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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since all attempts to infect animals of one species with virus derived from another species have failed. Serial transmission to animals of the same species has been accomplished with certain rodent strains; in young mice a fatal generalized infection is produced by intraperitoneal injection of infectious material(2). Tissue infection with these viruses results in cellular gigantism, with the formation of nuclear inclusions which, by their huge size, structure and staining characteristics, are set apart from those associated with other known viruses. In man and some other species basophilic inclusions also occur in the cytoplasm of many of the cells which have intranuclear inclusions. The incidence of intranuclear inclusions in the salivary glands of infants and young children, regardless of the cause of death, has been reported from different geographical areas as 10 to 30% in routine autopsies(3-5). The cytologic picture of the infected cells is considered pathognomonic(6), and the diagnosis of generalized human SGV infection is being made confidently and with increasing frequency on morphologic evidence alone; in some geographic areas, it is reported in approximately 1% of all infants and young children dying under the age of 5 years(7,8).

In vitro propagation of the species-specific virus is of obvious importance for further study of the disease. Propagation of the mouse SGV in cultures of mouse tissue has been reported(9). This paper is concerned with the propagation of a cytopathogenic virus from 2 cases of human SGV disease.

Material and methods. Tissue cultures. Pieces of human uterine wall were minced with scissors in Hanks' balanced salt solution and ox-serum ultrafiltrate (2:1). Fifteen to 20 implants were embedded in a thin layer of clotted chicken plasma on the walls of culture tubes (20 x 150 mm). The nutrient medium consisted of Hanks' balanced salt solution and ox-serum ultrafiltrate (2:1), chicken or beef embryonic extract (10%) and inactivated horse serum (10%). One-hundred units of penicillin and 100 μg of streptomycin per ml were added to the medium. The cultures were incubated at 34 to 35°C in a frame revolving 9x per hour, and the nutrient fluid was changed at intervals of 2 or 3 days. The cultures were maintained usually for 10 to 14 days before introducing the virus. *Origin of the Cytopathogenic Virus.* The agents were isolated from tissues obtained at autopsy. To obtain infective material both submaxillary salivary glands were removed at autopsy from infants under 3 years of age, using sterile instruments. One gland was fixed for microscopic study and the other placed in a sterile tube and frozen immediately in dry ice. The first infective agent to be isolated was from a submaxillary salivary gland of a 7-month-old infant dying from adrenal cortical carcinoma. In this case the other gland which was examined microscopically showed many characteristic inclusions, but no visceral involvement was found. The second agent was isolated from the kidney of a 1-month-old infant dying of generalized SGV disease (cytomegalic inclusion disease). The renal tissue was preserved in dry ice for 1 week before it was used for inoculation of cultures. Many characteristic inclusions were present in microsections of the kidneys and other viscera as well as in the salivary glands of this infant. *Preparation of Cultures for Histological Study.* All cultures for histological study were fixed in Bouin's solution and stained with hematoxylin and eosin. Many cultures were fixed and stained in the tubes. These preparations were cleared and preserved permanently in the tubes in xylol. They were studied with the low power objective (10 x). Other cultures were embedded, after fixation, in thin collodion in the tubes. The collodion cast was removed from the tube, cut into pieces of desired size, stained and mounted, according to the technic described by Enders(10).

Experimental. The salivary gland from which the first agent was isolated was ground in a mortar with a small amount of sterile alundum and diluted 1:5 in Hanks' solution and ox-serum ultrafiltrate. Penicillin and streptomycin were added. After centrifugation, 1 ml of the supernatant fluid and 1 ml of nutrient medium, containing twice the usual amounts of embryonic extract and horse

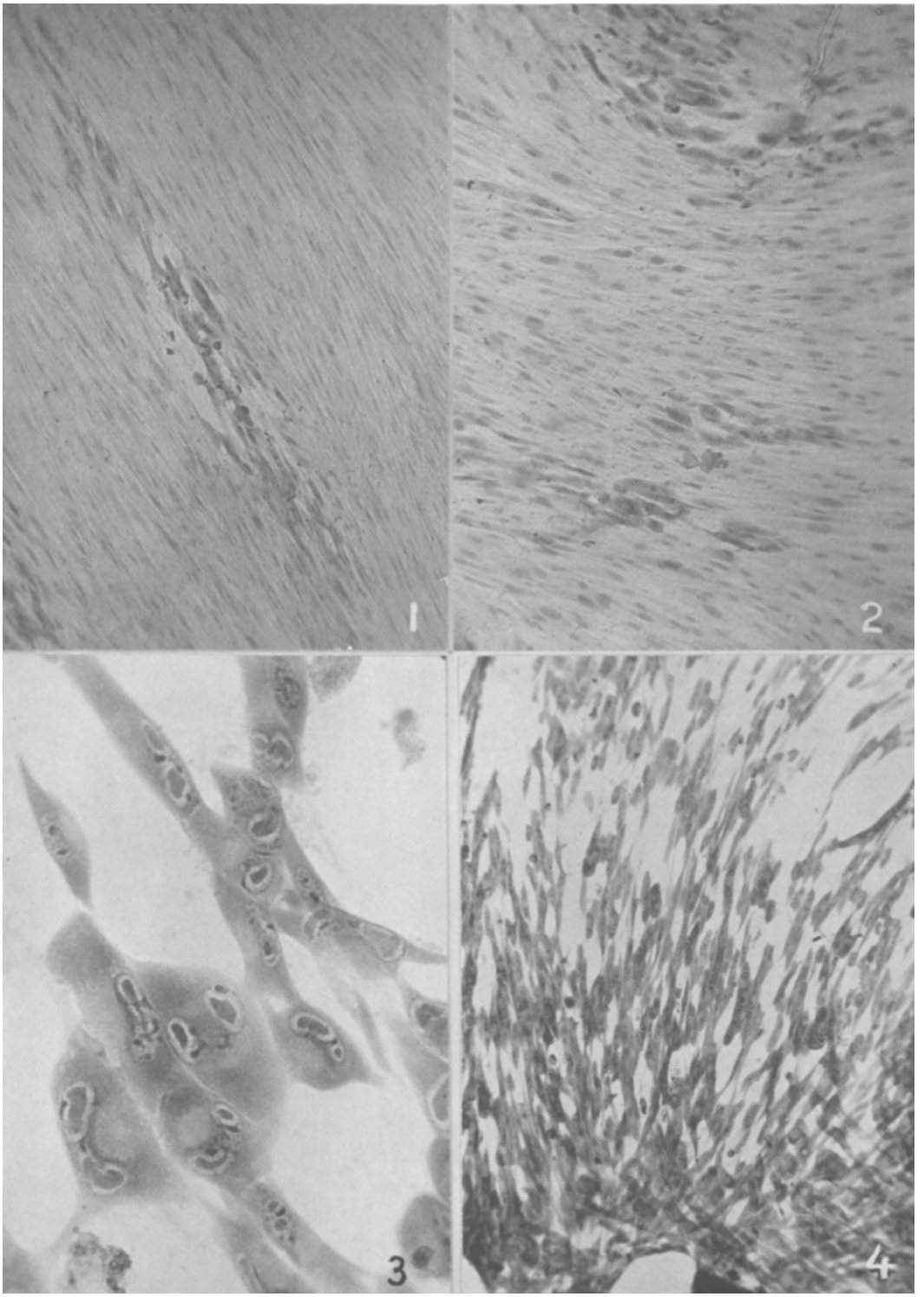

FIG. 1. Elongated focal lesion in culture of human uterine tissue, 29 days following inoculation of human salivary gland agent, original passage. Fixed and stained in culture tube, hematoxylin-eosin stain. × 90.

FIG. 2. Multiple small focal lesions from same culture as Fig. 1. × 90.

FIG. 3. High magnification of enlarged cells from a viral culture, showing intranuclear inclusions. Collodion embedded section, hematoxylin-eosin stain. × 515.

FIG. 4. Diffuse cytopathologic changes in culture, 16 days following inoculation with human salivary gland agent, 6th passage. Intranuclear inclusions are seen in many of the enlarged cells. Collodion embedded section, hematoxylin-eosin stain. × 90.

serum, were used to replace the nutrient fluid on four 14-day-old cultures of human uterine tissue. An equal number of uninoculated control cultures of the same age were maintained under the same conditions as those to which the human material had been added, 1 for 29 days and the other 3 for 45 days. Eight other uninoculated cultures, implanted 4 days before isolation of the virus was attempted, served as additional controls. These were observed for periods varying from 30 to 45 days. In the isolation of the agent from renal tissue preparation of the inoculum from the tissue and the inoculation of 4 cultures were carried out in the same manner as in the isolation of the 1st agent from salivary gland tissue. Control uninoculated cultures were maintained under the same conditions as those inoculated with the renal tissue.

Cytopathologic Changes in Cultures. The early cytologic changes in the cultures consisted of small, round or oval foci in which the cells were enlarged, rounded or oval, and somewhat refractile in contrast to the normal fibroblasts (Fig. 1 and 2). On the 7th day following inoculation of the salivary gland material, 2 of these small foci were observed in one of the culture tubes, and on the 10th, 15th and 24th days, respectively, 2 or 3 similar small areas of altered cells were observed in the other 3 cultures. There was a continuous slow increase in the number and size of lesions in all tubes, followed by degeneration of cells in the centers of the lesions. The degenerating central cells became granular and disintegrated, leaving masses of dense refractile granules. One culture was fixed and stained on the 29th day after inoculation and the remaining cultures on the 56th day. Even at 56 days less than half of the cells of the cultures appeared to be affected.

Cytopathologic changes indistinguishable from those induced by the first agent appeared in the 4 cultures, inoculated with the kidney-material, on the 6th or 7th day following inoculation. The focal lesions increased in size and number somewhat more rapidly than those in cultures inoculated with the salivary gland-material. Thirty days after inoculation of the cultures many of the focal lesions had become confluent. In large areas of the cultures there were degenerating cells and dense granules from disintegrated cells, bordered by the altered large rounded or oval cells. Between these large areas apparently normal fibroblasts were still present. Cytologic changes of the same character occurred in subsequent serial subcultures of both viruses. No cytopathologic changes were observed in any of the control cultures.

In cultures of both viruses fixed and stained in the tubes large intranuclear inclusions were seen under low magnification (100x) in most of the enlarged rounded cells. Occasional cells had 2 or 3 nuclei, each containing an inclusion. The inclusions were amphophilic when stained with hematoxylin and eosin. In cultures embedded in collodion and observed under high magnification the inclusions were finely granular, and in some nuclei 2 or 3 separate masses were seen (Fig. 3). The inclusion was separated from the nuclear membrane by a distinct clear zone and the shape of the inclusion usually corresponded closely to that of the nucleus. Small masses of basophilic material were present in the nuclei, frequently in a marginal position. The nuclear changes closely resembled those seen in infected human tissues in cases of SGV infection and were indistinguishable from those induced in cultures of mouse tissue by the mouse salivary gland virus(9). In most of the affected cells there was a round eosinophilic area adjacent to the nucleus while the remainder of the cytoplasm was basophilic. The large basophilic cytoplasmic granules or inclusions that often occur in infected cells in the human disease were not observed.

Serial Passages. An attempt to subculture the first agent 23 days after inoculation was unsuccessful. However, successful transfers were made on the 30th, 37th and 40th days, and this virus has now been maintained through 29 serial passages over a period of 33 months (Table I, 12 serial passages). For the first 3 serial transfers, made at successive intervals of 37, 47 and 33 days, 2 ml of pooled fluids from preceding cultures were used to replace the entire amount of nutrient fluid on each new culture. The culture fluids were

TABLE I. Serial Propagation of a Cytopathogenic Agent Isolated from Salivary Gland of an Infant, in 12 Successive Passages.

Passage	Days in culture before transfer	ml of fluid transferred from previous cultures	Day cytologic changes noted	No. of cultures, cytologic changes / No. of cultures inoc.
1	0	.. ..	7, 10, 15, 24	4/4
2	37	2.0	7	2/2
3	47	2.0	5	2/2
4	33	2.0	2	2/2
5	30	.5	4	1/1
6	21	2.0	2	1/1
7	9	.2	2	2/2
8	14	.2	5	2/2
9	9	.2	3, 5	2/2
10	10	.2	5	3/3
11	21	.2	2	2/2
12	21	.2	2, 3	12/12

adjusted to pH 7.6 by the addition of 1 or more drops of 1.4% $NaHCO_3$, and 0.5 ml of 2% glucose per 2 ml of fluids was also added. In the successive serial passages cytopathologic changes appeared earlier, were more diffuse and spread more rapidly, involving the entire culture within 2 to 3 weeks (Fig. 4). The first 6 serial passages of the second virus were made at successive intervals of 30 to 56 days by replacing the entire medium on each new culture with 2 ml of pooled fluids from preceding cultures. In the first 4 serial subcultures the incubation period of the cytopathologic changes was 10 to 17 days and the focal lesions increased slowly in size and number. Thereafter the cytologic changes appeared earlier and, as with the first virus, were more diffuse and spread rapidly. The second virus has been carried through 12 serial passages.

At present the transfers of both viruses are made after 2 to 3 weeks of incubation by adding 0.2 ml of fluid from infected cultures to each new culture, and the cytopathologic changes appear in 2 to 5 days.

Infective Titre of Culture Fluid. The infective titre of the supernatant nutrient fluid from the 6th serial subculture of the first virus was studied. Cultures were inoculated with 0.2 ml of the undiluted fluid and with the same amount of each of 3 serial 10-fold dilutions. Two or 3 culture tubes were inoculated with each dilution. The characteristic cytologic changes occurred in all tubes, but the time at which these changes appeared increased progressively with the dilution of the inoculated infected fluid. Changes were observed in cultures inoculated with 0.2 ml of the undiluted fluid on the 2nd day but did not appear in 3 cultures inoculated with 0.2 ml of the 10^{-3} dilution until the 24th, 28th and 34th days.

Neutralization Tests. The source of virus used in neutralization tests was fluid from 3-week-old cultures of the virus which had shown cytopathologic lesions 2 or 3 days following inoculations. When 0.2 ml of fluid from such cultures was used as the inoculum, the incubation period of the cytopathologic change in several cultures rarely varied more than 2 days. Culture fluid was added to an equal amount of the serum to be tested. Nutrient fluid, in the amount of 1.6 ml, and 0.4 ml of the serum-virus mixtures was placed on each of 3 cultures. Horse serum-virus mixtures were used in the control cultures. The incubation period of the cytopathologic change and the progression of the lesions were noted. A serum was considered positive when the incubation period of the cytologic change in the test cultures was prolonged 5 days or more in comparison with the control cultures.

The first agent was neutralized by the sera from 2 infants who died in the newborn period of generalized SGV disease and by the serum of the mother of one of these infants. The latter serum was obtained 1 month after

birth of the infant. The sera of 2 infants, 1 day and 3½ months of age, who had no clinical evidence of SGV disease also neutralized this virus. Sera from 2 other infants, 6 months and 1 year of age, who had no evidence of SGV disease gave negative results. Three of the same sera were tested with the 2nd virus. The serum of one of the infants dying of SGV disease and the serum of the mother neutralized the 2nd virus, and a serum that failed to neutralize the 1st virus also failed to neutralize the 2nd virus.

Properties of the Viruses. Both viruses retained infectivity in tissues from autopsies after storage in dry ice, for 1 week and 2 months respectively. In some instances infective culture fluid has been preserved in dry ice for 6 months with no apparent loss of activity. However, preservation in dry ice has not been uniformly good. Both viruses pass through a Selas #015 filter (maximum pore radius, 1.4 microns) with no apparent loss of infectivity. In repeated experiments, infective fluids from cultures of either virus failed to induce cytopathologic changes in cultures of mouse embryonic tissue.

Groups of 8 suckling mice and of 8 mice, 4 weeks of age, were inoculated intraperitoneally and intracerebrally with infective fluid from cultures of each virus. The mice which had been inoculated intracerebrally remained well during an observation period of 30 days. Those which had been inoculated intraperitoneally showed no signs of illness and were killed 2 weeks following inoculation. No evidences of SGV infection or other disease were found at autopsy. In contrast to these results with the 2 strains of virus of human origin, the mouse SGV disease, as characterized by the specific intranuclear inclusions, was produced uniformly in mice from the same colony by intraperitoneal inoculation of infective fluid from cultures of the mouse SGV in homologous tissue(9).

One-tenth ml of infective fluid from cultures of each virus was placed on a scarified cornea of each of 2 rabbits. No lesions of the corneae or conjunctivae, or other evidence of illness developed. One-tenth ml of infective fluid from cultures of the 1st agent was also inoculated on the chorioallantoic membrane of each of seven 10-day embryonated hen's eggs. The membranes appeared normal when examined on the 3rd and 5th days following inoculation, and microscopic examination of the membranes revealed no pathologic changes.

Discussion. Two viruses, inducing identical cytopathologic changes in cultures of human fibroblasts, have been recovered; the first from the salivary gland of an infant in whom infection of the salivary glands with the SGV was established by microscopic examination and the second from the kidney of an infant dying of generalized SGV infection. In each instance the cytopathologic changes occurred in all of the 4 inoculated cultures but did not occur in any uninoculated control cultures which were observed over the same period. Therefore, it may be concluded that the cytopathogenic agents were derived from the human tissue. Neutralization tests were performed with only a small number of human sera but the results also indicate that the viruses are of human origin. Both viruses were neutralized by the serum of an infant dying of cytomegalic inclusion disease and by the serum of the mother of the infant. One human serum did not neutralize either virus. Moreover complement-fixing and neutralizing antibodies to our 1st virus have since been demonstrated in human sera by Rowe and associates(11). Their results strongly suggest that our 1st virus is closely related to or identical with 2 other recently isolated viruses; one recovered by Weller from a biopsy of the liver of an infant having clinical signs of cytomegalic inclusion disease and another isolated by Rowe and associates from adenoid tissue.

The large intranuclear inclusions produced in the cultures of human fibroblasts are like those which are pathognomonic of SGV disease, and the cytopathologic changes, including the intranuclear inclusions, are indistinguishable from those induced by the mouse SGV in cultures of mouse tissue(9). However, the 2 agents from human tissue are differentiated from the mouse SGV by their failure to induce cytopathologic changes in cultures of

mouse tissue or to produce SGV disease in mice.

The character and progression of the cytopathologic changes induced in cultures of human fibroblasts by the 2 viruses which we have isolated resemble somewhat those described by Weller(12) for the varicella virus. However, the behavior of these 2 viruses in cultures differs from that of the varicella virus in that they are present in the culture fluid and can be readily transferred by the fluid. In addition the conspicuous dense granules which occur in the centers of degenerating focal lesions are not described for the lesions produced by the varicella virus.

The restricted host pathogenicity of the 2 agents also differentiates them from the virus of herpes simplex, a common virus producing a large intranuclear inclusion which might be encountered in human tissue in the absence of recognizable infection.

Conclusions. A cytopathogenic virus inducing large intranuclear inclusions like those occurring in salivary gland virus disease has been isolated from the tissues of each of 2 infants. In both cases the virus has been propagated serially in cultures of human fibroblasts derived from uterine tissue. The distinctive cytopathogenic effects and the apparent species specificity of the 2 viruses, together with the isolation of each of the viruses from human tissue which contained the characteristic inclusions of salivary gland virus disease are substantial evidence that these viruses are strains of the human salivary gland virus.

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Sedimentation Behavior of Serum Lactic Dehydrogenase. (22499)

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The elevation of serum lactic dehydrogenase (LDH) activity has been observed in many patients with neoplastic diseases(1,2). Since 2 components with LDH activity have been found in heart(3,4) it was of interest to ascertain whether the LDH activity found in the sera of apparently healthy individuals or in patients with neoplastic diseases is attributable to one or more moieties. A technic has been described(5) which permits the determination of the sedimentation pattern of an enzyme in crude preparations. This method was applied in the present investigation to the study of the sedimentation behavior of LDH activity in serum as a first step in determining the physical properties of the serum enzyme.

Materials and methods. For investigation of sedimentation behavior one part of serum was diluted with 4 parts of saline-phosphate buffer mixture to give final solution that was 0.145M with respect to sodium chloride and