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Experimental Herpes Simplex Infection in the Owl Monkey.* (32100)

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Characteristic of the herpesvirus group is the wide range of infectivity for animals. Thus, *H. hominis* infects the rabbit, guinea pig, mouse, hamster and cotton rat when inoculated by various parenteral routes(1). After ocular inoculation the usual strain of *H. hominis* infects the cornea of the rabbit and guinea pig, produces keratitis and fails to spread to the central nervous system or systemically. Intracerebral inoculation usually results in encephalitis. Certain neurotropic strains spread to the CNS after ocular inoculation.

A number of early studies showed that *H. hominis* was infectious also for Old World monkeys, by intracutaneous or intracerebral inoculation(2,3). Infection of the squirrel monkey by ocular inoculation resulting in keratitis, was reported recently(4). The susceptibility of other small New World monkeys is apparently unknown.

Herpesvirus simiae (Herpes B) represents the natural herpesvirus infection of the Rhesus monkey and is infectious for the human as well as a variety of laboratory animals. In the human, *H. simiae* is typically neurotropic and infection results in extensive encephalitis. Widespread systemic disease, with involvement of various organs, including the liver, spleen, kidneys and adrenals may also occur(5).

A new simian herpesvirus has recently been identified. The new virus, designated *Herpesvirus tamarinus* was isolated from marmoset monkeys during an outbreak of disease among newly formed colonies in this country. In these small New World primates *H. tamarinus* infections were frequently fatal. Characteristically, infection involved the liver and adrenal(6). Other small primates, such as the squirrel monkey *(Saimiri sciureus)* apparently develop subclinical infections, become viral excretors and may represent a reservoir of infection to a colony of susceptible animals(7).

During experiments designed to study the effects upon the cornea of ocular inoculations of *H. hominis*, it was observed that the owl monkey *(Aotus trivirgatus)* was markedly susceptible to infection. This report describes various clinical, virologic and pathologic events occurring during a generalized infection of the owl monkey with *H. hominis*.

Material and methods. Monkeys. The owl monkeys *(Aotus trivirgatus)* employed in these experiments were adult males and females weighing between 0.6 and 0.8 kg. These animals were captured wild in the jungles of Colombia, and were quarantined in mosquito-proof cages for 14 days prior to shipment to the United States. At an animal farm in this country they were housed separately, except for an occasional contact with Capuchins *(Cebus capucinus)*.

In the laboratory, the monkeys were caged

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individually in an isolation room. Food and water were provided by an animal caretaker who did not handle the animals. The animals were handled only by the investigators with hand washing and instrument changes between animals. Fomites were scarce in the animal room but not absent and no attempt was made to control them. During the first week the monkeys general health was noted and morning rectal temperatures were recorded. Animals of Group II were subjected to more intensive study than those of Group I, the preliminary study group.

Inoculation and clinical observations. Prior to virus inoculation, specimens were taken of conjunctival (right) and nasopharyngeal secretions, feces, and blood for virologic and serologic studies. All experimental infections were initiated by dropping a suspension of virus into the conjunctival sac. After sedation, analgesia of the cornea was achieved with proparacaine hydrochloride (Ophthaine, Squibb). In some animals the right cornea was scarified with a 26 gauge needle; 0.05 ml of stock virus suspension was then instilled into each conjunctival cul-de-sac, the lids held closed and gently massaged. In other animals, neither cornea was scarified, and virus suspension placed into the right cul-de-sac only.

All animals in Group II were observed daily for clinical evidence of disease and morning rectal temperatures recorded. Conjunctival and pharyngeal secretions, rectal swabs and blood were collected for virologic studies at intervals from any monkey with evidence of ocular or systemic disease as soon as the clinical signs were apparent.

At the death of any animal an autopsy was performed as quickly as possible. Specimens from dfferent organs were obtained using separate surgical instruments. The tissues were not frozen before processing. The following tissues were obtained for virologic and pathologic study: cornea, lungs, liver, spleen, kidneys, adrenals, spinal cord, brain, heart's-blood, tracheobronchial secretions and lymph node.

Virus. Herpesvirus hominis (strain HS-1-65) was isolated in human amnion cells from an 11-year-old white male with recurrent herpetic keratitis. This virus produced typical cytopathologic changes (CPC) and inclusion bodies in KB cells and was neutralized by reference guinea pig antiserum, obtained from the Microbiologic Reagent Section, CDC, Atlanta, Georgia. A standard seed stock was prepared by inoculating amnion cultures with virus sufficient to cause complete CPC to develop in 3 days. Cells and fluids were then harvested together, dispersed in 1 or 2 ml aliquots into glass vials and stored at −90°C until used. The virus titer was $10^{7.5}$ tissue culture infectious $dose_{50}$ ($TCID_{50}$) per ml.

Tissue culture. Confluent primary amnion cell monolayers in 1.0 ml roller tubes and 3 oz prescription bottles were cultivated and maintained in Eagle's minimal essential medium (EMEM) supplemented with 10-20% fetal bovine serum (FBS). These monolayers were used for virus isolations, neutralization tests, and for preparation of virus stocks.

Virus titrations. Serial $\log_{10}$ dilutions of virus stock were made in sufficient maintenance medium to allow 1 ml aliquots of each dilution to be added to each of 4 fresh amnion cultures in roller tubes. Media were completely changed every 3-4 days and incubation at 37°C was carried out for at least 14 days. Endpoints were dependent upon development of 50% cell destruction or greater (CPC) in the cultures and were calculated by the method of Reed and Muench(8).

Virus isolations. Swabs of nasopharyngeal, tracheobronchial, conjunctival secretions, stools and urine were immediately immersed in 1 ml of EMEM supplemented with 5% fetal bovine serum and containing 1000 units of penicillin, 200 μg of streptomycin and 50 μg of amphotericin B per ml. After 1 hour at 22°C, specimens were centrifuged at 6000 RPM for 20 minutes, and 0.2 ml of the undiluted supernate inoculated directly into each of 3-4 amnion cultures. Serum was inoculated after antibiotic treatment directly. Organs and tissues obtained by autopsy were immediately minced and ground in a cold mortar and pestle, 20% suspensions made in tissue culture medium with antibiotics and centrifuged for clearing. Aliquots of the undiluted supernates (0.2 ml) were added to

each of 3-4 amnion cultures. All cultures were observed for 30 days or until CPC developed.

Virus neutralization. Approximately 30 $TCID_{50}$ of virus and 4-fold dilutions of serum were mixed in maintenance medium and allowed to stand for 1 hour at 37°C, after which 1.0 ml was added to each of 3-4 amnion monolayers. The test was interpreted when CPC of virus controls was complete (100%). The titer of the reference antiserum was 1:1024 against approximately 100 $TCID_{50}$ of the HS-1-65 strain of herpesvirus.

Histopathology. Organs were fixed immediately in 40% formalin for histologic examination. Sections were stained with hematoxylin and eosin.

Results. Clinical and virological observations. Two groups of animals have been studied.

Groups 1, consisting of 4 animals, was inoculated with approximately $10^{6.0}$ $TCID_{50}$ of virus into both conjunctival cul-de-sacs after scarification of the right cornea. In all animals, eye infections were obvious by the 4th day after inoculation, presenting as conjunctivitis and keratitis with periorbital edema and ptosis of the lids. By the 5th day, all animals had signs of systemic illness and all were dead by the 6th day. From 2 of these animals, the following tissues were obtained: brain, cornea, heart's-blood, liver, spleen and kidneys. Virus was isolated from the brain, heart's-blood and cornea of one and from the kidneys, spleen and liver of the other.

On the 4th day after the above group was inoculated, uninoculated owl monkeys housed in the same room developed lethargy, nasal discharge and died within 3 to 4 days after onset of clinical illness. In this room all animals which developed signs of systemic illness died; the clinical illness was different only in the lack of clinically obvious ocular involvement. Tracheobronchial secretions and lung tissues were obtained from 2 of these animals and herpesvirus was isolated from all 4 specimens.

Based upon these observations, a more systematic study was carried out.

Group 2 consisted of 6 animals caged separately in a single 3-tiered rack. Animals #1, 2 and 3 were inoculated in the right eye with approximately the same amount of virus used in the first experiment and then placed in the top tier of cages. Animals #4, 5 and 6 were not inoculated and placed in cages in the middle and lower tier of the rack. No animal in the group had corneal scarification.

By the second day after inoculation, 2 of the monkeys (#1, #2) developed keratitis and by the third day, had elevated temperatures and nasal discharge and on the fourth day, were lethargic. These clinical signs rapidly worsened and both animals died on the fifth day. Conjunctival and pharyngeal secretions contained virus 72 hours after inoculation, coinciding with the clinical finding of keratitis and nasal discharge. Fever and keratitis had been observed in animal #1 24 hours before and this may have indicated advancing local infection or systemic spread. Both animals continued to excrete virus into conjunctival and pharyngeal secretions and one animal was found to be viremic before death (Fig. 1).

Animal #3 died on day zero apparently from shock secondary to handling and vena puncture.

Two of the uninoculated animals (#5, #6) also developed viral infection. That infection was present as early as 4-5 days after other animals had been inoculated was proven by recovery of virus from the conjunctival secretions of one animal and from pharyngeal secretions of the other. The course of infection in one of these (#6) was very similar to that in the animals directly inoculated: fever, conjunctivitis, keratitis, nasal discharge, lethargy, hypothermia and death, with virus present in the eye, pharynx, feces and blood. Once fever developed, the duration of illness until death was about the same as in inoculated monkeys; however, the incubation period was probably longer by 3-4 days. During the course, a single labial lesion developed but cultures taken did not reveal the presence of virus.

The infection in the other exposed animal (#5) followed a different course. This monkey had been noticed to have a slight nasal discharge and to be of moderate activity at time of first exposure, and the temperature was and continued to be slightly less than normal. These signs continued until the eighth day

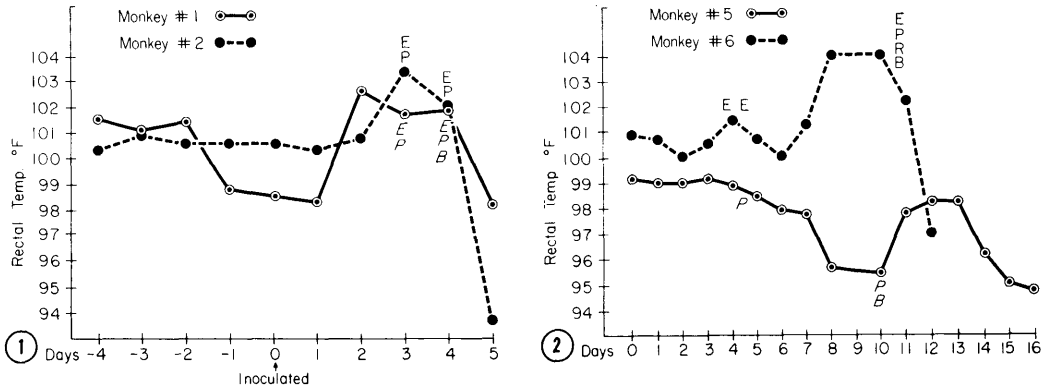

FIG. 1. Course and virus isolations in monkeys inoculated intraocularly with HS-1-65 herpesvirus. Italics equals isolations from animal #1. E - virus in conjunctival secretions from inoculated eye. P - virus in pharynx. B - viremia.

FIG. 2. Course and virus isolations in uninoculated monkeys in the same room with inoculated animals #1 and #2. Day 0 - time of inoculation of animals #1 and #2. Italics refer to animal #5. E - virus in conjunctival secretions of inoculated eye. P - virus in the pharynx. R - virus in feces. B - viremia.

when hypothermia developed. In spite of a transient return of the temperature to normal, the animal became progressively more lethargic, again hypothermic and died 16 days after exposure. During the period of maximal clinical illness, virus was again isolated from the pharynx and also from the blood. Subsequently the animal became virus-negative. The total clinical course in the exposed animals was apparently 8 days, as compared to 6 days in the inoculated group (Fig. 2).

The third animal exposed to the inoculated group failed to develop any signs of ocular or systemic illness during this study and survived for use in further experiments.

Pathologic and virologic studies of autopsy tissues. The animals which succumbed to acute infection (#1, 2, 6) all developed very similar gross and histopathologic changes in organs and tissues. Areas of focal necrosis were prominent in the peripheral and midzones of the liver, and in those areas, hepatic cell nuclei contained intranuclear inclusions. The adrenal glands had areas of focal cortical necrosis, in which there were many intranuclear inclusions (Fig. 3). Widespread interstitial pneumonitis was present and occasional amphophilic intranuclear inclusions were observed in the alveolar lining cells. Areas of acute focal necrosis were to be found in the congested splenic pulp as well as in several lymph nodes and intranuclear inclusions were present in these areas. The inoculated cornea showed complete desquamation of the epithelial layer, with diffuse stromal edema and focal infiltration of lymphocytes and neutrophils (Fig. 4).

The distal convoluted tubules of the kidneys of the exposed animals only were vacuolated with areas of desquamation.

None of these animals were found to have pathological changes in the central nervous system.

Isolation of herpesvirus from every organ and tissue obtained from the inoculated animals (#1 and #2) and from all except brain and spinal cord of the exposed animal (#6) confirmed that they died with an overwhelming generalized infection (Table I). The only urine specimen from any of the animals was obtained just after death of #6 by needle puncture, and this contained virus.

The other exposed monkey which developed infection was found to have only widespread hepatitis of a focal nature at post-mortem and all tissues were free of virus.

Serological studies. All viruses isolated from clinical as well as autopsy specimens induced typical rapidly progressive herpesvirus CPC in human amnion cells. Six of the virus isolates, 3 from clinical specimens and 3 from autopsy materials, were neutralized by pooled human gamma globulin and reference *Herpes-*

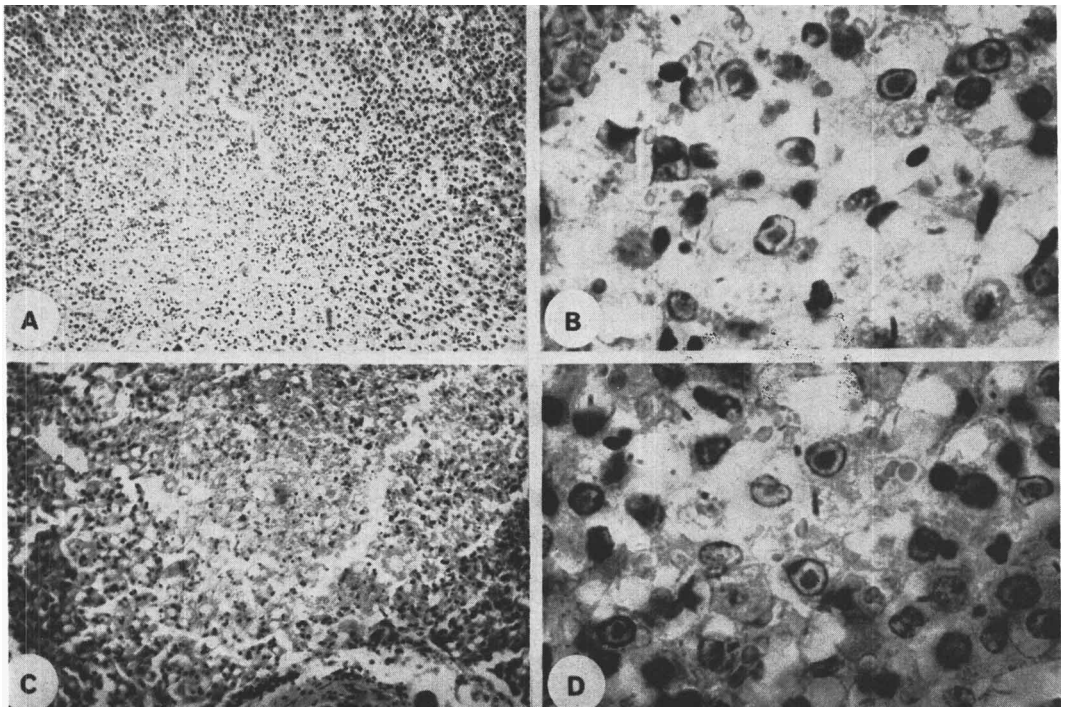

FIG. 3. (A) Area of focal necrosis of the liver 125 ×. (B) Type A & Type B intranuclear inclusions in the liver. (C) Area of focal necrosis of the adrenal cortex 125 ×. (D) Intranuclear inclusion in the ardenal cortex.

virus hominis antiserum. These viruses were not neutralized by antiserum to Herpesvirus "T", obtained from Dr. D. Deinhardt(5).

The sera of all Group 2 animals taken prior to infection, during the course of illness and at death failed to show any neutralizing activity to the virus inoculated. In addition, the sera of the animal which failed to become infected, taken during the time of exposure and 28 days afterward also had no neutralizing activity.

Discussion. These data provide certain evidence of susceptibility of the owl monkey to a strain of *Herpesvirus hominis*. The course of the disease observed in inoculated animals and also in animals developing infection by exposure was generally of short duration, terminating in death within 6-8 days in a majority. The onset was marked by conjunctivitis, nasal discharge, lethargy and fever and progression of these signs was characteristic of the infection. Death was usually preceded by marked hypothermia. During the course of illness, virus was usually present in conjunctival secretions of both eyes, in the nasopharynx, the blood and occasionally feces,

TABLE I. Virus Isolation From Autospy Material.

Animal #	Cornea	Tracheal secretion	Hearts blood	Lung	Liver	Adrenal
1	+	+	+	+	+	+
2	+	+	+	+	+	+
6	+	+	+	+	+	+
	Kidney	**Spleen**	**Lymph node**	**Brain**	**Spinal cord**	**Urine**
1	+	+	+	+	+	NS
2	+	+	NS	+	+	NS
6	+	+	+	−	−	+

+ = Virus Isolation, − = No Virus Isolation, NS = No Specimen Obtained.

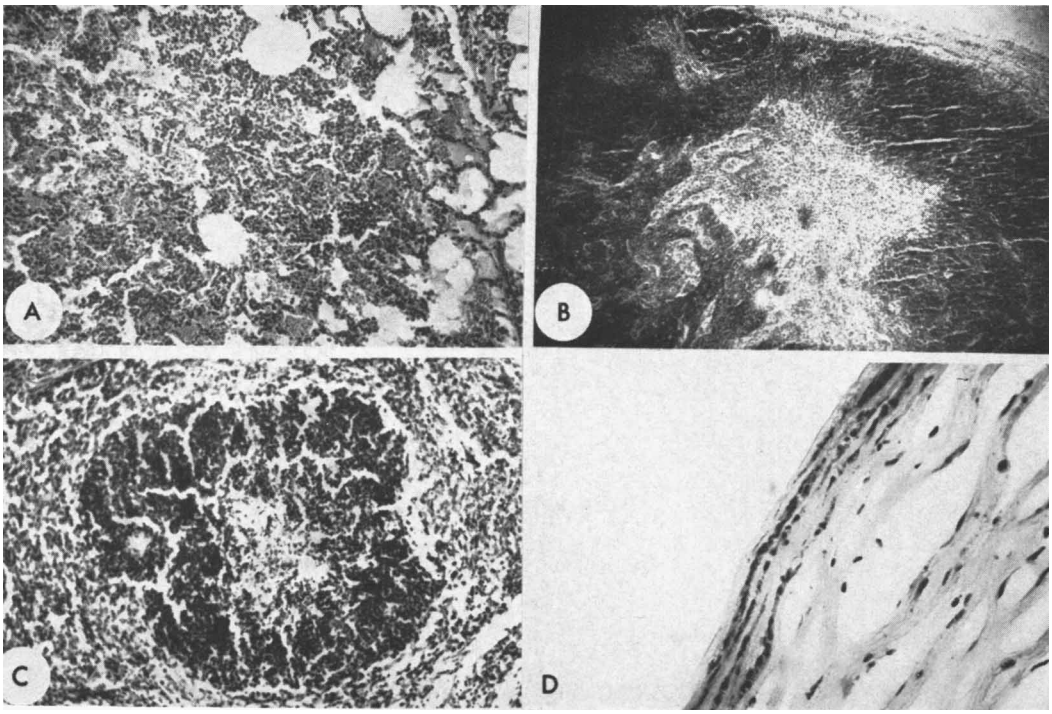

FIG. 4. (A) Interstitial pneumonia 125 ×. (B) Area of focal necrosis of the spleen 125 ×. (C) Focal necrosis in a 125 × lymphnode. (D) Desquamation of corneal epithelium with stromal edema and lymphocytic infiltration.

and in the one case where it was obtained, in the urine. These virologic findings along with the clinical signs of infection were considered to be consistent with generalized infection; early death of the animals was in line with this hypothesis. Pathological evidence of a widespread cytolytic process, particularly in the liver, adrenal and lung confirmed that death was a result of generalized disease. The presence of typical herpesvirus inclusion bodies, particularly prominent in the areas of greatest destruction, and the isolation of virus from these organs provided convincing evidence that the destructive changes were, in fact, due to infection with herpesvirus.

The presence of virus in many organs other than those in which gross destruction was evident points to the widespread nature of the infection. Isolation of virus from tissues of organs without pathology or in which mild or non-specific pathologic changes occurred emphasized the superiority of viral culture methods over pathologic examination as a means of proving infection.

The majority of animals succumbed to infection before a period long enough to allow primary development of specific antibody had elapsed. However, in some animals infected with viruses of human origin, specific antibody may appear at 7 days after inoculation (9) or sooner. In this case, it may have been that the reticuloendothelial organs of the monkeys were so markedly involved as to block formation of antibody by immunologically competent cells. Since amounts of sera collected from the sick animals allowed only tests for neutralizing antibody, a rise in CF antibody may have been missed.

No attempts were made in these experiments to establish the animal infectious dose. However, the dose of virus used to initiate infection in these animals may have represented a multiplicity of the minimal infecting dose, since in the unscarified, inoculated cornea, complete epithelial denudation occurred, whereas, in the cornea of animals developing infection after contact, only conjunctivitis and circumscribed keratitis developed. In further

studies to be reported, other animals developed generalized infection after ocular inoculation with 175-3000 $TCID_{50}$ of the same virus stock, providing further evidence of the susceptibility of the owl monkey to the HS-1-65 strain of *H. hominis*.

Exposure of susceptible monkeys to those inoculated resulted in infection in the contacts and development of severe, generalized illness followed by death in a majority. The means by which the infection was spread was not determined; the presence of virus in the excreta, urine, pharynx and conjunctiva of sick animals readily provided the source. Direct contact between the animals did not occur. The same caretaker and general facilities for food and water replacement were common to the animals and the spacing of cages did not prohibit aerosol contamination.

The primary sites of infection in the 2 contact animals were the conjunctiva and the nasopharynx. During the height of infection in these and inoculated animals, virus was present in both eyes. This suggests the sensitivity of the conjunctival membrane to the HS-1-65 virus. Infection of the nasopharynx may have resulted from direct drainage through the nasolacrimal duct after direct or contact infection, or have been primary by aerosol contamination. Further spread of an infection from either of these sites may have been through the lymphatics or veins, resulting in viremia or viremia may have developed secondary to viral pneumonitis.

Death probably was a result of overwhelming infection and necrosis of liver and adrenals. Although hemorrhagic pneumonitis was present, signs of lower respiratory tract disease were not prominent during life. The presence of virus in various areas of the brain of the inoculated animals, without specific pathological changes may be interpreted as low grade or early infection, and probably was not lethal.

Rabbits inoculated by the conjunctival method, after corneal scarification with strain HS-1-65 herpesvirus, developed discrete and extensive keratitis but failed to develop any evidence of generalized disease, and survived. This, and the lack of extensive neurologic infection in the inoculated monkeys provides some evidence that the virus used in these experiments was of limited neurotropism.

Infections with *H. hominis* in the newborn human infant frequently become generalized, severe and may be lethal. The most frequent type of infection is that in which multiple organs are involved, and necrosis of liver and adrenals is observed at post-mortem. Such infections may extend to the central nervous system. The generalized disease in the exposed owl monkey thus closely resembles the newborn infection(10).

Evidence that the generalized disease was a result of infection with the *H. hominis* inoculated and not to a latent herpesvirus, such as "T" virus lies in the observation that disease never developed spontaneously or sporadically, but directly after inoculation or exposure; that "pre-inoculation" specimens of secreta and excreta failed to reveal adventitial viral agents and that the 6 isolates selected at random and studied here were neutralized by reference *H. hominis* antiserum and not by antiserum to Herpesvirus "T".

Summary. The owl monkey, *Aotus trivirgatus*, was found to be extremely susceptible to infection with a strain of *Herpesvirus hominis*. The infection was established by ocular inoculation without corneal scarification, rapidly involved the upper respiratory tract, blood, and became widespread, systemically. Generally animals died within a period of 6 days after inoculation and virus was recovered from all organs and the CNS. Exposure of well to sick animals resulted in similar generalized disease and death in a majority of those exposed. The pathology of generalized infection closely resembled *H. hominis* infection of the human new born infant.

Addendum. Since the submission of this manuscript, a report by Sheldon, W. G. and Ross, M. A., U. S. Army Medical Research Laboratories, U. S. Army Medical Research and Development Command, Report No. 670, 3 of June 1966 describing a very similar fatal generalized infection in owl monkeys with Herpesvirus "T" has come to our attention.

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Effects of pH and Urea Concentration on Uptake of Fluid and Urea by Solutions Containing Serum Albumin.* (32101)

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It was recently reported(1) that approximately 30% of the total albumin content of the kidney is present in the interstitial space. Since urea concentration is high(2) and pH may be alkaline(3) in the renal medullary interstitium, it was of interest to find out how albumin affects the transport of fluid and urea into an environment of high salt and urea concentrations and of high pH.

Phosphate buffer solutions were arranged in the manner of a 2-way, 3 by 4 contingency table: pH values were adjusted to 5.0, 7.4 and 9.5, and urea concentrations were set at 0, 0.005 M, 0.3 M and 1.0 M, respectively. All solutions contained NaCl at a concentration of 0.4 M and approximately 2 μC/liter of C^{14}-urea. A volume of one hundred ml of each of these solutions was used as the bathing fluid during dialysis. A 30% bovine serum albumin in Tyrode's solution (National Biochemical Corp.) was diluted with the buffers to obtain 5% albumin solutions. The pH values were readjusted after addition of the albumin. Eight ml samples of the albumin solutions were placed in cellophane bags. The bags were made of cellophane tubing (Union Carbide Corp.) which was soaked in a sodium phosphate solution of pH 10 for 24 hours prior to experiment, then copiously washed with tap water and distilled water. Ample space was provided in the bags for an increase in volume and no hydrostatic pressure developed in the albumin solutions. Dialysis was carried out for 72 hours and in triplicate for each pH and urea concentration. The samples were gently and continuously shaken in stoppered containers at 25°C. The net inflow of solution was determined as the difference between initial and final weights of the bags containing the albumin. This quantity was expressed as a percentage fraction of the initial weight, and no correction was made for the weights of the bags themselves. The amount of bound C^{14}-urea was calculated as the excess radioactivity per ml in the albumin solutions. This quantity was arrived at by assuming that in the water phase of both solutions separated by cellophane, the concentration of C^{14}-urea was identical. The water content of each solution was determined by gravimetry and drying at 104°C.

Table I shows the weight increments of the albumin containing bags at 72 hours. Averages of triplicate determination are shown in both tables. An analysis of variance of the data indicated that at pH 9.5 the net inflow of solution was significantly higher ($p < 0.001$) than that at pH 7.4 or 5.0. In a

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