aortas of these animals than in the controls. Greatest protection against atherosclerosis was observed when the cholesterol KI ratio was approximately 2:1. Turner(1,2) fed equal amounts of cholesterol and KI, and Page and Bernhard(3) fed approximately equal amounts of cholesterol and diiodoricinsterolic acid. Moses and Longabaugh(9) employed ratios of 7:1 and 100:1, with the former giving relatively greater protection.

In Exp. 3 there was a slight weight loss in groups receiving 0.1% KI, 1% KI and thyroxine, but the overall weight difference in all groups was small. Firstbrook(11) has pointed out the high correlation between relative weight gain and severity of lesions in experimental atherosclerosis in rabbits, but the gains and losses recorded in Exp. 3 are too small to have affected the results. Both KI and control groups gained in weight, and there seemed to be no correlation in individual animals between atheromata and weight change. The 1% KI diet, while causing no hypocholesterolemia, lowered average liver cholesterol content significantly.

Summary. 1. Groups of rabbits were fed a 2% cholesterol diet augmented with 0.001%, 0.01%, 0.1% and 1% potassium iodide, 0.1% diiodotyrosine, and one group received 0.15 mg of thyroxine weekly by intraperitoneal injection. 2. Serum cholesterol and lipoprotein levels of all animals were elevated, but atheromata were significantly reduced in those on diet containing 1% KI. The S_f 0-12 lipoprotein levels were also higher in animals of this group than in controls, but on the whole levels of liver cholesterol were lowest.

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Cytopathogenic Agent Resembling Human Salivary Gland Virus Recovered from Tissue Cultures of Human Adenoids. (22497)

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Smith(1) recovered a cytopathogenic virus from a human salivary gland demonstrating intranuclear inclusions characteristic of the human salivary gland virus. Subsequently, Weller(2) isolated a similar virus from a liver biopsy from an infant with a clinical diagnosis of cytomegalic inclusion disease. During the course of studies in this laboratory of the presence of adenoidal-pharyngeal-conjunctival (APC) viruses(3-5) in human adenoids, 3 strains of an intranuclear inclusion body-producing virus were isolated from adenoid tissue cultures in which the fibroblasts underwent spontaneous degeneration. This report describes some of the properties of the virus, the development of a complement fixation test, and the serological relationship of this virus to the strains isolated by Smith and Weller.

Materials and methods. Adenoids were obtained from children undergoing tonsillectomy-adenoidectomy at the Children's Hospital, Washington, D.C., and the Clini-

FIG. 1. Ad. 182 strain. First passage, adenoid tissue, 14th day after inoculation. Focal area of rounding in sheet of fibroblasts. Unstained, ×125.

cal Center of the National Institutes of Health. Roller tube cultures of adenoids and embryonic tissues were prepared as described previously(3). Trypsin-dispersed cultures of human fibroblasts were prepared from embryonic skin-muscle, using a modification of the Youngner procedure(6). These cells were maintained in serial passage by Microbiological Associates Inc., Bethesda, Md., from whom the majority of cultures were obtained. The cells were grown directly on glass in stationary tubes and 32 oz. bottles, using a growth medium consisting of 10% to 20% human serum in Eagle's basal medium (7). Prior to inoculation, the cultures were washed four times with Eagle's medium and changed to a maintenance medium consisting of 5% horse serum in Eagle's medium; tube and bottle cultures received 1.0 and 40.0 ml respectively. All media contained penicillin (50-250 u/ml) and streptomycin (50-250 μg/ml). Nutrient fluids of tube cultures were changed once or twice weekly; bottle culture fluids were changed less often. Complement fixation (CF) tests were performed with a modified Bengston procedure(5). Neutralization tests were done in trypsinized human embryonic fibroblast cultures, using the neutralization procedure employed in APC virus studies(5). Seed viruses consisted of freshly prepared tissue suspensions from cultures showing severe cytopathogenic changes; lightly centrifuged preparations gave more reproducible results than uncentrifuged preparations, but the incubation period of the cytopathogenic effects was often unduly prolonged. A serum was considered positive if in two consecutive readings made several days apart, the cytopathogenic changes were less than half the degree of those in the virus control tubes.

Isolation of Strains. The 3 strains were recovered from adenoids taken from an 11-year-old boy (Ad. 162), a 7-year-old girl (Ad. 169) and a 6-year-old girl (Ad. 182). After 22 to 51 days in culture, epithelial sheets in several tube cultures of each adenoid underwent spontaneous degeneration characteristic of APC virus, and APC viruses were demonstrated in the culture fluids. Other culture tubes from each adenoid did not show APC virus effects, and after 71, 64, and 34 days in culture, respectively, one culture of each set showed focal areas of degeneration in the fibroblasts. Subsequently, one or 2 additional tube cultures of each adenoid developed identical

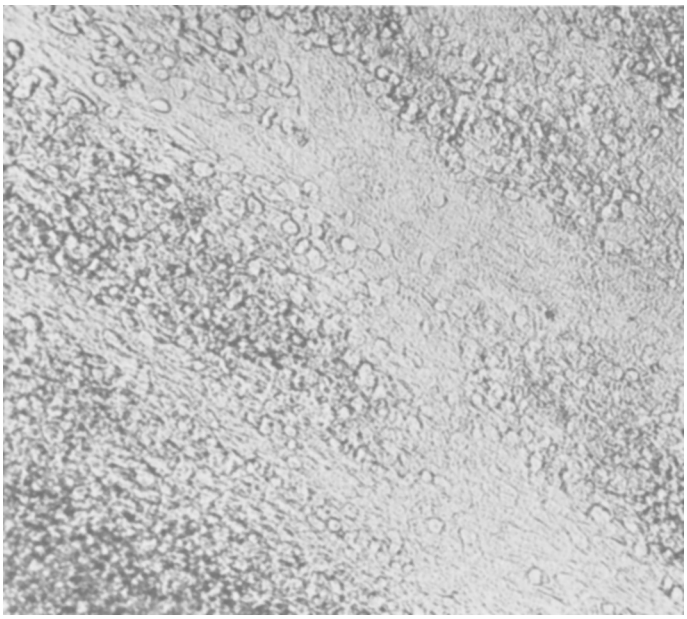

FIG. 2. Ad. 169 strain. Second passage, tonsil tissue, 56th day after inoculation. Numerous oval foci with dense pigmentation. Unstained, ×100.

changes, in one instance after 120 days in culture. The changes consisted of sharply delineated oval areas of clear, smoothly outlined oval or round cells. As the foci enlarged, the central cells became necrotic and densely pigmented, eventually leaving a granular mass in the center of the focus, surrounded by a thin border of rounded cells. Progression of the effects was very slow, usually requiring six to eight weeks for complete destruction of the fibroblasts. First passage was carried out by scraping off the involved tissues, grinding in a tissue grinder, and resuspending in a small volume of supernatant fluid. This material was immediately inoculated into roller tube cultures of adenoids and tonsils showing predominantly fibroblastic outgrowth, no other fibroblast cultures being available. Cytopathogenic effects identical to those in the original cultures developed after an incubation period of 7 to 50 days (Fig. 1 and 2). All three strains were carried through at least 2 serial passages by this method. Table I shows a representative passage series of the Ad. 169 strain. At the fourth passage, the incubation period shortened markedly, and the virus passed well subsequently.

Properties of Ad. 169 Strain. Cytopatho-

TABLE I. Representative Passage Series of Ad. 169 Strain.

Passage No.	Tissue	Type of culture	Day of first CPE	Day when CPE became generalized	CF antigen titer (cell susp.)
	Ad. 169	Roller tube (explant culture)	64		
1	Adenoid	Roller tube	7	45	
2	Tonsil	*Idem*	13	43	
3	Human embryo skin	"	20	61	>1
4	*Idem*	"	2	12	
5	Trypsinized H.E. skin-muscle	Bottle	4	6	6
6	*Idem*	"	4	9	6
7	"	"	3	5	2
8	"	"	5	11	>8
9	"	"	<5	10	16
10	"	"	1	4	

TABLE II. Virus Activity and CF Antigen Titer at Intervals after Inoculation of Bottle Culture with 8th Passage Ad. 169 Strain.

	Material tested	Time of sampling (days after infection of bottle culture) 3	5	7	9	11	13	15	17	20
Incubation period of cytopathogenic effects (duplicate cultures), days	Culture fluid	>47	10	>48	13	>44	8	7	6	7
		>47	>45	>48	>46	>44	9	7	6	
	Cell susp.					4	1	5	1	4
						4	2	5	1	5
CF antigen titer	Culture fluid	0	0	0	0	0	0	1:1	1:1	1:1
	Cell susp.					1:1	1:2	1:4	1:4	1:8
Cytopathogenic effects in bottle culture		±	+				3+			4+

genic changes were produced in all types of human fibroblast cultures tested, including tonsils, adenoids, foreskins, and embryo skin-muscle. In early passages, the changes were identical to those seen in original cultures; in later passages distribution of round cells occasionally became generalized, and only minimal granulation was observed. Hematoxylin-eosin stained preparations revealed that numerous cells in the foci contained large eosinophilic intranuclear inclusion bodies with margination of chromatin and halo formation. In Giemsa stained cultures, the nuclei containing inclusions also demonstrated one or two deeply basophilic bodies in the clear zone or on the nuclear membrane. In cultures containing both epithelium and fibroblasts, the epithelium remained unaffected, except in rare instances when cells immediately adjacent to affected fibroblasts underwent clumping and inclusion body formation. Cytopathogenic changes were not induced in cultures of HeLa cells, KB cells(8), monkey kidney, or rabbit trachea. Adult and suckling mice injected intracerebrally and intraperitoneally and rabbits inoculated intradermally and onto the scarified cornea showed no sign of illness. The virus passed through a Selas .015 filter (maximum pore diameter 2.8 mu) with no loss of activity, but filtration through a Selas .03 filter (maximum pore diameter 1.2 mu) prolonged the incubation period. Infectivity was destroyed by exposure to 20% diethyl ether for two hours. Aureomycin, 50 μg/ml, did not affect virus activity. There was no loss of infectivity in cell suspensions held for 3 hours at 37°C, room temperature, or 4°C. The virus did not withstand storage for a week at 4°C, and much virus was lost after quick freezing and storage at –20°C or –40°C; slow freezing with storage in a dry ice chest appeared more satisfactory for virus preservation. Supernatant culture fluids from early passages did not contain detectable virus, but in later passages the fluids were generally infectious. Table II demonstrates the pattern of virus release in a bottle culture. Five of 6 bottles tested, in 2 experiments, demonstrated peaks of virus activity on the 5th and 9th days, with markedly prolonged incubation periods of fluids removed on the 3rd, 7th, and 11th days. The occasional variation in incubation period of cytopathogenic changes in duplicate tubes was possibly due to the presence of virus in cell clumps, rather than as a uniform suspension. On the 13th day, regardless of whether the bottle had been partially scraped for testing of cell suspensions, the quantity of virus in the fluid increased markedly, and tended to remain elevated for the duration of the experiment. However, in 3 of the 6 bottles, the virus content of the culture fluid was again very low when sampled after all cells had been affected. The cell suspensions consistently produced cytopathogenic changes after a shorter incubation period than the culture fluids, and complement fixing antigen was likewise present in higher titer in the cells.

Serological Tests. Initial experiments to detect the presence of complement fixing antigen were done by testing culture fluids and cell suspensions against the serums of the children from whose adenoids the new agents had been recovered; a number of preparations gave positive reactions with the serums of the donors of Ad. 162 and Ad. 169, but not of Ad. 182. Known positive antigens were then used

TABLE III. Relationship of Neutralizing to Complement Fixing Antibodies in Human Serums by Age Group.

CF antibody (1:8)	Neutralizing antibody (1:4) 1-5 yr			18-25 yr			> 35 yr		
	+	±	—	+	±	—	+	±	—
Positive (≥ 1:8)	7	0	0	3	0	0	11	0	0
Partial at 1:8 (1-2+)	1	0	0	0	0	0	2	0	0
Negative (<1:8)	0	0	7	0	1	5	3	1	1

to test a number of human serums, and a high titer serum which was available in large quantity was selected as a standard serum for titrating antigens. This serum reacted in CF with antigens prepared from each of the three adenoid isolations. A number of positive human serums were tested against a control antigen consisting of a suspension of uninfected human embryo fibroblasts, and no reactions were obtained. Antigens were routinely heated at 56°C for 15 minutes, since this procedure generally removed anticomplementary activity without affecting antigen titer. CF antigen activity was not affected by storage at −20° for 3 months. The occurrence of CF antibodies in relation to neutralizing antibodies in human serums is indicated in Table III. In children and young adults there was very close agreement between the two tests, but in older adults serums negative in CF were often positive in neutralization. Serums positive in CF were in all instances positive in the neutralization test.

Relationship of Ad. 169 Strain to Other Viruses. Because of their similar cultural behavior, the agents recovered by Smith(1) and by Weller (Davis strain)(2) were compared with the Ad. 169 strain in this laboratory and in that of Dr. Weller; only the results obtained in this laboratory will be mentioned here. The general behavior of the 3 viruses appeared very similar, including incubation periods, patterns and rate of progression of cytopathogenic changes, and presence of virus in higher titer in cell suspensions than in culture fluids. Eight human serums having CF antibody to the Ad. 169 strain, and 8 serums having no CF antibody were tested for neutralizing and/or complement fixing antibodies to the Ad. 169, Smith, and Davis agents (Table IV). The majority of serums tested were from young adults. There was excellent correlation between the tests with the 3 viruses. The same association of antibodies was observed in 6 additional serums on which all tests were not completed. Thus, the close cultural and immunological similarity between these strains strongly suggests that they are closely related viruses, and that the Ad. 169 and Smith strains may be identical. Paired acute and convalescent serums from three children with herpetic gingivostomatitis showed antibody rises to herpes simplex virus, but no antibody response to the Ad. 169 virus; a herpes simplex rabbit antiserum with a homologous CF titer of 1:64 gave no CF reaction when tested at a 1:8 dilution against a known positive Ad. 169 antigen. The patterns of cytopathogenic changes produced by the Ad. 169 virus suggested a possible relationship to varicella virus(9); however, in the opinion of Dr. Weller(2), the presence of pigment in the foci and the presence of virus in the culture fluids sharply distinguish the Ad. 169 and Davis agents from varicella. In addition, paired serums from three children with clinical varicella showed no CF antibody response to the Ad. 169 virus. Similarly, paired serums from 10 cases of measles showed no CF antibody rise against the Ad. 169 virus. Because of their recovery from adenoid tissue, it was important to exclude a relationship to the APC virus group. There was no cross reaction between the Ad. 169

TABLE IV. Tests of 16 Human Serums for Neutralizing and/or Complement Fixing Antibodies to Ad. 169, Smith, and Davis Strains.

Ad. 169 CF	No. of serums showing following pattern	Smith CF	Smith Neut.	Davis CF
+	5	+	+	+
(8 serums)*	3	±	+	+
—	7	—	—	—
(8 serums)†	1	±	±	±

* Seven tested in neutralization vs Ad. 169; all positive.

† *Idem*; all negative.

Serums tested at 1:8 in CF and at 1:4 in neutralization.

+, positive test; ±, partial reaction (1-2+ CF reaction, partial delay in neutralization); —, negative test.

TABLE V. Occurrence of CF Antibodies to Ad. 169 Virus by Age and Sex.

Age group	♂ No. positive/No. tested	♂ % positive	♀ No. positive/No. tested	♀ % positive	Total No. positive/No. tested	Total % positive
Birth (cord blood)					12/17	71
6 wk					5/17	29
6-23 mo	3/15	20	0/ 6	0	3/21	14
2- 4 yr	8/17	47	3/19	16	11/36	31
5- 9 "	4/16	25	7/17	41	11/33	33
10-15 "	10/24	42	12/25	48	22/49	45
18-25 "	33/73	45	19/25	76	52/98	53
> 35 "	11/15	73	31/37	84	42/52	81

virus and APC viruses in CF tests; also, the lack of cytopathogenicity for epithelium and the sensitivity to ether treatment have not been observed with any APC virus.

Distribution of Antibodies to Ad. 169 Strain. Complement fixing antibodies were found in a high proportion of human serums (Table V). Cord blood serums and serums from 6-week-old infants were obtained from the same children. Children 6 months to 15 years of age recently admitted to a foundling home provided the majority of children's serums tested. The serums from young adult populations were obtained from student nurses, pregnant women, military recruits, and male penitentiary inmates. The persons over 35 years of age who were tested were employees or patients at the National Institutes of Health, the latter usually having infectious or malignant diseases. Antibodies were frequently present at birth, were less frequent at 6 weeks, and after the age of 6 months, tended to become increasingly prevalent with age. Because of the differences in composition of the sampled populations, the sex differences cannot be regarded as highly significant. Occurrence and development of CF antibodies were observed in serial bleedings of infants. In addition to cord serum and the 6 week bleeding recorded in Table IV, a number of subsequent serums obtained up to the age of 4 were available on 15 of the children.* Ten of the 15 had antibody in the cord serum; 5 were still positive at 6 weeks, but in all instances the titer was lower than in the cord serum. By the age of one or two years, 3 of the 10 had reappearance of antibodies or a 4-fold rise in titer; in all 3 instances, the first serum obtained after the 6 week bleeding was positive. Of the 5 children with no antibody in the cord serum, all were still negative at 6 weeks; however, one of the 5 became positive by the age of 11 months. Three of the positive children were followed for several years with serial titrations; in all instances, the CF titer decreased markedly within 2 to 3 years. In contrast to the decline in antibodies in the children, titrations of serial serums of 3 adult males indicated that CF antibodies remained elevated for at least 4 to 7 years.

Discussion. Several findings suggest a relationship of the newly isolated agents to the pathological entities of human salivary gland inclusions and cytomegalic inclusion disease, which have been suspected to be manifestations produced by a single virus(10). The recovery of the Smith virus from a salivary gland containing typical nuclear inclusions, and the isolation of the Davis virus from cytomegalic inclusion disease strongly support this hypothesis. Also, the inclusions produced in tissue culture by the Ad. 169 strain were reported by Dr. Henry Pinkerton(11) to be pathognomonic of the salivary gland virus. Finally, the type of rounding produced and the strict tropism for fibroblasts are similar to the effects produced by the mouse salivary gland virus in tissue culture(12).

The close correlation observed here between the presence of complement fixing and neutralizing antibodies in human serums is somewhat unique, and resembles the antibody patterns found with herpes simplex(13). This finding suggests that there is but one serological

* The serums were kindly supplied by Dr. Robert H. Parrott.

type of this agent, and is compatible with the concept that the virus persists in the body as is true of herpes simplex and the salivary gland viruses of rodents. However, inclusions are rarely found in salivary glands of adult humans(14). In view of its recovery from adenoid tissue, it appears possible that the virus may persist in lymphoid tissue.

The age distribution of antibodies and the pattern of acquisition of antibody after birth, as reported herein, is somewhat at variance with the usual concept of human salivary gland virus infection, which on the basis of morphologic studies appeared primarily to be an infection of infancy, with many infections acquired *in utero*(10).

The value of the procedure of growing tissues in culture for unmasking indigenous cytopathogenic agents is exemplified here as in similar studies of APC viruses.

Summary. Three strains of an intranuclear inclusion body-producing virus were isolated from spontaneously degenerating tissue cultures of human adenoids. One strain was studied in detail and appeared to be closely related to or identical to viruses isolated in other laboratories from a human salivary gland and a case of cytomegalic inclusion disease. It seems likely that these agents are representatives of the human salivary gland virus. Antibodies were found in a high proportion of human serums.

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Propagation in Tissue Cultures of a Cytopathogenic Virus from Human Salivary Gland Virus (SGV) Disease.* (22498)

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It is now well recognized that there exist in man, monkeys and several rodents closely related viruses, which lie dormant in the salivary glands, but are capable of causing fatal generalized infections with visceral necrosis (1). A general term for these agents is salivary gland virus (SGV). Each virus or strain of the virus is probably species-specific,

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