[10] Chance, B., A. Ghosh, P. K. Maitra, and J. J. Higgins, *Abstracts*, Conference on Computers in Biology and Medicine, May 27–29, 1963; book in press.

[11] Ghosh, A., and B. Chance, *Biochem. Biophys. Res. Commun.*, in press.

[12] Higgins, J. J., these PROCEEDINGS, **51**, 989 (1964).

[12a] Wilson, A. T., and M. Calvin, *J. Am. Chem. Soc.*, **77**, 5948 (1955).

[13] Chance, B., and B. Hess, *J. Biol. Chem.*, **234**, 2421 (1959).

[14] Ghosh, A., F. Charalampous, Y. Sison, and R. Borer, *J. Biol. Chem.*, **235**, 2522 (1960).

[15] Chance, B., *Rev. Sci. Instr.*, **22**, 619 (1951).

[16] Boyer, P. D., and H. Theorell, *Acta Chem. Scand.*, **10**, 447 (1956).

[17] Chance, B., H. Conrad, and V. Legallais, *Program and Abstracts, Biophysical Society Meeting* (Cambridge, Massachusetts, 1958), p. 44.

[18] Gamertsfelder, G. R., and J. V. Holdam, in *Waveforms*, ed. B. Chance (New York: McGraw-Hill, 1949), vol. 19, p. 142.

[19] Brooks, H. B., in *Electrical Engineers' Handbook*, ed. H. Pender, W. A. Del Mar, and K. McIlwain (New York: John Wiley, 1936), vol. 4, p. 5–03.

[20] Chance, B., B. Hess, and A. Betz, *Biochem. Biophys. Res. Commun.*, in press.

[21] Denbigh, K. G., and F. M. Page, *Discussions Faraday Soc.* **17**, 145 (1954).

[22] Hastings, J. W., *Ann. Rev. Microbiol.*, **13**, 297 (1959).

[23] Pittendrigh, C. S., and V. G., Bruce, *Rhythmic and Synthetic Processes of Growth* (Princeton; Princeton University Press, 1957), p. 75.

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*BIOCHEMICAL STUDIES ON ADENOVIRUS MULTIPLICATION, VI. PROPERTIES OF HIGHLY PURIFIED TUMORIGENIC HUMAN ADENOVIRUSES AND THEIR DNA's**

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Communicated by Edward A. Doisy, April 21, 1964

Preparations containing adenovirus types 12[1, 2] and 18[2] have been found to induce tumors in newborn hamsters. We have previously studied the biochemical events associated with the replication of adenovirus type 2 in cell culture[3–8] and several of the chemical and physical properties of adenovirus type 2. In this paper, we present a comparison of some of the biological, chemical, and physical properties of highly purified tumorigenic adenovirus types 12 and 18 and their DNA's with those of nontumorigenic[9] adenovirus types 2 and 4. Preliminary reports of some of this work have appeared.[10–12]

Experimental Methods.—Cells, media, and virus: See Green and Piña for methodological details.[6] Seed cultures of the adenoviruses were generously supplied by Dr. R. J. Huebner. Stock virus was subsequently prepared by passage in KB cell monolayers. Plaque titration of the adenoviruses was performed by a modification of the procedure of Rouse, Bonifas, and Schlesinger.[13]

Virus purification: Adenovirus types 2 and 4 were cultivated, isolated, and purified as previously described.[6] In addition to the prototype strain of adenovirus type 12 (designated type 12A), three new strains kindly provided by Dr. Huebner were cultivated, purified, and studied (12B, 12C, and 12D). The following two modifications were employed in the purification of adenovirus types 12 and 18: (1) suspension cultures of KB cells were infected at 4–5 $\times$ 10^6 cells/ml and diluted to 3 $\times$ 10^5 cells/ml at two hr after the addition of virus; (2) infected cells were broken by sonic disruption for 5 min in the Raytheon 10-kc sonic oscillator.

The purified virus preparation (after the 2nd RbCl gradient) was made up to 1.0 ml with RbCl

(ρ = 1.34) in 0.01 *M* Tris buffer (pH 8.1 at 25°) and stored at 4°. Prior to use in various studies, the virus was dialyzed for two hr against two changes of 200 × volume of 0.01 *M* Tris buffer, pH 8.1. Purified adenoviruses tend to precipitate and lose infectivity in dilute buffer; therefore, experiments were carried out on freshly prepared (less than 5 days old) and dialyzed material.

Chemical analysis: The protein, DNA, RNA, and P content of the virus preparations were determined by micromethods previously described.[3, 4]

Tumorigenicity measurements: Virus preparations were diluted in 0.01 *M* Tris buffer, pH 8.1, and 0.05 ml was injected subcutaneously over the abdomen into newborn hamsters (less than 24 hr old). Penetration of virus into the abdominal cavity occurred by this route as indicated by the formation of tumors in the cavity. Animals were examined daily for tumors, and autopsies were performed on dead animals.

Preparation of viral DNA: Numerous attempts to isolate viral DNA from adenovirus type 2 by the phenol method[14] and several modifications gave low yields (5–20%). The method finally developed is as follows. Virus was dialyzed against 0.01 *M* Tris, pH 8.1 to remove RbCl and treated with crystalline papain (0.6 mg/ml) in 0.1 *M* acetate, pH 5.1 containing 0.1 *M* cysteine and 0.005 *M* versene. (The papain had no detectable DNase activity; H^3-thymidine-labeled DNA from adenovirus type 2 was incubated with papain under these conditions for 18 hr with less than 0.1% breakdown to an acid-soluble form.) After one hr at 37°, one fifteenth the volume of 5% sodium lauryl sulfate in 45% ethanol was added and the sample further incubated at room temperature for 30 min. Protein was removed by either of two procedures: (1) solid CsCl was added to provide a density of 1.45 and the solution centrifuged for 20 hr at 35,000 rpm in the SW 39 rotor of the Spinco preparative centrifuge. The DNA pellet was dissolved in 0.15 *M* NaCl-0.015 *M* sodium citrate. (2) The preparation was shaken with an equal volume of phenol (saturated with 0.1 *M* NaCl-0.05 *M* sodium phosphate, pH 6.7) for 15 min at 4°. After centrifugation at 14,000 × *g* for 10 min, the aqueous layer containing the DNA was removed and treated twice more with phenol. The final aqueous solution was dialyzed for about 24 hr against five changes of buffer (0.1 *M* NaCl-0.05 *M* sodium phosphate) to remove all traces of phenol. Viral DNA prepared by procedure (1) contained up to 5% protein, while that prepared by procedure (2) generally contained less than 1% protein. The data reported here were obtained with DNA prepared by the phenol procedure, although no differences have been observed in DNA prepared by either method. Yields of 50–80% of the DNA's from the various adenovirus types were obtained.

Physical studies: The buoyant density of viral DNA was determined by density-gradient equilibrium centrifugation as previously described.[12] The buoyant density of adenovirus (5–10 μg/ml) was analyzed in a similar manner using CsCl solution at ρ = 1.34 in 0.01 *M* Tris, pH 8.1.

Sedimentation velocity measurements on viral DNA were performed in 12-mm Kel-F cells at 35,600 rpm using the Spinco E analytical ultracentrifuge with ultraviolet absorption optics. Densitometer tracings of the photographic films were made with the Spinco analytrol and $S^0_{20,w}$ values calculated by standard procedures.[15] DNA was sedimented in 1 *M* NaCl-0.01 *M* Tris buffer, pH 7.5 to provide the $S_{20,w}$ values recorded here. As reported by Davison and Freifelder[16] with bacteriophage T7 DNA, and also observed here, variable results were frequently obtained with lower ionic strength solvents (0.15 *M* NaCl-0.015 *M* sodium citrate).

The thermal denaturation curves of viral DNA were determined as previously described.[12]

Results and Discussion.—Virus isolation: Yields of 0.5–2.0 mg of highly purified adenovirus type 12A were isolated from 5–10 × 10^8 infected cells (0.5–2 × 10^{10} plaque-forming units). Similar yields of adenovirus type 18 were obtained while 2 and 10 times the amount of adenovirus types 4 and 2, respectively, were isolated. Differences in the proliferative capacities of the adenovirus types probably account for the different yields.

Chemical analysis of purified adenoviruses: Fresh virus preparations were analyzed simultaneously to minimize possible analytical variation and facilitate comparison of one virus with another. Values for protein, DNA, and P of adenovirus types 2, 4, 12A, and 18 are presented in Table 1. Adenovirus types 2 and 4 contain 13.3 and 13.8 per cent DNA (average values) in agreement with previous analyses.[6] However, the results of analysis of 10 different preparations of adeno-

TABLE 1

CHEMICAL ANALYSES OF ADENOVIRUS TYPES 2, 4, 12, AND 18

Preparation	Protein (μg/ml)	DNA (μg/ml)	P (μg/ml)		% DNA*
Type 2					
(1)	1110	165	16.7		12.9
(2)	2820	443	43.2		13.6
(3)	3900	602	55.9		13.4
(4)	2000	299	—		13.0
(5)	750	121	—		13.8
				Avg.	13.3 (±0.4)†
Type 4					
(1)	507	83	8.19		14.1
(2)	1940	305	28.5		13.6
(3)	2850	455	46.8		13.8
				Avg.	13.8 (±0.3)
Type 12A					
(1)	2410	359	34.2		12.9
(2)	1250	185	18.0		12.9
(3)	487	65	—		11.6
(4)	438	53	—		10.8
(5)	525	69	—		11.6
(6)	412	55	—		11.9
(7)	613	79	—		11.4
(8)	638	85	—		11.9
(9)	562	71	—		11.2
(10)	525	69	—		11.6
				Avg.	11.8 (±0.5)
Type 18					
(1)	538	62	5.86		10.4
(2)	760	88	9.90		10.4
(3)	875	132	14.2		13.0
				Avg.	11.3 (±1.3)

* DNA/DNA + protein × 100.
† ± Standard deviation.

virus type 12A indicate only 11.8 per cent DNA. Analyses of three preparations of adenovirus type 18 suggest that this virus also has a lower DNA content. Thus, tumorigenic adenovirus types 12A and 18 appear to contain about 12 per cent less DNA than the nontumorigenic adenovirus types 2 and 4.

Several lines of evidence indicate a high degree of purity for adenovirus type 2 isolated by this procedure.[6] Other adenovirus types, which are anticipated to possess a similar chemical and physical makeup, should be of similar purity. The following facts support this expectation: (1) the P content (Table 1) is close to 10 per cent of the DNA content suggesting the absence of appreciable RNA and phospholipid; no RNA was detected by colorimetric analysis (less than 1%). (2) Serological analyses[6] of four adenovirus type 12A preparations did not detect any host cell antigen (less than 0.3%). Less than 0.01 per cent host cell contamination of adenovirus type 2 was detected (larger amounts of virus were tested, thus increasing the sensitivity).

Buoyant density of adenoviruses: Chemical analyses (Table 1) indicate that tumorigenic adenovirus types 12A and 18 have less DNA than nontumorigenic adenovirus types 2 and 4. Virus particles with different DNA contents would be expected to possess different buoyant densities.[17] Density-gradient equilibrium centrifugations were performed on paired mixtures of tumorigenic and nontumorigenic adenovirus types, i.e., type 2 with type 12A, type 2 with type 18, and type 4 with type 12A. In each case, two well-separated bands were formed (Fig. 1); types 12A and 18 banded at a lower density than types 2 and 4. Each of the four

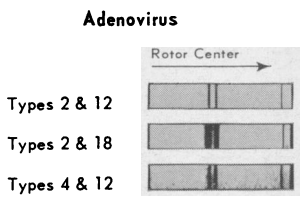

FIG. 1.—Analytical density-gradient centrifugation of tumorigenic and nontumorigenic adenoviruses. UV photographs of virus bands were made after 15 hr of centrifugation at 44,770 rpm. The bands on the left are adenovirus types 2 and 4; those further toward the rotor center are adenovirus types 12A and 18 (line on extreme right is the meniscus).

strains of adenovirus type 12 banded at the lower position. The lower buoyant density of the tumorigenic adenoviruses confirms the lower DNA content obtained by chemical analysis.

Different preparations of the same virus type were found to have the same buoyant density. The possibility exists, however, that the virus preparations are composed of mixtures of adenovirus particles of different buoyant densities. To ascertain if density heterogeneity occurs, the following fractionation procedure was employed with preparations of adenovirus types 2, 4, 12A, and 18. The virus band obtained in the final preparative RbCl density gradient was separated into four successive fractions by drop collection. An aliquot of each fraction was centrifuged with a reference adenovirus of different buoyant density as described above. The four fractions of each virus were found to have the same buoyant density. It is concluded that each virus type has a uniform buoyant density.

Tumor-inducing capacity of highly purified adenoviruses: Type 12A (prototype strain) induced tumors as early as two to three weeks after inoculation of *newborn hamsters* (Table 2). Animals died with tumors measuring 1–8 cm in diameter within 25–60 days after inoculation. Generally from 1 to 6 tumor masses were found in the abdominal cavity. It is not known whether tumors occasionally observed in the liver and lungs are the result of virus infiltration or metastasis. The tumors were classified as sarcomas by Dr. H. Pinkerton of our Pathology Department. Cancer formation was prevented by rabbit antiserum prepared against purified adenovirus type 12A but not by normal serum (Table 2).

As little as 0.2 μg of adenovirus type 12A induced tumors in newborn hamsters;

TABLE 2

TUMOR INDUCTION IN HAMSTERS INJECTED WITH PURIFIED ADENOVIRUS TYPES 12 AND 18

Amount injected	Adenovirus	No. positive/total*	Age at death (days)
6 μg	Type 12A (prototype)	6/6	25–43
2.5	"	6/6	28–51
1	"	8/8	28–50
0.25	"	6/6	39–70
0.20	"	4/5	42–90
0.15	"	2/3	35–80
0.02	"	0/10	
1.5	" + normal serum†	5/5	33–60
1.5	" + anti-12 serum	0/6	
2.5 μg	Type 12B	4/5	74–180
2.5 μg	Type 12C	3/4	79–108
2.5 μg	Type 12D	2/3	50–73
4.3 μg	Type 18	3/4	56–65
1.6	"	1/4	93

* Number of hamsters dying with tumors/number of animals surviving a minimum of 15 days. Animals were observed for at least 120 days.

† Incubated 30 min at 37° with equal volume of normal rabbit serum or antiserum prepared against purified type 12A adenovirus.

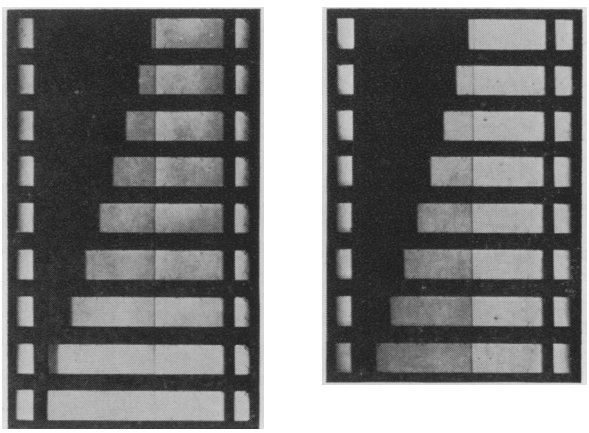

FIG. 2.—Analytical sedimentation patterns of DNA's from adenovirus types 2 and 12A. UV photographs were taken every 8 min at 35,600 rpm.

larger levels were tumorigenic in 100 per cent of the animals injected. Higher doses and longer time periods (Table 2) were required to induce tumors with the other three strains of adenovirus type 12 (12B, C, and D) and with adenovirus type 18.

Tumors have not developed within 16 months after injection of newborn hamsters with 3–30 μg of purified adenovirus type 2. Sufficient time has not elapsed since the inoculation of newborn hamsters with purified adenovirus type 4[9] to permit a definitive statement as to its tumorigenicity.

In additional experiments, *adult hamsters* were inoculated with 5 μg of adenovirus type 12A, six subcutaneous, six intrapulmonary, and six by the intraperitoneal route; no evidence of tumor formation was found 15 months after inoculation.

Sedimentation velocity analysis of adenovirus DNA's: Two alternative explanations may be suggested for the lower DNA content of adenovirus type 12A as compared to type 2, namely, (1) the DNA of adenovirus type 12A has a lower molecular weight than that of type 2, or (2) the DNA's have the same molecular weight, but the particle size of adenovirus type 12A is larger than that of type 2. To test these possibilities, sedimentation velocity measurements were performed on the DNA's of adenovirus types 2 and 12A. The sharp sedimenting boundaries (Fig. 2) indicate a high degree of homogeneity in both DNA's (examination of the analytrol tracings showed a boundary spread of less than 1 S after sedimenting 6 mm). The $S_{20,w}$ values at zero concentration were 32.0 and 30.7 for the DNA's of adenovirus types 2 and 12A, respectively, as determined in *M* NaCl (Fig. 3). $S^0_{20,w}$ values were 7–10 per cent lower in 0.15 *M* NaCl–0.015 *M* sodium citrate; the reason for this is not known but presumably some change in molecular configuration is involved.

The lower sedimentation coefficient of the DNA of adenovirus type 12A as compared to the DNA of adenovirus type 2 was verified by zone centrifugation analysis.[18] Mixtures of DNA's of adenovirus types 2 and 12A, differently labeled with P^{32}-phosphate or H^3-thymidine, were centrifuged in density gradients of sucrose

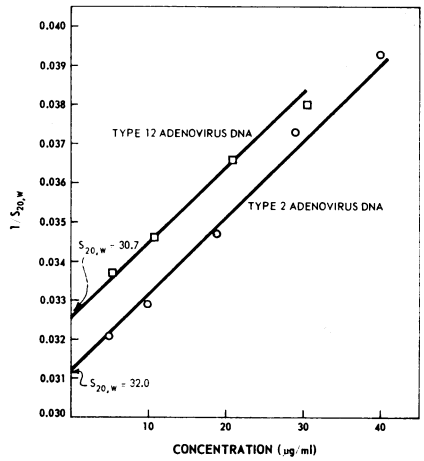

FIG. 3.—Relationship between $1/S_{20,w}$ and the concentration of DNA from adenovirus types 2 and 12A; solvent, *M*-NaCl.

and relative sedimentation rates determined. The DNA of adenovirus type 12A consistently sedimented at a 2–3 per cent lower rate than did the DNA of type 2. According to the relationship described by Burgi and Hershey,[18] a 2–3 per cent difference in S value corresponds to a 6–9 per cent difference in molecular weight of DNA. Considering the errors involved in comparing small differences in physical and chemical measurements, the 6–9 per cent difference in molecular weights of the DNA's of adenovirus types 12A and 2 is consistent with the 12 per cent diffference in the DNA content of the viruses (Table 1). We conclude therefore that the lower DNA content of the tumorigenic adenoviruses is due to a lower molecular weight of the viral DNA.

An $S^0_{20,w}$ value of 32 in 1 *M* NaCl and 30 in 0.15 *M* NaCl-0.015 *M* sodium citrate was determined by analytical centrifugation for the DNA of adenovirus type 2 (the S value of 25 previously reported[8] was not corrected to standard conditions and zero concentration). The sedimentation coefficient was further determined by zone centrifugation of a mixture of P^{32}-labeled DNA of adenovirus type 2 with H^3-labeled DNA of phage lambda (generously supplied by Drs. Burgi and Hershey). By comparison with the reference lambda DNA ($S^0_{20,w}$ of 33.6[18]), a $S^0_{20,w}$ of 30 was measured for the DNA of adenovirus type 2, a value which is in fair agreement with that determined by analytical centrifugation. The molecular weight of the DNA calculated from $S^0_{20,w} = 30$ by the formula of Burgi and Hershey[18] is 22.6 million. This value for the molecular weight of the DNA would appear to be too large since it leads to an unreasonably high molecular weight for the virus particle, calculated as follows: 22.6×10^6 (mol. wt. DNA) divided by 0.133 (fractional DNA content) = 169 million for the molecular weight for adenovirus. Minimum and maximum molecular weight estimates[8] for this virus are 90 and 150 million, 120 million being a reasonable approximation. Since the relationship[18] between $S^0_{20,w}$ value and molec-

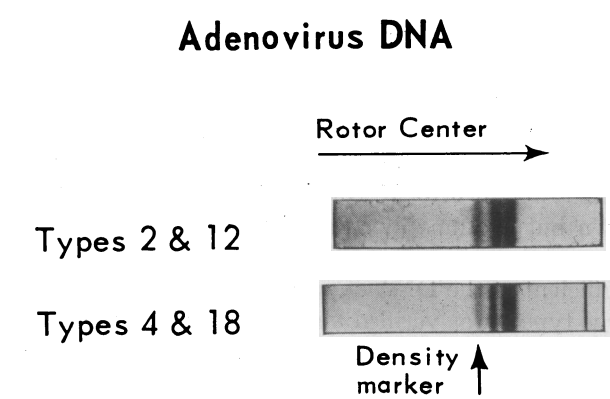

FIG. 4.—Analytical density-gradient centrifugation of the DNA's from tumorigenic and nontumorigenic adenoviruses. UV photographs were taken after centrifugation in CsCl solution at 44,770 rpm for 20 hr. The bands labeled density marker are the reference *Ps. aeruginosa* DNA's (buoyant density of 1.727), the middle bands are the DNA's of adenovirus types 2 and 4, and the bands on the right are the DNA's of adenovirus types 12A and 18.

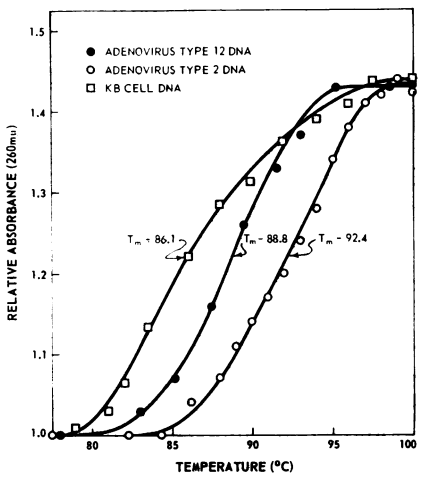

FIG. 5.—Thermal denaturation curves for the DNA's of adenovirus types 2 and 12A and the KB cell.

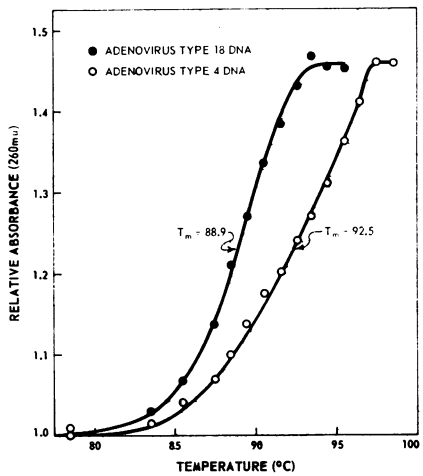

FIG. 6.—Thermal denaturation curves for the DNA's of adenovirus types 4 and 18.

ular weight has been verified for several well-characterized, linear, double-stranded, uniform DNA's, it is suggested that the higher-than-expected $S^0_{20,w}$ value for the DNA of adenovirus type 2 is indicative of a different molecular configuration, perhaps a ring structure. Evidence for a ring structure has been reported for the DNA's of polyoma virus[19, 20] and bacteriophage ϕX-174 DNA;[21] the faster sedimenting form of the DNA of rabbit papilloma virus[22] presumably also exists in a cyclic form. In addition, phage lambda DNA possesses two cohesive sites[23] and can undergo reversible transitions from a linear to a folded form.

Density-gradient equilibrium centrifugation and thermal denaturation profiles of adenovirus DNA's: The DNA's of tumorigenic adenovirus types 12 and 18 have lower buoyant densities (1.708 and 1.709) than that of nontumorigenic adenovirus types 2 and 4 (1.716 and 1.718) and are readily separated from the latter in a CsCl gradient in the analytical ultracentrifuge (Fig. 4). The DNA's of all four strains of adenovirus type 12 (12A, B, C, and D) banded at the lower buoyant density.

The thermal denaturation curves of the DNA's of adenovirus types 2, 4, 12, and 18 show two interesting features: first, the relatively broad melting curves for the DNA's (Figs. 5 and 6) suggest intramolecular heterogeneity of base composition, an unusual property of small homogeneous DNA's, which may indicate the existence of discrete segments of the viral genome with specialized functions; secondly, the DNA's of the tumorigenic adenovirus types 12 and 18 have T_m values 3.5° lower than the DNA's of the nontumorigenic adenoviruses.

Based on calculations from buoyant density and T_m values,[12] the DNA's of adenovirus types 12 and 18 contain 48–49 per cent guanine-cytosine in contrast to the DNA's of adenovirus types 2 and 4 which contain 56–57 per cent guanine-cytosine. The lower content of guanine-cytosine of the tumorigenic adenoviruses may indicate that a specific base composition is associated with or essential for viral tumorigenicity. To establish or disprove this correlation between base composition and tumorigenicity, it is important to perform base composition analyses

and viral tumorigenicity tests on purified preparations of the 28 human adenoviruses.

Summary.—Tumorigenic human adenovirus types 12 (four strains) and 18 have been purified and studied. Adenovirus types 12 and 18 contain less DNA (11–12% DNA) than do nontumorigenic adenovirus types 2 and 4 (13–14% DNA). The difference in DNA content was further shown by the physical separation of the tumorigenic from the nontumorigenic adenoviruses by analytical density-gradient equilibrium centrifugation in cesium chloride; as predicted from a lower DNA content, tumorigenic adenovirus types 12 and 18 have lower buoyant densities than nontumorigenic adenovirus types 2 and 4.

Purified adenovirus type 12 (prototype strain) induced tumors within three weeks after inoculation of newborn hamsters. Tumors were induced with 0.2 μg of virus. The induction of tumors with three other strains of adenovirus type 12 and adenovirus type 18 required larger amounts of virus and longer time periods.

Analytical centrifugation and zone centrifugation measurements on the DNA's from adenovirus types 2 and 12 provided evidence for a high degree of homogeneity. DNA from adenovirus type 12 sedimented at a 2–3 per cent lower rate than the DNA of adenovirus type 2. These data are consistent with the results of chemical analyses and indicate that the molecular weights of the DNA's of the tumorigenic adenoviruses are about 6–12 per cent lower than those of the nontumorigenic adenoviruses.

Thermal denaturation and buoyant density measurements on the viral DNA's showed that the DNA's of tumorigenic adenovirus types 12 and 18 possess markedly lower denaturation temperatures and buoyant densities than the DNA's of nontumorigenic adenovirus types 2 and 4. From these data, it is calculated that the DNA's of adenovirus types 12 and 18 contain 48–49 per cent guanine plus cytosine, while those of adenovirus types 2 and 4 contain 56–57 per cent guanine plus cytosine. This remarkably different base composition for genetically related viruses is unexpected, and its significance remains to be established.

We are pleased to acknowledge the fine technical assistance of Mrs. Clara Boudreau.

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† Research Career Awardee of the National Institutes of Health.

[1] Trentin, J. J., Y. Yabe, and G. Taylor, *Science*, **137**, 835 (1962).

[2] Huebner, R. J., W. P. Rowe, and W. T. Lane, these PROCEEDINGS, **48**, 2051 (1962).

[3] Green, M., *Virology*, **9**, 343 (1959).

[4] Green, M., and G. E. Daesch, *Virology*, **13**, 169 (1961).

[5] Green, M., *op. cit.*, **18**, 601 (1962).

[6] Green, M., and M. Piña, *Virology*, **20**, 199 (1963).

[7] Green, M., M. Piña, and V. Chagoya, *J. Biol. Chem.*, **239**, 1188 (1964).

[8] Green, M., in *Basic Mechanisms in Animal Virus Biology*. Cold Spring Harbor Symposia on Quantitative Biology, vol. 27 (1962), p. 219.

[9] Adenovirus types 2 and 4 are termed "nontumorigenic" based on the failure of preparations of adenovirus type 2[1, 2] and adenovirus type 4 (Trentin, J. J., private communication) to induce tumors in newborn hamsters. However, the possibility that these viruses are weakly tumorigenic and may induce tumors if inoculated in higher doses is not ruled out.

[10] Green, M., and M. Piña, *Bacteriol. Proc.*, **63**, 159 (1963).

[11] Green, M., M. Piña, and H. Polasa, *Federation Proc.*, **22**, 645 (1963).

[12] Green, M., and M. Piña, these PROCEEDINGS, **50**, 44 (1963).

[13] Rouse, H. C., V. H. Bonifas, and R. W. Schlesinger, *Virology*, **20**, 357 (1963).
[14] Gierer, A., and G. Schramm, *Nature*, **177**, 702 (1956).
[15] Schachman, H. K., in *Methods in Enzymology* (1957) vol. 4, p. 32.
[16] Davison, P. D., and D. Freifelder, *J. Mol. Biol.*, **5**, 643 (1962).
[17] Kellenberger, G., M. L. Zichichi, and J. Weigle, *J. Mol. Biol.*, **3**, 399 (1961).
[18] Burgi, E., and A. Hershey, *Biophys. J.*, **3**, 309 (1963).
[19] Dulbecco, R., and M. Vogt, these PROCEEDINGS, **50**, 236 (1963).
[20] Weil, R., and J. Vinograd, these PROCEEDINGS, **50**, 730 (1963).
[21] Fiers, W., and R. L. Sinsheimer, *J. Mol. Biol.*, **5**, 424 (1962).
[22] Watson, J. D., and J. W. Littlefield, *J. Mol. Biol.*, **2**, 161 (1960).
[23] Hershey, A., E. Burgi, and L. J. Ingraham, these PROCEEDINGS, **49**, 748 (1963).

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*THE NATURE OF AITKEN CONDENSATION NUCLEI IN THE ATMOSPHERE**

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Read before the Academy April 27, 1964

We know that in normal air thousands of submicroscopic particles occur per cubic centimeter which are capable of condensing water droplets from supersaturated water vapor. These so-called Aitken nuclei can conveniently and simply be counted with a Rich condensation-nuclei counter (made by Gardner Associates, Inc., Schenectady, N. Y.). Their physical and chemical nature is only partly known. It is for instance accepted that some of them are hygroscopic salt particles. But it is also known that cigarette smoke, or match or gas flames, or automobile exhaust are very rich sources of condensation nuclei (hereafter referred to as cn). And cn produced in a flame are not likely to be inorganic and hygroscopic.

There are several sets of data which make it possible to say something more about the nature of these condensation nuclei. Their concentration is very low in the higher atmosphere (at 3,000–4,000 m alt, 200 cn/cc), in high mountains, and in northern airmasses during winter (in Yellowstone also ±200 cn/cc toward the end of January at temperatures of −30°C). During summer there are in the morning 1,000–5,000 cn/cc in pure country air, which increases during the day. This increase is primarily due to small nuclei ($< 0.1\ \mu$ in diameter). This means that they are being produced in the air, for small nuclei can only develop by coagulation or conglomeration of smaller particles or by condensation of orginally molecularly disperse material. In cities the concentration of cn fluctuates between 10,000 and well over 100,000 with maxima at hours of peak traffic.

The condensation of organic molecules to submicroscopic particles was demonstrated 100 years ago by Tyndall (1869). He showed that in a beam of strong actinic light (from a carbon arc) vapors of amyl nitrite or allyliodide would produce a "blue cloud," consisting of submicroscopic particles reflecting particularly the short wavelengths, explaining the polarized blue sky light. Lord Rayleigh tried to explain this blue sky light by molecular reflection. But this can account at most for the deep blue sky color under the clearest sky conditions (such as in Jerusalem, or occasionally in the desert or at high altitudes, as in high mountains or in jet