or slightly less than that of the control tumor. In the irradiated nuclei the concentration is about 60 per cent greater than the control at 2 hours after irradiation, then decreases to a value 10–20 per cent greater than the control at 5–24 hours. The irradiated cytoplasm on

the other hand has 20 per cent less than the control at 2 hours and decreases to 40–60 per cent less than the control at 5–24 hours.

Increase in permeability cannot be invoked as an explanation for the increased nuclear P_{32} content since the total P_{32} content of the tissue is not increased. The altered distribution of the P_{32} may then be attributed either to increased uptake by the nucleus or to decrease in movement from the nucleus to the cytoplasm. Since the irradiated liver nuclei show less than the controls the rate of movement of P_{32} out of these nuclei cannot be inhibited unless we assume a simultaneous decrease in rate of movement into the nuclei.

Whatever may be the immediate cause of the altered distribution of P_{32} in the cell it is clear that the x-ray effect is non-existent or small in the liver but pronounced in the tumor. As previously pointed out the difference in normal P_{32} uptake by nuclei of liver and lymphoma is to be attributed to active

TABLE IX

Distribution of P_{32} in Lymphoma following X-Ray
Per Cent P_{32}/Gm.

Time after irradiation	Tissue			Nuclei			Cytoplasm		
	Control	X-ray	X/C	Control	X-ray	X/C	Control	X-ray	X/C
hrs.									
2	3.9	3.5	0.90	1.9	3.0	1.6	3.1	2.5	0.8
3	3.9	3.5	0.90	3.0	4.3	1.5	2.9	2.1	0.7
6	3.9	3.5	0.90	5.8	6.3	1.1	2.2	1.3	0.6
12	3.9	3.8	0.98	7.2	8.8	1.2	1.1	0.8	0.7
24	3.9	3.3	0.85	8.0	8.5	1.1	1.1	0.5	0.4

mitosis in the latter. The concentration of phosphorus in the lymphoma nuclei after irradiation may therefore be associated in some way with mitotic activity. X-rays will inhibit the onset of mitosis, arresting the nuclei in the resting stage. Sometime later than 3 hours after irradiation metaphase and anaphase nuclei begin to reappear. The results therefore suggest a correlation between the increased concentration of phosphorus in the nuclei and the inhibition of mitosis.

DISCUSSION

Hahn and Hevesy (13) studied the rate of turnover of nucleic acid in rabbit liver with the aid of P_{32}. They found that although other organic compounds of the liver were completely renewed in a few days only 1/3 of the nucleic acid phosphorus is replaced after as much as 50 days. They also suggest that the low specific activity of the nucleic acid may be due to a possible slow penetration of inorganic phosphorus into the nucleus. This is not supported by the results of the experiments reported here, which show that the nuclei

contain 4.1 per cent/gm. of the injected P_{32} at 1 hour after injection and 65 per cent of this is already converted into nucleoprotein. The rate of P_{32} uptake by the liver nuclei is greater than that of the lymphoma. In the latter there is complete P_{31} replacement in about 1 day. It follows that in the liver nuclei there is complete replacement in 1 day or less. In these experiments it has been shown that there is little or no exchange between inorganic phosphorus and the nuclei. Hahn and Hevesy have shown that there is no *in vitro* exchange between thymus nucleic acid and inorganic phosphorus. From the results of both experiments we may conclude that phosphorus enters the liver nucleus by replacement of nucleic acid or portions of it. An explanation of the apparent discrepancy in the specific activities of nucleic acid found in the two experiments may lie in the fact that the liver nuclei not only pick up phosphorus rapidly but give it up rapidly as well and since Hahn and Hevesy's conclusion was based on calculations of the nucleic acid P_{32} found in the liver at 24 hours after it was administered, the maximum rate was not observed.

Tuttle, Erf, and Lawrence studied the phosphorus metabolism of normal and leukemic tissues with the aid of P_{32}. They found a rapid conversion of the inorganic phosphorus to nucleoprotein by the lymphoma and were of the opinion that this was too rapid to be accounted for by growth alone (14). In these experiments it has been demonstrated that there is good agreement between the rate of P_{32} uptake by nuclei and the rate of growth of the tumor. Furthermore, when a tissue such as the liver is stimulated to a high rate of mitotic activity there is a corresponding rise in the rate of P_{32} incorporation into nuclei. However, it is also true that a tissue (liver) showing no mitosis will also convert inorganic P_{32} to nucleoprotein and also, a rapidly growing tissue (lymphoma) has cytoplasm with a high specific activity. Apparently synthetic processes and glycolytic processes in the cell are interrelated and cannot be treated as though independent. Such synthesis is evident in the replacement of nucleoprotein even in nuclei which do not undergo mitosis.

SUMMARY

1. A method for isolating nuclei in quantity from mammalian tissues is described.

2. The rate of uptake of radioactive phosphorus by nuclei is found to be quite rapid. The phosphorus was shown not to be taken up by exchange.

3. Nuclei of tumors accumulate more radioactive phosphorus than normal liver nuclei. This was shown to be due to mitotic activity and not a form of metabolism peculiar to tumor cells.

4. The specific activities of nuclei and cytoplasm are compared.

5. 60 to 70 per cent of the nuclear radioactive phosphorus is present as nucleoprotein from 1 hour to 5 days after it is administered. In the lymphoma

nuclei 90–95 per cent of the phosphorus is in the nucleoprotein fraction from 1-5 days after it is administered.

6. The specific activities of the nucleoprotein, lipid, and acid-soluble fractions of liver and tumor nuclei are compared.

7. From the rate of P_{32} uptake by nuclei it is calculated that a new lymphoma nucleus is synthesized on the average once every 27 hours. This is in agreement with the observed rate of growth of the tumor.

8. In the lymphoma nucleus it is calculated that 7×10^4 molecules of tetranucleotide are synthesized per second.

9. Irradiation with 200 r. x-rays alters the distribution of P_{32} in the lymphoma cell, markedly increasing the concentration in the nucleus shortly after irradiation. The P_{32} concentration in the cytoplasm decreases with time after irradiation. It is suggested that the altered distribution is correlated with the inhibition of mitosis produced by the x-rays.

10. Continual synthesis of nucleoprotein takes place even in nuclei of cells which do not undergo mitosis.

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