COMPOSITIONAL HETEROGENEITY OF NORMAL AND MALIGNANT TISSUE DEOXYRIBONUCLEIC ACIDS (DNA)

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Received September 16, 1960

The experiments to be described show that the range of DNA heterogeneity of composition is the same for 10 normal and malignant mouse tissues and that this range is similar to that of certain bacterial DNA preparations. Previous paper chromatographic determinations which demonstrated that the mean molar purine and pyrimidine base compositions of normal and malignant mouse DNA preparations were the same (Kit, 1960b) are confirmed by an alternate method. The latter experiments had left unanswered the question whether the results on the mean base compositions masked significant differences between specific DNA fractions in individual tissues (Bendich, Pahl, and Beiser, 1956; Chargaff, Crampton, and Lipshitz, 1953; Kit, 1960c).

An ingenious approach to the study of DNA heterogeneity of composition has been developed by Doty, Marmur, and Sueoka (1959) and Marmur and Doty (1959). These investigators have shown that the thermal denaturation temperature (Tm), defined

*Aided in part by grants from the American Cancer Society, The Leukemia Society, Inc. and the National Cancer Institute (C-4064, C-4238). The experiments were performed with the expert technical assistance of Andrew Cox and David Cullop.

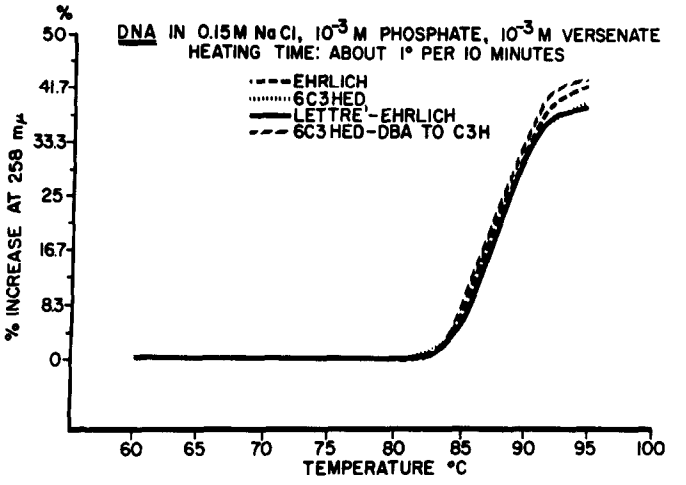

Figure 1: Per cent increase of optical density with temperature for DNA of ascites tumor cells. DNA concentrations: 0.002%.

as the midpoint of the optical density-temperature curve (Figure 1), increases in linear fashion with the guanine and cytosine (G + C) content of DNA. From the breadth of the thermal transition, estimates of the standard deviation ($\sqrt{\ }$) expressed in terms of mole % (G + C) may be made (Doty, Marmur, and Sueoka, 1959; Marmur and Doty, 1959).

Mouse DNA was prepared by p-aminosalicylate - phenol extraction of 1-5 grams wet weight of tissues (Kit, 1960a). Extinction coefficients, intrinsic viscosities, and sedimentation constants of the preparations are shown in Table I. These parameters were very similar for all the mouse tissues and are of the same order of magnitude as those obtained for high molecular weight DNA by other investigators (Butler, 1958; Laurence, Robins, and Shooter, 1957; Doty, McGill, and Rice, 1958). From the equations derived by Doty,

Table I

PROPERTIES OF THE DNA OF NORMAL MOUSE TISSUES AND TUMORS

Tissue		Tm	$2\sqrt{}$	% Hyperchromicity	$E^{1\%}_{1cm}$	$[\eta]^{26^\circ}$	$S_{20,w}$ +
Lung	(4)	87.9	5.4	45.1	193	49.5	24.1
Brain	(4)	87.7	5.4	44.0	207	65.0	24.5
Liver	(4)	87.8	5.7	39.2	207	56.0	25.7
Spleen	(4)	87.8	5.5	45.0	185	53.5	23.8
Kidney	(4)	87.7	5.7	44.2	191	53.8	23.3
6C3HED (diploid lymphoma)	(6)	87.8	5.5	45.2	181	52.3	23.9
6C3HED - DBA to C3H (tetraploid lymphoma)	(6)	87.7	5.5	45.1	186	58.4	25.4
Ehrlich (hypotetraploid carcinoma)	(7)	87.9	5.4	42.1	186	62.1	24.9
Lettre-Ehrlich (hyperdiploid carcinoma)	(7)	87.8	5.5	43.1	195	57.6	24.3
S91A (tetraploid amelanotic melanoma)	(2)	87.7	5.4	43.8	196	53.0	23.5

Values in parentheses signify the number of optical density-temperature curves carried out.

+ Sedimentation constants of DNA at 0.005% concentration obtained using the Spinco Model E analytical ultracentrifuge equipped with ultraviolet optics.

McGill, and Rice (1958) and Spitkovskii (1958), which relate intrinsic viscosity to weight average molecular weight, one may estimate the weight average molecular weights of the mouse DNA preparations to range from 5.3 - 7.2 x 10^6. Colorimetric analyses indicated that the present samples were contaminated by not more than 2-8% RNA.

The similarity of the thermal denaturation curves for four tumor DNA preparations is shown in Figure 1. The Tm values (Table I) were practically the same for all tissues. The precision of the measurements may be appreciated from the fact that the lowest Tm value of all measurements was 87.3 and the highest 88.1. The % hyperchromicity varied from 39.2% for mouse liver DNA to 45.2% for tumor 6C3HED DNA.

The slope of the curve relating the Tm values to the mole % (G + C) was 2.2 mole % (G + C) per degree increase in Tm. Doty, Marmur, and Sueoka (1959) and Marmur and Doty (1959) observed this slope to be 2.5. The middle 2/3 of the absorbance rise (17-83% of the total hyperchromicity) took place over a temperature range ($2\overline{\tau}$) of 5.4 - 5.7° (Table I). This figure may be corrected for the natural transition width by subtracting the corresponding quantity for the transition of an adenine-thymine DNA polymer. Doty, Marmur, and Sueoka (1959) have estimated the latter value to be 3.0. By multiplying the difference (5.5 - 3.0) by 2.2 mole % (G + C) per degree, one obtains an estimate of the mole % (G + C) corresponding to 2/3 of the transition [approximately 5.5 mole % (G + C)]. Heterogeneity of composition, as estimated in this way, was the same within experimental

error for all mouse DNA samples.

The following conclusions may be drawn: 1) Heterogeneity of composition of mouse DNA is similar to that of many bacterial DNA preparations and is less than that reported (Doty, Marmur, and Sueoka, 1959) for salmon sperm or calf thymus DNA. With DNA dissolved in 0.15M NaCl plus 0.015M sodium citrate, Doty and co-workers reported the following $2\sqrt{\ }$ values: D. pneumoniae, 4.4; E. Coli, 5.2; calf thymus, 7.4; and salmon sperm, 6.0. The $2\sqrt{\ }$ values for Serratia and D. pneumoniae DNA* dissolved in 0.15M NaCl, 10^{-3}M versenate, 10^{-3}M phosphate, pH7, and determined in this laboratory were 5.7 and 4.9, respectively, while the mean for all mouse preparations of Table I was 5.5. The small discrepency for D. pneumoniae DNA is not unexpected since the Tm values of a given DNA preparation are reduced and the $2\sqrt{\ }$ values increased by lowering the ionic strength of the solution. For example, the Tm and $2\sqrt{\ }$ of liver DNA dissolved in 0.15M NaCl, 0.015M citrate were 88.5° and 5.1°, respectively, whereas in 0.15M NaCl, 10^{-3}M versenate, 10^{-3}M phosphate, pH7, the values were 87.8° and 5.7°. Thus, the concept that a progressive increase in compositional heterogeneity necessarily occurs in going from bacteriophage to bacterial to vertebrate DNA appears to be invalid. The reduced heterogeneity of composition of mouse DNA preparations as compared with calf thymus or salmon sperm may be due to species differences, but might also be ascribed to recent improvements in the methods of preparation (Kit, 1960a). 2) A number of bacterial species and mice appear to have few if

* Bacterial DNA preparations were obtained through the kindness of Doctor Julius Marmur.

any DNA molecular types in common. 3) Hypothetical differences between the DNA of differentiated tissues of adult mice and various tumors cannot be attributed to differences in average (G + C) content or to the distribution of molecules having increased or decreased (G + C). Finally, the ratio of $\frac{G + C}{A + U}$ for the mixture of ribonucleic acid (RNA) molecules comprising about 90% of the total mouse tissue RNA was found to be about 1.8 (Kit, 1960c). The range of heterogeneity of RNA composition is not known as yet. The present study shows, however, that 4) the majority of DNA molecules do not have a base composition which reflects that of the RNA on the basis of complementary base pairing or identical base pairing.

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