Incorporation of ^{32}P *in vivo* into deoxyribonucleic acid (DNA) of rabbit appendix*

Evidence has been accumulated indicating the presence of different DNA molecules in a single tissue[1]. This paper reports that different kinds of DNA as fractionated by anion exchange chromatography[2] are indistinguishable with respect to the rate of incorporation of ^{32}P. Furthermore, no heterogeneous labelling of DNA fragments was found, in sharp contrast to the well-known unequal distribution of ^{32}P in RNA molecules under similar experimental conditions[3-6].

Inorganic ^{32}P (0.5 to 1 mc per animal) was injected intravenously to albino rabbits (2000 g) which were sacrificed 2 h later**. The appendix was then quickly removed and washed thoroughly with cold physiological saline. The essential steps of preparing the DNA labelled with ^{32}P included (1) isolation of nucleohistone by the method of MIRSKY AND POLLISTER[8], (2) precipitation of DNA from the nucleohistone solution in 2 *M* NaCl by addition of an equal volume of ethanol, and (3) further deproteinization of the crude DNA with duponol[9]. 200 to 230 mg of DNA were obtained from 50 g of fresh tissues; the product was a white fibre, highly polymerized, and almost completely free from protein. (It is not unreasonable to suspect that non-specific incorporation and/or adsorption of active phosphate to DNA might occur during the course of isolation. This possibility was excluded by the following test: appendix tissue from a rabbit that had not received ^{32}P was homogenized in the comparable volume of saline containing about 5 times as much ^{32}P as should be found in the acid-soluble fraction of the tissue when the isotope is administered to the animal. DNA was then isolated in exactly the same way as mentioned in the text. Only a negligible radioactivity was found in this DNA preparation.)

TABLE I. INCORPORATION *in vivo* OF ^{32}P INTO DIFFERENT DNA FRACTIONS OF RABBIT APPENDIX

Fraction No.			*1*	*2*	*3*	*4*	*5*	*6*	*7*
Elution medium		*Unfractionated*	*0.1 M NaCl pH 7*	*0.2 M NaCl pH 7*	*0.2 M NaCl-II pH 7*	*0.4 M NaCl pH 7*	*0.6 M NaCl pH 7*	*0.5 M NaCl pH 9*	*0.2 N NaOH*
R.S.A.§	Expt. I	100	- -	112	- -	-	112	112	110
	Expt. II	193	204	184	187	189	186	- -	160

§ Relative specific activity = $\frac{\text{c.p.m./}\mu\text{g DNA phosphorus}}{\text{c.p.m. administered into single animal}} \times 10^8$.

In the first experiment, the DNA labelled with ^{32}P was fractionated by means of ECTEOLA-cellulose[10]. Two methods were used. First, 18 mg of DNA were allowed to be adsorbed by 5 g of resin, and four classes of DNA were obtained by the fractional extraction with 0.2*M*, 0.6*M* NaCl both at pH 7.1, 0.5*M* NaCl at pH 9.0 and finally with 0.2*N* NaOH (Expt. 1 in Table I). Secondly, another preparation of DNA labelled with ^{32}P was submitted to chromatographic fractionation on a column following the method of BENDICH *et al.*[2]. The elution pattern shown in Fig. 1 was closely similar to theirs. In Table I are listed the relative specific activities of various fractions together with those of unfractionated samples.

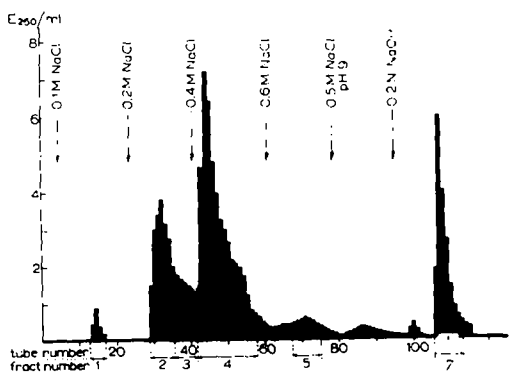

Fig. 1. Chromatography of DNA by method of BENDICH *et al.*[2]. 44.5 mg DNA on 8 g ECTEOLA-cellulose (17 × 1.55 cm). Flow rate, 1 tube (5 ml)/30 min. The recovery exceeded 94%.

* Supported by a grant from the Rockefeller Foundation, and the grant for Fundamental Scientific Research of the Ministry of Education awarded to "The Cooperative Research on The Nucleic Acids and Nucleoproteins".

** SMELLIE *et al.*[7] showed that the specific activity of DNA of rabbit appendix reaches a maximum 8 to 10 h after the injection of 1 mc ^{32}P. A higher isotope content in the sample naturally facilitates the counting procedure, but in order to detect a non-uniform labelling of DNA, if any, it seems preferable to adopt very short time intervals; thus the period of 2 h was chosen as a compromise between these two requirements.

The result demonstrates a remarkable uniformity of ^{32}P incorporation into different classes of DNA, suggesting that all DNA molecules are synthesized at the same rate in the course of cell division. It should be noted, however, that a considerable degree of heterogeneous labelling of different DNA fractions has been recently observed[11] when the cell suspension of the rabbit bone marrow or isolated nuclei of the rabbit thymus are incubated *in vitro* with thymidine-^{14}C. The discrepancy might be attributed to the difference in the isotopic precursors used or merely to the difference of experimental conditions such as between those *in vivo* and *in vitro*.

In the next experiment, a preparation of DNA labelled with ^{32}P was digested with crystalline deoxyribonuclease, and the fragments were separated on a Dowex-1-formate column using formic acid and formic acid-ammonium formate mixture as eluants. The effluent was roughly classified into six fractions according to the elution concentrations. No particular effort was made to identify each fraction obtained. Here again, no heterogeneous distribution of ^{32}P in different DNA fragments was found (Table II) despite the fact that the ratio of E_{260} to E_{275} varied widely through the fractions. The result obtained here is in good agreement with that of MOLDAVE AND HEIDELBERGER[5] who reported intramolecular homogeneity in DNA synthesis as seen by the incorporation of ^{32}P and glycine-2-^{14}C. Their results together with ours would indicate that phosphorylation of DNA precursors takes place in a very short period so that non-uniform incorporation of ^{32}P is not detectable in such a time interval studied. However a definite conclusion on this matter will have to be left until a more precise fractionation of ^{32}P-labelled DNA digests is undertaken.

TABLE II

DISTRIBUTION OF ^{32}P AMONG DIFFERENT FRACTIONS OF FRAGMENTS OF RABBIT APPENDIX DNA DIGESTED WITH DEOXYRIBONUCLEASE

Fraction No.		*1*	*2*	*3*	*4*	*5*	*6*	*7*
	Unfractionated							
R.S.A.	174	157	157	149	176	170	133	199

Biological Institute, Faculty of Science, Nagoya University, Chikusa-Ku, Nagoya (Japan)

SYOZO OSAWA
TOMOHIKO SAKAKI

[1] E. CHARGAFF, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press, Inc., New York, 1955, p. 307.
[2] A. BENDICH, J. R. FRESCO, H. S. ROSENKRANZ AND S. M. BEISER, *J. Am. Chem. Soc.*, 77 (1955) 3671.
[3] P. BOULANGER AND J. MONTREUIL, *Biochim. Biophys. Acta*, 9 (1952) 619; *Compt. rend.*, 236 (1953) 2275.
[4] J. SACKS, P. D. HURLEY AND Y. M. YOUNG, Jr., *J. Biol. Chem.*, 214 (1955) 723; J. SACKS AND K. D. SMARTH, *J. Biol. Chem.*, 223 (1956) 423.
[5] K. MOLDAVE AND C. HEIDELBERGER, *J. Am. Chem. Soc.*, 76 (1954) 679.
[6] L. E. HOKIN AND M. R. HOKIN, *J. Histochem. Cytochem.*, 2 (1954) 395.
[7] R. M. S. SMELLIE, G. F. HUMPHREY, E. R. M. KAY AND J. N. DAVIDSON, *Biochem. J.*, 60 (1955) 177.
[8] A. E. MIRSKY AND A. POLLISTER, *Proc. Natl. Acad. Sci. U.S.*, 28 (1942) 344.
[9] E. R. M. KAY, N. S. SIMMONS AND A. L. DOUNCE, *J. Am. Chem. Soc.*, 74 (1952) 1924.
[10] E. A. PETERSON AND H. A. SOBER, *J. Am. Chem. Soc.*, 78 (1956) 751.
[11] F. FRIEDKIN AND H. WOOD, IV, *J. Biol. Chem.*, 220 (1956) 639.

Received March 23rd, 1957

The conversion of 1-^{13}C-D-glucuronolactone to 5-^{13}C-L-xylulose in a pentosuric human

ENKLEWITZ AND LASKER[1] reported in 1935 that D-glucuronolactone greatly enhances the excretion of L-xylulose in individuals with essential pentosuria. More recently, we confirmed their finding and also showed that small increases in urinary xylulose levels occur when normal humans and guinea pigs are fed glucuronolactone[2]. The high yield of xylulose in pentosuric individuals suggests