BOVINE PAPULAR STomatitis

Dissertation

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by

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INTRODUCTION AND OBJECTIVES

Bovine papular stomatitis has not previously been recognized in the United States. The few reports in the literature are from Europe where, around the end of the nineteenth century, the disease was encountered during outbreaks of foot and mouth disease. The recent discovery of the mucosal complex of diseases has again focused attention on diseases of the mouth in cattle. The recognition of papular stomatitis and the development of diagnostic criteria are particularly significant since the lesions resemble those of some stages of foot and mouth disease, the other vesicular diseases, and the mucosal disease complex. The naturally occurring disease was encountered by the author as an intercurrent infection during the study of other diseases in calves in Ohio and Maryland.

The purposes of this study were -

1. To report, for the first time, the presence of bovine papular stomatitis in Ohio.

2. To characterize the naturally occurring disease.

3. To reproduce the disease in calves.

4. To characterize the experimentally produced disease.

5. To test the pathogenicity of the causative virus for laboratory animals and for tissue cultures.

6. To differentiate papular stomatitis from other diseases with which it might be confused.

7. To review the literature on non-vesicular viral stomatitides in cattle and to classify the diseases according to our present understanding of this group of diseases.
In 1884 Degive observed stomatitis in four heifers characterized by the presence of wart-like lesions on the buccal mucosa, inner surface of lower lips, muzzle, and margins of lips without any other signs of disease. Microscopically there was hyperplasia of the rete pegs. The lesions developed slowly and then quickly regressed and were replaced by red or yellow spots which persisted for several weeks. Degive found no reference to this stomatitis in the literature. He named it "la stomatite papillaire ou papillomateuse."

Utz (1891) observed a similar disease in six young cattle in one herd. In addition to warty granulations there were numerous red ulcerations on the palate and to a lesser extent on the tongue which persisted as yellowish brown spots for several weeks after healing. There were no vesicles and no lesions on the feet.

Several young animals in two large shipments of cattle were found by Peters (1892) to have warty nodules, ulcerations, and red spots on the oral mucosa, especially on the upper and lower gums and hard palate. Some of the ulcers were partly covered by adherent brownish crusts with a "dead" appearance which Peters thought were formed from the dried tips of warty projections of the lamina propria. The course was mild and afebrile and lesions were confined to the mouth.
Hess (1899) mentions a similar disease in young cattle, with nodules and erosions up to one centimeter or more in size, which were found on the lower edges of the nostrils, muzzle, inner surface of the lower lips, dental pad, hard and soft palate, upper and lower gums, edges of the lips, mucosa of the cheeks, in the pharynx, on the tip of the tongue, and in front of the frenum linguæ. He observed tiny vesicles in the centers of the nodules which quickly ruptured but which were not like those of foot and mouth disease. The disease was not reproduced in cattle exposed artificially to saliva from affected animals.

Deppe (1899) saw a similar disease in cattle and mistakenly considered it to be "sporadische Aphthenseuche" (vesicular stomatitis) described by Dieckerhoff (1903) in his textbook. Tiarks (1904) apparently reported papular stomatitis but the original article was not available for review.

Because of the appearance of the lesions, Ostertag and Bugge (1905) proposed the name "stomatitis papulosa bovis specifica." They successfully reproduced the disease in cattle with portions of oral lesions, whole blood, and filtered serum. Lesions were present in the mouths of some animals as long as four months. Esophageal lesions were found in two experimental calves. Several animals had tiny crusts in the skin during the disease. Microscopically they found ballooning degeneration of the cells of the stratum granulosum.
Cadéac (1906) reviewed the literature but presented no original material. Pusch (1906) found the disease only in animals with light colored muzzles. Transmission attempts with saliva were unsuccessful. Haag (1907) and Kern (1907) reported cases which were probably papular stomatitis. Reinhardt (1914) was unsuccessful in transmitting the disease to sheep, goats, pigs and one calf. According to Udall (1915) papular stomatitis had not yet been seen in the United States in 1915. An article by Göhre (1917) is often referred to in the literature but it could not be found at the locus cited.

Schaaf et al. (1910) could easily transmit the disease by scarification of the oral mucosa but transmission attempts were all unsuccessful on the teats, udder, penis, scrotum, and the skin of the abdomen and neck. Lesions in naturally occurring cases appeared first on the muzzle in the region of the nostrils. Acidophilic intracytoplasmic inclusion bodies of various sizes were found in degenerating epithelial cells in the mucosal lesions. A suspension of virus in glycerine under vaseline at 2°C. was still infective after five months. No disease was produced by the virus in horses, sheep, goats, rabbits, guinea pigs, mice, or embryonated eggs. Material from the fifth egg passage was inoculated into a cow but no lesions resulted.

Yosikawa, cited by Hutyra et al. (1949), was able to transmit the disease subcutaneously, intravenously and
intramucosally. Runge (1951) described several cases of papular stomatitis in Poland under the name "pseudo-aphthous stomatitis of cattle." He was unable to demonstrate the inclusion bodies reported by Schaaf et al. (1940). Mollaret et al. (1953a and 1953b) reported isolation of viruses from cases of meningitis in man and of "epizootic pseudoaphthous stomatitis" in cattle. They claimed the viruses were the same serologically. The disease in cattle, from the description, was apparently papular stomatitis but insufficient evidence was presented to confirm an etiological relationship with disease in man.

While searching for a possible viral agent in bovine hyperkeratosis, Olson and Palionis (1953) discovered a transmissible proliferative stomatitis in cattle that were on a hyperkeratosis-producing diet. With the exception of young calves, the proliferative stomatitis did not usually develop when hyperkeratosis-producing food stuffs were not fed prior to, or in conjunction with, exposure of test animals. No reference was made to papular stomatitis but the diseases are clearly the same. Attempts to produce disease with the virus in horses, sheep, pigs, rabbits and guinea pigs were unsuccessful even though some of them were on hyperkeratosis-producing diets. One of six dogs developed proliferative lesions at the inoculation sites in the mouth. Local proliferative lesions developed on the hands of two people who had been working with affected calves.
Olafson and Fincher were cited by Hagan (1943) to have encountered wart-like lesions in the mouths of calves during experiments on hyperkeratosis. Jansen et al. (1955) reported the presence of papular stomatitis in young cattle with naturally occurring hyperkeratosis. Papular stomatitis occurring in cattle with a chronic disease of unknown etiology was described by Pallaske (1955). He found lesions in the esophagus in two cases and one papule in the rumen in one case in addition to the oral lesions.
MATERIALS AND METHODS

Preparation of Tissue Cultures

Preparation of glassware.—Immediately after use all glassware was completely submerged in tap water. As soon as possible, individual pieces were dipped in a 0.5 per cent solution of 7X detergent\(^1\) and scrubbed with a portable motor-driven brush.\(^2\) The glassware was then placed in running tap water for two to three hours. Next it was soaked in \(\text{N/100} \) sodium hydroxide for 30 minutes at \(40^\circ\text{C}\). Then the glassware was rinsed in running tap water for 24 to 36 hours, followed by at least three rinses in triple distilled water and six rinses in double distilled demineralized water containing less than 0.1 p.p.m. of impurities. Pipettes were cleansed in a similar way except that the scrubbing was replaced by a 15 to 20 minute soak in 0.5 per cent 7X detergent and the rinsing in tap water was performed in an automatic pipette washer.

After the glassware had drained dry, pipettes were plugged with cotton, culture tubes were covered with stainless steel caps, and all other glassware was covered with aluminum foil. The use of cotton was restricted since

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\(^1\) Limbro Chemical Co., New Haven, Connecticut.
\(^2\) The Lofstrand Co., Rockville, Maryland.
cotton products may contain volatile oils harmful to tissue cultures. Either hot dry air or steam under pressure was used for sterilization.

Preparation of rubber stoppers.—Both non-toxic white\(^3\) and conventional black rubber stoppers were used. New stoppers were boiled for one hour in 0.1 per cent sodium carbonate and rinsed well before use. Used stoppers were cleansed by the same method used for glassware, drained, packaged in test tubes covered with aluminum foil, and autoclaved to be ready for use again.

**Phosphate buffered saline** was prepared according to the following formula:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.00 Gm.</td>
</tr>
<tr>
<td>KCl</td>
<td>0.20 Gm.</td>
</tr>
<tr>
<td>Na(_2)HPO(_4)</td>
<td>1.15 Gm.</td>
</tr>
<tr>
<td>KH(_2)PO(_4)</td>
<td>0.20 Gm.</td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>0.10 Gm.</td>
</tr>
<tr>
<td>MgCl(_2).6H(_2)O</td>
<td>0.10 Gm.</td>
</tr>
<tr>
<td>Demineralized water</td>
<td>1,000.00 ml.</td>
</tr>
</tbody>
</table>

All chemicals were reagent grade. The phosphates and calcium chloride were dissolved separately. The final solution with a pH of 7.5 was sterilized by filtration through a Seitz ST-1 filter pad under positive air pressure and stored at 4°C.

\(^3\) The West Co., Phoenixville, Pennsylvania.
Phenol red solution.—Phenol red solution was prepared by dissolving 1.0 Gm. of phenol red in 57 ml. N/20 sodium hydroxide. Demineralized water was added to bring the final volume to 100 ml. One ml. of this stock solution was added to each liter of phosphate buffered saline, trypsin solution, and nutrient medium to serve as a pH indicator.

Trypsin solution.—A 0.25 per cent concentration by weight of 1:250 trypsin in phosphate buffered saline was prepared. Penicillin, 500 units/ml., streptomycin, 0.5 mg./ml., and nystatin, 4 250 units/ml. along with 0.001 per cent phenol red were added prior to filtration. The filtered solution was stored at -20°C.

Nutrient medium.—The nutrient medium routinely used contained lactalbumin hydrolysate and Tris buffer. 5

Solution I.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris buffer</td>
<td>2.40 Gm.</td>
</tr>
<tr>
<td>Demineralized water</td>
<td>100.00 ml.</td>
</tr>
<tr>
<td>Adjusted to pH 7.4 with N/1 hydrochloric acid.</td>
<td></td>
</tr>
</tbody>
</table>

Solution II.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>14.35 Gm.</td>
</tr>
<tr>
<td>KCl</td>
<td>0.80 Gm.</td>
</tr>
<tr>
<td>CaCl₂ (anhydrous)</td>
<td>0.40 Gm.</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.40 Gm.</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.25 Gm.</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.00 Gm.</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.20 Gm.</td>
</tr>
<tr>
<td>Phenol red solution (10 mg./ml.)</td>
<td>2.00 ml.</td>
</tr>
<tr>
<td>Demineralized water</td>
<td>1,700.00 ml.</td>
</tr>
</tbody>
</table>

4 (Mycostatin) E. R. Squibb and Sons, New York, N.Y.
5 (Sigma 7-9) Nutritional Biochemical Corp., Cleveland, Ohio.
Solution III.

Lactalbumin hydrolysate (enzymatic) 10.0 Gm.
Demineralized water 200.0 ml.
Autoclaved at ten lb. pressure for ten minutes.

The three solutions were combined and sterilized by filtration under positive air pressure. Penicillin, 500 units/ml., streptomycin, 0.5 mg./ml., and nystatin, 250 units/ml. were added prior to filtration and if more than three days elapsed before use, fresh penicillin was added.

Serum.—Ovine serum was routinely added to the nutrient mediums. Pools of fresh blood from at least eight lambs were collected at local meat packing companies. The serum was forced through a Seitz ST-1 filter under positive pressure and stored at -20°C.

Method of tissue culture preparation.—Stationary trypsinized tissue cultures were prepared in a manner similar to that of Madin et al. (1957). The excised tissue to be cultured (usually bovine renal cortex) was aseptically minced with scissors and scalpel blades within a few hours after collection of the tissue. The minced fragments were washed several times in nutrient medium containing two per cent serum or in phosphate buffered saline. Then they were placed in an Erlenmeyer flask and sufficient trypsin solution was added to cover the tissue. The trypsin solution had been prewarmed to 32°C. The flask was tightly stoppered and the tissue-trypsin mixture was agitated on a magnetic stirrer at
about 32°C. Every 20 or 30 minutes the trypsin solution was discarded and replaced with fresh fluid. The supernatant fluid was examined microscopically at intervals. When small tissue fragments began to be liberated, the supernatant was collected at successive periods of trypsinization until the desired volume of packed cells was obtained.

The supernatant was centrifuged in 50 ml. graduated centrifuge tubes at 500 r.p.m. in an International PR-1 centrifuge for two minutes. The fluid was poured off and the cells resuspended in nutrient medium containing two per cent serum. This washing procedure was repeated twice to remove most of the trypsin. On the third wash the volume of packed cells was noted and the cells were resuspended in an Erlenmeyer flask in enough nutrient medium (containing ten per cent serum) to complete a 1:200 dilution. The cells were agitated magnetically to maintain a uniform dispersion while they were dispensed into the culture containers. Just before dispensing the pH was adjusted to 7.2 since it rapidly became more alkaline upon incubation. One ml. was put in 150 by 15 mm. culture tubes, one and one-half ml. in 150 by 15 mm. tubes flattened on one side to receive a narrow cover glass, and 75 ml. in 32 ounce prescription bottles.

The containers were incubated in a stationary position at 38°C. The culture tubes were slightly inclined. The cultures were undisturbed for 48 hours until the cells were attached to the glass and beginning to multiply. The
media was always replaced at 48 hours and again as needed to maintain a pH near 7.4, which was usually in about six days and every two days thereafter. After three to seven days of incubation the glass surface was usually covered with a confluent sheet of cells and the cultures were ready for use.

Staining.—In each experiment half of the culture tubes contained 50 by 10 mm. cover glasses which were removed at various intervals, and fixed and stained for study. Cultures growing on the side of round containers were removed by a collodion method modified from Melnick (1956). All cultures were routinely fixed in Bouin's fluid for five minutes followed by 70 per cent ethanol for five minutes with several changes. In the tubes without cover glasses the cultures were dehydrated in graded alcohols ending with five minutes in a 1:1 alcohol-ether mixture. This was replaced with two per cent collodion in alcohol-ether for 90 minutes and then six per cent collodion for 90 minutes. The collodion was poured out and the tube rotated until the collodion had almost dried in a thin film. The tube was filled with cold water and if the film was not easily removed it was kept at 4°C. for 30 minutes. After the film was peeled from the glass with a small spatula it was pressed firmly with filter paper, with the cell surface down, onto a glass slide. The film was cleared with oil of cloves, placed in absolute ethanol at 4°C. until the collodion dissolved, and then stained.
Either hematoxylin and eosin or May-Grünwald-Giemsa stain was used routinely.

**Experimental Calves**

Calves from one week to four months of age were purchased from farms near Columbus, Ohio. Most of them were male calves of dairy breeds but a few were female and two were the results of Angus-Holstein matings. The herds from which these calves were selected were known to have been healthy (except for mastitis and occasional calf scour) for at least the two preceding years. All the calves in each herd were examined for the presence of stomatitis and rhinitis before calves were purchased and again near the end of each experiment to serve as additional controls. Of six herds investigated in this way two were found to contain several calves naturally affected with lesions typical of papular stomatitis and calves from these herds were not used. The presence of the disease in these two herds was completely unsuspected by their owners and the local veterinarians in spite of constant competent supervision.

The calves were transported in a chemically disinfected, covered horse trailer to the isolation rooms where they were housed in individual temporary pens. In the transmission experiments calves were housed in rooms in which there had never been any calves. Routine isolation procedures included the changing of clothes and boots at the entrance to the room, additional chemical disinfection of
boots, the wearing of sterile surgical gloves while in the room, and the restriction of admission to the room to one caretaker and the author.

The calves were fed a powdered milk substitute in water with a nipple pail and free choice alfalfa hay. No whole milk was given. After three weeks of age small amounts of grain were given.

Baseline studies were conducted for from one to three weeks at the beginning of each experiment. Daily procedures included the following: examination of general body health, appetite, stools, pulse, respiration rate, rectal temperature, auscultation of heart, lungs, and rumen, and determination of the total circulating leukocyte and differential counts. At least twice during this period determinations were made of hemoglobin, total erythrocyte count, and sedimentation rate. Ophthalmoscopic examination, fecal flotation, complete urinalysis, and the collection of a preinoculation serum sample were all done at least once. Serum samples were stored at -65°C. Hemoglobin was determined by the oxyhemoglobin method and the hematocrit by both microhematocrit and Wintrobe tube methods.

After necropsy of experimental and naturally occurring cases the following tissues were routinely examined microscopically:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>skin</td>
<td>soft palate</td>
</tr>
<tr>
<td>muzzle</td>
<td>tonsil</td>
</tr>
<tr>
<td>lip</td>
<td>nostril</td>
</tr>
<tr>
<td>tongue</td>
<td>turbinate</td>
</tr>
<tr>
<td>hard palate</td>
<td>trachea</td>
</tr>
</tbody>
</table>
bronchial lymph node
testes or ovaries
lung, one section from each
lobe
seminal vesicle or uterus
heart
thyroid glands
aorta
adrenal glands
iliac lymph node
thymus
mandibular lymph node
pituitary gland
parotid salivary gland
rib, costochondral
esophagus
junction
rumen
bone marrow
reticulum
psoas muscle
comasum
diaphragm
omasum
eyelid
abomasum
eyes
duodenum
cerebrum
pancreas
thalamus
jejunum
midbrain
ileum
pons
cecum
cerebellum
colon
medulla
mesenteric lymph node
cervical spinal cord
liver
thoracic spinal cord
gall bladder
lumbar spinal cord
kidneys
all macroscopic lesions
urinary bladder
mammary gland

Tissues were routinely fixed in ten per cent buffered, isotonic formalin, embedded in paraffin, sectioned at six microns, and stained with hematoxylin and eosin. Special stains and histochemical methods included Giemsa, Grocott, Gridley fungus, Wilder's reticulum, and periodic acid-Schiff.

Laboratory Animals

Experimental mice.—The mice used in this study were BAGG strain albino mice (BALB/c) in the 69th generation of brother-sister matings. Several serial passages of brain intracerebrally and lung intranasally were performed in this mouse colony with no evidence of disease. This inbred colony was kept strictly isolated in a separate building from all other laboratory animals; mice to be inoculated were moved to another room.
In each experiment using mice, a parallel control series was made with uninoculated mice. To obtain tissues aseptically at necropsy, paraffin at 190°C. was applied to the skin with a swab. Tissues for microscopic examination were fixed in formalin and prepared in the same manner as those from the calves.

**Embryonated eggs.**—Fertile white hen's eggs were obtained from a flock on the Ohio State University poultry farm. They were incubated at 37°C. with a humidity of 55 per cent and turned every three hours. In each experiment using chicken embryos a parallel control series was made with uninoculated chicken embryos.

Inoculations were made via the yolk sac, amnionic cavity, and chorioallantoic membrane (Rivers, 1952). The shells were routinely sterilized with seven per cent tincture of iodine before drilling. Inoculations were usually made with 20 gauge, 1½ inch needles in 0.1 ml. amounts. Intramnionic inoculations were made under direct observation through a triangular one cm. opening. All inoculation sites were covered with sterile transparent cellophane tape.

**Guinea pigs.**—Young adult guinea pigs were purchased from a commercial source. Small cervical abscesses were not uncommon but the animals appeared healthy otherwise. In addition to a commercial pelleted laboratory diet, fresh
green vegetables were given three times a week. Plantar intradermal injections were made with 27 gauge, 1/4 inch needles.

Handling of Materials Containing Viruses

Tissues containing or suspected of containing viruses were collected aseptically when possible, ground in a mortar or a TenBroeck glass tissue grinder with phosphate buffered saline pH 7.4 to make a ten per cent or 20 per cent suspension, and frozen rapidly in an electrical freezer. The temperature in the freezer varied from -63°C. to -66°C. but was almost always at -65°C. Liquid nitrogen or dry ice and alcohol was not used. Penicillin, 500 units/ml., streptomycin, 0.5 mg./ml., and nystatin, 250 units/ml. were added to contaminated tissue suspensions. Tissue culture mediums were simply frozen undiluted. Chick embryo yolk sac contents were centrifuged at 1,000 R.P.M. for five minutes in an International PR-1 centrifuge and the layer just under the surface lipid was collected and frozen undiluted. Nutrient medium containing two per cent serum was mixed 1:1 with chicken embryo amnionic fluid which is low in protein. Serum proteins exert a protective effect on suspensions of viruses. For this reason and for its better buffering ability, nutrient medium with two per cent serum was used in place of phosphate buffered saline in the last several experiments. When removed from the freezer virus
suspensions were completely thawed as rapidly as possible under a hot water tap. All glassware used in storing or handling tissues or fluids containing viruses was cleansed in the same way as glassware used for tissue cultures.
THE NATURALLY OCCURRING DISEASE

For over thirty years there has been a sudden outbreak of a highly fatal disease in calves each winter at The Ohio State University Beef Farm. The disease was often referred to locally as "the shakes" because neurological signs were a prominent feature. The most likely diagnosis was considered to be enterotoxemia. The disease occurred explosively at about the same time each year, however, and usually all those that showed signs died within a few hours with severe neurological signs. Treatments with bivalent enterotoxemia antitoxins were unsuccessful. At necropsy few macroscopic lesions were found. Toxins could not be demonstrated in the intestinal contents. Microscopically, a viral etiology was suggested by the presence of acute interstitial pneumonia.

Experiment 1.

In January and February, 1958, five Angus calves (B137, B148, B167, B170, and B206) from two to four weeks of age died within an eleven-day period. They had been in a pen with four other Angus, two Hereford, and two Shorthorn calves and their nurse cows. Two months later a sixth Angus calf (B617), three-months-old, that had been in the same pen, died. The animals in this herd were closely supervised and all died within a few hours after signs were first noticed except calf B167 which lived for three days.
All six calves showed nervous signs characterized by constant rapid tremors of the head and neck and periodic convulsive seizures with muscular rigidity and vertical or horizontal nystagmus. Seizures were easily induced by mild stimulation such as being forced to move. They were often accompanied by coarse fasiculations of groups of muscles and the animals shook all over. The seizures became rapidly and progressively more severe until there was complete prostration, coma, and death.

Three calves had mild neutrophilia. Serum calcium, magnesium, and copper and urine sugar were within normal limits. There was no respiratory distress or diarrhea. All the calves had been treated with Clostridium perfringens type C antitoxin subcutaneously and types B, C, and D antitoxin intravenously when signs were first noticed. Calves B206 and B617 had also received transfusions of whole blood pooled from the dams of several calves.

At necropsy the intrathoracic portion of the thymus was sprinkled with petechiae in all calves. The anterior ventral portions of the lungs showed lobular atelectasis suggestive of viral pneumonia. There was frequently a faint reddish brown discoloration of the surface at the hilus of the anterior lobes. Petechiae were lightly scattered over the surface of the lungs and within occasional lymph nodes. The renal cortices were sometimes slightly pale. In all animals the brain was slightly swollen.
No macroscopic lesions were found in the digestive system except in calf B617. In this calf, twenty nodules, from two to four mm. in diameter and raised from one to two mm. were found in the mucosa of the esophagus within 12 cm. of the cardia (fig. 1). The nodules were semispherical, sharply delimited and dark red in color. In addition there were several circular, roughened, slightly depressed areas where nodules had apparently sloughed off. The nodules were slightly roughened on the surface and the cut surface had a papillomatous appearance.

Microscopically the most striking change was in the kidneys where in all six calves there was severe necrosis of tubular epithelium, especially of the convoluted tubules. In most of the lymph nodes and in the thymus were severe diffuse congestion, hemorrhage, and edema. Calf B167 had, in addition, focal necrosis of lymphocytes and phagocytosis of nuclear debris in the tonsils, thymus, spleen, Peyer's patches, and bronchial, mandibular, and mesenteric lymph nodes. Microscopical examination of the areas of the lungs that appeared atelectatic macroscopically revealed acute, non-suppurative, interstitial pneumonia without peri-bronchiolar lymphoid hyperplasia. The areas observed grossly to be reddish brown were found to be diffusely congested and edematous with frequent tiny, focal hemorrhages. The brains of all six calves were edematous. There was necrosis of many neurons, especially of Purkinje cells. Microglial
Fig. 1.—Papillary nodules in the mucosa of the esophagus of calf B617.

Fig. 2.—Papillary thickening and ulceration of the surface of one of the nodules shown in fig. 1. H and E stain. X30.
nodules in the parenchyma and intramural lymphoid infiltrations in small vessels were rarely observed.

The papillary nodules in the esophagus of calf B617 were found microscopically to consist of local areas of greatly thickened mucosa (fig. 2). The thickening appeared to be due to hyperplasia of the stratum spinosum although the nodules were also ulcerated and infiltrated with large numbers of neutrophils. In the mucosa at the margins of the ulcerations were small foci of hydropic degeneration. The subjacent lamina propria was congested and slightly edematous.

The only other significant microscopical finding was the presence in calf B617 of multiple tiny foci of granulomatous inflammation in the musculature of the tongue. Tiny intracellular bodies resembling Histoplasma capsulatum were demonstrated in these foci.

The lesions in these six calves were very suggestive of enterotoxemia. Although no attempts were made to demonstrate toxins in the intestinal contents of these calves, toxins that neutralized Clostridium perfringens type C and D antitoxin were later demonstrated in calves with similar signs and lesions (calf B782, experiment 6.). It was later learned that these calves had had access to fresh silage for a few days before death and "couldn't seem to get enough."

The nodular lesions in the esophagus of calf B617 were very similar to those that occur occasionally in papular stomatitis. No macroscopic or microscopic lesions
were detected, however, in the oral mucosa, lips, muzzle, or edges of the nostrils. Five months after calf B617 died, The Ohio State University beef herd was examined for papular stomatitis and nearly all the calves had typical lesions. Macroscopic lesions of papular stomatitis were not found in calves over four months of age. Two calves (B1344 and B1345) were obtained for further observation (experiment 2). It is interesting that calf B617 had been inoculated with pooled blood from several cows two months prior to death. Ostertag and Bugge (1905) and Schaaf et al. (1940) showed that the blood of infected cattle sometimes contains papular stomatitis virus.

Experiment 2.

Calves B1344 and B1345, both male calves from Hereford-Holstein matings, were obtained from the same farm as the calves in experiment 1. Although they were only eighteen and fourteen days old respectively, they weighed 135 and 100 pounds.

Calf B1344. On the day of admission calf B1344 had slightly roughened, reddened, almost hairless, oval areas below the medial canthus of each eye. The lesion below the right eye was three by five cm. in size and the lesion below the left eye was circular and one cm. in diameter. Between the lesion and the eyelid of the left eye was a strip of pigmented skin, two cm. wide, that appeared normal. The lesions were entirely confined to non-pigmented skin and probably represented the common "sunburns" found in white
faced calves born, as this calf was, during the hot summer.

The calf appeared well until the fourth day after admission when an elliptical lesion was present on the hard palate five mm. medial to the first cheek tooth on the left side. The lesion, three by one mm. in size, had a tiny, blanched, slightly raised center and a bright red but poorly demarcated periphery. The next day the center of the lesion on the hard palate was oval, two mm. in diameter, definitely raised, white and opaque. It was surrounded by a three mm. wide ring of moderate hyperemia.

On the muzzle at the edge of the medial wing of the right nostril was a circular lesion that had a yellowish, slightly roughened and slightly raised center, nine mm. in diameter, and a very narrow, red, peripheral ring. Around this was another concentric ring which was white, slightly raised and one mm. wide. This in turn was surrounded by another outer one mm. wide concentric ring of hyperemia. There was no fever, no leukopenia and no interference with eating or drinking.

On the sixth day, the lesion on the muzzle was round, yellowish gray, slightly elevated above the surface, greatly roughened and one cm. in diameter with a faint, pink, narrow border. The roughening and the color gave the appearance of a flat papilloma. On the outer edge of the upper lip were two round, red foci. They were not raised, but level with the surface and smooth. Each involved three lobules of skin
and were over-all about two mm. in diameter. On the inner surface of the lower lip, near its margin, was a similar lesion. There was also an area, five mm. in diameter, of diffuse reddening on the inside of the lower lip near the commissure of the lips. The lesion on the hard palate had not changed in size, but was now pale red in color with a tiny, dirty white center. On a ridge on the opposite side of the hard palate, three cm. behind the dental pad, was a red, smooth spot one mm. in diameter.

By the seventh day the lesion on the muzzle was still flat, greatly roughened, and yellowish gray with very little reddening at the periphery. A new lesion had appeared on the muzzle, midway between the large one at the edge of the nostril and the two smaller ones on the edge of the upper lip. Like the latter it was bright red, smooth, circular and two mm. in diameter. The small lesion on the hard palate behind the dental pad had disappeared. The lesion first observed on the hard palate near the first cheek tooth was still white and opaque in the center with a definite elevation of about one mm. above the surface. The lesion on the edge of the nostril was only mildly roughened and lighter in color on the following day. It appeared to be regressing. The lesions on the muzzle below the nostril, on the inside surface of the left lower lip near the commissure of the mouth, and on the hard palate near the left cheek tooth were also light pink in color and apparently disappearing.
However, the two lesions near the edge of the right upper lip on the muzzle were still bright red around the periphery. They were definitely raised, yellowish gray in the center and five mm. in diameter. The lesion on the hard palate just behind the dental pad had reappeared. It was bright red, 2½ mm. in diameter, and extended over one ridge of the hard palate onto the next one.

On the ninth day after admittance of the calf all the lesions were fading. Only the large lesion on the nostril was still yellowish gray and slightly roughened but even this lesion had lost its hyperemic border. On the following day the lesion on the muzzle at the edge of the nostril was scarcely visible. It was still slightly yellowish and very slightly roughened. The other lesions on the muzzle and upper lip were pale red, poorly defined spots, each about five mm. in diameter. The insides of the cheeks around both commissures of the mouth were diffusely, but moderately reddened. The gingival border posterior to the middle pair of incisors was very bright red and swollen, forming a one mm. wide border on the edge of these two teeth. No macroscopic lesions could be recognized on the hard palate or tongue.

On the eleventh day of observation all lesions had subsided. Externally only mild reddening of the two foci on the lower edge of the muzzle persisted. However, on the anterior margin of the dental pad, opposite the right, second
intermediate incisor, was a new lesion that was bright red, oval in shape and measured two by one mm. On the following day the two lesions on the lower muzzle near the upper lip were moderately reddened and enlarged to eight mm. in diameter, but very poorly defined. Remnants of the other previous lesions were still present - pale red, not raised, not roughened. The lesion at the edge of the nostril on the muzzle was now scarcely visible as a slightly yellowish, very slightly roughened area, five mm. in diameter.

On the thirteenth day the three remaining lesions on the muzzle were rust colored discolorations, one or two mm. in diameter. The lesion at the edge of the nostril was nearly healed. On the hard palate were six reddish brown foci, one or two mm. in diameter, scattered over the surface without respect to ridges. A seventh focus was present on the soft palate a few mm. from the last ridge of the hard palate. These lesions had probably been present for some time, but had not been detected until the rusty brown color appeared. They were smooth and neither raised nor depressed. The reddening of the buccal mucosal around the commissures of the lips had disappeared. The following day it was observed that the lesions on the muzzle, although pale reddish brown in color, blanched on digital pressure.

By the fifteenth day, three reddish brown spots one to two mm. in diameter on the lower muzzle and two similar foci on the hard palate were the only remnants of disease.
On the sixteenth day an irregular, poorly defined, five by
two mm., pale red focus appeared on the right edge of the
dental pad. Just to the left of the midline on the dental
plate was a roughly triangular, yellowish red, poorly de-
 fined area about five mm. in its greatest dimension. On the
next day the lesions on the dental pad and dental plate were
scarcely visible as areas of faint reddening. The lesions
on the lower muzzle near the margin of the upper lip were
again moderately reddened, three mm. in diameter, and smooth
and level with the surface. They blanched readily on digi-
tal pressure. On the outer edge of the lower lip near the
midline and just above the junction with the haired skin was
a round red focus two mm. in diameter. A round, red, level,
smooth and shiny focus, one mm. in diameter, was present on
a ridge of the hard palate five mm. from the left first
cheek tooth. On the nineteenth day the lesion on the outer
surface of the lower lip was pale red with fading, poorly
defined edges and it measured two mm. in diameter. All
other lesions had disappeared. The following day the lesion
on the lip was pale red and scarcely noticeable.

On the twenty-first day the lesion on the lower lip
consisted of two pale, one mm. in diameter, red spots and in
the skin on the chin just below the margin with the lip were
five poorly defined pink spots 0.5 to 1 mm. in diameter. On
the twenty-second day the lesions in the skin on the chin
had nearly disappeared; only two pale red foci one mm. in
diameter were visible. One was on the outer edge of the lower lip and the other was on the lower muzzle. These two lesions were in apposition when the mouth was closed. The next day reddish brown foci were still visible on the muzzle and lip. A small cluster of similar foci was present on the hard palate just anterior to and one cm. medial to the first left cheek tooth. On the twenty-fourth day a round focus, 2.5 mm. in diameter, with a white, slightly raised, one mm. in diameter, smooth center, and a bright red border was present on the edge of the upper lip. The lateral margin of the right nostril was faintly and diffusely hyperemic. The old foci on the lower muzzle, lower lip and hard palate were still visible and brownish red. All were less than two mm. in diameter. The next day the lesions had again regressed and were scarcely visible. The lesion on the upper lip had lost its white center and was uniformly pale red in color. The nostrils appeared normal. On the twenty-seventh day brownish red discolorations were still visible in two sites on the lower muzzle, in one on the lower lip, and scattered over the hard palate. Previously only a few foci had been observed on the hard palate, but now there was a sprinkling of one to two mm. in diameter, rust-colored spots. There were about 40 of them, located mostly on the posterior third of the hard palate. The next day only ten foci could be seen on the hard palate.
By the thirtieth day, tiny reddish brown discolorations were still visible on the lower lip and hard palate. The tip of one papilla in the cheek at the right margin of the hard palate was moderately reddened. In the upper lip opposite the dental edges of the left central and first intermediate incisors were two oval lesions, seven and four mm. respectively. They had pale pink centers and bright red peripheries that faded on the edges and did not blanch on digital pressure. On the thirty-first day a lesion appeared on the dental pad. It was oval, and eight mm. across, with a pale brownish red center and a poorly demarcated, yellowish brown periphery. The surface was very smooth and neither raised nor depressed. This lesion covered about one-third of the left half of the dental pad. A similar, smaller lesion, five mm. in size and roughly triangular in shape, was present on the right side of the dental pad. It extended anteriorly onto the mucosa of the upper lip for two mm. The two foci noticed the day before on the upper lip were moderately reddened, but the whitish centers could scarcely be seen. Small, brownish red foci were still present on the hard palate, and yellowish brown circles, remnants of previous lesions, were still present on the dental plate. On the following day the lesions on the dental pad were still white and smooth but were definitely convex.

All the lesions were very mild by the thirty-third day and might easily have been overlooked on casual
examination. There was scarcely any discoloration of the dental pad. The larger lesion on the dental pad was grayish white, smooth and translucent. There was no interference with prehension or mastication. Two days later only a yellowish brown streak, a remnant of the periphery of the smaller lesion, was present on one side of the dental pad. The larger lesion had not changed in appearance; it had a leathery feel to the touch and the calf acted as if the examination were painful. However, eating was not impaired. On the thirty-sixth day oval, three by one mm., roughened, raised papillomatous areas were present on the dental plate behind both fourth incisors. Two days later the lesions on the dental plate were still yellow, very roughened, firm foci without any marginal hyperemia. The lesion on the dental pad had disappeared except for a pale, yellowish brown, narrow ring that marked the periphery of the previous lesion. On the floor of the left nostril was a circular focus, two mm. in diameter, with a minute, roughened center and a red, faded periphery. By the next day, however, the lesion in the nostril was scarcely visible.

Pale yellowish brown discolorations were still visible on the dental pad on the fortieth day. Reddish brown, smooth, focal discolorations, one mm. in diameter, were present on two ridges of the hard palate, just anterior and medial to the first, right cheek tooth. They were almost confluent on each ridge for areas of about one cm. in
length. Six similar foci but without confluence were present on the fifth and sixth ridges from the dental pad on the left side. On the next day the lesions on the dental plate behind the fourth incisors appeared to be healing. They were still roughened and raised with yellowish brown centers but measured only two mm. in largest dimension. The foci on the hard palate were very pale. On the forty-second day of observation, two lesions were present on the ventral margin of the left nostril. One was a poorly defined area of mild reddening, two mm. in size. The other was less than one mm. in size, moderately reddened, and had sharp, irregularly shaped margins. Remnants of lesions were still visible on the hard palate. The next day the lesions on the floor of the left nostril were papular. They were semi-spherical, mildly reddened, and three and four mm. in diameter respectively. The redness was uniform over the entire surface and they blanched very readily on palpation. The only other macroscopic lesions were the persistent, yellowish brown, short streaks that marked part of the peripheral boundary of the previous lesion on the dental pad.

On the forty-fourth day, there were three foci on the floor of the right nostril, four, three, and one mm. in diameter and on the floor of the right nostril was a similar focus two mm. in diameter. On the upper lip just in front of the dental pad was a one mm. in diameter focus of reddening. All of these lesions were smooth on the surface. The
largest papule in the floor of the left nostril was raised about one mm. above the surface of the surrounding mucosa. The next day the foci in the floor of the left nostril were only slightly raised with smooth, curved surfaces. All were round and moderately but uniformly reddened. On the floor of the right nostril was a sprinkling of tiny, mildly reddened spots. The dental pad was still partly discolored. On the following day the nostrils appeared normal. Two days later (forty-eighth) the three foci in the floor of the left nostril and one on the upper lip were again visible as slightly raised, mildly reddened, well defined foci, three mm. in diameter. The surface was smooth and they did not blanch easily upon digital pressure. A lesion appeared on the medial wing of the right nostril on the forty-ninth day. It was a sharply delimited, round, red focus, two mm. in diameter, which appeared very slightly roughened on the surface.

By the fifty-first day the lesions in the left nostril had again disappeared. The lesion in the floor of the right nostril was a sharply defined, round, reddish brown focus, three mm. in diameter. The lesion on the upper lip was also reddish brown, but only 0.5 mm. in size and very slightly roughened on the surface. On the hard palate a new lesion appeared. It was a round, red spot one mm. in diameter located just anterior to the pale brownish remnants of the old lesions near the first left cheek tooth. By the fifty-third day, the discoloration of the dental pad was
almost entirely gone. The only other visible lesion was a circular three mm. area of mild reddening with a pronounced brownish red periphery on the dorsal margin of the medial wing of the right nostril. On the margin of the lower lip a circular, red, one mm. spot appeared on the fifty-fifth day. The reddish brown discolorations persisted for several days. On the fifty-eighth day the focal lesion on the margin of the upper lip and the lesion on the dorsal margin of the right nostril were also only pale, brownish red remnants of lesions. The lesion on the margin of the lower lip was a smooth, raised, almost conical, white spot which was surrounded by a one mm. wide zone of moderate reddening. Later on the same day the center of the lesion on the lower lip became finely granular and uniformly but mildly reddened. It was surrounded by a two mm. wide zone of reddening that faded at the edges and blanched upon slight pressure. The over-all size of this lesion was seven mm. with most of the lesion on the inner surface of the lip and a smaller portion extending for about two mm. onto the outer edge of the lip. Euthanasia was performed on the fifty-eighth day of observation, fifty-four days after lesions had first been seen. Throughout the observation period, lesions had been confined to the palate, dental pad, dental plate, lips, muzzle and margins of the nostrils.

At necropsy there was a trace of gray, sticky, translucent exudate which coated the floors of both nostrils.
On the upper margin of the right nostril was a faint trace of a rusty brown streak. Upon close examination of the sites of previous lesions on the muzzle, only a lighter gray color on the pigmented parts could be detected. The floors of the nostrils were very slightly excoriated but not reddened. On the margin of the lower lip was a nearly round, flat lesion, 3.5 mm. in diameter. It appeared to be very slightly depressed from the surrounding tissues but close examination revealed that it was actually level with the surface. The center was mildly reddened and slightly granular. It had been surrounded by a narrow zone of hyperemia that blanched after death. Two round, red, sharply delimited, smooth spots, one and 1.5 mm. in diameter respectively, were found in the mucosa at the depth of the angle between the lower lip and the mandible. The reddening extended almost through the mucosa. On the dental plate on either side of the mid-line just behind the central incisors, were two irregular, one mm. areas of faint brownish discoloration. Very faint remnants of discolorations were present on the lateral edges of the dental pad. The posterior half of the hard palate was sprinkled with yellowish brown spots about one mm. in size each. On the eighth ridge from the dental pad was a circumscribed focus, three mm. in diameter, of intense reddening.

In the proximal esophageal mucosa, 2.5 cm. from the prominence of the arytenoid cartilages, were two foci of
erosion. They were roughly elliptical with yellowish brown, roughened, depressed bases and rounded, white margins. The edges of the erosions were very sharp. They measured four by two and three by two mm. respectively and were oriented with the long axis of the esophagus. Two cm. farther down the esophagus were two similar three by one mm. erosions located very close together. Near the middle of the esophagus on a longitudinal ridge of mucosa was a long elliptical, raised, yellowish brown, caseous lesion which measured ten by 0.5 mm. Nearby was a five mm. long, yellowish gray, raised, roughened line suggestive of the same process. In the distal esophageal mucosa, 8.5 cm. from the diaphragm, was a long, narrow, sharply defined, yellowish brown, roughened and very deep erosion that measured eight by two mm. The mucosa on either side formed a rounded boundary. In the abomasum just posterior to the omasal-abomasal opening were irregular, faint, light brown discolorations of the mucosa suggestive of healed erosions. At the bases of all seven lobes of the lungs were scattered lobules of atelectasis. They were most numerous in the apical lobes where nearly one-fourth of the volume of the lobes were affected. There were no atelectatic lobules in the distal two-thirds of any lobe.

Microscopically, healing foci of papular stomatitis were found in the mucosa of the margins of the nostrils, on the lower lip, dental plate, rumen and reticulum. They
were characterized by focal hydropic degeneration in the superficial stratum spinosum and mild hyperplasia of the mucosa. Often there was local infiltration of neutrophils into the foci of degeneration and the subjacent papillary lamina propria. Some of the lesions consisted only of small foci of acellular proteinaceous globules or of nests of cellular debris in the stratum corneum (fig. 3). The latter probably represent nearly healed lesions. The lesions in the rumen and reticulum were discovered in sections that had been selected randomly.

The lesions in the esophagus were abrupt, steep walled ulcerations. The mucosal defects were filled with neutrophils, fibrin, cellular debris, and bacterial colonies but the inflammatory response was limited, at the base of each lesion, to a one mm. wide local zone of granulation tissue. There was mild pseudoepitheliomatous hyperplasia in the mucosa at the margins of the ulcerations. These lesions may have started as foci of papular stomatitis.

In the lobules of the lungs that were atelectatic macroscopically, was severe peribronchial lymphoid hyperplasia with mild secondary bronchopneumonia and partial collapse (typical viral pneumonia). There was mild extramedullary hematopoiesis in the liver and spleen, a common finding in young calves.
Fig. 3.—A nearly healed lesion of papular stomatitis in the lip of calf Bl344. Acellular, amorphous globules and a few neutrophils in the stratum corneum. H and E stain. X405.
Calf Bl345. Upon admission no macroscopic lesions were detected in calf Bl345. Four days later a circular, bright red spot, two mm. in diameter, was noted on the eighth ridge of the hard palate, one cm. to the left of the midline. The next day the lesion on the palate was brownish and barely perceptible. No other macroscopic lesions were detected until the fourteenth day after admission when an oval, two mm., pale red focus was present on the hard palate, 1 cm. to the right of the midline and at a level with the first cheek tooth. The following day there were three foci of reddening on the hard palate. Two were at the level of the first cheek teeth and the third about half way from the dental pad to the cheek teeth. Each was about one cm. from the midline. All were very bright red in color. One lesion was brighter red on the periphery and pale red and slightly roughened in the center.

On the eighteenth day the two foci were pale and still two mm. in diameter. The third focus was four mm. in diameter and brightly reddened. The slight roughening of the center was no longer noticed. On the following day the posterior portion of the hard palate was sprinkled with more than a dozen bright red, one mm. spots. The larger lesion on the anterior portion of the hard palate was now five mm. in diameter with a whitish, slightly roughened center that was surrounded by a one to two mm. wide, bright red ring that faded on the edges. One papilla in the right cheek
near the commissure of the mouth was moderately reddened. The next day (twentieth) all the lesions on the hard palate were pale red and poorly delimited. No other macroscopic lesions were detected. On the twenty-first day bright red foci were again very numerous on the posterior half of the hard palate. Many of them were on the ridges and partly confluent. The papilla in the buccal mucosa was again brightly reddened. The following day the papilla was no longer reddened but the lesions on the palate were still bright red.

On the twenty-third day the lesions on the hard palate had practically disappeared. Six red, one mm. in diameter foci were located within one cm. of an oval, red, smooth lesion, seven mm. in size, which had previously been the only focus on the anterior half of the hard palate. The edges of these seven lesions were pale and fading. The next day the lesions on the palate appeared as small, poorly defined reddeninggs over a small portion of the surface of the hard palate. All the lesions suddenly regressed on the twenty-fifth day and were visible only as pale, red, tiny discolorations. On the twenty-sixth day there were ten red foci on the hard palate near the posterior and left margins which measured one or two mm. in size. On the upper lip, opposite the dental edge of the second intermediate incisor, was a two by one mm., oval, pale gray lesion with sharply delimited but irregular edges. It was surrounded by a bright
red, two mm. wide zone of hyperemia that faded slightly on the edges and blanched on pressure. On the twenty-eighth day the lesion on the upper lip had a white, very slightly raised, smooth center, one mm. in diameter, surrounded by a ring of slightly roughened, gray tissue and then a pink circle of hyperemia. Pale lesions were still visible on the hard palate. By the twenty-ninth day the lesion on the lip had disappeared and lesions on the palate could scarcely be seen.

On the thirtieth day the lesion on the upper lip was again visible as an oval area, four mm. in size, of moderate reddening. Directly opposite this lesion on the margin of the lower lip was a round lesion, three mm. in diameter, that was pink in the center and red in the periphery. It extended onto both the inside and outside edges of the lip. On the posterior half of the hard palate were six well defined foci with white conical centers, 0.5 mm. in diameter, and narrow, moderately reddened peripheries. On casual observation they resembled pustules except that the centers were white instead of gray. The next day all of the lesions suddenly regressed again. Only faint reddening persisted on the lips and palate. The white centers of the lesions on the hard palate had disappeared and the foci were now smooth and poorly defined. Only four, faint, brown spots were present on the posterior hard palate on the thirty-second day.
On the thirty-third day the lesions on the palate had entirely disappeared. On the upper lip at the margin of the dental pad on the midline was a moderately reddened focus two mm. in diameter with poorly defined margins. The following day the lesion on the upper lip was 1.5 mm. in diameter. It had a sharply delimited, narrow, brownish edge and was moderately reddened and smooth in the center. A day later, thirty-five days after the first observations, the lesion on the upper lip had disappeared and the calf appeared entirely healthy. On the thirty-sixth day the lesion on the upper lip at the margin of the dental pad reappeared as a one mm. focus of reddening with a minute, roughened center. The dental plate was again mottled with faint, yellowish brown, poorly defined, linear discolorations, remnants of previous lesions. On the thirty-eighth day an erosion developed on the center of the left cornea with extreme edema of the bulbar and palpebral conjunctivas and moderate congestion. The face below the lid was soiled with tears. During the next three weeks the lesion progressed to ulceration with keratoconus and there was healing with scar formation following a severe inflammatory response. This lesion was considered to be infectious keratitis (pinkeye) and was probably not related to the papular stomatitis. Similar lesions were not found in the other eye or in calf Bl344 which came from the same source and was stabled in an adjoining pen.
Lesions of papular stomatitis reappeared on the forty-second day. A few mildly reddened foci, one mm. in size, were scattered over the hard palate. On the floor of the left nostril was an area of mild reddening that appeared to have resulted from the confluence of four poorly defined foci, each of which measured about three mm. in diameter. Two days later a circular, smooth, red spot, one mm. in diameter, appeared on the upper lip at the margin at the dental pad near the midline. Yellowish brown linear discolorations were still present on the dental plate from the previous lesions. The reddening in the floor of the nostril had disappeared. However, on the next day (forty-fifth) one poorly defined, mild focus of reddening, two mm. in size, was present in the ventral margin of each nostril. The pigmented portions of the muzzle were patchy with gray-white areas that indicated the sites of previous lesions. The lesion on the upper lip was only one mm. in diameter but roughened and yellowish brown with very little peripheral reddening. The next day the reddening in the floors of the nostrils disappeared.

On the forty-seventh day the lesion on the upper lip near the midline and anterior to the dental pad had reached five mm. in diameter and was slightly roughened. It was surrounded by a concentric, two mm. wide zone of moderate reddening. The following day the lesion on the upper lip was smooth, white and slightly convex. The hyperemic border
varied from two to four mm. in width and faded at the edges. This lesion was very similar to the lesion present at the same time on the dental pad of calf B1344. On the fortieth day, the periphery of the lesion was brownish red and slightly roughened. On the floor of the right nostril there were again three, poorly defined, reddened foci, two to three mm. in size. By the fiftieth day the lesion on the upper lip appeared nearly healed. The center appeared normal and the peripheral ring was brownish in color and very narrow. On the following day the left ventral nostril was mildly excoriated and covered with a thin layer of sticky, translucent white exudate. A brownish ring persisted on the upper lip at the edge of the dental pad until the fifty-fourth day when the center of the lesion was again slightly convex, white, smooth and glistening. However, the convexity disappeared the following day leaving only the narrow, yellowish brown ring.

On the sixtieth day small, reddish brown discolorations were still present on the dental plate and upper lip. A few brown specks were visible on the hard palate. On the upper lip opposite the lateral corner of the right, second intermediate incisor, was a bright focus of reddening, one mm. in diameter, with a tiny, white, raised center. The convexity reappeared in the center of the brownish ring at the anterior margin of the dental pad. Around it was a cluster of smaller brownish rings, 0.5 to two mm. in
diameter, which were also white, smooth and raised in the center. On the following day the centers of the lesions on the dental pad and upper lip again appeared normal. Reddish brown remnants of lesions persisted on the upper lip and dental plate until the seventy-seventh day when the calf was slaughtered. No other macroscopic lesions were found at necropsy.

The course, and the macroscopic and microscopic appearance of the lesions, in these two naturally occurring cases were very similar to that of calves B777 and B778 (experiment 8.), experimentally infected animals. The course and macroscopic appearance of the lesions in these two naturally occurring cases have been described in detail to emphasize the long mild course of the disease and the proliferative nature of the lesions. Fever or leukopenia was never detected during the period of observation.
PRELIMINARY TRANSMISSION EXPERIMENTS

Experiment 3.

The only calf immediately available for preliminary attempts at transmission was a four-months-old male Jersey. One hundred mg. of cortisone acetate had been injected twice daily for two weeks prior to inoculation and this was continued throughout the experimental period. Under local anesthesia a 3/16 inch trephination was made through the frontal bone and 0.5 ml. of a fresh 20 per cent suspension in nutrient medium of brain from calf Bl67 (experiment 1.) was inoculated into the left parietal cerebrum. At the same time 20 ml. of a two per cent suspension of the brain from calf Bl67 was instilled by the intranasal, oral, and conjunctival sac routes.

Nine days later the same calf was inoculated intramuscularly with 0.5 ml. of a 20 per cent suspension of lung from calf Bl67 and 0.5 ml. of a 10 per cent suspension of lung from calf Bl37. Careful observations were made for 16 days after the first inoculation. No signs of disease were detected.

Experiment 4.

In an attempt to isolate a viral agent, serial passages of lung, brain, and spleen from calf Bl67 (experiment 1.) were made on the chorio-allantoic membranes of
embryonated eggs (experiment 10.). No evidence of a virus was detected. Because of the possibility that a viral agent might have propagated even though it wasn't detected in the chick embryos, a pool of the chorio-allantoic membranes from the second, third, and fourth passages was inoculated into three calves (B427, B428, and B429).

The chorio-allantoic membranes had been stored as ten per cent suspensions at -65°C. for from two to four weeks. All were thawed rapidly, pooled, and centrifuged at 1,000 r.p.m. for four minutes. The supernatant was used directly for intravenous and intramuscular injection but was diluted 1:10 with nutrient medium containing two per cent ovine serum for intranasal, oral, and conjunctival sac instillation.

Five Guernsey calves, all from the same farm, were used in this experiment. Calf 425, one month of age, and calf B426, four months of age, served as uninoculated controls and were placed in a separate building. Calves B427, B428, and B429, one month, three months, and four months of age respectively were housed in an isolation room of The Ohio State University Veterinary Clinic. The room had a separate entrance and a separate caretaker but on two sides at the top of an eight feet high partition was a two feet wide space below the ceiling which communicated with a large isolation ward of the clinic. The closest cattle to the partition, about 15 feet away, were a Holstein cow with a
fractured leg and her one-week-old calf. At no time during the experiment were macroscopic lesions of papular stomatitis detected in the cattle in this ward.

After baseline studies were performed, calves Bu27, Bu28, and Bu29 were all challenged in the same manner with 2.0 ml. intravenously, 2.0 ml. intramuscularly, 10.0 ml. intranasally, 9.0 ml. orally, 0.5 ml. via conjunctival sac, and a few drops on small scarified areas of the buccal mucosa and ventral tongue. Oral and intranasal exposures were made by forceful expulsion from a syringe through a 23 gauge needle. The scarifications were made with the same hypodermic needle. In order to decrease resistance calf Bu29 was injected intramuscularly with 25 mg. prednisolone twice a day beginning 48 hours prior to inoculation and continuing through the entire course of the experiment.

Calf Bu27. On the third day before inoculation calf Bu27 had loose, nearly liquid stools. On the next day it was discovered that the three calves in the pen had mistakenly been getting twice as much grain as desirable. Although little or no grain was fed through the remainder of the experiment, loose stools occurred intermittently until the day before death.

Twenty-eight hours after inoculation the rectal temperature went up to 103.5°F. The highest recording was 104.2°F, made 51 hours after inoculation and by the third day it was back to normal. Total circulating leukocytes
were determined twice daily and as the temperature rose the total leukocyte count dropped from a baseline average of 6,000/mm$^3$ to a low of 4,300/mm$^3$ after 40 hours. The total leukocyte count also returned to normal by the third day. The change in total leukocytes was due entirely to lymphopenia. The calf continued to eat and appeared healthy except for the loose stools.

On the eighth day after inoculation the temperature suddenly dropped to 99.2°F. and was below 100°F. for over seven hours. The total circulating leukocytes remained normal. On the ninth post-inoculation day the ventral edges of the nostrils were slightly reddened, dry, and dull as if lightly eroded. The calf was rather thin by this time, apparently from diarrhea and an inadequate intake of grain. The nostrils appeared normal the next day but on the following day, 11 days after inoculation, they were again reddened along the ventral edges. On the hard palate, at the level of the first cheek teeth and near the midline, was a bright red, circular, smooth spot, three mm. in diameter and level with the surface. On the next day the ventral edges of the nostrils were lightly eroded but the lesion on the palate was pale red and scarcely visible. At the same time the calf was weak and stiff-legged and trembled when forced to move. He frequently stood with his neck extended.

On the thirteenth day after inoculation the calf was very depressed. When aroused he ran aimlessly around the
pen, running into the other calves, falling to his knees, and bellowing. After about three minutes the calf alternately fell down and got up and staggered for another five minutes. Lateral nystagmus was noticed for a short time during the latter period of ataxia. Suddenly the calf recovered, showed only depression and fatigue, and resumed eating. No other nervous signs were observed that day. On the next day the calf was listless and stood for minutes at a time staring into space. The ventral edges of the nostrils were still reddened and three red spots, two mm. in diameter were present on the hard palate.

The following day, 15 days after inoculation, the calf was comatose with flaccid muscles and slow respirations and pulse. Rapid vertical nystagmus was present. Occasional, ineffectual, weak righting movements were attempted followed in an hour by death. Starting on the eighth post-inoculation day there had been a gradual decrease in lymphocytes in the blood until the total leukocytes reached 3,600 and 3,350/mm$^3$ on the day before death and the day of death respectively.

At necropsy the most significant findings were emaciation, areas of lobular atelectasis in the anterior and ventral portions of the lungs, and tiny clusters and streaks of congestion and hemorrhage in the mucosa of most of the small and large intestines. The ventral and ventrolateral margins of both nostrils were shallowly eroded. The areas
of erosion were circular, two cm. in diameter, and extended for only 0.5 cm. into each nostril. On each side of the hard palate was a row of shallow but sharply defined, non-hyperemic erosions along the medial borders of all the cheek teeth. On the palate, one cm. to the left of the midline at the level of the first cheek teeth, was a shallow, circular, pink erosion, three mm. in diameter, with sharp, steep margins.

Microscopically, peribronchiolar lymphoid hyperplasia and lobular areas of acute bronchopenumonia were found in the lungs. In the kidneys were severe degeneration and necrosis of the cortical tubules, particularly the convoluted tubules. Edema was found in the brain and spinal cord associated with endothelial swelling, adventitial hyperplasia, and oligodendroglial and neuronal degeneration. Microscopic sections were prepared from 61 * blocks from all parts of the central nervous system. Edema without evidence of inflammation was found in all of them. Although no macroscopic lesions were found in the upper respiratory tract, microscopically, numerous neutrophils were present in the mucosa and submucosa of the nasal septum and turbinates. The lesions in the kidneys and brain were compatible with enterotoxemia.

The microscopic appearances of the lesions at the edges of the nostrils and on the palate were very similar (fig. 4 and 5). Sections taken at random from the dorsal
Fig. 4.—Thickening of the mucosa and focal hydropic degeneration. Hard palate of calf B427. H and E stain. X127.

Fig. 5.—Higher magnification of the hydropic degeneration in fig. 4. Note the disruption of the stratum corneum. H and E stain. X465.
edges of the nostrils and from the upper lip also showed these changes. In the superficial layers of the stratum spinosum were foci, about 200 or 300 microns in diameter, of hydropic degeneration. The mucosa (or epidermis) was often thicker at this place and the rete pegs long and narrow. The cells in the stratum corneum over the foci of degeneration retained their nuclei. The superficial layers were often slightly disrupted and partly desquamated giving roughness to the surface that could scarcely be seen macroscopically. There was mild hyperemia in the papillary lamina propria (or papillary dermis) with a slight increase in numbers of histiocytes. Very frequently the subjacent lamina propria and the focus of hydropic degeneration were infiltrated with neutrophils. In apparently older lesions only a focal accumulation of cellular debris and neutrophils remained in the stratum corneum. In the edges of the nostrils there was coalescence of foci which formed the larger lesion seen macroscopically. In these areas the stratum corneum was frequently eroded and one 600 micron area of ulceration with scarcely any inflammatory response was found. It is interesting that the lesions on the hard palate which were interpreted macroscopically as erosions were found microscopically to be level with the surface or even slightly raised.

The viral pneumonia is very often observed in calves just as it is in pigs and lambs and represents an
intercurrent incidental infection. The causes of rhinitis in calves are still obscure. The possibility exists that the lesions in the edges of the nostrils are excoriations resulting from the presence of exudate there from rhinitis. No exudate was ever observed in the nostrils in this case but it could have been removed by frequent licking. However, in one series of naturally occurring cases (Griesemer and Maurer, 1956) the lesions of papular stomatitis were observed to appear first in the edges of the nostrils. Moreover, the macro- and microscopic appearance of the lesions is similar to that found in the lesions in the mouth.

**Calf Bli28.** Like calf Blj27, calf Blj28 developed fever between 104.0 and 104.8°F. from 28 to 51 hours after inoculation accompanied by a slight decrease in circulating lymphocytes which promptly returned to normal. Low rectal temperatures and gradual absolute lymphopenia likewise occurred from the eleventh to the sixteenth post-inoculation days followed by a return to more normal limits.

On the sixth day after inoculation a tiny, red erosion appeared on the inside of the lower lip. Two days later it was 1.5 mm. in diameter, reddened, smooth, and very slightly depressed. By the tenth day the lesion could scarcely be seen and on the next day it was gone. No other lesions were seen.

The calf appeared healthy and gained weight during the experiment. Observations were continued for 28 days
after inoculation to obtain serum that might contain antibodies and then euthanasia was performed. No macroscopic lesions were found at necropsy. However, tiny foci in the stratum corneum similar to those in calf B427 were found in sections taken at random from the dorsal and ventral edges of the nostrils. In a randomly selected sample of skin from the lumbar area was found a very small focus of ballooning degeneration and infiltration of neutrophils in the epidermis. In the mucosa of the omasum were several foci of ballooning degeneration and infiltration of neutrophils (fig. 6). Evidence of the lesion previously seen in the mucosa of the lower lip could not be detected.

Sarcosporidia were very numerous but, in addition, small interstitial foci of infiltration of lymphocytes were found in the heart, psoas and cutaneous muscles, and in the periportal areas of the liver. In the lungs was mild peribronchial lymphoid hyperplasia.

Calf B429. The third calf in this experiment, B429, had been prepared with prednisolone. Like calves B427 and B428 there was transient fever from 29 to 50 hours after inoculation reaching 104.4°F. and transient leukopenia on the next day. However, there were wide fluctuations in the circulating leukocyte counts in this calf during the experiment. From the fourth day after inoculation until death the total leukocyte count ranged from 10,600/mm.³ to 13,750/mm.³ with slightly more neutrophils than lymphocytes.
Fig. 6.—An intramucosal focus of ballooning degeneration and infiltration of neutrophils in the omasum of calf B428. H and E stain. X560.

Fig. 7.—Foci lesions, slightly raised and hyperemia, in the floor of the nostril of calf B425.
The only other evidence of disease was mild reddening and roughening of the ventral edges of the nostrils from the eleventh day after inoculation until the eighteenth day when euthanasia was performed.

At necropsy the lesions in the edges of the nostrils were nearly healed and could scarcely be seen. At the base of the tongue on the lateral surface near the ventral attachment was an elliptical ulceration, seven mm. long, two mm. wide and 1 mm. deep, with a yellow, rough surface and slightly raised, white margins. There were several thin fibrous adhesions from the anterior lobes of the lungs to the parietal pleura and pericardium. Two atelectatic lobules were found near the hilus on the ventral surface of the left diaphragmatic lobe of the lung. The thymus was large but entirely within the thorax.

Microscopically only one tiny nest of neutrophils and cellular debris was found in the stratum corneum in the floors of the nostrils. Scattered neutrophils were found in the mucosa in the nasal septum, turbinates, and trachea. The lesion in the tongue was a deep ulceration filled with neutrophils and cellular debris. The base was a wide zone of granulation tissue and the sides consisted of hyperplastic epithelium. There was no hydropic degeneration or remnants of cellular debris in the mucosa to suggest a previous viral infection.
In the lungs were mild peribronchial lymphoid hyperplasia and slight interstitial pneumonia. Many arterioles had thick, partially hyalinized medias and one artery had a greatly thickened intima and a fragmented, mineralized, partly reduplicated internal elastic membrane. This change was not seen in arteries in other organs. The thymus was greatly involuted with mineralization of many of Hassel's corpuscles. There was no apparent involution of the lymph nodes. Many of the mesenteric and mediastinal lymph nodes had small accumulations of neutrophils at the cortico-medullary junctions and there was a circle of neutrophils around each lymphatic follicle in the spleen. The zona fasiculata of the adrenals appeared normal in width.

Mild papular stomatitis developed at about the same time in all three calves but may not have been a result of the inoculation. Calf Bl67, the source of the inoculum, came from a known infected herd but showed no evidence of the disease. The tissues from an infected animal are potentially infective since the virus has been demonstrated in the blood. However, Schaaf et al. (1940) were unsuccessful in one attempt to reproduce the disease with egg passaged material. The incubation period in this experiment was longer than in other experimental cases where lesions usually appeared within two or three days. The possibility of exposure to an infected animal in the nearby veterinary clinic cannot be excluded. The fever and lymphopenia
observed in all three calves were not observed in any other experimental animal and are probably not related to papular stomatitis.

**Calf B425.** Calf B425, an uninoculated control, remained clinically healthy until the twenty-first day after inoculation of the three other animals when severe depression and loss of weight was noticed. The ventral edges of the nostrils showed foci, two to four mm. in diameter, of mild reddening. The next day the depression was extreme. In the nostrils, the reddened areas were sharply delimited, very slightly elevated, and smooth on the surface (fig. 7). The next day the calf was found dead.

The only lesions found at necropsy were in the floors of the nostrils. Microscopically the stratum corneum was slightly thickened and contained small nests of neutrophils and cellular debris similar to those in calves B427, B428, and B429. Subjacent to one of these foci was once focus of hydropic degeneration in the stratum granulosum. The capillaries in the papillary lamina propria were distended with blood containing many neutrophils. Severe edema and slight interstitial pneumonia were found in the lungs. Severe edema in the brain and severe nephrosis were compatible with enterotoxemia.

**Calf B426.** Calf B426 remained clinically healthy until the twenty-fourth day after inoculation of calves B427, B428, and B429 when slight reddening of the ventral edges of
the nostrils was observed. A shallow, elliptical erosion with a yellow, roughened base was present on the hard palate. It was interpreted as a healing erosion, probably traumatic in origin. For the next three weeks the floors of the nostrils varied from normal to moderately reddened and roughened in appearance. On the forty-sixth day after the inoculation of the other three calves, six circular, red, sharply defined foci, one to two mm. in diameter, were present widely spaced on the hard palate. During the following week more and more focal lesions appeared in the mouth until, on the fifty-third day when euthanasia was performed, lesions were nearly confluent in some areas.

At necropsy focal lesions were present on the edges of the nostrils, on the muzzle, especially on its lower half, on the skin adjacent to the muzzle, on the upper lip, dental plate behind the incisors, floor of the mouth and on the mucosa of the cheeks (fig. 8). The lesions were most numerous on the lips and least numerous on the dental pad. No lesions were found on the mucosa of the tongue, pharynx, or esophagus. Most of the lesions were pale brownish red spots, circular or somewhat polyhedral, two to five mm. in diameter, smooth, and level with the surface. A few were slightly roughened. Some of them, however, especially on the palate and behind the dental plate, appeared as grayish white or pinkish, circular, shallow erosions which were sharply delimited and surrounded by a narrow zone of slightly thickened white mucosa. Upon close examination it was found that they
Fig. 8.—Foci of papular stomatitis on the upper lip and hard palate of calf Bt26. Although the lesions are level or slightly elevated they give the misleading impression of erosions.

Fig. 9.—Nuclear fragments and "inclusion bodies" in a focus of papular stomatitis. Lower lip of calf Bt26. H and E stain. X910.
were not erosions but were level with the surface. The il-
illusion was created by the sharp outline and the slightly
darkened color of the lesion which contrasted with the ad-
jacent white mucosa. No other significant macroscopic
lesions were found.

Microscopically, the lesions were all very similar.
The affected mucosa or epidermis was often but not always
greatly thickened in focal areas up to four or five mm. in
size. Most of the thickening was in the stratum spinosum.
The rete pegs were frequently elongated and variable in
width, often quite thin. Within the thickened mucosa were
foci of hydropic degeneration. They were usually about 200
or 300 microns in diameter and located in the superficial
stratum spinosum. The total cell size was the same or
slightly decreased and the nuclei, shrunken and wrinkled,
were in empty-appearing spaces. There were many small vacu-
oles between the cells in these areas which contained nuclear
fragments (fig. 9). Occasionally, eosinophilic, well-defined,
homogenous bodies, varying from two to twenty microns in di-
ameter, were present in the vacuolated cytoplasm. The
stratum corneum over the areas of hydropic degeneration was
sometimes thickened and slightly roughened. The papillary
lamina propria or dermis was usually hyperemic and slightly
increased incellularity. The latter was apparently due to
neovasculogenesis and hyperplasia of the rather primitive
mesenchymal cells usually found there.
In nearly half the foci and particularly in those in the nostrils, the foci of hydropic degeneration were infiltreated with neutrophils and the capillaries in the lamina propria or dermis were distended with neutrophils. Very frequently lesions which were apparently more advanced were present. They consisted of globular eosinophilic masses containing remnants of epithelial cells and neutrophils in a thickened, disrupted stratum corneum. This change rather than hyperemia apparently was responsible for the pale, brownish red color seen macroscopically, for hyperemia was often not present at this stage. There was often a mild infiltration of lymphocytes around the vessels in the lamina propria or dermis beneath these foci. Infrequently the basilar layers beneath foci of hydropic changes also showed hydropic changes and were slightly disrupted by migrating neutrophils that were in various stages of necrobiosis.

Findings considered incidental were extramedullary hematopoiesis in the spleen and liver, mild peribronchial lymphoid hyperplasia in the lungs, and mineralization of an occasional collecting tubule in the kidneys. There were several foci of infiltration of lymphocytes and eosinophils in the portal and centrolobular areas of the liver which may have been caused by migration of parasites. In the thalamus was one focus, 300 by 100 microns, of granulomatous encephalitis. No microorganisms were detected. In the eyelid was a small granuloma around a cluster of nematodes, possible *Stephanofilaria* spp.
Lesions of papular stomatitis were first observed in calves Bl4.27, B4.28, B4.29, B4.25, and B4.26 on the eighth, sixth, eleventh, twenty-first, and twenty-fourth days after the day of inoculation respectively. It is probable that calves B4.25 and B4.26 became infected through indirect transmission from the three inoculated calves since the facilities were not adequate for complete isolation. It is not likely that the five calves were all naturally exposed before the start of the experiment because the incubation period is usually short and the disease has not been discovered in the herd from which these calves came.

Experiment 5.

**Calf B6.38.** Thirty-one days after calves B4.27, B4.28, and B4.29 had been inoculated and seven days after the first signs of papular stomatitis appeared in calf B4.26, calf B6.38, a male, two-months-old Guernsey from The Ohio State University dairy herd, was placed in the same pen with calf B4.26. At the time of admittance a smooth, reddish brown discoloration, irregular in shape, and 3 by 1.5 cm. at its widest dimensions was present on the dorsal muzzle. On the next day the area appeared as a thin dried brown scab. It almost completely rubbed off on the following day leaving a small brown spot on a pink, smooth surface. On the third day after exposure to calf B4.26 the ventral edges of both nostrils were greatly reddened and roughened. On the fifth day two elliptical erosions, eight by three mm., and yellow
and rough in the center with narrow hyperemic margins, were present on the dental pad. They may have been traumatic in origin. The ventral edges of the nostrils were lightly reddened but the roughening extended around nearly the entire circumference. The brown spot on the muzzle was seven mm. in diameter. On the ninth day the lesions in the edges of the nostrils were still severe and there was a striking four or five mm. wide line of reddening extending from each nostril down the junction of muzzle and haired skin to the lip. The next day the reddening was less severe but the areas were roughened. The degrees of reddening and roughening varied widely from day-to-day until on the nineteenth day the muzzle appeared nearly normal and the edges of the nostrils only slightly reddened. On the twenty-second day there was a tiny bright red spot on the hard palate and another on the dental plate. On the twenty-fifth day the ventral edges of the nostrils were bright red just as they had been on the third day.

Variable mild reddening and roughening of the edges of the nostrils persisted for weeks. On the seventy-eighth day there were six bright red smooth foci on the palate and one on the upper lip. This calf was then inoculated with suspected mycotic stomatitis material in another experiment (experiment 7) along with calves B777 and B778. Inoculation was performed by scarification of the lip, tongue, and nostril and by intranasal instillation. No macroscopic
lesions developed at the inoculation sites. Twenty days later on the ninety-eighth day of observation when euthanasia was performed, there were still macroscopic lesions in the mouth and nostrils. Throughout the experimental period there was no fever or leukopenia. A sporadic non-productive cough which persisted throughout the entire course was the only other significant sign of disease.

At necropsy several circular areas of brownish discoloration, one or two mm. in diameter, were found on the hard palate, soft palate, inside the lower lip, on the gingiva medial to the mandibular cheek teeth, on the tongue, and on the ventral edges of the nostrils. Similar foci from three to ten mm. in diameter were on the gingiva and mucosa lateral to the lower cheek teeth. The apical and cardiac lobes of the lung were almost entirely collapsed. Small amounts of thick, opaque, yellow exudate could be expressed from the cut surfaces. A few lobules of the diaphragmatic lobes were also affected.

Microscopically the lesions in the mouth and nostrils were very similar. Most of them were tiny nests of cellular debris and neutrophils in the stratum corneum or, less often, in the superficial stratum spinosum. Foci up to 200 microns in diameter were present but most were about 50 microns in diameter. In a few areas the subjacent basilar layers showed hydropic degeneration. There was little or no thickening of the mucosa. In one focus in the hard palate
the stratum corneum was slightly disrupted and in the superficial layers were numerous curved or "s"-shaped microorganisms about ten microns long and one micron wide. In the submucosa were small perivascular collections of lymphoid cells.

In the turbinates and trachea were many lymphocytes and occasional neutrophils in the submucosa. In the lungs was chronic, lobular, purulent bronchopneumonia. All portions of the lungs showed extensive peribronchial and peribronchiolar lymphoid hyperplasia. In the heart were found three small foci of interstitial, non-suppurative myocarditis which were probably not related to the stomatitis. In the omasum were several tiny microabscesses in the mucosa. Sarcosporidia were very numerous in the skeletal and cardiac muscles.

This calf might have been already infected before exposure to calf Bh26. The herd from which it came was known to have been infected and there was a lesion present on the muzzle at the time of admittance. Lesions were present for more than three months. During this time there was no sign of systemic disease. There was no decrease in appetite or excessive salivation.

Experiment 6.

In experiment 4, both papular stomatitis and a disease resembling enterotoxemia occurred in calves after inoculation with egg passaged material from a naturally
occuring case of enterotoxemia. In order to test the possibility that we were dealing with a viral encephalitis or pneumoencephalitis instead of enterotoxemia another group of calves was inoculated with tissue suspensions from one of the naturally occurring cases.

The inoculum was prepared from 20 per cent suspensions (in phosphate buffered saline pH 7.4) of brain, lung, kidney, liver and mesenteric lymph node of calf B617 (experiment 1.). Tissue suspensions from calf B617 were chosen because they had been stored at -65°C. for the shortest time (38 days) of all the materials available from the first group of calves. The brain suspension was thawed and centrifuged at 1,000 r.p.m. for three minutes in an International PR-1 centrifuge and the supernatant was used for intracerebral inoculation in one calf. Part of the same supernatant was diluted 1:9 in phosphate buffered saline pH 7.4 to make a 1:50 suspension. This was forced through a Seitz ST-1 sterilizing filter pad under a positive pressure of less than four pounds per square inch and the filtrate used to inoculate another calf intracerebrally. The suspensions of lung, kidney, liver and mesenteric lymph node were pooled and centrifuged at 1,000 r.p.m. for five minutes. Some of the supernatant was used directly, part was diluted to make 1:50 and 1:00 suspensions and filtered in the same way as the suspension of brain, and part was combined with formalin to make 0.5 per cent formalin. The latter was held at 25°C.
for 45 minutes before inoculation. All inocula contained 50 units penicillin, 25 units nystatin, and 0.5 mg. streptomycin per ml.

Five male Guernsey calves (B778, B779, B780, B781, and B782) and one male Jersey calf (B777), from two to four weeks of age, were used. Just before inoculation, calves B780 and B781, the formalin-inactivated and uninoculated controls respectively, were moved to a separate building and strict isolation maintained.

Inoculations were made according to the schedule in Table 1. Intracerebral inoculations were made into the left parietal lobes through 1/8 inch trephinations under local anesthesia. Instillations into the nostrils and conjunctival sacs were performed by forceful expulsion from a syringe through a 23 gauge needle. Scarifications were made through drops of inoculum with a 20 gauge hypodermic needle on the inner surface of the lower lip and on the ventral surface of the tongue.

Calves B777, B778, and B779 were observed for 44 days. Then they were used in a mycotic stomatitis experiment which provided an additional 19 days of observation. Calves B780, B781, and B782 died from enterotoxemia on the twenty-first, thirteenth and twelfth days after inoculation respectively.

**Calf B777.** Throughout the entire experimental period calf B777 had a total circulating leucocyte count
<table>
<thead>
<tr>
<th>Calf</th>
<th>Route</th>
<th>Amount</th>
<th>Dilution</th>
<th>Filtered</th>
<th>Source of Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>B777</td>
<td>Intracerebral</td>
<td>0.25 ml.</td>
<td>1:5</td>
<td>no</td>
<td>brain</td>
</tr>
<tr>
<td>B778</td>
<td>Intranasal</td>
<td>16.00 ml.</td>
<td>1:100</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Conjunctival sac</td>
<td>1.00 ml.</td>
<td>1:100</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Scarified lip and tongue</td>
<td>1.00 ml.</td>
<td>1:100</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>1.00 ml.</td>
<td>1:50</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>1.00 ml.</td>
<td>1:50</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td>B779</td>
<td>Intracerebral</td>
<td>0.25 ml.</td>
<td>1:50</td>
<td>yes</td>
<td>brain</td>
</tr>
<tr>
<td>B780</td>
<td>Intravenous</td>
<td>1.00 ml.</td>
<td>1:50</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Intramuscular</td>
<td>1.00 ml.</td>
<td>1:50</td>
<td>yes</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>(formalin inactivated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B781</td>
<td>Uninoculated control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B782</td>
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<td>16.00 ml.</td>
<td>1:100</td>
<td>no</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Conjunctival sac</td>
<td>1.00 ml.</td>
<td>1:100</td>
<td>no</td>
<td>pooled viscera</td>
</tr>
<tr>
<td></td>
<td>Scarified lip and tongue</td>
<td>1.00 ml.</td>
<td>1:100</td>
<td>no</td>
<td>pooled viscera</td>
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<td>Intravenous</td>
<td>1.00 ml.</td>
<td>1:5</td>
<td>no</td>
<td>pooled viscera</td>
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<tr>
<td></td>
<td>Intramuscular</td>
<td>1.00 ml.</td>
<td>1:5</td>
<td>no</td>
<td>pooled viscera</td>
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between 14,000 and 21,000/mm. with at least 90 per cent lymphocytes. No evidence of disease was detected until 45 days after inoculation when a small accumulation of gray-white, opaque, thick exudate was observed in each nostril. The floors of the nostrils were slightly excoriated. This mild condition persisted for several weeks. There was no coughing or any other signs of disease.

**Calf B778.** The only evidence of disease detected during the observation period in calf B778 was a sporadic non-productive cough which was first noticed on the forty-sixth day after inoculation and continued very infrequently for several months.

**Calf B779.** No evidence of disease was detected in calf B779 during this experiment.

**Calf B780.** Sporadic non-productive coughing started the day before inoculation and continued until death. There was no local or systemic reaction to the injection of the formalinized tissue suspension. On the eighteenth day after inoculation the appetite was good but the calf appeared weak. The next day it was noticed that the calf was very thin in spite of a vigorous appetite. On the twentieth day after inoculation the calf was very weak and unable to rise from sternal recumbency. The stool was semi-formed. Later the same day the head was held against the left thorax. There was good tonus in the tail but postural thrust was very weak in the rear legs. Generalized flaccid paralysis
developed and the calf died on the twenty-first day. There had never been any macroscopic lesions in the mouth. The body weight was three pounds less than at the start of the experiment.

The only lesions found at necropsy were lobular areas of atelectasis and consolidation in the anterior ventral portions of the lungs. Microscopically in the lungs were lobular interstitial pneumonia and mild peribronchial lymphoid hyperplasia. There were large numbers of lymphocytes and a few plasma cells and neutrophils in the lamina propria of the trachea, turbinates, and nasal septum. In the mucosa of the nostrils were two foci, 300 microns in diameter, of microabscessation. They were each characterized by focal necrosis in a rete peg and intense infiltration of neutrophils into the necrotic area and subjacent lamina propria. There was almost no vacuolation of epithelial cells and the overlying stratum corneum appeared normal. There was extra-medullary hematopoiesis in the spleen and liver. In one adrenal was extensive cortical hemorrhage. There was edema in the brain and severe bilateral nephrosis.

The diagnosis was enterotoxemia. There had never been fever or leukopenia. Inoculation of mice intravenously with intestinal contents did not reveal a toxin. The reaction in the lungs was compatible with a viral etiology.

Calf B781. This uninoculated control calf, was well (except for a mild, sporadic, non-productive cough)
until the seventh day after the other calves had been inoculated when the stool was slightly loose. By the ninth day the stool was fluid. For the next several days the stool was only slightly loose. On the twelfth day the stool had a strong unpleasant odor; the calf was inactive, had a poor appetite, and had suddenly become very thin. The next day there was generalized flaccid paralysis with occasional, very feeble righting movements. The neck was extended. There was no nystagmus. The paralysis gradually progressed to coma and death. There had never been fever or leukopenia.

At necropsy the body weight was 64 pounds, 11 pounds less than at the start of the pre-inoculation, baseline period at one week of age. Several loops of small intestine were flaccid. Small areas of mucosal hyperemia were present in the small intestine, colon, and rectum. In several renal lobules the cortical radiations were obscured by finely granular, yellowish, friable tissue. The brain was very soft with flat, broad gyri and flattened sulci.

Microscopically the intestine was mildly congested and in the mucosa of the cecum and colon were coccidia. Like in calf B780 there was severe nephrosis, edema of the brain, and hemorrhage in the adrenal cortices. Although no macroscopic lesions were seen in the lungs, microscopically there was diffuse interstitial pneumonia. Several incidental focal granulomatous reactions to inhaled, tiny, acellular foreign bodies were present. In the floor of one nostril
was a microabscess in a rete peg similar to that in calf B780. In addition, for over five mm. in one area, the stratum corneum was replaced by a zone, 50 to 100 microns wide, of cellular debris, neutrophils, mucus, and colonies of bacteria. A few neutrophils were scattered throughout the mucosa and clustered around capillaries in the papillary lamina propria. In the mucosa of the lower lip, three mm. from the margin, was one small ulceration. The defect in the mucosa was 200 microns wide and the base was filled with neutrophils and granulation tissue.

Attempts to demonstrate a toxin in the intestinal contents by inoculation of mice were unsuccessful. The calf had never been fed any grain.

**Calf B782.** This calf was the same age and came from the same farm as calf B781. On the fourth day after inoculation the stools were semi-formed. Slight looseness of stools persisted through the eleventh day. On the seventh day after inoculation the calf appeared slightly weak and a little thin. On the ninth day the stool was very foul smelling. The calf was very thin and weak. By the eleventh day assistance was necessary for the calf to get up. Weakness became rapidly very severe. Respirations were very noisy as if the upper respiratory tract contained fluid. On auscultation bubbly sounds were present in the trachea. Rapid fasciculations of the flank muscles were observed for a one minute period. In spite of the weakness the
expression was alert and after the calf was helped to stand it appeared normal. On the next day (twelfth) the calf was prostrate with the head thrown sharply back and with weak running movements of all four legs. The eyeballs vacillated slowly in all directions. This progressed quickly to complete flaccid paralysis with slow vertical nystagmus, and finally to death.

At necropsy the calf was thin. There was a small amount of frothy fluid in the bronchi. Multiple lobules of atelectasis were present in the lungs in the right apical and cardiac lobes and in the left apical and diaphragmatic lobes. The brain was swollen with wide, flat gyri and the mamillary body was dark gray and translucent. Microscopically, there was severe nephrosis, edema of the brain, diffuse mild interstitial pneumonia, and hemorrhage in the cortices of the adrenal glands, like in calves B780 and B781. No lesions of papular stomatitis were found. A toxin was detected in the intestinal contents by inoculation of mice. It was neutralized by either *Clostridium perfringens* type C or type D antitoxin.

**Experiment 7.**

Forty-four days after the inoculation of calves in experiment 6, calves B777, B778, B779, and B638 were used in an experiment on mycotic stomatitis. This experiment is described here briefly since calves B777, B778, and B779 were later used in experiment 8 and also because this
experiment extends the observation period of experiment 6.

Swabs from the mouths, nostrils, and conjunctival sacs from six cattle with mycotic stomatitis had been stored in nutrient medium with two per cent ovine serum for ten months at -65°C. Calves B779 and B638 were exposed by spraying (through a 23 gauge needle) five ml. of the nutrient medium into each nostril, 0.25 ml. into each conjunctival sac, and eight ml. into the mouth and pharynx. Scarifications were made through drops of inoculum on the inside lower lip, ventral tongue and the edge of one nostril. Calf B778 was injected with 2.5 ml. intramuscularly and 2.5 ml. intravenously of whole blood from a cow that had had early signs of mycotic stomatitis and a mild fever. The blood had also been stored for ten months at -65°C. Calf B777 served as uninoculated control.

Calf B777. Observations were made for 19 days. The day after inoculation of the others calf B777 had a small amount of viscous, opaque, gray-white exudate in each nostril. The floor of the nostrils was slightly reddened and excoriated and this condition persisted throughout the observation period. The mucosa looked as if many tiny scratches had been made in it, in contrast to the appearance in papular stomatitis where discrete foci of reddening or slightly raised papules are seen.

Calf B778. Calf B778 developed a mild, sporadic, non-productive cough which persisted throughout the
observation period. On the seventeenth day an oval, two by one mm. erosion with a yellowish rough base was observed just anterior to the extreme left margin of the dental pad. It was apparently produced by the sharp corner of an incisor tooth.

Calf B779. At the time of inoculation calf B779 had a one mm., mildly reddened spot on the edge of the right nostril which disappeared the next day. On the fourth day two reddened areas less than two mm. in size appeared on the edge of the right upper lip opposite the corners of an incisor tooth. They were irregularly round, smooth, and the edges merged gradually with the surrounding tissues. On the next day they were no longer visible. On the sixth day the spot in the floor of the right nostril reappeared as a slightly reddened papule, one mm. in diameter. It disappeared again the next day. On the seventeenth day there was faint, poorly defined reddening of a small area of gingiva in front of the left, second intermediate incisor tooth. On the muzzle near its right margin, and five mm. dorsal to the edge of the lip was a red spot, one mm. in diameter. The next day no lesions could be found. No lesions developed at the inoculation sites. The lesion on the muzzle resembled those of papular stomatitis but it was the only one observed and, unlike the lesions of papular stomatitis, it was very transient.
Calf B638. Calf B638 already had lesions of papular stomatitis on the edges of the nostrils, on the hard palate, and on the right upper lip at the time of inoculation. No new lesions developed at the scarification sites. The lesions of papular stomatitis persisted until the twenty-first day when euthanasia was performed. At necropsy no evidence of mycotic stomatitis was detected.

In none of the calves did lesions of mycotic stomatitis develop. Inability to transmit the disease has been observed by many workers and, indeed, is an important criterion for diagnosis.
DIRECT REPRODUCTION OF THE DISEASE

Experiment 8.

Calves B777, B778, and B779 were used in an attempt to reproduce papular stomatitis. The inoculums were prepared from 20 per cent suspensions (in phosphate buffered saline pH 7.4) of the lower lip and hard palate of calf B426 (experiment 4) that had been stored at -65°C. for 76 days. The suspensions of lip and palate were pooled and diluted with phosphate buffered saline pH 7.4 to make a 1:50 suspension which was used for inoculation. Particles of cells could be seen floating in the final suspension.

Calf B778 served as an uninoculated control. Calf B777 was exposed by forcing from a syringe (through a 23 gauge needle) six ml. into the mouth, three ml. into the nostrils, and one ml. onto the conjunctivas. Calf B779 was treated in a similar way but with three ml. in the mouth and six ml. in the nostrils. Both were also inoculated by scarifying the mucosa of the lower lip through drops of inoculum.

Both calves B777 and B779 developed primary lesions of papular stomatitis at the scarification sites and calf B778 was later successfully infected by direct transfer of material from calf B777.

**Calf B777.** On the day after inoculation the scarifications were still visible. In the nostrils were small.
amounts of clear tenacious exudate containing small opaque white flecks. Microscopically the exudate consisted of mucus with small clumps of neutrophils. Small amounts of similar exudate were frequently present throughout the first several weeks of observation.

On the fifth day there was a reddened, circular area, three mm. in diameter at the scarification site on the inner surface of the lower lip. The next day the lesion resembled an edematous plaque. The shape was that of two ovals slightly overlapping at the ends. The larger was seven by four mm. and the smaller, five by three mm. They were quite sharply delimited. The whole lesion and particularly the margins were slightly raised above the level of the adjacent mucosa. The color was light pink. In the larger oval portion of the lesion was a central, tiny, white, depressed area. The gingiva at the anterior margins of the central pair of incisors was reddened. The inoculation had been made at the level of the right, second incisor tooth.

Two days later the lesion was irregular in shape and 1.5 cm. at its widest place (fig. 10). It consisted of a pink, edematous, slightly raised, flat plaque with a two mm. wide, bright red, sharply demarcated margin. In the center was an area, five mm. in diameter, that was yellowish brown and roughened, resembling coagulation necrosis. However, it was raised above the surface of the plaque. Inferior and medial to the large lesion, a few mm. away, were two gray
Fig. 10.—Early lesion of papular stomatitis at the inoculation site on the lower lip of calf B777. There are secondary lesions in the gingiva.
oval, umbilicated foci, one mm. in diameter. Directly opposite the primary lesion and in apposition with it when the mouth was closed was a lesion just inferior to the right, second intermediate incisor. It was an ovoid, reddened, edematous, slightly raised area, about four mm. in its largest dimension.

The next day, the eighth day after inoculation, the primary lesion was less prominent but the lesion on the mandible below the second intermediate incisor was bright red and raised one mm. above the surface. On the tenth day the center of the primary lesion was still five mm. in diameter and appeared slightly eroded. The narrow hyperemic border was still present. The lesion opposite it on the mandible was still red and raised but had no necrosis in the center. On the eleventh day the periphery of the primary lesion was a three mm. wide band of brownish, raised, very roughened tissue separated from the similar central area by a two mm. wide zone of smooth, moderately reddened mucosa that was also raised. The over-all size of the primary lesion was still 1.5 cm. in diameter.

On the twelfth day small portions of the center of the primary lesion had desquamated. The ventral margin of the right nostril was slightly reddened and appeared lightly eroded. By the fourteenth day the primary lesion had regressed until it was only seven mm. in diameter. In addition to the lesion in the gingiva at the base of the right,
second intermediate incisor, there was now a similar one, five mm. long and two mm. wide, at the base of the fourth right incisor. All three lesions were very rough, definitely raised, dirty gray, sharply delimited areas with very little hyperemia. Just lateral to the primary lesion was a smooth, white, very slightly raised nodule, three by two mm. in size. The lesions were apparently not painful and did not interfere with eating. There was no excessive salivation. The lesions would not have been noticed if the mouth had not been opened and examined.

The next day (fifteenth) the three lesions were raised one to two mm. above the surface and were shaggy and yellowish gray. In addition, two red spots, two mm. in diameter, were present on the outer mucosal surface of the lower lip near the mucocutaneous junction - one near the mid-line, the other near the right commissure of the lips. The floor of the right nostril was still lightly reddened and roughened.

On the sixteenth day along the margin of the lower lip on the right side were two rectangular brownish spots, two by one mm., which involved individual lobules of mucosa. They were smooth and not raised. A semi-spherical nodule, five mm. in diameter and two mm. high, firm, smooth, and yellowish brown was located at the mucocutaneous junction on the lower lip near the right commissure of the lips. The next day the nodule (fig. 11) was seven mm. in diameter,
Fig. 11.—Papillary nodule at the mucocutaneous junction of the lower lip of calf B777.

Fig. 12.—Photomicrograph of the nodule in fig. 11. Papillomatous thickening of the mucosa and focal hydropic degeneration. H and E stain. X48.
five mm. high, nearly spherical, very slightly roughened on the surface, and yellowish brown. Medial to the nodule on the mucocutaneous junction of the lower lip, was a row of sharply defined, circular, red spots from one to three mm. in size. Some of them had tiny, raised, very roughened, brown centers similar to the primary lesion. The primary lesion was now irregularly circular, sharply demarcated, 12 mm. in diameter, firmly attached, and appeared as a brown, shaggy, raised membrane. In the gingiva at the buccal margin of all the right incisors was a similar non-hyperemic, gray, shaggy, raised layer. In the gingiva at the buccal margin of the left second intermediate incisor and on the right upper gingiva at the margin of the dental pad, with one focus on the dental pad itself, were many shaggy gray raised foci, one to three mm. in size and irregular in outline. The gingiva of the incisors for a narrow line at the dental border was intensely reddened. The lesions did not appear to be painful. There were no other signs of disease.

A biopsy was performed under general surgical anesthesia. The large nodule on the lower lip was carefully washed with 70 per cent alcohol and allowed to dry. It was removed completely with a scalpel and the incision closed with stainless steel wire sutures. The nodule was cut in half revealing a greatly thickened mucosa. Half of the nodule was preserved in formalin and the other half cultured for bacteria.
The entire specimen for culture was ground and cultured both aerobically and anaerobically in several mediums including blood agar and thioglycollate. Many bacteria were isolated but two forms predominated: a facultative, alpha hemolytic streptococcus and an unidentified aerobic, gram negative rod. Neither of the two organisms was considered a pathogen.

Microscopically there was papillomatous thickening at the mucocutaneous junction (fig. 12). Much of the thickening was due to hyperplasia of the epithelium with elongation of the rete pegs. In the stratum spinosum were several foci up to 400 microns in diameter of hydropic degeneration giving a spongy appearance at low magnifications. Some of the vacuolated cells had small, round, eosinophilic bodies in the vacuoles. The stratum corneum was thickened and disrupted by large collections of neutrophils and fibrin. Bacterial colonies were present in the superficial layers. The papillary lamina propria and dermis were very cellular with capillaries, histiocytes, numerous lymphocytes and plasma cells, and scattered neutrophils. In one area there was ulceration with an intense local neutrophilic reaction and many bacteria in the cellular debris adherent to the surface.

On the nineteenth day there was a somewhat triangular area of reddening, one cm. across, with a central two mm. area that was white but raised and very roughened, located
on the floor of the mouth behind the dental plate. The other lesions on the lips and gingiva were still prominent and slowly increasing in size. There were no macroscopic lesions on the skin including the feet, teats, and scrotum. The calf resisted examination of the surgical site but there was no hesitancy or difficulty in eating or drinking.

By the twenty-first day the primary lesion on the lower lip was a gray plaque, two by 1.5 cm., uniformly elevated, firm, and very rough and papillomatous on the surface. Similar but smaller lesions, from one to five mm. in diameter, were very numerous on both lips, the dental pad, the gingiva, the muzzle, and in both nostrils. There was one suspicious reddened focus on the buccal mucosa of the right cheek. No macroscopic lesions were present on the hard palate, floor of the mouth, dental plate, tongue or conjunctivae.

The next day the primary lesion had extended to the margin of the lip (fig. 13) and the lip margin was thickened. The skin on the chin (fig. 14) was sprinkled with more than 20 foci, each with a small, yellowish, roughened center and a bright red peripheral ring. They measured up to five mm. in diameter. On casual examination the chin appeared covered with many red circles. Just to the right of midline over the transverse lumbar processes, three raised plaques in the skin, each about five mm. in size, were detected. They were firm, roughened on the surface, and partly denuded. There was no hyperemia.
Fig. 13.—Large papillomatous plaque on the lower lip of calf B777. Compare with fig. 10.

Fig. 14.—Hyperemic circles on the skin of the chin of calf B777.
The next day (twenty-third) a twelve by seven mm., oval, firm, gray plaque, raised two mm. above the surface, was present in the skin on the outer surface of the right upper lip, directly across from the biopsy site. The edges of the biopsy site were rounded with granulation tissue. All the lesions were progressively enlarging; the one at the edge of the dental pad was thickened but involved less than one-tenth of the dental pad itself. In addition to the circles there were many small red spots on the chin, which resembled the reaction to pricks by straw stubble. Each nostril contained about one ml. of thick, translucent, gray-white exudate. When the hair was clipped on the lumbar skin, thin dried, brownish scabs came off at the sites of the lesions observed the day before leaving a level, denuded, white surface.

By the twenty-sixth day gray papillomatous plaques were confluent over about half the mucosal surfaces of the upper and lower lips and dental pad. Sharply demarcated, light yellow, five mm. foci, resembling shallow erosions, were scattered over the muzzle and dorsal edges of the nostrils. The lesions were difficult to see because the mucosa here was pigmented. The ventral edges of the nostrils were roughened and granular and covered with a thin layer of sticky, opaque, gray-white exudate. The lesions on the right upper lip were particularly prominent resembling a row of beads.
A gray, thickened plaque, 2.5 by 0.5 cm., was present behind the right incisors on the dental plate on the twenty-seventh day. It apparently consisted of several coalesced foci. Lesions were rather uniformly distributed over the chin, edges of the lips, inside the lips, on the dental plate, on the muzzle and in the nostrils. There was no interference with eating.

On the twenty-eighth day the primary lesion began to decrease in size. About half the muzzle, particularly around the edges, was covered with brownish gray circles and spots which were difficult to see because of the normal pigmentation. The next day an oval, four mm., raised, papillomatous lesion was present on the ventral surface of the tip of the tongue. All the other lesions were rapidly improving. There was less reddening on the chin. Regression of the lesions left grayish white areas in the black muzzle. On the thirtieth day the primary lesion was still large but only slightly raised, gray, and smoothly granular instead of papillomatous. All lesions were regressing simultaneously.

The next day the normally black pigmented mucosa was light gray where raised lesions had been on the ventral and dorsal edges of the nostrils, the ventral half, midline, and dorsal edge of the muzzle, both lips (especially on the margins), part of the dental pad, and the ventral tip of the tongue. These areas were smooth and glistening. A few foci including the primary were still raised about 0.5 mm. By
the thirty-third day only discoloration was present except for two semi-spherical nodules, five mm. in diameter and two mm. high, on the margin of the right upper lip, which persisted until the fortieth day.

On the forty-third day a red spot, one mm. in diameter, appeared on the midline of the floor of the mouth, one cm. behind the dental plate. It disappeared the next day. On the fifty-second day the light gray discoloration of the black mucosa had not changed. One cm. below the edge on the outside of the lower lip, was an oval, dark brown, three by two mm. discoloration in the skin. The next day it was reddish brown and still smooth and it disappeared on the following day. One wire suture had been purposefully left in the skin at the biopsy site but there was only slight formation of granulation tissue around the edges where the wire entered the lip.

The discoloration of the pigmented mucosa gradually became less noticeable. On the seventieth day after inoculation a round, pale red erosion, two mm. in diameter, with shallow, sloped edges and without hyperemic borders was present in the upper lip opposite the dental edge of the fourth right incisor. It was probably not a viral lesion and it appeared healed the next day.

On the ninety-sixth day there was suspicious reddening of two areas, one mm. in diameter each, on the margin of the lower lip. The next day they were rusty red in color
and very slightly raised but smooth and the following day they vanished. Observations were continued until the 119th day after inoculation when the calf was slaughtered. No macroscopic lesions were found.

**Calf B778.** This uninoculated control calf remained well. On the tenth day after inoculation of calf B777, calf B778 was inoculated in the right nostril. The ventral edge of the right nostril was abraded with a 23 gauge needle and a fresh swab from the primary lesion in calf B777 was rubbed into the abraded area. Cortone acetate, 100 mg. twice a day intramuscularly, was started the day before inoculation and continued for 21 days.

The day after inoculation (eleven days after inoculation of calf B777) there was a shallow erosion, four mm. in diameter, with irregular but sharply delimited borders at the inoculation site. On the second day only mild hyperemia could be seen which persisted for four days. There appeared on the fifth day, a nearly circular, one cm. in diameter area of severe reddening and slight roughening in the floor of the right nostril. The next day (sixth) a similar lesion but only two by three mm. in size, was present in the ventral edge of the left nostril. By the seventh day the lesion in the right nostril had a seven by two mm., yellowish brown, central area that was raised one mm. above the surface and very rough and papillomatous. It was surrounded by bright red zone of hyperemia that faded on the edges. The following
day a similar, raised, yellowish brown, central, papillomatous area, two mm. in diameter, was present in the margin of the left nostril. There were no macroscopic lesions in the mouth.

The primary papillomatous area in the right nostril was ten by three mm. in size surrounded by slight hyperemia on the ninth day. On the dorsal margin of the nostril on the medial nasal wing was also a slightly raised, yellowish brown, roughened, circular area, nearly one cm. in diameter, with a mildly reddened surrounding area that faded on the edges. On the eleventh day the lesion in the floor of the right nostril was greatly thickened, very irregular on the surface, one cm. in diameter, and extended into the nostril a short distance. On the dorsal aspect of the muzzle and in the skin immediately lateral to the muzzle were six red, round spots, one mm. in diameter. Two similar dark red spots were present on the chin. The lesion in the left nostril was very slightly reddened and thickened and could scarcely be seen. The next day there were nine smooth, red spots, 0.5 to two mm. in diameter, on the chin. On the left nasal septum was a four mm. area of intense reddening that disappeared the following day. No lesions were detected in other portions of the skin or in the mouth.

The lesions slowly and progressively enlarged. By the sixteenth day the primary lesion in the right nostril was 1.5 by one cm. and still gray, thick, and papillomatous.
Multiple papular lesions, one to three mm. in diameter were present along the lateral margins of the muzzle at the junctions with the haired skin. They were nearly confluent on the right side. On the right lateral corner of the muzzle above the nostril was a circular, yellow-gray, very slightly roughened focus, three mm. in diameter. In the middle of the medial margin of the right nostril and on the outer margins of the upper lip, just below the right lateral edge of the muzzle, were two foci that were oval, five mm. in diameter, sharply demarcated, slightly raised, and yellowish gray in color. On the chin were six faded red spots level with the surface.

On the seventeenth day there was a three mm. red spot behind the fourth right incisor on the dental plate and later the same day there was a chain of such spots, two to five mm. in diameter behind all the incisors. The chin, muzzle, nostrils, and lips looked very similar to those of calf B777. The next day there was regression of the lesions. On the chin were only a few tiny red spots and one lesion, one mm. in size, with a flat, gray, raised center and a narrow, red rim. Many of the larger lesions on the dorsal nostril, muzzle, and upper lip were yellowish gray and little or not at all raised. By the nineteenth day there were very few raised lesions left. Often only a dirty, yellowish gray discoloration marked the site of a previous
lesion. The nostrils and muzzle were dry but slightly sticky as if a thin layer of serum had dried there.

On the twentieth day the lesion at the edge of the nostril on the muzzle was circular and 11 mm. in diameter. The center was gray and smooth but the outer 1.5 mm. was a dirty, brownish gray ring (fig. 15). The muzzle was variegated with yellowish, whitish or grayish spots up to five mm. in diameter. Beads of perspiration which had been absent for several days reappeared on the muzzle. Three gray, raised nodules, two or three mm. in diameter, were still present on the margin of the lower lip.

The next day (twenty-first) cortone acetate administration was discontinued. The large, circular, yellowish brown discolorations on the muzzle were covered with thin films of sticky serum. The lesions on the dental plate were also circular and yellowish brown. They were all about five mm. in diameter and nearly contiguous. The ventral border of the muzzle was marked by dirty, thin, crusty, brownish lesions. A similar circular, yellowish brown lesion, seven mm. in diameter, was present on the midline of the hard palate at a level with the first cheek teeth. It probably represented an older lesion that had not been noticed until it developed the distinctive color. By the twenty-third day only yellowish brown rings less than one mm. wide were left. Most of the muzzle was gray-white and contrasted with the normal black muzzle. A few rings were reddish brown,
Fig. 15.—Late stage of papular stomatitis in calf B778. Regression of lesions left a mottled muzzle. At the edge of the nostril is a brownish ring.

Fig. 16.—A focus of papular stomatitis in the lip of calf B779, sharply delineated by the presence of PAS-positive granules in the affected cells. PAS reaction. X113.
particularly on the outer edge of the lower lip. The lesion on the hard palate was scarcely perceptible. Discoloration of the nostrils and muzzle gradually became fainter during the next week.

On the twenty-ninth day faint, red, partial rings were still present on the muzzle at the edges of the nostrils. Most of the muzzle was black again. However on the dental plate and on the entire dental pad was brownish yellow, papillomatous tissue. About one-fourth of the ridges of the hard palate had red, poorly defined foci, one to two mm. in diameter, which gave the palate a splotched appearance. Prehension and mastication were not impaired. The following day the original inoculation site in the floor of the right nostril was again brightly reddened and excoriated as it had been soon after inoculation. The lesions on the hard palate were now very pale. On the inner surface of the lower lip was a raised, white, rectangular, sharply defined lesion, three by one mm., which had a very red, two mm. wide border that faded at the edges.

Portions of two brownish red rings were still present on the muzzle at the edge of the nostril on the thirty-second day. The dental pad was covered with a thin, rough, yellowish membrane that was not nearly so thick as the typical lesion of mycotic stomatitis. Bright red, poorly defined discolorations of the ridges of the hard palate were still present but not obvious because they were
very small and fading at the edges. The lesion inside the lower lip was scarcely visible as a very narrow red line around a blanched, slightly roughened area, 2.5 by 0.5 mm. in size. The following day reddened areas could be seen on only two ridges of the hard palate and on the next day, 34 days after inoculation only two, short, reddish brown lines on the muzzle and slight reddening of the floor of the left nostril persisted externally. The dental pad and dental plate were both still yellowish gray and very roughened.

One papilla in the cheek opposite the fourth right incisor was bright red on the thirty-sixth day. The next day the base of the papilla was only mildly reddened and the tip was white. On the following day it appeared normal. The dental pad was still uniformly yellowish gray, thickened, and slightly roughened. On the thirty-ninth day one red spot, one mm. in diameter, appeared on a ridge of the hard palate. The next day the floor of the right nostril was again greatly reddened over an area about eight mm. across. The dental pad was still slightly roughened but normal in color on the forty-first day. The reddening of the nostril extended for a few mm. onto the muzzle. The next day the reddening in the nostril regressed and involved exactly the area of inoculation and the following day the redness disappeared leaving only a slightly roughened mucosa.

No macroscopic lesions were observed after the forty-fourth day until the fifty-ninth day when both nostrils
again were reddened and very slightly roughened on their ventral margins. The next day the reddening extended part way down the junction of muzzle and haired skin from the edge of the right nostril. Moderate or mild reddening and slight roughening of the floors of the nostrils persisted variably until the seventy-eighth day. No macroscopic lesions were observed during the remainder of the observation period. The calf was slaughtered on the 109th day. No macroscopic lesions were detected.

**Calf B779.** On the third day after inoculation, there was an irregularly shaped, white, raised area, five mm. in diameter, at the inoculation site on the inside surface of the lower lip. It was surrounded by a pale, poorly defined, one mm. wide zone of hyperemia. The next day the peripheral zone was bright red but the center was still white. By the fifth day there was a tiny, yellowish gray spot in the very center. The lesion was still nearly white but had a very roughened surface. The surrounding border of hyperemia was more extensive giving the entire lesion a diameter of about one cm. Nearby, also on the inner surface of the lip, was an oval focus one mm. in diameter consisting of a pale, red, raised, umbilicated center with a very narrow, yellowish gray, peripheral ring that appeared slightly depressed from the normal mucosa. On the sixth day, while preparing for a biopsy, an overdose of anesthetic was administered.
At necropsy the primary lesion was found to be two mm. thick on cross section. The hyperemic border had disappeared when the animal died. Three tiny red spots including the one that had appeared on the fifth day were present on the inside surface of the lower lip. On the muzzle, five mm. from the edge of the upper lip and near the lateral margin, was a brown spot that involved three lobules of skin. On the cut surface the brown color was superficial and the underlying tissues appeared normal. No macroscopic lesions were present on the edges of the nostrils. On the dorsal surface of the tongue, four cm. from the tip, was one bright red papilla. Near the base of the tongue on the dorsal surface were several irregular areas of brownish discoloration up to five mm. in size. The intrathoracic portion of the thymus was sprinkled with petechiae. There were about 20 lobules of atelectasis scattered throughout the lungs with a few in each lobe.

Microscopically the mucosa at the site of the primary lesion was nearly doubled in thickness for several millimeters. The rete pegs were elongated and slender and the papillary lamina propria appeared very cellular. The cellularity of the latter was due to an increase in capillary endothelium and in histiocytes along with occasional lymphocytes and plasma cells. There was no apparent inflammatory reaction in the submucosa. The basilar layers of the mucosa appeared normal but the cells in the middle of the stratum
spinosum remained polyhedral and did not gradually flatten as the cells did in the adjacent unaffected mucosa. The cytoplasm had large granular PAS-positive areas (fig. 16) that stained bluish with hematoxylin and contained fine fibrils. The nuclei were larger and more vesicular than that of the unaffected cells. Near the surface of the stratum spinosum there was transition to several areas of hydropic degeneration. The cytoplasm of these cells appeared smaller than normal and mostly empty. Intercellular bridges were indistinct. The nuclei were irregular in shape, condensed, and sometimes consisted of a clump of very fine granules. In the cytoplasm and sometimes in the nucleus of these cells were frequently round, eosinophilic, sharply defined bodies from one to ten microns in diameter. The stratum corneum was increased in thickness. The fibers were often disrupted by eosinophilic, acellular, amorphous globules of 50 microns or more in diameter. The surface of the stratum corneum was slightly ragged since the desquamating fibers were not parallel with each other. There were no neutrophils in the lesion.

Microscopic examination of the red spots seen grossly on the lower lip and of a section of hard palate selected at random revealed similar focal hydropic degeneration in the stratum spinosum with thickening and roughening of the overlying stratum corneum and without infiltration of neutrophils.
Focal ulcerations with intense local neutrophilic infiltration were found in the buccal mucosa and in a papilla on the dorsum of the tongue. A few microabscesses were present in the mucosa of the reticulum and omasum. In the thymus were many foci of congestion and hemorrhage. Moderate peribronchial lymphoid hyperplasia, slight interstitial pneumonia, and partial atelectasis were present in a few lobules of the lung. In the spleen and liver was extramedullary hematopoiesis even though there had never been clinical anemia during the experiment.

Papular stomatitis developed readily and similarly in all three calves in this experiment. The administration of cortone acetate to calf B778 had no apparent effect on the course of the disease even though the drug was administered until after recovery had started. Signs of systemic illness did not occur in any animal.
REPRODUCTION OF THE DISEASE WITH VIRUS PASSAGED THROUGH TISSUE CULTURES

Experiment 9.

Although no cytopathogenic effect had been detected in tissue cultures inoculated with papular stomatitis virus (experiment 12), it was still possible that the virus had propagated in the cells without injuring them. To test this possibility, calves were inoculated with nutrient medium from the fifth tissue culture passage of bovine papular stomatitis virus in bovine kidney cells. The original source of the virus was a tissue suspension from the lip of calf B1425 (experiment 4). In order to prevent the possibility of the virus's being carried from one culture to another without multiplying, the nutrient medium was diluted 1:100 between each tissue culture passage. In addition, the nutrient medium was exchanged at least twice during each passage. The nutrient medium from the fifth passage represented a dilution of at least $1 \times 10^8$ of the original virus suspension. The inoculum had been stored at -65°C. for one week prior to use. Nutrient medium from serially passaged, uninoculated bovine kidney tissue cultures served as the control inoculum.

Four Jersey calves (B1685, B1686, B1687, B1688) were selected for this experiment. All were from the same farm. They varied in age from ten days to four weeks at the time
of inoculation. Calf B1687 had congenitally flexed tendons in both front legs and a loud systolic murmur. There was no evidence of hypoxia following forced exercise. Calf B1688 also had congenitally flexed tendons of the front legs but the condition was mild in one leg and the animal was able to walk fairly well on three legs.

All four calves were inoculated in the same manner. Calves B1685 and B1686 received undiluted nutrient medium from the tissue culture tubes. Calf B1688 was inoculated with a 1:10 filtrate of the nutrient medium that had been forced through a Seitz ST-1 filter pad under positive pressure. Calf B1687 was placed in a separate building before inoculation and received the control inoculum. Seven routes of inoculation were used in each calf (Table 3). Papular stomatitis developed in calves B1685, B1686, and B1688.

Calf B1685. On the day after inoculation the site of scarification on the inner surface of the lip was mildly hyperemic and abraded. By evening of the same day, 36 hours after inoculation, there was an elliptical, two by 0.75 mm., shallow, pinkish white erosion surrounded by a pale, one mm. wide zone of hyperemia at the scarification site on the lip. On the next day only slight reddening was present. The inoculation site on the tongue was also reddened but no change could be seen in the pigmented nostril. The third day after inoculation no macroscopic lesions were present.
TABLE 2.—SCHEDULE OF INOCULATIONS FOR EACH CALF, EXPERIMENT 9

<table>
<thead>
<tr>
<th>Site</th>
<th>Route</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Inner surface, lower lip</td>
<td>Intramucosal</td>
<td>0.03 ml.</td>
</tr>
<tr>
<td>2. Inner surface, lower lip</td>
<td>Submucosal</td>
<td>0.10 ml.</td>
</tr>
<tr>
<td>3. Inner surface, lower lip</td>
<td>Scarified</td>
<td>0.20 ml.</td>
</tr>
<tr>
<td>4. Ventral surface, tongue</td>
<td>Scarified</td>
<td>0.20 ml.</td>
</tr>
<tr>
<td>5. Ventral margin, right nostril</td>
<td>Scarified</td>
<td>0.20 ml.</td>
</tr>
<tr>
<td>6. Skin of neck, two sites</td>
<td>Intradermal</td>
<td>0.05 ml.</td>
</tr>
<tr>
<td>7. Right shoulder</td>
<td>Intramuscular</td>
<td>2.00 ml.</td>
</tr>
</tbody>
</table>

On the fourth day a lesion appeared in the lower lip opposite the dental edge of the fourth incisor. It was located five mm. lateral to the scarification site and was oval, seven by five mm., slightly raised, and yellowish white with a central, sharply defined, elliptical erosion, measuring three by one mm. The scarification site in the lip was again slightly reddened and there was an oval, three by four mm., smooth, white area of depigmentation at the scarification site in the floor of the nostril. The next day the large lesion in the lip had a reddish brown center. At the inoculation site on the floor of the right nostril was an oval, flat plaque, white and definitely raised above the surface. It measured three by four mm. The surrounding mucosa was black. By the sixth day the lesion in the
lower lip near the scarification site had nearly disappeared.

The raised smooth plaque in the edge of the nostril remained unchanged for several days. On the eighth day it had enlarged to eight by three mm. and in the center was a tiny rough area that was barely visible. Adjacent to this lesion was a similar but smaller oval, white, raised plaque, three by one mm. in size. The next day a lesion appeared on the muzzle near the medial wing of the left nostril. It was a smooth, circular, three mm. in diameter, sharply demarcated area of light gray depigmentation in the black muzzle. The lesions on the muzzle and margin of the nostril slowly enlarged during the next several days.

On the twelfth day five separate, slightly elevated, circular, pinkish white foci, from one to two mm. in diameter, appeared at the scarification site in the floor of the right nostril. The lesion on the muzzle was now five mm. in diameter but still smooth, not elevated, nearly white, and sharply defined. Two days later the floor of the nostril was covered with small, rounded, raised, gray papules which were partly confluent. They were slightly roughened on the surface so that the over-all appearance was that of a large, flattened, warty growth with an irregular knobby surface. The lesion on the muzzle was also slightly raised and slightly roughened in the center.

The next day (fifteenth) three rounded papules appeared on the floor of the left nostril. They were each
one mm. in diameter and raised 0.5 mm. above the surface. Euthanasia was performed the following day, sixteen days after inoculation for microscopic study.

At necropsy the area of confluent papules in the floor of the right nostril was one cm. wide and 1.5 cm. long and extended anteriorly onto the muzzle. The rough, nodular, light gray surface was conspicuous in the previously smooth, black mucosa. The individual papules varied from one to three mm. in diameter and were raised semi-spherically from 0.5 to one mm. above the surface. No erosion or hyperemia was detected. Three definitely raised nodules were still present in the floor of the left nostril. The lesion on the muzzle was circular, light gray, sharply defined, smooth, level with the surface and four mm. in diameter. No other macroscopic lesions were found. No macroscopic lesions were found at the inoculation sites in the lower lip, tongue, skin, or muscle.

Microscopically, the thickening of the mucosa in the floor of the nostrils was found to be only partly due to epithelial hyperplasia. The superficial stratum spinosum and the stratum corneum were thickened and the layers partly separated by many nests of neutrophils, vacuolated epithelial cells, and acellular, amorphous, eosinophilic, globular masses. Microscopic lesions of papular stomatitis were also found in the mucosa of a buccal papilla and a pillar of the rumen. No lesions were found in the lungs.
Calf B1686. On the third day after inoculation an oval, three by two mm., white, flat, slightly raised area of depigmentation was present at the scarification site in the floor of the right nostril. Two days later it had enlarged to six by three mm. and nearby was a similar but smaller (two by one mm.) lesion. The following day (sixth) both lesions were slightly smaller and slightly but distinctly raised above the surface. On the seventh day the larger lesion was only four by two mm. in size but on the next day it measured eight by three mm. By the tenth day after inoculation the lesion was composed of confluence of several foci. It was irregular in shape and over one cm. in its largest dimension. There was also a round lesion three mm. in diameter in the floor of the left nostril. Both lesions were smooth and very little raised; in the center of the larger was a tiny area of reddening.

The lesions in the floors of the nostrils quickly regressed on the fourteenth day and were scarcely noticeable. At the intramucosal inoculation site on the lower lip was a narrow, three mm. long, brown discoloration in the mucosa. The next day the lesions in the nostrils were again prominent and unchanged in size. By the seventeenth day there were still only a few papules in the floor of the left nostril but the lesion in the floor of the right nostril at the inoculation site was 1.5 by one cm. The latter was composed of confluent papules which were one to three mm. in
diameter and raised as much as 1.5 mm. above the surface. During the next several days the larger lesion gradually regressed and the individual papules became flatter and less distinct.

On the nineteenth day a semi-spherical papule, four mm. in diameter, appeared on the lateral margin of the right nostril. In the floor of the left nostril were four papules. The largest was three mm. in diameter and raised one mm. above the surface. The large lesion on the floor of the right nostril was only ten by seven mm. in size. The lesions continued to regress gradually. The papules became more and more flattened and indistinct at their margins until on the twenty-sixth day only a one cm., smooth area of depigmentation was left on the floor of the right nostril and a few circular depigmented spots on the floor of the left nostril.

On the twenty-seventh day, a round, roughened, red focus, one mm. in diameter, appeared on the inner surface of the lower lip, five mm. from the intramucosal inoculation site. On the dental pad was a firm flattened papule, two mm. in diameter. These two lesions were no longer visible the next day. During the next three weeks the lesions in the nostrils varied almost daily from smooth, depigmented areas to raised, knobby areas as papules appeared and regressed.
On the fortieth day the inside of the lower lip was again scarified and rubbed with a swab from the floor of the left nostril. Two days later there was a shallow, yellowish brown erosion, five by one mm., with a one mm. wide, blanched border, at the inoculation site. Eight days later the lesion on the lower lip appeared completely healed. On the next day, however, forty-nine days after the first inoculation, there was a slightly raised, white, four by 2.5 mm. depigmented area at the scarification site. The margins of the nostrils appeared normal except for a slight decrease in pigmentation. The lesion on the lower lip persisted until euthanasia was performed on the ninety-second day after the first inoculation. Starting on the sixty-third day erosion began in the center of the lesion which gradually developed until, at the time of death, an elliptical ulceration with a caseous base was present. No other macroscopic lesions were observed from the forty-ninth to the ninety-second day.

At necropsy an elliptical ulceration with a five by two mm., yellowish gray, crumbly, depressed center and thickened, raised, white, two mm. wide margin was present at the scarification site in the mucosa of the lower lip. Microscopically, focal lesions in the mucosa of the omasum were the only remaining evidence of papular stomatitis.

Calf B1688. Twelve hours after the inoculation of calf B1688 (with the filtrate), there was slight erythema at
the injection sites in the lower lip. On the day after inoculation the erythema disappeared but at the scarification site in the mucosa of the lower lip was a zone of hyperemia, seven mm. in diameter, with a central, three by 0.5 mm., raised, white area. This lesion persisted until the eighth day after inoculation when it quickly regressed. On the same day two smooth, level areas of depigmentation, three by two and two by one mm., were present at the scarification site in the floor of the right nostril. The lesion on the lower lip was again visible on the ninth day after inoculation and it persisted until the calf died on the thirteenth day of inoculation. There was progressive weakness and flaccid paralysis for the three days before death accompanied by constipation and infrequent muscular fasciculations.

At necropsy there was edema in the brain, necrosis in the renal cortices, congestion in the lungs, and irregular congestion in the intestines. Microscopically severe nephrosis and edema of the brain supported a diagnosis of entero-toxemia. Foci of hydropic degeneration, epithelial hyperplasia, and infiltration of neutrophils, similar to those in the preceding cases of papular stomatitis, were found in the margins of the nostrils and in several places in the mucosa of the lower lip.

Ten calves served as controls for this experiment. Unfortunately, calf B1687 died during the night after the inoculation with uninfected tissue culture medium. The moving
of the calf to the isolation building and the restraint
during inoculation may have contributed to cardiac failure.
At necropsy the heart was enlarged with an interventricular
septal defect and a patent foramen ovale. The lungs were
congested and edematous. On the twentieth day after inocu-
lation, the calves on the farm from which the four calves
had been purchased were examined. Nine calves from three
weeks to five months of age, including two calves that had
been in the same barn when the four experimental calves were
purchased, were examined but no evidence of disease was
detected in these uninoculated controls.
PATHOGENICITY OF THE VIRUS FOR LABORATORY ANIMALS

AND TISSUE CULTURES

The few previous attempts to infect laboratory animals with bovine papular stomatitis were unsuccessful. Reinhardt (1914) was unable to infect three sheep, two goats, and two pigs by introducing a fresh suspension of affected tissues from the mouth of a calf into the scarified mucosa. The same material kept for two days did not produce disease in a young calf. Schaaf et al. (1940) made three serial blind passages via oral scarification in two horses, two sheep, and two goats. A series of rabbits were inoculated by the intracutaneous, corneal, intratesticular, and intracerebral routes. Guinea pigs were inoculated intracerebrally, intradermally on the foot pad, and on the scarified oral mucosa, and mice were inoculated intracerebrally. All were unsuccessful. On the first intracerebral passage in mice "cerebral excitement" occurred between eight and 2½ hours after inoculation and one mouse died. All mice remained healthy on the second passage. Many attempts to infect embryonated eggs were reported to be negative. The fifth passage on the chorio-allantoic membranes did not produce disease when inoculated into a cow.

Mollaret et al. (1953b) reported successful transmission to newborn mice, guinea pigs and chick embryos and

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perhaps to sheep but not to horses. However, it is not clear what viruses were being dealt with. Some of their material came from people with oral ulcers resembling herpetic ulcers and it is likely that the lesions in rabbits, suckling mice, guinea pigs and chick embryos were caused by *Herpes simplex*. The lesions described in rabbits were often pustules.

Olson and Palionis (1953) were unable to transmit a disease similar to papular stomatitis to horses, sheep, pigs, rabbits, or guinea pigs. In one of six dogs proliferative lesions developed in the mouth at both inoculation sites after 13 days. They were excised on the eighteenth day and did not recur. Two of the refractory dogs were later found susceptible to oral papillomatosis. One calf inoculated with material from a canine oral papilloma remained well.

In the experiments described below, inoculation of suckling and weanling mice, guinea pigs, and embryonated eggs were also unsuccessful. The virus was propagated in tissue cultures but produced no cytopathogenic effect.

**Experiment 10.**

A series of experiments originated with tissue from calves B137 and B167. It is not known if these tissues contained bovine papular stomatitis virus. Calves B427, B428, and B429 were inoculated with egg passaged material from
calf B167 and developed papular stomatitis after a relatively long incubation period (experiment 4.).

Three groups of four embryonated eggs, on the eighth day of incubation, were inoculated via the yolk sac with 0.25 ml. of a 20 per cent suspension of brain, spleen, and lung respectively from calf B167. Two embryos died the next day from bacterial contamination. No elementary bodies were found in smears stained by the Macchiavello method. After eight days of observation the remaining embryos were still alive and normally developed.

Groups of three bovine kidney tissue culture tubes were inoculated with uncentrifuged, 20 per cent suspensions of spleen, brain, and lung from calf B167 and spleen, brain, lung, and thymus from calf B137. The materials for inoculation had been stored at -65°C for nine and 12 days respectively. In each tube 0.05 ml. was added to the 1.0 ml. of tissue culture medium containing two per cent ovine serum. After 24 hours the nutrient medium was exchanged. Observations were made for 12 days; no cytopathogenic effect was observed.

The same seven suspensions were inoculated onto the dropped chorio-allantoic membranes of nine-day embryonated eggs. Each embryo received 0.06 ml. of the uncentrifuged, 20 per cent suspensions; there were from two to four eggs in each group. Three embryonated eggs were dead the next day from bacterial contamination. The chorio-allantoic
membranes were harvested on the sixth day after inoculation. Each had a few, white or gray, sharply defined, flat nodules at the inoculation site. One had, in addition, whitish streaks consisting of rows of tiny white spots radiating for several centimeters along the vessels from the inoculation site. Similar lesions, however, were present in control eggs, both inoculated and uninoculated.

Serial passages were made with three groups of chorio-allantoic membranes: those inoculated with lung suspension from calf Bl67, a pool of those inoculated with spleen and brain suspensions from calf Bl67, and a pool of those inoculated with suspensions of spleen, brain, lung, and thymus from calf Bl37. After three serial passages the first two groups were combined and continued for a total of seven passages, all on the chorio-allantoic membranes of nine-day embryonated eggs. The third group, from calf Bl37 was continued through five serial passages. Six embryonated eggs were usually used for each passage in each group.

Membranes were harvested five days after inoculation, ground to make 20 per cent suspensions in phosphate buffered saline pH 7.4, and stored at -65°C. Passages were made at weekly intervals. Tiny gray or white spots less than one mm. in diameter were present in variable numbers on 90.9 per cent of the several hundred chorio-allantoic membranes, including the control membranes, in this experiment. They did not increase in numbers after serial passages. The chick embryos
remained alive and developed normally. Some of the controls, inoculated with phosphate buffered saline pH 7.4, were allowed to hatch and appeared normal. Microscopic examination of affected membranes revealed focal necrosis of the epithelium with little or no inflammatory reaction. The cause of the lesions is not known.

Calves Bt27, Bt28, and Bt29 (experiment 4.) were inoculated with a combination of the second, third, and fourth egg passages from both series that originated in calf Bl67. The fifth egg passage which originated with calf Bl67 was inoculated into 12 tubes each of bovine kidney and feline kidney tissue cultures without any cytopathogenic effects after 21 days. The same fifth egg passage material was inoculated intranasally, under ether anesthesia, into a litter of five three-week-old mice. After five days the lungs were collected and ground to make a ten per cent suspension in phosphate buffered saline pH 7.4. The tissue suspension was centrifuged at 1,000 r.p.m. for five minutes in an International PR-1 centrifuge and the supernatant was used to inoculate (intranasally) another litter of eight three-week-old mice. Five days after inoculation the lungs were harvested. There were no macroscopic lesions.

One tenth ml. of a ten per cent suspension of chorioallantoic membranes from the sixth egg passage was inoculated into each of five nine-day-old embryos via the amnionic route. No evidence of disease was detected during the next
ten days. The embryos developed normally. Four per cent suspensions of brain and spleen from both calves Bl67 and B427 were inoculated into feline kidney tissue cultures. Four tubes were used for each of the four groups. No cytopathogenic effect was detected during the next six days. The nutrient mediums from these groups were used to inoculate 12-day embryonated eggs. One-tenth ml. was placed on the dropped chorio-allantoic membrane of each of six embryos in each of the four groups. Eight days later no significant lesions were present.

Experiment 11.

Another series of experiments originated with tissue suspensions from calf B426 (experiment 4*).

A two per cent suspension of lip lesions from calf B426 in phosphate buffered saline pH 7.5 was used to inoculate six embryonated eggs, seven days old, via the yolk sac. The inoculum had been stored for 62 days at -65°C. Each embryo was inoculated with 0.2 ml. After five days the yolk sacs were harvested, drained and rinsed to remove excessive yolk, and ground with phosphate buffered saline pH 7.5 in a glass TenBroeck grinder to make a 20 per cent suspension. The suspension was centrifuged in an International PR-1 centrifuge for five minutes at 1,000 r.p.m. and the middle layer used in the next passage. Three similar passages were made. All the embryos remained alive and developed normally. A few in the second passage had subcutaneous hemorrhages,
especially around the head, but no other macroscopic lesions were detected.

The same two per cent suspension of lip from calf B426 was inoculated into three litters of three-week-old mice. One litter of six received 0.03 ml. each intracerebrally through a 27 gauge needle. Another litter of seven received 0.2 ml. each intraperitoneally, and a third litter of seven received 0.2 ml. each intranasally under ether anesthesia. There were no clinical signs of illness. Five days later euthanasia and necropsy were performed. No macroscopic lesions were found.

Experiment 12.

In the next experiment, ten different materials were passaged through five successive tissue cultures. The tissue cultures were all bovine kidney cells from young adult cattle. The nutrient medium contained ovine serum. The materials used for inoculation are shown in Table 3. The swabs of lesions were placed in two or three ml. of diluent and expressed. This fluid, with an unknown concentration, was used for inoculation. Nutrient medium containing two per cent ovine serum was the diluent except for series number ten where phosphate buffered saline pH 7.4 was used. The swabs used in series 8 and 9 came from two Angus calves, 21 and 11 days old respectively, from the Ohio State University beef farm. The older calf had moderate reddening and roughening of the floors of both nostrils. On the skin,
<table>
<thead>
<tr>
<th>Calf Number</th>
<th>Tissue</th>
<th>Per cent suspension</th>
<th>Days stored at -65°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. B426</td>
<td>lip</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td>2. B426</td>
<td>hard palate</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td>3. B137</td>
<td>lung</td>
<td>1</td>
<td>218</td>
</tr>
<tr>
<td>4. B167</td>
<td>lung</td>
<td>2</td>
<td>215</td>
</tr>
<tr>
<td>5. B167</td>
<td>whole blood</td>
<td>10</td>
<td>215</td>
</tr>
<tr>
<td>6. B777</td>
<td>lip</td>
<td>swab</td>
<td>7</td>
</tr>
<tr>
<td>7. B778</td>
<td>nostril</td>
<td>swab</td>
<td>7</td>
</tr>
<tr>
<td>8. Calf from OSU(^1) beef farm</td>
<td>nostril</td>
<td>swab</td>
<td>none</td>
</tr>
<tr>
<td>9. Calf from OSU beef farm</td>
<td>palate</td>
<td>swab</td>
<td>none</td>
</tr>
<tr>
<td>10. B1344</td>
<td>palate and muzzle</td>
<td>swabs</td>
<td>none</td>
</tr>
</tbody>
</table>

\(^1\)The Ohio State University
one cm. below the right commissure of the mouth, was a bright red papule, two mm. in diameter. Swabs were taken from the nostrils. The younger calf had six scattered areas of reddening, about eight or ten mm. in diameter, on the hard palate. The ventral margins of the nostrils appeared excoriated but not reddened. Swabs were rubbed against the lesions on the palate.

Eight tissue culture tubes were inoculated in each group by adding 0.1 ml. of the inoculum to the nutrient medium in each tube. After 24 hrs. the mediums were routinely exchanged. After five days the tissue culture mediums were harvested, diluted 1:100 with nutrient medium, and inoculated into the next series of tubes. It was necessary to change the tissue culture medium at least twice in each passage. The fifth passage represented a dilution of at least $1 \times 10^8$. In half of the tubes the cells were growing on cover glasses which were periodically removed, fixed, and stained. No cytopathogenic effects were observed (fig. 17). No inclusion bodies were detected. However, in experiment 9, calves B1685, B1686, and B1688 were unsuccessfully infected with nutrient medium from the fifth tissue culture that started with the lip of calf B126 (experiment 4.). It is very probable that the virus propagated in the tissue cultures even though it could not be detected.
Fig. 17.—Infected but normal-appearing bovine kidney tissue culture cells. H and E. X610.
Since the virus was demonstrated by inoculation of calves in the fifth tissue culture passage of material from the lip of calf Bl426, this material was used for further study. Three blind passages each were made in embryonated eggs via the yolk sac, amnionic cavity, and chorio-allantoic membrane. Microscopical examination of embryos from the third intraamnionic passage revealed no lesions. Three-week-old mice were inoculated with the same material intracerebrally and intraperitoneally. Blind passages of brain and pooled viscera respectively were made after five days. No clinical signs or macroscopic evidence of disease resulted.

Litters of suckling mice that were less than 24 hours old were inoculated by intracerebral and intraperitoneal routes with the tissue culture-passaged virus suspension. Three passages were made with brain and pooled lung, liver, spleen, and kidney at five day intervals. Serially passaged, uninoculated tissue culture medium served as inoculum for parallel control inoculations. There was no evidence of disease. Three young adult guinea pigs were inoculated intradermally in the foot pad but showed no evidence of disease during the next two weeks.

Tissue cultures inoculated with the known infective bovine kidney tissue culture medium included: newborn bovine kidney, newborn bovine lung, porcine kidney, HeLa cells, monkey kidney, and guinea pig kidney. All these
cells were maintained in nutrient medium containing bovine plasma. The plasma had been collected from a newborn calf that had never nursed, to minimize the possibility of specific antibodies being present in the nutrient medium. No cytopathogenic effects or inclusion bodies were observed in any of the tissue cultures.
DISCUSSION

Bovine papular stomatitis is a distinct infectious disease entity. The clinical course and the macroscopic and microscopic appearance of the lesions were very similar in all the naturally occurring cases observed. An identical disease has been reproduced by contact, by inoculation of portions of lesions, and by inoculation of the virus isolated in tissue cultures. The disease was also reproduced with a bacterial free filtrate indicating that the etiological agent is a virus.

Bovine papular stomatitis has not previously been recognized in Ohio. It is a mild, afebrile viral disease of young cattle characterized by the presence of focal proliferative lesions around the mouth, muzzle, and nostrils without any other signs of disease. It has been reported from France, Germany, Belgium, Poland, and Holland. Typical macroscopic lesions have been found by the author in a few young animals in nearly every herd examined critically in northern, central, and southern Ohio over a six year period. The disease is probably very widespread in the United States and Europe and perhaps over the entire world where cattle are kept. Synonyms that have been used include papillary stomatitis, proliferative stomatitis, pseudo-aphtous stomatitis, sporadic stomatitis, follicular stomatitis, and pseudo-foot and mouth disease.

So far cattle have been the only species of animal to be naturally affected. Olson and Palionis (1953),
however, observed the development of localized papular lesions on the skin of the hands of two people who had been working with calves affected with a disease similar to papular stomatitis. Attempts have been made to infect horses, sheep, goats, pigs, dogs, rabbits, guinea pigs, suckling and weanling mice, and embryonating eggs. Except for the experiment of Olson and Palionis (1953) in which one of six dogs developed lesions at the inoculation sites in the mouth, all attempts have been unsuccessful. Following their unsuccessful attempts, Schaaf et al. (1940) concluded that the virus is extraordinarily species specific. However, it is possible that the virus multiplies in species other than cattle without destroying cells and therefore is not detected. The mild prolonged course in naturally affected animals suggests very good adaptation of the virus and host to each other.

Lesions are most often observed in cattle from a few weeks to one year of age. Ostertag and Bugge (1905) found adult animals more difficult to infect. Two cows six and nine years of age were infected directly only on the second attempt and only very mild disease resulted. The disease has not been reported in newborn animals. Both dairy- and beef-type animals are affected and there are no apparent differences associated with sex.

The incubation period in both naturally occurring and experimentally produced cases is one to several days. A crop of secondary lesions, apparently disseminated hematogenously, appears from a few days to several weeks after
the appearance of the initial lesion. The first appearance of lesions might be easily overlooked since they are often very small and transitory. Although lesions may be seen in only a few calves in a herd at any one time, the morbidity within a herd is probably 100 per cent. Some animals may never have macroscopic lesions but still be resistant to exposure after they are several months old. Fatalities have not yet been reported.

The first lesions are often found in the ventral edges of the nostrils, even in animals never restrained by the nose. Lesions are confined to the edges of the nostrils, muzzle, lips, the skin around the muzzle and lips and possibly other parts of the skin, the inner surfaces of the lips, the hard palate, dental pad, gingiva, dental plate, the floor of the mouth between the rami of the mandibles, the frenum linguae, tongue, the papillae of the buccal mucosa, soft palate, esophagus, rumen, and probably the reticulum and omasum. They are most numerous on the edges of the nostrils, the hard palate, inner surface and margins of the lower lip, and muzzle. Lesions are frequently not detected if the mouth is not opened. On the tongue, lesions are seldom present on the posterior dorsal portion. No macroscopic or microscopic lesions were found in the eyes or oular adnexa.

Lesions were found in the esophagus in two of the 24 cases in this study (Table 4). The lesions in calf B617 (experiment 1.) were papillary like the two cases described by Ostertag and Bugge (1905), the one case (probably papular stomatitis) of Olson and Paliones (1953), and the two cases
of Pallaske (1955). The lesions in the esophagus of calf B1344 were partly proliferative and partly ulcerative. Lesions were found in the rumen, reticulum, or omasum in nine of the 24 cases studied (Table 4). All these lesions were microscopic and found in sections taken at random; no macroscopic lesions were found in these organs. Pallaske (1955) found one papule in the rumen of one of his cases whose papillomatous character, however, was modified by fungi. Microscopically a few of the lesions were identical with those in the mouth but most were infiltrated with large numbers of neutrophils so that they resembled intramucosal microabscesses.

Lesions in the skin away from the region of the mouth and muzzle were found macroscopically in calf B777 (experiment 8) and microscopically in calf B428 (experiment 4). Dirty, gray-brown crusts in the skin were found in a few cases by Ostertag and Bugge (1905). Attempts to produce lesions by intradermal inoculation in the neck were unsuccessful (experiment 9).

One cannot be certain that the lesions in the rumen, reticulum, omasum, and skin were caused by the papular stomatitis virus. A similar problem exists with the interpretation of erosive lesions in the nostrils because of the possible complications by infectious rhinitis (Jones and Little, 1922). These questions can only be answered by the use of disease-free cattle, which, unfortunately, were not available for this study. The lesions in the lungs were probably not related to papular stomatitis since not all the calves had pneumonia. Viral pneumonia is present in nearly
TABLE 4.—INCIDENCE OF LESIONS IN THE ESOPHAGUS AND THE FIRST THREE COMPARTMENTS OF THE STOMACH

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Experiment No.</th>
<th>Esophagus</th>
<th>Rumen</th>
<th>Reticulum</th>
<th>Omasum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>B167</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>B617</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>B1344</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>B428</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>B429</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>B638</td>
<td>5</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>B779</td>
<td>8</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>B1685</td>
<td>9</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>B1686</td>
<td>9</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

every calf presented for necropsy at the Department of Veterinary Pathology of The Ohio State University, just as it is in young pigs and sheep. There is probably no direct relationship between viral pneumonia and papular stomatitis. Ostertag and Bugge (1905) were unable to transmit the disease by inoculation of the nasal mucosa, conjunctiva, and vaginal mucosa. Schaaf et al. (1940) were likewise unsuccessful with inoculations on the teats, udder, penis, scrotum, and the skin of the neck and abdomen of cattle.

The lesions apparently start first with a focus of hydropic degeneration in the middle or outer layers of the stratum spinosum. The foci, which are often only about 50 microns in diameter, are found in individual papillae.
Macroscopically they might appear as tiny, white or, in pigmented mucosa or skin, as light gray spots. However, they are usually not detected until the focus has enlarged to nearly 0.5 mm. and the papillary submucosa (or dermis) is hyperemic. Macroscopically these appear as small, circular, smooth, red spots. As the focus of hydropic degeneration enlarges there is usually concomitant focal hyperplasia of the mucosa which becomes thickened with long slender rete pegs. The macroscopic appearance is that of a smooth, raised, white plaque with a peripheral red rim where the congested capillaries show through the mucosa.

The epithelial cells in the center of the focus of degeneration have vacuolated cytoplasm. The total size of the cells is normal or decreased. Intercellular bridges become indistinct. The cytoplasm appears empty or contains a few reticular strands. The nuclei are often shrunken and distorted and displaced to the cell membranes. Sometimes only a clump of chromatin granules persists. Between the cells are small vacuoles containing nuclear debris. Within the cytoplasm there are often circular, eosinophilic, smoothly contoured bodies, one to ten or more microns in diameter which Schaef et al. (1940) and Pallaske (1955) considered viral inclusion bodies. Occasionally there are two or more such inclusions in a cell. They are easily demonstrated in routine preparations stained with hematoxylin and eosin. Similar inclusion bodies are found in
many viral diseases where hydropic degeneration of epithelium takes place, such as ovine contagious ecthyma. Their presence suggests a viral reaction but in papular stomatitis they have yet to be demonstrated as specific viral inclusions. The epithelial cells at the deep transitional margin of the hydropic focus frequently have granular cytoplasm that is basophilic in preparations stained with H. and E. The granules show a positive periodic acid Schiff reaction but little PAS-positive material is present in the cells with hydropic degeneration. There is never lysis of cells with the formation of intramucosal fluid filled cavities (vesicles).

Healing may occur, of course, at any stage of the development of the lesions. Regression at the early stage of hydropic degeneration and hyperemia may be very rapid and bright red lesions may become impossible to detect macroscopically within a few hours.

As development of the lesion progresses, the focus is moved closer and closer to the surface of the mucosa by proliferation of epithelium below. By the time the focus of hydropic degeneration reaches the stratum corneum, the dead cells have formed globular, acellular, proteinaceous masses of about 50 microns in diameter. The tinctorial qualities of the globules and their apparent formation by granular epithelial cells suggest abortive keratin formation. The normally parallel laminations of the stratum
corneum are disrupted by the globules and the surface macroscopically appears slightly roughened. At the same time there is usually concomitant local parakeratosis and hyperkeratosis which contribute to the macroscopic elevation of the lesion.

Frequently the focus of hydropic degeneration is infiltrated by neutrophils. This is apparently a reaction to the dead epithelial cells rather than to secondary infection because bacteria are confined to the superficial layers of the stratum corneum. The capillaries in the papillary lamina propria (or dermis) become distended with neutrophils, and migration of neutrophils from the subjacent capillaries to the intraepithelial focus may slightly disrupt the basilar epithelium. There is no detectable neutrophilia in the circulating blood. When foci containing neutrophils move to the surface of the mucosa, the globular masses in the stratum corneum contain remnants of dead leucocytes. The presence of neutrophils and perhaps also of the globular masses in the stratum corneum gives the lesion a macroscopic yellowish brown or yellowish gray color which persists for a long time, even if the inflammation should suddenly subside.

In the areas of mucosa and skin where lesions develop, there are many focal lesions, within small areas, in various stages of development at the same time. Even when macroscopic discolorations have been present for many weeks,
small developing foci of hydropic degeneration are still present. It is likely that there is a very poor immune response to the virus and that the long course with many mild exacerbations represents a continuing infection.

Occasionally and not in every case, there is coalescence of foci in several papillae with severe epithelial hyperplasia that results macroscopically in a warty nodule, up to two or three cm. in diameter. The long fronds of papillary lamina propria are very cellular with capillaries and histiocytes. Not infrequently there is erosion or even ulceration of such a lesion with secondary bacterial infection and a local reaction of infiltration of neutrophils and formation of granulation tissue. The presence of exudate on the floors of the nostrils and frequent licking may cause excoriations which are slightly reddened and easily confused with papular stomatitis. The focal raised lesions of papular stomatitis are not present, however, and there are no lesions in the mouth.

There is no doubt that papular stomatitis is caused by a virus. Ostertag and Bugge (1905), Schaaf et al. (1940), and Olson and Paliones (1953) successfully reproduced the disease in calves with filtrates that had been passed through Chamberland, Berkefeld-N, and Seitz and Selas filters respectively. Schaaf et al. (1940) were able to store the virus in glycerine at 2°C. for at least five months. Olson and Paliones preserved the viral activity of
biopsied material from cases resembling papular stomatitis for seven months in 50 per cent glycerine at 4°C. The same material when lyophilized was still active one year later.

The course of the disease is often three or four months but there is no interference with the health of the animal. The appetite is normal and normal gains in body weight are made. There is neither fever, nor leukopenia. There is no excessive salivation or pain in the mouth. Lesions are absent on the feet and teats. In fact, the disease is so mild it is seldom detected except in the course of examination for another disease such as foot and mouth disease, the mucosal complex, or hyperkeratosis.

The warty, papillomatous appearance of the lesions may lead to diagnostic confusion with papillomatosis. However, the lesions of papular stomatitis are not generally found in the skin except around the mouth and muzzle and the more common tiny, smooth red foci are usually also present in the mouth. Schaaf et al. (1940) were unable to produce lesions by inoculating papular stomatitis virus into the skin of calves. Olson and Paliones (1953) were also unsuccessful with intradermal inoculations of calves with their virus. They injected the same calves intradermally and orally with bovine papillomatosis material and produced papillomatosis. The lesions of papillomatosis are characterized by marked papillary acanthosis and hyperkeratosis with little focal hydropic degeneration. In
addition no lesions are produced by papular stomatitis virus on the chorio-allantoic membranes of embryonating eggs. The two diseases are clearly distinct but have enough similarities to suggest that the two viruses are closely related.

The skin and oral mucosa, however, are limited in their possible reactions to viruses. Focal hydropic degeneration is common to many viral dermatitides including the pox group where intracytoplasmic inclusion bodies are also observed. Papular stomatitis is clearly different from cowpox. Elementary bodies are not present in affected epithelial cells and the virus does not produce lesions on the chorio-allantoic membranes of embryonated eggs.

Papular stomatitis is different from foot and mouth disease and vesicular stomatitis. Vesicles are never observed. Lesions are absent from the teats and feet. There is no fever, excessive salivation, smacking of the lips, or painful reddening of the mouth. Guinea pigs are not susceptible. The lesions in cattle may, however, be confused with the early papular stages of vesicular diseases. Papular stomatitis does not resemble rinderpest or the mucosal disease complex because there is no fever, there are no lesions in the abomasum or intestines, the lesions are raised rather than sharply eroded, and lymphoid tissue is not affected. Malignant catarrhal fever, with its serious course, excessive fluids from the mouth and nostrils,
enlargement of lymph nodes, ulcerations in the mouth (and often intestines), corneal opacities, and inclusion bodies in the brain, is easily distinguished from papular stomatitis. Raised nodular lesions occur in the mouth in Knopvelsiekte (lumpy skin disease) but they also are widespread in the skin (Henning 1956).

The diseases with which papular stomatitis is most likely to be confused are erosive stomatitis and mycotic stomatitis. For this reason and because there is a great deal of confusion in the literature about terminology, these two diseases will be briefly described and the pertinent literature reviewed.

Much of the early terminology reflects the evolution of the understanding of foot and mouth disease. Foot and mouth disease was already present in Europe in the seventeenth century (Dieckerhoff, 1903). In the early nineteenth century Maulseuche, Klausenseuche, and Zungenseuche (Zungenanthrax) were considered separate diseases in the French and German literature. Later all three were recognized as foot and mouth disease. Many authors made a distinction between "bosartigen Maulseuche" (Aphthae malignae) and "leichte" or "gutartige Maulseuche" (Aphthae benignae). The former, which was foot and mouth disease, was misconstrued as a form of anthrax. The latter was also sometimes called "Blasenfieber" or "fever with metastatic outbreak of vesicles" and probably included mild foot and
mouth disease, vesicular stomatitis (Iwerson, 1890), papular stomatitis, and other unrecognized diseases. Ostertag and Bugge, who first named stomatitis papulosa bovis specifica (papular stomatitis), gave "gutartige Maulseuche" as a synonym.

Révész (1914) observed an erosive stomatitis that affected half of a herd of 750 young and adult female cattle in Hungary. The disease was most severe at first. There was no fever but the animals stopped eating, salivated profusely, and smacked their lips. Round or elliptical, grayish red, painful erosions and ulcerations were found on the edges and inner surfaces of the lower lips, gingiva, dental pad, hard palate, dental plate, and tongue. The edges of the lesions were sharply defined and vertical. There were no vesicles, no lesions on the feet, and no diarrhea. After several days the lesions healed by granulation with some scar formation. Révész called the disease benign infectious ulcerative stomatitis of cattle. The report of ulcerative stomatitis by Bedel (1904) is incomplete and cannot be interpreted.

During an outbreak of foot and mouth disease in South Africa, Mason and Neitz (1940) encountered two forms of stomatitis in cattle. One, called "scaly" or "furry tongue" affected only the tongue. The epithelium peeled off the tongue and, if complete, the tongue was smooth and eel-like. The other was an erosive stomatitis observed in
young cattle in which oval or elongated erosions, with ragged edges, non-bleeding papilliform bases, and filled with gray, cheezy material, occurred on the tongue, muzzle, dental pad, and lower lip. There was no fever, no vesicles, and no lesions on the feet. The lesions healed in a week or two after a mild course with no signs of systemic disease. The disease was reproduced in calves with gradocol filtrates of lesions and with the second and fourth passages on the chorio-allantoic membranes of embryonated eggs. Sheep, rabbits, rats, guinea pigs and mice were not susceptible.

Gibbons (1949 and 1954) encountered an erosive stomatitis in calves while studying hyperkeratosis. The disease was characterized in addition by fever, diarrhea, and leukopenia. It may have been a viral diarrhea in the mucosal disease complex Carlson, et al. (1957). Schaaf (1955) described endemic erosive stomatitis in cattle which also had enteritis. The description is that of virus diarrhea (which has not yet been reported in Germany). In India, Pande and Krishnamurtz (1956) encountered a mild, erosive stomatitis in calves from three months to two years of age. Discrete erosions were found on the dental plate behind the incisors, upper and lower lips, and dental pad. No lesions were found on the tongue. One experimental animal had a fever. The lesions healed in about a week. The disease was, unfortunately named parotido-stomatitis in
calves on the basis of paroditis which was apparently only an incidental finding in the first case.

Pritchard et al. (1958) described an infectious ulcerative stomatitis in cattle in which the lesions in the mouth resembled those in virus diarrhea. There was anorexia and loss of body weight but no fever, leukopenia, or diarrhea. Lesions were found on the tongue, lips, buccal mucosa, palate, muzzle, nostrils, anterior turbinates and the skin around the mouth. The lesions healed in two or three weeks and no animals died. The microscopic appearance of the lesions was similar to that of papular stomatitis but the lesions progressed to ulceration. The horse, pig, sheep, guinea pig, and mouse were not affected when exposed to the virus. Particles between 125 and 150 nm in diameter were demonstrated under the electron microscope in purified suspensions of triturated lesions.

Erosive stomatitis resembles papular stomatitis in that it is a relatively benign disease with lesions confined to the area in and around the mouth. The only significant difference is the presence of erosions and ulcerations in ulcerative stomatitis. Many of the diseases reported in the literature under the names erosive or ulcerative stomatitis are clearly either mycotic stomatitis or papular stomatitis. Only the diseases reported by Révész, Mason and Neitz, Pande and Krishnamurtz, and Pritchard et al. comprise a group that might be considered distinct. It
has already been mentioned, however, that some of the lesions of papular stomatitis must be very carefully examined to distinguish them from erosions. Furthermore, secondary ulcerations, especially in the nostrils, buccal mucosas, and esophagus are not uncommon in papular stomatitis. The existence of erosive stomatitis as a disease entity must still be determined by pathological and specific serological means.

Diseases resembling mycotic stomatitis were observed by Bang (1899), Anderson (1901), and Stribolt in Denmark. Adult cattle were affected during the summer months. An exudative eczema was present on the udder, teats, and between the toes and there was a croupous stomatitis on the inner surfaces of the lips, and palate. The animals were off feed, salivated excessively, and dropped in milk production.

Our present concept of mycotic stomatitis is very little different from the original description of Mohler (1904, 1924). Adult pastured cattle were affected in the late summer. They were unable to eat because of lesions in the mouth. Ulcers were most frequently present on the gingiva, dental pad, inside surfaces of the lips, and the tip of the tongue. They also occurred on the cheeks, palate, and dorsum of the tongue. The ulcers had a hemorrhagic border and a depressed, suppurating surface, and contained a brownish or yellowish adherent debris. The
muzzle became dry and parched followed by erosions and exfoliations of the superficial layers. Adherent brownish crusts and scabs formed over the lesions, and similar lesions were seen around the nostrils and external lips. In some cases swelling of the pasterns and fissuring of the skin around the coronets occurred with resulting pain and lameness in walking. Superficial erosions were sometimes present on the teats. Cracks in the skin filled with serum and formed brownish scabs. The teats were tender and milk production decreased. Fissures and scabs sometimes formed in the skin on the neck and shoulders. There was a slight fever in some early cases. Recovery soon followed and was often complete within a week. The course was from seven to 15 days with less than 0.5 per cent mortality. Mohler ascribed the cause to fungi on the pasture. This has never been confirmed.

Kantorowicz (1906) described typical mycotic stomatitis in Germany but called it pseudo-foot and mouth disease. In Hungary, Vigadi (1906) reported an ulcerative stomatitis in cattle that was probably mycotic stomatitis. Keppel and Robinson (1932) described mycotic stomatitis under the name ulcerative stomatitis in South Africa. The presenting sign was often lameness. They were unable to transmit the disease. Bekker (1934) reported signs and lesions of bluetongue in cattle which were identical with mycotic stomatitis. That bluetongue virus can be recovered...
from the blood of cattle is known (Spreull, 1905). Mason and Neitz (1940), however, were unable to produce lesions in cattle with Bekker's own strain of bluetongue virus. Wheeler et al. (1945) and Belonje (1952) also described but did not recognize mycotic stomatitis in South Africa. Blumer and Hindmarsh (1938) described mycotic stomatitis in Australia. Schneider (1955), Scheidy et al. (1956), and Hollister et al. (1956) in the United States described mycotic stomatitis under the names necrotic stomatitis and muzzle disease. It should be noted that candidiasis (moniliasis, thrush, Soor, stomatitis oidica) is also often referred to, and perhaps more correctly, as mycotic stomatitis.

The most important features of mycotic stomatitis for differential diagnosis are - adult cattle are most often affected, other species are not affected, the morbidity is low with an average of two to five per cent, it occurs seasonably in the late summer, lesions are present on the teats, udder, and skin as well as in the mouth, lameness, rapid loss of weight, and abrupt drop in milk production are prominent signs, and it has not yet been transmitted.

Mycotic stomatitis is thought by some authors to be the same as vesicular stomatitis. Hanson (1952) stated, "It is difficult to find any significant way in which the disease described by Mohler differs from vesicular stomatitis whether it be clinical symptoms, course, or epizootiological
factors." However, the lack of vesicles in mycotic stomatitis, inability to transmit the disease, and failure to isolate a virus in guinea pigs, mice, or embryonated eggs are sufficient reasons to consider mycotic stomatitis distinct from vesicular stomatitis. The true nature of mycotic stomatitis will not be known until histopathological studies are performed.

Prentice (1913), during an investigation of foot and mouth disease in County Armagh, Ireland, encountered cases of "peeling tongue" in cattle. Mettam and Norris (1913) saw similar cases in the same county and successfully transmitted the disease to calves but not to sheep and pigs. Most of the affected cattle were under two years of age. Lesions were present on the tongue, upper gingiva, upper lip, dental pad, and muzzle. The portion of the tongue with hairy papillae was affected. Lesions were not present on the feet or skin. Dirty, yellow-brown, very thin flakes of epithelium sloughed off the mucosa of the tongue. The lesions were large and limited by an irregular ragged fringe of epithelium, outlined by a dark brown line. The epithelium was readily removed in flakes with the fingernail. In ten to 14 days the entire dorsal surface of the tongue was sloughed leaving a soft, clean surface. There was no soreness or salivation. Evidence of any constitutional disturbance was lacking and the animals appeared to be in no way inconvenienced by this trifling ailment. The
disease has since been referred to in the literature as "Armagh disease." Mason and Neitz (1940) and Wheeler et al. (1945) observed similar cases in South Africa. Mason and Neitz referred to the disease as "furry" or "scaly tongue." This disease is apparently infectious and probably viral but the relationships with other disease entities have not yet been determined.
SUMMARY

Bovine papular stomatitis has not been previously reported in the United States. This study represents the first recognition of the disease in Ohio. Naturally occurring papular stomatitis was encountered as an intercurrent infection during the study of another disease of calves. The identity of the disease was confirmed by histopathologic study and reproduction of the disease.

Papular stomatitis is a mild, afebrile, viral disease of calves. Lesions are found in the mouth, on the margins of the nostrils, on the muzzle and skin around the mouth, and sometimes in the esophagus and first three compartments of the stomach. The principal microscopic changes are focal hydropic degeneration and acanthosis in the mucosa or epidermis. Macroscopically these foci are raised and often irregular on the surface forming brownish papillary nodules and papillomatous plaques. The lesions often persist for several months. Affected animals do not have fever, anorexia, excessive salivation, leukopenia, or depression. There are no lesions on the feet or teats or in the abomasum or intestines. The morbidity is nearly 100 per cent in affected herds; fatalities have not yet been recorded.

The disease was reproduced in calves by contact, inoculation of portions of lesions, and inoculation of the virus isolated in tissue cultures. The experimentally
produced disease was identical with the naturally occurring disease. Guinea pigs, weanling and suckling mice, and embryonated eggs were not susceptible to the disease. No cytopathogenic effects were detected in tissue cultures of bovine kidney, bovine lung, porcine kidney, monkey kidney, Hela, or guinea pig kidney cells. However, nutrient medium from the fifth passage in bovine kidney tissue culture cells, representing a dilution of at least $1 \times 10^6$ of the original affected tissue, produced the disease when inoculated into calves.

The literature of the world on non-vesicular viral stomatitisides of cattle was reviewed and the significant differential features of the diseases briefly discussed.
BIBLIOGRAPHY

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I, Richard Allen Griesemer, was born in Andreas, Pennsylvania, May 8, 1929. I received my secondary school education at North Ridgeville High School, North Ridgeville, Ohio, and my undergraduate and professional training at The Ohio State University, which granted me the degree Doctor of Veterinary Medicine in 1953. I was appointed Instructor in the Department of Veterinary Pathology at The Ohio State University in June, 1953, and held this position for six years while completing the requirements for the degree Doctor of Philosophy. During this time, a two year military leave of absence was spent in full-time research in veterinary pathology at the Armed Forces Institute of Pathology, Washington, D.C., under Colonels F. D. Maurer and T. C. Jones. I have completed the board examination in veterinary pathology and have been certified by the American College of Veterinary Pathologists to practice the specialty of Veterinary Pathology.