The incorporation of ^{14}C-deoxynucleotides into deoxyribonucleic acid by soluble mammalian enzymes

Previous studies on the properties of the soluble supernatant fraction obtained from rat-liver homogenates by centrifugation at 106,000 × g[1,2,3] have now been extended to the synthesis of DNA by this cell fraction. It has been found (a) that incorporation of deoxynucleotides into DNA occurs in this soluble fraction of rat liver, (b) that addition of rat-liver DNA to the incubation mixture increases the incorporation, (c) that an increased incorporation is observed when all four nucleotides are present in the incubation mixture, and (d) that addition of an extract of regenerating rat-liver nuclei stimulates the incorporation.

Expt. 1, Table I, shows that the incorporation of deoxyadenylic acid into DNA is enhanced by dialysis of the enzyme system; this probably reflects the greater conversion of the deoxymononucleotides to the di- and triphosphate stage which occurs after dialysis of this system, as has been shown previously[3]. Expt. 2 shows that addition of DNA, prepared from rat liver by the method of ZAMENHOF[4], results in a 2-fold increase in the incorporation of the deoxymononucleotides into DNA, suggesting that a primer may be required, as was observed by KORNBERG *et al.*[5] in a bacterial system. Expt. 3 shows that all four deoxymononucleotides are incorporated into DNA to a similar extent. It is of particular interest to note that the incorporation of deoxyadenylic acid is not reduced by the presence of a 1000-fold excess of adenosine triphosphate, indicating a complete specificity of the incorporating system for the deoxynucleotide.

TABLE I

INCORPORATION OF ^{14}C-DEOXYNUCLEOTIDES INTO DNA BY SOLUBLE MAMMALIAN ENZYMES

The incubation mixture is as described under ref. [3] (Table I). At the end of the incubation the DNA was extracted by the method of HECHT AND POTTER[7] and counted. Supernatant fraction (106,000 × g) of a homogenate of regenerating rat liver 36 h after hepatectomy, dialyzed for 30 h against two 4-l changes of 0.05 M tris(hydroxylmethyl)aminomethane buffer, pH 7.4. Abbreviations: C, deoxycytidylic acid; T, thymidylic acid; A, deoxyadenylic acid; G, deoxyguanylic acid.

Experiment	*Treatment*	*Total c.p.m. incorporated into DNA in the presence of the following precursor ^{14}C-deoxynucleotides*			
		C	*T*	*A*	*G*
1	Dialyzed	—	—	30	—
	Non-dialyzed	—	—	5	—
2	Dialyzed; with added DNA	35	60	—	—
	Dialyzed; without added DNA	20	29	—	—
3	Dialyzed; with added DNA	54	58	52	63
4	Dialyzed; with added DNA	50	100	130	261
5	Non-dialyzed; with added DNA	—	—	—	27
	Non-dialyzed; with added DNA and nuclear extract	—	—	—	76

Expt. 4 shows that when two or more ^{14}C-deoxymononucleotides are incubated concurrently, an additive effect is obtained, with some stimulation being observed when all four nucleotides are present together. Expt. 5 shows that addition of a soluble extract of nuclei of regenerating rat liver greatly stimulates the incorporation of the four deoxynucleotides into DNA.

The evidence that the radioactivity is associated with deoxyribonucleic acid is as follows. The incorporated radioactivity is acid-insoluble and is resistant to hydrolysis with 1.0 N NaOH or to heating with 0.1 N NaOH at 80° for 20 min. It can be extracted from the precipitate with hot 10% NaCl, and can be precipitated from this NaCl solution with 2.5 volumes of 95% alcohol. It is non-dialyzable, both against water and against 3% NaCl. The radioactivity can be liberated from the bound form by treatment with deoxyribonuclease and snake-venom phosphodiesterase and can be isolated from the enzymic hydrolysate in the form of the original radioactive deoxyribonucleotide by column chromatography under the conditions previously described[3].

The results so far obtained are in general agreement with those obtained in the bacterial system by KORNBERG *et al.*[5] and with those obtained with tritiated thymidine in a similar mammalian system recently developed by BOLLUM AND POTTER[6] although certain variations, particularly the effect of the nuclear extract, are evident.

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The molecular weight of ribonucleic acid prepared from ascites-tumor cells

Light-scattering measurements have been carried out on highly polymerized ribonucleic acid (RNA) prepared from ascites-tumor cells by the method of COLTER AND BROWN[1]. After extraction, the high molecular weight RNA was separated by precipitation with 1 *M* NaCl and kept at —40° until 12 h before the experiment. It was then thawed and dialyzed against pH 7.2 buffer (ionic strength (*I*), 0.02 sodium phosphate, 0.08 NaCl). The measurements were carried out in a 20 ml WITNAUER cell[2] with a BRICE photometer[3] with narrow-slit optics at angles between 25° and 145°. The solutions were clarified by centrifugation and filtration through a special filter[4]. Successive concentrations between 0.11 and 0.73 g/l were measured in a single cell using a micro burette[5] and mixing with a magnetic stirrer. Concentrations were measured by absorption at 260 mμ using the absorptivity of 21.0 lg^{-1} cm^{-1} obtained on an RNA sample dried to constant weight at 105°. The value of dn/dc (refractive index increment) was found to be 0.1716 and to be independent of concentration.

The results obtained gave a value of $10.05 \cdot 10^{-7}$ for $(Kc/R_{90})^{*}_{c=0}$ and an intrinsc dissymmetry of 1.14, resulting in a molecular weight of 1,090,000. (The experimental value for depolarization was found to be not greater than that obtained with bovine-serum albumin and, therefore, was not used in the calculations[6].) When the data were plotted according to ZIMM[7], the molecular weight was found to be 1,180,000 and the initial slope yielded a radius of gyration of 320 A. The curvature found in the zero-angle extrapolation plot suggests that the RNA is polydisperse. Analysis of the results in terms of the usual molecular models (random coil, sphere, rod and ellipsoid) showed that the data could be fitted reasonably only by rods or prolate ellipsoids. Using the light-scattering molecular weight of $1.1 \cdot 10^{6}$ and average particle lengths of 700–1,100 A, deduced from the intrinsic dissymetry and angular envelope of the scattering, along with a partial specific volume of 0.52[8], it is possible to calculate for prolate ellipsoids particle widths of 51–41 A This results in axial ratios of 14–27.

Electron micrographs, taken on similar preparations of RNA, have shown elongated particles which vary in both cross-section and length. The lengths of these particles ranged from 800 A to 2,000 A. They appeared to be tapered at the ends and the diameters measured at the center were between 40 and 70 A. These dimensions are considered to be in good agreement with those deduced from the light-scattering data.

A similar RNA preparation had an intrinsic viscosity of 0.40. Assuming 20% hydration and taking the light-scattering molecular weight of $1.1 \cdot 10^{6}$ and prolate ellipsoidal shape, one calculates from the data an axial ratio of 28 and dimensions of 40 A × 1,120 A. Such a hypothetical molecule would have a sedimentation constant of 29. Ultracentrifugal analysis at 0.03 g/l using ultraviolet optics presented a simpler picture than that obtained by schlieren optics[1]. Although there was some spreading of the boundaries, the RNA sedimented as two components, 60% with $s = 32$ and 40% with $s = 15$. The weighted average sedimentation coefficient ($s = 25$) of the

* K is an optical constant, c is the RNA concentration and R_{90} is the Rayleigh ratio at 90°.