REFERENCES

[1] S. J. SINGER, *Proc. Nat. Acad. Sci. U.S.*, 41 (1955) 1041.
[2] S. J. SINGER, *J. Cell. Comp. Physiol.*, 50, suppl. 1 (1957) 51.
[3] A. F. S. A. HABEEB, H. G. CASSIDY AND S. J. SINGER, *Biochim. Biophys. Acta*, 29 (1958) 587.
[4] S. I. EPSTEIN AND S. J. SINGER, *J. Am. Chem. Soc.*, 80 (1958) 1274.
[5] D. PRESSMAN, A. L. GROSSBERG, L. H. PENCE AND L. PAULING, *J. Am. Chem. Soc.*, 68 (1946) 250.
[6] H. FRAENKEL-CONRAT, R. S. BEAN AND H. LINEWEAVER, *J. Biol. Chem.*, 177 (1949) 385.
[7] W. L. HUGHES, H. A. SAROFF AND A. L. CARNEY, *J. Am. Chem. Soc.*, 71 (1949) 2476.
[8] A. NISONOFF AND D. PRESSMAN, *Science*, 128 (1958) 659.
[9] R. A. KEKWICK AND R. K. CANNAN, *Biochem. J.*, 30 (1936) 227.
[10] M. C. BAKER, D. H. CAMPBELL, S. I. EPSTEIN AND S. J. SINGER, *J. Am. Chem. Soc.*, 78 (1956) 312.
[11] D. H. CAMPBELL, R. H. BLAKER AND A. B. PARDEE, *J. Am. Chem. Soc.*, 70 (1948) 2496.
[12] H. FRAENKEL-CONRAT, J. I. HARRIS AND A. L. LEVY, in D. GLICK, *Methods of Biochemical Analysis*, Vol. 2, Interscience, N.Y., 1955, p. 370.
[13] J. R. MARRACK AND E. S. ORLANS, *Brit. J. Exptl. Pathol.*, 35 (1954) 389.
[14] P. H. MAURER, J. SRI RAM AND S. EHRENPREIS, *Arch. Biochem. Biophys.*, 67 (1957) 196.
[15] W. A. KLEE AND F. M. RICHARDS, *J. Biol. Chem.*, 229 (1957) 489.

INCORPORATION OF ^{32}P INTO ISOLATED NUCLEI OF RABBIT APPENDIX: THE ROLE OF DEOXYRIBONUCLEIC ACID

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SUMMARY

1. Nuclei isolated from rabbit appendix could incorporate ^{32}P into DNA, RNA and organic acid-soluble phosphorus fraction (OASP) *in vitro*.

2. The removal of the bulk of DNA from the nucleus by pancreatic DNase abolished the incorporation into DNA and RNA almost completely, while that into OASP was reduced only to about 60%.

3. Appreciable restoration of the impaired incorporation activity was observed when salmon sperm DNA or yeast RNA was added to the nuclei treated with DNase. However, if an extensive removal of DNA had been achieved, the restoration occurred only in the case of RNA, and not DNA, of the nucleus on addition of exogenous nucleic acids.

4. Some indication was obtained that certain polyanionic compounds, such as chondroitin sulphate or hyaluronate, could replace the nucleic acids in restoring the impaired activity of DNase-treated nuclei, provided the action of DNase was not very extensive.

5. It was suggested that in the observed restoration added nucleic acids acted merely as polyanions which might eliminate the imbalance of electrostatic charges resulting from the removal of the bulk of DNA from the nucleus.

Abbreviations: ATP, adenosine triphosphate; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; DNase, deoxyribonuclease; OASP, organic acid-soluble phosphate fraction.

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INTRODUCTION

Much information has been collected in recent years about the synthetic activities of isolated nuclei of animal cells. They can phosphorylate adenosine monophosphate to adenosine triphosphate[1], and incorporate various labelled precursors into proteins[2], ribonucleic acids[2-4], and deoxyribonucleic acid[5-7]. The removal of the bulk of DNA by deoxyribonuclease impairs the incorporation of the precursors into RNA and protein, and the lost activity is restored by various nucleotide compounds of relatively high molecular weight, including DNA and RNA[2]. The formation of ATP in the nucleus is also dependent on the presence of DNA, which can likewise be replaced by exogenous nucleic acids[8]. It has thus been presumed that one of the roles of DNA is to provide energy-rich nucleotides needed for the synthetic activity of the nucleus[8].

Enzymes catalysing the incorporation of thymidine or deoxyribonucleotide triphosphates into DNA were found in the supernatant fraction of certain animal cells; they require DNA for their full activity[9,10].

In the present study we have attempted to determine (1) whether the preformed DNA in the nucleus is indispensable for the incorporation of inorganic ^{32}P into DNA in isolated nuclei, and (2) whether the incorporation activity of isolated nuclei depleted of their DNA can be restored by some anionic compounds of non-nucleotide nature. In doing this we are looking for some relevant information concerning the possible role of DNA in the anabolic processes exhibited by the isolated nuclei, either as a carrier of information needed for fabrication of macromolecules, as a potential source of high-energy phosphate compounds, or as a polyanionic compound the removal of which will cause some disturbance in the electrostatic charge distribution within the nucleus.

A preliminary note on this work has been published[11].

EXPERIMENTAL

Chemical compounds

Yeast RNA was a gift of Dr. KUROIWA, Kirin Research Institute, Kirin Brewery Co., Ltd., Amagasaki. Highly polymerized salmon sperm DNA was received from Prof. I. WATANABE, University of Tokyo. A sample of chondroitin sulphate was obtained from Dr. T. MAKI, Minophagen Pharmaceutical Co., Tokyo, and another sample purchased from Wako Pure Chemical Industries, Ltd., together with a sample of heparin. A highly purified preparation of hyaluronate was a generous gift of Dr. M. ISHIMOTO, University of Tokyo. A crystalline preparation of egg albumin was kindly supplied by Dr. Y. FUJISAKI, Yamaguti Medical School. The mixed 2',3'-mononucleotides were obtained by hydrolysing yeast RNA in 0.1 *N* NaOH at 80° for 20 min and neutralising with HCl; the content of NaCl was calculated and substituted for the NaCl in the incubation medium. ^{32}P was received from the Radiochemical Centre, Amersham, England, on allocation of Japan Isotope Association. Preparations of pancreatic DNase, once crystallized for treatment of nuclei, and not crystallized for hydrolysing a DNA preparation, came from the Worthington Biochemical Corporation. A part of them was made available through the courtesy of Dr. K. SUZUKI and Dr. H. UCHIDA of the University of Tokyo.

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Preparation of nuclei and lymphatic cells

Appendices and Peyer's patches of adult rabbits served as the starting material. Sucrose nuclei were prepared essentially according to ALLFREY *et al.*[2]; they were finally suspended in 0.25 *M* sucrose–0.002 *M* $CaCl_2$ at a density of $1.5 \cdot 10^9$ nuclei/ml. Lymphatic cells were obtained as described in a separate communication[12].

Incubation with ^{32}P

Usually the final incubation mixture consisted of 1 ml of a 1:1 mixture of 0.25 *M* sucrose–0.002 *M* $CaCl_2$ containing $3 \cdot 10^8$ nuclei and Ca-free Tyrode solution. This suspending medium will henceforward be called the sucrose-Tyrode medium. The suspension also contained 5 μg of streptomycin, 10 μC of ^{32}P-orthophosphate and other appropriate additions where indicated. The mixture was equilibrated in a 15-ml centrifuge tube at 37° for 5 min and then shaken at the same temperature. If not specified, the incubation lasted 1.5 h.

Experiments with lymphatic cells were conducted as described elsewhere[12].

Individual tests were run in duplicate tubes. In most experiments, where total activity of nucleic acids per tube had to be determined, another set of duplicate tubes without ^{32}P was run for each of the varied experimental conditions, and subjected to the determination of nucleic acids by the Schneider method at the end of incubation without separating the nuclei from the incubation medium.

Digestion of nuclei with DNase

The nuclei (0.2 ml of the original suspension) were mixed with 0.2 ml of Tyrode solution containing 0.0125 *M* $MgCl_2$ with and without an appropriate amount of crystalline DNase. The mixture was incubated at 37° for 15 min, chilled in ice, and added to 5 ml of ice-cold 1:1 mixture of 0.25 *M* sucrose–0.002 *M* $CaCl_2$ and Ca- and Mg-free Tyrode solution containing 0.01 *M* sodium citrate. The nuclei were sedimented by centrifuging, and resuspended in the final incubation mixture as described above.

Preparation of nucleic acids and organic acid-soluble phosphate fraction

Incubation was terminated by chilling in ice; the contents of individual tubes (nuclei plus medium) along with the appropriate zero-time control were immediately subjected to the procedure described elsewhere[12] for the preparation of nucleic acids and OASP.

Determination of nucleic acids and total phosphorus

DNA-P and total P were determined as in a previous report[13]. RNA-P was measured by the phloroglucinol test according to HAHN AND VON EULER[14].

Measurement of radioactivity

This was made as in a previous report[13] using an end-window Geiger-Müller counter. In each experiment the radioactivity of the samples of zero-time control were measured, and when not negligible, subtracted from the values of the tests. This was necessary because the activities obtained were rather low, especially with DNA, in many of the experiments reported here, although the contamination found in zero-time samples was usually very slight, if any. Specific activity was expressed

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in counts/min/μg P. In most cases, however, the total radioactivity (counts/min/tube) was employed to express the extent of incorporation into nucleic acids. This was calculated from the specific activity of purified samples and the total amount of respective nucleic acid P (μg/tube).

Chromatographic procedure

A large-scale incubation of the nuclei was conducted using 100 ml of nuclear suspension with the same composition as already described. The mixed nucleic acids were obtained by the routine procedure[12]. They were fractionated into DNA and RNA in the usual way by alkaline hydrolysis with 0.3 *N* KOH at 37° and acidification with 10 *N* perchloric acid[15]. The RNA fraction was adjusted to pH 3 and treated with charcoal, and the adsorbed mononucleotides were eluted with 20 % aqueous pyridine according to VAN BEKKUM[16], in order to eliminate the eventual contamination of inorganic ^{32}P. They were then chromatographed on a Dowex-1 column (X-10, formate form) according to OSAWA *et al.*[17].

With intact lymphatic cells, 40 ml of cell suspension ($4 \cdot 10^8$ cells/ml of Ca-free Tyrode solution) was used and the charcoal treatment was omitted because the ^{32}P contamination could be neglected in this case.

The DNA precipitated from the acidified solution of the alkaline digest of crude nucleic acids was washed twice with cold 10 % trichloroacetic acid, redissolved in a dilute NaOH, neutralised with HCl, precipitated with ethanol, washed in ethanol and ether, and dried. Twenty mg of DNA thus obtained was dissolved in 4 ml of 0.008 *M* $MgCl_2$ solution containing 4 mg DNase and incubated at 37° for 80 min and the digest was acidified with cold perchloric acid and centrifuged. The mixture of nucleotides of various sizes in the supernatant was chromatographed on a Dowex-1 column (X-10, formate form) according to the method of SAKAKI[18], using 0.36–7.0 *N* formic acid in the reservoir of a graduated elution system.

RESULTS

Incorporation activity of the isolated nuclei

When the nuclei were incubated with ^{32}P in the sucrose–Tyrode medium, ^{32}P was incorporated into DNA, RNA and OASP, their specific activities increasing in the order mentioned (Table I). As shown in Fig. 1, the incorporation into these fractions proceeded linearly for about 1 h and then tended to level off. In subsequent experiments the period of incubation was fixed at 90 min.

TABLE I

In vitro INCORPORATION OF ^{32}P INTO LYMPHATIC CELLS AND SUCROSE NUCLEI OF RABBIT APPENDIX

Cells and nuclei were obtained from the same source of pooled tissues and incubated for 1.5 h at 37°.

Material	*Incubation medium*	*Concentration of ^{32}P (μC/ml)*	*Specific activity (counts/min/μg P)*			
			OASP	*RNA*	*DNA*	*RNA/DNA*
$3 \cdot 10^8$ cells	2 ml Ca-free Tyrode	5	4550	322	48.5	6.6
$6 \cdot 10^8$ nuclei	2 ml Sucrose–Tyrode	10	1420	56.2	2.15	26.2

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Compared with the results on lymphatic cells prepared from the same tissue, the incorporation into the nuclei was considerably less (Table I). The specific activity ratio RNA/DNA was much higher in the nuclei than in the whole cells, and this was in agreement with the current view that the isotopic uptake by nuclear RNA was much higher than that by cytoplasmic RNA[19]. These observations, together with the fact that DNase treatment reduced the DNA content and ^{32}P uptake of the nuclear preparation (Table VI) but not of the intact cells, indicate that the observed incorporation was due to the real activity of isolated nuclei rather than of contaminating intact cells.

In the course of the work it was noticed that the incorporation activity of the nucleus was subject to seasonal variation, being highest in summer and decreasing gradually to reach a complete loss of activity in midwinter. A similar trend was also noticed with lymphatic cells of rabbit appendix[12].

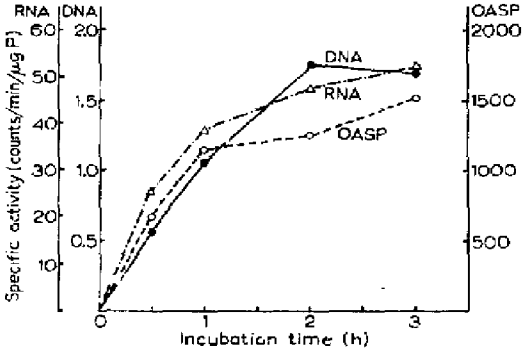

Fig. 1. Time course of ^{32}P incorporation into DNA, RNA and OASP of isolated nuclei of rabbit appendix.

Labelling of nucleic acids

The specific activity patterns of individual 2′,3′-mononucleotides obtained by alkaline hydrolysis of RNA's of isolated nuclei and intact cells were very similar (Table II). It may be noted that the results closely paralleled those obtained with RNA of the rat liver *in vivo* (1 and 24 h)[20].

The fractions obtained chromatographically from a DNase-digest of DNA of the incubated nuclei showed a much more diverse distribution of specific activities than that observed *in vivo*[18,21], but appreciable quantities of radioactivity were recovered

TABLE II

LABELLING OF INDIVIDUAL 2′, 3′-MONONUCLEOTIDES OF RNA IN NUCLEI AND LYMPHATIC CELLS OF RABBIT APPENDIX INCUBATED *in vitro*

Nuclei and cells originated from separate sources. Duration of incubation: 1.5 h

	Specific activity (counts min μg P)			
	Adenylic acid	*Guanylic acid*	*Cytidylic acid*	*Uridylic acid*
Lymphatic cells	75.2	55.0	54.5	92.0
Isolated nuclei	7.05	4.25	4.70	7.84

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from all the ultraviolet-absorbing fractions examined (Table III). The observed radioactivities of the nucleic acid fractions obtained from the nuclei incubated with ^{32}P can therefore be taken to indicate the incorporation of ^{32}P into polynucleotides of DNA and RNA.

TABLE III

DISTRIBUTION OF RADIOACTIVITIES IN DIFFERENT FRACTIONS OF A DNASE-DIGEST OF DNA OF RABBIT APPENDIX NUCLEI INCUBATED *in vitro*

DNA was obtained from the same material as RNA of Table II.

	Original DNA	*Fraction**									
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Specific activity (counts/min/μg P)	0.38**	0.82	0.71	0.36	0.31	0.57	0.31	0.37	0.54	1.56	0.43
Relative P content (% of total)		0.0	1.5	8.7	2.0	4.5	41.0	23.0	8.7	0.6	7.0

* Fractions 1–8 were obtained chromatographically; fraction 9 was extracted with hot 5% trichloroacetic acid from the resin after termination of elution with 7 *N* formic acid; fraction 10 was the acid-insoluble residue of DNase-digest.

** Weighted mean of specific activities of fractions 1–10: 0.30.

Use of the total activity as a measure of incorporation

In experiments involving the removal of DNA from the nucleus by DNase and/or the addition of nucleic acids, the use of the specific activity to express the magnitude of incorporation is obviously misleading, because the amount of nucleic acids in different tubes may vary considerably and cannot be used as the common reference standard for the incorporated radioactivity. It was therefore decided to express the magnitude of incorporation by total activity. For OASP this was determined directly. For DNA and RNA it was calculated from the content of respective nucleic acids per tube and the specific activities of the purified samples under the assumption that the latter were representative samples of the nucleic acids present in respective tubes. Such an assumption may be justified because the relative yield of nucleic acids in our fractionation procedure was largely independent of the absolute amount of the acids (Table IV). It was also found that the magnitude of contaminating ^{32}P was independent of the amount of nucleic acids present.

Effect of DNase treatment

In order to examine the effect of the removal of DNA on the ^{32}P uptake, the nuclei were incubated for 15 min with varying concentrations of DNase in sucrose–Tyrode medium fortified with magnesium. The control tubes were also incubated with the same medium lacking DNase. As shown in Table V, a selective destruction of DNA in DNase-treated nuclei could be detected. However, during the subsequent incubation a considerable amount of DNA was released from the control and treated nuclei. In subsequent experiments, the extent of the removal of DNA by DNase was determined (somewhat arbitrarily) from the whole content of the tubes at stage C of Table V, *i.e.*, at the end of incubation for ^{32}P incorporation, the possible release of DNA from the nuclei into the medium during this incubation being disregarded.

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TABLE IV

AMOUNT AND RADIOACTIVITY OF DNA FROM THE NUCLEI SUBJECTED TO DNASE TREATMENT OR ADDITION OF SALMON SPERM DNA

Nuclei were digested with DNase (125 μg/ml), washed, and suspended in sucrose–Tyrode medium with and without DNA (4 mg/ml).

DNase treatment	Addition	μg DNA-P/tube			Specific activity (counts/min/μg P)	Total activity (counts/min/tube)
		Original	Extracted	Relative yield (%)		
—	—	110	66	57	1.63	189
	DNA	342*	209	61	0.40	136
+	—	42	21	50	0.02	1
	DNA	323*	202	63	0.41	131

* The reason why the effect of DNase treatment was not reflected by the amount of DNA in the tubes supplemented with DNA is not clear.

TABLE V

CHANGE IN QUANTITY OF DNA AND RNA DURING THE TREATMENT WITH DNASE AND SUBSEQUENT INCUBATION OF NUCLEI

Samples were taken at the following stages. A: Original nuclear suspension. B: After incubation with or without DNase (125 μg/ml) for 15 min; the whole content of the tubes was subjected to analysis. Then the nuclei were separated by centrifugation, resuspended in sucrose–Tyrode medium with or without additions and incubated for 90 min. C: The whole content of the tubes as well as the nuclei separated from the medium by centrifugation was analysed.

Stage	Addition	Fraction	μg P/tube incubated:			
			without DNase (control)		with DNase (test)	
			DNA	RNA	DNA	RNA
A. Initial	—	Total	170	33	170	33
B. After first incubation (DNase)	—	Total	157	29	42	31
C. After second incubation (^{32}P)	—	Total	116	30	42	29
		Sediment	89	18	42	29
	Salmon sperm DNA	Total	342	26	323	36
		Sediment	—*	—*	67	25
	Yeast RNA	Total	121	228	44	235
		Sediment	67	38	22	90

* Owing to the high viscosity of DNA solution added the nuclei could not be separated by ordinary centrifugation.

Table VI shows the extent of removal of DNA and the change in total activity of OASP, RNA and DNA of the nuclei digested with different concentrations of DNase. The incorporation into DNA and RNA was progressively impaired by the increase in the extent of the removal of DNA. Apparently the incorporation into DNA was more sensitive to the removal of DNA than that into RNA. The incorporation into OASP was much less sensitive in this respect; it decreased only to 60% of the control when the incorporation into nucleic acids was abolished almost completely

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TABLE VI

EFFECT OF REMOVAL OF DNA ON ^{32}P INCORPORATION OF ISOLATED NUCLEI

Expt. No.	Concentration of DNase (μg ml)	Amount of DNA (% of control)	Total activity (counts min tube)		
			OASP	RNA	DNA
M23	0	100	13,950	1,160	137
	16	97	18,600	1,180	115
	63	92	19,000	782	83
	125	64	11,500	452	4
M21	0	100	15,810	645	204
	125	31	15,010	150	21
	250	29	12,400	78	17
	500	27	9,500	0	18
	1,000	26	8,000	11	0

by the removal of about 70 % of the DNA, and even a slight stimulation of incorporation was observed with OASP when small amounts of DNA were removed.

During the course of experiments it was noticed that it was the concentration of DNase used rather than the extent of the removal of DNA that determined the extent of impairment of the incorporation. Also the different batches of DNase could differ markedly in their effect on the nuclei.

Restoration of the impaired incorporation activity of DNase-treated nuclei by addition of nucleic acids

Since it was anticipated that the effect of DNA in the incubation medium on the incorporation activity of DNase-treated nuclei was non-specific, no attempt was made to test the effect of homologous DNA. Salmon sperm DNA and yeast RNA were used throughout. From Table V it is seen that the nucleic acids added may be taken up and retained by the nuclei; the effect was especially noticeable with DNase-treated nuclei (lines 6 and 8). There was also some indication that a part of DNA in the nuclei was dislodged by the RNA added (line 8).

TABLE VII

EFFECT OF NUCLEIC ACIDS AND SOME OTHER COMPOUNDS IN THE INCUBATION MIXTURE ON THE INCORPORATION OF ^{32}P INTO CONTROL NUCLEI

Expt. No.	Addition (per tube)	Total activity (% of control)		
		OASP	RNA	DNA
M24	4 mg DNA	70	88	72
M25	1 mg RNA	133	146	119
	2 mg RNA	132	127	110
	4 mg RNA	151	78	84
M38	2 mg RNA	84	96	29
M26	2 mg 2',3'-mononucleotides	398	88	57
	2 mg chondroitin sulphate	107	115	97
M30	4 mg chondroitin sulphate	117	86	114
M38	2 mg hyaluronate	40	33	22
	2 mg heparin	61	41	39
	2 mg egg albumin	91	112	80

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Usually the presence of nucleic acids in the incubation medium did not very much affect the total activity of the control nuclei (Table VII), but occasionally there was a remarkable inexplicable diminution of the incorporation activity (Table VII, M38). In experiments involving the DNase digestion of, and addition of nucleic acids to, the nuclei, the total activity of individual fractions was usually expressed in % of the undigested controls receiving the same addition, but in some experiments such "addition controls" were not run: the undigested control without addition served as the reference standard (values in parentheses in Table VIII).

TABLE VIII

EFFECT OF NUCLEIC ACIDS AND SOME OTHER COMPOUNDS IN THE INCUBATION MEDIUM ON THE INCORPORATION OF ^{32}P INTO NUCLEI TREATED WITH DNASE

Expt. No.	Concentration of DNase (μg/ml)	Fraction of DNA removed (%)	Addition (per tube)	Total activity (% of control*)		
				OASP	RNA	DNA
M24	125	64	—	97	17	1
			4 mg DNA	107	176	90
			4 mg RNA	82	97	44
M20	1000	84	—	72	0	8
			2.5 mg DNA	(89)	(106)	(16)
			2.5 mg RNA	(110)	(46)	(9)
M25	125	63	—	66	15	0
			1 mg RNA	61	41	35
			2 mg RNA	60	57	34
			4 mg RNA	57	83	64
M26	125	74	—	64	10	11
			2 mg RNA	(114)	(30)	(33)
			2 mg 2′,3′-mononucleotides	125	15	42
			2 mg chondroitin sulphate	69	27	44
M19	1000	66	—	150	3	1
			2.5 mg chondroitin sulphate	(184)	(9)	(1)
M40	250**	38	—	138	38	21
			2 mg RNA	115	48	118
			2 mg chondroitin sulphate	117	16	58
M39	63**	23	—	107	87	75
			2 mg RNA	107	115	136
			2 mg chondroitin sulphate	124	83	132
			4 mg chondroitin sulphate	79	96	101
M38	125**	17	—	81	43	57
			2 mg RNA	131	87	191
			2 mg heparin	120	83	58
			2 mg hyaluronate	149	157	215
			2 mg egg albumin	95	67	84

* Undigested controls also received additions except for the cases in parentheses, where undigested controls did not receive additions.

** In these experiments a different batch of DNase from that used in other experiments was employed. The results with this batch were much inferior to those obtained with the previous batch as regards removal of DNA and impairment of incorporation.

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The results summarised in Table VIII now clearly indicate the following points.

(1) Both DNA and RNA, when present in the incubation mixture of DNase-treated nuclei, were effective in restoring the lost incorporation activity, provided the digestion with DNase was not very extensive (M24).

(2) This effect of nucleic acids increased with their concentration* (M25).

(3) When a higher concentration of DNase was used and the removal of DNA was exhaustive, both RNA and DNA restored the incorporation into RNA, but not into DNA (M20).

Attempts to restore the lost incorporation activity by addition of compounds other than nucleic acids

The next step was to examine non-nucleotide polyanions and some other compounds for the ability to restore the lost incorporation activity of the DNase-treated nuclei. Unfortunately the results were in most cases not clear-cut. For one thing, a new batch of DNase was used in a few experiments, and this was found to be not so effective in removing the DNA from the nuclei as that used in preceding experiments. Further, some compounds tested brought about a profound modification of the incorporation activity in the control nuclei, making the results ambiguous (Tables VII and VIII).

Chondroitin sulphate did not significantly affect the incorporation into the control nuclei. Its effect on the DNase-treated nuclei was either almost equal (M26, M39) or inferior to (M19, M40) that of RNA. Hyaluronate inhibited the incorporation into the control nuclei rather severely. But the activity of the DNase-treated nuclei was higher than that of the control nuclei in the presence of hyaluronate. It seems therefore that the depression of incorporation caused by the depletion of DNA was relieved by hyaluronate**. Heparin also had an inhibitory effect on the control nuclei and its effect on the DNase-treated nuclei was not very conspicuous.

Egg albumin, as an example of non-anionic compounds having no significant effect on the control nuclei, might have a slight restoring ability, but compared with RNA and hyaluronate, its effect was not significant. The effect of a mixture of 2',3'-mononucleotides was also ambiguous, because it caused a moderate inhibition of the incorporation into DNA of the control nuclei together with a considerable enhancement of the incorporation into OASP in both control and DNase-treated nuclei.

DISCUSSION

The results of the present investigation confirmed in essentials the findings of the previous investigators. As was indicated by FRIEDKIN AND WOOD[7], BREITMAN AND WEBSTER[6] or FICQ AND ERRERA[23] with calf thymus nuclei, DNase impaired the incorporation of the precursor into DNA in isolated nuclei. It also affected the incorporation into RNA, and to a lesser extent, that into OASP. Such an impairment could be relieved by exogenous nucleic acids. These results closely parallel the

* However, higher concentration of RNA might affect the incorporation into the nucleic acids of the control nuclei (Table VII, M25).

** In this particular experiment (M38) even added RNA inhibited the incorporation into DNA of the control nuclei (Table VII).

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pioneering findings of ALLFREY *et al.*[1] concerning the effect of various nucleotide compounds on the restoration of impaired incorporation of labelled precursors into protein and RNA of the DNase-treated nuclei.

However, an extensive removal of DNA ultimately resulted in a different response of nuclear DNA and RNA with regard to their ability to recover the incorporation activity on addition of exogenous nucleic acids; while incorporation into RNA was partially or fully restored, that into DNA was scarcely regained. This indicates that the bulk of the ^{32}P incorporation into nuclear RNA can proceed without an amount of pre-existent DNA which is indispensable for the ^{32}P incorporation into DNA. Such findings may at first sight seem to be in line with the synthesis of deoxypolynucleotide by the polymerising enzyme in the supernatant fraction of mammalian cells[9, 10], which, like the corresponding bacterial enzyme[22], requires preformed DNA as the primer. However, since even the addition of heterologous DNA failed to restore the incorporation into DNA in the nuclei treated extensively with DNase, the significance of pre-existing DNA in the incorporation into DNA in isolated nuclei must be something more complicated than its function simply as a "primer".

Although some of the results of the present investigation were not very conclusive, the main body of results would suggest that the favourable action of the exogeneous DNA and RNA on the DNase-treated nuclei is not peculiar to nucleotide compounds. The hypothesis previously proposed by ALLFREY AND MIRSKY[8] ascribing the action of added nucleic acids to their function as the potential source of high-energy nucleotides becomes thus rather unlikely.

In animal cells the DNA is "neutralised" by basic proteins such as protamines and histones, and the removal of DNA without touching the basic proteins will leave the nuclei positively charged, and this will probably be the cause of the reduction of the ability of DNase-treated nuclei to form ATP and of the consequent blockade of synthetic acitivity[8]. Polyanions of either nucleotide or non-nucleotide nature may then "neutralise" "basic" nuclei depleted of DNA.

SPIEGELMAN[24] showed that in lysed protoplasts of *B. megaterium* the depletion of DNA did not abolish the ability to synthesise DNA as well as RNA or protein. This was confirmed by OTSUJI AND TAKAGI[25] with lysed protoplasts of *E. coli*. The difference between animal cells and bacterial cells may partly be explained by the fact that DNA in bacterial cells is, unlike in animal cells, apparently complexed with some amines of relatively low molecular weight such as putrescine or spermidine[26], which, on elimination of DNA, may easily be lost from the protoplast and will not cause a serious disturbance of electrostatic charge distribution around the synthetic mechanisms still operative on the lysed protoplasts.

Considerable incorporation of ^{32}P into OASP was noticed in our present study, and it was not as sensitive to the removal of DNA as ^{32}P incorporation into nucleic acids. However, the nature of this incorporation into OASP was not studied, and what fraction of it represented the formation of nuclear ATP therefore remains obscure.

The results reported here are consistent with the idea that for DNA synthesis in the nucleus preformed specific DNA is needed for transmitting the genetic information to the progeny molecules, but it is not required for the synthesis of nuclear RNA for which the genetic information is transmitted from DNA only indirectly *via* some sort of macromolecules other than DNA. But it must be emphasized that the present study cannot be taken even as a partial proof of this. Firstly, only the incorporation

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of ^{32}P, not the net synthesis, was followed *in vitro*; secondly, no information was collected about the formation of specific macromolecules. Further, the removal of DNA was not absolutely complete, and one does not know what role the DNA remaining after DNase treatment played in the observed anabolic processes of the nuclei which were restored by the polyanions.

NOTE ADDED IN PROOF

After this paper had been submitted for publication, ALLFREY AND MIRSKY[27] reported an extensive investigation on the effects of various non-nucleotide polyanions, including chondroitin sulphate and polyethylene sulphonate, upon the DNase-treated nuclei. Their results very clearly confirmed our suggestion as to the role of DNA as a polyanion in the anabolic processes of isolated nuclei. Thanks to the improvement of technique indicated by the American authors, it has also been demonstrated in this laboratory[28] that in the presence of chondroitin sulphate the nuclei can be digested with DNase without their ability to incorporate ^{32}P into RNA and DNA being affected; but in this case also, an exhaustive removal of DNA completely abolished the incorporation into DNA, whereas that into RNA was fully retained under the same conditions. Thus, ambiguous points of the present paper have now largely been eliminated.

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REFERENCES

1 S. OSAWA, V. G. ALLFREY AND A. E. MIRSKY, *J. Gen. Physiol.*, 40 (1957) 491.
2 V. G. ALLFREY, A. E. MIRSKY AND S. OSAWA, *J. Gen. Physiol.*, 40 (1957) 451.
3 R. LOGAN, *Biochim. Biophys. Acta*, 26 (1957) 227.
4 V. G. ALLFREY AND A. E. MIRSKY, *Proc. Natl. Acad. Sci. U. S.*, 43 (1957) 821.
5 T. R. BREITMAN AND G. C. WEBSTER, *Biochim. Biophys. Acta*, 27 (1958) 408.
6 T. R. BREITMAN AND G. C. WEBSTER, *Federation Proc.*, 17 (1958) 194.
7 M. FRIEDKIN AND H. WOOD, IV, *J. Biol. Chem.*, 220 (1956) 639.
8 V. G. ALLFREY AND A. E. MIRSKY, *Proc. Natl. Acad. Sci. U. S.*, 43 (1957) 589.
9 R. MANTSAVINOS AND E. S. CANELLAKIS, *Biochim. Biophys. Acta*, 27 (1958) 661.
10 F. J. BOLLUM AND V. R. POTTER, *J. Biol. Chem.*, 233 (1958) 478.
11 M. SEKIGUCHI AND A. SIBATANI, *Biochim. Biophys. Acta*, 28 (1958) 455.
12 A. SIBATANI, *Exptl. Cell Research*, (in the press).
13 H. K. KIHARA, M. AMANO AND A. SIBATANI, *Biochim. Biophys. Acta*, 21 (1956) 489.
14 L. HAHN AND H. VON EULER, *Arkiv Kemi, Mineral. Geol.*, A22 No. 23 (1946).
15 A. SIBATANI, *Biochim. Biophys. Acta*, 25 (1957) 592.
16 D. W. VAN BEKKUM, *Biochim. Biophys. Acta*, 25 (1957) 487.
17 S. OSAWA, K. TAKATA AND Y. HOTTA, *Biochim. Biophys. Acta*, 28 (1958) 271.
18 T. SAKAKI, *Proc. 7th Symposium on Nucleic Acids, Nagoya, Japan, 1958*, p. 11.
19 R. M. S. SMELLIE, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. 2, Academic Press, Inc., New York, 1955, p. 393.
20 P. BOULANGER AND J. MONTREUIL, *Biochim. Biophys. Acta*, 9 (1952) 619.
21 S. OSAWA AND T. SAKAKI, *Biochim. Biophys. Acta*, 25 (1957) 195.

[22] M. J. BESSMAN, I. R. LEHMAN, E. S. SIMMS AND A. KORNBERG, *J. Biol. Chem.*, 233 (1958) 171.
[23] A. FICQ AND M. ERRERA, *Exptl. Cell Research*, 14 (1958) 182.
[24] S. SPIEGELMAN, in W. D. MCELROY AND B. GLASS, *The Chemical Basis of Heredity*, Johns Hopkins Press, Baltimore, 1957, p. 232.
[25] N. OTSUJI AND Y. TAKAGI, *Proc. 7th Symposium on Nucleic Acids, Nagoya, Japan, 1958*, p. 6.
[26] D. L. KEISTER, *Federation Proc.*, 17 (1958) 84.
[27] V. G. ALLFREY AND A. E. MIRSKY, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 981.
[28] Y.-T. TCHOE AND A. SIBATANI, *Nature*, in the press.

STUDIES ON THE CARBOHYDRATE METABOLISM OF THE LIVER FLUKE *FASCIOLA HEPATICA*

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SUMMARY

Fasciola hepatica utilizes carbohydrate anaerobically at a high rate. When glucose is omitted from the medium, the amount of glycogen utilized varies from 84–97 μmoles of glucose units/g wet wt./6 h. The rate of glucose utilization from the outside medium varies from 110–180 μmoles/g wet wt./6 h. Production of volatile fatty acids accounts for almost all of the carbohydrate utilized anaerobically. These acids were identified as propionic and acetic acids in an approximate ratio of 3:1. Only 4–9% of the metabolized carbohydrate is converted to lactic acid. The rates of glucose utilization, lactic acid and volatile fatty acid production are only slightly decreased in the presence of atmospheric oxygen. Oxygen is utilized by these organisms when available. The respiratory quotient varies from 1.56–2.2. Anaerobic metabolism of flukes with ligatured oral openings is identical with intact organisms. This indicates that neither the absorption of glucose from outside medium, nor the excretion of the metabolic products are carried out through the gut of the organism.

INTRODUCTION

Previous studies indicated that carbohydrate is the major, if not the exclusive source of energy for many parasitic helminths[1,2]. The end products of carbohydrate metabolism vary from one species to another. As early as 1926, WEINLAND AND VON BRAND[3] observed that the utilization of carbohydrate by *Fasciola hepatica in vitro* is associated with the production of higher fatty acids and possibly butyric acid. Their experiments were carried out on the European variety of these organisms. The experiments reported in this paper were carried out on a variety of *Fasciola hepatica* which is predominant on the Gulf Coast of this country. In these experiments, the rate of endogenous carbohydrate utilization and of glucose uptake were measured under

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