Non-human primates are known to be susceptible to at least two herpes viruses; herpes-B (herpesvirus simiae) and herpes-T (herpes-M). Herpes-B affects old world primates, principally the rhesus monkey (Macaca mulatta) in which it usually produces a subclinical disease, and is therefore somewhat analogous to herpes simplex infection in man. A small percentage of infected rhesus monkeys may exhibit clinical signs of disease, characterized principally by lingual ulceration, however a variety of visceral lesions have also been reported as a result of both natural and experimental infection. Although the number of reported cases in man is few, herpes-B produces a highly fatal systemic disease characterized chiefly by encephalomyelitis. It is reasonably well established that rhesus monkeys are the natural host for this virus, allowing its persistence in nature, and serving as the source of infection for man.

Herpes-T is a more recently described agent which produces a systemic, fatal disease in marmosets, a group of New World primates. This virus has been isolated from the white-lipped tamarin (Tamarinus nigricollis) and the cotton-topped marmoset (Oedipomidas oedipus). Herpes-T does not have the same relationship to marmosets as herpes simplex does to man, or herpes-B to rhesus monkeys, in that herpes-T produces a fatal disease in marmosets. We have recently demonstrated that herpes-T virus can exist as a latent infection in the squirrel monkey (Saimiri sciureus). This suggests that the squirrel monkey is a natural host for herpes-T virus. It is not known if man be susceptible to herpes-T.

This report describes the pathological features and the viral isolation and identification of spontaneous herpes-T infection in 6 owl monkeys (Aotus trivirgatus), a New World species.
Materials and Methods

Pathology

Six owl monkeys which died while being maintained as experimental animals in a research laboratory in Boston were submitted to the New England Regional Primate Research Center for study. Tissues representative of all organ systems were fixed in 10% neutral, buffered formalin or in Zenker’s solution, paraffin embedded, sectioned at 5μ, and stained with hematoxylin and eosin. One or more tissue sections were prepared from each organ examined, with the exception of the brain where tissue sections were prepared from full cross sections taken every 0.5 cm from the olfactory lobes to the cervical spinal cord. The remainder of the spinal cord was not examined. Selected tissue sections were also stained with Masson’s trichrome and Gomori’s iron stains, and by the Feulgen reaction.

Viral Isolation

Cells: Rabbit, rhesus monkey (Macaca mulatta) and African green monkey (Cercopithecus aethiops) kidney cells were employed for viral detection. The rabbit kidney cells were prepared in the laboratory from 1 to 2 week-old rabbits using standard techniques. The African green monkey and rhesus monkey kidney cells were obtained from commercial suppliers. A second or third transfer of these cells was employed for viral detection.

Media: Eagle’s minimum essential medium (MEM) was employed as a basal nutrient. The growth medium was prepared by adding 10% fetal calf serum to MEM, and maintenance medium was prepared by adding to MEM 2% fetal calf serum. The pH was adjusted to 7.2–7.4 with a 7.5% bicarbonate solution. Two-hundred and fifty mg of streptomycin and 250 O.U. of penicillin per ml were added to the media.

Tissue samples: Tissues obtained during necropsy of 5 owl monkeys described below provided inocula for viral detection on cell cultures. Single samples were prepared from liver, brain, lung, spleen and tongue. A 20% suspension of each sample was prepared in maintenance medium. Supernatants were obtained by centrifugation of this suspension at 1500 rpm for 10 minutes.

Inoculation procedure: One tenth ml of undiluted sample suspension was inoculated in cells growing in slant tubes. They were previously washed with Earle’s balanced salt solution (EBSS). The tubes were then kept at 37° C for 1 hour to allow viral adsorption. After this period the cells were washed 5 times with EBSS and 1 ml of maintenance medium was added. The tubes were then kept at 37° C for an observation period of 7 to 10 days. Aliquots of each sample were kept with an equal volume of a mixture of glycerine and skim milk at −20° C. This mixture was prepared by adding 1 volume of neutral glycerine to 1 volume of 20% skim milk in deionized water.

Viral isolates: The fluids from the cells that showed cytopathic effect (alteration and destruction) by the action of the tissue samples constitute the viral isolates.
Herpes-T Infection in the Owl Monkey

These were kept at -20°C as indicated for the tissue samples. Each time these isolates were inoculated into cell cultures the same procedure was followed.

**Viral titration and neutralization tests:** These procedures were conducted *in vitro* in rabbit kidney cell cultures following the standard virological techniques. Normal and immune sera against herpes-T virus and the owl monkey isolate were prepared in rabbits. Infectious bovine rhinotracheitis antiserum was also employed for the neutralization tests. The sera were used in a final dilution of 1:6.

**Experimental infection:** Four owl monkeys were inoculated via the intramuscular route with 100 tissue culture infective doses 50% (TCID<sub>50</sub>) of the virus isolate. Prior to inoculation undiluted sera from these monkeys was tested against 100 TCID<sub>50</sub> of the virus isolate. Only one of the monkeys did not have antibodies to the virus.

**History of Outbreaks**

In July 1964 a research laboratory in Boston received 12 owl monkeys from a commercial supplier. These 12 animals were housed with 23 other owl monkeys that had been in the laboratory for varying periods of time, up to 6 months. One animal from the new shipment arrived dead and 6 others became ill and died 2 days after receipt. The only clinical observations recorded were anorexia, dehydration and depression. During the month of August, 11 monkeys all of which had been in the laboratory for 6 months died. One male from this group (*Case 1*) was submitted to the New England Regional Primate Research Center for histopathological study, the remaining animals being discarded. Viral isolation was not attempted in this animal. No other death occurred in the colony until November 1964. In November a new shipment of 10 owl monkeys was received, 1 animal arriving dead. Six of these monkeys died over a period of 10 days after arrival. Five of these were submitted to the New England Regional Primate Research Center for study (*2 females, Cases 2, 3; and 3 males, Cases 4, 5, 6*).

Throughout the period of time covered above, the owl monkeys were housed in the same quarters in which squirrel monkeys were also maintained. However there was no direct physical contact. None of the squirrel monkeys exhibited signs of disease.

**Pathologic Findings**

Due to the similarity of the findings in all 6 monkeys they are described together rather than individually. Dissimilarities are discussed when present and the case numbers (see above) indicated.
Inclusion bodies

Inclusion bodies were observed in epithelial and/or reticuloendothelial cells in all the various organ systems in which lesions occurred. In order to avoid repetitious descriptions, the character of the inclusion bodies will be described prior to the presentation of the gross and histopathological findings.

Two morphological types of inclusion bodies were seen. In one group there was distinct margination of the nuclear chromatin and the inclusion bodies were acidophilic to slightly basophilic in staining reaction, and surrounded by a clear halo (Fig. 1). These inclusion bodies were either homogeneous or granular in structure and often appeared to be composed of several smaller units. They generally were of the shape of the nucleus in which they occurred, most often being round or oval in tissue section. Most of these inclusion bodies did not stain by the Feulgen technique, however a few were slightly positive.

The second type of inclusion body completely filled the nucleus, there being no clear halo, however there was margination of nuclear chromatin (Figs. 2, 3). These inclusion bodies were either homogeneous or granular and were amphoteric in staining reaction, appearing steel blue to lavender in hematoxylin and eosin-stained tissue sections. Many of these inclusion bodies contained inner basophilic granules and others appeared to be composed of several smaller units which had a basophilic margin and a nearly clear center which gave the entire structure a vacuolated appearance. An occasional inclusion body of this type had a single or several very clear vacuoles which appeared to be true vacuoles and not sub-units. Most of these inclusion bodies stained by the Feulgen reaction, either diffusely or as small granules within the inclusion body; however a few were entirely negative.

Skin

Gross

Only in Case 2 were gross lesions observed in the skin. In this monkey there was a marked swelling of the eyelids and periorbital tissue which resulted in complete closure of the eyelids. There was alopecia and the surface was encrusted with a thick layer of grumous,
Fig. 1. Eosinophilic intranuclear inclusion bodies surrounded by clear haloes in hepatocytes. Hematoxylin and eosin stain (also in all subsequent photomicrographs).

Fig. 2. Intranuclear inclusion bodies completely filling the nuclei of several epithelial cells in the lingual mucosa. Note the inner granulation and the vacuoles in some of the inclusion bodies. The cells are separated from one another due to intercellular edema.
tan material that could easily be removed with a blunt instrument. This appearance extended over the cheek to include a small portion of the upper lip. On cut surface the greater portion of the epidermis was occupied by the above type of material and the subcutaneous tissues were thickened, and oozed a slimy, viscid, clear to pink fluid on slight pressure. Throughout the subcutis in this area petechiae and ecchymoses were present. Lesions of the skin were not present in any other location.

Microscopic

Tissue sections of the eyelid and lip were examined from each case but lesions were confined to Case 2. In tissue sections of skin from these areas the entire thickness of the epidermis was necrotic except for small groups of cells beneath the necrotic membrane. In place of the epidermis was a thick layer of brightly eosinophilic, homogeneous to slightly granular material which was for the most part completely acellular save for nuclear debris, isolated groups of pyknotic nuclei, and variable sized colonies of bacteria. Beneath this layer, sebaceous glands, hair follicles and apocrine sweat glands appeared normal. There was parakeratosis and intercellular edema of the small islands of viable epidermis and of the epidermis adjacent to the necrotic tissue. In a few epidermal cells in these latter areas, in the cells of ducts of sebaceous and sweat glands and in scattered epithelial cells beneath the necrotic membrane there were intranuclear inclusion bodies of the two types described above. Multinucleated epithelial giant cells were present among the scattered individual epithelial cells beneath the necrotic membrane. In most examples, each nucleus of these giant cells contained an inclusion body, generally of the type that completely filled the nucleus.

The dermis and subcutis were infiltrated by a minimal number of polymorphonuclear leukocytes, principally located around venules and arterioles. The endothelial cells of these vessels were enlarged with an occasional cell containing an intranuclear inclusion body. Several of these vessels contained fibrin thrombi. The collagenous and adipose tissue of the subcutis as well as the periorbital skeletal muscle were separated by numerous clear spaces, a light pink fibrillar material, and extravasated erythrocytes. Occasional bundles of collagen and skeletal muscle were necrotic.
Oral cavity

Gross

A necrotizing and ulcerative stomatitis was observed in all 6 cases. The lesions ranged in size from about 1 mm in diameter to large irregularly shaped areas greater than 2 cm in diameter. They appeared as either round or irregular raised plaques which varied in color from grey to greenish grey to round or uneven grey-red crateriform ulcers with a slightly raised border (Fig. 4). These later lesions were presumed to represent a later stage of the plaques in which the necrotic membranes had been disrupted. The lesions were randomly distributed throughout the oral cavity. They were most numerous over the entire dorsal and ventrolateral surfaces of the tongue, the lips and the soft palate, but were also seen over the hard palate, lingual and buccal gingiva, the floor of the mouth and the pharynx.
Microscopic

These lesions consisted of zones of partial to complete necrosis of the oral mucous membrane. A layer of necrotic debris which contained a few scattered neutrophils and lymphocytes as well as isolated epithelial cells was present over the surface of the ulcers which extended directly to the submucosa or were interrupted at irregular intervals by islands of mucosa consisting chiefly of the basal layers (Fig. 5). The epithelial cells in these areas and adjacent to the ulcers were larger than normal, round to polygonal in shape had increased cytoplasmic eosinophilia and often individualized, separated from their neighbors by a thin clear space of intercellular edema (Fig. 2). Ballooning degeneration was seen in a few epithelial cells but their number were few and in only one lesion observed microscopically was a vesicle seen. Here the most superficial layers of epithelium were nearly normal, with spongiosis of the middle layers and necrosis of the basal layers. Several multinucleated
Fig. 5. A zone of necrosis on the dorsum of the tongue. Most of the mucosa at this site is necrotic except for portions of the basal layers. A moderate infiltration of neutrophils is present in the necrotic tissue and in the adjacent submucosa.

Fig. 6. Necrosis of the entire thickness of the mucosa in the colon. Partially viable mucosa is present in the upper right hand corner.
epithelial cells were present throughout the lesions (Fig. 3). Intra-
nuclear inclusion bodies of the two types described in the skin were
present in many of the epithelial cells and giant cells (Fig. 3). Colonies
of bacteria were present in the necrotic membrane but in the sub-
mucosa there was only a slight inflammatory reaction primarily com-
posed of neutrophils and a moderate degree of hyperemia and edema.

Several early lesions were seen in tissue sections in which necrosis
had not yet occurred. In these areas the mucosa was thicker than
normal as a result of both an increase in the number of epithelial cells
and ballooning degeneration. The cells had become disoriented from
one another disrupting the usual classical arrangement of maturation
from the basal layer to the surface. Numerous intranuclear inclusion
bodies were seen in these cells principally of the type surrounded by a
clear halo. In the pharynx the submucosal lymph follicles were con-
gested and there was focal necrosis characterized by karyorrhexis of
lymphocytes and their replacement by a pink granular to fibrillar
material. Many reticuloendothelial cells within and immediately adja-
cent to the necrotic tissue contained eosinophilic intranuclear inclusion
bodies most of which were surrounded by a clear halo. No lesions were
seen in the submucosal salivary glands and mucous glands of the cheek,
tongue or pharynx.

Esophagus

Gross

In Cases 3 and 5 numerous 1 to 3 mm in diameter yellow white
plaques, some with red umbilicated centers and others with a red
border were present throughout the length of the esophagus. Numerous
1 to 3 mm yellow white erosions were also present.

Microscopic

In these 2 cases lesions essentially identical to those described in
the oral cavity were seen. The only significant differences were that
there was less necrotic material overlying the ulcers and that in many
of the lesions the necrosis did not involve the entire thickness of the
mucosa, the basal layers being spared. Giant cells and both types of
intranuclear inclusion bodies were present. Bacterial colonies were not
seen but there was hyperemia and a minimal neutrophilic infiltration in the submucosa. In both Cases 3 and 5 and also in Case 4 early pre-necrotic foci were seen where the epithelial cells were disoriented, swollen, had indistinct cellular membranes and contained intranuclear inclusion bodies. Multinucleated epithelial giant cells, bearing intranuclear inclusion bodies were also present in these foci.

Small Intestine, Cecum and Colon

Gross

In all monkeys except Case 2 serosal petechiae and ecchymoses were seen. On the mucosal surface corresponding to the serosal lesions were irregularly shaped, depressed, roughened, tan to red-brown patches from 1 mm up to 3 cm in greatest dimension. The larger of these ulcerative appearing zones were surrounded by a distinct red border.

Microscopic

No microscopic lesions were seen in Case 2. In other animals lesions were seen at all levels of the small intestine, cecum and colon with the exception of the duodenum. Within the mucosa there were numerous foci of coagulation necrosis affecting both the epithelium and the supporting tissues, extending through its entire thickness to the muscularis mucosa (Fig. 6). Over most of the lesions the necrotic tissue either remained in place, maintaining a resemblance to the original architecture or as a nearly homogeneous plaque or membrane; but in some it had sloughed exposing the muscularis mucosa to the lumen. Within the necrotic tissues scattered epithelial cells could be seen and occasionally the greater part of an entire gland or villus was unaffected. There were also many karyorrhektic nuclei, scattered nuclear debris, free erythrocytes, and bacterial colonies intermixed with the necrotic tissues, however few inflammatory cells were present except for a small number of neutrophils. In a few foci the necrotic tissue, whether it maintained its original morphology or not, was nearly entirely acellular and eosinophilic, even lacking any significant amount of nuclear debris. Intranuclear inclusion bodies were present in the mucosal epithelial cells remaining within the necrotic sites and in the mucosa
immediately adjacent to these lesions. The inclusion bodies were of both types described above; however, those surrounded by a clear halo and brightly eosinophilic in staining reaction were the most numerous.

In addition to these larger areas of necrosis there were numerous small lesions scattered along the entire intestinal tract in which groups of epithelial cells of a single villus or gland were necrotic as evidenced by karyorrhexis, lack of cytoplasmic boundaries, or complete loss of cells. Both types of intranuclear inclusion bodies were present in these foci. Accompanying these changes, was a marked karyorrhexis of the leukocytes usually seen in the lamina propria. An occasional multinucleated epithelial giant cell was seen adjacent to the necrotic tissue, similar to those described in the tongue and esophagus, but they were fewer in number.

The submucosa beneath the mucosal lesions was congested, edematous and hemorrhagic. There were a few scattered neutrophils and mononuclear cells, but no significant inflammatory cell infiltrate. In some of the mononuclear cells intranuclear inclusion bodies of both types were seen. In a few areas the necrosis of the mucosa extended through the muscularis mucosa to also affect the submucosa, in which case all of the submucosal tissues were necrotic including the walls of arterioles and venules. Groups of non-hemolyzed erythrocytes remained within the lumens of these necrotic vessels. In the submucosa not directly adjacent to the mucosal lesions, similar changes were seen but to a lesser degree. For the most part the muscularis was unaffected but there were scattered zones of hyperemia and in one tissue section examined (Case 4) the muscularis was necrotic. The serosa was essentially normal except for scattered zones where the mesothelial cells were cuboidal in shape. The Peyer’s patches were hyperemic and there was diffuse karyorrhexis of the lymphocytes and their replacement by eosinophilic granular to fibrillar material. Intranuclear inclusion bodies of the 2 types described were present in reticuloendothelial cells.

Nasal Cavity

Gross

In all 6 monkeys a small amount of white to gray mucus was present at the external nares. After fixation gray mucus was seen throughout the nasal cavity. The mucosa contained roughened irreg-
ular foci 1 to 2 mm in diameter, which varied in color from gray-white to deep red.

Microscopic

In all 6 monkeys lesions were seen in the respiratory mucosa from its origin at the nares to and including the respiratory pharynx. Within the lumen there were varying quantities of mucus mixed with numerous neutrophils and occasional desquamated epithelial cells, rare multinucleated giant cells and colonies of bacteria. For the most part the mucosa was intact, however randomly distributed there were ulcers characterized by almost complete absence of the epithelium except for a few remaining viable cells and small aggregates of necrotic epithelium clinging to the surface. There were no necrotic membranes overlying the ulcers as seen in the gastro-intestinal tract, the dead tissue having almost entirely sloughed to the lumen. Intranuclear inclusion bodies of the 2 types previously described were present in epithelial cells admixed with the luminal exudate, within and adjacent to the ulcers and scattered haphazardly about the intact mucosa. In scattered foci the necrosis had occurred in the deeper cells of the pseudostratified epithelium but not in the more superficial cells, however inclusion bodies were present in these superficial cells. There were also foci where the pseudostratified epithelium was disoriented and thicker than normal as a result of an increased number of cells, swelling of the cells, and the presence of multinucleated epithelial giant cells. Cilia were absent over most of these foci and many cells in these areas contained inclusion bodies. All of these lesions occurred throughout the nasal cavity and respiratory pharynx with no particular distribution.

The submucosa was markedly hyperemic and there was a slight infiltration with neutrophils and macrophages. Some of the macrophages contained intranuclear inclusion bodies. There was no necrosis of the mucosal mucus glands within the nasal cavity or pharynx but intranuclear inclusion bodies were present in both acinar and ductular cells.

Larynx and Trachea

Gross

The mucosae were hyperemic but otherwise not affected.
In all 6 animals the mucosal vessels were hyperemic. In Case 3, 5, and 6 there were small areas where the pseudostratified columnar epithelium was disoriented, with an increase in thickness and number of cells which were cuboidal to polygonal in shape, and which did not have cilia. Most of the epithelial cells in these disorganized plaques contained intranuclear inclusion bodies of the 2 types described above. Mitotic figures were observed in several of these plaques. There were also small ulcers characterized by necrosis of the epithelium, exposing the underlying connective tissue in which a few neutrophils were present. Inclusion bodies were present in the epithelial cells adjacent to these ulcers. The submucosa was congested, but no lesions were seen in the submucosal glands or cartilage.

Lung

Gross

In all 6 cases the lungs were uniformly red-brown and sub-crepitant. Collapse was nearly complete and no gross consolidation was evident. In Cases 3 and 5 there were scattered 1 to 3 mm in diameter, slightly raised, gray and red circular foci on the pleural surface of the lung.

Microscopic

In all 6 monkeys there was a mild pneumonitis affecting all lobes but sparing some lobules in which few or no lesions could be seen. The reaction was characterized by thickening of the alveolar septae with mononuclear cells, a few neutrophils, hyperemia and serous exudate within the walls. The alveolar lining cells were often prominent and in some alveoli these cells were free in the lumens. Scattered groups of alveoli were filled with eosinophilic homogeneous, or fibrillar material within which there were usually a few macrophages and neutrophils, but in no area was a dense cellular exudate seen in the alveoli. Free erythrocytes filled some alveoli.

The circular foci observed grossly in Cases 3 and 5 were focal areas of coagulation necrosis in which the original alveolar architecture
was preserved by irregular strands of eosinophilic material in which non-hemolyzed erythrocytes were present. The lumens of these dead alveoli were filled with eosinophilic fibrillar material, nuclear debris and a few mononuclear cells and neutrophils. A few alveolar septae within these foci still contained viable cells, some of which contained intranuclear inclusion bodies similar to those described in other tissues. There was no inflammatory cell reaction surrounding these necrotic foci. Although their appearance suggested infarcts, no thrombi or other vascular changes which could have resulted in such a lesion were seen.

Within the bronchioles and bronchi lesions similar to those described in the larynx and trachea were seen, consisting of foci of mucosal necrosis and small plaques where the pseudostratified columnar epithelium was disorganized being composed of a stratified cuboidal epithelium which lacked cilia (Fig. 7). Mitotic figures were seen in these plaques. In these lesions and adjacent to the necrotic tissues intranuclear inclusion bodies of the 2 types described in other tissues were seen. There was no inflammatory exudate within the bronchi and bronchioles, however in some desquamated epithelial cells were present.

Liver

Gross

In each monkey on the capsular and cut surface of all lobes of the liver were myriads of foci 0.5 to 3 mm in diameter and pale brown to gray in color. A lesser number of foci, 2 to 5 mm in diameter were also present throughout each liver. These lesions did not alter the contour of the capsule. In addition, in Case 2 there were two large, pale brown, raised capsular lesions, that extended into the parenchyma, one measuring 0.5 × 0.5 × 2 cm, and the other 0.5 × 0.3 × 1 cm; and in Case 6 there were several dark red-brown depressed lesions up to 1 cm in diameter with irregular outlines. No lesions were seen in the gall bladder.

Microscopic

The lesions observed in the gross consisted of areas of necrosis which varied from involving a few hepatocytes to large foci encompassing 1 to several lobules. Their distribution was focal in character.
with no obvious lobular pattern. In most of the smaller foci complete necrosis had not yet occurred, but the hepatocytes were swollen, had increased eosinophilia and homogeneity to their cytoplasm, and the cytoplasmic membranes of most of the cells were indistinct. Almost every hepatocyte in these areas contained an intranuclear inclusion body, the majority of which were brightly eosinophilic and surrounded by a clear halo, however a lesser number were present which completely filled the nucleus (Fig. 1). Within these foci the cytoplasmic membranes forming sinusoids and an occasional membrane between two adjacent hepatocytes were markedly thickened. The larger, and more advanced lesions, consisted of almost complete necrosis of the hepatocytes, there only remaining amorphous accumulations of eosinophilic material within which there was considerable nuclear debris, a moderate number of neutrophils, and an occasional Kupffer cell, but no recognizable hepatocytes. A lesser number of the larger foci were characterized by a more classical type of coagulation necrosis, in which no viable hepatocytes were seen but the normal cytoarchitecture was partially preserved. Surrounding these foci the hepatocytes resembled those described in the early pre-necrotic lesions, but interestingly most of the inclusion bodies in these cells were of the type that completely filled the nucleus.

Scattered throughout the liver isolated hepatocytes and rare Kupffer cells contained inclusion bodies of both types. Evenly distributed in all tissue sections examined, most of the hepatocytes contained one to several fat vacuoles and a yellow-brown, iron negative pigment interpreted as bile pigments. Biliary canaliculi were not visible. Though not numerous, intranuclear inclusion bodies of both types were also present in the bile duct epithelium. In these ducts there were also foci of necrosis and hyperplasia characterized by a lining epithelium several cells thick and disoriented in direction. Multinucleated epithelial giant cells were seen in some of these ducts. In the periportal tissue there was an increase in number of bile ductules. No lesions were seen in the gall bladder.

Spleen

**Gross**

The spleen of each monkey was small, had a slightly wrinkled capsular surface and was variegated in color from pale red to tan.
Fig. 7. Necrosis of bronchial mucosa. The necrotic tissue has remained as plaques lining the bronchus. Note the increased number of epithelial cells adjacent to the lower necrotic plaque.

Fig. 8. Karyorrhexis of lymphocytes in a lymph node. Several intranuclear inclusion bodies are present in reticulum cells.

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Microscopic

In each spleen there was a dramatic paucity of lymphocytes; no Malphigian corpuscles were present. The lymphocytes that were present were diffusely scattered without forming any compact groups. The bulk of the lymphoid follicles and much of the intervening tissue were replaced by hemorrhage and varying sized irregular masses of necrotic tissue, which was principally composed of granular to homogeneous eosinophilic material containing scattered nuclear debris, erythrocytes and reticulum cells. By the size, shape and contour of the necrotic zones it was evident that both the lymphoid and the intervening reticulum were necrotic. Unaffected central and sheathed arteries were present within the necrotic tissue. The intervening viable tissue was primarily composed of reticuloendothelial cells, erythrocytes and a small number of lymphocytes as well as a few plasma cells. In a few reticulum cells intranuclear inclusion bodies of both types were seen, but their numbers were not numerous. The splenic trabeculae appeared normal, however many of them ended abruptly at a zone of necrosis and could not be traced further.

Lymph Nodes

Gross

Most of the body lymph nodes were enlarged (at least clearly visible) red and moist. On cut surface most of the nodes were pink to red but in some of them small bright red or gray spots less than 1 mm in diameter were present.

Microscopic

Tissue sections were examined from the submaxillary, bronchial and mesenteric lymph nodes. In almost all of the nodes examined, and representing the only change seen in some nodes there was hyperemia, and marked dilation of the sub-capsular, trabecular and medullary sinuses. These sinuses were filled with numerous reticuloendothelial cells, and a lesser number or erythrocytes, lymphocytes, plasma cells, and neutrophils, as well as granular eosinophilic material. In nodes
where these were the only changes noted, the lymphoid follicles appeared normal in size and morphology, but germinal centers were not present. In other lymph nodes, in addition to these changes, and constituting the bulk of some lymph nodes, there were irregular foci of necrosis, principally but not entirely confined to the cortex. These foci varied from recognizable lymphoid follicles in which there was karyorrhexis of anywhere from 50% to nearly 100% of the lymphocytes, to masses of amorphous or fibrillar eosinophilic material in which there were nuclear debris, erythrocytes and a few viable reticuloendothelial cells (Fig. 8). Within these latter cells and in reticuloendothelial cells surrounding necrotic follicles were intranuclear inclusion bodies of the 2 types previously described (Fig. 8).

Adrenal

Gross

No lesions were seen on the capsular surface of the adrenals in any of the cases, nor on the cut surface of Cases 1, 2, 3, and 6. However in Cases 4 and 5 within the cortex were numerous pale tan irregularly shaped zones surrounded by dark red borders.

Microscopic

In Cases 1, 2, 3 and 6 within the zona fasiculata there were numerous small foci of necrosis and pre-necrotic lesions almost identical to those in the liver. The pre-necrotic foci consisted of small groups of cortical cells that were swollen, and had either increased eosinophilia to their cytoplasm or increased vacuolation with little stainable cytoplasm visible. Intranuclear inclusion bodies of the 2 types described previously were seen in nearly every cell of these foci. The more advanced lesions consisted of small zones of coagulation necrosis in which the original cytoarchitecture was partially preserved. Most of the cortical epithelial cells surrounding these foci contained intranuclear inclusion bodies. Nuclear inclusions were also scattered randomly throughout the zona fasiculata and the zona reticularis with no
relation to necrosis. In Cases 5 and 6 there was extensive hemorrhagic necrosis of most of the adrenal cortex. The zona glomerulosa was least affected and for the most part remained intact. The zona fasiculata was almost entirely necrotic except for small clumps of viable cells and scattered cells throughout the necrotic tissue. The zona reticularis was partially necrotic but not to the extent of the zona fasiculata. Intranuclear inclusion bodies of both types were numerous in the zona reticularis and in the remaining viable cells of the zona fasiculata but were sparse in the zona glomerulosa. The original architecture could still be seen in the necrotic tissue. The sinusoids in these zones were markedly dilated and filled with erythrocytes but their lining endothelium was necrotic. In the medulla of all 6 animals intranuclear inclusion bodies of both types were present in the chromaffin cells, but no necrosis was seen.

Bone Marrow

Gross

The bone marrow was not examined until after fixation and no lesions were observed at this time.

Microscopic

The bone marrow was examined in tissue sections of decalcified bone. Tissue sections were prepared from the sternum, rib at the costochondral junction, distal femur, patella and tibia together with the stifle joint, mandible, maxilla, palatine, ethmoid, sphenoid, occipital and turbinate bones. Hematopoietic marrow was observed in all of these bones excepting the palatine, maxilla, mandible and turbinates. In the cellular marrow of all cases numerous irregularly shaped areas of necrosis were present which were characterized by karyorrhexis of nuclei and replacement of the marrow by amorphous eosinophilic material containing nuclear debris, and erythrocytes. In these foci there were scattered viable cells with oval, leptochromatic nuclei, surrounded by a large clear zone of cytoplasm. These cells were interpreted as reticulum cells. Many of these latter cells in and adjacent to the necrotic foci, and scattered randomly throughout the marrow...
contained intranuclear inclusion bodies of the 2 types previously described. Occasional multinucleated giant cells were associated with necrosis. These cells were not considered megakaryocytes in that their nuclei were individually discrete and leptochromatic, and the cells were nearly circular in outline. No lesions were seen in any of the bones or joints examined.

Eye

Gross

Only in Case 2 were gross lesions of the eye noted. These were described under Skin in a preceding paragraph. Examination after fixation did not reveal any lesions of the intraocular tissues but a yellow brown (Zenker’s fixation) granular exudate was seen overlaying the conjunctiva.

Microscopic

No lesions were seen in the intraocular tissues in any case. Preservation was not adequate to thoroughly evaluate these tissues, however, no inflammatory reaction was seen, nor any inclusion bodies. In Case 2 the necrosis of the skin of the eyelids described above extended uninterrupted to the palpebral and bulbar conjunctiva which in multiple sections was found to be entirely necrotic. In its place was a thick eosinophilic, fibrillar membrane containing nuclear debris, a moderate infiltration of neutrophils and a few epithelial cells and multinucleated giant cells. A similar material which had apparently sloughed was present in the conjunctival sac. Many of the remaining epithelial cells and the giant cells contained intranuclear inclusion bodies of the 2 types previously described. The sub-conjunctival tissues (except the cornea) were congested and edematous and contained a moderate number of neutrophils, lymphocytes, macrophages and a few multinucleated giant cells. Many of the latter 2 cell types contained intranuclear inclusion bodies. The corneal epithelium was entirely absent and the superficial portion of the substantia propria was infiltrated with numerous neutrophils. The fibers of the deeper portions were separated by clear clefts, but these were interpreted as artefact as they were seen in the other eyes examined.
Other Organs

In the following tissues examined from each animal no significant lesion was observed: submaxillary salivary gland, heart, aorta, stomach, pancreas, kidney, urinary bladder, testicle, epididymis, ovary, fallopian tube, uterus, cervix, vagina, skeletal muscle, bone, joint capsule, brain and cervical spinal cord.

Viral Isolation and Identification

Liver samples from Cases 2, 3, 4, 5 and 6 produced a marked cytopathogenic effect 5 days after inoculation in rabbit kidney cell cultures. A similar effect was observed with samples from brain, spleen, lung and tongue. Liver isolates from Cases 2, 4 and 5 were demonstrated to have a tissue culture infective dose 50% (TCID\textsubscript{50}) titers of $10^{5.7}, 10^{5.3}$, and $10^{5.7}$ per ml respectively in rabbit kidney cells. The same isolates when inoculated in African green monkey cells reached a titer of $10^{4.2}, 10^{7.3}$ and $10^{4.5}$ respectively. None of these isolates produced cytopathogenic effect on rhesus monkey cells, with the exception of Case 6, which had a very low cytopathogenic effect on rhesus kidney cells when inoculated undiluted.

The cytopathogenic effect in rabbit kidney cells was first apparent 48 to 72 hours after inoculation with undiluted virus on the first passage. The alteration began with the presence of small foci of round and bizarre refractile cells and the formation of multinucleated giant cells (Fig. 9). These changes occurred earlier with virus that had been passed 2 or 3 times in cell cultures. In stained preparations intranuclear inclusion bodies of the 2 types described above were seen (Fig. 9).

Owl monkey virus antiserum and herpes-T virus antiserum diluted 1:6 neutralized 100 TCID\textsubscript{50} of owl monkey virus and herpes-T virus. Infectious bovine rhinotracheitis virus and herpes simplex virus at the same concentration were not neutralized by the former antisera dilutions. Infectious bovine rhinotracheitis virus antiserum neutralized 100 TCID\textsubscript{50} of the homologous virus but none of the heterologous viruses tested. By employing the owl monkey virus antiserum in the dilution 1:6 it was found that the neutralization indices were 3.0, 3.5, 0.0, and 0.0 for owl monkey virus, herpes-T virus, herpes simplex virus and infectious bovine rhinotracheitis virus respectively.
Clinical signs of disease were not observed during the first 5 days after inoculation. On the sixth day 1 owl monkey had a marked tendency to scratch all parts of the body and became less responsive to noise or handling. This animal was found dead the following day. The other monkeys developed a similar picture, dying between the 8th and 9th day after inoculation. Herpes-T virus was isolated from liver, brain, spleen and kidney of these animals. The viral content of these tissues will be described in a separate report. Gross and histologic changes observed in these animals were essentially the same as those described in detail above in the spontaneous disease, with the following exceptions. In none of the animals was gross oral ulceration observed. Small 1 to 2 mm foci of congestion were the only changes observed in the oral cavity. Microscopically these foci consisted of areas of early necrosis of the oral mucosa. In one animal hemorrhagic necrosis of corpora lutea was observed in the ovary with intranuclear inclusion.

Fig. 9. A multinucleated epithelial giant cell containing intranuclear inclusion bodies in rabbit kidney tissue culture 5 days following inoculation with herpes-T virus.

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bodies in the luteal cells. The majority of the inclusion bodies observed in all affected tissues in the experimentally infected animals were of the type that completely filled the nuclei.

Discussion

The results of the viral studies clearly indicate that the spontaneous disease observed in at least 5 of the owl monkeys described in this report was due to herpes-T virus. As the lesions observed in the first owl monkey examined were essentially identical to those observed in the 5 owl monkeys from which herpes-T virus was isolated and in the 4 owl monkeys experimentally infected with herpes-T it is reasonable to believe that this animal also was infected with herpes-T virus. The fact that the virus was isolated from all tissues tested, whether or not lesions were observed probably indicates that viremia was present, however no attempt was made to isolate the virus from blood.

The gross and histological lesions observed in the owl monkeys are similar to those described in spontaneous and experimental herpes-T infection of the marmoset. The lesions that have been reported in marmosets consist of rhinitis, necrotizing pneumonitis, necrotizing hepatitis, necrotizing splenitis, necrotizing nephritis and necrosis of the adrenal. Encephalitis has not been reported. These lesions, and the nature of the inclusion bodies are all similar to our findings in the owl monkey. The obvious similarity of herpes-T in the owl monkey and the marmoset is that in both species the infection is severe and results in death of the host.

Allowing some variations, the pathological process is also similar to that reported in spontaneous and experimentally induced herpes-B infection in the rhesus monkey. The significant difference is that spontaneous herpes-B infection of the rhesus monkey is a relatively mild infection and systemic lesions are usually not extensive. In herpes-B infection encephalitis is a frequent occurrence in both the spontaneous and experimental diseases. Thorough examination of the central nervous system disclosed no lesion in the owl monkeys. Despite these differences in the two diseases the general pathological processes and the nature of the inclusion bodies are similar.

The 2 types of inclusion bodies which we observed in the owl monkey tissues and tissue culture, 1 eosinophilic, Feulgen negative and surrounded by a clear halo and the other slightly basophilic to
amphoteric, Feulgen positive and completely filling the nucleus are characteristic of herpes virus infections. The inclusion bodies that completely fill the nucleus have been shown to contain viral antigen, whereas the eosinophilic inclusions have been shown to lack viral antigen and are interpreted as the end stage in the development of the inclusion body after the viral particles have been released.

The original source of the infection in the colony discussed in this report cannot be determined, however 2 possibilities exist. One: the owl monkeys were infected en route to the laboratory. Secondly, the squirrel monkeys housed with the owl monkeys could have served as the source of the virus and/or to perpetuate the virus in the colony, especially since latent herpes-T has been demonstrated in this species. Unfortunately the first owl monkey we obtained for examination died one month after the first series of deaths occurred in the colony. The cause of death in this first group is unknown which makes accurate speculation on the role of the squirrel monkey in the epizootiology of herpes-T in this colony difficult.

Summary

The pathological features of spontaneous herpes-T infection in 6 owl monkeys (*Aotus trivirgatus*) were characterized by the presence of intranuclear inclusion bodies in epithelial and mesenchymal cells associated with necrosis of the skin, oral mucous membranes, esophagus, small intestine, cecum, colon, nasal mucous membranes, larynx, trachea, lung, liver, spleen, lymph nodes, adrenal gland, and bone marrow. The inclusion bodies and the lesions are similar to herpes virus infections of other animal species. A virus was isolated from 5 monkeys, which on the basis of serum neutralization tests in *vivo* was identified as herpes-T.

Zusammenfassung


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