THE INCORPORATION OF ^{32}P INTO THE NUCLEIC ACIDS OF LYMPHATIC CELLS *IN VITRO*. EFFECT OF ADRENAL CORTICAL HORMONES (COMPOUND F) *

by

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It is well established that adrenal cortical hormones—compound F (17-hydroxycorticosterone) in particular—produce involution of the lymphatic tissue accompanied by early degeneration of the lymphatic cells[1]. On the assumption that this hormonal effect may be due to inhibition of protein synthesis, experiments were performed whereby the rate of incorporation of ^{14}C-labelled amino acids into the proteins of lymphatic cell suspensions was measured in the presence of compound F and of corticosterone (compound E). There was a definite inhibition under conditions where only a slight inhibition on respiration was observed[2].

It is maintained by BRACHET[3] and by CASPERSSON[4] that protein synthesis and ribonucleic acid content in the cells are closely related, there being an increase of this nucleoprotein when there is increased protein synthesis. It was important, therefore, to study the effect of corticoid hormones on the synthesis of nucleoproteins by suspensions of lymphatic cells. SKIPPER *et al.*[5] found that cortisone inhibits the incorporation of labelled formate by both desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the viscera of mice; KASS AND KENDRICK[6] reported that the injection of cortisone into rabbits produced a decrease of RNA in the poplitheal lymph nodes; and CAVALLERO *et al.*[7] have recently reported that the injection of cortisone into chicken embryos produced a significant decrease in protein and RNA, whereas the DNA content was practically unchanged. This problem has been studied by measuring the incorporation of ^{32}P into the organic phosphorus compounds of the lymphatic cells, since it was known that the injection of ^{32}P into animals resulted in its rapid incorporation into the nucleoproteins of lymphatic tissue[8].

METHODS

The lymphatic cell suspensions were prepared as previously described[2], and the procedure for preparing the various tissue extracts was based mainly upon the methods of OGUR AND ROSEN[9] and of SCHMIDT AND THANNHAUSER[10] as modified by SCHNEIDER[11].

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Appendix and tumor cells were twice washed with 0.154 *M* NaCl containing 0.025 *M* $NaHCO_3$; the spleen cells were washed only once. The cells were subsequently suspended in this medium. They were kept either in glass-stoppered Erlenmeyer flasks or Warburg vessels, and were shaken in a water bath at 38° C. At the end of the incubation period, the flasks were quickly removed to the cold room (4° C), and one volume of 0.4 *N* $HClO_4$ was added to each vessel or flask. The contents were then transferred into 12 ml graduated centrifuge tubes; flasks and vessels were rinsed with 2 ml of 0.2 *N* $HClO_4$.

OAS-P fraction

The tubes were centrifuged for five minutes at 2,000 rpm (International Clinical Centrifuge), and the supernatant was transferred into centrifuge tubes containing 5 micromoles of phosphate carrier and 1 ml of magnesia mixture[12] to precipitate the inorganic phosphate. The residue was resuspended in 2 ml of 0.2 *N* $HClO_4$, centrifuged for five minutes, and the supernatant then added to the centrifuge tubes containing the first supernatant fluids. After addition of a drop of phenolphthalein, 5 *N* NH_4OH was added to a faint pink, and the precipitation was allowed to proceed overnight in the cold room (4° C)*. After centrifugation, the fluid was poured into graduated centrifuge tubes containing 5 more micromoles of phosphate carrier and 0.05 ml of 10 *N* H_2SO_4. Ammonia was added again to a faint pink colour and the tubes were left at 4° C for four hours. After centrifugation, the supernatant, designated as the organic acid-soluble phosphate fraction (OAS-P), was collected for analysis of inorganic P and total P and for ^{32}P counts. (OAS-P was calculated by difference of total-P and inorganic-P.)

RNA fraction

The residue from the acid-soluble fraction was washed twice with 0.2 *N* $HClO_4$, twice with ethanol, three times with a mixture of ethanol ether (3:1) for five minutes at 65° C, and finally with 0.2 *N* $HClO_4$. The residue was extracted overnight at 4° C with 4 ml of 1.2 *N* $HClO_4$. This was found necessary because when the alkaline digests of RNA were obtained by the SCHMITD-THANNHAUSER method, there were present protein breakdown products which interfered with the application of the ERNSTER procedure[14] in which molybdic acid is employed. This fluid, together with the fluid of three additional washings with 1 ml of 1.2 *N* $HClO_4$ was designated the ribonucleic acid fraction, and was used for the determination of ribonucleic acid phosphorus and for ^{32}P counts. The RNA fraction obtained by the $HClO_4$ extraction gave a positive orcinol and negative diphenylamine test[15].

DNA fraction

The residue from the RNA extraction was washed with ethanol, 1 ml of 1 *N* KOH was added, and the mixture was kept for 18 hours at 38° C. DNA and protein were precipitated with 0.2 ml of 6 *N* HCl and 1 ml of 4% CCl_3COOH. The DNA was extracted from the precipitate with 5 ml of 4% CCl_3COOH for 15 minutes at 90° C and centrifuged; the residue was washed at room temperature with 2 ml of 4% CCl_3COOH, and the supernatant fluid, after centrifugation, was added to the first acid fraction. This fraction was used for the determination of desoxyribonucleic acid phosphorus (DNA-P) and for ^{32}P counts. The DNA fraction gave a positive diphenylamine test.

Phosphoprotein fraction

The residue from the last centrifugation was designated the phosphoprotein fraction. This fraction does not contain all the phosphoprotein, since the treatment with KOH hydrolyzed it partially, as emphasized by DAVIDSON *et al.*[16]. The figures obtained are, therefore, unreliable and no conclusion has been drawn from the data.

The phosphorus determinations were made by the method of GOMORI[17] with the addition of 10% ethanol, which facilitates the colour development[18]. Digestions were performed in an oven at 140° C after addition of 0.4 ml of 10 *N* H_2SO_4 and 0.1 ml of 72% $HClO_4$ into the DNA and OAS-fractions. All digested fractions, including standards and blanks, were neutralized to phenolphthalein with NH_4OH. Prior to the addition of the reagents, 0.1 ml of 10 *N* H_2SO_4 was added. A 3 ml aliquot of OAS-P fraction was taken for digestion and a 3 ml aliquot for inorganic P determination. For the RNA digestion a 3 ml aliquot was also used, and a 1 ml aliquot for the DNA digestion.

The ^{32}P counts were made according to ERNSTER *et al.*[14].

(a) For OAS-P: An aliquot of 0.5 ml of the OAS-P fraction together with 0.1 ml of 0.1 *M* Na_2HPO_4, 0.5 ml of 10 *N* H_2SO_4, 0.5 ml of 10% ammonium molybdate, 3.4 ml of water, and 5 ml of a 1:1 mixture of *iso*butanol-benzene was shaken 100 times. The alcohol phase was discarded, and 0.1 ml of 0.1 *M* Na_2HPO_4, 0.25 ml of 10% ammonium molybdate, and 5 ml of *iso*butanol-

* ENNOR AND ROSENBERG[13] have shown that the extent of coprecipitation is a function of the amount of carrier phosphate added; the precipitate of magnesium ammonium phosphate is probably contaminated with ATP.

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benzene were added. This mixture was shaken 100 times and the alcohol phase again was discarded. The solution was shaken 100 times more after addition of 5 ml of *iso*butanol-benzene. Then 0.3 ml of the aqueous phase was transferred into planchets containing two dried drops of 0.05% Lakeseal. The planchets were dried overnight at 105° and subsequently counted for at least 2,000 counts in a gas-flow counter. Under these conditions no absorption correction was necessary. (b) For RNA: An aliquot of 3 ml of the RNA fraction, together with 0.5 ml of 10% ammonium molybdate, 0.14 ml of 10 *N* H_2SO_4, 0.1 ml of 0.1 *M* Na_2HPO_4, 1.26 ml of water and 5 ml of *iso*butanol-benzene was shaken 100 times to extract traces of inorganic phosphate. After further addition of carrier and washings, as in the OAS-P fraction, 0.5 ml of the water fraction phase was taken for ^{32}P counts. (c) For DNA: An aliquot of 4 ml of the DNA fraction, together with 0.1 ml of 0.1 *M* Na_2HPO_4, 0.4 ml of 10 *N* H_2SO_4, 0.5 ml of 10% ammonium molybdate, and 5 ml of *iso*butanol-benzene was shaken 100 times. After further treatment, as in the other fractions, 0.3 ml of the aqueous phase was taken for ^{32}P counts.

AEC $H_3{}^{32}PO_4$ was a gift of Dr. BERTHARD of the Department of Medicine of this University. It was hydrolyzed in 1 *M* HCl for one hour, and after the addition of a small amount of carrier phosphate, the phosphate was precipitated with the magnesia mixture. The precipitate was dissolved in a small volume of HCl and was neutralized to pH 7. Compound F was generously provided by Merck and Company.

Traces of inorganic ^{32}P contaminating any of the organic-phosphate fractions cause large errors, due to the extremely high specific activity of the inorganic phosphorus. It is therefore necessary to remove this contaminant as completely as possible. The method of ERNSTER[14] has been useful for this purpose. Routine checks showed that when acid was added to inactivate the tissue prior to the addition of the ^{32}P, the specific activity of this zero time blank was of the order of 1% of the control, as can be seen in this experiment: Appendix cells, OAS-P after 2 h incubation a 38° C, 4,017 c/m/mg P; at zero time, 40. RNA-P after incubation 451; at zero time, 3. DNA-P, after incubation 142; at zero time 1.5.

A comparison of nucleic acid phosphorus turnover by the SCHMIDT-THANNHAUSER, and by the combined SCHMIDT-THANNHAUSER and the OGUR-ROSEN methods employed in these experiments is given in Table I.

TABLE I

NUCLEIC ACID PHOSPHORUS TURNOVER IN THE LYMPHATIC CELLS OF RABBIT APPENDIX ESTIMATED WITH THE SCHMIDT-THANNHAUSER METHOD (I) AND WITH THE COMBINED PROCEDURE USED IN MOST EXPERIMENTS IN THIS STUDY (II)

Cells suspended in Saline-bicarbonate, pH 7.4, containing 0.01 *M* glucose and O_2; CO_2 as gas phase. Incubation time, 2 h. Temp. 38° C. Specific activity of the added ^{32}P, 180,000 c/m/microgram P. Figures give counts per minute per microgram of P-compound.

P-Compound	*I*	*II*
OAS-P	9,979	8,786
RNA-P	931	874
DNA-P	261	224

The Nucleic Acid Content of the Lymphatic Cell Suspensions

From the cell counts, dry weight measurements, and determination of nucleic acids in all the experiments performed in this study it has been found that 10^6 rabbit appendix lymphatic cells give a dry weight of 64 micrograms, while 10^6 mouse spleen and mouse lymphosarcoma cells gave a dry weight of 112 micrograms. All three kinds of cells contained about $5.5 \cdot 10^{-12}$ g DNA per cell, a value which agrees with data reported for other diploid cells[19, 20]. The RNA-P content per cell was as follows: $0.12 \cdot 10^{-12}$ grams for mouse spleen cells, $0.16 \cdot 10^{-12}$ grams for appendix cells, and $0.37 \cdot 10^{-12}$ grams for lymphosarcoma cells. The RNA-DNA ratio was 0.33 for appendix lymphatic cells, 0.2 for the spleen, and 0.86 for lymphosarcoma. These results are of the same order of magnitude as those summarized by DAVIDSON[20] for other lymphatic tissues where different methods were employed. It is to be noted that losses of RNA-P occurring during the preparation of the cell suspensions.

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The Uptake of ^{32}P by Lymphatic Cells

In previous experiments on the uptake of ^{32}P by lymphatic tissues, the labelled substance was always administered *in vivo*.—It was therefore necessary to study the conditions under which ^{32}P is taken up by these cells *in vitro*. The uptake of ^{32}P was increased in the presence of glucose, a phenomenon perhaps correlated with the penetration of phosphorus across the cell membrane. Addition of succinate had no influence at all in one experiment (Table II).

TABLE II

THE UPTAKE OF ^{32}P BY LYMPHATIC CELLS
EFFECT OF DINITROPHENOL, ANAEROBIOSIS, FLUORIDE, GLUCOSE, AND SUCCINATE

The final volume in the flasks with O_2: CO_2 as gas phase was 1 ml; of the flasks with N_2: CO_2 as gas phase, 1.5 ml. The phosphate concentration varied from $6.4 \cdot 10^{-4}$ *M* to $9.4 \cdot 10^{-4}$ *M*. Substrate, glucose 0.01 *M*. Incubation, 2 h, 38° C. The figures give counts/min/microgram P.

Tissue	*Experimental conditions*	*OAS-P*		*RNA-P*		*DNA-P*	
		Control	*Exp. Cond.*	*Control*	*Exp. Cond.*	*Control*	*Exp. Cond.*
Appendix	No glucose	2670	2063	238	107	72.4	11.1
Lymphosarcoma	No glucose	6301	1507	247	40	44	6.8
Lymphosarcoma	No glucose (succinate)	6301	1393	247	29	44	8.8
Appendix	N_2: CO_2 gas phase	2670	1032	238	74	72.4	10
Appendix	N_2: CO_2 gas phase	3003	1307	306	80	133	11.4
Lymphosarcoma	N_2: CO_2 gas phase	13,752	19,839	281	470	52.9	50.9
Lymphosarcoma	NaF, $5 \cdot 10^{-3}$ *M*	13,752	15,078	281	113	52.9	28.1
Appendix	DNP, 10^{-3} *M*	—	—	246	117	50.3	7.8
Appendix	DNP, $5 \cdot 10^{-5}$ *M*	4017	3794	451	390	142	103
Appendix	DNP, $5 \cdot 10^{-5}$ *M*	2670	2294	238	200	72.4	69.4

Dinitrophenol, $5 \cdot 10^{-5}$*M*, increased the respiration of appendix lymphatic cells. When the concentration was increased to 2 and $5 \cdot 10^{-4}$ *M*, the initial respiratory stimulation was followed by inhibition. Inhibition was marked with 10^{-3} *M* (Fig. 1). Dinitrophenol, $5 \cdot 10^{-5}$ *M*, produced a slight inhibition on the incorporation of ^{32}P into the three fractions, OAS-P (10%), RNA-P (15%), and DNA-P (16%). A concentration of $1 \cdot 10^{-3}$ *M*, produced marked inhibition (Table II).

Fluoride, which inhibits some enzymes concerned with phosphorus exchanges (enolase, phosphatases), inhibits also amino-acid incorporation by the cells of lymphosarcoma[21]. In the same cells, sodium fluoride $5 \cdot 10^{-3}$ *M*, increased slightly the incorporation of ^{32}P into OAS-P whereas the incorporation into RNA-P

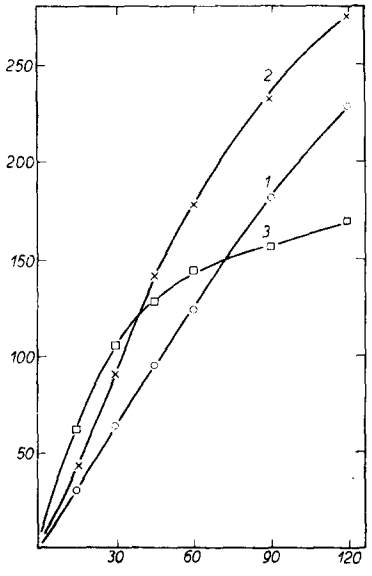

Fig. 1. Effect of dinitrophenol (DNP) on the respiration of lymphatic cells (rabbit appendix). Abscissa, time in minutes. Ordinate, O_2 uptake in μl. Buffer, Ringer-phosphate, pH 7.4. Substrate, glucose, 0.01 *M*. 1. Control: 2. DNP, $2 \cdot 10^{-5}$ *M*: 3. DNP, $1 \cdot 10^{-4}$ *M*.

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and DNA-P was definitely inhibited 60% and 47% respectively (Table II). The stimulation of OAS-P formation may be due to the accumulation of triose and hexose phosphates with simultaneous inhibition of phosphatases (Table II).

In the lymphocytic cells of the appendix the uptake of ^{32}P was markedly inhibited by anaerobiosis when the cells were incubated in Ringer-bicarbonate saturated with $N_2 : CO_2$ instead of $O_2 : CO_2$. Incorporation of ^{32}P into OAS-P was reduced by 58%; into RNA-P, 71%; and into DNA-P, 88%. No such inhibition was observed in lymphosarcoma cells which took up ^{32}P and incorporated it into the three P-fractions at a slightly higher rate. This difference may be due to the higher anaerobic glycolysis of lymphosarcoma (Table II).

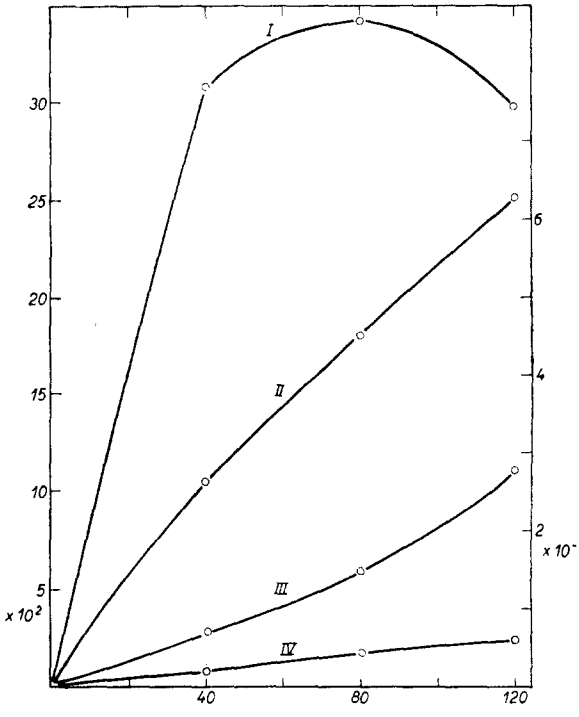

Fig. 2. Incorporation of ^{32}P into Organic Phosphorus Compounds by the Lymphatic Cells of the Appendix (rabbit). Abscissa, time in minutes. Ordinate, right; ^{32}P counts per min per micrograms P of OAS-P. Ordinate, left; ^{32}P c/m/μg of P in Phosphoprotein, RNA, and DNA. I. OAS-P; II. Phosphoprotein; III. RNA-P; IV. DNA-P.

Rate of ^{32}P Incorporation

In the cells of the appendix the incorporation of ^{32}P into the nucleic acids and phosphoprotein increased progressively with time during the two hours of the incubation period. However, the specific activity of the OAS-P, after reaching a high value at the end of 40 minutes, increased slightly 40 minutes later and decreased at the end of two hours (Fig. 2). In a similar experiment with the cells of lymphosarcoma, most of the ^{32}P incorporation into the nucleic acids occurred in the first 80 minutes. However, as in the appendix, maximum incorporation into the OAS-P fraction took place in the first 40 minutes, after which there was some diminution.

Comparative ^{32}P Uptake

It is known that the rate of renewal of ribonucleic acid is higher than that of desoxyribonucleic acid and that turnover in regenerating liver and hepatoma is more rapid than in resting liver[22]. In Table III are compared the specific activity ratios for OAS-P, RNA-P, and DNA-P as related to the initial ^{32}P and to OAS-P. The formation of OAS-P in the lymphatic cells of the appendix, spleen and lymphosarcoma was about 5% of the specific activity of the inorganic phosphorus added to the flasks.

The specific activity of the organic acid-soluble fraction increased somewhat as the concentration of phosphate was increased. The specific activity of RNA-P was greater in the cells of the appendix, the per cent ratio to the OAS-P being 1%, while in spleen, and in lymphosarcoma, it was 5%. The cells of the appendix showed also greater

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TABLE III

NUCLEIC ACID PHOSPHORUS TURNOVER IN LYMPHATIC CELLS

Cell suspensions in saline-bicarbonate with 0.01 *M* glucose and $O_2 : CO_2$ as gas phase. pH 7.45. Incubation, 2 h, 38° C.

Tissue	*PO_4 M Conc.*	$\frac{OAS\text{-}P\ c/m/\mu g}{c/m/\mu g\ In.P} \cdot 100$		$\frac{RNA\text{-}P_1\ c/m/\mu g}{AS\text{-}P,\ c/m/\mu g} \cdot 100$	$\frac{DNA\text{-}P_1\ c/m/\mu g}{AS\text{-}P,\ c/m/\mu g} \cdot 100$
Appendix (rabbit)	$7 \cdot 10^{-4}$	$\frac{7418}{320{,}000}$	2.3	14.9	3.0
	$7 \cdot 10^{-4}$	$\frac{8786}{180{,}000}$	4.9	9.9	2.5
	$7 \cdot 10^{-4}$	$\frac{4017}{90{,}000}$	4.5	11.0	3.5
	$7 \cdot 10^{-4}$	$\frac{2768}{60{,}000}$	4.9	9.3	2.7
	$8 \cdot 10^{-4}$	$\frac{2670}{47{,}633}$	5.6	9.0	2.6
	$1.4 \cdot 10^{-3}$	$\frac{3657}{60{,}000}$	6.1	8.8	2.4
	$3.5 \cdot 10^{-3}$	$\frac{4612}{60{,}000}$	7.7	7.4	2.2
Spleen (mouse)	$7 \cdot 10^{-4}$	$\frac{11{,}151}{252{,}800}$	4.4	~6.0*	0.74
	$7 \cdot 10^{-4}$	$\frac{7{,}783}{107{,}687}$	7.2	4.4	0.52
	$1.2 \cdot 10^{-3}$	$\frac{6{,}074}{70{,}000}$	8.7	4.8	1.11
Lymphosarcoma	$7 \cdot 10^{-4}$	$\frac{6{,}301}{123{,}726}$	5.1	3.9	0.70
	no carrier	—	—	2.6	0.43
	$6.4 \cdot 10^{-4}$	$\frac{13{,}752}{356{,}200}$	3.9	2.1	0.39
	$1.2 \cdot 10^{-3}$	$\frac{3{,}733}{58{,}000}$	6.4	4.6	0.85
	$2.4 \cdot 10^{-3}$	$\frac{5{,}290}{58{,}000}$	9.1	3.0	0.57

* Estimate only. Colorimetric determination lost.

turnover for DNA-P, the per cent being 2.7, as compared with 0.8% in spleen, and 0.6% in lymphosarcoma. It must be emphasized that a small contamination of the DNA fraction by the RNA fraction would introduce a serious error in the specific activity of the latter. For this reason, the residue containing DNA and protein was subjected to alkaline treatment and reprecipitation with acid[10] to remove the traces of RNA. Nevertheless, the DNA-P specific activities might be considered as possibly high.

Phosphoprotein-P

The specific activity of the phosphoprotein obtained by the SCHNEIDER procedure or by the OGUR-ROSEN procedure was 33 to 45% of the specific activity of the esterified acid-soluble fraction. Measurements of phosphoprotein made soluble by the SCHMIDT-THANNHAUSER's procedure with subsequent precipitation by $CaCl_2$-$MgCO_3$[23] were not accurate because only traces of P were recovered. The phosphoprotein fraction probably

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has a specific activity approaching that of the OAS-P fraction, since the contamination of this fraction with small amounts of DNA-P would lower considerably the specific activity*.

Effect of Compound F on the Uptake of P

When Compound F (100 micrograms) was added to the lymphatic cells there was in general a decrease in the incorporation of ^{32}P into the three P-fractions. In the lymphatic cells of the appendix inhibition of the OAS-P fraction was small (8%), whereas the RNA-P and DNA-P fractions were inhibited by 16 and 23%, respectively. (The experiment with 200 micrograms of Compound F was not considered.) The cells of the spleen were less sensitive to the inhibiting effect; there was practically no effect on OAS-P, and RNA-P and DNA-P were inhibited by 5 and 12%, respectively. The lymphosarcoma cells were more sensitive, and inhibition of the three fractions was around 30% (Table IV).

TABLE IV

EFFECT OF COMPOUND F ON NUCLEIC ACID-P. TURNOVER ON LYMPHATIC CELLS

In all experiments 100 micrograms were used, except on the experiment marked with asterisk where 200 micrograms were added. Incubation time, 2 h. Figures give counts per minute per microgram P.

Tissue	OAS-P		RNA-P		DNA-P	
	Control	Comp. F	Control	Comp. F	Control	Comp. F
Appendix (rabbit)	—	—	17.5	15.1	4.8	3.66
				11.8*		3.45
Appendix (rabbit)	—	—	246	210	50.3	36.4
Appendix (rabbit)	4017	3859	451	378	142	104
Appendix (rabbit)	2670	2350	238	192	72.4	62.5
Spleen (mouse)	7978	9389	—	—	184	171
Spleen (mouse)	7783	6550	341	323	39.5	34.4
Spleen (mouse)	6074	5897	289	276	67.6	56.7
Lymphosarcoma (mouse)	6301	3696	247	151	44	27
Lymphosarcoma (mouse)	3733	2208	168	95	31.5	16.8
Lymphosarcoma (mouse)	13752	10995	281	244	52.9	38.0

DISCUSSION

The experiments presented here are, we believe, the first *in vitro* demonstration of ^{32}P uptake by lymphatic cells in suspensions. These cells washed with Ringer solution and kept in a non-protein medium, were in a resting condition, *i.e.*, non-dividing. This resting condition must be the reason why there was no difference in the turnover of the RNA and DNA between the lymphatic cells of the appendix and those of tumor (lymphosarcoma). The classic observation of BRUES *et al*[22]. of increased turnover of nucleic acids in the regenerating liver and in hepatoma were made *in vivo*, where the cells were undergoing their normal processes of multiplication and development. However, since the tumor cells contained more RNA *per cell* than did the other tissues, the total RNA synthesized *per unit* cell must have been greater in the tumor than in spleen though not greater than in the appendix.

* See R. M. JOHNSON AND S. ALBERT, *J. Biol. Chem.*, 200 (1953) 335, which was published after this paper was written.

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There were striking differences in the incorporation of ^{32}P into OAS-P fraction under anaerobic conditions: the lymphatic cells of appendix showed less incorporation of ^{32}P in anaerobiosis and this incorporation was largely aerobic (60% inhibition in absence of oxygen), whereas in the cells of lymphosarcoma, anaerobiosis had no effect at all. Dinitrophenol, a known inhibitor of aerobic phosphorylation, produced an acceleration of respiration without however inhibiting phosphorylation markedly. The increased incorporation of ^{32}P into OAS-P produced by fluorides is in agreement with similar findings of KAPLAN *et al.*[24] in rat kidney and liver slices.

Addition of Compound F at levels which in earlier experiments had little effect on respiration significantly inhibited the incorporation of ^{32}P into the three fractions of the tumor. However, if, instead of measuring inhibition of the individual fractions, the ratio of RNA-P/OAS-P or DNA-P/OAS-P is compared, there would appear only a small inhibition of the RNA-P/OAS-P ratio in appendix cells, while both the ratio DNA-P/OAS-P and RNA-P/OAS-P in all other cells remained constant and were not affected by Compound F. On the assumption that the esterified acid-soluble fraction is the presursor of the RNA-P and DNA-P, it would appear as if Compound F inhibited the initial phosphorylation process only. The greater extent of inhibition observed in tumor cells remains without explanation.

SUMMARY

Addition of ^{32}P to suspensions of lymphatic cells of normal and tumor tissues produced its incorporation into the organic acid-soluble phosphorus compounds and into ribonucleic and desoxyribonucleic acids. Lack of oxygen inhibited the incorporation of ^{32}P in the cells of appendix, whereas it had little effect in the tumor cells of lymphosarcoma. Fluoride had no effect on the synthesis of organic acid-soluble phosphorus compounds, whereas it inhibited the synthesis of nucleic acids. Dinitrophenol, at concentrations which increased respiration had little effect on nucleic acid synthesis. The relative specific activity of the esterified acid-soluble-P fraction was the same in all three kinds of cells (appendix, spleen, lymphosarcoma), whereas the activity of the nucleic acids was greater in the cells of the appendix.

Compound F inhibited the uptake of ^{32}P into the three fractions of phosphorus compounds.

RÉSUMÉ

^{32}P introduit dans des suspensions de cellules lymphatiques de tissus normaux et tumoraux, est incorporé dans des composés organiques phosphorés acido-solubles et dans les acides ribonucléique et désoxyribonucléique. L'absence d'oxygène supprime l'incorporation de ^{32}P dans les cellules de l'appendice, alors qu'il n'a guère d'effet dans le cas des cellules tumorales de lymphosarcome. Les fluorures n'ont pas d'action sur la synthèse des composés phosphorés organiques acido-solubles, alors qu'ils suppriment la synthèse des acides nucléiques. Le dinitrophénol, à des concentrations qui accroissent la respiration, n'a guère d'effet sur la synthèse des acides nucléiques. Les activités spécifiques relatives des fractions de phosphore acido-soluble estérifiées sont les mêmes dans les trois types de cellules étudiés (appendice, rate, lymphosarcome) alors que l'activité des acides nucléiques est plus grande dans les cellules de l'appendice.

Le composé F inhibe l'incorporation de ^{32}P dans les trois fractions des composés phosphorés étudiés.

ZUSAMMENFASSUNG

Der Zusatz von ^{32}P zu Suspensionen von Lymphzellen von normalen und Tumorgeweben verursachte seinen Einbau in organische, säurelösliche Phosphorverbindungen und in Ribonuclein- und Desoxyribonucleinsäure. Das Fehlen von Sauerstoff hemmte den Einbau von ^{32}P in die Zellen der Appendix, während es wenig Wirkung auf die Tumorzellen der Lymphsarcome hat. Fluoride üben keinen Einfluss auf die Synthese der organischen säurelöslichen Phosphorverbindungen aus, dagegen

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hemmen sie die Nucleinsäuresynthese. Dinitrophenol, in Konzentrationen, die die Respiration erhöhen, zeigte keinen Einfluss auf die Nucleinsäuresynthese. Die relative spezifische Aktivität der esterifizierten säurelöslichen P-Fraktion war die gleiche in allen drei Arten von Zellen (Appendix, Milz, Lymphsarcome), dagegen war die Aktivität der Nucleinsäure in den Zellen der Appendix grösser.

Verbindung F hemmte die ^{32}P-Aufnahme in die drei Fraktionen der Phosphorverbindungen.

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