

Part II.STUDIES UPON EXPERIMENTAL VARIOLA IN MONKEYS
(*Macacus cynomologus* and *M. nemestrinus*) AND IN
THE ORANG UTAN (*Simia satyrus*.)

1. Variola inoculata following inoculation of the skin of the monkey.
2. Variola inoculata in the orang utan.
3. Variolous keratitis in the monkey.
4. Variola inoculata following inoculation of the mucous membrane of the monkey.
5. On the occurrence of variola vera in monkeys and in the orang utan.

W. R. Brinckerhoff and E. E. Tyzzer.

I. VARIOLA INOCULATA FOLLOWING INOCULATION OF
THE SKIN OF THE MONKEY.

In these experiments particular attention was paid to the evolution of the specific lesion at the site of the inoculation, to the general exanthem, and to the constitutional reaction of the animal. Material was collected for the histological study of the primary lesion of the exanthem, and of the internal organs.

TECHNIC. The different strains of virus used were obtained from cases of smallpox occurring among the European and native population of Manila and of the Provinces. So far as circumstances permitted, the virus was kept on ice from the time of collection until used for inoculation.

In some experiments the contents of the pustule or the dry disk was used, but as a rule the contents of the unclouded vesicle was selected for inoculation. Each strain of virus, except one, was inoculated at least once upon the skin of a fresh monkey to test its potency. The different strains of virus used were as follows:

- No. 21. — Collected April 6, 1904, from a case of severe variola vera in a native male, aged about twenty years, on the twelfth day of the disease.
No. 52. — Collected May 4, from a case of severe variola vera in an

American negro in Bilidid Prison, aged about thirty years, on the tenth day of the disease.

No. 167. — Collected from a case of severe variola vera in a native male, aged about twenty years. The contents of unclouded vesicles was collected on the eighth day of the disease, August 6, and again on the following day. The contents of the pustules was collected on the fifteenth day, and the disks from the palms and soles on the nineteenth day of the disease.

No. 199. — Collected on September 18, from a case of severe variola vera in an American infant on the ninth day of the disease.

No. 200. — Collected on September 17, from a case of severe variola vera in a Spanish-Filipino infant, on about the ninth day of the disease.

No. 252. — Collected October 7, from a case of severe variola vera in a native infant, at autopsy, on about the tenth day of the disease.

No. 307. — Collected on October 23, from a case of severe variola vera in an adult native male, on about the ninth day of the disease.

No. 325. — Collected November 3, from a case of mild variola vera in a native boy, aged eight years, on about the tenth day of the disease.

No. 326. — Collected November 6, from a case of severe variola vera in a native boy, aged twelve years, on about the eighth day of the disease.

No. 327. — Collected on November 9, from a case of severe variola vera in a native girl, aged eleven years, on about the ninth day of the disease.

Method of inoculation. The introduction of the contagion into the animal was effected by making a number of separate shallow scratches with the point of a scalpel on the previously shaved and clean skin of the abdomen, and rubbing the virus into the wound with the back of the instrument. The inoculations were placed at least two centimeters apart. The usual precautions were observed to avoid the introduction of extraneous matter. An anesthetic was employed whenever discomfort might otherwise be caused to the animal.

The animals were observed daily during the course of each experiment. The naked eye appearance of the lesions at the sites of inoculation was recorded, together with the occurrence and evolution of the exanthem, the constitutional reaction, the body temperature taken per rectum, and the reaction of the lymph nodes.

Material for histological study was obtained either by excision of the lesions during the course of the experiment, or by autopsy when the animal was killed. All tissues were

fixed in Zenker's fluid for twenty-four hours, washed in running water over night, and then hardened by passing through alcohols of graded strength. Tissues were embedded by the chloroform-paraffin method. Sections were cut on the Minot microtome of a thickness of from three to six microns, and were stained in a variety of ways.

In certain experiments the immunity of the animal, resulting from the first inoculation, was tested by subsequent skin inoculations with vaccine or variola virus.

This section is based upon the study of sixty-five monkeys inoculated on the skin with variola virus, of which the following experiments are selected to be given in detail:

I. Clinical course of disease.

No. 114. — Adult male, *Macacus cynomologus*. Monkey was inoculated in twelve places on the skin of the abdomen with virus No. 167 (vesicle contents). Body temperature 37.6° C.

Twenty-four hours after inoculation the scratches show a narrow, dry, crust, about which the skin is opaque, and slightly elevated for a distance of one or two millimeters. Body temperature 39.2° C.

Forty-eight hours. The skin for a short distance about the crusts is white, but this area fades, without a definite line of demarcation, into the surrounding normal skin. Body temperature 38.2° C.

Three days. The elevated skin about the crust is pink for a distance of two millimeters. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. The lesions present as rounded pink elevations with fairly definite borders, and surmounted by delicate yellow crusts. The lesions average seven millimeters across. In some there is a narrow translucent zone immediately around the crust. Axillary lymph nodes somewhat enlarged and firm. Body temperature 40° C.

Five days. The central crust is depressed, and is surrounded by a definitely elevated vesicular ring, which is bordered externally by a red areola, fading outwards into normal skin. There is much edema of the subcutaneous tissue beneath the lesions, producing a broad, indurated base on which the lesions are individually prominent. The lesions average eight millimeters in width. Axillary lymph nodes distinctly enlarged and hard. Body temperature 41.5° C.

Six days. Both the crust and the vesicle have increased in extent. The contour of the lesion shows an abrupt elevation in the zone of the areola. Average width ten millimeters. A few small, red papules are noted on the shaved area of the abdomen near the belt line. Axillary lymph nodes enlarged and hard. Body temperature 40.8° C.

Seven days. The lesion presents as a flat-topped elevation with a central brown crust, and an opaque vesicular ring. Average width eight millimeters. The lesions are less prominent to-day owing to the subsidence of the subcutaneous edema. Two of the papules noted near the belt line have increased somewhat in size. Axillary lymph nodes as before. Body temperature 40°C .

Eight days. Primary lesions are beginning to undergo involution, the crust is spreading at the expense of the vesicular ring. Subcutaneous edema has almost disappeared. On the face there are a dozen or more papules and vesicles from two to four millimeters in diameter. Similar lesions are present on the abdomen, the scrotum, the inner aspect of the thighs, and on the palms. Axillary lymph nodes as before. Body temperature 40°C .

Nine days. The vesicular element in the primary lesions has been obliterated by the spreading of the central crust. The lesions of the exanthem present as filled out vesicles, some of which have opaque white or yellow contents, and all are surrounded by a bright red areola. An abundant eruption is noted on the tail and the skin about its base. Axillary lymph nodes slightly enlarged and hard. Body temperature 39.5°C .

Ten days. Many of the primary lesions have been scratched and present as shallow ulcerations over which the epithelium is spreading from the edge. The lesions no longer present specific characters. The lesions of the exanthem are dry and crusted. The eruption is noted to-day on the soles and on the dorsal aspect of several of the fingers and toes. Axillary lymph nodes of almost normal size but still firmer than normally. Body temperature 38.5°C .

From this time on the specific lesions healed without complications. Material was collected at intervals for the histological study of the primary lesions.

No. 115. — Half grown male, *Macacus cynomologus*. Inoculated in twelve places on the abdomen with virus No. 167 (vesicle contents). Body temperature 38°C .

Eighteen hours after the inoculation a narrow brown crust surrounded by a sharply circumscribed, elevated, opaque white area, five millimeters across, marks the site of the inoculation. Body temperature 39.4°C .

Forty-eight hours. The skin about the crust is elevated and pink for a distance of two millimeters. Body temperature 37.8°C .

Three days. The elevated area has increased in extent and is distinctly red in color. Axillary lymph nodes slightly enlarged. Body temperature 38.8°C .

Four days. Immediately about the crust there is a distinct translucence of the skin. Average width of lesions seven millimeters. Axillary lymph nodes distinctly enlarged. Body temperature 39.5°C .

Five days. The lesion presents as a rounded elevation surmounted by a narrow brown crust about which there is a distinct vesicular ring, translucent near the crust and shading insensibly into a pink zone which fades

out into the surrounding normal skin. Considerable subcutaneous edema. Average width of lesions eight millimeters. Axillary lymph nodes distinctly enlarged and hard. Body temperature 41.5° C.

Six days. The central crust is surrounded by a narrow opaque white vesicle on a pink elevation measuring from nine to ten millimeters across. Axillary lymph nodes as before. Body temperature 39.8° C.

Seven days. The lesions present as flat-topped, sharply circumscribed elevations from nine to ten millimeters in diameter. The central crust, somewhat depressed, is surrounded by an elevated vesicular ring which is opaque white in color. On the upper lip, the abdomen, the arm, and the scrotum are seen small, red, papular elevations. Axillary lymph nodes as before. Body temperature 39.8° C.

Eight days. Yellow turbid fluid oozes from beneath the central crust. The vesicular ring has been obliterated in places by the spreading of the crust. The lesions of the exanthem are somewhat larger and vesicular. Three new lesions have appeared on the face and one in the groin. Lymph nodes as before. Body temperature 39.5° C.

Nine days. The vesicular zone of the primary lesion has been entirely obliterated. The subcutaneous edema has almost disappeared. To-day an exanthem is noted at the edge of the nostril and in the vestibule. The tail and the skin at its base present numerous small papules and vesicles, each surrounded by a distinct areola. Axillary lymph nodes enlarged and hard. Body temperature 39.5° C.

Ten days. Primary lesions are healing and present no specific characters. The lesions of the exanthem have dried and are healing. One eruptive lesion found on the sole of the foot and several on the dorsal aspect of the toes. Axillary lymph nodes of almost normal size but still firm. Body temperature 39.5° C.

No. 116. — Young adult male, *Macacus cynomolgus*. Inoculated in twelve places on the skin of the abdomen with virus No. 167 (vesicle contents). Body temperature 38.5° C.

Eighteen hours after the inoculation the skin about the scratch is slightly elevated and opaque for a distance of two millimeters. Body temperature 38.6° C.

Forty-eight hours. The site of inoculation is marked by a narrow yellow crust surrounded by a pink elevation three millimeters in width. Body temperature 37.8° C.

Three days. The primary lesion as before, save that the elevation is more marked and the color deeper. Axillary lymph nodes slightly enlarged. Body temperature 40° C.

Four days. The brown central crust is seated upon a pink elevation six to ten millimeters across, which fades out into the normal skin. Immediately about the crust there is a narrow zone of translucence suggesting vesicle formation. A marked subcutaneous edema renders the lesions prominent. Axillary lymph nodes enlarged and hard. Body temperature 40° C.

Five days. Primary lesion as before, but it has increased in size. Axillary lymph nodes markedly enlarged and hard. Body temperature 41° C.

Six days. The crust is surrounded by a definite vesicular ring which, in turn, is surrounded by a dull pink areola. Average width of lesions nine millimeters. Axillary lymph nodes as before. Body temperature 39.5 C.

Seven days. The primary lesions present as circumscribed elevations with a central, depressed, brown crust, an elevated opaque white, vesicular ring and a bright red areola. Average width of lesions nine millimeters. On the face, the abdomen, the scrotum, the thighs, and the arms are numerous small papules or vesicles, each surrounded by a red areola. Axillary lymph nodes as before. Body temperature 39.8° C.

Eight days. Primary lesions no longer show a vesicle. Many of the papules have become vesicles and the vesicles pustules. There is some increase in size of the lesions of the exanthem. Axillary lymph nodes as before. Body temperature 40° C.

Nine days. Some subcutaneous edema persists beneath the primary lesions which, however, have lost their specific character. The exanthem is beginning to dry on the face. Many papules and vesicles, not noted before, are visible on the tail and on the skin at its base. Axillary lymph nodes enlarged and hard. Body temperature 39.5° C.

Ten days. The primary lesions are healing. Eruptive lesions found to-day on soles and palms. Axillary lymph nodes still enlarged and hard. Body temperature 39.6° C.

No. 117. — Adult male, *Macacus cynomologus*. Inoculated with same virus and in the same way as the preceding animals. Body temperature 37.8° C.

Eighteen hours after inoculation. A narrow yellow crust is seen upon an opaque, slightly elevated area. Body temperature 39.2° C.

Forty-eight hours. The elevation and opacity have extended somewhat. Body temperature 37.2° C.

Three days. The lesion presents as a linear crust on a slightly elevated pink area four millimeters across. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. About the crust is a narrow translucent zone which merges with the pink elevation which, in turn, fades off into the surrounding skin. Average width of lesions seven millimeters. Axillary lymph nodes distinctly enlarged and hard. Body temperature 39.5° C.

Five days. A distinct but narrow vesicle is evident around the crust. Considerable edema beneath the lesions. Areola well marked. Axillary lymph nodes as before. Body temperature 40.5° C.

Six days. Certain of the lesions show a typical development presenting a crust, a definite vesicle, and an areola, the whole lesion being sharply elevated from the surrounding skin. Other lesions show departure from the normal type in that the vesiculation is less marked and the lesions are

not as sharply circumscribed. Lymph nodes enlarged and hard. Body temperature 40.2° C.

Seven days. Some lesions typical, others show a spreading of the crust without macroscopic evidence of vesicle formation. Average width of lesions nine millimeters. On the face, abdomen, scrotum, inner aspect of thighs, axillæ, and arms are numerous pink papular elevations, two millimeters in diameter, some of which present translucent central points. Axillary lymph nodes as before. Body temperature 39.6° C.

Eight days. Certain of the primary lesions show remnants of the vesicle at the edge of the spreading central crust. The lesions of the exanthem have increased in size, and the contents of many of the vesicles has become cloudy. New eruptive lesions are present on the abdomen. Axillary lymph nodes as before. Body temperature 39.8° C.

Nine days. The primary lesions show a narrow zone of translucent epithelium close to the crust. This appearance suggests the beginning of the healing of the lesion by the ingrowth of new epithelium rather than vesicle formation. Lesions of the exanthem have dried up with or without rupturing. Axillary lymph nodes slightly enlarged but firmer than normal. Body temperature 39.5° C.

Ten days. The healing of the primary lesions is well under way and they have lost all specific character. To-day an exanthem is noted in the region about the ischial tuberosities and beneath the tail. Eleven eruptive lesions found in the palms and soles and many are also present on the fingers and toes. Axillary lymph nodes slightly enlarged and hard. Body temperature 39° C. Specific lesions healed without complications.

No. 118. — Adult male, *Macacus cynomologus*. Inoculated with the same virus and in the same manner as the previous animals. Body temperature 38.8° C.

Eighteen hours after inoculation. Some elevation and opacity is evident about the scratch. Body temperature 38.5° C.

Forty-eight hours. Elevation and opacity more marked. Body temperature 37.4° C.

Three days. Lesion presents as a narrow yellow crust on a pink elevation five millimeters across. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. A narrow zone of translucence is visible near the central crust. The lesion is roundly elevated, of a pink color, and merges without sharp line of demarcation with the surrounding skin. Average width of lesion seven millimeters. Considerable subcutaneous edema. Axillary lymph nodes enlarged. Body temperature 39.6° C.

Five days. A definite vesicular ring surrounds the crust and is surrounded in turn by a pink areola. The lesion is not sharply circumscribed. Axillary lymph nodes enlarged and hard. Body temperature 41.2° C.

Six days. The vesicular ring has become opaque and has increased in extent. There is some diversity in the size of the lesions. Axillary lymph nodes as before. Body temperature 40.6° C.

Seven days. The central crust is surrounded by an elevated ring in which evidence of vesiculation can be made out in places. The edge of the lesion is sharply circumscribed and rises abruptly from the surrounding skin. A definite pink areola is present. Average width of lesions nine millimeters. On the face, inner aspect of arms, thighs, and abdomen are red papular elevations two millimeters in diameter. Some of these present a minute translucent center. Axillary lymph nodes as before. Body temperature 40.2° C.

Eight days. Involution of primary lesions has begun. The vesicular ring has been entirely obliterated by the spreading of the central crust. The subcutaneous edema has disappeared. The lesions of the exanthem have increased in size and are definitely vesicular. Some new papules are present to-day upon the abdomen and face. Axillary lymph nodes of normal size but firmer than normal. Body temperature 40.2° C.

Nine days. Primary lesions beginning to heal. The exanthem is dry and no new lesions have appeared. Axillary lymph nodes as before. Body temperature 39.6° C.

Ten days. Primary lesions not notable. Eruptive lesions, not noted before, are present on the palms. Axillary lymph nodes as before. Body temperature 39° C.

No. 170. — Adult male, *Macacus cynomologus*. Inoculated in six places on the skin of the abdomen, with a suspension of pulverized disks (virus No. 167) in sterile salt solution.

Three days. An opaque elevation three millimeters across is present at the site of inoculation. Body temperature 39° C.

Five days. Lesion presents as a small crust on a pink elevation five millimeters across. A narrow zone of translucence borders the crust. Body temperature 39° C.

Six days. The central crust is depressed and about it there is irregular vesicle formation. The lesions vary much in size. Body temperature 40° C.

Seven days. Primary lesions have increased slightly in extent. Considerable subcutaneous edema is present. Body temperature 39.5° C.

Eight days. Some of the primary lesions show a distinct but irregularly developed vesicular zone. Near anus is a single vesicle two millimeters across surrounded by a red areola.

Nine days. The central crust has encroached upon and partly obliterated the vesicular zone. Single small vesicles are present on the arm, the thigh, beneath the tail, and on the ankle.

Ten days. Primary lesions healing. No new exanthem.

No. 171. — Inoculated with the same virus and in the same manner as the preceding monkey.

The evolution of the primary lesion was delayed and the vesiculation irregular as in the previous experiment. No general exanthem was observed. The temperature reaction was indefinite.

No. 164. — Half grown male, *Macacus cynomologus*. Inoculated in six places on the skin of the abdomen with a suspension of pustule contents (virus No. 167) dried with *Lycopodium* powder in sterile salt solution.

Twenty-four hours after inoculation there is slight elevation and opacity about the scratches.

Subsequent to this for a period of five days after the inoculation there was no evidence of a process.

Six days. Lesions present a narrow crust on a pink elevation. The appearance is the same as that seen on the third day after an inoculation with fresh vesicle contents.

Seven days. The lesion has increased somewhat in extent and is sharply circumscribed.

Eight days. An irregular vesicular zone is developed about the crust.

Ten days. Vesiculation no longer apparent. But slight induration can be made out in the lesion. No general exanthem was observed either before or after this date. The animal was subsequently inoculated with vaccinia virus No. 1, but failed to react.

No. 194. — Young male, *Macacus nemistrimus*. Inoculated in twelve places on the skin of the abdomen with virus No. 199. Body temperature 38.8° C.

Twenty-four hours after inoculation there is slight elevation and opacity about the scratch. Body temperature 37.8° C.

Forty-eight hours. Marked elevation and opacity around the linear crust. Body temperature 39.1° C.

Three days. There is marked subcutaneous edema beneath the lesion. The elevation about the crust is distinctly pink in color. Body temperature 38.9° C.

Four days. The lesion consists of a rounded pink elevation five millimeters across surmounted by a crust about which is a narrow zone of translucence, the whole lesion being underlaid by a broad indurated area of subcutaneous edema. Body temperature 39.3° C.

Five days. An irregular development of the vesicular ring is apparent in certain of the lesions. Body temperature 39.5° C.

Six days. Many of the primary lesions have been scratched by the animal and present as excoriated areas surrounded by a red elevation and seated upon a broad indurated base. Body temperature 39.6° C.

Seven days. Primary lesions as before. A profuse exanthem consisting in small red papules and vesicles is present upon the face, trunk, and extremities. Body temperature 38.7° C.

Eight days. The primary lesions have crusted, and in places there is slight vesicle formation. Several new papules and vesicles are present on the face and thighs. One small vesicle is visible on the hard palate. Body temperature 38° C.

Animal killed and autopsy done at once. Material saved for histological examination of specific lesions and of the viscera. Axillary lymph

nodes enlarged and red. On section much blood stained fluid exudes from the cut surface.

II. Histological examination.

The primary lesion eighteen hours after inoculation. Microscopic examination of sections from the site of the inoculation shows a solution of continuity which involves the epithelium and may or may not extend into the corium beneath. The defect is filled by an exudate composed of polynuclear leucocytes which lie in a meshwork of fibrin. On the surface the elements of this exudate are fused into a crust. On either side of the defect the epithelial cells of the rete are more or less swollen and some present clear circular areas in their protoplasm, suggesting hydropic degeneration. The nuclei of these cells are swollen and the chromatin tends to collect in masses. Polynuclear leucocytes are present in considerable numbers in and around the vessels of the corium and in the connective tissue beneath the defect. The leucocytes are streaming into the defect and into the adjacent epithelium.

Forty-eight hours. The sections present the same general characters as those from the earlier lesions. The defect in the epithelium is sometimes obliterated, in such wise that a layer of epithelium is interposed between the exudate collected beneath the crust and the injury to the corium. The polynuclear leucocyte infiltration is more intense. The epithelial cells of the lower layers of the epidermis about the line of inoculation show more marked degeneration, and the cells are frequently separated one from another by fluid.

Three days. The crust has increased in thickness and, with the destruction of the epithelial cells in the central line of the lesion, may fuse with the corium. The epithelial cells of the lower layers adjacent to the crust show various forms of degeneration. Some present ballooning degeneration, and the accumulation of fluid between the cells is more evident than in the earlier lesions. In places vesicle formation is foreshadowed by the appearance of small, irregular cavities formed either by the accumulation of fluid between the cells or by the bursting of hydropic cells. The corium is the site of a definite reaction shown by an enlargement of the endothelial cells of the blood and lymph vessels. These enlarged endothelial cells may contain Cytoryctes. There is a marked polynuclear leucocyte infiltration of the corium and the epithelium about the crust. In some cases a mass of leucocytes, with granular precipitate and fibrin, is found in the corium just beneath the center of the lesion. The cellular sheaths of the hair follicles show changes similar to those seen in the cells about the crust, and an abundant polymorphonuclear infiltration is present. Some lesions of this age show definite vesicle formation such as will be described later.

Four days. Lesions of this age may present vesicular cavities under the lateral expansions of the crust. In some lesions the whole crust is elevated and the lateral vesicles communicate with one another. The epithelium

at the sides of the lesion and below the lateral portions of the vesicle is more or less swollen and show various degenerations, the most marked forms of which are found in those cells nearest the center of the lesion. The epithelium is completely destroyed in the axis of the lesion. The corium beneath shows proliferation of the endothelial cells of the blood vessels and of the lymphatics, together with an enlargement of the connective tissue cells. A polymorphonuclear leucocyte infiltration is apparent in the corium and in the thickened epithelium about the vesicle. In some lesions the contents of the vesicle shows a predominance of polymorphonuclear leucocytes under the crust, while the outer portions are all but free from these cells, containing only granular precipitate and fibrin.

Five days. The topography of the lesion at this stage shows considerable variation due to differences in the extent of the vesicle formation. The typical picture is similar to that in the four-day lesion with some increase in the extent of the vesicle, and in a more marked reaction in the corium beneath the lesion. The polynuclear leucocyte infiltration of the corium is less intense, but the vesicle cavity shows a more even distribution of the pus cells. The reaction of the corium is more marked, being shown by the presence of edema, necrosis, and an increase in the cellular content of the tissue. Aside from the polynuclear leucocyte the dominant element is a large cell with a vesicular nucleus surrounded by an abundant reticular protoplasm. These cells show a great variation in form, some being rounded, others irregular, the outline seemingly being conditioned by the space in which the cell lies. Many of these cells are found just outside of the capillaries and in the lymph spaces of the tissue, and by comparison of their morphology and staining reaction with that of the swollen endothelial cells, in situ in the capillaries and in the lymph spaces, it seems certain that they are identical with them. Many of these cells are phagocytic and in some mitosis is seen. In certain of these cells early stages of the cytoplasmic phases of *Cytoryctes variolæ* are present. In addition to these endothelial cells, which have often wandered for some distance from their place of origin, a certain number of elements are found of the lymphoid and plasmic cell series. The connective tissue cells all through the corium about the lesion are swollen, and in some mitosis is in progress. The nerve bundles are frequently invaded by polymorphonuclear leucocytes. The cellular reaction of the corium is shared by the adjacent subcutaneous tissue, and extends for a considerable distance from the line of inoculation. Necrosis is seen in the tissue beneath the center of the lesion, the cells losing their basic affinity and undergoing more or less fragmentation or solution. Many deeply stained spherules of various sizes are scattered through the necrotic tissue, evidently the nuclear fragments of polynuclear leucocytes.

Six days. The general relations of the vesicle cavities and the crust remain as before, although the outer limits of the vesicle often extend beyond the limits of the crust, being roofed by a layer of cornified epithelium which sweeps downward to form a part of the lateral wall of the

cavity. In some instances the beginning of vesicle formation, shown by collection of fluid between the cells, is apparent a short distance beyond the outer limits of the main vesicle. A similar condition is met with in the depths of the cellular sheaths of the hair follicles. The fusion of the middle portion of the crust with the underlying corium is often apparent, although in many places a collection of leucocytes is present here which is continuous with the lateral vesicles and with the purulent focus which forms in the corium along the line of inoculation. In such a lesion a roughly T-shaped cavity can be made out, the verticle portion being filled with leucocytes and extending from the crust for a variable distance into the corium, while the cross arm has for its extremities the vesicular cavities which extend laterally under the crust. The verticle portion of this T can often be traced to the focus of necrosis which lies in the lower layers of the corium and the upper portion of the subcutaneous tissue. The reaction of the corium is very marked at this stage. Study of the outer limits of the necrotic area shows that the large cells, which we believe to be endothelial in origin, seem to be resistant to the agent which is causing the necrosis. The large cells are frequently to be seen in an apparently normal condition in an area where all other cellular elements have been destroyed. It is hard to determine whether this apparent immunity from destruction is due to qualities of the cell, or whether it is because the cells have migrated into the necrotic area. In one instance a small capillary was found in the corium near the necrotic area lined by swollen endothelial cells, many of which were infected with early stages of the cytoplasmic phases of *Cytoryctes variolæ*.

Seven days. The lesions of this age and those collected later show a progressive healing of the process. The vesicle soon disappears and the polynuclear leucocyte infiltration of the whole field becomes less intense and eosinophiles appear. The epithelium grows inward under the crust and is joined by that which comes from the proliferation of cells of the hair sheaths. The blood vessels of the corium send out prolongations and the usual phenomena of repair dominate the picture.

The exanthem. Sections of lesions collected on the first day of the appearance of the exanthem show a vesicular cavity of variable size in the epidermis. This cavity is roofed by a layer of cornified epithelium, and laterally and below is surrounded by thickened epithelium. The cells adjacent to the cavity show varying degrees of degeneration. In every case the layer of the rete which forms the floor of the vesicle is wanting at one or more points, so that the cavity in the epidermis is in communication with the corium. The blood vessels and the lymphatics of the corium beneath the lesion present marked changes. These are in part due to the migration of polynuclear leucocytes which is going on from the vessels into the corium, the vesicle, and the epithelium about it. Besides this purely exudative phenomenon the endothelial cells of the capillaries and of the lymphatics show marked swelling and some proliferation. The normal relationships of structure in the corium are much disturbed by this combination of exudation, proliferation, and swelling. A careful search of such

areas failed to show any Cytoryctes, although they were numerous in the cells of the rete which formed the floor of the vesicle.

Sections from the exanthem collected later in the evolution of the lesion show an increase in the size of the vesicular cavity, but no other characters than those found in the early lesions.

The study of sections from the primary lesions and of the exanthem in *M. nemestrinus* show variations in degree, but not in kind from the picture seen in the corresponding lesions in the Philippine monkey. The reaction in the subcutaneous tissue and in the corium is more marked, and edema plays a more important rôle. The vesicle is not so well developed, but is similar in all fundamental characteristics to that in *M. cynomologus*.

Axillary lymph nodes. The sinuses are dilated, sometimes to a high degree. The cell content of the sinuses shows various deviations from the normal. The most prominent character is an increase in the number of endothelial cells. These cells increase greatly in size, become free in the sinuses, and show marked phagocytic properties. In the nodes from monkeys killed on the sixth and eighth day of the disease, the included cells are in part red blood corpuscles and in part polynuclear leucocytes. Later the polynuclear leucocytes are the common inclusion, though lymphoid and other cells may occasionally be found within the phagocytes. Besides the endothelial phagocytic cells, red blood corpuscles and polymorphonuclear leucocytes are found in considerable numbers free in the sinuses. The latter cells predominate in the nodes from animals killed on the ninth day of the disease and later. Eosinophile cells are frequently encountered, but do not as a rule occur in such numbers as do the polymorphonuclear leucocytes.

The follicles show many phagocytic endothelial cells, singly or in small groups, scattered through their substance. In nodes from animals killed on the eighth day of the disease, small areas of hemorrhage were frequently found. In these areas red blood corpuscles were present in the follicular tissue about a small capillary, many of them having been taken up by phagocytes. In nodes collected later in the disease, large phagocytic endothelial cells were demonstrable whose whole cell body was crowded with red blood corpuscles in various stages of dissolution. In one node, collected on the eighth day of the disease, masses of eosinophile leucocytes were present in the follicles and in the sinuses. These cells differed from the usual eosinophile cells in that the granules were elongated. Eosinophile cells having round or slightly oval granules were also present. The cells with the long granules differed from the usual eosinophile cells in having a more distinct cell membrane than the regular type of eosinophile. In this respect they conformed more closely to the type of the polymorphonuclear leucocyte.

Bone marrow and testicle. As these organs present the only specific visceral lesions of smallpox in man, they were scrutinized with care in our variolated monkeys. No macroscopic evidence of focal lesions in these organs were seen at autopsy, and the bone marrow from four cases and

the testicle from twelve were negative in this respect when studied microscopically.

Liver, spleen, kidney, and lung. No pathological process was demonstrable either macroscopically or microscopically in these organs.

SUMMARY.

1. The macroscopic appearance of the primary lesion in *Macacus cynomologus*. — Twenty-four hours after inoculation there is some elevation and some opacity of the skin about the scratch. The process has rarely a lateral extent of more than two millimeters.

After forty-eight hours the appearance is usually the same.

After three days the elevation is more marked and the opacity gives place to a pink or red appearance. So far as can be discovered by the naked eye the specific process may now be said to have begun. Previous to this the reaction has been in no way different from that which follows a simple scratch, without the introduction of virus.

After four days the lesion usually attains a breadth of six or seven millimeters and presents as a distinctly pink or red elevation which is firm to the touch, and bears on its summit a narrow crust which has its origin in the drying of the serum exuding at the time of inoculation. The crust has, however, somewhat increased in extent. At this time the skin about the crust is more or less translucent. It is often impossible to say whether or not this translucence is, at this time, the site of a definite vesicle.

After five days the lesions show the characteristics of a pock, being resolvable into rather distinct zones which correspond with those that are distinguishable in the microscopic sections. Going from the center of the lesion towards the periphery we recognize in turn first a crust, second a vesicular ring, third a zone of elevation and hyperemia. The lesion now has a lateral extent of from six to eight millimeters. The whole lesion is more or less elevated upon a broad indurated base due to edema of the skin and subcutaneous tissues.

After six days the picture is the same, save that each of

the zones has extended peripherally. At about this time the profile of the lesion undergoes a change in that it loses its flowing outline, as a more or less hemispherical elevation, and takes on a flat-topped plateau-like appearance. In going from the center outwards we pass along the fairly flat crust, then over the vesicular ring, which attains a greater elevation than the crust, forming a rampart, and then come to an abrupt declivity where the zone of hyperemia or areola extends into normal skin.

After seven days the crust and the vesicular ring will be seen to have extended somewhat, but the former has encroached more or less upon the area occupied by the latter. The subcutaneous edema is less marked.

After eight days the lateral excursion of the lesion has definitely ceased and involution begins. This process of involution is evidenced by the spreading of the crust so that it comes to occupy all the territory held by the vesicular ring. At the same time the zone of hyperemia fades, the subcutaneous edema disappears, and the lesions come to consist of a crust of variable thickness beneath which the normal epithelium is slowly spreading. If a few days later the crust be forcibly removed either a small pocket of pus or a tough mass adherent to the crust will be found beneath, bordered by pink, new-formed epithelium which is growing in from the periphery. In some lesions the microscopic characters of the process are reflected in the naked eye appearances to a greater extent than in others. Thus in the vesicular ring two zones can, at times, be made out, an inner opaque zone and an outer translucent one. This agrees with the microscopic findings in some lesions where the leucocytes are seen beneath and to one side of the crust, while there is only clear serum in the peripheral part of the vesicle.

Departures from the type lesion were seen in certain of our animals. In some cases the vesicle ring does not form completely around the crust, so that at the height of the process the crust may be bordered in part by elevated and hyperemic skin. In other lesions a vesicle may not at any time become recognizable to the naked eye. A variation

which is not uncommon is for the lesion to undergo typical evolution but for the different phases to be more or less delayed or accelerated. For example, a vesicular ring may be made out after three days or it may not be evident until after six days. In some lesions the process appears to start from a number of separate points instead of spreading symmetrically from the scratch.

The typical process is modified considerably by the character of the skin at the site of inoculation. Thus on the thick and hairy skin of the back the several zones of the lesion, which are clear enough in an inoculation on the abdomen, are not distinguishable. The same phenomenon is seen when the skin of the tail is inoculated.

2. The occurrence and macroscopic appearance of the exanthem. — In sixty-five monkeys inoculated on the skin of the abdomen with variola virus, in one form or another, a general exanthem was noted in fifty (seventy-seven per cent). In the animals in which vesicle contents was used for inoculation the exanthem occurred in thirty-two out of forty (eighty per cent). The exanthem was first noted on the seventh day of the disease in seven animals, on the eighth day in twenty-six, on the ninth day in fourteen, and on the tenth day in three. When vesicle contents was used for the inoculation, the exanthem was first noted on the seventh day of the disease five times, on the eighth day eighteen times, on the ninth day eight times, and on the tenth day once.

The extent of the exanthem varied greatly. In some animals only one typical lesion was present, while in others over a hundred were found.

The distribution of the exanthem showed a partiality for certain regions. The face was most often the site of an eruption. Elsewhere, roughly in the order of frequency, it was present upon the wrists; the scrotum of the male; the region about the anus and base of the tail; on the palms of the hands and the soles of the feet; on the inner aspect of the arms and thighs. The eruption seemed to avoid the trunk and the outer hairy surfaces of the limbs.

The evolution of the exanthem was rather constant. The

first appearance of the eruption was as minute pink papules, rarely exceeding one millimeter in diameter. On the next day this papule was larger, often measuring two millimeters in diameter and showing a vesicular structure. In the majority of cases the fluid contents of the lesion became cloudy on the next day, and the lesion was completely dried in another twenty-four hours. The exanthem, therefore, has its complete evolution in about four days. In some animals the lesions pass through a longer period of development. In such lesions the papular, vesicular, and pustular stages should be recognized with as much certainty as in the primary lesions, or as in the eruptive lesions of variola vera of man. The phenomena was noted of the lesions appearing first on the face and later on other parts of the body, and of their drying up in the order of their appearance.

3. The constitutional reaction, aside from the temperature, which might be taken as an indicator of the general reaction of the inoculated animal, showed little of a definite nature. At a time when the primary lesion is in its active stage, about the sixth to the eighth day of the disease, the animal sometimes shows some degree of anorexia and a tendency to droop, but at no time does it present such a constitutional reaction as is seen in the case of variola vera, of even moderate severity, in man.

The temperature reaction in variola inoculata in the monkey, unlike its companion disease vaccinia, presents a very definite curve. A comparison of the temperature charts of twenty animals, inoculated on the skin of the abdomen with vesicle contents, shows a marked rise in the body temperature on the sixth day of the disease in fourteen, on the seventh day of the disease in two, and was indefinite in four. In only three of the animals was there no distinct elevation. This onset temperature may reach 41° C. In most cases the fever declines by lysis. The temperature reaction precedes the appearance of the exanthem by twenty-four to forty-eight hours.

4. The lymph nodes, which are interposed between the area of skin on which the primary lesion develops and the

main lymph trunks, show a definite reaction. On the fourth or fifth day of the disease they are increased in size. This tumefaction increases and may result in an enlargement of the individual nodes in the axilla, when the inoculation is on the abdomen, to a diameter of one centimeter or more. At the time of the greatest swelling the nodes are markedly tender. After the ninth or tenth day the nodes become smaller, but remain for a considerable time firmer than normally.

5. The viscera of the animals killed during the disease showed no macroscopic lesions. Particular attention was paid to the bone marrow and testes, as these organs are the site of the only specific visceral lesion in variola vera in man.

Inoculations of the skin with variola disks. — The inoculations in this series of animals resulted in a primary lesion which conformed to the type described above, save that the whole process was retarded in its evolution. The occurrence of the exanthem is, however, in sharp contrast to that on the animals inoculated with the contents of the variola vesicle. The exanthem occurred in only three of the five animals, appearing in one on the eighth day, in the other two on the ninth day of the disease. In these three animals a total of only seven eruptive lesions were found. The exanthem passed through its evolution rapidly, and the individual lesions were small. The temperature reaction was like that in the preceding experiments in three of the animals, while in two it was indefinite. In other respects the results were similar to those in which the contents of the variola vesicle was used for inoculation.

Inoculation of the skin with dried pustule contents. — In both animals of this series the primary lesion was typical in its development, but somewhat delayed in its evolution. No exanthem was observed. The temperature reaction was the same as in the animals inoculated with the contents of the variola vesicle.

Inoculation of *Macacus nemestrinus*.— In this monkey there is considerable variation from the type of the disease seen in *Macacus cynomologus*. This difference is shown principally in the evolution of the primary lesion. In this species the vesiculation of the lesion is much less definite, and there is an exaggeration of the edema beneath the lesion. The exanthem differed in no way from that observed in the Philippine monkey. The temperature reaction was less definite. In other respects the results of the inoculations were similar.

Histological examination of the primary lesions.— The evolution of the lesion at the site of inoculation is characterized by a combination of degenerative, exudative, and reparative processes. Some one of these phenomena dominates the picture at different stages of the lesion, and in these phenomena various cell types figure.

The earlier lesions, collected during the first, second, and third days of the disease, present primarily the process of repair of a simple wound of the skin, but to this is added a change in the epithelial cells which border the incision. In these cells some degree of swelling and of degeneration is evident, although the usual reparative power of the rete is retained in sufficient degree to bridge the defect caused by the inoculation during the third day of the experiment. The cytoplasmic phases of *Cytoryctes variolæ* are found in the cells of the rete during these early stages of the lesion.

From the third day of the disease onward the picture becomes more complicated. The degeneration of the epithelial cells is quite extensive and is evident for some distance from the line of inoculation. This degeneration is preceded by more or less swelling of the individual cells and some, though not wide spread, proliferation. The thickening of the epidermis about the inoculation is due in the main to the former process. Concomitant with the appearance of these phenomena we find fluid collected between the epithelial cells which finally leads to the formation of definite cavities, the vesicular space being partly contributed to by

the solution of the swollen and degenerated epithelial cells. The polymorphonuclear leucocytes pass into the fluid of this vesicle, and their increasing numbers finally give to the lesion the macroscopic character which we designate as a pustule. While this vesiculation has been in progress the crust, which originated in the inspissated exudate in the inoculation scratch, increases in extent and in thickness. The degeneration of the epithelial cells about the lesion is most marked where they form the floor of the vesicle, and becomes less and less as we pass to the peripheral portions of the lesion. Similar cell changes are found in the sheaths of the hair follicles close to the lesion.

While the vesicle has been forming in the epidermis a reaction in the corium has become manifest. The first change, besides the collection of polymorphonuclear leucocytes in the vessels and their migration towards the vesicle, is enlargement and proliferation of the endothelial cells of the lymphatics and blood vessels. The connective tissue cells in the corium undergo similar changes. Later in the disease a definite edema of the corium and of the adjacent subcutaneous tissue is present, and an area of necrosis can be made out beneath the center of the lesion. Associated with this edema and necrosis there is a large increase in the number of endothelial cells. These cells appear not only in and around the lymph vessels and capillaries but are found in the surrounding tissue. These cells contain cytoplasmic phases of *Cytoryctes variolæ* both when in situ on the walls of capillaries and when free in the tissue. Mitoses in these cells are frequent, and some cells are met with containing two nuclei. The phagocytic properties of these cells are shown by their including leucocytes and other cells. Lymphoid and plasma cells, together with a certain number of eosinophile leucocytes, are found in the corium at this time.

During the later stages of the lesion the process of repair dominates the picture. The epithelium grows in from the sides and up from the hair sheaths, and finally closes the defect caused by the variolous process. In the corium the solution of the necrotic tissue and the new formation

of blood vessels and connective tissue shows repair to be active.

The exanthem. — The process seen in the epithelium, in the development of the exanthem, agrees with that found in the primary lesion. In the corium, however, the process of edema and necrosis is lacking. There is, however, some degree of cell reaction evidenced by the enlargement and proliferation of endothelial cells. The emigration of polymorphonuclear leucocytes is a prominent feature of the process.

The lymph nodes associated with the primary lesion show a marked reaction, consisting in proliferation of endothelial cells in the sinuses and in the follicles and in the active phagocytic properties of these cells. The presence of red blood corpuscles and of polymorphonuclear leucocytes in the sinuses, and the small areas of hemorrhage in the follicles appear also to be a part of the process.

The internal organs show nothing that can be interpreted as manifestations of the disease produced in the animal by the inoculation.

DISCUSSION. — We have seen that the inoculation of the skin of the monkey with variola virus brings about a disease which exhibits characters relating it at once to variola in man and to vaccinia in man and in animals. The disease which follows an inoculation of the skin of the abdomen of *Macacus cynomologus* with fresh variola virus consists essentially in:

- (1.) The development of a lesion at the site of inoculation.
- (2.) The appearance of a general cutaneous eruption of vesicular lesions.
- (3.) The enlargement of lymph nodes in the axillæ and groin.
- (4.) The constitutional reaction.

When we examine the primary lesion microscopically we find it to be a self-limiting process which passes through certain definite phases which are reflected in the gross appearances and are described as vesiculation, pustulation, and crusting.

When we turn to the lesions of the exanthem we find that the characteristic phases of the primary lesion are produced in them.

As we have said, the disease presents a series of characteristic phenomena, and when a number of animals are simultaneously inoculated we see that these phenomena bear a definite time relation to one another. If we emphasize this time element we find that the phenomena of the disease occur as follows:

The evolution of the primary lesion covers a period of about fourteen days from the time of inoculation. The first portion of this period comprises the active evolution of the local process. This period of active growth terminates on about the seventh day of the disease. It is difficult to say exactly when the lesions stop developing, but, after combining various observations, this date is selected as the probable average time for the acme of the active evolution of the primary lesion. During the remainder of the period the phenomena of repair is dominant in the lesion.

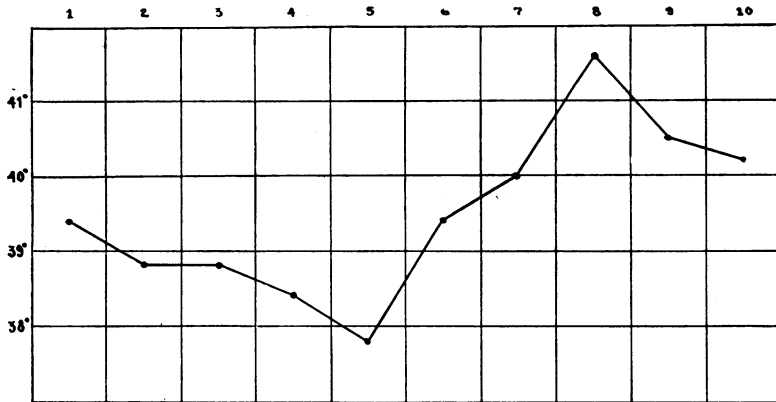
The general exanthem appears in the majority of cases on the eighth day of the disease and has a duration of about five days, the first two of which are employed in growth and the last two in healing.

The lymph nodes show an enlargement on the fourth day of the disease and are always markedly enlarged on the fifth day. They decrease in size during the crusting of the primary lesion, but they are firmer than normal for a considerable time after the healing of the process.

The constitutional reaction occurs at about the height of the active phase of the primary lesion, that is, on the sixth and seventh day of the disease. The temperature reaction begins, in a great majority of cases, on the sixth day of the disease, and persists for two or three days. It is difficult to say just how long the fever lasts, as the decline is by lysis, but it is almost always within normal limits by the twelfth day.

If we compare the disease produced in the monkey by cutaneous inoculation with variola virus with the disease

which follows inoculation of that animal with vaccine virus, we see at once that the processes are closely related. They are similar in that a self-limiting lesion appears at the site of inoculation, that the development of this lesion is associated with more or less constitutional reaction, and that certain lymph nodes become enlarged. The disease produced by inoculation with variola virus differs from vaccinia in that the primary lesion is usually followed by a general cutaneous eruption of lesions similar in many respects to the primary lesion, and in that the temperature reaction is more abrupt in its onset and more intense.



No. 118. VARIOLA INOCULATA IN THE MONKEY.

If we compare the disease produced in the monkey by cutaneous inoculation with variola virus with the various manifestations of variola in man, we see that the disease produced is more like variola inoculata than any of the other forms. In fact the only differences that we find between the two lie in the time of occurrence of the general exanthem and in the duration of the temperature reaction. Thus in the monkey we have the exanthem appearing on the eighth day of the disease and the temperature, which appeared on the sixth day, quickly falling by lysis, while in variola inoculata in man the exanthem appears on the eleventh day, and the temperature persists from the seventh to the ninth day.

The development of the primary lesion and of the exanthem is practically the same in both.

When we compare variola inoculata in the monkey with variola vera in man we find that only certain characteristics of the disease type are held in common.

Different forms of virus, as vesicle contents, pustule contents, and disk, were used for inoculations. On the skin all these forms of virus produced typical primary lesions. It is to be noted, however, that the nature of the contagium seemed to have an influence upon the occurrence and extent of the exanthem. We feel that these differences are explainable upon physical grounds and have to do with the reaction of the virus to external conditions rather than to any difference in the virus inherent to different ages of the lesion from which it is collected. This question will be taken up in another article in this series.

In the course of our experiments monkeys of all ages and of both sexes were employed. We did not observe any difference in the reactions of these animals which could be attributed to these factors. The general condition of the animal did not seem to affect the results of the inoculations.

The histological study of the specific lesions and of the viscera in variola inoculata in the monkey adds some details to our picture of the disease. We see that the specific lesions are similar in most respects to the vaccine lesions of man and of animals, and to the lesions of the exanthem of variola vera in man. As has been pointed out,* the specific lesions of variola inoculata in the monkey differ from the lesions of the exanthem in variola vera in man in the greater prominence of the polynuclear leucocytes in the former. The primary lesions of variola inoculata in the monkey differ from the vaccine lesion of that animal in the extent and character of the process in the corium beneath the lesion, it being more intense in variola inoculata.

It is evident that the lymph spaces of the skin are flooded with virus at the time of the inoculation, and this fact may be a factor in the early development of the exanthem in the

* Magrath and Brinckerhoff, Jour. Med. Research, xi, 230.

inoculated disease. From the fact that Cytoryctes are demonstrable in the endothelial cells of the capillaries in the corium beneath a primary lesion of five days duration, it seems probable that at the time of inoculation cells of this type become infected. Such infected cells in the lymph spaces or in the capillaries might easily be swept away in the circulation, and lodging in skin capillaries become the focus for an exanthem.

The absence of focal lesions in the bone marrow and testicles in variola inoculata in the monkey emphasizes the difference already mentioned between the disease experimentally produced in the monkey and variola vera in man.

CONCLUSIONS.

1. Inoculation of the skin of the monkey (*M. cynomolgus* and *M. nemestrinus*) with variola virus produces a disease in which all the essential characteristics are identical with those of variola inoculata in man.
2. Variola inoculata in the monkey differs from variola inoculata in man in that the fever has a shorter duration and the exanthem appears at an earlier date.
3. Variola inoculata in the monkey is as distinct a clinical entity as is variola inoculata in man.
4. Cytoryctes variolæ are found in the endothelial cells of the capillaries in the corium beneath the primary lesion of variola inoculata.

2. VARIOLA INOCULATA IN THE ORANG UTAN.

INTRODUCTION.—The experiments here reported were performed to determine the reaction of the orang utan to inoculation with variola virus, and to obtain material for the microscopic study of the specific lesions, and of the morphology of the causative organism in this species of animal. Four orang utans were procured for this work, but unfortunately only two of them survived long enough for the experiments to be carried out. These experiments are the first in which anthropoid apes have been used as the experimental animal in a study of smallpox. The systematic position of the orang utan in the animal kingdom makes it of peculiar value in the comparative study of a disease such as smallpox. The orang utan is susceptible both to diseases peculiar to animals (hemorrhagic septicemia) and to diseases common to man and to animals (amebic dysentery). This makes the animal an ideal one for bridging the gap between the monkey and man in the study of the reactions of various mammalian hosts to a given disease-producing parasite. We regret that owing to the difficulty of acclimating these animals our data is not abundant as might be desired.

TECHNIC.—Two young female orang utans were inoculated on the skin of the abdomen with variola virus in the same way as were the monkeys already described. The animals were observed daily, and the evolution of the lesion at the site of inoculation and the constitutional reaction was recorded. Material for the histological examination of the specific lesions was collected. The details of the experiments are as follows:

No. 197.—Young female orang utan. The animal was first exposed to smallpox fomites. The results of this experiment will be considered in another article.

Twenty-one days after the exposure the animal was inoculated in twelve places on the abdomen with virus No. 252. Forty-eight hours after inoculation there was slight elevation about the scratches. The deep

pigmentation of the skin made it impossible to tell whether or not hyperemia was present.

Five days. Along each line of inoculation there was a narrow yellow crust seated upon a vague elevation. On gentle pressure turbid fluid exuded from beneath the crust. A small amount of this fluid was used to inoculate a Philippine monkey on the skin of the abdomen. This animal developed a typical pock at the site of inoculation and subsequently a profuse general exanthem.

Six days. The primary lesions were somewhat more prominent and the whole of the area beneath them was indurated. Axillary lymph nodes enlarged, firm, and tender.

Seven days. The animal had scratched the lesions and they presented as shallow ulcerations with ragged and often bleeding edges. The subcutaneous edema had increased in extent, causing a brawny induration over the whole field of inoculation. Animal died during the night of an intercurrent infection.

No. 198. — Young female orang utan. Inoculated in twelve places on the skin of the abdomen with virus No. 199. After twenty-four hours slight elevation was apparent along the lines of inoculation. Body temperature 36.1° C.

Four days. A narrow crust marked the scratch and was bordered by a distinct elevation. No change of color could be made out on account of the deep pigmentation of the skin. Body temperature 35.9° C.

Five days. The elevation of the lesions had increased and they had become circumscribed. Body temperature 36.6° C.

Six days. The zone of elevation was distinctly vesicular near the crust and clear serum oozed out on general pressure. Body temperature 36° C.

Seven days. Lesions had increased somewhat in extent and the crust had spread. Body temperature 35.7° C.

Eight days. Animal found dead in cage early in the morning. Rigor mortis present, body still warm. Autopsy at once. Skin: the inoculation sites present crusts about which is a shallow cavity filled with turbid fluid. This vesicle appears to lie between the true skin and the epidermis. The border of the lesion shows some thickening of the skin, but this is not nearly so marked as at a corresponding period in the primary lesion of variola inoculata in the monkey. No evidence of a general exanthem is present. Axillary lymph nodes enlarged, measuring one to one and one-half centimeters in diameter, in color deep red, and on section rather dry. Peritoneal cavity contains one liter of clear straw-colored fluid. Surfaces of normal color and texture. Pleural and pericardial cavities normal. Heart: valves and cavities normal; myocardium red brown in color and of firm consistency. Lungs normal. Spleen: capsule smooth, purple, on section pulp rather dry, color deep red brown. Malpighian bodies and trabeculae not prominent. Liver: general surface smooth and yellow-brown in color. On section, markings distinct and consistency normal.

Pancreas normal. Gastro-enteric tract: stomach normal. The mucosa of the cecum and of the colon presents numerous punctate hemorrhages. Kidneys: capsule strips readily from a smooth yellow-brown surface. On section general color opaque yellowish-brown with irregular areas of injection in the cortex. Glomeruli visible as bright red points. Genital organs and bladder normal. Bone marrow of femur deep red, homogeneous, and of firm consistency. Brain and meninges normal. Smears from the heart's blood show immense numbers of small, short bacilli which take a polar stain with Loeffler's methylene blue.

Bacteriological examination. — Cultures from the heart's blood, the liver, and the spleen yield an organism which was identified by Dr. W. B. Wherry, bacteriologist of the Government Biological Laboratory, as belonging to the group of organisms causing hemorrhagic septicemia in animals.

Histological examination.

Primary lesion. — Six days. The point of inoculation is marked by a complete destruction of the epithelium. The material present here consists of a lamella of cornified epithelium which fuses with a more or less homogeneous crust in which cell elements can occasionally be recognized. The upper layers of the corium at this place are more or less extensively necrosed, the connective tissue fibers are swollen and fused with one another and with the crust. Cell detritus is scattered through this tissue. A fibrin network lies in the lacunæ of the corium in this region. On either side of the line of inoculation, immediately adjoining the crust, the cells of the rete are recognizable, but are very much degenerated. Between these degenerated cells and the cornified layer are finely granular areas of the size of epithelial cells, each outlined by a membrane, but not containing recognizable cell structure. Polymorphonuclear leucocytes are present in this tissue, their nuclei often fragmented or show other signs of degeneration. As we pass outward from the line of inoculation the degenerated rete splits horizontally, one layer more or less imperfect, following along the surface of the corium, while the other, still merging superficially with the degenerated strata above, curves up to form an almost complete band across the vesicle. Between this superficial layer of the rete and the corium is a lenticular cavity filled with a fine granular material and containing a fibrin network. At the outer limits of the vesicle the cornified layer sweeps downward to merge with the thickened epidermis which marks the outer limits of the lesion. The layer of rete which forms the floor of the vesicle likewise runs into this thickened region. Beyond the main vesicle small cavities are to be seen in the middle layers of the epidermis. Some of these are evidently formed by hydropic degeneration of the cells, the cell membranes persisting as partitions across the cavity. In others these partitions have disappeared, and the process seems in part due to an accumulation of fluid within and between these cell cavities. On the other side of the line of inoculation, vesicle formation is evident for a considerable distance. Here, however, the rete remains intact, and

the collection of fluid is between and within the cells of the stratum granulosum and stratum spinosum. In places this vesicle is traversed by more or less vertical partitions composed of compressed epithelial cells.

The reaction of the corium is evidenced by some infiltration with polymorphonuclear leucocytes and a certain degree of swelling of the endothelial cells lining the blood vessels and lymphatics of the corium adjacent to the site of the inoculation. Immediately beneath the lesion endothelial cells of lymphatics and of capillaries were found which contained in their protoplasm cytoplasmic phases of *Cytoryctes variolæ*.

The epithelial cells of the lesions contain many stages of the parasite. Both the cytoplasmic and the nuclear phases were well represented.

Seven and eight days. Lesions of these durations showed the same characteristics as did that described above. The vesicle becomes more extensive and the necrotic area beneath the crust larger. The coalescence of the lateral vesicles often left single cells or islands of cells in a fair state of preservation, which were to be found in various stages of agglomeration on the way to the formation of trabeculæ, or partitions in the large vesicle.

Viscera. — Histological study of the internal organs did not reveal any lesions of a variolous nature. All through the organs, wherever blood vessels were cut, short bacilli, having the morphology and staining peculiarities of the bacillus of hemorrhagic septicemia, were readily demonstrable.

SUMMARY.

The evolution of the primary lesion at the site of inoculation with variola virus in the orang utan is similar to that which follows the inoculation in the monkey and in man. The thickness and the deep pigmentation of the skin of this animal rendered the naked eye appearance less characteristic than in *Macacus cynomologus*. The death of the animals, of intercurrent disease, before a general exanthem might be expected to develop deprived us of data upon this point. Both animals showed a marked constitutional reaction, but this cannot be interpreted as resulting from the smallpox process on account of the existence of an intercurrent disease.

The histological study of the primary lesions and of the viscera of the orang utan inoculated with variola virus shows the process to be essentially similar to that which follows inoculation of the monkey with the same virus. There seems to be some difference in the degree of reaction in the

corium beneath the primary lesion, it being notably less in the orang utan. The histogenesis of the cutaneous vesicle is similar and we note the absence, as in the monkey, of focal lesions in the bone marrow. The most striking thing about the primary lesions seems to be their richness in nuclear forms of Cytoryctes. This matter will be taken up in detail in another paper, and so will not here be further commented upon.

CONCLUSIONS.

1. The orang utan is susceptible to variola inoculata.
2. The evolution of the specific lesion at the site of inoculation is comparable with that which follows similar inoculations in the monkey (*M. cynomologus* and *M. nemestrinus*).
3. The primary lesions of variola inoculata in the orang utan stand closer to the cutaneous lesions of variola vera in man than do the primary cutaneous lesions of variola inoculata in the monkey in respect to richness in forms of Cytoryctes variolæ, and particularly in the number of nuclear forms present.

3. VARIOLOUS KERATITIS IN MACACUS CYNOMOLOGUS.

INTRODUCTION. — In this section will be considered the results of a series of inoculations of the cornea of the Philippine monkey with variola virus. These experiments were undertaken to determine what variations in the type of disease might result from a change in the locus of inoculation. They also yielded material for the histological examination of the specific lesion in tissue which is ideal for the study of cell changes, owing to perfect preservation and the relative simplicity of the tissue elements.

TECHNIC. — The methods of inoculation and of observation were the same as those described in the section upon vaccinal keratitis in the monkey.

DETAILS OF EXPERIMENTS. — Eighteen animals were employed in this series, of which the following experiments are selected to be given in detail :

No. 223. — Monkey inoculated on both corneas with virus No. 200. After forty-eight hours slight unevenness was apparent along the line of inoculation. After ninety-six hours a minute defect in the corneal epithelium was present in the inoculated area. Chloroformed after eight days. The cornea presents a small defect in the epithelium with very slight unevenness about it. No photophobia was observed. There was no general exanthem.

Histological examination showed proliferation of the epithelium about the inoculation wound and the presence of Cytoryctes.

No. 224. — Monkey was inoculated on the cornea with virus No. 200. After forty-eight hours the cornea was slightly uneven along the line of inoculation. Photophobia and haziness of the cornea was observed after ninety-six hours. Conjunctivitis was not present. On the sixth day of the disease a general exanthem consisting of five small vesicles was observed on the face and extremities. On the next day five new vesicles appeared. The animal was chloroformed on the seventh day. At autopsy no evidence of an initial lesion, other than that on the cornea, was demonstrable.

Histological examination. — At the site of inoculation enormous numbers of Cytoryctes were present in the epithelial cells. No leucocytes were found in the lesion or in the corneal substance about it.

SUMMARY.

The inoculation of the cornea with vesicle contents was followed by the development of a lesion which had much in common with that which follows a similar inoculation on the rabbit. The following summary of the macroscopic appearances is based upon the observation of eighteen experiments similar to those given above.

After twenty-four hours there is some roughening of the surface along the line of inoculation. After forty-eight hours there follows more or less loss of substance at the site of the inoculation incision. This loss of substance is not so great as in vaccination of the cornea. When pyogenic infection does not complicate the process there is no opacity of the cornea or conjunctivitis, and the lesions heal after a variable period. In two animals, which were allowed to survive long enough for the eruption to appear, an exanthem was seen on the sixth and seventh day of the disease. The evolution and extent of the exanthem was like that following the skin inoculation. Another animal, kept under observation for a long period, did not develop an exanthem.

Histological examination shows that the process at the site of inoculation with variola virus, on the cornea of the monkey, consists primarily in degeneration and in proliferation of the epithelial cells. Seventy-two hours after the inoculation, when the process is at its height, the line of inoculation is marked by a defect in the epithelium, below which there may be a slight destruction of the corneal substance. The epithelial cells about this defect may be swollen and separated one from another, and show various degrees of degeneration. As we pass from the center of the lesion towards the periphery we find the epithelium much thickened. This increase in the thickness is in part due to swelling of the individual cells, shown particularly by those of the lower layer which appear pale and assume a cuboidal or cylindrical form, and in part to an increase in the number of cells. At the point of greatest thickening the epithelium may measure twice its normal depth. In lesions of greater duration the degeneration

of the individual cells and collection of fluid between the cells may occasionally result in the formation of minute cavities in the thickened epithelium which are analogous to the vesicle of the specific skin lesion.

Polynuclear leucocytes do not form a prominent feature of the corneal lesions. In many sections a prolonged search is necessary to find a single cell of this type. When the inoculation wound penetrates the corneal substance polynuclear leucocytes are more numerous. The paucity of these cells in the lesion is in strong contrast with the condition in the cutaneous smallpox lesions in the monkey.

Cytoryctes variolæ were present in all the lesions examined. Their morphology and staining reactions were identical with those found in the skin lesions. No nuclear phases were present. The parasites were found as early as eighteen hours after the inoculation and persisted through the eleventh day, that date being the last on which a microscopic examination was made.

DISCUSSION. — When we compare the lesion produced on the cornea of the monkey by inoculation with variola virus with vaccinal keratitis in the same animal, we see at once that we have to do with a similar process. The only striking difference that these two lesions present is that in the variolous keratitis there is less exudation, and the epithelium of the lesion does not become detached and cause a large superficial defect as in the vaccinal lesion. In this respect variolous keratitis in the monkey approaches more nearly to vaccinal or variolous keratitis in the rabbit than does vaccinal keratitis in the monkey.

The small part played by the polynuclear leucocyte in the variolous keratitis contrasts strongly with the prominence of this cell in the specific cutaneous lesions of the disease in the monkey. It seems probable that in the skin inoculation the presence of a large number of these cells is conditioned by the destruction of tissue incident to the inoculation, and that they continue to be attracted, not so much by the variola organism or its products, as by the presence of substances set

free in the process of cell destruction incident to the activity of the parasite and the other organisms which gain access to the lesion. As all such degeneration products are retained at the site of inoculation by the crust in the case of a cutaneous lesion, while they readily escape in the case of the corneal lesion, it is easy to understand why in the former case large numbers of leucocytes pass from the vessels to the lesion.

The absence of nuclear phases of *Cytoryctes variolæ* in the corneal lesion was disappointing. It is possible that there is some inherent difference in the cells of the cornea which makes them unsuitable for the development of the parasite beyond the cytoplasmic stage, though we are inclined to regard the absence of nuclear forms in these lesions as being due to the action of physical factors. In a typical corneal lesion the cells are probably cast off from the surface before the nuclear forms are produced. It is possible that oblique incisions into the corneal substance, in which islands of epithelial cells would be retained, might show a development of the nuclear forms of the parasite.

In a previous section we have shown that the endothelial cells of capillaries beneath the primary skin lesions of variola inoculata are invaded by *Cytoryctes*. From this we have been led to believe that some such process is involved in the dissemination of the organisms to form the exanthem. From the consideration of the time elements it seems probable that such infected endothelial cells are set free at an earlier stage in the disease than the infected endothelial cells have been demonstrated beneath the primary lesion. The fact that an exanthem follows the development of a lesion in the non-vascular cornea, where extension of the process to blood vessels does not occur, suggests that the distribution of the organism, if brought about through the intermediation of endothelial cells, is due to an infection of the cells lining lymph channels.

CONCLUSIONS.

1. Inoculation of the cornea of the monkey (*M. cynomolgus*) with variola virus produces a specific lesion characterized by swelling, proliferation, and varying degrees of degeneration of the epithelial cells.

2. The lesion is similar to that produced by inoculation of the cornea of the rabbit with vaccine or with variola virus.

3. The lesion results in less destruction of the corneal epithelium than follows similar inoculations of the cornea of the monkey with vaccine virus.

4. The lesion on the cornea differs from the variolous lesion on the skin of the monkey in that exudation does not play as prominent a part, and that true vesicle formation does not occur.

5. Cytoryctes variolæ are present in the lesion up to eleven days after the inoculation, but nuclear forms of the parasite are not found.

6. A variolous lesion on the cornea of the monkey may be followed by a general exanthem which appears on the same day as after skin inoculation. We therefore identify the disease produced in the monkey by variolation on the cornea as variola inoculata.

4. VARIOLA INOCULATA FOLLOWING INOCULATION OF THE MUCOUS MEMBRANE OF THE MONKEY.

INTRODUCTION. — In the preceding sections of this paper we have detailed the results which follow the inoculation of the skin and the cornea of the monkey with variola virus. We will present here a series of experiments which show the results of inoculation of that animal upon the mucous membrane of the nose, the lip, and the palate. The experiments also yielded material for the study of the variola organism and the histology of the specific lesion on the mucous membrane.

TECHNIC. — The method of inoculation and of observation was the same as that followed in the series of inoculations of the mucous membranes of the monkey with vaccine virus.

CLINICAL COURSE OF THE DISEASE. — Twenty-nine animals were used in this series of inoculations, of which the following are selected to be described in detail:

No. 125. — Adult male, *Macacus cynomologus*. Inoculated on the left side of the nasal septum, on the inner side of the lower lip, and on the left palate with variola virus No. 167 (vesicle contents). Body temperature 40° C.

Twenty-four hours after inoculation a slight elevation is noted on the nasal septum. The lip and palate are negative. Body temperature 38.8° C.

Forty-eight hours. On the lip a narrow white line surrounded by hyperemic mucous membrane marks the site of inoculation. Nose and palate negative. Body temperature 40° C.

Three days. The nose shows considerable swelling of the septum, but no distinct lesion can be made out. The lip presents a white area, one by three millimeters, which is slightly elevated and has a translucent appearance. The palate shows a small gray spot on the line of inoculation. Body temperature 40° C.

Four days. Nose negative. The lip shows a white opaque area, one by three millimeters, with a ragged, elevated edge about which the mucosa is distinctly reddened. The palate presents a white, slightly elevated area,

two by four millimeters, the surface of which is unbroken. Body temperature 40° C.

Five days. Nose negative. The lip shows an opaque white area, covered in part by the remains of the macerated epithelium, and surrounded by a dull pink elevated border. The palate presents an elevated area, two by four millimeters in extent, and of a gray white color. About this are smaller similar spots. Body temperature 39.5° C.

Six days. The nose presents an opaque area on the septum surrounded by a bright red areola. The lip is much swollen, and the lesion shows an area of erosion surrounded by a ragged edge of elevated and hyperemic mucous membrane. The lesions on the palate have increased somewhat in size, and show a distinct red areola. Body temperature 40.2° C.

Seven days. Considerable muco-purulent discharge from the nostril; swelling of the mucous membrane prevents inspection. Lesion on lip as before. The palate shows some increase in the size of the lesion. Body temperature 40° C.

Eight days. The swelling of the mucous membrane in the nose continues. On the lip the lesion presents as an ulceration with marked inflammatory reaction about it. On the palate the lesion shows no erosion and has ceased to spread. Body temperature 40.4° C.

Nine days. Lesions as described yesterday. A general exanthem was carefully searched for but not found. Animal killed and autopsy done at once. No macroscopic evidence of disease, except at the sites of inoculation.

No. 126. — Half grown male, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding animal. Body temperature 39° C.

After twenty-four hours the nose shows a delicate crust on a slightly elevated area of mucous membrane. On the lip a small lacerated wound marks the point of inoculation. The palate presents some reddening of the mucosa about the scratch. Body temperature 38.6° C.

Forty-eight hours. Lesions as above described. Body temperature 39° C.

Three days. In the nose the elevation about the crust has increased. The lip shows an elevated opaque white area, three by four millimeters, surrounded by a diffuse red flush. On the palate is a slightly elevated grayish area, two by four millimeters in extent, surrounded by hyperemic mucosa. Body temperature 40.4° C.

Four days. Nose as before, save that the elevation has taken on a gray tint. The lip presents a macerated appearance over the area occupied by the lesion. The lesion on the palate has increased in size. Body temperature 40.5° C.

Five days. On the palate the lesion is eroded, otherwise there is no change. Body temperature 39.5° C.

Six days. The nose presents a narrow crust on a pink elevation. The lesion on the lip shows signs of healing. Palate as before. The top of

the tongue near its tip presents a gray elevated area, two millimeters across, surrounded by a pink areola. Lesions show no change since yesterday. Animal killed and autopsied at once. Viscera show no macroscopic lesions. A single small vesicle outside the left eye is shown to be specific by microscopic examination. No other exanthem found.

No. 127. — Young female, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding monkeys. Body temperature 39.2° C.

Twenty-four hours after. Slight elevation and reddening at the points of inoculation. Body temperature 38° C.

Forty-eight hours. In the nose there is reddening of the mucosa on the septum. On the lip a yellow line surrounded by slightly elevated and pink mucosa marks the site of inoculation. Palate negative. Body temperature 39° C.

Three days. Nose negative. Lip shows a gray line on a white elevated area, two and one-half by five millimeters, the edge of which is translucent. Palate presents a grayish-white line three millimeters long. Body temperature 39.5° C.

Four days. Nose negative. The lip shows a gray-white area, two by five millimeters, with a pink edge and a granular surface. Palate as before. Body temperature 39.5° C.

Five days. Nose negative. The lip is swollen and presents an erosion, with a yellow surface and a thickened pink edge, two by four millimeters in extent. Palate presents an eroded area, two by five millimeters, with a slightly depressed gray base and an elevated margin, which is surrounded by reddened mucosa. Body temperature 38.5° C.

Six days. Lesions as before but somewhat increased in size. Body temperature 39.5° C.

Seven days. Nose presents a narrow crust about the orifice of the left nostril. The lip shows a large erosion, one centimeter across, the base of which is covered by yellow, macerated material, and is surrounded by an elevated, ragged, pink border. Lesion on palate has increased in size. Body temperature 38.8° C.

Eight days. Nose shows an eroded area extending from the septum out upon the surrounding skin. Lesion on lip as before. Palate presents a long, narrow ulceration, two by eight millimeters in extent, with a white, elevated edge and surrounded by a red areola. One small, pink, papular elevation on the inner side of each thigh and on right arm. Histological examination shows this lesion to be variolous. Body temperature 38.5° C.

Animal killed; at autopsy organs appear normal.

No. 128. — Full grown female, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding monkeys. Body temperature 39° C.

After twenty-four hours there is slight reddening of the mucosa about the scratches. Body temperature 38.2° C.

Forty-eight hours. Lesions as before. Body temperature 38.2° C.

Three days. The lip is swollen and presents a white area, one by three millimeters, slightly elevated and surrounded by a pink areola. Palate presents two yellow elevations, two by four millimeters in extent, with a red periphery. Body temperature 39.5° C.

Four days. Lesion on lip is excoriated. Palate as before, but lesion has spread somewhat. Body temperature 39° C.

Five days. Lip shows an eroded area with a gray base and a white elevated edge surrounded by reddened mucous membrane. The whole lower lip is distinctly swollen and indurated. Palate as before. Body temperature 38.2° C.

Six days. Swelling of the lip more marked. The base of the lesion is yellow and the edge ragged. A minute pink papular elevation appears on the gum opposite the lesion. Probably an auto-inoculation. Palate as before. Body temperature 39° C.

Seven days. Lesions as described but somewhat increased in extent. Body temperature 38.5° C.

Eight days. From this time on the primary lesions healed without complication. A general exanthem was not found either before or after this date. Body temperature 37.5° C.

No. 129. — Full grown male, *Macacus cynomolgus*. Inoculated in the same way and with the same virus as the preceding animals. Body temperature 38.5° C.

Twenty-four hours after inoculation the lip and palate show a white line surrounded by a hyperemic mucosa. Nose negative. Body temperature 39.8° C.

Forty-eight hours. Lip and palate show irregular elevation with opacity and a peripheral flush at the site of inoculation. Nose negative. Body temperature 38.2° C.

Three days. Lip presents a yellow eroded area, two by five millimeters, surrounded by a pink areola. Palate shows an opaque white area, two by five millimeters, surrounded by reddened mucosa. Body temperature 39° C.

Five days. Lesions on lip and palate somewhat increased in extent. The inoculation in the nose evidently did not take. Body temperature 39.4° C. Animal killed and autopsied at once. Viscera present no macroscopic lesions.

A series of ten monkeys were inoculated on the palate alone and a series of nine upon the nasal mucosa alone. The results of the inoculations were similar to the experiments detailed above.

SUMMARY.

The lesion which develops on the inner side of the lip of the monkey following inoculation with vesicle contents presents the following macroscopic characters:

After twenty-four hours the site of inoculation shows, at most, a slight reddening of the mucous membrane about the scratch.

After two days a narrow white line is seen which is surrounded by a faint red flush.

After three days there is a definite opaque white area, two or more millimeters in extent, slightly elevated above the general surface. This area is more or less eroded and is surrounded by a distinct zone of hyperemic mucous membrane.

After four days the opaque area is somewhat eroded and presents as a shallow ulcer with an elevated white, often sinuous edge, which is bordered externally by reddened mucosa.

From this time on the lesion presents the same characteristics, the only change being due to a gradual extension of the process. After eight or nine days the peripheral flush fades and healing begins. This process results in complete repair after about a week.

Inoculation of the palate causes a similar lesion to that following inoculation of the lip, and the lesion runs essentially the same course. In this situation the lesion is less apt to become eroded.

After inoculation of the mucous membrane of the nose it is difficult to follow the process from day to day, as at the time when the lesion is undergoing its active evolution the swelling of the mucous membrane which accompanies the process prevents inspection during life. From study of the site of inoculation in animals killed at various periods it is seen that the evolution of the lesion in the nose differs principally from those on the lip and palate, in that there is less tendency to form an ulcer. When the inoculation is near the anterior nares the process tends to spread out on the skin about the nostril and then takes on the characteristics of a skin inoculation.

No notable constitutional reaction followed the inoculation of the mucous membrane. The temperature reaction was indefinite. A general exanthem was observed in two of the monkeys.

Histology of the primary lesion. — Lip. — Sections from lesions collected twenty-five and fifty-three hours after inoculation show no evidence of a specific process. The defect in the epithelium caused by the inoculation has been completely repaired, and the only evidence of the wound is a small collection of fibrin and polynuclear leucocytes just beneath the epithelium.

Three days after inoculation the lesion shows specific characters, and we find an area in which the epithelium presents a pathological change, and beneath this the tissue is infiltrated with polynuclear leucocytes. The changes in the epithelium consist in degeneration and disintegration of the epithelial cells, together with more or less accumulation of fluid in and between the cells. The accumulation of fluid occurs not only in the degenerating portion of the epithelium, but also at the sides of the lesion. The process is similar to that seen in a skin inoculation of the same duration.

The degeneration of the epithelium is not uniform, and we find islands of comparatively normal cells in the midst of areas where the affinity of the nuclei of the epithelial cell for basic stains is lost. Polynuclear leucocytes are present in large numbers. The epithelium at the edge of the lesion is somewhat thickened, apparently as a result of the swelling of individual cells.

After four days the lesion shows the same characteristics save that there is considerable loss of substance in the area of degenerated epithelium, and the lesion is more extensive. At this time the reaction in the tissue beneath the lesion is well marked, and proliferation and enlargement of the endothelial cells of the lymphatics and blood vessels is apparent. Many elements of the lymphoid and plasma cell series are present about the vessels beneath the lesion.

Five and six days after inoculation the lesions are similar in character to those just described, but the necrotic area

becomes sharply limited and the inflammatory reaction beneath is more intense.

Lesions of seven days duration show evidence of beginning repair. The epithelium at the edge of the lesion is normal, and the lesions consist in a sharply circumscribed ulceration in the depths of which repair is active. Later lesions show the epithelium growing inward to close the defect, and new-formed blood vessels and young connective tissue cells are much in evidence in the tissue beneath.

In the lesions of three, four, five, six, and seven days duration epithelial cells containing cytoplasmic phases of *Cytoryctes variolæ* are of frequent occurrence. The earlier forms of the parasite occur at the margin of the lesion, the latter forms nearer the center. Nuclear phases of the parasite are also found, but they occur later than the cytoplasmic phases.

Nose. — The primary lesions in the nose vary in character according to the locus of inoculation. When the incision is near the orifice of the nostrils, on a stratified epithelium, the lesion conforms to the type described on the lip. When the lesion is seated higher up, upon a columnar epithelium, the process in the submucous tissue is most marked. In such a situation the bulk of the degenerated epithelial cells seems to be carried off almost at once, and we find but little thickening of the mucous layer. The submucous tissue, however, shows accumulations of lymphoid and plasma cells, enlargement and proliferation of the endothelial cells of the lymphatics and blood vessels, and a marked polynuclear leucocyte infiltration.

Both cytoplasmic and nuclear phases of *Cytoryctes variolæ* are present in the epithelial cells. In one lesion, of five days duration, an endothelial cell was found on the wall of a lymphatic, just beneath the epithelium, which contained a cytoplasmic form of the parasite.

Palate. — The histology of the primary lesion in this situation is similar to that seen in the lesion on the lip. A lymphatic with cytoplasmic forms of *Cytoryctes*, in the endothelial cells lining its wall, was found beneath a lesion of five days duration.

Histological examination of the viscera showed no lesions. The bone marrow and testicle were carefully examined for focal lesions, such as are found in these organs in variola vera in man, but none could be demonstrated.

DISCUSSION. — The disease produced by variolation of the monkey upon the mucous membrane of the lip, nose, and palate is characterized by the development of a self-limiting lesion at the site of inoculation, which may be followed by a general cutaneous exanthem, and be associated with an indefinite constitutional reaction.

If we compare the initial lesion produced on the mucous membrane with that which follows a similar inoculation on the skin, we see that the two processes are similar in that they run a definite course and tend to heal after about the same interval of time. The microscopic study of these lesions shows them to be the result of similar cell changes, and in each the parasite, *Cytoryctes variolæ*, is found associated with the process. The lesions differ in that on the mucous membrane the absence of a crust prevents the development of a vesicle or pustule, although an accumulation of fluid between the cells is in evidence at certain stages of the lesion. The primary lesion on the mucous membrane, of four or five days duration, simulates closely a skin lesion of the same duration which has lost its crust through rough manipulation. The primary lesion on the mucous membrane also differs from that upon the skin in that the process in the tissue beneath the lesion is more exudative and proliferative than necrotic.

When we compare the other manifestations of the disease following variolation of the mucous membrane with that following skin inoculation, a decided difference is found. We see that a general exanthem is much less apt to follow the inoculation of the mucous membrane. Of nineteen animals inoculated in the nose, on the lip, or on the palate, only two showed a general exanthem. In both these animals the lesion of the eruption were few in number, and required microscopic study for their positive diagnosis. The exanthem

occurred in a trifle over ten per cent of the animals. This is in sharp contrast with the occurrence of the exanthem in *variola inoculata* from skin inoculation, where an eruption develops in from seventy to eighty per cent of the animals.

The constitutional reaction in monkeys variolated upon the mucous membrane differs in degree from that in the animals variolated upon the skin. We do not find such an abrupt elevation in the body temperature, and malaise and anorexia are absent. It seems reasonable to suppose that this relatively slight constitutional reaction is conditioned by the physical conditions at the locus of inoculation. The initial lesion on the mucous membrane is practically an open wound from the first and, consequently, the products of cell destruction and any toxins produced by the specific organism can readily escape. The systemic absorption from these lesions on the mucous membrane must be quantitatively much less than from the lesions upon the skin.

We are inclined to attribute the infrequency of the exanthem in this series of animals to physical conditions at the locus of the primary lesion. At the time of inoculation the amount of virus which enters and remains in the scratch must be notably less than when the skin is inoculated, owing to the fact that the surface inoculated is bathed with fluids, and any exudation stream set up by the trauma of the inoculation would be assisted by the moist condition of the surface and the action of similar opposed surfaces to carry off the bulk of the virus. In these inoculations there can be no such flooding of the lymphatics about the scratch as must occur after similar inoculations of the skin.

The type of disease produced by variolation upon the mucous membrane conforms in general to that which follows inoculation of the skin. The nature of the primary lesion and the time of occurrence of the exanthem relate the disease at once to *variola inoculata*. The absence of focal lesions in the bone marrow and testicle differentiate the disease from *variola vera* in man.

CONCLUSIONS.

1. Inoculation of the mucous membrane of the lip, the nose, or the palate of the monkey (*M. cynomologus*) with variola virus produces a disease which conforms to the type of variola inoculata.

2. The primary lesion on the mucous membrane is similar, cytologically and histologically, to that which follows variolation of the skin.

3. *Cytoryctes variolæ*, in both the cytoplasmic and the nuclear phases, are present in the lesions.

4. Cytoplasmic forms of the parasite are found invading endothelial cells of lymphatics beneath the lesions of five days duration.

5. ON THE OCCURRENCE OF VARIOLA VERA IN MONKEYS
AND IN THE ORANG UTAN.

In the preceding sections we have shown that the monkey and the orang utan react in a definite manner to inoculation with the virus of smallpox. This reaction consists in the development of a disease which conforms closely to the type of smallpox in man which follows deliberate inoculation of the skin with variola virus. In short, we can produce in the monkey and in the orang utan the homologue of human variola inoculata. We have also shown that if the epithelium of the cornea or of the mucous membrane of the nasal, buccal, or oral cavity be chosen as the locus of inoculation the same type of disease is produced. The experiments upon which this section is based were devised in an attempt to reproduce in the monkey, and in the orang utan, a disease having the clinical features of variola vera. We have sought to attain this end by changing the locus of inoculation and by subjecting the animals to conditions in which man contracts the disease. The experiments which we have chosen to give in detail will be grouped according to the manner in which we have sought to bring about the infection.

(a.) Inoculation of the tracheal epithelium through a tracheotomy wound with the contents of the variola vesicle.

These experiments were performed to determine if the course of the disease would be modified by the initial lesion being seated upon the columnar epithelium of the trachea. The generally accepted hypothesis of smallpox in man supposes a "protopustule" in the respiratory tract, and we proposed to deliberately produce such a lesion by direct inoculation with variola virus.

No. 144. — Adult male, *M. cynomologus*. Monkey anesthetized with chloroform and a median incision made in the neck over the upper part of the trachea. Trachea exposed by blunt dissection. The upper ring of the trachea cut and, through the opening so made, the epithelium on the posterior wall slightly scarified and inoculated with variola virus No. 167

(vesicle contents). The skin incision closed with silk sutures. After four days the operation wound showed a marked inflammatory reaction.

On the ninth day of the experiment six red, papular elevations, two to three millimeters in diameter, were seen on the scrotum. On the next day papules and small vesicles were found on the face, trunk, and extremities. On the eleventh day of the experiment the lesions on the scrotum presented as flat-topped, umbilicated vesicles with an opaque center and surrounded by a bright red areola. The evolution of these lesions was comparable with that seen in the exanthem of a case of discrete variola vera in man. A distinct pit remained after the healing of the lesions. One lesion was found in the palm of the hand. The body temperature rose to 40° C. on the sixth day of the experiment and remained elevated until the thirteenth day.

No 139. — Adult male, *M. cynomologus*. Inoculated in the same manner as previous animal. A profuse general exanthem appeared on the seventh day of the experiment. Animal died on the ninth day. At autopsy extensive cellulitis of the neck about the operation wound. Smears from this region show many streptococci. Mucous membranes: in cheek pouches about a dozen opaque, elevated, sharply-circumscribed lesions, three to four millimeters in diameter and one millimeter high. One similar lesion on under surface of tongue. Similar, though smaller lesions are found scattered over the surface of the esophagus, some of which are eroded. In the trachea, at the site of inoculation, is a red, slightly elevated area, due apparently to thickening of the epithelium. Viscera: no macroscopic lesions found. Skin: vesicles and pustules, from three to four millimeters in diameter, are found scattered over the face, trunk, and extremities. The exanthem consists of over a hundred lesions.

Histological examination. — Operation wound: subcutaneous tissue contains much fibrin and finely granular precipitate. There is considerable necrosis of the underlying muscle. Polymorphonuclear leucocytes in all stages of degeneration are present in large numbers. Many small blood vessels are thrombosed, and in them and in the surrounding tissue streptococci are demonstrable. The epithelium at the edge of the wound is much degenerated, but no evidence of a variolous process can be made out. Cytoryctes were, however, found in the cells of the thickened corium. Trachea: the epithelium is wanting in places, and in other areas it is somewhat thickened. In many places the basement membrane is broken. The epithelial cells show various degenerations. In many groups of cells cytoplasmic phases of *Cytoryctes variolæ* are demonstrable. The vessels of the submucous tissue are much injected and in one is a mass of fibrin in which are phagocytic cells containing streptococci. The tissue is infiltrated with polynuclear leucocytes. The endothelial cells of a capillary, immediately below an area in which the epithelium shows *Cytoryctes*, are prominent and contain cytoplasmic phases of the parasite. Skin: the

lesions of the exanthem present the characters before described in similar lesions occurring in the course of variola inoculata in the monkey. Mucous membrane: the lesions in the cheek pouches consist in areas of degenerated epithelium in which groups of cells are stained red, the nuclei having lost their affinity for basic dyes. The cytoplasm is finely granular, but the cell outlines are retained. Other cells are present whose staining properties are unimpaired, and in them are found various cytoplasmic phases of *Cytoryctes variolæ*. In the upper layers of the degenerated epithelium, particularly towards the edge of the lesion, the cells are separated by fluid. In some places the appearance is similar to that seen near the edge of a four-day primary lesion in the skin. Polynuclear leucocytes are present in the epithelium and in the tissue beneath, although in the latter situation the reaction is much less intense than it is beneath a primary lesion of the mucous membrane. Esophagus: small areas are present in which the epithelial cells are swollen and their nuclei shrunken. Evidence can be made out of accumulation of fluid in and between the cells. Cytoplasmic forms of *Cytoryctes variolæ* are present in many of the cells. Comparatively few polynuclear leucocytes are present in the lesion, and there is practically no reaction in the tissue beneath. Seminal vesicles: focal lesions are present in the septa of the tubules. The lesions consist of collections of degenerated cells and fibrin which lie in the connective tissue stalks of the septa. Associated with this there is more or less degeneration of the adjacent epithelial cells. A few polynuclear leucocytes are present. Many of the epithelial cells contain cytoplasmic phases of *Cytoryctes variolæ*. Testicle: normal. Bone marrow: no focal lesions are demonstrable. The lung, liver, spleen, and kidney show no lesions.

SUMMARY. — From observation of the trachea in animals, killed at various times after the inoculation, it was seen that a distinct process occurs at the site of inoculation. Our data on this point seems to us insufficient to form the basis of a description of the stages in the evolution of the specific lesion in this situation.

A general exanthem was observed in three of the four monkeys of this series. The single animal that did not show an eruption died on the eighth day of the experiment. The exanthem appeared on the seventh day in one animal, on the ninth day in the other two. The exanthem was very extensive and, in the two animals in which it appeared on the ninth day of the disease, it passed through an evolution similar to that of the eruption of variola vera in man.

The constitutional reaction was very marked, the animals showing anorexia and weakness during the active stage of

the disease. This marked constitutional reaction may be, in part, explained by the intensity of the pyogenic process in the operation wound. The temperature reaction was similar to that seen after skin inoculation, showing a marked rise on the sixth or seventh day.

The histological examination demonstrates that the lesion at the site of inoculation, and the lesion of the exanthem on the skin and the mucous membranes, was variolous in type. In one animal focal lesions were demonstrated in the seminal vesicles, and they were shown to be specific.

The method of inoculation used in this series of experiments was abandoned, as it was impossible to exclude a variolous infection of the operation wound. Obviously if this were to take place the experiments would be merely repetitions of the skin inoculation complicated by a coincident infection of the trachea.

(b.) Inoculation of the epithelium of the trachea, without tracheotomy, with the contents of the variola vesicle.

These experiments were devised to overcome the difficulty experienced in the previous series resulting from infection of the tracheotomy wound. This series consists of ten monkeys (*M. cynomologus*), of which one will be described in detail. Six animals were killed at various times after the inoculation to obtain material for histological study.

No. 339.—Adult male, *M. cynomologus*. Monkey anesthetized with chloroform and a large glass tube, having a fire-burnished end, introduced into the larynx through the mouth. By way of this tube instruments were introduced to scratch the wall of the trachea and to inoculate it with the virus. Variola virus No. 328 was employed.

On the seventh day of the disease the body temperature rose to 41° C., and a pink papular elevation, one millimeter in diameter, appeared on the scrotum. On the next day small vesicles, surrounded by a bright red areola, were present upon the face, scrotum, hands, and feet. On the following day, the ninth of the experiment, new eruptive lesions were found on the face, the scrotum, and the palms and soles. Animal chloroformed and autopsied at once. Skin lesions as described above. The mucous membrane of the trachea is congested throughout and presents several opaque spots on which are minute granular elevations. The trachea and large bronchi contain much slimy mucus. The left lung presents an area of

consolidation, five millimeters in extent, about the primary bronchus. On section this area is reddish brown in color and finely granular. Other organs appear normal.

Histological examination. — The microscopic study of the organs from these monkeys yielded data upon the variolous lesion in situations not previously described. The following descriptions are selected as types of the lesions found:

No. 345. — Trachea: Lesion of three days duration. The basement membrane is intact. There is no reaction in the submucous tissue. The epithelial cells in a small area, which can be included in a single field of the oil immersion lense, contain various stages of the cytoplasmic forms of *Cytoryctes variolæ*. Aside from the vacuole about the parasite these cells appear normal.

No. 348. — Trachea: Lesion of six days duration. The epithelium is thickened in places and frequently tongue-like projections, composed of epithelial cells, project into the lumen. There is widespread infection of the epithelial cells with cytoplasmic phases of *Cytoryctes variolæ*. In places groups of epithelial cells have lost their selective staining affinity, and are colored an even red. Occasionally there is evidence of the collection of fluid within and between the degenerated cells. Polynuclear leucocytes are numerous and are found in the epithelium as well as in the submucous tissue. In places there is necrosis and accumulation of fibrin in the submucous tissue, such areas occurring beneath breaks in the basement membrane. The endothelial cells of the lymphatics and blood vessels of the submucosa are prominent and frequently contain cytoplasmic forms of *Cytoryctes variolæ*.

Lung: Six days after inoculation. The section shows a large bronchus and the surrounding lung substance. The epithelium of the bronchus is much thickened at two points in its circumference. In these areas the cells are more or less swollen and degenerated. The majority of the cells contain cytoplasmic forms of *Cytoryctes variolæ*. Polynuclear leucocytes are present in large numbers in the lumen of the bronchus, in the epithelium, and in the peribronchial tissue. The air cells for a considerable distance from this bronchus are filled with cells, fibrin, and granular precipitate. The relative amount of each of these constituents varies in different air cells, but in general the granular precipitate predominates in those remote from the bronchus. The cellular elements present are in the main polynuclear leucocytes and epithelial cells. The latter are found free in the air cells and also attached to the walls. In the latter situation they are frequently cuboidal in form and lie one beside another. Mitoses are frequently present in these cells. Cytoplasmic phases of *Cytoryctes variolæ* are present in large numbers in the epithelial cells of the air spaces. The capillaries in the septa between the affected air cells are injected with blood, and many polynuclear leucocytes are to be seen in them and migrating through their walls.

Two other monkeys, killed seven and nine days after inoculation, showed small areas of pneumonia in their lungs. In both cases the process was characterized by proliferation of the epithelial cells lining the alveoli. No Cytoryctes were demonstrable in these lesions.

The exanthem which occurred in this series of monkeys was examined histologically and found to be similar in all respects to that which developed in the other variolated monkeys.

SUMMARY.—Four animals were allowed to survive long enough to show an exanthem. Six were killed at various times after the inoculation in order to inspect the initial lesion and to obtain material for histological study. In each of the former animals an exanthem appeared; in one on the eighth day, in two on the ninth day, and in one on the tenth day. The exanthem was of moderate extent and showed an evolution intermediate between that in the monkeys inoculated through a tracheotomy wound and the average case of variola inoculata following variolation of the skin.

The constitutional reaction was marked. The temperature reaction was definite, consisting of a distinct rise with or immediately before the appearance of the exanthem.

The histological examination of tissues from these animals shows that a lesion similar to that which follows variolation of the mucous membrane of the nose, the lip, and the palate can be produced by inoculation of the epithelium of the trachea. In one animal a variolous bronchitis and a variolous pneumonia was demonstrable. No focal lesions were found in the bone marrow or testicle.

A series of five Java monkeys (*M. nemestrinus*) were inoculated in the same manner and with the same virus. The results of these experiments were unsatisfactory. No exanthem or constitutional reaction was observed, and we were unable to demonstrate a specific lesion at the site of inoculation.

(*c.*) Inoculation of the lung by inhalation of a spray of the contents of the variola vesicle.

These experiments were performed to approximate the conditions under which natural infection with smallpox in

man might be supposed to occur. Of course the dose of virus is much larger than it possibly could be under natural conditions, but at least this method approaches more nearly epidemic conditions in that no deliberate trauma to an epithelial surface precedes the distribution or application of the contagium. Five animals were employed in this series, of which the following is selected to be described in detail:

No. 207. — The monkey's mouth was held open and a fine spray of vesicle contents, virus No. 199, was thrown into the throat from an atomizer. The animal was observed to breathe while the spray was acting.

No distinct temperature reaction was noted during the seventeen days that the animal was kept under observation. No lesion was observed to develop on the visible mucous membranes. On the seventh day of the disease a papule appeared on the inner aspect of the right arm, at the bend of the elbow. This lesion increased in size, became vesicular, and on tenth day was distinctly umbilicated. The contents of this lesion was used to inoculate a fresh monkey upon the skin of the abdomen. A typical primary lesion developed, and was followed by a profuse general exanthem.

SUMMARY. — One of the animals of this series was killed five days after inoculation. Inspection of the mucous membrane showed no evidence of a specific lesion. Sections from various parts of the lungs and the trachea were studied microscopically, but no lesions were found. The four animals remaining were under observation for sixteen days. In one of them a single eruptive lesion appeared on the seventh day of the experiment. This lesion was shown to be specific by inoculation of its contents upon the skin of a fresh monkey.

No constitutional reaction or rise of temperature was observed in this series of monkeys.

(*d.*) Inoculation of the lung by inhalation of dry variola virus.

These experiments were devised to determine if variola vera could be produced in the monkey by employing the contagious material in a dry condition. Two series of experiments were done, in one dried pustule contents was used, and in the other a powder prepared from desiccated crusts or disks of a case of variola vera.

A preliminary experiment was performed to determine how far a powder blown into the larynx would enter the lung. A large monkey was anesthetized with chloroform and a glass tube containing a mixture of lycopodium and methylene blue powder was introduced into the larynx. During an inspiration the powder was forcibly blown into the lungs. The animal was killed at once, and on dissection the smallest bronchi which could be made out with the naked eye were found stained distinctly blue. Histological examination of the lung showed lycopodium spores in the bronchi and the alveoli.

No. 160. — A monkey was put under chloroform anesthesia and the same procedure was followed as above described, except that dry pustule contents was substituted for the methylene blue. The animal was kept under observation for sixteen days. The monkey had a cough from the fifth to the eighth day after the inoculation. On the eighth day of the experiment the body temperature rose to 40.5° C., and an abundant general exanthem appeared. The lesions of the eruption passed through an evolution which closely simulated that seen in the lesions of a discrete variola vera in man. On the sixteenth day of the experiment the animal was vaccinated on the abdomen with virus No. 1. No reaction followed.

Two other monkeys were inoculated in the lung in the same manner and with the same virus. One of these developed symptoms like that above described. The third animal showed a distinct rise of temperature on the third day of the experiment, and was immune to subsequent vaccination, but did not develop an exanthem. The same method of inoculation was followed in another series of five monkeys, but powdered variola disk was substituted for the mixture of pustule contents and lycopodium.

SUMMARY. — Two of the animals in the series inoculated with dried pustule contents showed an exanthem. The exanthem appeared on the eighth day of the disease and was profuse. In one of these animals the evolution of the eruption was similar to that seen in variola vera in man. A cough was noted in the two monkeys, which developed an exanthem.

The constitutional reaction was not marked, but in each animal a distinct rise in body temperature was observed. The fever began on the eighth day in two, and on the sixth day in one.

The monkeys inoculated by inhalation of pulverized variola disks showed no exanthem, no constitutional reaction, and an indefinite temperature reaction. One animal was found to be refractory to a subsequent skin inoculation with variola virus.

(*e.*) Attempts to inoculate the monkey by exposure to smallpox fomites.

In the series of inoculations previously described we employed material the infectiousness of which was demonstrable. In all these experiments, although they approximate somewhat the conditions in which man contracts smallpox, the amount of contagious material employed was excessive. In the series to be described we attempted to place the animals under conditions which experience has shown would result in an attack of smallpox if man was subjected to the test, although we were unable to demonstrate the presence of a contagium.

No. 243. — Adult male, *M. cynomolgus*. This monkey, together with four others, was kept in a cage in which a blanket was placed that had been wrapped around a smallpox patient. The blanket had been in contact with the patient for six hours and was carried from the ward to the animal room in a light-proof sterile bag. The blanket was left in the cage with the monkeys over night. Observations were recorded upon the monkeys daily for a period of sixteen days, during which time no eruption and no temperature reaction appeared. The animal was variolated on the skin on the seventeenth day of the experiment with virus No. 260. A typical primary lesion developed at the site of inoculation.

One of the remaining animals of this series died on the fifteenth day of the experiment, before the immunity had been tested by skin inoculation. At autopsy there was no evidence of variola in this animal. The three other monkeys were shown to be susceptible to variolation by skin inoculation.

SUMMARY. — Five monkeys showed no clinical symptoms of variola after exposure to a smallpox infected blanket. Four of these animals were subsequently shown to be susceptible to variola by skin inoculation. The fifth animal died

before its immunity was tested, but no anatomical evidence was found that it had contracted variola.

(*f.*) Attempts to inoculate the monkey by exposure to a smallpox patient.

This experiment was tried as a further test of the reaction of the monkey to conditions which would bring about an infection with smallpox in man.

No. 240. — Adult male, *M. cynomologus*. This monkey, and four others, were placed in a cage which was kept in a room for sixteen hours with a smallpox patient in the vesicular stage of the disease. The animals were kept under observation for sixteen days. No eruption and no temperature reaction was observed. On the seventeenth day of the experiment the animal was inoculated on the abdomen with variola virus No. 260. A typical primary lesion developed at the site of inoculation.

Of the four remaining monkeys of this series two reacted positively to subsequent variolation on the skin, while two gave no reaction.

SUMMARY. — Five monkeys exposed to a smallpox patient did not develop symptoms of variola. Three of these animals were subsequently shown to be susceptible to variolation by skin inoculation.

(*g.*) Attempt to inoculate the orang utan by exposure to smallpox fomites.

This experiment was planned to determine if an animal higher in the scale than the monkey would contract smallpox, under conditions in which man becomes infected.

No. 179. — A young female orang utan was given a blanket which had just been taken from a case of smallpox in the vesicular stage of the disease. The animal at once wrapped herself in the blanket and used it until the following day when a clean blanket was substituted for the infected one. During the following eighteen days the animal was kept under constant observation. No exanthem appeared, and the variations in the body temperature which occurred were explained by intercurrent infection. The animal was subsequently variolated on the skin and yielded a positive reaction.

SUMMARY. — An orang utan exposed to smallpox fomites did not develop symptoms of variola. The animal was shown

subsequently to be susceptible to variola inoculata by skin inoculation.

DISCUSSION.—The experiments detailed above were devised to produce variola vera in the monkey or in the orang utan. An analysis of the results of these experiments shows that of twenty-nine monkeys ten developed a distinct group of symptoms. Seven of the remaining animals showed no symptoms, and twelve were affected in an indefinite way. All the positive results were obtained where some product of the cutaneous lesion of human smallpox was introduced into the animal. All of the negative results were in experiments where smallpox fomites or the air of a smallpox ward was depended upon to carry the contagium. Fifteen experiments, in which vesicle contents or pustule contents was either inoculated upon the tracheal mucous membrane or blown into the lung, yielded ten positive results. The disease produced in these animals was characterized by the development of an exanthem, some degree of constitutional reaction, and fever. The evolution of the exanthem was usually similar to that seen in variola inoculata, but in three animals it resembled the eruption in a mild variola vera. The exanthem appeared between the seventh and tenth day of the experiment. The constitutional reaction and the fever appeared on the sixth, seventh, or eighth day of the experiment.

The disease which occurred in these animals agrees with that type which follows variolation of the skin of the monkey, and we have no difficulty in recognizing it as variola inoculata.

The negative results, which followed exposure of the monkey and the orang utan to smallpox fomites and smallpox patients, show that these animals do not develop a recognizable form of variola when placed under conditions that we believe would produce smallpox in man. We are unable to exclude the possibility of the occurrence of variola vera sine exanthem or variola inoculata sine exanthem in these animals. The two monkeys that were refractory to variolation after exposure to a smallpox patient might owe their

immunity to such unrecognizable forms of variola, but we are inclined to regard this phenomena as due to an individual peculiarity of the animals (natural immunity).

The histological study of the tissues from these series of monkeys brings out certain points of interest. The epithelium of the trachea is shown to be capable of harboring the parasite of the disease, and we see that a lesion can develop there which has features in common with the lesions produced by variolation of other mucous membranes.

The occurrence of a variolous lesion in the bronchus, and associated with it a pneumonia in which the parasite is present, shows that the organism is capable of multiplying in the deeper parts of the respiratory tract. This fact bears upon the pathogenesis of variola vera in man. It is quite conceivable that such a lesion might run its course unnoticed, and serve as a focus for multiplication of the organism during the incubation period of the disease. We have shown that *Cytoryctes variolæ* is capable of infecting the endothelial cells of capillaries and lymphatics. It is easy to understand that the organisms in a focus of variolous pneumonia might invade the adjacent blood vessels, infect endothelial cells, and so be carried throughout the vascular system. If such an infected endothelial cell were to lodge in a capillary adjacent to an epithelium hospitable to the organism, an eruptive lesion would result. The focal lesions in the seminal vesicles of one of the monkeys inoculated in the trachea is doubtless due to such a process, although in this case the infected endothelial cells probably came from vessels beneath the tracheal inoculation. In variola inoculata the tissue at the site of the inoculation is flooded with organisms at the time of inoculation, and it seems probable to us that the exanthem developing in these animals results from endothelial cells infected at this time. In variola vera the exanthem appears four or five days later than in variola inoculata. We believe this interval to be associated with the development of a focus of variolous pneumonia during which the organisms multiply, and finally invade the vessels.

CONCLUSIONS.

1. Inoculation of the mucous membrane of the trachea of the monkey (*M. cynomologus*) with variola virus produces a variola inoculata in that animal.

2. Inhalation of variola virus by the monkey (*M. cynomologus*) produces a variola inoculata in that animal.

3. Exposure of the monkey (*M. cynomologus*) and the orang utan (*Simia satyrus*) to smallpox fomites and to a smallpox patient does not produce variola vera, or any other recognizable form of variola, in these animals.

4. Inoculation of the mucous membrane of the trachea of the monkey (*M. cynomologus*) with variola virus is followed by the development of a variolous lesion on the mucous membrane which is similar to that produced on other mucous membranes by similar inoculations. A variolous lesion may develop in the bronchi and be associated with a pneumonia in which *Cytoryctes variolæ* are present. The development of the specific lesion in the trachea may be followed by a general cutaneous exanthem, and also by focal lesions of a variolous nature in the seminal vesicles.

5. *Cytoryctes variolæ* can invade the epithelial cells of the trachea, the bronchi, the alveoli of the lung, and the seminal vesicles.

6. *Cytoryctes variolæ* can invade the endothelial cells of lymphatics and blood vessels. This property of the organism probably plays an important part in the production of the exanthem in variola.