on AHF in stabilized ($BaSO_4$-adsorbed) plasma. Only reagents that provided a source of thrombin caused AHF to disappear after recalcification. Materials tested that produced loss of AHF were: normal, hemophilic, PTC-deficient and Stuart factor deficient plasmas and hemophilic serum. All contained prothrombin. Those that produced no significant loss were: dicumarol plasma, normal serum, SAF, PTC and adsorbed serum; they contained no detectable prothrombin. Slow prothrombin conversion (*e.g.* Stuart plasma, platelet-poor plasma) was accompanied by slow AHF utilization. Removal of fibrinogen from reaction mixtures did not abolish AHF loss. Purified prothrombin caused utilization of AHF in proportion to its concentration if cephalin and calcium ions were present to cause conversion to thrombin. Preformed thrombin was also effective in proportion to its concentration; its effect was accelerated by calcium ions. The significance of these findings is discussed in relation to blood preservation, intravascular coagulation and basic clotting reactions.

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Serial Propagation of the Guinea Pig Salivary Gland Virus in Tissue Culture. (23455)

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The use of tissue culture methods for isolation and propagation of the viruses of mouse(1) and human(2-4) salivary gland disease and the attendant development of technics for their serological study suggested similar efforts to establish the salivary gland virus of guinea pigs. Andrewes(5) found that the inoculation of suspensions of infected salivary glands into modified Maitland cultures of guinea pig testis regularly resulted in development in 3 to 6 days of intranuclear inclusion bodies in mononuclear cells in the in-

terstitial tissues. Active virus was recoverable for at least 5 days by subcutaneous inoculation of young guinea pigs with culture fluids. However, all attempts to pass the virus serially in tissue cultures failed. The present report is concerned with the successful propagation of a strain of the guinea pig salivary gland virus in tube cultures of guinea pig tissue, and the application of serological technics to its identification.

Methods. Tissue cultures. Explant cultures of guinea pig submaxillary gland and embryonic guinea pig muscle were prepared by the plasma clot technic and were grown and maintained in a modification of Enders' beef amniotic fluid medium(6,7). Trypsin-dispersed stationary tube and 8 oz. bottle cultures were prepared from embryonic guinea pig muscle and were grown in 15% guinea pig serum in Eagle's basal medium(8), to which soybean trypsin inhibitor (0.005%) was added; after satisfactory outgrowth of cells, usually in 4 to 7 days, cultures were washed 3 times in Eagle's basal medium and thereafter maintained in 5% horse serum in Eagle's basal medium. The cell sheet consisted almost exclusively of elongated polygonal or spindle-shaped cells, which appeared to be fibroblasts; occasional small islands of epithelium were present in a few cultures. All media contained penicillin (250 units per ml) and streptomycin (250 μg/ml). Tube cultures contained 1 ml of maintenance fluid and were changed twice weekly; bottle cultures contained 20 ml and were changed once weekly. *Virus. Strain 1.* Healthy guinea pigs harboring the salivary gland virus were supplied by a dealer in Yonkers, N.Y. The virus was established in serial passage in guinea pigs from the N.I.H. colony ("N.I.H." strain) by subcutaneous injection into the region of the salivary glands using 0.2 ml of a 10% suspension of submaxillary glands from 2 infected animals; passages were made at 3- to 4-week intervals. Histological examination of submaxillary glands harvested at each passage revealed typical intranuclear inclusion bodies within enlarged duct cells, with varying degrees of mononuclear cell infiltration. No overt disease was associated with infection by this route. The virus carried in serial passage may represent a mixture of strains since 7.7% (3/39) of control "N.I.H." strain guinea pigs aged 5 months or older were naturally infected, as evidenced by infrequent salivary gland inclusions. The tissue culture passage line was initiated by inoculation of guinea pig embryo muscle explant cultures with 0.2 ml of a freshly prepared, lightly centrifuged 10% submaxillary gland suspension from the second guinea pig passage. Serial passages in tissue culture were made with 0.1 or 0.2 ml of culture fluid containing whole or ground cells harvested at the time of severe cytopathogenic effects. *Strain 2.* Infected submaxillary gland material from guinea pigs supplied by another dealer in New York State, was the source of a second serial passage line initiated and carried in "N.I.H." strain animals. *Complement Fixation Test.* The technic was a modification of the Bengtson procedure(9,10). The source of complement was serum from 6- to 8-week-old guinea pigs of a strain ("Hartley") apparently free of infection; the serum was preserved at 4°C in hypertonic salt solution, one part of saturated NaCl to 9 parts serum(9); activity comparable to that of commercial lyophilized complement was maintained for at least 6 weeks. Virus and control antigens consisted of tissue culture cells ground in a TenBroeck grinder and suspended in culture fluid; antigens were usually heated at 56°C for 30 minutes. Standard positive serum for titrating antigens was hyperimmune guinea pig serum or commercial complement having high titer antibody. Serums were inactivated by heating at 56°C for 30 minutes immediately before use. *Neutralization test.* Virus preparations consisted of supernatant fluid of fresh homogenates of severely affected cells in culture fluid, centrifuged at 1000 rpm for 10 min. Tests were performed in trypsin-dispersed cultures, using the technic described for the adenoviruses(11). Because of difficulty in obtaining complete neutralization, the criterion of neutralization was cytopathic effects less than one-half the virus control reading on the day when the latter first showed 2-plus or greater involvement. *Histology.* Tissues were fixed in Zenker's-formalin and stained with Giemsa stain.

Results. Tissue culture isolation of strain 1. Primary inoculation of explant cultures of guinea pig embryo muscle with infected submaxillary gland suspension produced in 10 days small foci of enlarged, round, refractile, smoothly outlined cells in the fibroblast outgrowth. By the 20th day, the focal areas had extended to involve more than one-half the fibroblasts; at 28 days, there was almost complete involvement and many necrotic cells were present. The characteristic changes were reproduced on transfer of fluid containing ground cells to both trypsin-dispersed and explant cultures. Cytopathic changes were not seen in explant cultures until the 24th day after inoculation, but the incubation period in trypsinized cultures was 6 days and all cells were involved in 12 days; consequently, trypsin-dispersed cultures were used for all subsequent passages. After 10 passages, the incubation period shortened to 2 or 3 days, with complete degeneration occurring within 6 to 8 days. The virus was carried through 22 serial passages, representing a theoretical dilution of the original inoculum of 10^{-33}. In stained preparations made at intervals throughout the passage series, the majority of cells contained large, elongated, often kidney-shaped, eosinophilic intranuclear inclusion bodies with marginated chromatin and a clear halo. The inclusions closely resembled those produced by the human salivary gland virus. Definite cytoplasmic inclusions were not observed.

Repeated attempts to infect tissue cultures of human cells with the guinea pig agent, and vice versa, were completely negative.

Complement fixing antigen was demonstrated with regularity in cultures showing severe cytopathic changes; highest antigen titers (1:8 or, rarely, 1:16) were obtained by grinding the cells and suspending in supernatant fluid. Commercial guinea pig complement from each of 5 companies was found to contain antibody against the guinea pig salivary virus antigen prepared in tissue culture, in titers of 1:64 to 1:256. Consequently, serum from young guinea pigs, demonstrated to be free of antibody, was used for complement. The commercial complement served as a positive standard reference serum.

CF antibody-free guinea pigs inoculated with either strain 1 or strain 2 guinea pig passage virus regularly developed CF antibody titers of 1:64 within 3 weeks. Titers of 1:128 or greater were found in guinea pigs hyperimmunized with submaxillary gland suspensions infected with either strain. No reactions were obtained when control guinea pig tissue culture antigens were tested against a number of strongly positive antiserums. No serological relationship could be demonstrated to the human or mouse salivary gland viruses.

Poorly adapted virus in early tissue culture passage was generally unsatisfactory for tissue culture neutralization tests, because of extended incubation periods of cell-free virus suspensions and the difficulty in neutralizing virus in cell suspensions. After the 7th tissue culture passage, there was generally enough virus activity in centrifuged cell homogenates to permit satisfactory tests. Development of neutralizing antibody was demonstrated with regularity in guinea pigs infected with either animal passage or tissue culture passage virus.

Identification of the tissue culture isolate as the virus of guinea pig salivary gland disease was indicated by the characteristic inclusion bodies produced in tissue culture cells and the ability of tissue culture virus to demonstrate neutralizing and complement-fixing antibody responses in animals infected with submaxillary gland virus from two animal passage lines. In order to provide supportive evidence, attempts were made to reproduce with tissue culture passage virus the pathognomonic features of spontaneous salivary gland disease and the acute meningitis resulting from intracerebral inoculation of virus-containing submaxillary gland suspensions (12). Virus from the 10th tissue culture passage was used; this material produced characteristic cytopathic effects in 2 days when inoculated into tissue culture and represented a 10^{-20} theoretical dilution of the original submaxillary gland suspension. Four 4- to 5-week-old guinea pigs from the virus-free colony were anesthetized with ether, a portion of the right submaxillary gland was removed for sectioning, and 0.2 ml of virus inoculated subcutaneously into the area of the left submaxillary gland. At 13 and 22 days, 2 animals

were sacrificed and the left gland was removed. Blood specimens were obtained before inoculation and at sacrifice. Preinoculation submaxillary gland biopsies were uniformly negative, while post-inoculation sections showed characteristic salivary gland disease histopathology, varying in intensity from scattered foci of mononuclear infiltration with few inclusions to more widespread infiltration with moderate numbers of inclusions, several ducts containing 5 to 8 inclusions in a single cross section. Virus was recovered from all 4 glands in tissue culture either on primary isolation or after one blind passage. Both animals sacrificed at 22 days demonstrated CF antibody responses, the titer increasing from $<1:8$ to 1:8 in one animal, and from $<1:8$ to 1:64 in the other. Neutralizing antibody in the latter animal rose from $<1:5$ to 1:40.

Two guinea pigs of the same age and strain as the above animals were inoculated intracerebrally with 0.05 ml of the tissue culture virus and were observed daily for fever and symptoms. Fever was noted in 48 hours and typical symptoms of weakness, lack of coordination, and convulsive movement were evident in 4 days, accompanied by a marked fall in temperature; the animals were sacrificed when moribund, on the 4th and 5th day respectively. Brain sections of both animals revealed marked mononuclear infiltration of the meninges, with typical intranuclear inclusions in mononuclear cells. A suspension of meninges and superficial brain tissue from one animal produced typical cytopathic changes in 6 days after inoculation into tissue cultures. Neither animal had detectable CF antibody in the preinoculation serum specimen.

Additional isolation attempts. In the course of serial passages of the 2 strains of salivary gland virus in guinea pigs, 12 suspensions of submaxillary glands with inclusions were inoculated into explant or trypsin-dispersed tissue cultures of guinea pig embryo muscle. Ten produced cytopathic effects identical with those seen with the strain 1 tissue culture line, with incubation periods of 3 to 10 days; at least 1 subsequent serial passage was possible with 5 of the 7 isolates so tested.

One of the strain 2 isolates has been carried through 8 serial passages with findings completely comparable to those with strain 1; much difficulty was encountered in maintaining the virus during the first 5 passages, but at the 6th passage the incubation period shortened, and the virus has since passed readily. Strain 2 was not distinguishable from strain 1 by CF or reciprocal tissue culture neutralization tests.

No cytopathogenic effects were produced by one suspension of glands which did not contain demonstrable inclusions.

Spontaneous degeneration of tissue cultures of infected salivary glands. Since the human salivary gland virus has been recovered from spontaneously degenerating tissue cultures of human adenoids(3), attempts were made to recover guinea pig virus by cultivating salivary gland tissue. In two experiments, explant cultures were prepared from inclusion-containing submaxillary glands removed on the 17th and 21st day after animal inoculation with guinea pig passage virus. The first of these represented portions of the glands used in suspension to initiate the tissue culture passage line of strain 1. Both sets of cultures grew well, producing primarily fibroblastic outgrowth. On the 28th and 29th days, respectively, cytopathic changes developed which were identical with those produced by infected gland suspensions and tissue culture passage virus. Both sets of cultures produced complement-fixing antigen.

Discussion. The study presented here has indicated that the behavior of the guinea pig salivary gland virus in tissue culture is strikingly similar to that of the human salivary gland virus(2-4). The cytopathic effects of the agents are very similar, and are produced only in fibroblasts. In early passages, the viruses produced effects only after a prolonged incubation period, the rate of progression of cytopathic effects was slow, and the virus could be passed with ease only by using ground cells in the inoculum. In later passages the virus was better adapted to growth in culture and could be passed with cell-free fluids. These similarities suggest that the guinea pig agent would be a useful model in studies designed to anticipate the natural history of human salivary gland virus infection.

The successful passage of the guinea pig

virus in tube cultures, in contrast to the unsuccessful attempts of Andrewes with suspended fragment cultures(5), perhaps is directly comparable to the experience of Weller with varicella virus. Inoculation of tissue fragments with varicella or herpes zoster materials resulted in inclusion body formation which could not be reproduced on serial passage(13); however, using tube cultures and inocula containing cells, Weller was able to carry the viruses in serial passage(14).

Summary. The guinea pig submaxillary gland virus has been successfully propagated in tissue culture, one strain having been carried through 22 serial passages. The tissue culture passage virus produced characteristic intranuclear inclusion bodies in tissue cultures, demonstrated complement fixing and neutralizing antibody responses in guinea pigs infected with animal passage virus, and produced characteristic disease and histopathology in susceptible guinea pigs. Commercial complement consistently contained high titer CF antibody.

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Differential Photoreactivation of *Escherichia coli* after Exposure to 2650 and 2250 A Ultraviolet.* (23456)

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The authors have reported(1) that the fraction of the mutagenic and lethal effects of ultraviolet (UV) radiation in *Escherichia coli* cells that is photoreactivable is the same after exposure to 6 wave lengths ranging from 2378 to 2967° A. The fraction of the effect that was photoreactivable was significantly greater for mutation than for inactivation. Extension of these studies to 2250 and 2180 A has revealed some interesting differences in the response to photoreactivating (PR) light and in the response of different strains of *E. coli*.

Inactivation and mutation to streptomycin independence in *E. coli* SD-4 was studied initially. *E. coli* SD-4 is particularly well suited for tests of photoreactivation of mutations since a linear mutation-dose relation is obtained(1,2). The methods of preparation of cells for irradiation and of exposure to UV and PR light have been described(1). Muta-

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