*SIMILARITY OF DNAs ISOLATED FROM TUMOR-INDUCING VIRUSES OF HUMAN AND ANIMAL ORIGIN**

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Preparations containing type 12[1,2] or 18[2] adenovirus have been recently reported by Trentin, Yabe, and Taylor[1] and Huebner, Rowe, and Lane[2] to induce tumor formation in newborn hamsters. These are the first "human" viruses reported to possess carcinogenic activity. In confirmation of these reports, we have isolated types 12 and 18 adenovirus in highly purified form and have induced tumor formation with as little as 0.2 μg of type 12 adenovirus.[3, 4] The induction of tumors with "pure" virus provides further convincing evidence that the virus itself, rather than some adventitious contaminant, is actually responsible for tumor induction. In the present communication, we wish to report on several properties of the DNAs isolated from tumorigenic types 12 and 18 adenoviruses and from nontumorigenic types 2 and 4 adenoviruses. A comparison of these properties suggests several provocative hypotheses.

Experimental Methods.—Virus: Seed cultures of the various adenoviruses were kindly given to us by Dr. R. J. Huebner. Types 2, 4, 12, and 18 adenoviruses were grown, isolated, and purified by minor modifications of the procedure described for type 2 adenovirus.[5, 6]

Viral DNA: Highly purified adenovirus (0.5 to 2 mg) was (1) incubated with crystalline papain, followed by (2) Duponol treatment and centrifugation in CsCl solution as described by Watson and Littlefield[7] for Shope papilloma virus (the salt precipitation step was deleted). Treatment with papain was required to separate viral DNA from a viral protein component. Yields of 50–80 per cent of the viral DNA were obtained. Examination of the viral DNA preparations in the analytical ultracentrifuge revealed one very homogeneous sedimenting component with $S_{20,w}$ of 30–32.[8]

Buoyant density of viral DNA: Viral DNA at 0.5–2 μg per ml in CsCl solution (density 1.71, in 0.01 *M* Tris buffer, pH 8.1) was centrifuged in the Spinco E analytical ultracentrifuge[9] together with a density reference of 1 μg/ml of *Ps. aeruginosa* DNA (kindly given to us by Dr. N. Sueoka). Ultraviolet absorption photographs of the DNA bands were taken after 20 hr at 44,770 rpm and traced with a Spinco Analytrol. Densities were calculated[10] by comparison with the *Ps. aeruginosa* DNA standard. The latter was assigned a buoyant density of 1.727 to facilitate comparison with the DNA buoyant densities compiled by Schildkraut, Marmur, and Doty.[11]

Denaturation temperature (T_m): The T_m value for each viral DNA was determined in saline citrate (0.15 *M* NaCl plus 0.015 *M* sodium citrate, pH 7.4) with a thermostatically controlled Beckman DU spectrophotometer as described by Marmur and Doty.[12]

Results and Discussion.—Table 1 summarizes the buoyant density and T_m values that we have found for the DNAs isolated from types 2, 4, 12, and 18 adenoviruses. Also included are the corresponding values for the DNAs of the two

tumor-inducing animal viruses thus far reported: polyoma virus[13, 14] and Shope papilloma virus.[7] The base composition listed for each double-stranded viral DNA (expressed as per cent guanine-cytosine) was either determined by direct base analysis or calculated from its buoyant density[11] or T_m.[12] The base composition calculated from the buoyant density and T_m are in good agreement with each other and with the results of direct analysis (where carried out).

TABLE 1

SIMILARITY IN BASE COMPOSITION OF DNA-CONTAINING MAMMALIAN TUMOR VIRUSES

Virus	Tumor induction	Buoyant density	Denaturation temperature (T_m)	% Guanine-cytosine
Adenovirus type 2	No	1.716	92.5°	56*
Adenovirus type 4	No	1.718	92.5°	57*
Adenovirus type 12	Yes	1.708	89.0°	48†
Adenovirus type 18	Yes	1.709	89.0°	49†
Polyoma virus	Yes	1.709[13]	89.0°[14]	48†
Shope papilloma virus‡	Yes	1.711[7]	89.5°[7]	49§

* Base composition by direct chemical analysis.[5, 6]
† Calculated from buoyant density[11] and denaturation temperature.[12]
‡ The buoyant density reported for the DNA of Shope papilloma virus[7] (1.714) was changed to 1.711 for this comparison to compensate for the different buoyant density values assigned the reference DNA standard.
§ Base composition by direct chemical analysis.[7]

A comparison of the base compositions of these viruses reveals several interesting and startling features. First, it should be noted that the DNAs of the nontumorigenic types 2 and 4 adenoviruses have 56–57 per cent guanine-cytosine, while the DNAs of the tumorigenic types 12 and 18 adenoviruses have 48–49 per cent guanine-cytosine. Thus, both types 12 and 18 adenovirus DNA have base compositions quite different from that of types 2 and 4 and closer to that of cell DNA (mammalian cell DNA has 42–44 per cent guanine-cytosine).[15] It is possible that tumorigenic adenoviruses evolved from nontumorigenic adenoviruses by deletion of a piece of DNA rich in guanine-cytosine. This possibility is consistent with data showing that the DNA of type 12 adenovirus has a lower molecular weight than that of type 2 adenovirus.[8] An alternate possibility, suggested by the large differences in base composition, is that the tumorigenic and nontumorigenic adenoviruses are quite unrelated genetically. Further evidence is needed to settle this point.

Perhaps of much greater interest is the fact that the four tumor-inducing DNA-containing mammalian viruses, namely, adenovirus type 12, adenovirus type 18, polyoma virus, and Shope papilloma virus, have very similar buoyant densities, T_m values, and therefore base compositions. This similarity is conceivably fortuitous. Alternatively, it may reflect a common evolutionary origin for these viruses. A third and more exciting and speculative possibility is that virus tumorigenicity may depend upon its DNA having regions homologous in structure to a segment of host cell DNA. Thus, it may be hypothesized that a region of cell DNA of approximately one millionth that of the whole genome has a base composition similar to that of the tumor viruses. (There is approximately one million times as much DNA per cell as per adenovirus particle.) Tumor virus DNA may be able to replace or combine with this area of host cell DNA. This process would subvert the function of this region of host cell DNA whose role may involve the control of cell division.

Summary.—Tumorigenic and nontumorigenic types of human adenovirus were isolated; the buoyant density, denaturation temperature, and base composition of their DNAs were determined. The DNAs of nontumorigenic types 2 and 4 adenoviruses contained 56–57 per cent guanine-cytosine, while those of tumorigenic types 12 and 18 adenoviruses were surprisingly different and contained 48–49 per cent guanine-cytosine. A striking similarity between the DNAs of the tumor-inducing viruses of human origin, i.e., adenovirus types 12 and 18, and those of animal origin, polyoma virus and Shope papilloma virus, is noted: all four tumor viruses have very similar buoyant densities, denaturation temperatures, and base compositions. The possible implications for the evolution of tumor-inducing viruses and for the mechanisms of virus-induced tumorigenicity are briefly discussed.

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*DIALYSIS STUDIES, VIII. THE BEHAVIOR OF SOLUTES WHICH ASSOCIATE**

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The recent rapidly expanding interest in hemoglobin stems in part from the fact that, in addition to its interesting function, it is a readily available protein of the largest size thus far to yield to the detailed structural investigations of the chemist. It has become a useful model for many different types of biochemical investigations. These in turn have often contributed to a better understanding of the over-all structure of the molecule.

A study of hemoglobin was undertaken in this laboratory several years ago for several reasons. Apart from the challenge presented by an attempt to elucidate the sequential arrangement of the amino acids, it offered an excellent model with