to the metal, it follows that, granting this mechanism, erythrocytes should be more stable in the presence of metals of low work function (least electron affinity). Currently, experiments are being carried out *in vivo* to check this hypothesis.

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[1] Sawyer, P. N., and J. W. Pate, "Bio-electric phenomena as etiologic factors in intravascular thrombosis," *Surgery*, **34**, 791 (1953).

[2] Sawyer, P. N., and J. W. Pate, "Bio-electric phenomena as an etiologic factor in intravascular thrombosis," *Am. J. Physiol.*, **175**, 113 (1953).

[3] Sawyer, P. N., B. Deutch, and J. W. Pate, "The relationship of bio-electric phenomena and small electric currents to intravascular thrombosis," *Thrombosis and Embolism*, Proceedings of the First International Conference on Thrombosis and Embolism, Basel, Switzerland (Basel: Benno Schwabe and Co., 1955).

[4] Harshaw, D. H., H. S. Ziskind, and P. N. Sawyer, "The ionic structure of the blood-intimal interface as an aid in the development of vascular prostheses," Amer. Soc. for Artificial Internal Organs, vol. 9 (1963), p. 317.

[5] Abramson, H. A., and L. S. Moyer, "The electrical charge of mammalian red blood cells," *J. Gen. Physiol.*, **19**, 601 (1936).

PRODUCTION OF TYPE-SPECIFIC C ANTIGEN IN VIRUS-FREE HAMSTER TUMOR CELLS INDUCED BY ADENOVIRUS TYPE 12

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Previous reports in these PROCEEDINGS presented evidence that virus-free cancer cells induced by adenovirus 12[1-3] produced antigens which in hamsters and rats resulted in complement-fixing antibody responses to viral antigens associated with the growth of adenovirus type 12 in tissue culture systems. These antibodies seemed largely type-specific, occurring earlier and reaching higher titers with the homologous viral antigen than they did with the heterologous, but related, adenovirus 18 serotype. Similarly, antigen preparations from hamster tumors and tissue culture-grown cells derived from them reacted with serums of hamsters carrying adenovirus type 12- or type 18-induced tumors, but not with a pool of human serums used in our laboratory as a standard representative of adenovirus group-reactive antibody.[1, 2] In addition, serums of hamsters containing high-level antibodies to adenovirus 12 and/or type 18 viral antigens revealed little or no antibody response to the group-reactive antigen of other adenoviruses, the latter an important characteristic of all adenoviruses. These data led to the hypothesis that the virus-free but adenovirus-induced tumor cells probably replicated the type-specific C (or E) antigen, but not the group-reactive A (or L) antigen described for various adenoviruses by Klemperer and Pereira,[4, 5] and Wilcox and Ginsberg.[6]

This communication describes the fractionation of type 12 adenovirus into its

A and C antigen components, and observations on the development of serum antibodies to the type-specific C component in hamsters and rats carrying progressively growing transplanted adenovirus-induced cancers. The total absence in hamster tumor cells of demonstrable infectious virus and the failure of the neoplastic cells to replicate A antigen suggest that the information from the viral genome inherited by these cells responsible for production of C antigen must either be defective or incompletely expressed. Although in this paper we place major emphasis on the C antigen, we call attention to the presence in the adenovirus-induced tumor cells of additional specific antigens other than C which on further study may be found to have equivalent significance.

Materials and Methods.—Methods for preparing viral antigens, hamster tumor antigens, for collection and testing of hamster and rat serums, and the procedures used for the complement fixation (CF) and neutralization tests were described previously.[1, 2]

Methods used for assay of infectious virus in viral preparations and for establishing the absence of virus from the adenovirus 12-induced hamster tumors were described previously.[2, 13]

Preparation of purified A and C antigens from prototype adenovirus 12: Fractionation of adenovirus type 12 antigens was achieved by chromatography on DEAE-cellulose (Fig. 1).[4-7] Concentrated and purified virus material extracted from infected HeLa cells by fluorocarbon treatment[8] was dialyzed against 0.01 *M* phosphate buffer pH 7.0 and placed on a column equilibrated against the same buffer. Stepwise elutions were accomplished with 20 ml (top curve) or 10 ml (lower curves) of NaCl solutions of increasing concentrations in 0.01 *M* phosphate buffer, pH 7.0. The points in Figure 1 represent complement-fixation titers of each fraction against serum from a rabbit given two doses of fluorocarbon-treated adenovirus type 12 material in complete Freund's adjuvant 10 days apart and bled 10 days later. In Figure 1, the top curve represents fractionation of original virus material; the lower curves represent rechromatography of pool *A* (0.025 + 0.05 *M*), pool *B* (0.075 + 0.1 *M*), and pool *C* (0.125 + 0.15 *M*) fractions. These pools were concentrated 10 × by Kohn's technique[9] and dialyzed against 0.01 *M* phosphate buffer pH 7.0 before rechromatography. The final antigens represented 10 × concentrates of 0.025, 0.05, and 0.075 *M* fractions of rechromatographed pool *A* (antigen 12 A) and 0.15, 0.175, and 0.2 *M* fractions of rechromatographed pool *C* (antigen 12 C). Antiserums specific for A and C antigens were prepared in rabbits using antigens purified by a second rechromatographic run. The immunization schedule was as described above for the unfractionated virus antigen.

Results.—The separation of adenovirus type 12 complement-fixing antigens A and C is illustrated in Figure 1. Adenovirus type 12 differs from other serotypes in its failure to produce antigen B (the cell-detaching factor) and in the reversal of the order of elution of the group-specific (A) and type-specific (C) antigens. The reactivity of the purified and unfractionated antigens and their respective antisera is illustrated in Table 1. It is apparent that the A and C antigen fractions are highly reactive with the homologous antiserums, but nonreactive with heterologous serums. Using these antigens, we found that a human serum pool representative of adenovirus group-specific antibody[10] reacted with A antigen but not with C antigen, and that a serum pool[11] taken from hamsters with maximal antiviral titers, late after onset of virus-induced tumors contained antibody reactive with C antigen but not with A antigen (Table 2). Thus, the group-reactive human serum behaved like the rabbit antiserum to A and adenovirus type 2 antigens (Table 1) and the hamster serum reacted like the specific C rabbit antiserum.

Individual serum specimens taken at different intervals from hamsters carrying transplanted type 12 tumors revealed the gradual development in some of the hamsters of CF antibodies to C antigen, but none to A antigen when 8 or more units[12]

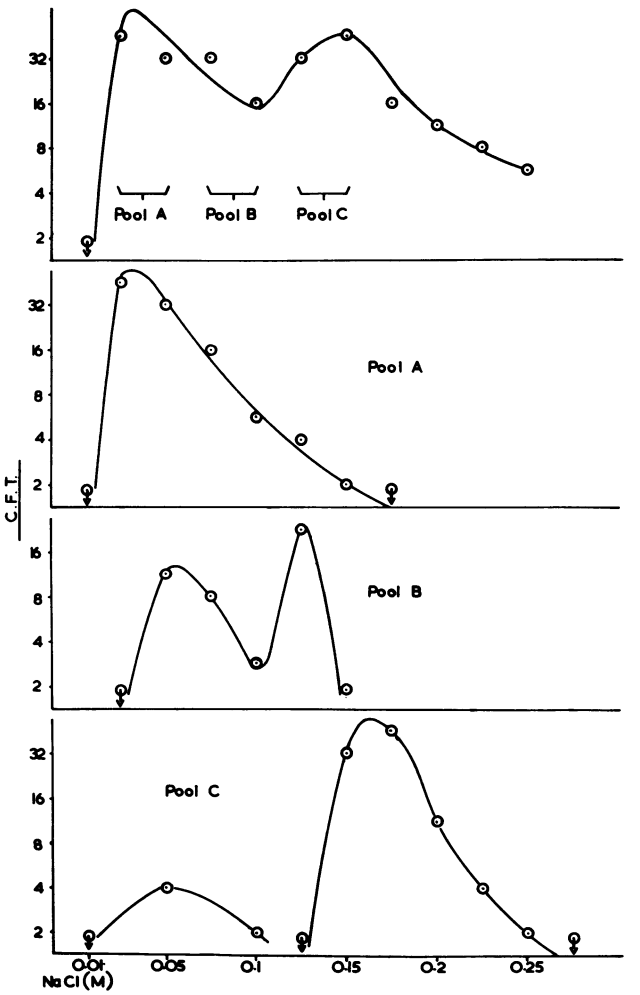

FIG. 1.—Chromatographic fractionation of adenovirus type 12 antigens derived from infected HeLa cells. Top curve: fractionation of original virus preparation. Lower curves: rechromatography of pool *A* (0.025 + 0.05 *M*), pool *B* (0.075 + 0.1 *M*), and pool *C* (0.125 + 0.15 *M*). Fractions—each 10 × concentrated by Kohn's technique[8] before rechromatography. CFT = complement-fixing titers of fractions versus hyperimmune serum of a rabbit immunized with unfractionated virus.

of each antigen was used (Table 3). Additional similar experiments suggested a relationship between antibodies to the C antigen and antibodies to the crude viral antigen, and indeed, in Figure 2, it can be seen that every hamster serum containing C antibodies also reacted with the viral antigen; on the other hand, many serums having antiviral antibodies failed to react with C antigen. Since earlier studies suggested a positive correlation between antiviral CF antibodies and neutralizing antibodies,[2] it was not surprising to find a similar correlation between anti-C and neutralizing antibodies in the hamster serums (Fig. 3). Although a significant proportion of hamster serums containing neutralizing antibodies did not show anti-C reactions, 15 of the 16 with C antibodies had neutralizing antibodies. It seemed likely from these data that the tumor cells replicated the specific C antigen of adeno-

TABLE 1

COMPLEMENT-FIXING REACTIONS OF TYPE 12 AND TYPE 2 ADENOVIRUS ANTIGENS AND PURIFIED A AND C FRACTIONS OF TYPE 12 ANTIGEN WITH IMMUNE SERUMS PREPARED IN RABBITS USING THE RESPECTIVE VIRUSES

Antigens	Antisera	Adenovirus 12							
		20	40	80	160	320	640	1280	2560
Adeno 12 crude antigen	10	4	4	4	4	4	4	2	1
	20	4	4	4	4	4	4	0	0
	40	4	4	4	4	4	4	0	0
	80	4	4	4	4	4	4	0	0
	160	4	4	4	4	4	4	0	0
	320	4	4	4	4	4	4	0	0
	640	3	3	3	3	3	2	0	0
	1280	0	0	0	0	0	0	0	0
Adeno 12-A DEAE (2) 0.05 + 0.075 *M*	10	4	4	4	4	4	4	0	0
	20	4	4	4	4	4	4	0	0
	40	4	4	4	4	4	4	0	0
	80	4	4	4	4	4	3	0	0
	160	4	4	4	4	4	1	0	0
	320	2	4	4	2	2	1	0	0
	640	0	0	0	0	0	0	0	0
Adeno 12-C DEAE (2) 0.1 + 0.125 *M*	10	4	4	4	4	4	3	0	0
	20	4	4	4	4	4	3	0	0
	40	4	4	4	4	4	3	0	0
	80	4	4	4	4	4	2	0	0
	160	4	4	3	2	2	0	0	0
	320	3	3	1	0	0	0	0	0
	640	0	0	0	0	0	0	0	0
Adeno 2	10	4	4	4	4	3	0	0	0
	20	4	4	4	4	4	1	0	0
	40	4	4	4	4	4	1	0	0
	80	4	4	4	1	1	0	0	0
	160	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0
	640	0	0	0	0	0	0	0	0

Antigens	Antisera	Adenovirus 12-A							
		10	20	40	80	160	320	640	1280
Adeno 12 crude antigen	10	4	4	4	4	4	0	0	0
	20	4	4	4	4	4	0	0	0
	40	4	4	4	4	4	1	0	0
	80	4	4	4	4	4	2	0	0
	160	4	4	4	4	3	0	0	0
	320	2	2	2	0	0	0	0	0
	640	1	0	0	0	0	0	0	0
	1280	1	0	0	0	0	0	0	0
Adeno 12-A DEAE (2) 0.05 + 0.075 *M*	10	4	4	4	4	4	0	0	0
	20	4	4	4	4	4	0	0	0
	40	4	4	4	4	4	2	0	0
	80	4	4	4	4	4	1	0	0
	160	4	4	4	4	2	0	0	0
	320	2	2	2	0	0	0	0	0
	640	1	0	0	0	0	0	0	0
Adeno 12-C DEAE (2) 0.1 + 0.125 *M*	10	2	0	0	0	0	0	0	0
	20	1	0	0	0	0	0	0	0
	40	1	0	0	0	0	0	0	0
	80	1	0	0	0	0	0	0	0
	160	1	0	0	0	0	0	0	0
	320	1	0	0	0	0	0	0	0
	640	1	0	0	0	0	0	0	0
Adeno 2	10	4	4	4	4	4	0	0	0
	20	4	4	4	4	4	0	0	0
	40	4	4	4	4	4	0	0	0
	80	4	4	4	4	2	0	0	0
	160	2	2	2	0	0	0	0	0
	320	1	0	0	0	0	0	0	0
	640	1	0	0	0	0	0	0	0

Antigens	Antisera	Adenovirus 12-C							
		10	20	40	80	160	320	640	1280
Adeno 12 crude antigen	10	4	4	4	4	4	1	0	0
	20	4	4	4	4	4	0	0	0
	40	4	4	4	4	4	0	0	0
	80	4	4	4	4	4	0	0	0
	160	4	4	4	3	2	0	0	0
	320	4	4	4	1	0	0	0	0
	640	3	3	3	1	0	0	0	0
	1280	1	1	1	0	0	0	0	0
Adeno 12-A DEAE (2) 0.05 + 0.075 *M*	10	0	0	0	0	0	..	..	..
	20	0	0	0	0	0	..	..	..
	40	0	0	0	0	0	..	..	..
	80	0	0	0	0	0	..	..	..
	160	..	..	..	..	..	..	..	..
	320	..	..	..	..	..	..	..	..
	640	..	..	..	..	..	..	..	..
Adeno 12-C DEAE (2) 0.1 + 0.125 *M*	10	4	4	4	4	4	0	0	0
	20	4	4	4	4	4	0	0	0
	40	4	4	4	4	3	0	0	0
	80	4	4	4	2	1	0	0	0
	160	3	3	3	0	0	0	0	0
	320	0	1	1	0	0	0	0	0
	640	0	0	0	0	0	0	0	0
Adeno 2	10	1	0	0	0	0	..	..	..
	20	0	0	0	0	0	..	..	..
	40	0	0	0	0	0	..	..	..
	80	0	0	0	0	0	..	..	..
	160	..	..	..	..	..	..	..	..
	320	..	..	..	..	..	..	..	..
	640	..	..	..	..	..	..	..	..

Antigens	Antisera	Adenovirus 2							
		20	40	80	160	320	640	1280	2560
Adeno 12 crude antigen	10	4	4	3	0	0	0	0	0
	20	4	4	4	0	0	0	0	0
	40	4	4	4	0	0	0	0	0
	80	4	4	1	0	0	0	0	0
	160	2	1	0	0	0	0	0	0
	320	1	0	0	0	0	0	0	0
	640	1	0	0	0	0	0	0	0
	1280	1	0	0	0	0	0	0	0
Adeno 12-A DEAE (2) 0.05 + 0.075 *M*	10	4	4	4	0	0	0	0	0
	20	4	4	4	1	0	0	0	0
	40	4	4	4	2	0	0	0	0
	80	4	4	4	1	0	0	0	0
	160	4	4	3	0	0	0	0	0
	320	2	1	0	0	0	0	0	0
	640	1	0	0	0	0	0	0	0
Adeno 12-C DEAE (2) 0.1 + 0.125 *M*	10	1	0	0	0	0	..	..	..
	20	1	0	0	0	0	..	..	..
	40	1	0	0	0	0	..	..	..
	80	1	0	0	0	0	..	..	..
	160	1	0	0	0	0	..	..	..
	320	..	..	..	..	..	..	..	..
	640	..	..	..	..	..	..	..	..
Adeno 2	10	4	4	4	4	4	2	0	0
	20	4	4	4	4	4	3	0	0
	40	4	4	4	4	4	4	2	0
	80	4	4	4	4	4	4	2	0
	160	4	4	4	4	3	2	0	0
	320	4	4	3	0	0	0	0	0
	640	2	1	0	0	0	0	0	0

TABLE 2

CHESSBOARD REACTIONS OF A SERUM POOL FROM HAMSTERS WITH PRIMARY TUMORS, WITH FRACTIONATED ADENOVIRUS 12 C ANTIGEN AND OTHER ANTIGENS

	Serum Dilutions																			
	Adeno 12 Viral Antigen					A Antigen					C Antigen					Adeno 2 Viral Antigen				
Antigen dilutions	10	20	40	80	160	10	20	40	80	160	10	20	40	80	160	10	20	40	80	
10	4	4	4	4	4	0	0	0	0	0	4	4	4	4	4−	0	0	0	0	
20	4	4	4	4	4	0	0	0	0	0	4	4	4	4	4−	0	0	0	0	
40	4	4	4	4	3	0	0	0	0	0	4	4	4	4	4−	0	0	0	0	
80	4	3	1	0	0	0	0	0	0	0	2	2	2	1−	0	0	0	0	0	
160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

virus type 12; and although additional as yet uncharacterized viral antigens were also produced in these cells (Table 3 and Fig. 2), it also seemed clear that the group-reactive A antigen was not produced to any measurable extent.

Antibodies to C but not to A antigens were found in serums of Sprague-Dawley rats carrying transplanted tumors induced by the prototype 12 strain and in serums of hamsters carrying transplants of tumors induced by a 1961 isolate of adenovirus type 12. Serums with C antibodies invariably contained neutralizing and anti-viral CF antibodies to the prototype 12 virus.

To date, we have found no evidence of antibodies to type 12 C antigen in serums of hamsters carrying tumors induced by adenovirus type 18 prototype, even in those showing strong (1:80 or greater) reactions in the CF test with adenovirus type 12 viral antigen. However, negative data such as this cannot be fully evaluated until C fractions from type 18 virus are available for CF tests with serums of hamster carrying serotype 18-induced tumors.

C antigen in tissue cultures of hamster tumor cells: Adenovirus-induced hamster tumor cells grown serially in tissue cultures also appeared to replicate C antigen. Hamster tumor cells in 12th and 24th tissue culture subpassage (kindly furnished by Drs. Strohl, Rouse, and Schlesinger of St. Louis University),[13] and similar tissue cultures prepared after various subtransplants of hamster tumors by Dr. T. Ward and S. Cross of Microbiological Associates[14] grew progressively into large lethal tumors when transplanted back into newborn or weanling hamsters.[2] Hamsters surviving for 4 weeks or more and which developed large tumors (30 mm or more) were found to have high levels of serum antibodies not only to tumor and viral antigens, but also to the purified C antigen; reactions with A antigen again were not observed.

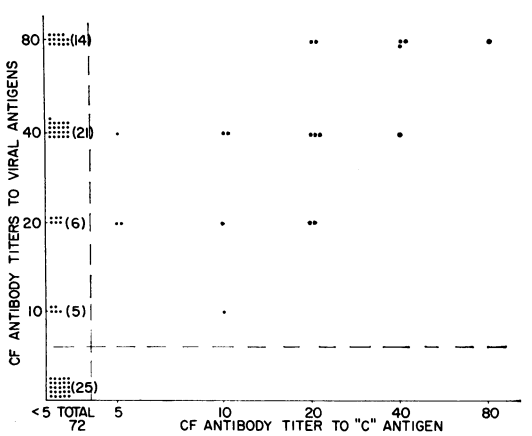

FIG. 2.—Serum antibody responses of tumored hamsters. Correlation of reactions to C and viral antigens.

Lack of A antigen in adenovirus-induced tumors: Tests of 80 individual serums from hamsters carrying adenovirus prototype type 12-induced tumors were negative when tested at a 1:10 dilution with 8 units of A antigen. Twenty-one of these sera had antibodies to C antigen, and 44 had neutralizing antibodies

to type 12 virus; virtually all had CF antibodies to tumor and viral antigens. However, one very high-titered hamster pool and one individual hamster serum (both from hamsters with primary tumors) showed low-level reactions with two A fractions. One reaction observed with the A antigen preparation used for the definitive tests reported herein was observed in one of our laboratories, but was not subsequently confirmed in the other. In the second instance, the reaction was observed with the A fraction only after initial fractionation; this reaction dropped to an insignificant level (less than 3+ at 1:10) after refractionation; on the other hand, following this second cycle, the A fraction increased in titer when tested with A rabbit serum, suggesting that whatever antigen the initial reaction was due to, it was probably not the group-specific A antigen.

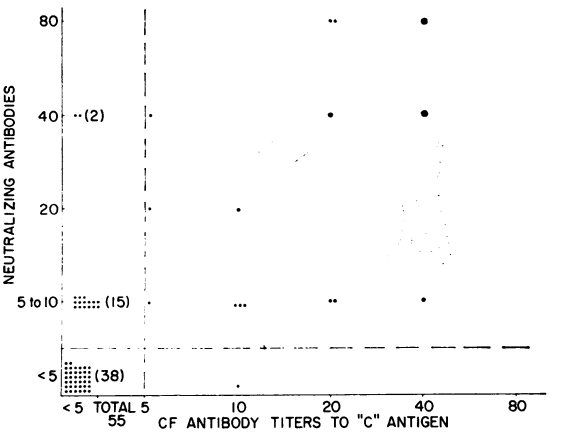

FIG. 3.—Serum antibody responses of tumored hamsters. Correlation of neutralizing antibodies and reactions with C antigen. The hamster with a 1:10 titer versus C antigen and < 5 in the neutralization test developed high neutralizing antibodies one week later.

Specificity of CF reactions with hamster tumor antigens shown by serums of rabbits immunized with purified A and C antigens: The reactions of A and C rabbit antiserums were quite specific when tested with purified (twice fractionated) A and C antigens (Table 1); however, reactions with hamster tumor tissue antigens gave very low antigen titers (1:2–1:4) which appeared to some extent to be nonspecific in reactivity. One antigen prepared from hamster tumors induced by SV40 virus reacted with both A and C rabbit serums and gave reactions equivalent to that

TABLE 3

SERUMS FROM HAMSTERS CARRYING TRANSPLANTS* OF ADENOVIRUS 12-INDUCED TUMOR TISSUE TESTED VERSUS ADENOVIRUS 12 AND 18 ANTIGENS AND ANTIGENS A AND C

Litter (L) and toe clip (TC) #	Serum #	Date	Antigens: Ad 12 V†	Ad 12 T‡	Ad 18 V	Ad 18 T	Ad 12 A	Ad 12 C	Tumor size
L #1 TC #1	11377	3/9	20	<10	0	0§	0	10	10 mm
"	11838	3/18	>80	20	20	<10	0	40	25 mm
"	12168	3/28	80	40	40	10	0	40	45 mm
L #2 TC #6	11390	3/9	0	0	0	0	0	0	0
"	11848	3/18	<10	10	0	0	0	0	5 mm
"	12172	3/28	40	>80	<10	10	0	5	20 mm
L #3 TC #4	11394	3/9	<10	<10	0	0	0	0	5 mm
"	11851	3/18	80	80	0	0	0	0	20 mm
L #3 TC #5	11395	3/9	<10	<10	0	0	0	0	5 mm
"	11852	3/18	40	80	20	0	0	0	15 mm
L #3 TC #8	11398	3/9	<10	<10	0	0	0	0	10 mm
"	11855	3/18	20	40	10	0	0	5	25 mm
"	12173	3/28	20	80	2	0	0	5	40 mm

* Transplanted 2/16/63.
† V = viral antigen.
‡ T = tumor antigen.
§ 0 = <10 with adenovirus 12 and 18 antigens, and <5 with A and C antigens.

shown by adenovirus-induced hamster tumor antigens; however, other SV40-induced hamster tumor antigens and normal hamster tissue antigens have generally been negative. Since rabbits are capable of producing Forssman antibodies and rodent tissue suspensions are known to contain them, it is not unlikely that the nonspecific reactions revealed by the rabbit serums were indeed due to a Forssman or Forssman-like immune response. It should be pointed out that the occurrence of a nonspecific reaction does not exclude a specific reaction, but it does make interpretation difficult. Adsorption of the serums with Forssman antigens will require larger amounts of A and C antiserums than are currently available.

Is the C antigen a subunit of the adenovirus particle? Wilcox and Ginsberg[6] concluded that the soluble antigens of type 5 adenovirus were predominantly protein in nature and represented "virus structural units produced in excess," a conclusion compatible with data reported by Pereira and associates.[4, 5] That this may be so for type 12 also is suggested by the fact that the A and C antigens used for the tests reported herein were prepared from highly purified and concentrated viral suspensions (see *Methods*), and by the fact that the specific A and C rabbit serums revealed little or no reactivity in the CF test with normal tissue culture antigens.

When another suspension of highly concentrated and purified type 12 adenovirus particles, kindly furnished by Dr. Maurice Green of St. Louis University of School of Medicine, was tested as antigen versus A and C antiserums, as well as with group-specific human and type-specific hamster serum, comparable positive reactions were obtained with all serums, including the hamster and the C serums. Since this virus preparation was virtually devoid of cellular materials,[15] the conclusion seems inescapable that both the A and C antigens of type 12 represent integral subunits of the virus particle, and that the inheritable component in hamster tumor cells responsible for the highly specific antibodies to the C antigen in hamster serums is very likely identical to the C component of the purified virus. This should be subject to direct demonstration through concentration and purification of tumor cell antigens; however, this may not be a simple procedure because all evidence suggests that the type-specific C antigen is not only one of the lesser components of purified adenovirus,[7] but in the type 12 tumor system this antigen appears to be present in extremely small concentrations, showing poor reactions with the C rabbit serum (*vide supra*) and being the last tumor antigen to be expressed in terms of antibody production in the hamsters (Table 3).

Discussion and Conclusion.—The findings reported herein can best be interpreted as follows: Virus-free tumor cells induced by adenovirus type 12 grown *in vitro* as well as *in vivo* carry viral information which codes for the production of several virus-associated antigens, at least one of which appears to be an integral component (or subunit) of the virus, namely, the type-specific C antigen. This antigen, which can be separated in a relatively pure state from other soluble antigens in purified virus preparations is either similar to or associated with the viral component responsible for the induction of type 12 neutralizing antibodies. The complete or nearly complete absence from the tumor cells of another integral component of the sedimentable adenovirus infectious particle, namely, the A antigen, very likely accounts in part at least for the failure of the cells to replicate complete infectious viral particles.

The persistent replication of soluble CF virus-specific antigens in cells trans-

formed by viruses into tumor cells is not limited to the adenovirus 12 and 18 systems; similar soluble CF antigens have also been demonstrated in hamster tumors induced by avian sarcoma and SV40 viruses.[2, 16, 17] The possible relationships of these soluble CF antigens to the tumor resistance-producing factors of Habel[18] and Sjögren[19] cannot of course be determined until the respective antigens responsible for the various CF and resistance-producing reactions are separately purified and characterized.

To explain the data in this paper, conventional concepts of microbial genetics would require that material from the adenoviral genome be incorporated somehow in the chromosomal network of the transformed cell; however, the apparent absence from the tumor cells of the group-reactive A antigen, an essential component not only of adenovirus 12 but of all other adenoviruses as well,[20] suggests that the incorporated viral DNA must either be defective or incompletely expressed.

Future studies are required to determine the precise nature and general meaning of the C antigen. The evidence does not prove that the noninfectious type-specific C antigen is in any way responsible for either initiating the oncogenic process or for its continuance. For the present, this antigen does serve as a specific viral marker, furnishing testimony concerning the integral role of viral information in this mysterious process.

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

[2] Huebner, R. J., W. P. Rowe, H. C. Turner, and W. T. Lane, these PROCEEDINGS, **50**, 379 (1963).

[3] Huebner, R. J., W. P. Rowe, and L. D. Berman, in *Viruses, Nucleic Acids and Cancer*, Proceedings of 17th Annual Symposium on Fundamental Cancer Research, M. D. Anderson Hospital (Baltimore: Williams & Wilkins Co., 1963), p. 564.

[4] Klemperer, H. G., and H. G. Pereira, *Virology*, **9**, 536 (1959).

[5] Pereira, H. G., A. C. Allison, and C. P. Farthing, *Nature*, **183**, 895 (1959).

[6] Wilcox, W. C., and H. S. Ginsberg, these PROCEEDINGS, **47**, 512 (1961).

[7] Wilcox, W. C., and H. S. Ginsberg, *Proc. Soc. Exptl. Biol. Med.*, **114**, 37 (1963).

[8] Pereira, H. G., and R. C. Valentine, *J. Gen. Microbiol.*, **19**, 178 (1958).

[9] Kohn, J., *Nature*, **183**, 1055 (1959).

[10] A pool of serum from volunteers experimentally infected with several different adenoviruses (but not types 12 or 18).

[11] Pool of 10 hamster serums having titers of 1:80 or greater versus adenovirus 12 viral antigen.

[12] When tested with 8 units of anti-C and A specific rabbit serums (see Table 1).

[13] Strohl, W. A., H. C. Rouse, and R. W. Schlesinger, *Virology*, **21**, 291 (1963).

[14] Furnished on contract SA-43-PH-81.

[15] Green, M., and M. Piña, these PROCEEDINGS, **50**, 44 (1963); Green, M., personal communication.

[16] Black, P. H., W. P. Rowe, H. C. Turner, and R. J. Huebner, these PROCEEDINGS, **50**, 1148 (1963).

[17] Huebner, R. J., D. Armstrong, M. Okuyan, P. S. Sarma, and H. C. Turner, unpublished data.

[18] Habel, K., *Proc. Soc. Exptl. Biol. Med.*, **106**, 722 (1961); Habel, K., *J. Exptl. Med.*, **115**, 181 (1962); Habel, K., *Fed. Proc.* (abstracts), **22**, 438 (1963).

[19] Sjögren, H. O., *Virology*, **15**, 214 (1961); Sjögren, H. O., I. Hellström, and G. Klein, *Cancer Res.*, **21**, 329 (1961).

[20] Pereira, H. G., R. J. Huebner, H. S. Ginsberg, and J. van der Veen, *Virology*, **20**, 613 (1963).