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ON THE

PATHOLOGY AND ON THE ETIOLOGY OF VARIOLA AND OF VACCINIA

BLICATION OFFICE OF THE JOURNAL OF MEDICAL RESEARCH:
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CONTENTS.

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PATHOLOGY AND ON THE ETIOLOGY OF VARIOLA AND OF VACCINIA

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From the Sears Pathological Laboratory, The Harvard Medical School

TO THE MEMORY OF

DR. MORRILL WYMAN

A PHYSICIAN OF CAMBRIDGE

WHO, AS AN INVESTIGATOR AND AS A PRACTITONER OF MEDICINE,
UNITED CAREFUL OBSERVATION, SOUND REASONING, AND
GOOD JUDGMENT, WE WHO HAVE KNOWN HIM
PERSONALLY AND HAVE RECEIVED INSPI-
RATION FROM HIS WORDS AND LIFE

DEDICATE

THIS SERIES OF PAPERS

INTRODUCTION.

The papers brought together in this report present the results of a study of variola carried on in Boston during the recent outbreak of the disease in that city.

The work, which was continued over a period of about two years, was in the hands of several men who, singly or together, studied different aspects of the disease, and one of whom studied vaccinia. It embodied no attempt at a complete review either of variola or vaccinia, but included in each of the subjects considered careful attention to previous work.

The study was undertaken with the belief that recent improvements in technical methods warranted a review of the pathological histology of variola, and with the hope of gaining some positive knowledge as to its etiology. We feel that the results of the work add something to the knowledge both of variola and of vaccinia, particularly with regard to the comparative relations of the diseases, and establish their etiology.

The work began in the summer of 1901, when material from a case of variola was placed at our disposal by Dr. Paul Carson, port physician of the city of Boston. In the fall of this year, the disease having become mildly epidemic in the city, we sought and obtained from the Board of Health Commissioners permission to make use of the opportunity for a thorough and systematic study of variola.

To explain the manner in which the work was carried on, there are needed a few statements relative to the epidemic itself. It began seemingly as a part of the pandemic of the past two or three years. Several cases of variola appeared in Boston in the summer of 1901, the number increasing during the fall, and reaching its maximum in the following winter. The disease showed a marked remission in the succeeding spring and summer months, but increased in prevalence again in the fall of 1902, and continued through the winter of 1902–3. The city became free from small-pox in April, 1903. The general character of the disease was moderately severe, and its various types were well represented.

The sick were cared for in the one or the other, or both, of two hospitals, in charge of the Board of Health, the Quarantine Hospital at Gallup's island, Boston harbor, and the Detention Hospital in Southampton street. During the first year of the epidemic both of the hospitals were in use; during the second, the Detention Hospital only.

With the beginning of a systematic study of variola, in December, 1901, the following objects were determined upon and occupied attention during the first year of the work: (1) the collecting of anatomical and histological material; (2) the study of vesicle contents and of the blood; (3) the acquiring of familiarity with the disease. For the first six weeks work was carried on at the Quarantine Hospital, with Dr. Brinckerhoff in residence throughout, Dr. Tyzzer for a part of the period, and Dr. Magrath in frequent attendance. Here a small temporary laboratory afforded what facilities were needed formicroscopic work incidental to the study of vesicle contents and of the blood. The Sears Laboratory at this time and throughout the research served as headquarters to which, from the temporary laboratories, all histological material was sent after fixation. This material was prepared for microscopic use under the direction of Dr. F. B. Mallory, and was studied and described by Dr. Councilman. The opportunity afforded at Gallup's island was invaluable, and did much in giving the several workers the acquaintance with the disease which is indispensable to its intelligent and effective study.

In the middle of January, 1902, the work was transferred to the Detention Hospital, and here consisted mainly in the study of the pathological anatomy of the disease, the collecting of anatomical and histological material, and in the study of the blood. It was carried on by Dr. Magrath, who for several weeks lived at the hospital. The opportunities at this hospital yielded the greater part of the material for anatomical and for histological study. Adequate laboratory facilities were provided for the incidental microscopic work.

The work of the following summer included the completing of the preparation of histological material and its study.

In the fall of 1902, with the increased prevalence of the disease, preparations were made for yet further studies at the Detention Hospital, and the following were taken up as subjects of the second winter and spring of the epidemic: (1) Experimental variola in the monkey; (2) the blood in variola; (3) the infectiousness of the "disc;" (4) the bactericidal complement of the blood. For the carrying on of this work more extensive laboratory facilities than had previously existed were needed, and were provided for in an out-building of the hospital by constructing a room in which were installed animal cages and all of the apparatus requisite for bacteriological and general microscopical work. This laboratory was under the immediate charge of Dr. Brinckerhoff, who lived at the hospital for five months. The studies on experimental variola in the monkey and upon the blood in variola were conducted by Drs. Magrath and Brinckerhoff; the study of the infectiousness of the "disc" was undertaken by Dr. Brinckerhoff; and that of the bactericidal complement of the blood by Dr. Thompson, who also lived at the hospital while carrying on his studies.

Coincidently with this work, Dr. Tyzzer, in the Sears Laboratory, took up the study of vaccinia, which from the beginning was felt to be an integral part of the study of variola.

In the summer of 1903 the construction of the life history of the variola organism, which we had described in a preliminary communication, was undertaken by Professor Calkins, who studied histological material already prepared and additional sections of the skin lesions.

At the same time, Dr. Southard, in the pathological laboratory of the Boston City Hospital, worked upon the pathological histology of the central nervous system in variola.

Throughout the epidemic Dr. Bancroft, resident physician at the Detention Hospital, studied the clinical aspects of the disease.

We recognize that in certain respects the work is incomplete. It will be seen that we have given comparatively little attention to the study of vesicle contents and to bacteriological examinations. Neither of these methods of research, pursued by competent observers, has led to positive knowledge of the etiology of the disease, and we assigned to them a place of minor importance in the research. The work on these subjects in our studies was entirely confirmatory of the results of the best investigators.

While it is true that much of the work upon special subjects depended upon the independent action of those in charge of it, it is certain that each man in many ways assisted the work of the others. In all cases it is difficult to say to whom should be given the credit for the most important contributions. The work of Dr. Tyzzer upon vaccinia was of the greatest value in the study of the parasite of variola, while the knowledge of the protozoa possessed by Professor Calkins made possible the grouping of forms of the parasite already observed, and others not detected in the original histological study of the skin lesions, but identified by him, into a life cycle.

We gratefully acknowledge our indebtedness, and desire to express our thanks to Dr. S. H. Durgin and his associates of the Board of Health Commissioners, for their hearty coöperation at all times; to Dr. Paul Carson, physician in charge of the Quarantine Hospital, and to Dr. T. B. Shea, physician in charge of the Detention Hospital, for their painstaking efforts to further the work of those of us making use of the material in their charge; to Dr. S. F. Cox, assistant physician at the Quarantine Hospital, and to Dr. I. R. Bancroft, resident physician at the Detention Hospital, for valuable assistance in collecting material; to the superintendents of both hospitals for many kindnesses; and to the health authorities of Fall River and of Philadelphia for timely favors.

We here express our thanks to the unknown benefactress, whose generous gift of one thousand dollars rendered possible the purchase of the apparatus and the supplies necessary for the research; to the generous donor of twelve hundred dollars whose gift provided for the illustrative plates accompanying this report; and to the Rockefeller Institute for Medical Research for two scholarships, held by Dr. E. E. Tyzzer and by Dr. R. L. Thompson.

Finally, we acknowledge our indebtedness to Dr. F. B. Mallory, for valuable suggestions, assistance, and criticism.

William T. Councilman.
George Burgess Magrath.
Walter Remsen Brinckerhoff.

Boston, Mass., Jan. 1, 1904.

I.

I. THE PATHOLOGICAL ANATOMY AND HISTOLOGY OF VARIOLA.

W. T. Councilman, G. B. Magrath, and W. R. Brinckerhoff.

Introductory. — This paper is based on material derived from fifty-four autopsies. Much of this was obtained during the hospital residence of those engaged in collecting it, a fact which insured prompt study and good preservation. In all cases the examination was as thorough as possible, and, as a rule, all of the organs of the body, regardless of the probable occurrence of lesions, were inspected.

Histological technic. — For histological study material was taken from the skin and from nearly all of the other organs of the body. As fixatives, alcohol, formaline, corrosive sublimate, and Zenker's fluid were used, in a few instances all of these; in the majority of cases, as the best for general histological purposes, Zenker's fluid alone. Material was embedded and cut in paraffine and stained by a variety of methods, chief among which were the eosin-methylene blue stain,[1] Mallory's iron hematoxylin followed by acid fuchsin and picric acid, Mallory's connective tissue stain, and a number of other special stains.

Bacteriological examinations. — Bacteriological examinations were made in several cases by means of cultures from the heart's blood, the spleen, the liver, and the kidneys.

Classification, statistics. — The forms of variola met with in this series of cases may be classified as follows:

A. Variola vera.
  (*a.*) Uncomplicated.
  (*b.*) With hemorrhage.

[1] Pathological Technique, Mallory and Wright, p. 296.

(*c.*) Abortive.
(*d.*) With complications.
B. Variola pustulosa hemorrhagica.
C. Purpura variolosa.

A. Variola vera (*a*) uncomplicated, presented in thirty-seven cases, nineteen males and eighteen females. Grouped according to age there were under five years of age, eleven; from five to twenty years, four; from twenty to fifty years, twenty; over fifty years, two. Grouped according to the duration of the disease, these cases fall into three periods: (1) of less than ten days' duration, seven cases; (2) from ten to thirteen days' duration, twenty-seven cases; (3) over thirteen days' duration, three cases. These periods correspond very closely with the first, the second, and the third weeks of the disease.

(*b.*) Variola vera, with hemorrhage, met with in two cases, both males about thirty years of age, who died on the tenth day of the disease.

(*c.*) Variola vera, abortive, met with in two cases, a female infant who died on the eighth day, from anemia, and a woman of forty-seven years who died on the thirteenth day from acute mania.

(*d.*) Variola vera with complications, represented by a group of six cases, in age ranging from infancy to thirty-nine years, fatal between the fourteenth and thirtieth day of the disease from such complications and sequelæ as noma, gangrene of the lung, pleuritis, broncho-pneumonia, and nephritis.

B. Variola pustulosa hemorrhagic, manifest in two cases, both males, one twenty-five years of age who died on the fourth day, and one thirty-seven years who died on the ninth day of the disease.

C. Purpura variolosa,[1] present in four cases, three males

[1] We include under this heading all cases of variola in which the purpura precedes the development of the eruption, and place in the class of variola pustulosa hemorrhagica those cases in which the eruption develops with or antedates the purpura.

and one female, from twenty-five to forty years old, who died between the fifth and seventh days of the disease.

The anatomical and histological findings will be presented under the head of the following organs:

I. Skin.
II. Mucous membranes.
III. Lungs.
IV. Liver.
V. Spleen.
VI. Lymph nodes.
VII. Bone marrow.
VIII. Testicles and ovaries.
IX. Kidneys.
X. Adrenal glands.
XI. Pancreas.
XII. Heart and arteries.

THE PATHOLOGICAL ANATOMY.

I. THE SKIN.

Variola vera (a) uncomplicated. — The lesions of the skin differ widely according to the duration of the disease at the time of death. Inasmuch as nearly seventy per cent of fatal cases die during the latter half of the second week, the general post-mortem picture is that of the late pustular or crusting stage. Seven of our series of thirty-seven cases were fatal during the first week. In the earliest of these the skin lesions consisted of papular elevations,[1] two to three millimeters in diameter, very slightly raised above the general level of the skin, and "shotty" to the feel. These lesions were fairly numerous on the forehead, cheeks, neck, shoulders, and the upper part of the chest, and were abundant on the backs of the hands and on the inside of the thighs; they were sparse on the abdomen. The palms and

[1] In the anatomical descriptions, the classical terms "papule" and "papular" will be used, although, as will be seen from the histological descriptions, the specific skin lesion of variola is from the first a vesicle.

the soles showed none. At this stage of the disease, about the fifth day, the lesions to the naked eye are not vesicular, but are opaque, reddish papules. Somewhat later the lesions on the face and the upper part of the trunk are vesicular, and papules appear on the lower legs. Toward the close of the first week confluence of the lesions on the face may be apparent. Edema of the face and the formation of crusts about the nostrils and the lips may also be observed at this time. In children who die during the first week the premature formation of crusts, due to the early, and in many instances the traumatic, rupture of vesicles formed in tender skin, is of frequent occurrence. In several children we observed the tendency of lesions upon the lower extremities to develop in groups, and also to differ in their size; in one instance the lesions upon the arms varying from one to four millimeters in diameter. A similar variability in size is also apparent in old people.

The second week includes what may be regarded as the lethal period of the disease, that from the tenth to the thirteenth day; within this, twenty-seven of our thirty-seven cases of variola vera died. At this time the face shows edema, often so marked as to produce great deformity. The prominent skin lesions of this region are crusts, which in size and in extent depend upon whether the pre-existing pustules were discrete or confluent. The face may show discrete crusts and scattered, filled out, or ruptured pustules, or a mask-like mat of crusts covering the face and the neck. Purulent conjunctivitis is sometimes present.

The skin of the trunk during this period presents as the predominant lesion the intact pustule, seldom umbilicated, interspersed with occasional ruptured pustules and crusts. The pustules are in general more numerous upon the upper than upon the lower part of the body. Upon the back they are evenly distributed. The axillæ, the flanks, and the groins are usually devoid of lesions. The number of the pustules depends on the severity of the case, and their condition in some degree on their position,—those on the dependent parts being ruptured earlier than others.

The skin of the extremities at this time presents likewise the pustule. Upon the arms the lesions are more abundant than elsewhere on the extensor surfaces and on the backs of the hands and wrists, in which position they show a marked tendency to confluence, a condition occasionally accompanied by the formation of large blebs filled with serous fluid. Palms, which possess a thick horny layer of the epidermis, show lenticular, copper-colored discs; those of children and others in whom this layer is thin show pustules, often more or less flattened. Upon the legs the lesions are most numerous on the inside of the thighs and upon the ankles and the dorsum of the foot, where they show a tendency to confluence and to the associated formation of blebs. Next in order to the regions named, the lesions are most numerous about the knees. They are few in number on the backs of the legs. The soles, like the palms, present discs, but elevated pustules are here comparatively rare.

Certain cases within this group presented characteristics differing from those of typical severe variola vera. In three such, an eruption of vesicles confluent in distribution was delayed in its evolution; the lesions did not fill out, and had failed to become pustular at the time of death. In these cases the lesions were small, about three millimeters in diameter, and so close-set as to leave practically none of the skin intact. Instances were observed of the grouping of lesions about an abrasion received during the stage of incubation, and in one case of the occurrence of numerous pustules in the flanks and the groins. The grouping of lesions along lines of pressure upon the skin, as beneath a waist-band, was repeatedly observed.

The third group of cases includes those fatal in the third week of the disease, and later. The skin of the face at this time is marked by copper-colored or reddish areas, at first more or less elevated and scaly, later becoming colorless pits.[1] The skin of the trunk and of the extremities shows

[1] This primary, irregular elevation of the skin at the site of the healed lesion was most marked in the negro.

markings of similar character, which gradually lose their color. Immediately after the falling off of the crust the sites of the lesions are somewhat elevated and rough. The palms and soles show usually reddish-brown discs, the color depending upon thickness of the epidermis.

The following are protocols of autopsies on typical cases of variola vera:

CASE NO. XLVIII. — Clinical diagnosis: variola vera (twelve days' duration).

Anatomical diagnosis. — Variola vera (late pustular, crusting); erosions of the palate; focal lesions of the testicle; edema of the lungs; cloudy swelling of the kidneys; sclerosis of the epicardium; chronic adhesive pleuritis.

BODY. — That of a man forty-five years of age; slender; length one hundred and seventy centimeters; fairly well nourished; rigor mortis present; some post-mortem lividity of dependent parts. Eyelids firmly interadherent; edges somewhat crusted. The skin lesions are fairly evenly distributed, and consist in general of umbilicated pustules, with occasional crusts.

The scalp and face present flattened and more or less umbilicated pustules, fairly thickly set, of an average diameter of five millimeters; the forehead and nose show many dry, yellowish crusts. The neck presents closely-set, umbilicated pustules without crusts.

The trunk is covered with pustules of the same general character as those above described, the frequency of the lesions is about one to every centimeter square; upon the back the lesions are evenly distributed; upon the anterior aspect of the body they are most abundant upon the upper part of the chest and the shoulders; the abdomen presents few lesions, about fifty pustules occurring upon the skin of the epigastric region; there are but few lesions in the flanks, and none in the groins. The prepuce, glans penis, and scrotum show flattened pustules and crusts.

The arms present pustules all more or less flattened and umbilicated, of an average diameter of five millimeters, most thickly set upon the forearms, where they are in places confluent; the back of the right hand shows an old abrasion covered by a hard, red crust; around this lesion pustules are crowded and confluent. The palms are covered with very thick epidermis, and show many brown discs.

The legs present lesions of the same sort as those upon the trunk, thickly set upon the front of the thighs, about the knees, and upon the dorsa of the feet. In the latter position some of the lesions are of large size, six to eight millimeters in diameter. The soles show a few discs, most numerous upon the hollow of the instep.

Upon section, subcutaneous fat three-tenths centimeters in thickness; muscles red and firm. Vermiform appendix normal.

Mesenteric lymph nodes normal.

THORAX. — Diaphragm in normal position.

Pleural cavities: left normal; right partially obliterated by old fibrous adhesions to the diaphragm, and in the anterior part of the cavity.

Pericardial cavity normal.

Heart: size, normal; upon the anterior surface is a circular white patch, three centimeters in diameter. Upon section, myocardium red and fairly firm; auricles contain a moderate amount of red and yellow clot. Valves and cavities normal. Coronary arteries normal.

Lungs: alike, present moderate carbon pigmentation; general color, gray-red; crepitant throughout; upon section, cut surface under pressure yields a moderate amount of bloody, frothy fluid, surface gray red and homogeneous. Bronchi upon section contain frothy mucus; mucous membrane red. Bronchial lymph nodes black.

ABDOMEN. — Peritoneal cavity normal.

Spleen: weight two hundred grams; capsule smooth; surface blue red; upon section cut surface presents a mottled appearance, the large and more irregular areas gray red, the smaller and round, or oval, intervening areas deep blood red; consistence slightly lax; pulp not increased.

Stomach and intestines negative.

Pancreas normal.

Liver: weight one thousand six hundred grams; surface smooth; general color brown; upon section markings visible; consistence normal. Gall bladder distended, with thin bile.

Kidneys: weight of both three hundred grams; capsule strips easily from a smooth surface; upon section, markings distinct; cortex six-tenths centimeters in thickness; glomeruli visible as pink points; cut surface, gray red, slightly opaque. Adrenal glands normal. Bladder normal.

Testicles: size, normal; to the touch they are distinctly nodular; upon section the cut surface presents multiple, not very sharply-defined areas which project above the level of the surface about one millimeter; these areas are of an average diameter of two to three millimeters; they are fairly evenly distributed throughout the body of the testicle, and their frequency is about one to the centimeter square; their contour is round or oval, sometimes irregular; in appearance they differ but little from the surrounding substance of the testicle.

ORGANS OF THE NECK.— The mucous membrane of the palate, pharynx, tonsils, and the root of the tongue is overlaid with thick, tenacious, yellow mucus; upon removal of this, the mucous membrane of the palate is thick and white, presenting here and there shallow erosions which have a reddish base. Similar conditions are present upon the uvula. Esophagus normal. Trachea shows a mucous membrane dark red, and overlaid with tenacious mucus. Larynx normal.

CASE No. XV. — Clinical diagnosis. —Variola vera (twelve days' duration).

Anatomical diagnosis. — Variola confluens, crusting on face, pustular and vesicular elsewhere; edema of the lungs; focal necrosis of the testes; focal lesions of the pharynx, trachea, esophagus, and urethra; acute laryngitis, tracheitis, and bronchitis; phlegmonous inflammation of neck and anterior mediastinum.

Body of a well-developed and well-nourished man, thirty-six years of age. Rigor mortis present and fully developed.

The face and neck present a confluent mass of soft, moist, brown crusts. The nostrils are almost occluded by crusts. Left eye is absent (old injury). Right eye and conjunctiva normal, pupil of normal size.

Over the trunk there are numerous late vesicles and early pustules. The lesions are four to eight millimeters in diameter, are of the color of the skin, show a marked umbilication, and sometimes have a slightly oval outline. These lesions are not very closely set (about one to a centimeter square).

On the arms and legs are many lesions like those on the body, but they are more closely set and somewhat larger, particularly on the dorsum of the hands. On the penis and scrotum are a considerable number of similar lesions.

On the thighs are closely set, umbilicated vesicles like those described on the body. The lesions here are about two or three to the centimeter square. On the legs and dorsa of the feet the lesions are fewer in number and are less umbilicated. There is no clustering of the lesions, and in the groins and flanks they are comparatively few in number. There is a purulent balanitis.

On section, subcutaneous fat three centimeters thick. Muscles deep red. Mesenteric lymph nodes and appendix normal.

THORAX. — Pleural cavities: surfaces normal. Pericardial cavity normal, contains a few centimeters of clear, straw-colored fluid.

Heart: weight, two hundred and sixty grammes. Myocardium pale brown red. Right ventricle flabby and filled with yellow clot. Left ventricle firmly contracted. Valves and cavities normal.

Lungs: on the outer aspect of the left lower lobe is an area of thickened pleura one and a half centimeters across, which is of a pearly-white color and with a serrated border. Lungs alike. Crepitant throughout. On section, cut surface mottled light and deep pink with distinct carbon markings. Surface, on gentle pressure, yields considerable clear fluid containing innumerable bubbles of air. Bronchial mucosa normal. Bronchi contain stringy mucus.

ABDOMEN. — Peritoneal cavity normal.

Spleen: weight, two hundred grams. Color, deep purple. On section cut surface deep red, Malpighian bodies visible as gray points; trabeculæ normal. Consistency is firm, and little substance adheres to knife on gentle scraping.

Pancreas normal.

Stomach: some diffuse ecchymoses along lesser curvature. Mucosa normal.

Intestines: lower part of ileum normal.

Liver: weight, two thousand six hundred and fifty grams. Surface smooth and of a yellow-brown color. On section, markings indistinct. Consistency normal. On the surface are irregular yellow areas which are contoured in such wise as to suggest aggregations of fatty lobules. On section similar markings are found. Gall bladder normal.

Kidneys: weight, three hundred and fifty grams. Surface yellow-brown. Capsule strips readily, leaving a smooth surface. On section markings are indistinct. Glomeruli visible as gray points. Cortex of normal thickness and of a yellow-brown color. Pyramids reddish brown.

Adrenals normal. Bladder normal.

Genitals: urethra. About midway in the penile portion is an elevated area two millimeters across, with an irregular, oval outline and with a central depression. Elsewhere urethra is normal. Seminal vesicles and epididymis normal. Prostate normal. Testes: Tunica vaginalis normal, slightly nodular to the feel. On section the markings are distinct, but scattered over the cut surface are nodular elevations one to three millimeters across, which are redder than the surrounding tissue. These areas are not sharply circumscribed, and in them the tubular markings are visible.

Lymph nodes of the groin are enlarged, red, and somewhat hard. (Largest, two centimeters across.)

Aorta normal.

Organs of Neck. — Soft palate, uvula and pharynx show low, gray, nodular elevations, some of which present a central superficial loss of substance.

Epiglottis thickened and similarly beset with nodules.

Larynx contains much gray mucus and its mucous membrane is reddened. In the trachea are numerous elevations from one to three millimeters in diameter, surrounded by a red ring. These elevations are conical and are occasianally eroded. About thirty such lesions of various sizes are present. Esophagus, upon its mucous membrane, presents many pale gray, oval or circular elevations from one to three millimeters in diameter. The largest of these is a low cone of a translucent gray color surrounded by a narrow elevated rim like the hull of an acorn; this surrounding rim is red.

The deep tissues of the neck on the right side are infiltrated with a grayish material, and on section a considerable amount of turbid, slightly blood-stained fluid exudes. The jugular vein is filled with a yellow clot and its wall appears normal. The muscles close about the larynx are in part friable and of an opaque, yellow-brown color. The fibrillary markings are indistinct. The tissue of the anterior mediastinum is edematous, and on section a cloudy, faintly blood-stained fluid exudes. This condition is most marked over the precordium and about the remnant of the thymus. Left side of neck normal.

Bone marrow: in general yellow with many areas of deep red color.

Variola vera (b) with hemorrhage (two cases, adult males, death on the tenth day). — Under this head we group cases in which the lesions of typical severe variola vera are complicated by hemorrhage. In one case the skin lesions were chiefly pustular, and in one vesicular. In the latter the lesions were for the most part filled with blood or bloody fluid, and scattered petechiæ were present on the legs. In the other, in addition to the close set pustules, were bullæ, two to three centimeters in diameter, and three to five millimeters in elevation, of dark reddish-brown or black color, filled with cloudy or with bloody fluid, and splitting groups of pustules horizontally.

Variola vera (c) abortive. — In this form of the disease death is due to some intercurrent cause. The skin lesions are exceedingly few in number, from twelve to forty by actual count in our cases (two), and show a tendency to rapid evolution from vesicle to crust.

Variola vera (d) with complications. — In four of the six cases falling within this group death occurred during or later than the third week; in the remaining two, fatal on the fourteenth day, the lesions of the skin were such as have already been described.

Variola pustulosa hemorrhagica (two cases, adult males; death on the fourth and on the ninth day). — The skin of the face is edematous; in the early cases it shows a bluish-gray pallor or a pale, gray-red or blue-red flush, together with red and blue petechiæ, one to five millimeters in diameter; in the later cases it presents gray-white papules and vesicles. Crusts are present about the eyelids, nares, and lips. The skin of the trunk shows vesicles, close-set and white, present on the chest and on the abdomen. The skin of the shoulders and the arms resembles that of the trunk; in the early cases petechiæ occur on the inner side of the arms. The palms in each instance showed no lesions. The skin of the lower extremities in the earliest cases is of a deep, purple-black color, due to the presence of close-set petechiæ, many of which are surmounted by gray vesicles. In both cases the skin of the lower legs was marked by punctate hemorrhages, which in some instances bore small vesicles. In the later cases the vesicular character of the lesions is more apparent. In all cases the soles showed no lesions.

The following is a protocol of an autopsy on a typical case of variola pustulosa hemorrhagica:

CASE No. XX. — Clinical diagnosis. — Variola pustulosa hemorrhagica; Hematuria.

Anatomical diagnosis. — Variola pustulosa hemorrhagica; multiple erosions of the palate and pharynx; ecchymoses of the stomach; hemorrhagic pyelitis; acute splenic tumor; cloudy swelling of the myocardium, the liver, the kidneys; edema of the lungs; acute bronchitis; acute vegetative endocarditis (mitral); infarction of the kidneys; tuberculosis of the lungs; chronic adhesive pleuritis.

BODY. — That of a man of twenty-five years of age; unvaccinated; length one hundred and eighty-five centimeters; powerfully built; well nourished; rigor mortis present; lividity of dependent parts. Face presents a bluish-gray pallor; the rest of the body shows a dusky bluish-red flush. The face is swollen, and there are numerous excoriations about the alæ nasi and lips. The skin of the face is thickly studded with grayish-white, extremely confluent, papular elevations two to four millimeters in diameter, in some instances slightly umbilicated; nearly all of these upon section contain a small amount of clear fluid. The skin of the neck and shoulders, and the upper and front part of the chest presents lesions similar in character to those upon the face, the vesicles so closely set that no normal skin intervenes; these regions present also occasional bluish-red elevations of about the same size. The lesions are sparse on the abdomen; they are infrequent in the flanks and in the groins; on the hands they are large and closely set, and are here notably transparent. Upon the prepuce the vesicles are large, thickly set, transparent, and more or less umbilicated.

The skin of the arms is thickly studded with papular elevations one to four millimeters in diameter, no normal intervening skin apparent. The skin of the thighs and legs presents lesions for the most part papular, two to eight millimeters in diameter, gray red, in some instances vesicular and umbilicated; interspersed with these are small, pale elevations about one millimeter in diameter; the larger vesicles are surrounded by a prominent red zone. The lesions are very closely set about the knees. Upon the legs the lesions are purpuric in character, consisting of red macules from pin-point size to four or five millimeters in diameter; some of them slightly elevated; they are most numerous upon the front of the legs. The dorsa of the feet show papules one to six millimeters in diameter. The palms and soles are negative.

Upon section, subcutaneous fat three centimeters in thickness; muscles thick, red, and firm. Vermiform appendix and mesenteric lymph nodes normal.

THORAX. — Height of diaphragm fifth rib on right side, sixth on left.

Pleural cavities: both partially obliterated by old fibrous adhesions, which are most marked in the upper part of the right cavity.

Pericardial cavity normal.

Heart: estimated weight three hundred and seventy-five grams; upon section, myocardium red, and somewhat opaque. The free edges of the curtains of the mitral valve present small, pink, granular elevations; otherwise valves and cavities normal. Coronary arteries normal.

Lungs: right upper lobe presents extensive thickening of the pleura, and is closely adherent to the wall of the chest; this part of the lung upon section shows several areas of caseation about three centimeters in diameter, invested in a tough, fibrous connective tissue; no tubercles are apparent. Both lungs upon section show considerable edema; the bronchi are of a dirty, gray-red color, the mucous membrane apparently thickened; upon pressure they yield considerable muco-purulent material.

ABDOMEN. — Peritoneal cavity normal.

Spleen: moderately enlarged and somewhat flabby; upon section, markings vague, pulp a little increased.

Stomach: upon section the mucous membrane presents numerous red areas, evenly distributed, two to eight millimeters in diameter; otherwise negative.

Intestines negative.

Pancreas normal.

Liver: size normal, brown red; upon section markings vague; surface of section opaque; consistence rather friable. Gall bladder normal.

Kidneys: rather large; upon section capsule strips easily from a smooth surface; markings distinct; surface of section opaque and pale, somewhat edematous; the pelvis of the right kidney contains a small amount of bloody fluid; its mucous membrane is smooth, glistening, and of a deep cherry-red color, upon section showing extensive hemorrhage into the submucosa; the left kidney shows a small, recent, cortical infarct; its pelvis normal.

Adrenal glands normal.

Bladder normal.

Testicles: upon section no focal lesions apparent; tubules unravel easily.

Aorta normal.

ORGANS OF THE NECK. — The mucous membrane of the soft palate, the tonsils, the uvula, and the upper part of the pharynx is swollen, rough, of a dirty, grayish-green color, and in places shows shallow erosions.

Larynx and trachea: mucous membrane red and edematous; that of the trachea presents appearances similar to those seen in the pharynx. Esophagus negative.

Bone marrow: that of the femur yellow, mottled with reddish areas of small size.

Bateriological examination. — Cultures from the heart, liver, and kidney show the streptococcus pyogenes.

Purpura variolosa (four cases; all adults, three males, one female, duration five to seven days). — The skin of the face shows a dusky, bluish-red flush, deepest about the neck and ears; a few small crusts may be present about the alæ nasi. The skin of the trunk presents a general terra-cotta-red or bluish-red flush, deepest upon the upper part of the chest and upon the dependent parts, together with sparse, vague, bluish splotches, two to four millimeters in diameter, rather more numerous than elsewhere in the flanks. The skin of the arms shows a faint red mottling; in one case that of the upper arms showed five or six small papular elevations. The palms in all showed no lesions. The skin of the legs resembles that of the arms, and like the palms, the soles are negative. The epidermis strips off readily in large sheets, leaving a moist, glistening surface.

The following are protocols of autopsies on typical cases of purpura variolosa:

Case No. XLIII. — Clinical diagnosis. — Purpura variolosa (five days' duration).

Anatomical diagnosis. — Purpura variolosa; subserous punctate hemorrhages; cloudy swelling of the heart, liver, and kidneys; punctate hemorrhages of the stomach; hemorrhagic pyelitis.

Body. — That of a man about forty years of age; length one hundred and seventy-five centimeters; powerfully built; well nourished; body warm; rigor mortis absent; pupils equal and widely dilated.

The skin of the entire body is of a dull, bluish, brick-red color; this color is somewhat deeper upon the dependent parts than elsewhere, and the dusky blue tinge most marked about the ears and upon the sides and back of the neck. The skin is dull and lusterless and presents a somewhat velvety appearance, although it is not especially rough to the touch. The epidermis is readily separable from the cutis, leaving a raw, moist surface.

Upon the arms and legs there is a suggestion of fine red mottling which upon pressure becomes more apparent. Here and there upon both trunk and extremities are faint bluish spots of irregular outline, three to five millimeters in diameter; these are most prominent in the flanks. No other lesions are present upon the surface of the body.

Upon section, subcutaneous fat one and five-tenths centimeters in thickness; muscles well-developed, red, and firm. Mesenteric lymph nodes normal. Vermiform appendix normal.

THORAX. — Height of diaphragm fourth rib on right side, fifth on left. Pleural cavities normal. Pericardial cavity normal.

Heart: estimated weight three hundred and seventy-five grams; the epicardium presents multiple punctate hemorrhages, most numerous along the course of the right coronary artery. Upon section, myocardium red, opaque; blood, fluid; valves and cavities normal. Coronary arteries normal.

Lungs: alike; crepitant throughout; the visceral pleura presents many punctate hemorrhages, most numerous upon the interlobar surfaces; upon section, moderately edematous; bronchi pink, containing frothy mucus.

ABDOMEN. — Peritoneal cavity normal.

Spleen: size normal; free surface bluish red; upon section dark red, follicles visible, but not prominent; surface moist; consistence firm; pulp not notably increased.

Stomach: the peritoneum presents fairly numerous punctate hemorrhages; upon section the mucous membrane is mottled with cherry-red spots two to three millimeters in diameter, and fairly thick set; these are distributed throughout the whole organ and apparently extend into the submucosa. Intestines negative. Pancreas normal.

Liver: surface smooth, presenting here and there small punctate hemorrhages; upon section, brown-red, cut surface opaque; markings vague; consistence normal. Gall bladder normal.

Kidneys: estimated weight three hundred and twenty-five grams; upon section capsule strips easily from a smooth surface; cortex five-tenths centimeter in thickness; markings fairly distinct; cut surface opaque gray-red. The pelvis of each kidney presents a deep, cherry-red surface, slightly roughened; the mucous membrane thickened and edematous; no free blood is present in the cavities. Adrenal glands normal. Bladder normal.

Testicles: upon section no focal lesions apparent. Aorta normal.

ORGANS OF THE NECK. — Mucous membrane of the pharynx and soft palate, uvula, and larynx injected and overlaid with mucus; no focal lesions apparent. Esophagus normal. Trachea shows injection of mucous membrane. Cervical lymph nodes somewhat enlarged and red.

Cranium: brain, moderately congested, otherwise not remarkable.

Naso-pharynx: mucous membrane normal.

Bacteriological examination. — Culture from the heart's blood shows streptococcus pyogenes.

CASE NO. LIV. — Clinical diagnosis. — Purpura variolosa (five days' duration).

Anatomical diagnosis. — Purpura variolosa; punctate hemorrhages of the serous membranes, stomach, intestines, bladder, and kidney; hemorrhagic pyelitis; hemorrhagic endometritis; multiple cysts of the ovaries; corpus hemorrhagicum; fatty degeneration of the kidneys.

BODY. — That of a woman about thirty-five years of age; length one hundred and sixty centimeters; well-developed and nourished; rigor mortis present; slightly warm; post mortem lividity of the back and other dependent parts; eyelids interadherent; conjunctivæ blood-shot; pupils equal three-tenths centimeter in diameter.

The skin of the face and neck presents a faint, general bluish-red flush, most marked in the lateral cervical region; the lips are covered with dry blood clot; the skin of these parts is further marked by rather sparse red spots from pin-point to five millimeters in size; interspersed with these are larger, somewhat vague, bluish areas two to four millimeters in diameter; these are rather more numerous than elsewhere upon the cheeks and beneath the chin.

The skin of the trunk shows upon the anterior aspect of the chest and upon the abdomen closely-set, punctate areas, pin-point to two millimeters in diameter, red, and without sensible elevation. The abdomen is marked by numerous striæ atrophicæ; the striæ are the seat of confluent areas of the same sort, so closely set as to give the appearance of red, interlacing bands, two to three millimeters across. Interspersed with punctate areas are bluish-red spots, ill-defined, two to seven millimeters in diameter, placed four to five centimeters apart; more numerous than elsewhere upon the abdomen, between the striæ. The skin in these regions exhibits, in addition, a faint, general, terra-cotta-red flush.

The skin of the arms is similar to that of the trunk; the forearms show a great number of bluish-red spots; in this region these occur at intervals of from one to two centimeters.

The lower extremities show upon the thighs confluent areas of red macules, the larger areas from five-tenths to one and five-tenths centimeters in diameter. These areas show a slight degree of elevation, and are sensibly rough. The fronts of the thighs, in addition, show, in the larger of these red areas, faint pearly elevations from one to two centimeters in diameter, barely visible, but distinct to the touch. The groins and the upper part of the thighs show a diffuse terra-cotta-red flush. The knees are free from lesions. The legs show sparse punctate areas, from one to two millimeters in diameter, interspersed with which are other lesions, some not hemorrhagic, slightly elevated, pearly, and of the same size. The backs of the legs are shotty to the touch. The ankles and dorsa of the feet show scattered punctate hemorrhages. The lesions are more numerous upon the inner than upon the outer aspect of the legs.

Upon section, subcutaneous fat two centimeters in thickness; vermiform appendix normal; mesenteric lymph nodes slightly reddened.

THORAX. — Position of diaphragm natural.

Pleural cavities normal.

Pericardial cavity contains about thirty cubic centimeters clear, straw-colored fluid.

Heart: weight, two hundred and seventy-five grams; epicardium shows sparse punctate hemorrhages; upon section cavities contain yellow clot; myocardium pale red, firm; wall of left ventricle shows an area of hemorrhage two millimeters in diameter. Valves and cavities normal. Coronary arteries normal.

Lungs: alike; pleural surface presents numerous areas, mostly cherry red, some blue-red, two to four millimeters in diameter, numerous upon the interlobar surfaces; the backs show a faint, general bluish-red color; crepitant throughout; upon section, red and moist; bronchi show some general reddening of the mucous membrane, with occasional areas of diffuse hemorrhage.

Diaphragm of the left side shows diffuse hemorrhages into its substance.

ABDOMEN. — Peritoneum shows sparse punctate hemorrhages.

Spleen: size normal; capsule tense; upon section dry, beefy red; trabeculæ visible, follicles indistinct, pulp not increased.

Stomach: upon section the mucous membrane shows evenly distributed punctate hemorrhages, from one to two millimeters in diameter; it is overlaid with tenacious brown mucus. The peritoneal surface of the anterior aspect near the pylorus shows an area of hemorrhage three centimeters in diameter, extending down into the duodenum.

Intestines: upon section, the lower part of the ileum shows faint punctate hemorrhages at the site of the solitary follicles; elsewhere the mucous membrane shows diffuse reddening. The colon shows fairly numerous areas of hemorrhage from one to three millimeters in diameter at the site of the solitary follicles; the follicles present no sensible elevation.

Liver: size normal; surface smooth, save at the anterior border where it is slighlty granular; upon section markings visible, general color liver brown, consistence normal. Gall bladder contains thick, dark bile.

Kidneys: size normal; free surface shows occasional capsular hemorrhages, from one to two millimeters in diameter; upon section capsule strips easily from a smooth surface; markings vague, pale; cortex seven-tenths of a centimeter in thickness; surface of section opaque; mucous membrane of the pelvis dark cherry red and slightly thickened; consistence flabby.

Adrenal glands normal.

Bladder: upon section mucous membrane shows sparse punctate hemorrhages, with a larger spot of cherry-red color, four millimeters in diameter, at the trigonum.

Genitalia: uterus upon section shows the endometrium overlaid with dark red clot. Ovaries, both cystic; the right contains a corpus hemorrhagicum.

Aorta upon section shows a few small yellow plaques just above the aortic valves.

ORGANS OF THE NECK. — Tongue: the outer half shows marked dessication of the dorsum, the surface dark brown-red, and crusted. Pharynx, soft palate: mucous membrane dusky gray-red, overlaid with brown mucus; no erosions present. Esophagus: mucous membrane shows oval, diffuse, bluish red areas, two to five millimeters in diameter. Larynx negative. Trachea: mucous membrane of the dorsal aspect shows extensive areas of hemorrhage, two centimeters above the bifurcation. Thyroid gland normal.

Bone marrow of femur for the most part yellow, flecked with red.

Cranium: brain shows moderate injection of the vessels of the pia; upon section normal. Spinal cord negative.

II. THE MUCOUS MEMBRANES.

Naso-pharynx. — Examination in three cases of variola vera fatal during the first week showed in two, some injection of the mucous membrane; in the other, and in one case fatal during the lethal period, the examination was negative. Examination in one case of purpura variolosa likewise gave negative results.

Palate, uvula, pharynx.[1] — In variola vera, in cases fatal during the first week, the mucous membrane of these regions is swollen and edematous, usually overlaid with tenacious mucus, and of a dirty, gray-red color. It may further show papules, more commonly shallow erosions, about two millimeters in diameter and slightly elevated. These may occur upon the uvula. In cases fatal during the second week the erosions are somewhat larger and more prominent; they are variously distributed, but are somewhat more numerous than elsewhere on the soft palate and on the posterior and lateral walls of the pharynx. In the later stages of the disease the conditions may be normal, or the lesions be limited to superficial erosions.

In variola pustulosa hemorrhagica the mucous membrane of these parts presents appearances varying between simple injection and edema to marked swelling with multiple erosions, occasional vesicles, and more or less general necrosis.

[1] In one case, that of a child, diphtheria was present as a complication.

In one case of purpura variolosa the mucous membrane was injected and overlaid with tenacious mucus.

Esophagus. — Early cases of variola vera usually show no lesions of this organ; in one such fairly numerous, grayish-white, papular elevations from two to four millimeters in diameter were distributed over the mucous membrane of its upper half. Of twelve cases fatal during the second week, in nine the esophagus was normal; in three it presented papular elevations, more or less eroded at the center. In the late stages erosions were seen once or twice.

In variola pustulosa hemorrhagica the esophagus is either negative or marked by injection and occasional small submucous hemorrhages.

In purpura variolosa no lesions were observed.

Larynx and trachea. — In the earlier stages of variola vera the mucous membrane of these organs is either normal or is swollen and injected. In those of our cases fatal during the lethal period the larynx was either normal or more or less edematous, and occasionally the seat of shallow erosions. Erosions upon both surfaces of the epiglottis were noted in a case dying on the twelfth day. The trachea is usually normal, in some instances its mucous membrane is injected and covered with frothy mucus; in others it presents either nodular elevations from two to three millimeters in diameter, or small, superficial erosions. The latter conditions were observed in four of our cases. In the later stages of the disease these organs are usually normal; lesions, if present, take the form of erosions.

In variola pustulosa hemorrhagica the trachea presents erosions and ecchymoses of its mucous membrane; in one instance it showed an extensive area of submucous hemorrhage just above the bifurcation.

In purpura variolosa these organs were negative.

Gastro-enteric tract. — The mucous membrane of the stomach and of the intestines in variola vera was normal in all but four of our cases; in three, fatal during the second week, punctate hemorrhages were present in the stomach along its lesser curvature, and in one the mucous membrane of the cardia showed slightly elevated, opaque, yellow areas, two to three millimeters in diameter.

In three cases of variola pustulosa hemorrhagica, in one the stomach and the intestines were negative; in two the stomach presented punctate hemorrhages two to eight millimeters in diameter, scattered over its mucous membrane; and in one similar hemorrhages appeared in the solitary follicles of the ileum and colon.

In purpura variolosa the mucosa of both the stomach and intestines shows thickly set, evenly distributed, punctate hemorrhages about two millimeters in diameter.

Bladder. — In variola vera the mucosa of the bladder is uniformly normal; in a case of variola vera, complicated with hemorrhage, it presented together with punctate ecchymoses red pointed elevations one to two millimeters in diameter. The latter may also be present in variola pustulosa hemorrhagica, although in two out of three cases of this form of the disease the bladder was normal. In purpura variolosa no lesions of the bladder were found.

Urethra. — Examination of the urethra in one case of variola vera fatal during the second week showed, in the penile portion, a colorless elevation of the mucosa two millimeters in diameter.

Uterus. — In general no lesions were apparent. In a case of variola vera, fatal from metrorrhagia, on the fourth day of the disease the uterus contained a moderate amount of red clot, the greater part of which was closely adherent to the endometrium. In purpura variolosa our single case of this type of the disease in the female showed submucous hemorrhages of the endometrium.

III. THE LUNGS.

Variola vera (*a*) *severe.* — In the cases fatal during the first week broncho-pneumonia (macroscopic) was observed twice, edema three times, and atelectasis with acute bronchitis once. In the cases grouped within the lethal period, the following pulmonary conditions occurred with the frequency indicated: edema (6), acute bronchitis (1), atelectasis and broncho-pneumonia (3), broncho-pneumonia (4), atelectasis (4), lobar pneumonia (2), peribronchial tuberculosis (1), broncho-pneumonia with multiple abscesses (1), atelectasis and edema (1), atelectasis and abscess (1), subpleural ecchymoses (1). In one instance a vesicular elevation in the mucosa of a bronchus was observed.[1]

In the later stages of the disease, constituting fatal complications, were seen pleurisy, broncho-pneumonia, and gangrene.

Variola vera (*b*) *with hemorrhage.* — In one instance the mucosa of the bronchi showed small, indefinite, pale yellow, slightly elevated areas.

Variola pustulosa hemorrhagica. — Among the cases of this group occurred broncho-pneumonia (1), edema (1), subpleural punctate hemorrhages (2), edema of the bronchial mucosa (2), occasional diffuse hemorrhages into the bronchial mucous membrane (1).

Purpura variolosa. — In this form of the disease the lungs show sub-pleural punctate hemorrhages and moderate edema, with some reddening of the bronchial mucosa.

IV. THE LIVER.

In every type of variola the liver may be somewhat increased in weight; in some instances this condition is well marked.

Variola vera. — In the uncomplicated form of the disease cases fatal during the first week present either no changes in

[1] Microscopic examination showed this to be an elevation of the epithelium caused by exudation, and not a specific lesion.

the liver, or cloudy swelling and fatty metamorphosis. During the second or lethal period these degenerated changes are more common and more pronounced. In rather less than one-third of the cases falling within this period the liver was normal. Later in the disease occur fatty metamorphosis and small, opaque areas suggesting focal necrosis. The gall bladder was normal in all cases. In abortive cases the liver showed no lesions. In variola vera complicated with hemorrhage the liver shows some cloudy swelling and fatty metamorphosis. In cases of variola vera with later complications the liver showed usually the same lesions.

Variola pustulosa hemorrhagica.—In this type of variola the liver shows few changes; in two of our three cases it presented cloudy swelling.

Purpura variolosa.— In one case the liver was of a dull, olive green color; in all it showed cloudy swelling.

V. THE SPLEEN.

Variola vera.— Of the cases fatal within the first week of the disease, in five the spleen showed no changes; in two slight enlargement. Of those fatal within the second week, in ten it showed no changes; in seventeen some enlargement, and in twelve enlargement, diminished consistency, and increase in pulp. The appearance of the follicles is not characteristic. Sometimes they are of ordinary prominence; sometimes vague. The color is within normal limits, varying from bright red to purple. Late in the disease the spleen is generally normal.

In the abortive type the spleen is normal.

In variola vera complicated with hemorrhage some increase in pulp and in the size of the follicles was observed.

In cases with late complications a moderate degree of splenic tumor was noted.

Variola pustulosa hemorrhagica.— In two cases the spleen showed some enlargement; in one the organ was normal.

Purpura variolosa. — In this type of the disease the spleen may show hemorrhages into the capsule and some increase in size.

VI. THE LYMPH NODES.

Variola vera. — In uncomplicated cases which died during the first week of the disease the mesenteric nodes were usually normal; in two instances they were somewhat enlarged; the cervical nodes in one case were tuberculous. When death occurs in the second week the mesenteric nodes, and occasionally the cervical, the aortic, and the inguinal, may show some swelling. In cases fatal during the third week changes in the lymph nodes are rare; in one the cervical nodes were somewhat enlarged.

In the abortive form of the disease the lymph nodes were normal.

In variola vera with hemorrhage the inguinal and cervical lymph nodes were in one case enlarged and red.

In variola vera with complications similar conditions were occasionally noted in the cervical and in the aortic lymph nodes.

Variola pustulosa hemorrhagica. — The bronchial and the cervical lymph nodes in two instances were enlarged and red; in other cases the lymph nodes in general were negative.

Purpura variolosa. — In this form of variola the cervical and inguinal lymph nodes presented conditions similar to those found in variola pustulosa hemorrhagica.

VII. THE BONE MARROW.

The marrow of the femur was examined in many of our cases, but, as a rule, segments of the vertebræ were relied upon for histological study, and these showed no macroscopic lesions.

Variola vera. — Uncomplicated cases fatal during the first week of the disease, in four instances presented changes in the bone marrow. The marrow of the femur was red, brown to reddish yellow in color, or was mottled with large, gray-red, soft spots. When death occurs in the second week the marrow may be normal. In nine cases fatal within this period it was of a gray pink, or dark pink color, or was marked by red streaks, or mottlings, occasionally with grayish-yellow areas two millimeters in diameter. Similar mottlings were observed in one case fatal in the third week.

In abortive variola the marrow was negative.

In variola vera with hemorrhage the yellow marrow in one case showed grayish red mottlings.

In variola vera with complications the marrow in three cases was red, and in two red and soft.

Variola pustulosa hemorrhagica. — The marrow in this type of variola is red, mottled with yellow splotches.

Purpura variolosa. — In this form of the disease no changes in the bone marrow were observed.

VIII. THE TESTICLES AND THE OVARIES.

Variola vera. — Among cases fatal during the first week the testicles in two instances were normal, and in one showed focal lesions apparent upon the cut surface in the form of slightly elevated, pink areas about two millimeters in diameter. At this stage of the disease the ovaries were in all cases normal. With death during the second week, the testicles in about half the cases showed focal lesions. These, in general, appear as areas from one to four millimeters in diameter, pink, gray, or grayish yellow, opaque, and slightly elevated above the surface of section; they are not sharply defined from the substance of the organ, are evenly distributed, and may have a shotty feel. The ovaries in four out of thirteen cases showed focal lesions of the same general character as those in the testicles. Among cases fatal late in the disease, focal lesions of the testicles occurred in two out of six; the ovaries were normal.

Our cases of abortive variola vera were females, and the ovaries in these were normal.

In variola vera with hemorrhage the testicles in both cases showed lesions; in one the surface showed nodules three millimeters in diameter and one millimeter in elevation, sharply defined, yellow and hard, upon section vague in outline, with a reddish center. In the other case, one testicle showed small, shotty nodules of the same sort.

Variola pustulosa hemorrhagica. — In one case of this type the testicles showed doubtful lesions in the form of small, opaque areas. In this form of the disease no lesions were noted in the ovaries.

Purpura variolosa.— In this type of variola the testicles and the ovaries were in all cases negative.[1]

IX. THE KIDNEYS.

The lesions of the kidney in variola consist mainly in diffuse parenchymatous degenerations and in cellular infiltrations, conditions macroscopically so indefinite as to render difficult their recognition by the naked eye. The changes in the organ will, therefore, be considered chiefly from a histological standpoint. (Vide p. 100.)

The only distinctly anatomical change in the kidney is seen in the hemorrhagic types of variola; in both variola pustulosa hemorrhagica and in purpura variolosa there is extensive hemorrhage into the submucosa of the pelvis.

X. THE ADRENAL GLAND.

In no form of variola were changes observed in the adrenal gland.

XI. PANCREAS.

The pancreas was invariably normal.

XII. THE HEART AND ARTERIES.

Variola vera. — Cases fatal during the first week of the disease occasionally show cloudy swelling of the myocardium;

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[1] The results of the microscopic examination of the testicle and of the ovaries show that lesions in these organs cannot always be detected by naked eye examinations.

one such presented punctate hemorrhages of the epicardium. Of the cases which died within the second week, or later, about one-half showed cloudy swelling of the myocardium.

The aorta and the coronary arteries were normal, save in one case where slight arteriosclerosis was present.

In abortive variola vera the heart and the aorta in both of two cases were normal.

In variola vera with hemorrhage both of two cases showed cloudy swelling of the myocardium, and no changes, save sclerosis, in the arteries.

Variola pustulosa hemorrhagica. — Among cases of this type cloudy swelling of the myocardium was invariably present; punctate hemorrhages into the pericardium and epicardium occurred twice, and hemorrhage into the myocardium and acute mitral endocarditis each once. In two of these cases there was early arteriosclerosis of the aorta.

Purpura variolosa. — All cases of this type showed cloudy swelling of the myocardium and punctate hemorrhages of the epicardium, the latter usually most numerous in the vicinity of the right coronary artery.

THE PATHOLOGICAL HISTOLOGY.

I. THE SKIN.

In the pathological anatomy of the infectious diseases there is no other organ in which the specific lesions of the disease are so characteristic in appearance, in situation, and in development as they are in the skin in small-pox. But little work has been done on the histology of the skin lesions, notwithstanding their striking character and the abundance of material given by the numerous epidemics in almost every country.

The literature of the histology of the lesions really begins with the great epidemics which appeared in Europe in the sixth and seventh decennia of the nineteenth century. The article by Auspitz and Basch may be said to mark the beginning of the study of the finer anatomy of the process.

They describe the vesicle as formed by a reticulum which includes in its meshes fluid exudate and cells. The reticulum is formed from the epithelial cells, and the contained pus cells are also formed endogenously from the epithelium. They also describe the formation of the central depression of the pock as due primarily to changes in the epithelium, and secondarily to removal of the exudate from the center or oldest portion of the lesion by evaporation.

Klebs, also, speaks of the reticular character of the lesion. The process begins in the middle layers of the epidermis, and the reticulum is formed by cells which are separated from one another by fluid and cellular exudate. The most valuable of these earlier articles is that by Weigert, who made two hundred autopsies in Breslau in the epidemic of 1871 and 1872. His description of the lesions remains the best, and is further interesting in that it formed the basis for his further studies on necrosis, and marks the beginning of the study of bacteria in relation with anatomical lesions. Weigert found that in every case the primary lesion consists in a peculiar degeneration of the lowest layer of the epithelial cells. The center is composed of irregular, reticular or thread-like masses formed of cells which have lost their nuclei. These cells have undergone a form of necrosis which he calls diphtheroid degeneration, which is distinguished from the ordinary forms of necrosis by the non-disintegration of the necrotic material. He regarded it as analogous to the degeneration of the epithelium described by Wagner in the formation of the diphtheritic membrane. This degeneration of the epithelium forms the primary and central focus of the pustule, and around it there are secondary lesions consisting chiefly in an exudate, which collects between the cells. The central depression or dell is due to the rigidity of the cells, which have undergone the primary necrosis, the tissue around this being distended by the collection of exudate. In the pustules without central depression there may be elevation of the connective tissue, or the depression may be obliterated by the rupture of the epithelial threads. The primary necrosis he regards as due to the direct action of the small-pox virus on the epithelial cells, the virus being brought to the cells by the blood. He opposes the views of Luginbühl, who asserted that the infection producing each pustule came from the outside. To this primary action of the virus is added a secondary or irritative action which leads to the production of the exudate and to proliferation of the epithelial cells. Weigert also was the first to describe and depict the cell inclusions which have such an important place in the later descriptions.[1] The newly-formed epithelial cells are more transparent than the others, and sometimes have a number of nuclei resembling giant cells.

In his first article on the structure of the pock, Unna regarded the primary lesion as due to hypertrophy and inflammatory swelling of the epithelial cells. The stratum lucidum is swollen and the cells separated, the whole changing into a transparent body shaped like a convex lens. Small cavities are finally formed in it. The diphtheroid change described by Weigert he found only in one case. Cornil describes a reticulum formed by the epidermis, the cavities are formed in the interior of the epithelial cells, and the reticulum represents the remains of the protoplasm. The cells in the cavities are due in part to emigration, in part to proliferation of the epithelium. He was the first to describe changes in the corium consisting in swelling of the lining endothelium of the capillaries. The vessels so altered offer little resistance to the passage of the leucocytes.

Renault gives an excellent description of the process. In the corium in the papular stage there is edematous infiltration with dilatation of the lymph capillaries. The reticulum first appears in the prickle cell layer and is due to distention of the clear circumnuclear part of the cell, the peripheral portions becoming united and forming the network. The

[1] In anderen Fällen finden sich ganz ähnliche kleinere, im ungefärbten Zustände glänzendere, im gefärbten dünklere, runde Körnchen neben den hell gefärbten Kernen. Ihr Glanz resp. ihre Fähigkeit, die Haematoxylinfarbe anzunehmen, steht gerade im umgekehrten Verhältnisse zu ihrer Grösse. Die kleinen, übrigens ziemlich seltenen Körnchen, gleichen etwa den kernen der weissen Blutkörperchen, aber sie sind kleiner, werden niemals von einem besonderen Protoplasma-Mantel umbegen und finden sich stets nur neben den grossen Kernen der Epithelien.

dell is formed by the atrophy of the upper epidermis cells in the center of the pustule, while those in the periphery undergo the same changes as the cells lower down. The cylindrical cells in the Malpighian layer undergo cloudy swelling, but form a continued layer, the lymph for the vesicle passing between them. The cells in the pustule are chiefly emigrated leucocytes. He describes the cell inclusions as round, refractive bodies in the interior of the distended epithelial cells, and thinks that they stand in causal relation with the anatomical changes. He was the first to regard these bodies as parasites. He also says that he has seen them germinate in the moist chamber.

With regard to the dell formation, Rindfleisch at first held the view that it was the result of the formation of the lesion around a resistant skin gland. Later he regarded the depression as really below the pustule and due to the destruction and depression of the corium. The pus cells, he thinks, are formed within the epithelial cells.

The latest work which deals with the histology of the complete process is that of Unna. In the formation of the pustule there is liquefaction of the swollen, edematous reticulum. The reticulum is formed by a series of epithelial cells compressed between the primary liquefied areas. The chief contribution of Unna is his description of a peculiar degeneration of the epithelial cells, to which he has given the inappropriate name of "balloon degeneration" (ballonartige). It affects chiefly the cells of the lower prickle layer. They become separated from one another and converted into large, round, solid masses staining with eosin, and the nuclei undergo degeneration. There is a gradual transition between these cells and the flattened cells of the upper layers which form the reticulum. Most of the cells finally undergo fibrinoid degeneration and give the reaction for fibrin. The dell-formation is due to the reticular degeneration and to the edema, which is most marked in the periphery of the pustule. Under the pressure of the peripheral edema, the papillæ gradually disappear, while in the center they are not only preserved, but generally edematous, and project into the middle of the pock. During the stage of vesicle formation the emigration of leucocytes is scanty, but becomes abundant with the conversion of the vesicle into the pustule. There is an accumulation of plasma cells in the adventitial sheaths of the vessels of the corium, which constantly increases with the change of the vesicle into the pustule. The abundance of these plasma cells, which are ordinarily found in the more chronic inflammations, is remarkable when the acuteness of the process is considered. Although Unna gives a good description of the process of healing, he does not mention the cell inclusions.

All of the more recent work, with the exception of Unna's, refers directly to the cell inclusions in variola and in vaccinia and will be considered in connection with that subject.

In our study of the skin lesions, a number of pieces of skin showing the most distinctive lesions were taken from every case. From the material hardened, a further selection of pieces was made, and these were embedded in paraffin and the sections stained with alkaline methylene blue and eosin as a matter of routine. Eight sets of complete serial sections were made through typical vesicles and pustules. The pieces for these serial sections were embedded in celloidin and the sections stained with hematoxylin. After a primary examination further sections were made when necessary.

In going over so large an amount of material as this, comprising, as it did, a number of sections from each case, in addition to the serials, there is an embarrassment from the variety of the lesions presented. It seems possible, in the first place, to distinguish two groups of lesions which are generally marked by distinctive histological changes: (1) lesions which are sharply circumscribed, and are characterized by a structure and a mode of development which, in whole or in part, are so frequently repeated that it is possible to construct a type. These lesions we regard as due to the direct action of a specific cause, and it is only in these that the various forms of the parasite of the disease are found. They are analogous to the tissue reactions which we find associated with the presence of bacteria. (2) Lesions which are more diffuse in character, which present more variations, and which we regard as due to more or less accidental conditions, possibly to the action of soluble-toxic substances, may be produced, either by the specific cause, or by bacteria. The same is true of the clinical aspects of the skin lesions. The preliminary rash appearing on the skin of the lower abdomen and the groin would certainly seem to be due to a cause different from that of the typical exanthema, which appears later, and which tends to avoid the places in which the rash has appeared.

The Pustule.[1] — The factors concerned in the production of the small-pox pustule are degeneration of the epithelial cells, associated with or followed by exudation. In the number of cases and sections at our command it was possible to trace the earliest and the simplest form of degeneration. This was found in three cases of purpura variolosa without vesicle formation; it was possible to find the same conditions also in the periphery of a growing vesicle. The

[1] Four layers of cells may be recognized in the epidermis, which though passing by gradations into one another, present types differing in structure and in relation. In the deepest or Malpighian layer the cells are placed perpendicular to the corium; they have relatively large, well-staining, vesicular nuclei with abundant chromatin, and a finely granular protoplasm. Between the cells are small channels crossed by fine protoplasmic prolongations, but these are not so distinct here as in the next or prickle cell layer, into which the Malpighian layer insensibly passes, the cells becoming larger, the nuclei rounder. Here the spaces between the cells are wider, and the connecting protoplasmic processes more pronounced. Above this layer the cells are flatter, and the nuclei pale and poor in chromatin. The protoplasm is homogeneous, and contains granules of kerato-hyalin. Above this the individual cells are fused together, the nuclei no longer stain, and the fused cells form a homogeneous mass, with continual disintegration on the surface. The mass of cells so arranged is permeable to fluids coming from below, the permeability gradually decreasing; the homogeneous, horny layer on the surface is but little or not at all permeable. The various epidermic appendages in the corium beneath are connected with the surface by ducts passing between these cells. The cells forming these ducts are closely connected with the epithelial cells in their vicinity, so that in these places the cells of the epidermis are more closely bound together. Beneath the epidermis is the corium, composed chiefly of white fibrous and elastic tissue, arranged in fine bundles, closely interwoven, and containing numerous epithelioid cells embedded in it. It is extremely rich in blood vessels and lymphatics. The portion of the corium which is in immediate contact with the epithelial cell is composed of closely compacted, very fine fibrillæ, and is analogous to the membrana propria of some mucous membranes.

same case of small-pox often showed in one section beginning vesicles, macroscopically not apparent, and more advanced vesicles.

What we regard as the earliest form of degeneration takes place in the nucleus. This becomes swollen, more vesicular, and the clumping of chromatin in the center more marked than in the normal. This condition was particularly apparent in one of the hemorrhagic cases, the nuclei here appearing as vesicles with but little chromatin around the periphery, and with a large, irregular mass of it at the center. This was more refractile than the normal chromatin, and in specimens stained with alkaline methylene blue it often assumed a distinct greenish tinge. It could not be said that this degeneration would always be followed by the further changes leading to vesicle formation. Cytoplasmic inclusions were often found in cells with such nuclei. Similar nuclei were often found in advanced lesions, together with cells in which were advanced forms of degeneration.

Associated with this in most instances, and always present in the lesions leading to vesicle formation, is a reticular degeneration of the cytoplasm with more advanced degeneration of the nucleus. This may affect all the lower cells of the epidermis, but in the typical vesicle it is best seen in the cells above the Malpighian layer. The cells are swollen, and the cytoplasm loses its normal character and becomes either a faintly-stained, pale mass, or distinct spaces may be formed in it.

The nuclei may lose their form and become irregular and shrivelled, assuming peculiar shapes. This shrivelling is apparently due to the passage of the intranuclear fluid into the space around the nucleus. The irregular rim of the nucleus is preserved, and in the early stages the chromatin does not seem to be diminished and the large central mass is seen. Advanced forms of cytoplasmic inclusions are common in the nuclear space and in vacuoles in the protoplasm. The protoplasmic processes connecting the cells disappear. The periphery of the cell remains and seems to undergo a peculiar condensation and may stain deeply with iron hematoxylin. It is this degeneration which causes the peculiar reticular appearance of the early vesicle. It is always better seen in the periphery than in the center of the vesicle. With the increase of the exudate coming from below, the spaces within the cells enlarge, finally rupture, and a network is formed by the coalescence of the cell borders. The typical small vesicle is always fan-shaped. The bottom of the vesicle may be seated on the corium, or be separated from this by a layer of comparatively intact cells. The lowest cells have undergone the hyaline fibrinoid degeneration, and form the handle of the fan. Above this and extending widely laterally is the reticular degeneration with the formation of large spaces. A section which does not go through the stalk of the lesions shows nothing but the spaces with an almost intact epithelium below. The cells of the kerato-hyaline layer take but little part in the formation of the reticulum, though they may become separated to some extent by the exudate. As the exudate increases the tension becomes so great that the adherent cell-remains rupture, and large spaces are formed, with the cell-strings extending irregularly between them. In other cases this typical process does not take place, and the spaces are formed by the exudate separating cells which preserve their characteristics. In yet other cases an indefinite degeneration affects all of the cells of the epidermis, and section of the vesicle shows degenerated epithelial cells lying in the exudate, in part adherent in masses or strings, in part separated.

A later form of degeneration is that to which Unna has given the name "ballonartige," and which may best be regarded as a hyaline fibrinoid degeneration. The Malpighian cells are chiefly affected. They become swollen, their protoplasm loses its granular character, becomes homogeneous and refractile, and stains more intensely with the acid dyes. The single cells so altered may be separated from each other, or masses of them may be joined together and lie more or less free in the exudate. In some cases, instead of such a total conversion of the cell into hyaline, droplets of this may appear, and, by their coalescence, fill the cell. The nuclei may entirely disappear, apparently undergoing the same change as the cytoplasm. In sections stained with carmine, followed by Weigert's fibrin stain, the homogeneous nucleus may be distinguished in the cell by its redder tinge. The periphery of the nucleus often forms a homogeneous red line. The nuclear chromatin rarely undergoes fragmentation. In cells in which the degeneration is slight and shown only by a more homogeneous appearance of the cytoplasm, degenerative direct division of the nucleus may take place, the chromatin first becoming arranged around the nuclear membrane. Cells may be found containing as many as four pale vesicular nuclei formed by direct division. In addition to the masses, lines of cells so altered may be found passing through the vesicle. These may give, with more or less distinctness, the reaction for fibrin, and in specimens stained with iron hematoxylin a dark band may be found around the periphery of some of the cells. This degeneration is found in the older and central parts of the lesion. It is not distinctive of small-pox. It has been best described by Unna, and is found in varicella and in other skin diseases associated with vesicle formation. In the only section of varicella which we were able to study it was not distinctly shown. Very much the same condition is found in diphtheria in the cells of thick mucous membranes, and is the main change leading to the formation of the hyaline diphtheritic membrane. (Councilman, Mallory, and Pearce, 1901.)

The fluid exudate begins early and in most cases probably simultaneously with the degeneration. In the smallest papule which is visible the swelling is due chiefly to the presence of exudate, and in no case have we found degeneration without any evidence of exudation. The character of the exudate varies. In the early stages of the lesion it is clear, without any admixture of cells, and may be very poor in coagulable constituents. In sections hardened in Zenker's fluid we have frequently found perfectly clear spaces in the epidermis without any granular precipitate. On the other hand, the exudate may have a peculiar appearance due to its being filled with small, definite circles; a similar condition is often seen in the kidney and in other places where an exudate comes in contact with epithelium. These small circles, which must be due to the presence in the exudate of globules which differ from the fluid around them and which do not give a granular coagulum, were almost constantly found in the exudate when it had lifted up the entire mass of epidermis. One of the things which is most impressive in the study of the small-pox process everywhere is the extreme paucity of cells in the exudate. We have found cases in which there was extensive degeneration of epithelium and abundant fluid exudation without a single foreign cell in the epidermis. The cells appear only in a late stage of the process, and are always fewer than would be found in any analogous process of degeneration and exudation due to bacterial infection. The condition commonly found in all sorts of skin lesions, that of polynuclear leucocytes advancing between the epithelial cells to the area of lesion, was never met with.

In the examination of all of the sections of the skin particular attention was paid to the character of the cells in the exudate. It seems probable that the cells appear when the specific character of the process has passed, they being then attracted by the necrosis. The cells in the exudate vary extremely and represent the different varieties of leucocytes. Polynuclear neutrophiles were the most numerous of the exudation cells. In but few cases were pustules found with a perfectly frank, purulent exudate within them. In the later stages of the process, all the cells, both those in the exudate and the degenerated epithelium, undergo fragmentation and give rise to granular masses which cannot be identified. One form of degeneration of the polynuclear leucocytes was particularly noticeable. The cell becomes swollen and clear, the granular contents seeming to have dissolved or passed out. The irregular nucleus is swollen, and the chromatin arranged in small masses, often of the same size, at the periphery of the nuclear membrane, in some cases assuming the form of rosettes.

The study of the vessels in the corium from which the emigration must take place, shows what we should expect from the character of the cells in the exudate. The mural accumulation of polynuclear leucocytes, which is such a common feature in inflammation, was never found in them. In the early lesions but few polynuclear leucocytes were found in the tissues about the vessels. In the late pustules, and in cases in which there is extensive necrosis extending deeply into the corium, they are found in larger numbers. In the late lesion there are accumulations around the vessels of the varieties of lymphoid cells which are so prominent in the lesions in internal organs, and which Unna has identified as plasma cells. These cells were rarely found in the early vesicles. Small lymphoid cells were often found in the intact epidermis between the cells and in the vesicle and the pustule. Mononuclear cells with phagocytic properties were found both in the corium and in the vesicle. Eosinophile cells in any considerable numbers were rarely found, either in the exudate in the vesicle, or the pustule, or in the corium, except in one case, that of an infant. As the result of our study of the cellular exudation in the small-pox lesion of the skin, we must conclude that there is no positive attraction between the virus and the polynuclear leucocytes. They are present in any considerable numbers only in the late stages of that lesion in which there is extensive necrosis, and in which a secondary infection with pyogenic cocci has in most cases supplemented the action of the small-pox virus. The diminution in the number of polynuclear leucocytes in the blood cannot account for the small numbers found in the skin, for in the lesions in the lungs and in the mucous membranes due to streptococcus infection the cellular exudate has the usual character.

Structures were occasionally found in the epithelium at the edge of both vesicles and pustules which were regarded as necrotic leucocytes. These were hyaline, structureless bodies of the size of leucocytes, showing no trace of nucleus, and found between the epithelial cells. A single such body sometimes extended between several adjoining cells and had an ameboid appearance. There was no difficulty in distinguishing these structures from the specific parasitic inclusions.

Red blood corpuscles were rarely found in the exudate in the youngest vesicles, nor in such had any hemorrhage taken place in the corium around the vesicles. In the cases of purpura variolosa red blood corpuscles were found in considerable numbers in the papillary layer, but were not present in the epidermis. Extensive hemorrhages were found in the corium, and numerous red corpuscles were found in the exudate in the marked hemorrhagic cases. In the exudate the red corpuscles were in some cases well preserved and stained in the usual manner, in others they were represented by shrivelled shadows. A peculiar staining reaction of the corpuscles in the exudate was often present, shown particularly well by the Weigert fibrin stain. The periphery was sharply stained, leaving an unstained homogeneous center. We must disagree with Ewing in reference to the importance of the part which red corpuscles play in the process.

Fibrin, in varying amounts, was always present in the exudate in the advanced vesicle and in the pustule. It was usually absent in the beginning vesicle. The greatest amount of it was formed in immediate contact with the papillary layer, and this was often connected with fibrin filaments in the pustule contents.

Repair of the lesion begins early. In an early stage of vesicle formation there is slight hyperplasia of the surrounding epidermis and an increase in the thickness of the horny layer. These newly-formed horny cells are edematous and swollen, and their nuclei are faintly visible. The contents of the vesicle or the pustule undergo condensation from the evaporation or absorption of the fluid, becoming finally changed into a solid, granular mass in which nothing can be recognized. While these changes in the pock contents are going on, regeneration of the epithelium is taking place. In the less severe cases the destruction of the epithelium is not complete, and there may be rows of cells at the bottom of the lesion little or not at all affected. New epithelium is constantly being produced from these cells, or is growing in from the sides beneath the outlying edges, where the process never extends to the papillæ. This regeneration is never absent in late lesions. In the most extensive processes, with great exudation and degeneration involving large areas of the skin, the destruction extending deeply into the corium, the ingrowing epidermis will always be found at the edge of the lesion. This regeneration is accompanied by nuclear figures, often abundant, in the surrounding intact epidermis. Few are found in the early stages in the ingrowing cells. Until these become established on papillæ and their relation becomes normal, proliferation is not active. In some of the newly-formed cells a degenerative, direct division of the nuclei may be seen. All layers of the skin are not formed simultaneously in the process of regeneration. The cells of the horny layer are formed in great numbers in the periphery of the lesion, and from this gradually extend over the deeper cells which have already been formed. The appearance is often given as though the horny cells were being formed only in the periphery, and from this were being pushed over the lower cells which are already in position; but the differentiation, although beginning at the edges, undoubtedly extends continuously over the base of the lesion. In the regenerated epithelium the formation of kerato-hyaline extends from the edges in the same way. In consequence of this regeneration, the mass of necrotic cells and exudate, which has become solid by evaporation and by absorption of the fluid, becomes enclosed within two layers of horny cells: the old layer which is elevated by the exudate, and a lower layer which is newly formed. The old horny layer is finally broken through, and the pustule contents, now a scab, comes away as a whole or in fragments. This process is somewhat modified in the lesions of the palmar and plantar surfaces. In these places the layers of horny cells are very thick, and the dried exudate enclosed within the mass of horny substance is not easily removed. It may remain for a long time, and becomes converted into a more or less translucent, firm mass, of the form of a lenticular disk, by the removal of which from the soles of each other patients while away the tedium of hospital sojourn.

A complete regeneration without cicatrization is possible when the lesions are not extensive and do not involve the entire epithelium, and also when the entire epithelium is destroyed over a small area only, the papillary bodies remaining intact. When the destruction extends into the corium and the entire architectural arrangement is destroyed, complete regeneration does not take place. This is usually the case in lesions more than one-half centimeter in diameter, the base or stalk of the lesion being one-half or one-third of this. These large lesions rarely show intact epithelial cells below them; the area to be covered by the epithelium growing from the edge is more extensive, and destruction of the papillæ is more apt to result than in the smaller lesions. Even in very small lesions there is sometimes destruction of the papillæ with inevitable cicatrization. After recovery the papillæ are absent or very imperfectly developed, and the connective tissue beneath has the characteristics of cicatricial tissue.

There are some very interesting minor modifications of the process, which are due to the character of the different parts of the epidermis. All parts of the epidermis, with the exception of the horny layer, are more or less penetrable by fluid, and the horny layer is more easily detached from its connection than are the other layers. It happens from this that when the exudate is abundant, further distention being prevented by the interlacing and connected reticulum, the tension within the vesicle may be considerable. In the larger vesicles, and always in the pustules, the reticulum is ruptured and large spaces are formed. In rare cases (in four only of the hundreds of lesions examined) space for the exudate is gained by its passage through the stratum lucidum and the elevation of the horny layer in mass. In this way a secondary vesicle may be formed on the surface of the true vesicle. Figure 1 (Plate III.) shows this process in a vesicle in which, judging from the extensive rupture of reticulum, the tension must have been considerable. The character of the exudate in the two places is interesting. In the true vesicle there is a great deal of detritus and of granular precipitate. In the secondary vesicle there is only a faint cloud of precipitate. In other cases the exudate in the primary and the secondary vesicles had the same characteristics. A condition just the opposite to this was found in one section. In this there was a typical vesicle in an early stage, but with a broad base. The entire vesicle with the lower layer of adherent Malpighian cells had been elevated by the exudation. The exudation beneath the epithelium contained more cells than the vesicle, Figure 2 (Plate III.). In three cases, all of them severe, and of vesicular type, there were numerous false vesicles due to the lifting of the epidermis by exudation. Some of the vesicles so formed were circumscribed and considerably elevated, most of them were not sharply circumscribed. No cell inclusions were found in such lesions. The secondary vesicle may form the more important feature of the lesion. Figure 3 (Plate I.) shows a small vesicle in the vicinity of a large one, but not connected with it. There is very little reticulum. Almost the entire exudate is contained beneath the elevated horny layer. This was in a child two and one-half years old, in whom the disease was very acute, and it is possible that the epithelium in the child is more penetrable than it is in the adult.

In going over the literature of the disease, we have found no mention either clinical or anatomical of this modification of vesicle-formation. The small elevation filled with clear fluid on the surface of the vesicle or the pustule should be apparent on close observation. An extreme type of this is seen in the formation of large bullæ. These were found in a number of the more severe cases. They occurred on various parts of the body, but were more common on the wrists and ankles than elsewhere. The epidermis was elevated over considerable areas by the accumulation of a serous or bloody fluid beneath. That it was the entire epidermis and not merely the horny layer which was elevated was shown by the presence in the elevated layer of well-marked, umbilicated pustules. No tissue from such lesions was obtained in a condition suitable for study.

The dell-formation or umbilication.—This is almost constantly present in the circumscribed typical lesions. It appears at a very early stage in the formation of the vesicle, and becomes accentuated as this increases in size. When the contents of the vesicle becomes purulent it is often especially evident, and it may persist when healing is taking place. The only lesions in which the umbilication is always absent are those on the palmar and plantar surfaces. Elsewhere there appears to be no difference in the umbilication of the discrete lesions. We have studied the sections with especial reference to the umbilication, and have here found the serial sections of typical lesions especially useful. In all of the other material examined, the sections were made in most cases through the center of the nodule. In the few figures given of sections through entire lesions, it happens that the umbilication is not present or is not marked, but this is not usual. In Weigert's studies of the lesion he regarded the umbilication as due to the diphtheroid degeneration of the epthelium in the center, which was the earliest lesion and prevented the distention of the center by the exudate. Weigert, however, was not able to exclude the part which the hairs or the ducts of the skin glands played in its formation. Before Weigert's work it had been generally assumed that the umbilication took place at such points. The mode of formation described by Weigert has been generally accepted.

We have not been able to attribute the umbilication to the action of any single cause, and in view of its almost constant presence it is remarkable that several factors should take part in its formation. The mode of formation described by Weigert is undoubtedly the correct explanation in many instances. In the early stages of vesicle formation the spaces containing the exudate are usually larger in the periphery than in the center. The reticulum may be absent in the center, all the cells here undergoing the other form of degeneration. Figure 4 (Plate I.), which shows one of the earliest typical lesions we have seen and one which would not have been apparent clinically, shows this very well. In the center the papillary layer is very close to the surface, and the epithelium is adherent and has undergone the hyaline fibrinoid degeneration. Outside of this the tissue is distended by the collection of the exudate in spaces. Such a condition as this was more often found in the chance sections than in the serials. Even in this figure it is not certain that further sections would not have shown either a hair or a sweat gland in the center, although there is no indication of this in the corium beneath. In the serial sections the presence of hairs and sweat glands at the point of umbilication was rarely missed. It might be supposed from this that the primary lesion was most often in the vicinity of one of these structures. This is not the case. All of our studies of primary lesions have shown the absence of any such relation. The hair follicles play a more important role than do the sweat gland. Where the latter are very numerous, as on the palmar and the plantar surfaces, they simply form a part of the reticulum, and rupture with the increase of tension. They are never sufficiently resistant to draw in the dense horny layer under the tension. The umbilication does not always correspond exactly with the center of the lesion, although it usually does. The serial sections even of small lesions often show several hair follicles or sweat ducts, those in the periphery having ruptured, while one in the center remains. It is possible that there may be a connection between the two conditions, a naturally more resistant center, which makes its resistance more manifest when it is further strengthened by the hair sheath. Where the hair passes through the epidermis the cells of the sheath form a compact layer closely connected with each other and with the surrounding cells. The same is true to a lesser degree of the ducts of the sweat glands. In one case a section through a large vesicle without umbilication showed a hair duct in about the center which had been pulled partly out of the corium where it had broken off. Without serial sections it is impossible to determine whether what appears to be a central depression may not represent the junction between two lesions. The specimen which best shows the relation between the hairs and umbilication is Figure 1 (Plate IV.), which represents a flat section of a small vesicle. The periphery of the vesicle is shown on one side, most of the section representing the space of the vesicle. In the center of this is a lanugo hair, apparently cut just beneath its exit from the skin and surrounded by the horny cells of the sheath.

The sweat glands may take part in the umbilication in another way, and the depression be formed in the secondary vesicle. We have seen this only in one instance in which serial sections were cut. Macroscopically there was well marked umbilication, and the section showed extensive separation of the horny layer and the formation of a secondary vesicle. At the center, corresponding to the depression, the duct of a sweat gland is seen, and at its emergence the horny layer was not separated, being apparently more firmly connected with the layer beneath. In some very small lesions which are but slightly elevated the central depression may be accentuated by the hyperplasia and swelling of the surrounding epithelium in the healing stage. That the hairs are not the exclusive agents in the umbilication is shown by the frequent presence of this in the lesions of the glans penis. Umbilication is not present in the typical lesions of the mucous membranes, for reasons which we will consider in connection with the description of these lesions.

So far we have described that lesion of the skin only which is the most typical of the disease, and which, in the lighter cases, is the only lesion present.

More extensive lesions, conforming to the type described, arise by confluence of adjoining areas, or by the simultaneous affection of large areas of the skin, and it is not always possible to determine by sections in which of these ways the lesions arise. In the large confluent areas the several points of umbilication may be preserved, or the surface may be smooth or irregular. Often vesicles, apparently confluent, will not be found to be so on examination, the apparently continuous vesicle being separated by a number of small partitions. This was often seen in the large lesions on the palmar and plantar surfaces. In some of the severe early cases very extensive lesions were found, due apparently not to confluence, but to the simultaneous affection of a large area. Some of those examined extended over an area two and one-half centimeters in diameter. In these large areas there is great irregularity, the process being more advanced in some places than in others. There is always a much greater degree of involvement of the corium. The entire papillary layer is destroyed, its place being indicated by a dark line composed of fibrin and degenerated epithelium. There is cellular infiltration extending throughout the entire thickness of the corium around all the vessels, hair follicles, and glands. In places the cells are entirely of the small lymphoid type, in others a mixture of these and large basophilic cells. There is rarely any extensive necrosis in the glands in these cases.

Edema of the corium, so far as could be estimated from the microscopic appearance, was generally absent. When acute exudation takes place in the corium it seems usually to find its way either into or beneath the epidermis or into the subcutaneous tissue. When edema was present it showed more in the papillary layer than elsewhere. In several cases the fibers of the tissue here were separated by clear spaces due to fluid. Fibrin, though often abundant in the vesicles, was rarely found in the exudation in the corium. When present it was found chiefly in the papillary portion, the entire tissue of this having undergone the same fibrinoid metamorphosis which is found in diphtheria. In the typical discrete lesions there was very little extension downwards along the hair follicles. In the severer lesions the cellular infiltration, combined with destruction of epithelium, may extend deeply into these. In one of the cases there was purulent infiltration with masses of streptococci in a hair follicle. In rare cases the same primary lesions as in the epidermis may take place in a hair follicle. We have seen hyaline fibrinous degeneration combined with numerous cell inclusions in the outer cells of the hair sheath.

The blood and lymphatic vessels of the skin show but little change The lymphatics were usually dilated and contained granular precipitate, some red blood corpuscles, and a few large mononuclear cells. The nuclei of the lining cells in some were large, the cells projecting into the lumen and apparently increased in number. No nuclear figures were found in them. The blood vessels showed less change than we expected to find. We have already alluded to the absence of accumulations of polynuclear leucocytes in them. Occasionally blood vessels were found which contained numbers of large and small lymphoid cells. Thrombi composed of fibrin or blood plates were found in the most superficial blood vessels, when the tissue around them was destroyed and had undergone fibrinoid degeneration. The thrombus-formation in these cases was secondary. The walls of the vessels underwent the same hyaline change as was found in the vessels beneath the diphtheritic membrane. They gave the fibrin stain and were connected with the fibrin around them.

The lesions in the four cases of purpura variolosa were much alike. Macroscopically they showed diffuse hemorrhages of the skin and an absence of characteristic lesions, save in two cases in which a few vesicles were visible. From the absence of typical eruption, from its extraordinary rapid and different clinical course, and from the alleged greater frequency in vaccinated subjects, some authors have considered it doubtful if this is a form of small-pox. In none of these cases were the hemorrhages circumscribed, but were so diffuse that they gave a dark plum color to almost the entire skin, the color being accentuated at certain places. We have not found in the literature any detailed anatomical study of the skin lesions, though a great deal of clinical study has been devoted to them. Numerous sections of the skin from all cases were examined, and all showed much the same condition. In two cases a few small beginning vesicles were found. There was no doubt, however, about the character of the process. The skin almost everywhere showed the earliest stage of true lesions. The lesions were extremely diffuse. Often the entire area of a large section showed the same condition, in other sections small areas of normal skin were found. The general condition was that of swelling and reticular degeneration of all the lower cells of the epidermis. There were large vacuoles in the cells, but rarely spaces between the cells resulting from their rupture. In the Malpighian layer there was some separation of the cells by the exudate, and in one section the entire epidermis over a small area was separated from the corium. The nuclei were degenerated, shrunken, and lay in large spaces. In all the cases there were numerous parasitic inclusions. In the earliest cases only early stages of the cytoplasmic inclusions were found, and in the later cases nuclear inclusions as well. Many of the degenerated nuclei had undergone direct division, and the nuclear space often contained several shrunken nuclei with almost no chromatin in them. In the cells least changed the clumping of chromatin in the nucleus was obvious. In the few areas of unchanged epidermis there were numerous nuclear figures. The corium showed little change, all the vessels were dilated, and there were numerous hemorrhages chiefly in the papillary layer. There was some edema particularly about the sweat glands and slight infiltration with mononuclear cells. The lymphatics were dilated. The most striking condition found in the corium was the enormous numbers of streptococci in the lymphatics and blood vessels and in the tissue. None were found in the epidermis. There was entire absence of polynuclear leucocytes in the epidermis, and but few were found in the corium.

On the microscopic examination of sections of both the vesicles and the pustules bacteria were generally absent. Every section was examined with reference to their presence, and the routine staining in methylene blue and eosin stained them vividly. The Gram-Weigert and other stains were also used in an attempt to demonstrate some bacterium which could be considered to stand in causal relation to the lesions. Cocci, usually definitely arranged in chains or in pairs, were the organisms usually found. In but six cases were organisms found in any considerable numbers in vesicles or pustules, — in all of these the organisms were streptococci. In one case it seemed possible that the skin might have been the point of invasion for a general septicemia. One section showed the corium laid bare, probably by the rupture of a pustule, necrosis of the corium extending a considerable distance; on the surface and in the necrotic tissue were masses of streptococci. Occasionally single threads of streptococci were found in pustules from the other cases. In one case streptococci were found in the deep lesions around a hair follicle. Organisms were found much more frequently in the corium than in the epidermis. We have already referred to the great numbers found in the purpuric cases. They were usually seen as embolic masses within the vessels, and occasionally in the tissues outside of them. They were not found more frequently, or in greater numbers in the vessels of the corium than in the vessels of any other part of the body. In order to show any possible relation between the skin lesions and the ordinary bacteria, we have preferred to place the chief reliance on the histological examination, rather than on cultures. As we shall see later, a general invasion of the tissues by cocci seems to be one of the most characteristic features of the disease. Bacteria were found much less frequently on histological examination than in either smears of pustule contents or cultures made from them, and it is probable that they were frequently missed in the study of the sections. In no case were they found in such relation with the early epithelial lesions that they could have been considered as a causal factor. When they are present they are brought to the lesions by the blood. As we shall see later, streptococcus septicemia is so generally present in fatal cases that it can almost be regarded as a part of the disease. It did not seem that the skin lesions were the usual place from which the streptococcus infection takes place.

We had one opportunity to investigate the lesions in a case of fetal small-pox. We have not been able to find in the literature one record of the histological examination of the skin lesions in a similar case, although fetal infection is probably more common in small-pox than in any other of the acute infectious diseases. The child, evidently at full term, was in process of birth at the death of the mother. It was not known whether the child was already dead when the labor began. The mother had given birth to five still-born children. There was no anatomical evidence of syphilis, either in the mother or the child. The mother died in the thirteenth day of the disease, dating from the onset, with chill. There was an abundant pustular eruption, partly confluent, which was crusting in most places. The child was a male, well developed and nourished. The appearance of the skin at autopsy was described as follows: "The skin of the neck, shoulders, arms, chest, and back presents small, closely-set elevations, from pin-point to one-half millimeter in diameter, with a transparent, vesicular center, and a white, opaque border. The lesions are largest and most marked upon the backs of the hands. The general surface of the body is covered with a moderate amount of vernix caseosa. The general color of the skin is dull violet." The other organs, with the exception of the testicle and bone marrow, which, on microscopic examination, showed characteristic lesions, presented nothing normal, save small petechiæ on the serous surfaces. Considerable tissue was saved from the case, which was regarded as important for the study of earliest lesions. We were greatly surprised to find an entire absence of typical lesions in the skin, and it was only after lesions of the same character were found in other cases in connection with typical lesions, and after finding characteristic lesions in the testicle and bone marrow, that we could be sure that the skin lesions were those of small-pox. Microscopically, what appeared as vesicles were represented by clear spaces within the horny layer, which was split, and the surface of the vesicle was formed by a thin layer of adherent horny cells, Figures 2 and 3 (Plate VI.). A great number of sections were examined, and they all showed the same formation. Often a row of vesicles, separated by thin partitions, was found. In addition to this, there were numerous areas where the epidermis was elevated with a granular coagulum filled with small, clear spaces beneath it. All the vessels of the corium were greatly dilated, and the fibers, particularly at the papillary border, separated by edema. There was no hemorrhage, and no cells were found in the exudate. The vesicles were never umbilicated. A close search was made for bacteria and cell inclusions. No bacteria were found, and nothing which we could be sure was a cell inclusion. In one cell, in the vicinity of a small vesicle, an enclosed body was found in the protoplasm adjoining the nucleus, but we could not be sure of its character. The vesicle formation beneath the horny layer, and the numerous cases in which the entire epidermis was elevated by the exudation, showed a similarity between this case and certain of the lesions in the others. In the absence of all characteristic lesions in the epidermis, and the absence of cell inclusions, it seemed probable that these changes, and similar ones in other cases, may have been due rather to the action of soluble toxines than to the direct action of parasites. The lesions were due entirely to the serous exudate, which in some cases passed through the lower layers of epithelium collecting in the horny layer, and in other cases lifted the entire epidermis from the corium. One of the vesicles in a child of two years was formed chiefly by the elevation of the horny layer, though the other characteristic changes accompanied it. This probably does not represent the usual condition in fetal small-pox. Characteristic vesicles, pustules, and cicatrices have been described, which could not have arisen in this way.[1]

II. THE MUCOUS MEMBRANES.

Eppinger was the first to call attention to the frequency and gravity of the lesions in the mucous membranes in small-pox, though their presence was known to the early investigators. Kendall, in 1881, described three forms of laryngitis which might appear: One pustular, with discrete pustules; another confluent pustular, with extensive

[1] In some sections of the skin of fetal small-pox, kindly shown me by the late Dr. Englemann, lesions of the same character were found consisting, in some degree, of hemorrhage and cellular infiltration of the corium, with lifting of the entire epidermis by a non-cellular exudation. The sections were made thirty years ago, and were not adapted for the study of the finer histological details, but the general character of the lesions was the same as in our case. Professor Hektoen also told me that in a case of fetal small-pox examined by him the lesions were similar.

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inflammation of the submucous tissue; and the third, the most serious and dangerous of all, a croupous or diphtheritic inflammation. Huguenin gives especial attention to the lesions in the mucous membranes of the nose, mouth, palate, and pharynx, which may all be affected at the same time. He also says lesions have been found in the rectum, urethra, and vagina. He thinks that the bronchitis and capillary bronchitis with the accompanying atelectasis of the lungs may be referred to the specific action of the small-pox virus. He says pus organisms are always present in the lesions, which run a more rapid course than they do in the skin. Roger says the eruption in the mouth and pharynx often precedes or at least appears at the same time with the skin eruption and passes through its evolution more rapidly. The eruption may produce dysphagia, and a tendency to the hemorrhagic form is often seen in the lesions in the throat, which may be sprinkled with small, black points. He found otitis media rare.

The lesions in the mucous membrane histologically showed a general resemblance to those in the skin, although the process is modified by the nature of the tissue. The epithelial cells are in much looser relation with one another than they are in the skin, and the impervious, horny layer which in the latter plays an important part in binding the mass of cells together is absent. There is also a greater vascular supply and a more active permeation of the tissues with fluid.

This being the case, the specific character of the lesion is lost in a very early stage. The epithelial cells which are affected and those which are adjacent are thrown off by the increasing exudate, leaving an area bare of epithelium. This takes place very much more easily on mucous surfaces covered with cylindrical epithelium, such as the trachea, than on such a surface as that of the tongue, lips, and vagina. The pharynx stands about midway between.

The mucous membrane was examined histologically from every case in which lesions were apparent, and was also taken in several of the cases in which no lesions were found at autopsy. In each case the mucous membrane from a number of different places was examined. From all these examinations it is possible to form a general conclusion as to the nature and progress of the lesions in the mucous membranes. Only in the earliest cases of the disease, in which the skin lesions were in the vesicular or in the very early pustular stage, were lesions found in a sufficiently early condition for their specific character to be recognized. They presented a widely varying degree of intensity. As far as could be seen, the earliest change consisted in an exudation into the epithelium leading to separation of the cells. Only small spaces, however, were formed between the cells in this way. The extensive fan-like arrangement of spaces, which is so characteristic of the skin lesion, was never seen in the mucous membranes. Even in this stage, numbers of superficial cells were exfoliated. Along with this there was degeneration of both cells and nuclei. Many of the changes in the cells corresponded with those seen in the skin. The same form of hyaline fibrinoid degeneration was found here as occurs in the skin (page 43). In other cases there was the same shrivelling of the nuclei of the cells with more or less disintegration of the cytoplasm. Complete necrosis extending over considerable areas was also found.

In connection with these early changes in the cells, the same forms of parasites were found as in the skin. The cytoplasmic parasites in the various stages leading to gemmule formation were the forms most commonly found, but they were usually not so numerous as in the skin. In one case, however, that of a woman dying on the fourth day of the disease, the lesions in the pharynx were very similar to the lesions of the skin in the cases of purpura variolosa. In this case there was a general swelling and distention of the epithelium over considerable areas by fluid imbibition, and almost every cell of the lower layers contained the large ameboid forms of the parasite. The intranuclear forms were found only in the smaller lesions in the squamous epithelium. They were never found in the lesions in the trachea or in the larynx. The most characteristic forms were found in the squamous epithelium of the tonsil and of the soft palate.

Of all the mucous membranes examined, that of the soft palate and uvula was most affected. It is very probable that it is affected in all cases of the disease. As a rule, however, the lesions which could be regarded as truly specific and due to the direct action of the parasite on the cells were not found. The changes consisted in exfoliation of the epithelial surface with very extensive lesions in the tissues beneath. This was particularly marked in the uvula. In places there were large losses of substance. In all these places there were masses of bacteria, chiefly streptococci. They grew in masses very much resembling the single colonies on a solid culture. Such masses were found on necrotic surfaces and also in tissues apparently but little altered. Large numbers of bacteria were also found between the cells and on the surface in the earliest specific lesions. It is very probable that the destruction of the epithelium and its exfoliation is due in large part to the action of the streptococci.

In connection with these very extensive lesions, small areas were often found showing the specific lesions. It is probable that the deep and extensive lesions in the mucous membrane are always preceded by the true specific lesions which form the atrium for the streptococcus infection. Our conclusions as to the importance and frequency of streptococcus infection in small-pox agree with those of Perkins and Pay, who regard the bronchial mucous membrane as the chief portal of entry. The tissue beneath the necrosis shows various degrees of alteration, the most common condition being extensive edema with a great deal of fibrinous exudate. Not only is the fibrin found infiltrating the edematous tissue, but often large masses of fibrin may be found beneath the surface, filling up the distended lymphatic vessels and forming a coarse network.

The specific lesions in the mucous membrane also differ from those of the skin in the greater number of polynuclear leucocytes which are present. Polynuclear leucocytes were found in every stage of the lesions of the mucous surfaces. There was also extensive infiltration of the tissue beneath with the various sorts of lymphoid cells, particularly with large basophile cells. The mucous glands of the tissue were variously altered. They were involved in the necrosis of the surrounding tissue and in addition to this lesion showed others closely corresponding to those found in the kidney and in the testicles. In these cases there was an interstitial infiltration of the gland with large basophilic mononuclear cells, which was combined with necrosis of the gland cells. There was no definite relation between the interstitial infiltration and the necrosis. The greatest degree of interstitial infiltration did not correspond with the greatest degree of necrosis. In several of the glands a considerable degree of interstitial infiltration had taken place without any or with but little necrosis. The blood vessels were dilated and contained usually a relatively greater number of polynuclear cells than do those in other situations, save the lungs.

Pyogenic organisms, chiefly streptococci, appear to grow in these lesions in much greater numbers than in lesions produced in any other disease. We have never seen in the lesions in diphtheria or in other infectious processes such masses of organisms as occur here. In one case, all the tissue around the tonsil was edematous, in part necrotic, and contained multiple masses of streptococci. The submaxillary gland in one case was affected, and numbers of organisms were found in the ducts.

It was very difficult to find, either in the larynx or the trachea, lesions which could be regarded as specific, owing probably to the rapidity with which the infected epithelial cells were cast off. In several cases there was very extensive necrosis of the surface epithelium with bacterial invasion. The bacteria in these cases, however, were chiefly bacilli, and formed in the methylene blue and eosin specimens a blue ring immediately above the membrana propria. In certain cases both in the larynx, trachea, and in the primary bronchi small foci of degenerated cells were found, and in these were the cytoplasmic parasite inclusions.

In addition to these lesions, which we regard as specific, vesicles produced by the total elevation of the epidermis by the exudation beneath were frequently found. Such vesicles were found in pharynx, uvula, tonsils, larynx, and trachea. It is probable that the majority of the unbroken elevations which were described in the mucous membrane at the autopsies are due to such vesicle formation and are not true lesions. There was frequently a great deal of fibrin on the eroded surfaces, in some cases closely resembling the membrane formation in diphtheria. In one case there was evidently a true diphtheria, although examination by culture was not made. In this a characteristic fibrinous hyaline membrane was present, and on the surface and within this were clumps and masses of bacilli, morphologically and in their situation similar to diphtheria bacilli. In this case similar bacilli were found in the bronchi and in the foci of broncho-pneumonia.

Degenerative changes were found in the nuclei of the epithelial cells in connection with the lesions and elsewhere. Within the lesions the grouping of the chromatin masses around the nuclear membrane was frequently found, and there was often direct division of the nuclei so altered, just as in the skin.

The other change which affected the nuclei is a more interesting one, and is difficult to explain. It was found not only in the nuclei in the vicinity of the lesion, but elsewhere, and chiefly in the pharynx and the esophagus. This consisted in a very much more marked degree of the same sort of lesion which we describe in the nuclei of liver and kidney epithelium. The size of the nuclei was not altered. The characteristic appearance of the chromatin was lost, it stained less intensely, and was homogeneous. The main mass of chromatin was collected in the center of the cell, forming there a mass sometimes round or oval, sometimes extremely irregular. What was most peculiar in these cases was the appearance of small refractive vesicles in the interior of the chromatin masses.

Especial interest is attached to the mucous membrane of the eye in small-pox in view of the frequency with which blindness results, especially in children. From the literature it appears that this must have been much more frequent formerly than it is now.

Adler speaks of the frequency of the eye affections during and after small-pox. He saw pustules appear on the conjunctiva before they appeared on the skin. He doubts whether there is a true pock-formation on the cornea, since the pustular keratitis only appears late in the disease. Hebra found the cornea constantly free from pustules in his cases. Roger found that conjunctivitis was frequent and severe, and that the infection often extended from the conjunctiva to the cornea; the keratitis resulting may be superficial or deep, and may end by perforation of the cornea and hernia of the iris, or by a more extensive infection with destruction or atrophy of the eye. In confluent small-pox he has seen an extension into the anterior chamber.

Conjunctivitis

In our autopsies there was a remarkable freedom of the eye from infection. Purulent conjunctivitis without specific lesions was found in but two cases, and in one, an infant of two years dying in the late stage of the disease, there was an ulcer of the cornea near the scleral edge. Microscopical examination of this showed entire absence of any specific lesion. There was a loss of substance extending about one-third the thickness of the cornea, and the base of the ulcer was densely infiltrated with polynuclear leucocytes and endothelial cells. There was some ingrowth of epithelium from the adjoining edge. No form of the parasite was found in connection with the ulceration.

Certain of the mucous membranes appear to be less susceptible to infection than others. We have never found any lesions in the ureters, nor have we found specific lesions in the bladder; in but two cases were early specific lesions found in the urethra, although the glands were frequently affected. The vagina was also usually found free from lesions. With the exception of hemorrhages in the mucous membrane of the stomach, which were found in all of the cases of purpura and in some of the others, and with the exception of erosions, which were evidently due to these, no change was found in the mucous membrane of the gastro-intestinal canal. In one case small opaque foci were found in the stomach on macroscopic examination, and these microscopically were found to be foci of necrosis. A few doubtful cytoplasmic inclusions were found in this. We have thought that this lesion was very probably specific, although the fact could not be positively determined.

GENERAL RELATION OF PARASITES TO LESIONS.

We have never found parasites except in the specific lesions of the skin and of the mucous membranes. Difficulty is experienced in determining the relation of the parasites to the duration of the lesions. We have computed the duration of the disease from the beginning of the initial fever. Patients usually came into the hospital with the eruption at various stages, and it was often difficult to determine from their statements or statements of friends the time of onset. Within certain limits the duration of the disease can be determined from the character of the skin lesions. Where it has been possible to determine the duration of the disease we can say with absolute certainty that parasites will be found in skin lesions up to the tenth day of the disease, this corresponding to about the sixth day from the appearance of the eruption. At this time, histologically, the lesions are clear vesicles, or beginning pustules. The uncertainty begins after this. At the eleventh and twelfth day of the disease parasites are sometimes found in the lesions, and rarely on the thirteenth day. When the disease is older than this, and when healing in the skin lesions is advanced, we have never found any parasites. The great majority of cases of smallpox die at about the twelfth day of the disease, and most of these cases are useless for investigation. In these later cases vesicles apparently delayed in their formation may be found, and in these parasites may be present in small numbers, or absent.

The cases which are most suitable for investigation are those of purpura variolosa, in which death usually takes place on or about the fourth or fifth day of the eruption, and the early cases of variola vera. In the fifty-four cases which we

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have examined, and which embraced all stages extending to complete convalescence, we have found the parasites present in twenty-nine cases, and in several of these in very small numbers.

The cytoplasmic forms appear first. We have found but two cases in which these appeared alone. These were cases of purpura variolosa in which there was no microscopic appearance of vesicle formation. The skin everywhere showed histologically the cell degeneration with edema, which precedes the formation of vesicles. In more advanced cases of purpura variolosa numerous early intranuclear forms accompanied the cytoplasmic. In nearly all of the other cases the intranuclear forms were found generally accompanied by the cytoplasmic forms.

When the vesicle is formed, the cytoplasmic forms of the parasite are found in the cells of the rete mucosum at the edges where the vesicle is extending. There is evidently a continuous infection of the surrounding epithelium by gemmules formed by the proliferating cytoplasmic forms. The intranuclear forms of the parasite are found in the epithelial cells at the bottom of the vesicle, this being the oldest portion. A point may be found where both forms are seen in the same field of the oil immersion lens. Parasites are rarely found in any of the epithelial cells above the rete mucosum, and they are often limited to the lowest cells of this layer.

III. THE LUNGS.

The frequency of bronchitis and broncho-pneumonia in small-pox is generally mentioned in the text-books. There have been but few articles which especially treat of the condition of the lungs. Ivanowski, in 1876, found in the lower parts of the lung small grayish or red nodules up to the size of a pea. The lung alveoli were filled with cells resembling white blood corpuscles, and also contained epithelial cells with granular, cloudy protoplasm. There were also red blood corpuscles in the alveoli. He regarded these nodules as true-small pox foci resulting from the inhalation of the germs of the disease.

Breynaert found that the bronchitis in small-pox was sometimes due to the development of definite pocks in the bronchi, which frequently did not reach the pustular stage. He described the pustules as becoming progressively smaller with the diminution of the bronchi in caliber, and found them even in bronchi of the third order. The pustules are sometimes delled and similar to the lesions of the skin, but may be quickly changed to ulcers with slightly overhanging edges. There is also bronchitis not due to the development of pustules which was marked in bronchi of medium caliber, but may also extend to the finest bronchi. Broncho-pneumonia was found in fifty per cent of seventy autopsies. It was less marked in children than in adults, and affected by preference the right lung. The beginning of the broncho-pneumonia takes place between the twelfth and fifteenth days. He has never seen lobar pneumonia in variola. Atelectasis was common in connection with the lesions. Joffroy found pustules in bronchi. These decreased in frequency with diminishing caliber. Along with these pustules there was an independent bronchitis with marked hyperemia of the mucous surface. Broncho-pneumonia was more common in the right lung. He found no lobar pneumonia; in cases in which it appeared to be present the consolidation was due to confluence of foci of broncho-pneumonia.

Roger says that pulmonary foci may be found which contain very few bacteria, but great numbers of his special parasite of the disease (see page 124). He found lung lesions more frequently and more extensive in infants and progressively less marked up to the adult age. The lung complications vary in different epidemics. He regards broncho-pneumonia as a grave complication. In the epidemic in Paris of 1900 there were twenty-four cases of lung complications, with only one recovery. The lung complications were especially prone to develop in cases profoundly affected with the disease in from twenty-four to forty-eight hours before death. Pneumococci are commonly found, though other bacteria may be seen, or bacteria may be absent and only his special parasites found.

We have never found in the lungs or in the bronchi any lesions which appeared to be specific for the disease, or which may be compared with the lesion in the skin and mucous membranes. True lesions, though modified by the character of the epithelium, have been found in the trachea, but never in the bronchi, save in one case in which cytoplasmic parasites were found in small necrotic foci in the primary bronchi. With this exception, we have never found in the lungs any form of the parasite of the disease. The most common lesion found in the lungs, and one which is very rarely absent, is bronchitis, usually combined with more or less extensive broncho-pneumonia. This was found microscopically in cases in which there was no macroscopic evidence of it. Both the larger and the smaller bronchi were affected. In all cases there was hyperemia of the mucous membrane of the larger bronchi and an increased secretion. In numerous cases there was a very marked degree of bronchitis, even in the larger bronchi. The lumen was obstructed by cast-off epithelial cells and exudate in which there was little or no fibrin. The epithelial covering in many cases without being entirely cast off is lifted by an exudate beneath it. The lesions in the smaller bronchi are always attended with foci of pneumonia as well. Some degree of bronchitis and broncho-pneumonia was found in forty-three of the fifty-four cases. It did not differ from the forms of broncho-pneumonia so commonly seen in diphtheria, although the exudate rarely contained fibrin in any considerable amount. In two cases lobar pneumonia was found. One of these a child who died on the eleventh day, and the other aged fifteen who died on the fifteenth day of the disease.

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The lung lesions differed from the lesions in other organs in the character of the exudate. The exudate in the lungs contained great numbers of polynuclear leucocytes. They were usually degenerated and the nuclei in various stages of

Great Numbers of polynuclear leucocytes

destruction. Along with these there were cast-off epithelial cells from the walls of bronchi and alveoli, and larger phagocytic cells. The basophilic lymphoid cells were rarely contained in the exudate.

In eight of the cases the exudate was largely hemorrhagic in character, and in the four cases of purpura variolosa hemorrhages were found, both within the lung and on the pleural surface, without any evidence of an accompanying pneumonia.

The different varieties of pyogenic organisms were constantly found in the foci of bronchitis and broncho-pneumonia. The most common organism was the streptococcus, and next to this the pneumococcus. These organisms were not only found in connection with the lesions, but also in parts of the lung which seemed normal. Along with the cocci, bacilli were frequently present, and in one case the bacilli had the morphology and characteristic grouping of diphtheria bacilli. This case appeared to be one of mixed infection with the diphtheria bacillus, although the organisms were not demonstrated by culture. In the pharynx of this case a diphtheritic membrane was found which contained similar bacilli.

These lung lesions were found in all stages of the disease, from the earliest to the latest. We should regard them as probably the most common and the most serious complication in small-pox. In many of the cases the lesions of the lungs were so marked that they constituted a sufficient cause for death of the individual without the accompanying specific infection. They were not more common in children than in adults, although atelectasis was much more marked in children.

Interstitial lesions consisting of focal infiltration of the tissue around the bronchi and around the blood vessels of the lung were found in a number of cases. These did not seem to have any relation with the purely exudative lesions. The cells found in these interstitial foci were the large basophilic cells and a small number of phagocytic cells. The lesions, however, never assumed the importance here that they had in the kidney and in the testicle, and were comparable to the unimportant interstitial lesions which were found in the portal spaces of the liver. In spite of the fact that the large basophilic cells were contained but rarely in the exudate in the lung, numbers of them were often found in the capillaries, and in two cases there were thrombi in the veins which were chiefly composed of these cells entangled in a fibrinous network.

The very small amount of fibrin in the exudation made a marked difference in the histological picture of the bronchopneumonia of small-pox, as compared with similar conditions in diphtheria and other infectious diseases. This was probably due to the character of the blood, for the organisms causing the lesions are the same in small-pox and in diphtheria.

IV. THE LIVER.

The first observations on the condition of the liver in variola are those of Quinquard, in 1870, who describes lobular foci of hepatitis with fatty degeneration of the cells. Wagner found no special conditions in the liver. Curschman found fatty degeneration in such degree that he compares the liver with the phosphorous liver. Weigert describes small nodules of the size of a miliary tubercle formed of cells similar to those in the cutaneous lesions. Barthelemy found extensive fatty degeneration nineteen times in twenty-three autopsies. Siredey found in the earliest cases lesions of the interstitial tissue and cloudy swelling of the liver cells which later gave place to fatty degeneration involving all the cells of the lobule. The most recent article is that by Roger and Garnier. They found the liver in the pustular cases enlarged up to thirty-one hundred grams; the average weight was two thousand. In hemorrhagic cases the weight was less, but still above the normal. They think that the over-weight is due to an increase in the quantity of hepatic tissue, "la suractivité fonctionnelle aboutit à une proliferation anatomique active." In the interstitial tissue, particularly in the portal spaces, they found foci of infiltration with mononuclear leucocytes, and, in several

fatty degenerat
23 | 1900 (82%
184
60
enlarged

cases, large foci of hemorrhage. In addition to fatty degeneration, they found vitreous or hyaline degeneration and dislocation and separation of the cells. In the less intense cases of fatty degeneration the fat is found chiefly in the periphery of the lobules; later it extends to the centers.

In all of our cases the liver was examined microscopically in the usual way. Only the outer portions of the sections where the tissues was immediately exposed to the action of the hardening fluid were considered, as Zenker's fluid gives perfect preservation of the liver not more than two millimeters from the edge.

At the autopsy enlargement of the liver was generally noted. The average weight in twelve adults was two thousand two hundred and sixty-six grams. The greatest enlargement of the liver (one thousand five hundred grams) was found in a child ten years old. In considering the weights in these cases, it must be remembered that the subjects, as a rule, were otherwise healthy, and that the weights would, therefore, be higher than those found at ordinary hospital autopsies. Orth gives one thousand five hundred and seventy-four grams as the average weight of the liver of adults. This must be considered as below the weight of the liver in acute infectious diseases. In sixty-four cases of typhoid in adults the weight of the liver averaged one thousand eight hundred and ninety-three grams. It is to be regretted that the conditions of the autopsies did not allow accurate weights of the organs to be taken in all instances. The largest livers were found in the late pustular and crusting stages.

increased weight

It is difficult to understand the condition producing this increased weight of the liver. In one liver which weighed two thousand seven hundred and fifty grams there were large fat vacuoles in the cells, in addition to intense degeneration with numerous small vacuoles in the very granular protoplasm. In the other cases there was but little fat. The over-weight was not due to distention of the vessels, for there was no evidence of this in the sections, and at autopsies the livers were generally described as pale. The only condition to which the increased weight could be ascribed was the very evident swelling of the cells. The cells are larger and more granular than normal. The granulation of the cells has generally no particular character and represents only an intense degree of cloudy swelling. We have been accustomed to regard the liver in typhoid as the type of cloudy swelling, but the increase in weight in typhoid is slight as compared with the increase in small-pox. In most of the large livers there is no edema shown by dilatation of the pericapillary space. We think this enlargement of the liver to be a practically constant condition in the typical small-pox autopsy, which commonly occurs when the skin eruption is in the pustular and crusting stage. It was less marked in the earlier cases.

Liver cells larger more granular

In seven cases a peculiar and intense form of degeneration was found to which the French investigators have given the most attention. In this the individual liver cells are separated from each other. The connective-tissue framework about the portal vessels is preserved, and these retain their relative positions, as do also the larger portal veins. In the most advanced cases all the other connective tissue, including the walls of the capillaries, has disappeared. The section represents simply a loose mass of liver cells such as would be given after the maceration and separation of the cells. There was but a small amount of blood in the tissues. The condition was found both in children and in adults and in most cases in the pustular or crusting period. It is not due to ordinary post mortem change. The liver cells and nuclei, though degenerated, gave no evidence of this. The autopsies were made in the winter and at an average of ten hours after death, except in one case in which sixty-five hours elapsed before autopsy. None of the other organs showed evidence of post mortem change. It does not, however, appear to us conceivable that the condition could have been present during life, for in such a liver the circulation would have been impossible. If this had been present in any great degree, the loose liver cells would have been carried in large numbers into the pulmonary capillaries, and in no case were emboli of liver cells found in the pulmonary vessels. We

separation of the cells

regard it rather as a post mortem change due to the presence of some substance in the liver which exercised a solvent action on the cement substance between the hepatic cells and on the capillary walls. In the slightest degree the condition was seen first around the hepatic veins. We have found here separation of the liver cells from one another without any lesion of the capillary walls. Embolic masses of pyogenic cocci were common in the livers, showing these changes, as they were in all, but there did not seem to be any relation between the cocci and the condition of the liver cells.

very little fatty degeneration

There was but a small amount of fat in the liver. In one adult there were large fat vacuoles, in addition to small vacuoles in the granular protoplasm. Even in the livers of children there was less than the ordinary amount of fat. In but five of the fifty-four cases could the fat be regarded as representing a degeneration.[1]

refractive hyaline bodies

In five cases hyaline degeneration of the cells was found. In this the cells chiefly at the center of the lobule contain refractive hyaline bodies, staining strongly with eosin, and lying in distinct vacuoles in the cells. They were round, and varied in size from those just distinguishable from the granules of the cytoplasm to those of the diameter of a red blood corpuscle. Two such bodies, attached to one another like diplococci, were often found in the same vacuole. The material resembles the hyaline formed in the kidney epithelium, but is more strongly eosinophilic, and is much smaller in amount, the cells never being filled with it as they are in the kidney. In one case only could the hyaline bodies be regarded as numerous. In this, an adult dying in a late stage of disease, the central cells of every lobule contained them in considerable numbers. The periphery of the bodies often stained more strongly than the center. The liver of this case weighed two thousand eight hundred and seventy grams. This degeneration is a condition not confined to small-pox, but seems to be more common in this than in other diseases. We are ignorant of its significance and character.

[1] By fatty degeneration we mean degeneration of the cytoplasm associated with the presence of fat.

In six cases necrosis varying in extent was found. In three this took the form of the ordinary central necrosis of the lobule, and in one case only was it extensive. The necrotic cells were small, eosinophilic, devoid of nuclei, the pericapillary space dilated. In two of the cases the necroses differed from those found in the liver in other diseases in the absence of polynuclear leucocytes in the necrotic cells. In one case, which died in a late stage of the disease, leucocytes were found. No local cause for the necrosis could be determined. In three cases there were no foci of necrosis, but single necrotic cells were found, without any relation to the lobule. The very small degree of necrosis was singular, in view of other evidences of degeneration and of the constant streptococcus infection, with which necrosis is so often associated in other diseases. Necrosis probably represents the "dégénérescence vitreuse" of the French authors.

The nuclei showed marked variations from the normal, both with and without evidences of cell degeneration. The most common evidence of nuclear degeneration was clumping of the chromatin. All the chromatin of the nucleus was collected usually into a single large clump lying in the center. Occasionally it was attached to the nuclear membrane, and sometimes two or more clumps were found. The clumps were usually round and stained less intensely than normal chromatin. In a few instances the chromatin was acidophilic. In these large chromatin clumps there were occasionally homogeneous, colorless, small, round or oval intensely refractive bodies. They were usually all of the same size, and few in number. In the nuclei so altered there was a considerable network of granular anastomosing threads which were acidophilic. They were too regular in number and arrangement to be regarded as coagulum. We have regarded this network as representing the linen of the nucleus. It was probably swollen and more than ordinarily apparent from the withdrawal of the chromatin from its connection with it. A similar condition was found in the nuclei of the convoluted tubules of the kidney. In a number of other cases the nuclei were greatly enlarged and perfectly

In nuclei round or oval refractive bodies homogeneous. In some cells the nuclei were to be distinguished from fat vacuoles in the cells only by the presence of the nuclear membrane. The entire contents of the nucleus had disappeared. The membrane of the nuclear vacuole was usually round or oval, but occasionally folded and irregular. A condition was seen, regarded as antecedent to this, in which the nuclear contents were converted into a hyaline material, staining very faintly. This, however, could be recognized as material, because of a small space occasionally discernible between it and the nuclear membrane. In a few cases an irregular, acidophilic mass was seen between the homogeneous material and the nuclear membrane. In many of the nuclei so altered a small, faint, hyaline, round body was found almost always attached to the nuclear membrane. A similar condition of the liver nuclei has been found in leucemia. Both of these conditions have especial interest, in that they are unusual forms of nuclear degeneration, and may present slight similarity to some of the forms of the intranuclear parasites.

a small faint hyaline round body.

In some of the cases dying late in the disease there was more or less central congestion of the lobules, but in general the capillaries contained but little blood. In all instances there was an increased number of white cells in the capillaries, and in one case they were present in great numbers. They were almost exclusively the large basophilic mononuclear cells which have been described in the spleen and in the lymph nodes. Nuclear figures were found in considerable numbers in these cells. In one case, that of a child of eighteen months, these cells were so numerous that small foci were found in which all the capillaries were filled with them. Polynuclear leucocytes were almost wholly absent in the cases dying at the height of the disease. In the hemorrhagic and other early cases degenerated polynuclear leucocytes with fragmented nuclei and free nuclear detritus were found. The numerous other cells in the capillaries were not degenerated. Large phagocytic cells were found, alike free and attached to the walls of the vessels, containing both cells and detritus.

In six of the cases there were interstitial changes, consisting in accumulations in the portal spaces of cells of the lymphoid type.

No parasites were found in the liver. Emboli of cocci were found more frequently in the liver than in any other organ.

cocci emboli

V. THE SPLEEN.

Golgi and Ponfick found a difference between the spleen in the pustular form and that of the hemorrhagic forms of variola. In the typical pustular form it was enlarged, and in the hemorrhagic form it was small, hard, and dark. Birsch-Hirschfeld, on the contrary, found the spleen constantly swollen in the hemorrhagic form, while in the pustular forms swelling was slight or absent. Roger found the spleen enlarged in sixteen confluent pustular cases, while in twelve hemorrhagic cases, it was enlarged only in four. The pulp was distended by cells belonging to the different varieties of leucocytes, but the mononuclear forms predominated. Some of these were non-granular and others contained neutrophilic granules. The hemorrhagic form of variola is especially characterized by the presence of nucleated red blood corpuscles. Arnaud found enlargement of the Malpighian bodies and infiltration of the pulp.

enlarged in pustular

In our cases enlargement of the spleen with a maximum of five hundred and fifty grams was found in seven cases, and in most of these death took place in the late pustular or crusting stage.

In one respect the spleen in small-pox differs from that of the other acute infectious diseases, and that is in the great reduction in numbers of the polynuclear leucocytes. The spleen in the hemorrhagic forms of variola and in the early period of the ordinary type of the disease up to the end of the pustular stage contains, in some cases, no recognizable polynuclear leucocytes, in others very few. In the period of desquamation polynuclear leucocytes are fairly abundant, and in one case of death during convalescence they were numerous. Degenerating polynuclear leucocytes and the nuclear detritus resulting from their necrosis are found in cases of purpura variolosa and in some instances in the vesicular stage of variola vera.

In the spleen, as in the lymph nodes and in the marrow, the formation of large cells of the basophile type is a prominent feature. It was particularly striking in the spleens of children up to the age of seven years in the early pustular stages. These cells are formed both in the spleen pulp and in the Malpighian bodies. The Malpighian bodies, like the follicles in the lymph nodes, are in certain cases almost wholly composed of these large cells. The follicles appear edematous, the cells being separated from each other. In such cases there seems to be a slight difference between the large cells in the Malpighian bodies and those in the pulp, the former being not so granular as the latter. There is also a difference in size between the large basophile cells in the spleen and similar cells in the lymph nodes. The very large cells with a loose, granular, vacuolated protoplasm, which are so common in the lymph nodes, are rarely found in the spleen. Nuclear figures are numerous in the basophile cells, both in the follicles and pulp. Occasionally in these cells there is a separation of the cytoplasm into a loose, outer, basophilic mass and a more compact, neutrophilic interior. Much more evidence of ameboid activity is seen in the cells of the spleen than elsewhere. They are frequently found engaged in the walls of the sinuses.

large cells of the basophile type

The numbers of eosinophile cells vary, but they are more numerous in the spleen than in either the lymph nodes or the marrow. They are more numerous in the spleens of children than in those of adults, and they usually are of the type of marrow eosinophiles.

Enlargement and multiplication of the cells in the sinuses, leading to the formation of large phagocytic cells, was also seen. Phagocytic cells from this source were found containing cell inclusions. Distinct nodules, similar to those described in the follicles of the lymph nodes, and so commonly found in diphtheria, were found in the follicles in seven cases. They are composed of aggregations of phagocytic cells formed from the cells covering the reticulum. In some cases they contained much nuclear detritus coming from lymphoid cells. Nucleated red blood corpuscles are rarely found, and only in the spleens of children. They were very numerous in the case of an infant of four months.

The hyaline globules which were described by Arnaud were found in four cases within large phagocytic endothelial cells. These globules are acidophilic, and vary in size. In some cases only one large globule is found, and in others the cell is filled with a mass of globules all of about the same size. Their formation does not seem to stand in any relation to the disease.

acidophilic globules

In addition to these purely cellular changes there is always a varying, and in some cases a high degree of congestion, but rarely sufficient to produce a considerable enlargement. Hemorrhages into the pulp are frequently found, and the rare cases of considerable enlargement of the spleen seem to be due chiefly to this.

enlargement

Fibrin, both within the sinuses and diffusely scattered in the tissue, is found with and without hemorrhage. It is not contained within the phagocytic cells as it is in the lymph nodes. In several of the cases in the late stage a considerable amount of pigment was found, both free and enclosed in cells.

Numbers of streptococci were found in twelve cases. The streptococci are both within the sinuses and in the pulp. They are especially prominent in the purpuric cases.

Hyaline degeneration of the arteries of the follicles and the formation of hyaline reticulum in the follicles was found in four cases, the most marked formation being in a child of seven. This formation of hyaline is identical with that found in the spleen in diphtheria, and is not specific.

VI. THE LYMPH NODES.

Very little attention has been paid to the condition of the lymph nodes in variola, notwithstanding the fact that the cellular changes in them are most important and throw much light on conditions found in other organs. The only special mention of the lymph nodes which we have found is by Wagner in 1872 and by Roger in 1902.

Congested & enlarged

Wagner merely says that the lymph nodes are hyperemic and hypertrophied. The only histological description of the lymph nodes is that by Roger. He says that the changes in them are very analogous to the changes in the spleen. There is hypertrophy in the pustular forms of the disease, but this is little apparent or absent in the hemorrhagic. The swelling is manifest in the beginning of the disease, increases and persists during the period of eruption, and slowly diminishes. Cell forms appear in the sinuses and about the follicles analogous to those which are met with in the marrow. The cells are principally neutrophile myelocytes, basophile myelocytes, and a few eosinophiles. Giant cells and nucleated red blood corpuscles may also be found. In the cellular infiltration about the node is a condition resembling leukemia.

The lymph nodes in our study of the disease were examined in thirty-five cases. In some of these lymph nodes from all regions of the body were examined; in others only those which macroscopically appeared to be the most affected. In the examination of single nodes the cervical were most often selected for study. The changes found varied according to the period and the character of the disease, but they are sufficiently constant to enable us to form a definite idea of their character and progress.

oedema

The condition which is most constant, it being absent only in some of the cases which died during convalescence, is a general edema, producing dilatation of the sinuses and separation of the cells. The enlargement of the node is due more to edema than to cellular hyperplasia. All the sinuses of the nodes are dilated, but the peripheral sinuses are most affected. Both the afferent and efferent lymphatics, particularly the former, are also dilated and filled with cells.

Roger has described cells in the sinuses and in the follicles similar to those in the marrow. In the absence of perfectly sharp granule staining, it is often impossible to decide as to the character of the granules in the cells, and so to differentiate them. In those preparations where the preservation of the tissue is best, the cells are mostly of the basophilic type. They agree substantially with the basophilic cells of the marrow, but are larger, and their nuclei are more vesicular and contain abundant chromatin. The chromatin often shows a peculiar arrangement, there being large masses from which finer filaments radiate for a short distance. One or several such large masses of chromatin are found in the nucleus or may be attached to the nuclear membrane. The cytoplasm has an irregular outline, this being especially marked in those cells which are undergoing division. In many of these there seem to be two kinds of cytoplasm. In the center, around the nuclear figure, the cytoplasm is dense, finely granular, and takes the eosin stain. Outside of this is a loosely arranged mass of blue-staining granules. In other cells, not in division, there is a similar separation of the basophilic part of the cytoplasm. In the nodes from three cases, and to some extent in others, there is a definite blue-staining cap on one or both sides of the cell, in rare cases forming a circle around the cell. The cap in some cases forms a part of the general outline of the cell. In others it gives the impression of a structure added to the cell, forming a crescentic prominence on the periphery. In one such large blue crescent there is a pale, oval area in the middle.

In some of the nodes, particularly in those from children in the early vesicular stage, these cells are the most abundant. The structural separation of follicles and sinuses has completely disappeared, and the node represents a loose mass of cells. In cases not so advanced as this, the large cells, though chiefly in the sinuses, are also in the follicles. Every transition can be seen between the large cells and the lymphocytes. In this transition there is shrinkage and gradual disappearance of the protoplasm. In the large cells it is abundant, contains numerous small vacuoles, and the granulation is not definite. As the cells diminish in size, the vacuoles disappear, and the granulation becomes more evident and more distinctly basophilic. The apparent separation of the cytoplasm into a neutrophilic central portion and a basophilic exterior disappears, all the cytoplasm being basophilic. The nucleus undergoes somewhat similar changes. Its vesicular character becomes lost, it becomes smaller, and the chromatin becomes arranged around the periphery of the nuclear membrane, forming solid clumps without the radiating filaments. Some of these cells have all the characteristics of Unna's plasma cells. In the smallest cells the cytoplasm almost disappears, forming an irregular outline close to the nucleus, and being without evident granulation. The nucleus is so contracted that it is almost filled with chromatin which is arranged in triangular masses around the membrane. No cell membrane can be distinguished in these cells, but the outline becomes sharper with the reduction in the size of the cell. Nuclear figures are found in great abundance and perfection in the largest of these cells. They become rarer as the cells decrease in size and none are found in the small cells.

Degeneration and necrosis are rarely found in the large cells, and progressively are more frequent in the smaller forms. In one of the late hemorrhagic cases nearly all of the small lymphocytes were degenerated. There are two forms of degeneration: one in which the nucleus becomes irregular, the chromatin less distinct, and finally disappears; and another and more common, in which the chromatin swells, forming a solid mass which assumes peculiar shapes. It is rarely homogeneous, usually it forms a circle with the bulk of the chromatin chiefly on one side, and only a thin rim of it on the other, and with an unstained, homogeneous material in the center. The whole may break up into small fragments, each of which assumes this shape, or perfect circles may be formed with a small, unstained area in the center. In some cases several small, unstained areas may be found, the nucleus representing, on a reduced scale, the masses formed by the degeneration of the cells in the testicle. It was often striking to see the most evident degeneration going on in the small cells with perfectly preserved and rapidly multiplying large cells present in the same field.

With these large basophilic cells and lymphocytes, phagocytic cells are always present. They are most numerous in the later stages of the disease, and are chiefly confined to the sinuses. They vary in size from that of the basophile cells (or smaller) to four or five times their diameter. They are phagocytic for all cells of the basophile type and for other material. They contain large vacuoles in which cells and other substances are included. The vacuole is sometimes out of all proportion to the size of the cell. Single or sometimes several cells are included in the vacuoles and undergo destruction, only fragments remaining. The vacuoles often contain granules which are arranged around the periphery. Granular material in varying abundance is also contained in the sinuses, and is regarded as coagulum or material from broken up cells. The granules within the vacuoles differ from the granules in the sinuses in being more sharply acidophilic, in showing greater irregularity in size, and in being definitely round. It is impossible to say whether the granules in vacuoles represent remains of cells, or granular material taken into the cell, or whether they are formed by the phagocytic cells. Fibrin filaments and beautiful stellate masses of fibrin were frequently found in the vacuoles.

Distinct nodules, composed of epithelioid or phagocytic cells similar to those so frequently found in the follicles of the nodes in diphtheria, are also seen in small-pox. They contain some fragmented chromatin from lymphoid cells, but there is not such a definite relation of these cells to necrosis as there is in diphtheria. Often the nodules are composed of groups of single cells which later undergo more or less fusion. The origin of the phagocytes from the endothelial cells of the reticulum is evident. The cells on the reticulum lining and crossing the sinuses are swollen and increased in number. Cells are found partially detached from the reticulum which agree in all repects with those in the sinuses. Nuclear figures are found in the cells attached to the reticulum, and in those lying in the sinuses. It was perfectly evident that only the forming and recently formed cells were undergoing division. When the cell enlarges and digestive vacuoles are formed in it, and its phagocytic function is established, the reproductive activity ceases. In the largest cells the nucleus is almost unrecognizable, it being formed of a thin, nuclear membrane, and almost devoid of chromatin. Hyaline globules are found in the phagocytic cells, either few in number, or filling the entire cell. There are also a few free hyaline globules.

In the convalescent cases great numbers of polynuclear leucocytes are found both in the sinuses and in the follicles. In all cases more polynuclear leucocytes are found in the nodes than in any other tissues save in the vicinity of the streptococcus infections. Occasionally they are absent. In one node the only polynuclear leucocyte found was within the vacuole of a phagocytic cell. Those in the sinuses often show degeneration and fragmentation. It seems probable to us that many of the polynuclear leucocytes in the sinuses have been brought there by the afferent lymphatics from some focus of bacterial infection. There is no evidence that they are formed in the nodes. Examination of the blood vessels in these cases does not show that they are carried into the node by the blood.

Varying amounts of fibrin are found in the sinuses, and a certain amount is almost universally present. Few of the nodes contain extensive hemorrhages. The cervical nodes of an adult, in the early vesicular stage with extensive lesions in the pharynx, were hemorrhagic in high degree, all the sinuses containing blood. In a node of one of the hemorrhagic cases there was disintegration of the red blood corpuscles leading to the formation of round bodies varying in size, with a bright acidophilic margin. A few scattered red blood corpuscles were also found in the sinuses, either free or enclosed in the phagocytic cells.

Eosinophile cells are fairly numerous, more so than they are in any other situation save in the marrow of children. They are found in all stages of the disease. They are principally of the mononuclear type, though polynuclear cells are occasionally found. In one infant of eighteen months numerous giant cells of the bone marrow type were found in the sinuses of a lymph node attached to the pancreas.

Parasites are never found in the nodes, nor are there forms of degeneration which resemble them. Streptococci are very commonly found in the sinuses. The most marked instance of streptococcus infection was found in the cervical nodes of an early hemorrhagic case. In these nodes the entire peripheral sinus and most of the other sinuses were blocked with them. Occasionally a few were found enclosed in the phagocytic cells, but these, in general, are not attracted by the bacteria. The cells are often found in the sinuses with masses of streptococci about them, yet they show no inclination to incorporate them. No reaction is found around the streptococci; they exert no influence on the number and the varieties of the cells found in the nodes, and they stand in no relation to necrosis, although both streptococci and necrosis are more abundant in early than in later cases.

VII. THE BONE MARROW.

The first investigation of the bone marrow in small-pox was made by Golgi in the epidemic of 1873. He examined the marrow in thirty-five cases and found different types of changes in the suppurative and hemorrhagic forms of the disease. In the suppurative, colorless cells predominated, and in the hemorrhagic, nucleated red corpuscles. Chiari, in 1893, investigated the marrow in twenty-two cases embracing all stages of the disease, and found changes in eighty-six per cent. He described under the name of osteomyelitis variolosa changes consisting in foci of necrosis and hemorrhage, associated with the presence of large cells. He considered these lesions to be of the same general character as the lesions in the skin and in the testicle. The necrosis was most marked in the more advanced cases, and occasionally he found fibrinous exudation associated with the necrosis. He regarded the large cells as formed from the marrow cells, their size being due to the absorption of fat. Mallory studied the anatomical distribution of the foci described by Chiari, and found that the marrow in all parts of the skeleton was affected.

The more recent studies on the marrow in small-pox have been in connection with the changes in the blood. Roger, Josué, and Weil combined their examination of the marrow with the ante mortem study of the leucocytes in the same individuals. They found in the adult a degree of reaction of the marrow corresponding with the number of leucocytes in the blood. In cases with a leucocyte count of from four to six thousand there was in the adult little or no reaction in the marrow. In children, and in adults with complications, the changes in the marrow were more marked and corresponded with the changes found in the blood. There was an increase in the mononuclear cells and a diminution or complete absence of the polynuclear. The essential condition is that the marrow is incapable of transforming the mononuclear myelocytes into the polynuclear neutrophiles. The authors also note karyolysis and karyorrhexis in the marrow cells. They make no mention of the focal changes described by Chiari. Courmont and Montagard come to essentially the same conclusions. Muir studied the marrow in eleven cases, using that of the rib for both sections and smears. The marrow showed about the normal amount of fat. There was a general diminution in cells, this being compensated for by vascular dilatation. Like other observers, he found the polynuclear cells practically absent. The neutrophile myelocytes were diminished in numbers, a few of them being in mitosis and occasionally degenerated. The eosinophile myelocytes were also scanty, but proportionately less diminished than the neutrophiles. The cell depletion of the marrow is due to the cells being carried off in the blood. He found none of the large necrotic foci described by Chiari.

The marrow was investigated in twenty-seven cases, embracing all ages and all varieties and stages of the disease. The examination was entirely confined to sections of marrow hardened and stained in the usual manner. The marrow was taken from the vertebræ and from the upper third of the femur. The best sections for cell study were obtained from hyperplastic marrow of the femur.

The most striking change, and one which has been noted by all observers, is the marked reduction, even to complete absence, of the polynuclear leucocytes. This was noted in all the cases, with the exception of one, in which death took place during convalescence. In this the marrow corresponded to that of an active leucocytosis. The absence of leucocytes does not seem to be due to their degeneration and destruction in loco, for, save in the hemorrhagic cases, degeneration forms and nuclear detritus, due to their destruction, are not found. The foci of necrosis are without the usual border of leucocytes, and there are none in the fibrinous exudate occasionally seen in the reticulum.

Extensive degeneration, both in definite foci and a diffuse degeneration affecting single cells, was found in the hemorrhagic and in other early cases. The foci of degeneration, taking the form of complete necrosis of the tissue, vary in size from those easily visible to microscopic areas. In the hemorrhagic cases the necrosis is not circumscribed, and in addition to actual necrosis there is a general diffuse degeneration shown by imperfect staining of cells. In other cases the foci of necrosis are more definitely limited. They are frequently associated with hemorrhages which extended beyond the necrosis. In the early cases there is no reaction around the necrotic areas. In the cases dying in advanced crusting stages, collections of large cells are found in the fat spaces around the necrosis and in the reticular tissue. These foci of necrosis and hemorrhage are the lesions described by Chiari, but the large cells, which he described as occurring in connection with them, do not constitute a necessary part of the process. We did not find the foci so commonly as did Chiari, probably due to the fact that our search for them was not so minute.

The abundance of cells in the marrow varied. It was not hyperplastic in the hemorrhagic and in the early vesicular cases. In the later cases it was generally hyperplastic, but there was considerable variation. In the hyperplastic marrow the most numerous cells were those which could be regarded as premyelocytes. They vary somewhat in size. The protoplasmic contour is irregular, and they are often provided with blunt processes suggesting an ameboid character. The protoplasm stains slightly pinkish with eosin, and lacks definite granules. The nucleus is round or oval, relatively large, with abundant chromatin, which is often in large masses in the interior of the nucleus. There are also small masses of chromatin attached to the nuclear rim. Definite myelocytes are not numerous, and the forms with incurved nucleus, generally regarded as transitional forms leading to polynuclear leucocytes, are also rare.

In most cases there are present variable numbers of cells of a different and more distinct character. These vary considerably in size. The cell outlines are distinct and sharp. The protoplasm is definitely granular, and has a pronounced blue color when stained with the methylene blue. The nucleus is more brightly stained than it is in the cells first described. The chromatin is abundant, and distributed chiefly at the periphery. The shape of the cells is somewhat irregular, but forms suggesting ameboid activity are rarely found. In some cases these cells are so abundant as to form the predominant type, but generally they are much less numerous than the premyelocytes. They are of the same character as, or are identical with, many of the cells found in the outer part of the follicles and sinuses of the lymph nodes and in the interstitial tissue of organs. Nuclear figures are always abundant in them in all situations. When undergoing division the cell is enlarged, and the granules in the protoplasm become larger and more conspicuous. In some of the dividing cells there may be a distinct line of granules around the periphery, and granules of the same character are often found around the outside of the cells, apparently being separated from the protoplasm. In marked type these cells are easily distinguishable from the premyelocytes, but forms are seen in which neither the granulation nor the color of the protoplasm is so distinct. Many of the cells approach very closely to the character of plasma cells, and occasionally it is possible to distinguish in them the small crescent at one side of the nucleus, in which the protoplasm takes more of the eosin stain. Cells of a typical lymphoid character are found in varying numbers, depending largely upon age, they being more abundant in children than in adults. They are often simply distributed among the other cells, but there is a tendency towards their occurrence in groups. We have never found groups of them representing definite follicles, such as are often found in the marrow in diphtheria. They are more abundant in those sections in which the large basophile cells are more abundant.

The eosinophile cells also vary greatly in number and are more abundant in children than in adults. They are much less numerous than in the hyperplastic marrow of other infections. In some cases they are absent. In type they correspond chiefly to the eosinophile myelocyte, and those with nuclei of the polynuclear type are as generally absent as are the polynuclear neutrophiles. In the cases in which they were abundant cells similar to premyelocytes were found with a varying number of eosinophile granules in them. These granules were never uniformly distributed in the protoplasm, but were in a group generally near the nucleus.

Nucleated red blood corpuscles are below the number found in healthy individuals of corresponding ages. In some of the latter cases they seem slightly to increase, but it is not possible to make this out definitely. No particular change can be distinguished in the giant cells.

Great interest is attached to the large cells first described by Chiari. He regarded them as formed from the myelocytes and as representing a reaction of the tissue around the areas of necrosis. We are not able to make out any constant relation between the necrosis and their presence. They are occasionally found at the edge of necrotic foci, but are more frequently independent of these. They vary greatly in size. Cells are seen from those of the size of a myelocyte to those nearly filling a fat space. The smaller cells have some resemblance to myelocytes, but could be distinguished from these by their having a more vesicular nucleus poorer in chromatin, and a more homogeneous protoplasm. The nucleus is generally more oval than round, sometimes irregular in outline, and the chromatin seems to diminish with the increase of the cell in size. In the very large cells, swollen from fat absorption, the faint outlines of the nucleus can with difficulty be distinguished. The protoplasm is distinctly acidophilic, but this is more marked in the smaller than in the larger cells. The outlines of the small cells are often irregular, with knob-like projections. The cells are in part distributed singly among the other cells, but more frequently are found in groups. The most common mode of occurrence is in foci about a number of fat spaces. In no case are these cell-groups large enough to have produced a macroscopic alteration. They often form a line around a fat cell, or the space which this formerly filled may be occupied by two or more very large cells. The sizes of the large cells is evidently due to fat absorption. There is no increase in the cytoplasm. This takes the form of a very fine reticulum enclosing wide spaces which are presumably filled with fat, for the fat cells disappear as these cells become larger. Cells are occasionally found with large vacuoles within them. These large cells often show a distinct line around the periphery where the protoplasm is denser. They are phagocytic for all the cells of the marrow, and cells are taken up which show no evidence of degeneration. When formed around the foci of necrosis, they seem to be absorbing the fat. Nuclear figures are rare, but in one case a tri-polar figure was found. The origin of these cells is obscure. Cells similar to them are found in the blood vessels, both free and attached to the wall. We believe them to be endothelial in origin. Occasionally, rows of smaller cells of the same character were found surrounding a small artery. They are similar to the phagocytic cells found in typhoid fever. They are most numerous in the cases in which the marrow shows the greatest hyperplasia, and it seems probable that their function is intimately associated with the hyperplasia, in that they remove the fat to make place for the multiplying cells. They have not, however, been described in the hyperplastic marrow under other conditions. Not their presence, but their number and their focal accumulations seem to be characteristic of small-pox. Their relation to fat absorption is further shown by the finding of similar cells in the same relation to the fat in fat necrosis and in inflammation of the mesentery and the omentum. They are also occasionally found in lipomas and in the fat when invaded by a carcinoma.

The blood vessels are generally prominent and filled with blood. Muir calls attention to this and considers it secondary to the cell depletion of the marrow. Thrombi are occasionally seen in the vessels, but this is not a prominent feature. In the cases of purpura variolosa, hemorrhages were found involving considerable areas, without any change in the surrounding tissue. As has been said, hemorrhages often complicated the necrosis. Fibrinous exudation was found both in connection with the hemorrhage and necrosis and without any relation to these.

We have never found any form of cytorycytes variolæ in the bone marrow. In our early investigations particular attention was directed to the marrow, for it was believed that the lesions described by Chiari were characteristic focal lesions, and that the organisms might be found in them. Streptococci were found in the marrow in many cases, particularly in purpura variolosa, but there does not seem to be any relation between their presence and the focal lesions.

VIII. THE TESTICLE AND THE OVARY.

The first definite description of the lesions of the testicle in small-pox was given by Beraud in 1859. He found in a number of cases an inflammatory affection of the tunica vaginalis and foci in the tail of the epididymis, and in one case an affection of the testicle itself, consisting in a parenchymatous inflammation. He describes in this rather dense yellowish foci, in size from that of the head of a pin to that of a pea. Beraud cites Velpeau and Gosselin, each of whom had noted the condition, and the latter says he has never known it to give rise to any symptoms during life. Trousseau says he has frequently been able to make out the condition clinically. Curshmann found orchitis in only four out of four hundred and thirty-two cases. Chiari cites a very interesting observation of Geraud, who found orchitis in a boy with small-pox, and also bilateral orchitis in two men who had been revaccinated. In all three cases the affection ran a rapid and favorable course. Wagner found in the testicle in a case of hemorrhagic small-pox, in addition to hemorrhages, peculiar, small, grayish red or yellowish, lymphoid nodules. Chiari has made the most thorough study of the condition. He found the lesions in fifteen cases of children, and in a further examination of sixty-three cases, mostly of adults, lesions were found in forty-five. The lesions showed a perfect agreement in their stage of development with the skin lesions. In the early stages of the skin eruption the lesions were slight; they increased in intensity during the pustular stage and diminished during desquamation. In well-marked foci he distinguished a central area of necrosis, around this a line of small cell infiltration, and outside this a broader zone, not so well defined, which he calls the zone of exudation. In the earliest cases the interstitial tissue only was affected, the necrosis appearing secondary to this. Chiari regards the affection as due to the direct action of the small-pox virus carried to the tissue by the blood. He thinks that in the testicle the conditions for the action of the virus may be just as favorable as in the skin. The lesions show in their histological details similarity to the skin pocks.

45 75% lesions in testicle

In his excellent description of the pathological anatomy of small-pox, Roger says that the lesions of the testicle are frequent, but rarely have any clinical importance. Exceptionally, there is slight swelling of the testicle with an increase of fluid in the tunica vaginalis. The fluid is remarkable in the large number of mononuclear cells which it contains, and in the absence of polynuclear cells. On section, the tissue is often reddish and sown with small hemorrhagic foci and yellowish nodules which have somewhat the appearance of small tubercles, and which do not project above the cut surface. The histological examination shows intense congestion of the vessels, and an infiltration of the interstitial tissue, with large mononuclear cells and cells of Turck. The epithelium of the tubules may be destroyed. Roger says nothing of the etiology of the process.

In our cases the lesions were found in such numbers, and in such different stages of their evolution, that it has been possible to follow their development. There is undoubtedly a relation between the stage of the lesions in the testicle and that of the lesions in the skin, the earliest lesions being found in the early vesicular stages of the skin eruption, but the relation is not so absolute as Chiari describes it. The tissue was stained in a variety of ways, none of which gave any advantage over the stain in methylene blue and eosin. The focal lesions were usually visible on macroscopic examination, but were found in a number of cases in which they were not seen at the autopsy.

a relation between the lesions of the testicle & of the skin

(1.) Diffuse lesions. — There was absence of spermatogenesis save in the cases in which convalescence was established. This was shown in most cases by absence of normal spermatozoa in the lumina of the tubules, and also by the absence of those appearances in the epithelium which indicate their formation. In the early hemorrhagic cases some normal spermatozoa were found in the tubules and occasionally in the cells, but they were generally degenerated. This absence of spermatogenesis is not confined to small-pox, but may be found in typhoid fever and in other infectious diseases. The absence of spermatogenesis is due to degeneration of the epithelial cells of the tubules, and is most marked in those cells most nearly related to the spermatozoa. In the large basic cells of the tubules the degeneration was absent or least marked. Next to these, the cells with spiral chromatin were least affected. The most marked degeneration was in the smaller inner cells of the tubules. The degeneration affected both cytoplasm and nucleus. The cytoplasmic degeneration was of no special importance. The cells were swollen, the outline ragged, the granules larger and less distinct. Fragments of granular material, evidently separated from cells, were found both in their vicinity and in the lumina. The entire cells were often cast off, either singly or in adhering masses. Hyaline globules in the cytoplasm were occasionally found. In a few cases all the interior cells were lost, the tubules being lined with a single row of swollen, irregular

Small round acidophilic bodies

cells, showing indefinite striæ. In the basic cells, in a few instances, single, small, round, acidophilic bodies were found, varying somewhat in size, and sometimes lying in an indefinite vacuole. They presented a slight similarity to the young cytoplasmic parasites in the skin. They were found most frequently in the epithelium about the focal lesions. They were distinguished from the parasites by differences in staining and by the absence of structure and indications of growth.

The nuclear changes were more striking. The simplest consisted in swelling of the nucleus producing a relative diminution in the amount of chromatin, which stained less sharply. In these swollen nuclei peculiar parallel lines were often found, giving the appearance of rod-shaped structures within them. These structures were inexplainable until fortunate sections showed that they were due to sharp infoldings of the nuclear membrane often extending completely across the nucleus. In some cases several such folds were found, appearing as rods in the optical cross section. Degeneration affecting the chromatin was most marked in the interior cells. There was but little chromatin clumping. The degeneration chiefly took the form of swelling and fusion of the chromatin. The nucleus was converted in some cases into a large, homogeneous mass, which stained intensely with basic stains. In other cases the degenerated chromatin contained clear or slightly stained spaces which sometimes were arranged around a larger, central space. The chromatin was refractive, and differed in no respect in staining and appearance from the chromatin in other forms of nuclear degeneration. These vacuolated masses were sometimes enclosed in cells, at others were free in the lumen, mingled with cell debris. A very similar condition was seen in the chromatin of the spermatozoa. These were swollen, dense, and refractive, and contained vacuoles. Vacuolated bodies of this character present a superficial resemblance to the intranuclear parasites. They are smaller, their framework stains more intensely with methylene blue and is more refractive, and they never lie within a nucleus, being formed of the nucleus and not in it. All these forms of degeneration, including the vacuolar degeneration of the nucleus, have been found to as great an extent in typhoid fever. They have nothing especial to do with small-pox. The lesions were absent in the undeveloped testicles of children. Even when the focal lesions were numerous and extensive, the remainder of the organ was normal. In a few cases, notably in that of a man of forty-five years dying in the late pustular and crusting stage, small, round, vesicular bodies were found in the nuclei. Only one was found in each nucleus and that generally in the middle. They consist of a ring which stains intensely with iron hematoxylin, and refractive, clear contents. At a certain focus they show a dark dot in the center. In many of the cells, in place of these rings, there are solid masses which stain with equal intensity, and it seems possible that the rings may have been formed by the vacuolization of the solid masses. Probability was lent to this by the fact that the rings were often thicker on one side than on the other. spores? The bodies varied only slightly in size and were a little larger than spores, which, in staining and refraction, they resembled. They were never found in the typhoid and other testicles which we examined, and with the exception of the instance mentioned, very rarely in the small-pox cases. They are different from the rings in the pansporoblast. We are unable to explain these structures. The possibility of their being spores must be admitted, but the weight of the evidence is against this interpretation. We are inclined to think that the rings represent a form of degeneration of some part of the nucleus, possibly the nucleolus. Although we have seen this condition only in small-pox, it is probable that if it be a degeneration, it is not confined to this disease.

In one case of convalescence from small-pox we found old lesions consisting in increase of connective tissue, thickening of the membrana propria, and atrophy and destruction of the tubules. Such chronic lesions were, however, so commonly found in connection with the acute that it was impossible to say that in this case they had been preceded by the specific acute lesions. In the histological examination of the testicles it is remarkable how often chronic lesions are found, and this in cases which give no macroscopic indication of them.

Accompanying this general degeneration of the epithelium, there is usually some change in the interstitial tissue, consisting in edema and a slight increase in the number of cells.

(2.) Focal lesions. — The focal lesions are more numerous close beneath the tunica than elsewhere. They vary in number, some testicles being thickly sown with the lesions, while in others a single lesion would be found only after a number of sections were examined. The foci vary in size from those which are microscopic to those which are five millimeters in diameter. The smallest lesions, and those best adapted for study, are in the undeveloped testicles of children. In these and in some adult cases the beginning of the lesion is found to consist in infiltration of the interstitial tissue with cells. The infiltrating cells are lymphoid, and large, basophile mononuclear cells. The vessels are dilated, and cells similar to those in the tissue are found in them. Such foci involve only the interstitial tissue between two or three tubules. No change can be demonstrated in the epithelium, beyond the common degeneration. Nuclear figures are found both in the cells in the interstitial tissue and in those in the vessels. No degeneration is found in the interstitial cells. From such lesions as these, which can best be compared with the small interstitial foci in the kidneys, the process extends. In the larger foci the cells in the interstitial tissue are more numerous and closely packed in the dilated septa. The lymphoid cells and the large mononuclear basophiles predominate, but among them phagocytic cells begin to appear. These vary in size here, as elsewhere, and their phagocytic qualities are shown by the red corpuscles and other cells enclosed within them. There is some difference between the lesions in children and those in adults in the greater numbers of eosinophile cells in children. The phagocytic cells seem to be less numerous in children. The blood vessels in and about the foci are greatly dilated, and contain cells of the same character as those in the interstitial tissue, and there is considerable hemorrhage from them. At the edge of the lesion the interstitial infiltration extends gradually. In those lesions of about the size of a submiliary tubercle some degeneration is seen in the tubules. In the undeveloped tubules of children the epithelium of the tubules, where the interstitial changes are most intense, becomes necrotic, the nuclei are lost, and the entire cell is changed into a smooth, hyaline mass staining strongly with eosin. The necrotic cells retain their connection with one another, and the necrosis is often limited to small groups of cells. Little or no nuclear detritus is formed. In the adult testicle the necrotic cells are cast off, filling the lumen with a mass in which the single cells and nuclei are no longer recognizable. There is usually a small amount of fibrin in the interstitial tissue, particularly in those cases in which hemorrhage is present.

In the larger lesions, embracing all those visible to the naked eye, there is extensive necrosis of the epithelium and to a less extent of the interstitial tissue. The tubules are filled with a mass of necrotic material composed of epithelium and phagocytes, the whole staining so strongly with eosin that the tubules stand out in sharp contrast to the blue intertubular tissue. In addition to the phagocytic cells, which form part of the necrotic mass, others are found containing cells and detritus. The phagocytes are never found in the tubules until necrosis has taken place, and they enter the tubules from the interstitial tissue. The membrana propria of the tubules becomes swollen, fibrillar in character, and the sharp separation between the tubule and the interstitial tissue is lost. With the increase of the intertubular tissue and the encroachment of this on the tubules, the latter may be represented only by the remains of the necrotic epithelium. The tubules are also invaded by the large basophilic cells which likewise undergo necrosis and add to the mass of detritus. The blood vessels in the foci are obliterated in some cases by thrombosis, but chiefly by the pressure of the infiltrating cells. The walls of the small veins are often completely infiltrated with cells; in some the lining endothelium being elevated by them. Endarteritis, marked by an accumulation of cells beneath the intima, may be found not only in the arteries in and around the foci, but also in those at a distance from the foci. The endothelial cells are hypertrophied, projecting into the vessels, and in many cases lying free. Hemorrhage was found around such vessels. There are always large numbers of nuclear figures, both in the phagocytes and in the large mononuclear basophiles in the interstitial tissue. Thrombi are occasionally found in the larger veins at a distance from the foci. These thrombi are remarkable for the character of the cells within them, polynuclear leucocytes being almost absent. In all the lesions polynuclear leucocytes play no part; it was only rarely that a single one was found either in the vessels, in the interstitial tissue, or in the tubules in any stage of the lesions. The phagocytes acted very much as did the polynuclear leucocytes, being attracted in the same way by the necrotic tissue. In the vessels remote from the lesions polynuclear leucocytes were found in small numbers. In a differential count of the leucocytes in several large veins beneath the tunica vaginalis the large mononuclear cells and lymphocytes formed eighty-three per cent, the polynuclear leucocytes seventeen per cent.

No parasites were found in the interstitial lesions, although careful search was made for them here as in the foci in the bone marrow. It is not absolutely possible to exclude the possibility of their being present, for single gemmules and spores could be overlooked, or not distinguished in the mass of cell and nuclear detritus in both tubules and interstitial tissue.

Streptococci were found in the testicles with about the same frequency as in other organs. They were most numerous in the cases of purpura variolosa. They were usually found in the vessels, and occasionally in clumps in the interstitial tissue, without any reaction of the surrounding tissues. In one case necrosis was found around them, and in the necrotic tissue here were numbers of polynuclear cells. The streptococci cannot be shown to stand in any relation to the interstitial foci. They are occasionally found in the foci, but not more often than in the tissue elsewhere.

Epididymis. — Lesions were found in the epididymis in three cases. In two they were small, but of the same character as those in the testicle. The interstitial infiltration preceded the degeneration of the epithelium. The necrosis of the epithelium was not so extensive as it is in the testicle. The epithelium in places was lifted in festoons formed by the entering of large mononuclear and phagocytic cells. In one case with normal interstitial tissue the lumina of a number of adjacent tubules were filled with phagocytic cells closely packed together. There was no epithelial degeneration and no evidence that these cells had entered the tubules at the place where they were found. It seemed to us that they had reached the epididymis from the lesions in the testicle.

Prostate. — The prostate was examined microscopically in only two cases. In one there were focal lesions of the same character as those in the testicle, and some general infiltration of the intertubular tissue with large, mononuclear, basophilic cells.

It is to be regretted that both epididymis and prostate were not included in the routine examinations.

The ovaries. — The only reference to lesions in the ovaries in small-pox is by Beraud. He gives three cases in which there was exudation on the surface of the ovary with hyperemia of the vessels. We have found nothing which conforms to this description by Beraud.

The ovaries were examined in all cases, and in but two were lesions found which were regarded as part of the disease. In these cases, one dying on the tenth day of the disease and one a case of purpura variolosa, lesions were found very similar to those in the testicle. In the first the ovaries contained a number of small, simple cysts, and the lesions were for the most part in the immediate neighborhood of these cysts. They consisted in an infiltration of the tissues with large basophilic cells similar to those found in the organs elsewhere, and among which were numerous phagocytic cells.

The cells were in places necrotic, but the necroses were not sharply marked and were confined to single cells rather than to areas. In these places there were a number of polynuclear leucocytes. The cysts were lined with simple epithelium and in the interior there was granular material and a number of large, swollen, epithelial cells, some of them containing hyaline. The foci could not be regarded as due in any way to changes which had taken place within the cysts. In one of the sections from the purpuric case a single similar focus was found. No parasites were found in the lesions.

The examination of the Fallopian tubes showed nothing abnormal, excepting in one case of hemorrhagic small-pox, in which death had probably taken place during menstruation; in this case hemorrhages were found in the lumen and in the tissue without any specific alteration.

IX. THE KIDNEYS.

Little attention has been paid either to the clinical examination of the urine in small-pox or to the anatomical study of the renal lesions. These lesions are extensive and interesting, both in relation to the specific disease and in relation to the general pathological anatomy of the organ.

Hemorrhages in kidney pelvis. — Unruh, in 1872, was the first to call attention to hemorrhage in the pelvis of the kidneys and in the ureters in small-pox. He found this in twenty-eight of two hundred and twelve cases. In sixteen cases the hemorrhage was slight; in twelve marked. The hemorrhage took place both in and below the mucous membrane, leaving the epithelium intact, and in some cases producing no clinical symptoms. This work of Unruh is the only one in which especial attention has been called to this interesting condition, though Ponfick, in 1875, says that hemorrhages may be found in the mucous membrane of the urinary tract.

Such hemorrhages were present in five of our cases. Like Unruh, we found the epithelial surface of the pelvis intact, the hemorrhage taking place in and beneath the mucous membrane. Free blood was present in the pelvis. Microscopically, the epithelium was in part absent, in part necrotic, and a few polynuclear leucocytes were found on or within it. In places it was fairly well preserved. No parasites were found, but in many sections there were masses of streptococci. Beneath the epithelium the tissue is infiltrated with blood and with cells chiefly of the lymphoid type. Very little fibrin is found in the tissue. This condition we consider as connected with the secondary infections, and not due to the direct action of the parasites. It was found in cases in which there were hemorrhages in other organs. It has no connection with hemorrhagic nephritis, this being a rare condition in the disease. The hemorrhage was confined to the mucous membrane of the pelvis and did not extend into the ureters or bladder, except in one case in which punctate hemorrhage in the mucous membrane of the bladder was associated with the condition in the pelvis.

Streptococci

Huguenin, in 1900, called attention to the cloudy swelling of the kidney and to the frequency of glomerular affections. He thinks that these changes are due to the passage of toxic material through the kidney. In some epidemics albuminuria is rare, in others it is common. Renal affections may appear in late periods of the disease.

Cloudy swelling

Arnaud, in 1898, found albuminuria in ninety-five per cent of his cases. The amount of albumen varied from day to day, making a very irregular curve. As a rule, it was more abundant in the severe cases. He found anatomical alterations of the kidney very frequently. The lesions are diffuse and consist chiefly in interstitial cellular infiltration due to diapedesis, and in alterations of the epithelial cells. The lesions of the epithelium are marked and there may be necrosis. Lesions of the glomeruli are frequently seen. In the acute period, even without any albuminuria, there may be marked lesions both of the interstitial tissue and of the epithelium. He describes both a general acute edema of the interstitial tissue and islands of cellular infiltration. The interstitial lesions predominate. He gives no description of the cells in the interstitial tissue, but says that they come there by diapedesis from the vessels. In eight out of the thirteen cases which he examined the lesions were chiefly of the interstitial type. He has also made the observation that in

Albumin in 95%

interstitial cases the albuminuria may be slight or entirely absent.

Roger, in 1902, found albuminuria not common. It was present in fifteen per cent of the light cases, in twenty-five per cent of the discrete, and in twenty-eight per cent of the confluent. In two cases he saw nephritis appear during convalescence. The lesions of the kidney presented no special features. In a hemorrhagic case the kidneys were large and covered with ecchymoses; there were parenchymatous hemorrhages and the pelves were filled with blood.

Auché et Jouchères, in 1895, found the toxicity of the urine diminished in the state of suppuration. Bowen found focal lesions in the kidney which he compared with the lesions in the skin. They consisted of a necrotic center, surrounded by a zone of pus corpuscles and detritus, and outside of this a zone of epithelioid cells.

In our investigation the kidneys were examined in all cases, sections being made from a number of pieces. The usual methods of hardening and staining were employed.

There is nothing in the condition of the kidney which points to any specific action of the small-pox virus, either as a parasite acting directly on the tissue, or by means of soluble toxine. There is a close analogy between the condition in the kidney in this and that in other infectious diseases, but the changes are more common and more severe in small-pox than they are in any other acute infectious disease which we have studied. It is possible that the lesions are due rather to the concurrent infections than to the small-pox virus.

In our series there were two cases of acute suppurative nephritis, both in adults, and both in late stages of the disease, with crusting well advanced in the skin lesions. There were five cases of acute glomerulo-nephritis which which were also in late stages of the disease, the earliest case being in an infant of nineteen months which died on the fourteenth day of the disease.

acute degeneration

In all cases the kidney was affected; the most common change was acute degeneration, often of an intense character, which occurred in all stages of the disease and at all ages. The kidneys were usually somewhat enlarged, rather edematous, and opaque on section. Leaving out of consideration the cases of acute diffuse and acute glomerulo-nephritis, the greatest weight of both kidneys found was four hundred and ninety grams in an adult of twenty-nine years, dying on the eighth day of the disease, and two hundred and fifty grams in a child of ten years, dying on the eleventh day of the disease. The average of the recorded weights of those kidneys which showed the most degeneration was three hundred and ninety-one grams. In all of these cases death occurred comparatively early in the disease, in the vesicular and beginning pustular stage of the skin lesions. In the cases in which the degeneration was not so marked, the weight of the kidney was only slightly above the normal. The degeneration in these cases differed but little from the degenerations found in other acute infections. The convoluted tubules were chiefly affected. The cells were swollen, almost occluding the lumen; their protoplasm was much more irregular in outline than normal, and the granules coarse and few in number. The striated border of the cells entirely disappeared. The outlines of many of the cells were lost. In some cases the cells contained large, irregular vacuoles, apparently due to edema. There was no examination for fat, and the presence of this was only judged by the appearance of a line of vacuoles along the membrana propria. It is very probable that a considerable degree of fatty degeneration was present in all of the severe cases. The desquamation of cells was not so marked in these as in some of the later cases.

enlarged oedematous

Vacuoles

fatty degener

The nuclei showed various changes corresponding with the degeneration of the protoplasm. These changes consisted in an apparent diffusion of the chromatin in the nucleus, giving to it a rather solid appearance. In the nucleus of the normal renal epithelial cells there is a very slight staining of the general mass of nuclear fluid, in which the chromatin lies, and there is no appearance of a nucleolus differing from the chromatin in staining, although there may be one or more clumps of chromatin in the nucleus. With this diffusion of the chromatin in the nucleus, there were found one or often two masses simulating nucleoli, generally lying at the periphery of the nucleus, and, as the degeneration became more advanced, showing a greater affinity for the acid stains. In the most marked degree of degeneration, the nucleus appeared solid and entirely devoid of chromatin filaments; the peripheral ring was less marked, the entire mass was distinctly blue, and in this there was found a lilac or bright red mass, often taking an indefinite ring form, the interior being less sharply stained than the circumference. The condition is very similar to that described in the epithelium of the testicle. Not infrequently, small, colorless, refractive masses were found in the chromatin clumps.

Hyaline degeneration of the epithelium, consisting in great swelling of the cells, which became filled with hyaline droplets, was often associated with this form of degeneration. In the minor forms of degeneration the condition consisted chiefly in swelling and increased granulation of the cells, with less evidence of destruction.

In certain cases the degeneration affected all parts of the kidney to an almost equal degree. The glomeruli in three cases were not affected, although there was often found a slight amount of granular material in the capsular space. In one case of very marked degeneration an adherent mass of cells was found in the capsular space, which had evidently come from the convoluted tubules, and had been forced into the capsular space by the swelling and tension of the tissue. This condition was first described by Welch in the cantharadin nephritis of the white rat. In the lumina of the tubules there were found hyaline casts, desquamated epithelial cells, and a peculiar circular reticulum, consisting of masses of circles varying in size.[1] Casts, however, were not so common in these early cases as later in the disease.

It is not evident how much such degenerations as these

[1] We have elsewhere called attention to this condition, for which no adequate explanation has been given. These extremely definite circles may form in an albuminous exudate in almost any situation where it is in contact with epithelium.

have to do with the death of the individual. They point to an extensive destruction of the kidney, probably due to the direct influence of a toxin or of toxins on the tissue. From the clinical reports it seems probable that such degenerations may take place without leading to albuminuria.

Total necrosis of the cells was rarely found to any considerable extent. Here and there single necrotic cells would be found, or a small area in which all the cells of a tubule were necrotic. In the two cases of acute diffuse nephritis there was extensive necrosis, and in one of them a beginning abscess. Most of the kidneys were hyperemic, though hemorrhages were not common.

One case in which the kidney was of considerable interest was that of the fetus with congenital small-pox. In this the epithelium of the convoluted tubules was enormously swollen and vacuolated, and the nuclei in the cells usually stained very imperfectly or not at all Post mortem change could be excluded. It is not impossible that the death of the fetus in such cases may be due to degeneration of the kidney from the effect of toxins in the mother's blood.

The interstitial tissue, in addition to containing the foci of cells about to be described, was often distended with fluid. The increased size of the kidney is due to this edema, as well as to the vascular congestion and to the swelling of the cells.

The five cases of glomerulo-nephritis were of the usual types. In the most marked case, in which the kidneys weighed seven hundred grams, a very imperfect history of the duration was obtained, but it was supposed from the character of the eruption that the duration of the disease was only ten days. In this case the glomerular vessels were occluded by cells, and there was extensive proliferation of the capsular epithelium. In another case of glomerulo-nephritis in which death occurred in the period of convalescence, the glomeruli were enlarged and contained great numbers of polynuclear leucocytes, which were present also in the capsular space, evidently reaching there by migration from the glomerular capillaries. This case is of special interest because it is the only one in which any considerable number of polynuclear leucocytes were found in the kidney. In several of the cases the vessels of the glomeruli contained hyaline thrombi. In two cases, in addition to the hyaline thrombi, true fibrinous thrombi were found in the glomeruli. Occasionally thrombi were also found in branches of the renal veins.

All of these lesions of the kidney, however, are of inferior interest when compared with those which owe their character to changes in the blood. Probably the most striking thing on an examination of the kidney, and that which is most frequently met with, is the appearance of small groups of cells in the interstitial tissue, in various places, but chiefly in the upper portion of the pyramid, in the cortex bordering on this, and around the glomeruli. These cell groups are usually quite small, but they may cover a considerable area. The cells lie both inside the capillaries, which are distended, and outside of them. The cells are almost universally of one sort. They comprise large numbers of lymphoid cells and cells offering every transition between these and the large, basophilic, mononuclear cells.

Cell groups

Cells

In addition to their occurrence in these definite interstitial foci, the long vessels of the pyramids are usually dilated and filled with these cells. Great numbers of cells may be found in the vessels without any being present in the interstitial tissue. They are found chiefly in the vessels of the pyramids, but they may be found elsewhere. Often large veins in the cortex contain great numbers of them. In the small foci only cells of this character are found. In the larger, along with them there may be numbers of phagocytic cells. In one case the blood vessels of the pyramids contained large numbers of the mononuclear cells, and in an adjacent lymphatic there were numbers of phagocytic cells evidently derived from the lining endothelium. The large mononuclear cells are distinctly ameboid. We have seen them in the kidney, as in the spleen, in the act of direct migration from the vessels, and in the interstitial tissue they show blunt processes and every morphological evidence of ameboid activity. Nuclear figures in considerable numbers are found both in the cells inside the vessels and in those in the interstitial tissue. The interstitial cell accumulations and the collections of cells in the vessels were most marked in the cases which died in an early period of the disease, and in one of the cases of purpura of five days' duration.

It seems to us evident that these cell accumulations in the vessels and in the interstitial tissue of the kidney depend directly upon the changes in the blood. Similar cells are present in the blood in large numbers, and for some reason they tend to accumulate in the vessels of the kidney, particularly in those of the pyramids. Here the vessels often present the appearance of those around an area of inflammation, filled with leucocytes. The number of cells found within the vessels certainly show that they continually accumulate, for we know that the circulating blood does not contain them in such proportions. Whether this is brought about by a purely physical condition due to the character of the pyramidal vessels, and the presence of these cells in the blood stream, is uncertain, but in most cases there does not seem to be a positive chemotaxis between the cells and the tissue. They lie at random in the vessels and have no tendency to a mural arrangement. In the interstitial tissue they have no relation whatever to alterations of the parenchyma. There is no special alteration of the parenchymatous cells where they are, and very extensive destruction and necrosis of the parenchyma may be found without them. The vessels in the glomeruli do not usually contain them, or certainly they are never found there in greater numbers than they are in the blood anywhere. Cell degeneration leading to complete destruction, with the presence of nuclear detritus, is not found to any considerable degree in the interstitial foci. In only one instance was there any considerable degree of this, a case of purpura variolosa of five days' duration. In this there was nuclear detritus, which came both from the cells within the vessels and from those in the interstitial tissue.

The absence of polynuclear leucocytes is possibly a more striking feature in the kidney than in any other organ. We have repeatedly gone over sections of kidney without finding a single polynuclear leucocyte. A few leucocytes were seen in some of the late cases, and, as has been remarked in one case of glomerulo-nephritis, they were present in great numbers. In several of the cases in which degeneration was marked, hyaline masses were found in the capsular spaces of the glomeruli. The hyaline often took the form of a solid ring around the capsule, with irregular projections.

Streptococci were in seven cases found on histological examination, both as distinct emboli and diffusely scattered in the vessels. No especial search was made for them, and they were probably present in a large number of cases. There was no relation between the bacteria and the lesions.

Another point of interest in these cases was the very good condition of the kidneys, as far as the presence of chronic lesions was concerned. In only one case was there a chronic lesion of the kidney, which consisted in slight atrophy of the parenchyma with focal increase of the interstitial tissue; and this was in a man of twenty-five years.

X. THE ADRENAL GLANDS.

The only reference which we have found to the adrenal gland in small-pox is by Oppenheim and Loeper. They describe as the most common lesion foci of interstitial infiltration with large mononuclear cells.

In our investigation the adrenal was examined microscopically in nineteen cases embracing all stages of the disease, and in but five of these could it be regarded as normal. The most common change found was degeneration, which was present in thirteen cases. This affected all parts of the gland, but was usually less marked in the labyrinth than elsewhere. The degeneration was in some cases slight, in others it had proceeded to complete necrosis of the cells. It was either more marked in foci, or assumed entirely a focal character. The degenerative changes affected both nuclei and cytoplasm. The cytoplasm showed the presence of hyaline in droplets or in irregular masses, vacuolation, and disintegration. In the degenerated cells the cytoplasm was more acidophilic than normal, and in the necrotic cells it was strongly acidophilic.

The nuclei showed nothing of the clumping of the chromatin with degeneration, which was so prominent in the nuclei both of the kidney and the liver cells. The change which was common to all of the degenerated nuclei consisted in an apparent diminution in the amount of chromatin, which for the most part was arranged in small masses around the nuclear membrane. At the same time the contents of the nucleus became more acidophilic and homogeneous. The chromatin after undergoing these changes disappeared and did not give rise to the well-known masses of nuclear detritus. An occasional nucleus was found converted into a homogeneous, sharply circumscribed, acidophilic mass. Another form of degeneration consisted in separation of the cells from their relations to one another in the same way as has been described in the liver. This was found most marked in the same cases in which the separation of liver cells occurred.

The other lesions of the gland consisted in foci of cellular infiltration. The interstitial cells were here, as in other organs, chiefly the large basophilic cells. The foci were usually small and were found chiefly in the medulla of the gland. These interstitial cells were derived from the blood, and in the blood vessels of the foci numbers of similar cells were found. There were no collections of these cells in the vessels in cases in which interstitial lesions were not found. Among the cells there was a varying number of phagocytic cells. Both the phagocytic cells and the basophilic cells were found not only in the interstitial tissue of the gland, but also between the parenchymatous cells. There was no relation between the degeneration of the cells and the interstitial foci. Each occurred independently of the other, although in general the interstitial lesions occurred in cases in which the degeneration was most marked. Neither in the degenerated parenchymatous cells nor in the interstitial tissue were there any parasites found.

XI. THE PANCREAS.

The pancreas was examined in all cases, and no lesions were found. The gland seemed to be especially well preserved, and even in the cases where autopsy was made some time after death the pancreas showed less change, referable to post-mortem alteration, than any other organ. In the course of the routine examinations of other infectious diseases, we have never found such perfect preservation of the pancreas tissue as we have in these cases.

XII. THE HEART AND THE ARTERIES.

We have found but few special references to cardiac lesions in variola. Huchard, 1871, considers that death frequently results from heart paralysis. In the lighter forms of the disease he found little or no affection of the heart. In more severe forms he found affections of both endo and epicardium, and sometimes of the valves. Roger says that there is an exaggerated idea of the frequency of the cardiac affections in the disease; on the contrary, they are extremely rare. Hayem, Desnos, and Huchard, cited by Roger, have described arteritis in small-pox, especially in the hemorrhagic form, in the period of eruption and later, affecting both the aorta and the coronary arteries. Brouardel, in 1874, described changes both in the endocardium and within the aorta consisting in the formation of small elevations, which he regarded as true pocks. The changes which he observed in the aorta were simply the forms of chronic endarteritis.

In our series of cases the heart was always examined. Acute vegetative endocarditis of the mitral valve was found in one case, an adult who died on the ninth day of the disease.

There was no enlargement of the heart save in the cases of acute nephritis. The microscopic examination of the heart gave little more than the macroscopic. There were no examinations of the fresh tissue for the presence of fatty degeneration, the conditions under which the autopsies were made interfering with this. From the macroscopic descriptions it was probably frequently present, and in four of the cases the examination of the hardened tissue showed the presence of fat vacuoles in the fibers. In three cases, two of them purpura variolosa, there were small, well-marked foci of degeneration of the muscle fibers limited to the region close beneath the endocardium. In two cases, each of them late, rather diffuse areas of necrosis of muscle fibers were found in one case accompanied by hemorrhages and emboli of streptococci.

In four cases there was interstitial cellular infiltration with the large basophile cells. In one case, that of an infant of four weeks who died on the twelfth day of the disease, there was a general infiltration beneath the entire endocardium with these cells. They were also found in small foci elsewhere. The greatest interest, however, was attached to the case of acute vegetative endocarditis of the mitral valve. The vegetations on the valve were composed of fibrin, most of it hyaline. At the base of the vegetations there was some infiltration of the tissue with large cells. The most peculiar aspect of the vegetation was the absence of polynuclear leucocytes. These were not found either within or on the fibrin or infiltrating the base of the vegetation. The blood count in this case showed a very marked diminution of the number of polynuclear cells. Neither parasites nor bacteria were found in the lesion.

Our experience is in accord with that of Roger in showing the infrequency of cardiac lesions in small-pox. The few lesions found were in no sense specific, and were of the same character as those which might be found in any infectious disease. This seems to us rather singular in view of the frequency and intensity of the secondary infection with the pyogenic organisms.

There is little to be said about the arteries. Acute lesions in the large arteries were never found. There was, in a few cases, hyaline degeneration of the arteries of the spleen follicles.

Bacteriological examinations. — In eleven cases cultures were made from the heart's blood at autopsy. The streptococcus pyogenes was found in nine, the pneumococcus in one, and the staphylococcus pyogenes aureus in one. Cultures from the other organs were in accordance with the results of cultures from the heart's blood. Our results agree with the findings of Perkins and Pay as to the occurrence of the streptococcus pyogenes.

PATHOLOGICAL ANATOMY AND HISTOLOGY.

Summary.

The lesions in variola may be grouped as follows:

A. Lesions in character and in distribution fundamentally specific and due to the presence of a parasite peculiar to the disease.

B. Lesions associated with the above, of indeterminate specificity, in kind analogous with those present in many of the infectious diseases, but in degree characteristic of variola.

C. Lesions caused by accessory etiological factors, bacteria, whose presence and activity are conditioned by the specific infection.

A. The specific lesion.

1. The specific lesion of variola is a focal degeneration of stratified epithelium, vacuolar in character, and accompanied by serous exudation and the formation of a reticulum.

2. The fully developed product of these processes is a characteristic multilocular pock, or pustule.

3. The occurrence of these lesions is sharply limited to the stratified epithelium of the skin and of the mucous membranes of the soft palate, the pharynx, and the esophagus.

4. In number the lesions of the skin may be very few or so numerous as to overspread the entire body. In distribution they are fewest on the lower part of the trunk and most numerous on the face and on the hands; they bear no fixed relation to any anatomical structure in the skin. In size,

even when fully developed, they vary widely. Adjacent pocks may become confluent.

5. The lesions appear on about the fourth day of the disease, and passing through progressive stages reach a climax of development on the eighth or ninth day. Their appearance upon all parts of the body is not simultaneous, but begins on the face and hands and extends thence to the trunk and to the extremities.

6. The typical lesion is best seen in the skin. It begins with degeneration of the cells of the lower layers of the epidermis, accompanied by exudation, at first serous, later more or less cellular, the products of which are contained within the spaces of a reticulum formed by the degenerated cells. The exudate increases in amount and the spaces of the reticulum enlarge until its fibers finally rupture, and the lesion becomes a filled-out pustule. This development may take place wholly within the epidermis, and the fluid contents of the pock be separated from the corium by comparatively intact cells; or the corium may form the bottom of the pustule, in which case there is usually necrosis of the papillary border.

7. The subsidence and the repair of the lesion are accomplished by the removal of the fluid portion of the exudate by absorption and by drying, and by the regeneration of the epidermis, in the course of which the residual mass of degenerated epithelial cells, leucocytes, and debris, enclosed between two layers of horny epidermis, the old and the newly-formed, is exfoliated. The complete evolution of the lesion occupies about two weeks.

8. Associated with the lesions of the epidermis may occur edema, cellular infiltration, and hemorrhage of the corium.

9. The lesions of the mucous membranes are in degree proportional to the extent and to the severity of those of the skin. At an early stage of development they resemble the lesions of the skin, but owing to the structure of the mucous membrane, the resemblance is lost in the course of their evolution. In the absence of a restraining horny layer, the degenerated epithelial cells are cast off, and the vesicle within the epidermis is rarely seen, the pustule never.

Parasites

10. Contained within these lesions of the skin and the mucous membrane and determining their specificity and occurring chiefly in the cells of the rete mucosum is the parasite peculiar to variola; in its younger or cytoplasmic form it is present in the protoplasm of the epithelial cells of early lesions and of such of the older lesions as are extending; in its intranuclear form it is for the most part in lesions more advanced. No parasites have been found in any lesions of the skin in which repair was well advanced.

11. Accompanying the specific lesions and simulating them in form are others, bleb-like, in which the entire epidermis is elevated by fluid exudate; in these the parasite is absent.

12. Variations from that form of the disease which presents typical specific lesions are determined by differences in the character and in the extent of the eruption. On the one hand, the lesions may be few in number or incomplete in evolution, as in the abortive types of variola; on the other, they may, very early in their development and before becoming visible to the naked eye, be accompanied by diffuse hemorrhage into the corium, as in purpura variolosa. In any case, forms exist intermediate between the representative type of the disease and the variation. Thus, between purpura variolosa and variola vera connection is established through types of the disease in which vesicles are developed subsequent to hemorrhage, others in which the specific lesion is followed by hemorrhage (variola pustulosa hemorrhagica), and yet others in which the lesions of a typical eruption after development became hemorrhagic (variola vera with hemorrhage).

B. Associated lesions of indeterminate specificity.

1. Proliferation within the hematopoietic organs is constant and well-marked, and gives rise in the spleen, the lymph nodes, and the bone marrow to the formation of mononuclear, basophilic cells, and in the lymph nodes and the marrow to phagocytic endothelial cells. The former pass into the blood in large numbers. This process is present to some

degree in other infectious diseases, but is here so prominent as to be well-nigh characteristic.

2. Cellular infiltration with the mononuclear basophilic elements above mentioned, focal and interstitial in distribution, occurs constantly in the testicle, and usually in the kidney, in the liver, and in the adrenal glands. In the testicle this infiltration, by pressure and by thrombosis, causes anemic focal necrosis, lesions which seem to be specific of the disease.

3. Degeneration, focal in character, apparently not anemic, but due to the action of toxines, and leading to necrosis, at times with hemorrhage, and accompanied by focal formation of phagocytic cells, is present in the blood-forming cells of the bone marrow, and constitutes a lesion almost pathognomonic, but devoid of parasites. Diffuse degeneration, toxic in character, is present in the liver, the kidney, the adrenal gland, and the testicle; in the liver cloudy swelling is more marked than it is in any other acute infectious disease. Otherwise, the degeneration is not to be distinguished from that due to bacterial infection.

4. Inhibition of cell differentiation by the action of toxines is evidenced in the bone marrow in the absence of complete transformation of antecedent cells into polynuclear leucocytes, and in the testicle in the absence of spermatogenesis. The first-mentioned is a condition seemingly peculiar to variola.

5. The paucity of polynuclear leuococytes, alike in the specific lesions, in the focal degenerations, and in the bone marrow, is a condition so constant and so pronounced as to render it a striking peculiarity of the disease.

C. Associated lesions, bacterial in origin.

Accompanying the lesions specific or characteristic of variola, are others due to bacterial infection through channels created by them or incidental to the lowering of resistance.

1. Acute parenchymatous degeneration of the liver, the kidney, the adrenal gland, and the testicle, already mentioned as associated lesions of the disease, may in part be due to the

action of the toxines of the pyogenic bacteria usually present in severe cases.

2. Inflammatory processes, constituting complications of varied and often of great importance, are present with a frequency dependent upon their position. Boils, erysipelas, and cellulitis are common but unimportant sequelæ. Pharyngitis and consequent broncho-pneumonia are frequent in severe cases, and the latter is often the cause of death. Lobar pneumonia, abscess, and gangrene of the lung occasionally occur. Endocarditis, pleuritis, and empyema are relatively uncommon. Acute lymph-noditis is present in all severe cases.

LITERATURE WITH REFERENCE TO PROTOZOA AND THE CELL INCLUSIONS OF VACCINIA AND VARIOLA.

The first reference to the presence of protozoa in vaccinia or variola is contained in an article of Grünhagen. He described in the lymph of vaccine vesicles, clear, refractive, sharply contoured, greenish bodies which were either free in the fluid or clinging to leucocytes. He was not able to show that they multiplied while being observed.

Following Grünhagen, the next description of organisms other than bacteria is that of Van der Loeff in 1887. He found, on examining animal lymph in hanging drops, ameba-like structures which he regarded as the virus. In a second article, appearing the same year, he describes in the contents of the pustules in two cases of confluent small-pox the same structures which he found in the animal lymph and which he regards as protozoa. There is little doubt that the discovery and the general confirmation of the protozoal origin of malaria at this time had a great influence in directing attention to the possible presence of similar bodies in other diseases. In the same number of the same journal which contained the second article of Van der Loeff there appeared the first of a long series of articles on the subject by L. Pfeiffer. In all there are eight publications of Pfeiffer's on the subject, extending from 1887 to 1896. In Pfeiffer's first article he describes an organism, the "monocystis epithelialis."

It is found in both vaccinia and variola and passes through its first stage of development in the epithelial cells of the Malpighian layer. The smallest examples are nine microns and show a clear nuclear-like spot five microns in diameter. A cyst enclosing several and surrounded by a thick membrane is found. After this encapsulation sporulation begins. The spores enter into the lymph and are converted into the ameba-like bodies ordinarily seen. They were found in the contents of the vesicles and were cultivated up to the third generation.

There can be little doubt that neither Van der Loeff nor Pfeiffer saw the inclusions which later attracted the chief attention, and they probably mistook degenerated epithelial cells, or leucocytes, or fragments of such for the organisms. The second article of Pfeiffer's shows still more clearly the influence of the malarial work. In this, apparently neglecting his first publication, he describe bodies similar to the malarial parasites in the red blood corpuscles of vaccinated individuals. He shows figures in the red blood corpuscles in the vaccinated dog which are in all respects similar to the appearance depicted in the red blood corpuscles in malaria. He was evidently unfamiliar with the appearance of the malarial parasite. The work is important in that it contains the first description of the warm box for the microscope which has so generally replaced the warm stage. In his third article, which contains an admirable contribution to our knowledge of the coccidia, he refers only briefly to smallpox, and is evidently unable to group his supposed organisms in this order. In a further article he considers the specific parasite to be identical with the coarsely granular ameboid elements of the contents of the pock, but says at the conclusion of the work that a classification of the structures in question cannot be given until the histological and staining reactions of the long series of degenerated cells can be ascertained, and the zoölogist can demonstrate motility and mode of reproduction.

These early papers throw little or no light on the subject. The literature with regard to the presence of protozoa really begins with the publication of Guarnieri in 1892. In this he describes bodies which stain with carmine, hematoxylin, and safranin in the deep epithelial cells of the skin in vaccine and small-pox pustules, as well as in the epithelial cells of the cornea of rabbits which were inoculated either with vaccine or with variola virus. These bodies lie in clear spaces which are much larger than they, and vary in size from that of a micrococcus to the nucleus of an epithelial cell. He thought he could recognize both nucleus and protoplasm in them, and gave them the name of "cytoryctes variolæ," the name having reference to the supposed property of the organism to devour the epithelial cells and in this way form a space around it. He believed multiplication took place by two methods: (*a.*) Binary division.— He saw several times the division of the nucleus in the organism. The chromatin was situated at opposite poles, between which extended indefinite threads. He found several organisms in which there were two nuclei, and others in which there was a cross line indicating a cleft in the protoplasm. (*b.*) Gymnospore formation.— Organisms of a mulberry form were seen by him, the peripheries of which were studded with small masses which he considered spores.

His description of the bodies is perfectly clear. There is no doubt, however, that similar bodies had been seen in the epithelial cells in the variola pustule both by Weigert and Renault. Since the publication of Guarnieri there have appeared a large number of articles which have been, in the main, confirmatory of his description and interpretation, but which, with few exceptions, have added nothing to our knowledge of the bodies. Guarnieri was not the first to use the cornea for the purpose of studying the vaccine process. This was done in 1890 by Straus, Chambon, and Menard, who described the gross changes in the cornea after vaccination without mentioning the inclusions described by Guarnieri. In a second article, in 1894, Guarnieri again gave the results of further investigations on the nature of the cell inclusions. He found ameboid motion in the bodies when they were observed in hanging drops of fluid taken from young vesicles of small-pox and of vaccinia. In Guarnieri's third article, in 1897, he adds little to his previous work. He describes the bodies as taken up by polynuclear cells, and by adding methylene blue to the fluid in which they were examined, he found in them a slightly stained body of round shape, surrounded by a clear edge. The clear area is not enclosed in a membrane. He thinks that the stained body is a chromatic nuclear substance. He considers the bodies similar to those which he had described in the cornea of a rabbit after vaccination, and places them among the rhizopods. In this communication he regards the spore forms described in his first article as degenerations.

At the same time with Guarnieri's second paper there appeared an important publication of Monti. He found no bacteria in the skin in cases of hemorrhagic small-pox, but in the epithelial cells of the Malpighian layer were corpuscles which stained with Biondi's hematoxylin. On inoculating the corneas of rabbits with pustular contents, there was a formation of transparent, small nodules which healed in ten days; and in the epithelial cells he found the same bodies as in the skin. He regarded these corneal lesions as perfectly characteristic, and inoculated corneas with various tissues from cases of variola with a view to ascertaining the distribution of the virus. He produced successful inoculation with skin, pharynx, larynx, and occasionally with lungs, testicles, and spinal cord. The blood and internal organs gave negative results.

Clarke, in 1894 and 1895, confirms the work of Guarnieri. He found the bodies both in the cornea and in small-pox lesions. In some of them there was segmentation of the nucleus and cellular division. The cornea of the guinea-pig he regarded as more suitable for study than that of the rabbit. He also found similar bodies in syphilis. The plates which accompany his article show the gradual development of these bodies up to the spore stage. It seems improbable, both from the description and the plates, that he has seen the specific inclusions described by Guarnieri. Some of his developing bodies can be regarded as degenerated cells, some as leucocytes, and the origin of the others it is impossible to determine.

Ruffer and Plimmer described bodies in vaccinia and variola which they regarded as protozoa, mainly because they had ameboid movement. The bodies lie in vacuoles in the epithelium. Piani and Galli-Valerio found in the pustules of small-pox bodies varying in size, partly hyaline and partly granular, and sometimes motile. They were both in and between the epithelial cells. Von Sicherer gives a good description of the cell inclusions, confirming the work of Guarnieri.

The most important work following that of Guarnieri is that of Wasielewski, which is contained in two publications. The article in the "Zeitschrift für Hygiene" may be regarded as the most important confirmatory article which has appeared on the subject. He regards the inclusions as parasites and the etiological factor of the disease, and says that the organism cannot be classified with either the gregarinida or the coccidida. Its first influence on the epithelial cell is to produce an increased nutrition and an enlargement of the cell. He regards the perinuclear space as due to shrinkage in fixation. Wasielewski showed that injuries to the cornea produced by sterile substances did not give rise to changes of the same character as those produced by the vaccine inoculations, and that in them the typical inclusions do not appear. The inoculations with the contents of the vesicles of foot and mouth disease give rise to lesions of a different character from those of vaccinia. He also inoculated corneas with blastomycetes and other organisms, but never found lesions similar to those of vaccinia. He showed that the virus increased in the lesions, for he could inoculate from eye to eye indefinitely, without any change in the character of the lesion. The re-inoculations were successful up to the tenth day, but the most constant results were obtained by inoculating from a lesion from two to six days old. He vaccinated from the cornea of the rabbit to the calf in the fifteenth and the twenty-fifth generation, and in each obtained perfectly typical vaccine pustules and immunity towards

The original was vaccine

further vaccination. The vaccination of children was interesting. For this a cornea which represented the thirty-sixth generation was used. The epithelium was scraped off, rubbed up with sterile bouillon, and found free from bacteria by cultures made on agar, blood serum, and bouillon. This material, free from bacteria, was found to be perfectly efficacious for corneal inoculation. The amount of material so obtained was very slight, but positive results were obtained in six out of seven children who were vaccinated with it. These children were not further vaccinated to show immunity, the reactions being so typical that the parents would not suffer it. He confirms Guarnieri's observations as to the ameboid movement of the supposed organism.

The general conclusions of Wasielewski are important. The vaccine bodies are the only characteristic structures which can be found in the skin and mucous membrane in variola and in vaccinia. They are absent in normal and in other pathological conditions of the skin. The vaccine bodies appear in the corneal epithelium of rabbits with certainty when active vaccine is placed in an epithelial pocket of the cornea. These structures in the epithelial cells of the rabbit's cornea cannot be produced in any other way. Their origin from leucocytes can be excluded, nor can they originate from the nuclei of epithelial cells. Their origin from the cell protoplasm, in consequence of a specific toxic action, can be denied, because they are retained by filters, because they appear in dividing cells with normal protoplasm, and because we know nothing of specific toxic action affecting certain cells. The changes which these bodies undergo and their granular degeneration have never been observed in degenerating cells. The process cannot be compared with any of the known processes of degeneration. The inactive filtrates of vaccine lymph produce no action on epithelium. Wasielewski leaves untouched the question of the production of immunity in the rabbits by these bodies.

Siegel finds in foot and mouth disease and in the vaccine vesicles of calves structures which he thinks belong to the coccidia, and has photographed these with a power of one thousand five hundred diameters. The photographs are not convincing.

Gorini, in a series of publications, confirms the work of Guarnieri. He finds the vaccine bodies specific, but thinks it possible that they may be derived from the nucleus, or if the body is to be regarded as a parasite, that it may be a nuclear parasite as well as a cell parasite. In his publication of 1901 he compares the vaccine changes with the changes produced by the inoculation of the cornea with the "plasmodiaphoria brassicæ." Macroscopically, there is a certain similarity, but the changes produced by the plasmodiaphoria develop much more slowly than those produced by vaccinia. Microscopically, the process is totally different. The plasmodiaphoria produces a greater proliferation of the corneal tissue than of the epithelium. His study of the plasmodiaphoria led him to the idea that the cavity in which the vaccine body lies may probably be regarded not as a cell vacuole, but as a part of the supposed organism itself. The work of Gorini on the results of plasmodiaphoria injection was induced by the article of Podwyssotzky on the etiology of tumors. In his last article, 1902, he describes coccus-like forms which precede the specific inclusions. With the appearance of the larger forms the coccus-like forms disappear. He has proved that these forms, as well as the true cytoryctes, are the products of active vaccine and are not foreign organisms. He thinks that the cornea of the rabbit is the most suitable agent for testing vaccine lymph. In the plates which accompany this last article of Gorini's, he shows small bodies of about the size of cocci in and between the cells. These bodies are often united to form tetrads similar to micrococcus tetragenus. Gorini leaves the question undecided as to whether the larger forms are derived from the coccus-like forms.

coccus forms

E. Pfeiffer inoculated the corneas of rabbits and guinea-pigs and investigated them after from one to one hundred and four hours. He found in the sub-epithelial tissue small, dark-staining bodies which afterwards invaded the epithelium, divided, and produced the small cell inclusions. He inoculated corneas with syphilitic products and obtained the same forms. The plates which he publishes with the article show that he has seen the vaccine bodies, but most of his description and figures refer to things which have nothing at all to do with them.

Lebredo, in 1903, published a series of experiments in which he confirmed the work of Guarnieri and recognized the great practical value of the corneal inoculation as a means for establishing the diagnosis of small-pox in doubtful eruptions.

In addition to these articles, which refer specifically to the cell inclusions found in the cornea, there have been a number of other publications which refer only in part to these, being articles generally of a more ambitious character, in which the attempt is made to construct a life history of a supposed unicellular organism which is regarded as the etiological factor of small-pox and of vaccinia. The most important of these attempts is probably that made by Pfeiffer in various papers, the results of which he brought together in a publication in 1895. In this he compares the lesions produced in the cornea of the rabbit by inoculation with the prepustular stage of variola. The body enters the cornea and produces a specific change in the epithelial cells. On shaving off the infected epithelium and examining it on the warm stage, in a weak methylene blue solution of anterior chamber fluid the bodies inside the epithelial cells show active ameboid motion. The parasites increase by direct division. Around the vaccine point there is a zone of formative irritation which may extend to the membrane of Descemet. He thinks that such a vaccine pustule seven days old can be compared with a variola pustule five days old. In human vaccination and in small-pox the bodies pass from the epithelial cells into the blood. The blood parasites are found in variola, in vaccinated children, and in calves. The ameboid cells, some adherent to the blood corpuscles, others free and sometimes ciliated, are found in the blood. These bodies may form a sort of cyst whose contents divide up into an indefinite number of spores. He thinks that in variola there may be a mother pustule on the internal epithelial surface of the body. The first fever is due to this and ends with the passage of the germ into the epithelium. The eruption is an embolic affection of the skin. The ameboid bodies in the blood are, in the calf one-half, and in the child one-fourth, the diameter of a red blood corpuscle.

Doehle, in an article embracing the etiology of several of the exanthemata, describes the condition found in three cases of small-pox. He found structures more or less refractive and one-half to one micron in diameter, in the blood. On some of these he found cilia. There were also dark granular structures of two and one-half microns which showed contraction, and, in their interior, refractive granules in irregular arrangement. The development of these parasites can be followed in the contents of the pustule. In the beginning of the pustule they were never in the epithelium. The bodies in the blood were found some days after the beginning of the appearance of the exantheme and continued from five to seven days. In the vesicle there were motile ciliated bodies corresponding to the bodies in the blood. The larger bodies increased in the pustule, and the smaller ciliated bodies disappeared. He found the same thing in the lymph of calves. Illustrative plates accompany the article.

Roger and Weil found in the pustule contents, both between and in the leucocytes, small, round, or oval bodies staining intensely with Loeffler's methylene blue. They found them also in the blood of variola and more abundant in severe cases than in the milder.

In the hemorrhagic form these bodies were found in the hemorrhages in the spleen, in other internal organs, and in the bone marrow. They found similar bodies in the amniotic fluid of two pregnant women who died of small-pox. They were also contained in the blood of variolated rabbits. There is little doubt that the bodies referred to represent degenerated nuclei of leucocytes or fragments coming from these.

The chief part of Bosc's work refers to the sheep-pox or clavelée. He gives a good description of the inclusions and has attempted to cultivate them in various ways. He compares the vaccine lesions with those of small-pox, and believes that they are the same. In his plate of the third-day cornea he shows larger nucleated inclusions and the segmentation of these into spores. Weber, in two articles, has described in the blood of small-pox structures which he regards as different stages of development of protozoa. There are small, round, refractile, greenish or bluish bodies with rotation or with ameboid motion, larger homogeneous bodies which contain in their interior one or two small granules, and also granules which are connected together with threads. He obtained cultures in alkaline agar. Funck found in vaccine lymph and in the contents of variola pustules when examined in hanging drops, cysts filled with small refractile spores. He succeeded in cultivating the spores on agar. The parasite of variola he regards as occurring in two forms, as cysts filled with spores and as free spores. Reed, in 1897, found in the blood of small-pox and in vaccinated monkeys and children small ameboid bodies one-sixth to one-half as large as a red blood corpuscle. Some of them were clear; some of them granular. In one case he found a flagellum attached to the body. He says that similar or closely-allied bodies were found in the blood in several normal children and in a monkey. Dombrowski found in the pustular contents of variola, small bodies, motile and with clear contour, and also larger, sharply-contoured, yellowish bodies. In the fresh blood of small-pox he found bodies similar to the smaller forms.

In blood of small pox

cysts with spores & free spores

normal children

The work of Guarnieri has also led to a number of articles opposing his interpretation. The first one of these is the article of Ferroni and Massari. They conclude that the supposed sporozoa of Guarnieri are merely derivatives of nuclei of epithelial cells or leucocytes. They obtained the same lesions in a rabbit's cornea by producing inflammation in other ways, but they rarely found the inclusions in such large numbers.

The next article in opposition is that of Salmon. He regards the lesions which are produced as specific in character and as inoculable from one animal to another, but regards the cellular inclusions not as parasites, but as derived from the wandering cells, *i.e.*, the polynuclear leucocytes. He excludes them from the protozoa because they have no nucleus or cell membrane. The shape and size of the inclusions are also too irregular for such an origin. He thinks they are derived from portions of the protoplasm of the leucocytes or portions of their nuclei. The article of Borrel follows that of Salmon. Borrel has grouped a number of exanthemata under the name of infectious epithelioses. He finds inclusions similar to those described by Guarnieri in all of them. In clavelée they are contained not only in the epithelial cells but in cells of mesodermic origin. He regards all these inclusions as originating from polynuclear leucocytes and shows transitions between them all.

The most extensive article in opposition to Guarnieri's view, and one which constitutes a valuable addition to our knowledge of cellular changes, is that of Hückel. He regards the lesions as specific in character, as inoculable from animal to animal, and as conferring immunity, in which he agrees with Straus Chambou and Menard. The work of Hückel was carried out with the utmost detail, and every possible change in the epithelium brought out by various methods of hardening and staining was depicted. Hückel believes that they represent degenerations in the cells brought about by vaccination, but they are not organisms.[1] It is very difficult to understand all of the things which he has described. Some of them are certainly included leucocytes or fragments of them. Some of them seem possibly to be artifacts, but it is difficult from examination of the true inclusions to which he has only given a small space in his second plate (see Plate II., Fig. 58) to see any relation between these and the various

[1] His best results were obtained with the Biondi staining method; he gives the following classification: 1. Naked bodies; 2. Bodies with blue center and erythrophilic mantle; 3. Spherical bodies with red granules; 4. Spherical bodies with threads radiating to cytoplasm; 5. Half moon, sickle or spindle-shaped bodies; 6. Bodies of triangular form; 7. Rare forms; and 8. Bodies in the connective tissue cells of the cornea.

other things depicted. The figures which he gives of the inclusions are not satisfactory because they show no structure within them.

Roux regards the virus of vaccinia and of small-pox as probably belonging to the invisible organisms, and accepts the work of Salmon and Borrel regarding their leucocytic origin. Borrel has filtered the organism of clavelée, the filtrate retaining its virulence, and thinks that vaccinia is probably of the same character, although he does not give any filtration experiments on this. Sikorsky thinks that the bodies do not have specific character and that they may be produced by the injection of various animal fluids and especially by diphtheria toxin. Besredka, who reviews the article, says that the view is universally accepted that there is nothing specific in the vaccine corpuscles. They should be regarded merely as a transformation of the migrating cells. The substances producing them are of the nature of toxins. Ewing found the inclusions present in all stages of the small-pox lesions and their numbers proportionate to the degree of hemorrhage in the lesions. He believes that both in the skin and in the cornea they are derivatives of the red blood corpuscles.

Ishigami has described bodies in vaccinea and in variola which he regards as protozoa and which pass through two stages. In one, the ameboid form, the organism multiplies by simple division. In the other form division takes place by cyst-formation and the production of spores. He has cultivated the organism in a special culture medium, whose chief constituent was epithelial cells. It is impossible to reconcile the description of the bodies which Ishigami has found with the work of any preceding investigator.

In 1903 Councilman, Magrath, and Brinckerhoff published a preliminary communication in which they described bodies similar to those described by Guarnieri in the cytoplasm of the epithelial cells of the skin in both variola and vaccinia. In addition to these and representing a further and complete development of the parasite there are forms which develop only in the nucleus. The cytoplasmic forms precede the intranuclear forms.

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. From a case of purpura variolosa. Degeneration of the epithelium preceding vesicle formation with cytoplasmic parasites in the reté mucosum. X 200.

FIG. 2. Degenerated cells showing vacuolation of protoplasm and shrivelling of nuclei. X 1,000.

FIG. 3. Beginning vesicle formation with splitting of stratum corneum by the exudation. X 120.

FIG. 4. Section passing through a young vesicle. X 40.

FIG. 5. Reticular degeneration of epithelial cell. X 1,000.

PLATE II.

FIGS. 1 and 5. Early vesicle showing formation of epithelial reticulum. X 120.

FIG. 2. Later stage showing enlargement of spaces and rupture of reticulum fibres. X 120.

FIG. 3. Early vesicle at edge of hair sheath. X 120.

FIG. 4. Hyaline (Ballonartige) degeneration of epithelial cells at bottom of vesicle with nuclear fragments lying between cells. X 450.

PLATE III.

FIG. 1. Late vesicle with formation of secondary vesicle by elevation of the stratum corneum. X 40.

FIG. 2. Vesicle showing formation of secondary vesicle beneath true vesicle by elevation of entire epidermis. X 40.

FIG. 3. Septa between adjacent pustules. X 40.

PLATE IV.

FIG. 1. Flat section of umbilicated vesicle showing sheath of lanugo hair in centre of vesicle. X 110.

FIG. 2. False vesicle due to uplifting of entire epidermis by exudation. X 110.

FIG. 3. False vesicle showing separation of gland duct from corium by the elevation of the epidermis. X 110.

Plate V.

Fig. 1. Section through healing pustule showing regeneration of stratum corneum. X 40.

Fig. 2. Remains of pustule showing scab or disk within the horny layer. X 40.

Fig. 3. Section through healed pustule of palm showing hyaline mass, remains of pustule, disk, or scab beneath the dense horny layer. X 40.

Plate VI.

Fig. 1. Section through healed pustule of plantar surface showing "disk." X 20.

Fig. 2. From case of congenital small-pox showing formation of small vesicle by splitting of the stratum corneum by exudation. This is analogous to the formation of secondary vesicle in Fig. 1, Plate III., and in Fig. 3, Plate I. X 110.

Fig. 3. From congenital small-pox showing elevation of entire epidermis by exudation; analogous to process in Plate IV., Figs. 2 and 3. X 200.

Plate VII.

Fig. 1. False vesicle in trachea showing separation of epithelium by exudation. X 200.

Fig. 2. Early lesion in mucous membrane showing cell degeneration and invasion by bacteria. X 200.

Fig. 3. Section of trachea showing necrosis of epithelium with masses of bacteria extending along membrana propria. X 200.

Fig. 4. From a section of uvula showing masses of streptococci in lymphatics. X 200.

Fig. 5. Section of uvula showing masses of streptococci in necrotic tissue. X 200.

Plate VIII and VIII a.

Cytoplasmic forms of parasite.

Fig. 1. Advancing edge of vesicle under low power showing young forms of parasites in the epithelial cells. X 200.

Fig. 2. From same region showing number and distribution of parasites. X 800.

Figs. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, to show growth and differentiation of cytoplasmic parasites. X 1,000.

In Fig. 10 an hour-glass form denoting either ameboid motion, or multiplication by simple division. X 1,000.

Fig. 13. Cytoplasmic parasite preceding gemmule formation. The dark points become the future gemmules. X 1,000.

Fig. 15. A later stage. X 1,000.

Fig. 14. Gemmule formation. A few gemmules are seen within the parasite and in the surrounding tissue. X 1,000.

Fig. 16. Framework of parasite after formation and escape of gemmules. X 1,000.

Plate IX and IX a.

Phases of the intranuclear form of the parasite.

Figs. 1, 2, 3, 4, 5, 6, show young forms and development of intranuclear parasites into sporoblasts.

Figs. 7, 8, 9, 10. Intranuclear sporoblasts.

Figs. 11, 12, 13, 14, 15, 16, 17. Free sporoblasts. In Fig. 16, on the right, is a group of pansporoblasts.

Fig. 18. Early intranuclear parasite probably microgammete, from case of purpura variolosa.

Fig. 19. Free spores within nucleus. Fig. 19 is magnified 3,000 diameters Figs. 18, 3, and 6, 2,000 diameters. Figs. 7, 8, 9, 1,500 diameters. The remainder 1,000 diameters.

Plate X.

Fig. 1. Masses of streptococci in uvula. X 200.

Fig. 2. Area of broncho-pneumonia. X 110.

Fig. 3. Section of pleura and lung in a case of empyema. The pleural surface is covered with a thick mass of streptococci. The lung below is necrotic, and the tissue is covered up by enormous growth of streptococci. X 110.

Fig. 4. Necrotic tissue adjoining submaxillary gland with masses of streptococci. X 200.

Fig. 5. A small area from Fig. 4 showing streptococci. X 1,000.

Plate XI.

Fig. 1. Section of liver showing large basophile cells in capillaries. X 1,000.

Fig. 2. Section of liver showing hyaline bodies in liver cells with cloudy swelling. X 1,000.

Figs. 3, 4, 5. Sections of spleen showing character of cells, principally of large basophilic variety in pulp and in sinuses. In Fig. 4, a large basophile cell is shown in the wall of sinus in the act of migration.

Plate XII.

Fig. 1. Section of cervical lymph node from case of purpura variolosa showing the sinuses of node filled with masses of streptococci. X 25.

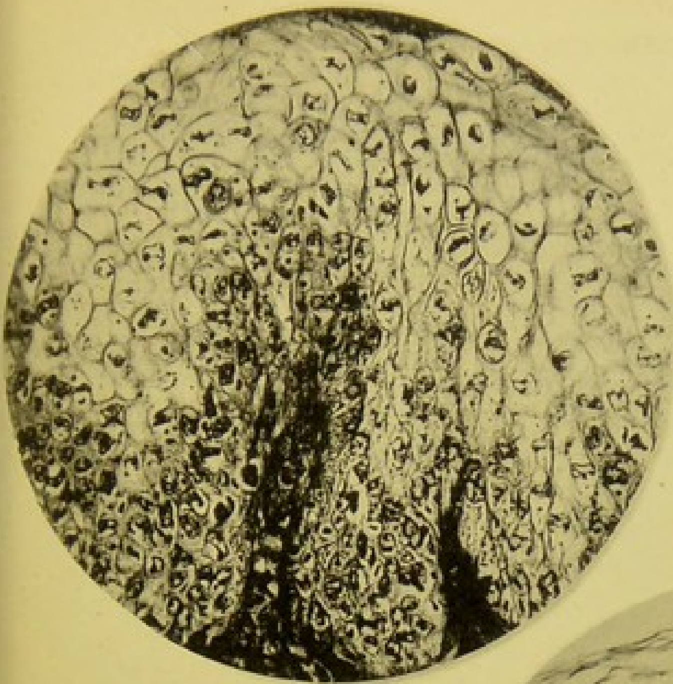
Figs. 2 and 3. From lymph node showing large basophile cells in sinuses. In the centre of Fig. 2 is a phagocytic cell with cell enclosed in the vacuole. X 1,000.

Fig. 4. Section of bone marrow showing large phagocytic cells in tissue. X 200.

Plate XIII.

Figs. 1, 2, and 3. Sections of bone marrow showing groups of phagocytic cells. X 200.

Fig. 4. Three cells of lymphoid type. The cell in the center has the characteristics of a plasma cell. X 1,000.



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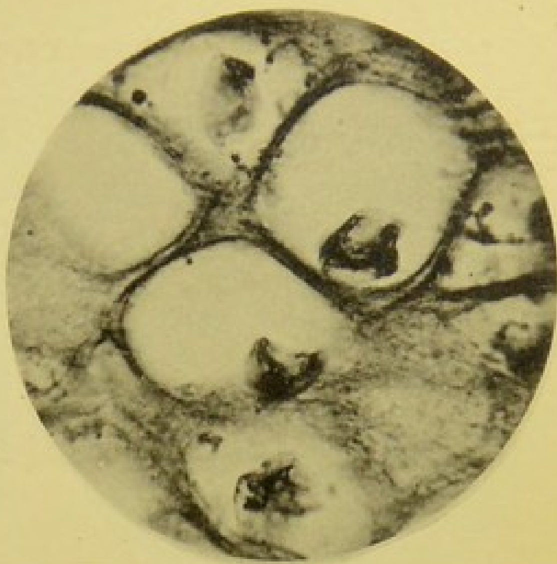

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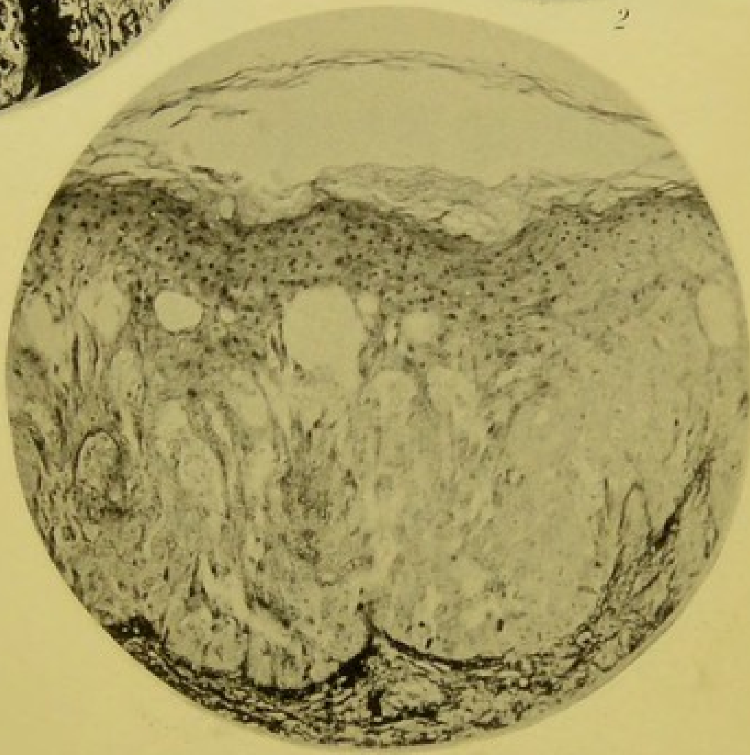

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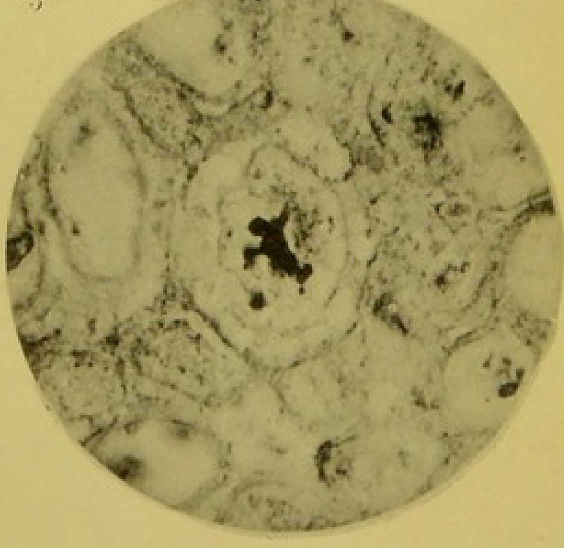

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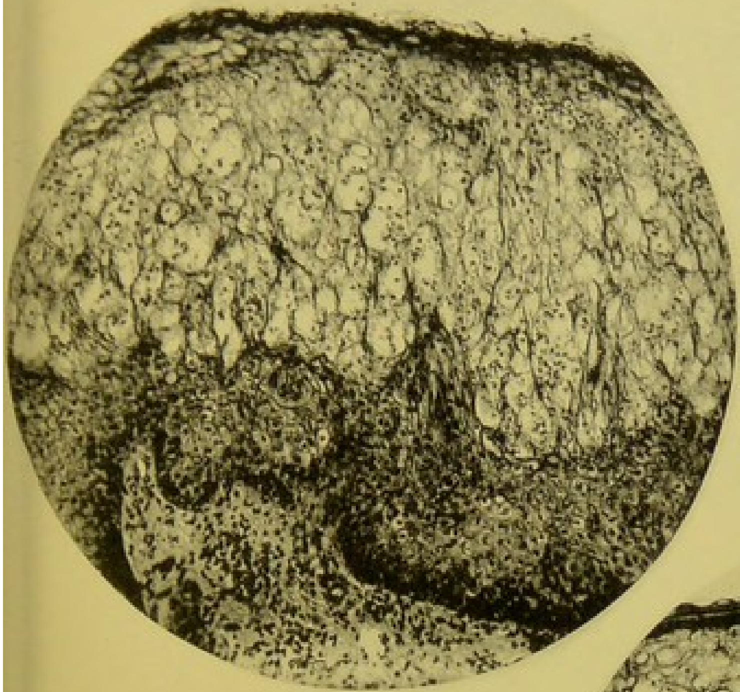

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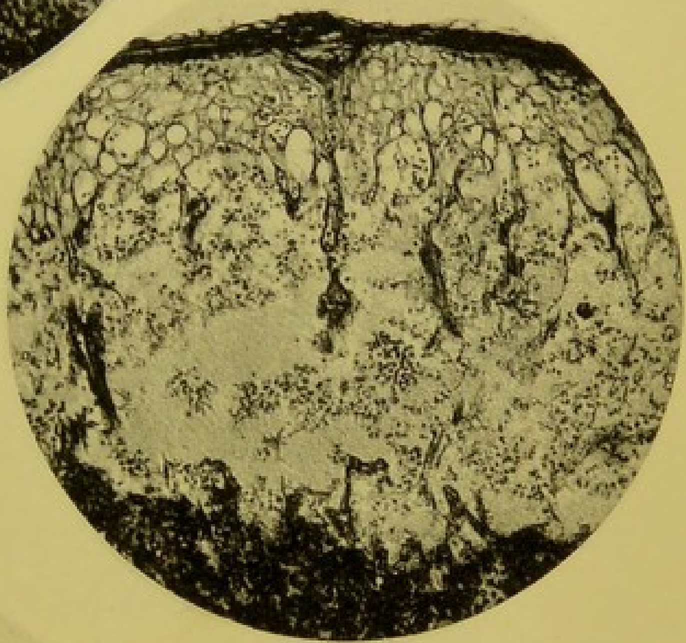

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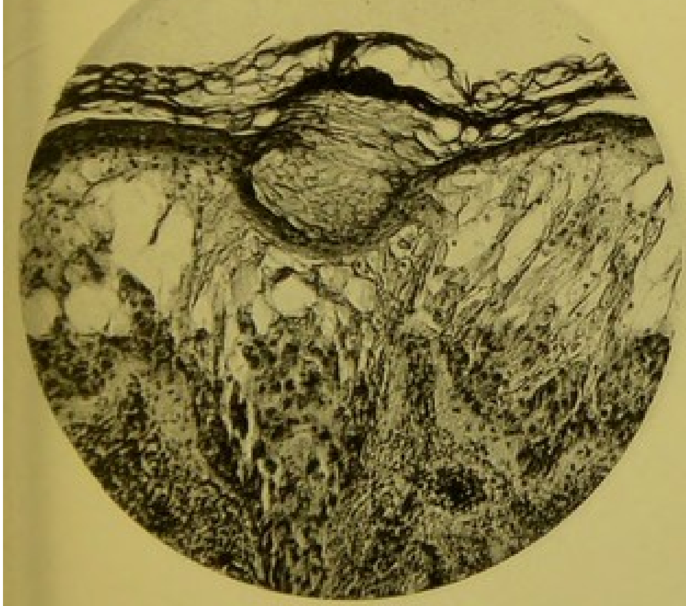

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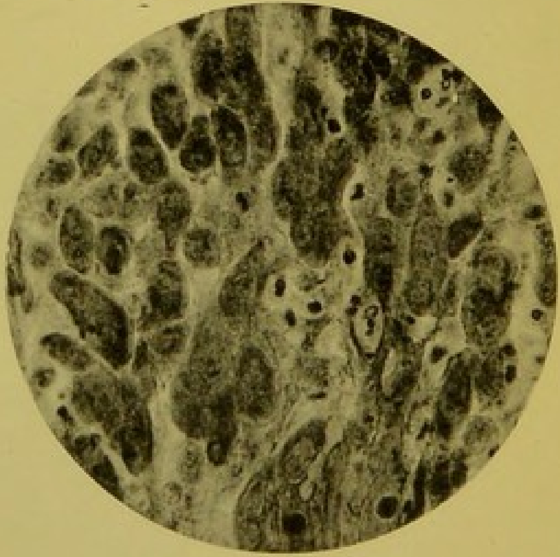

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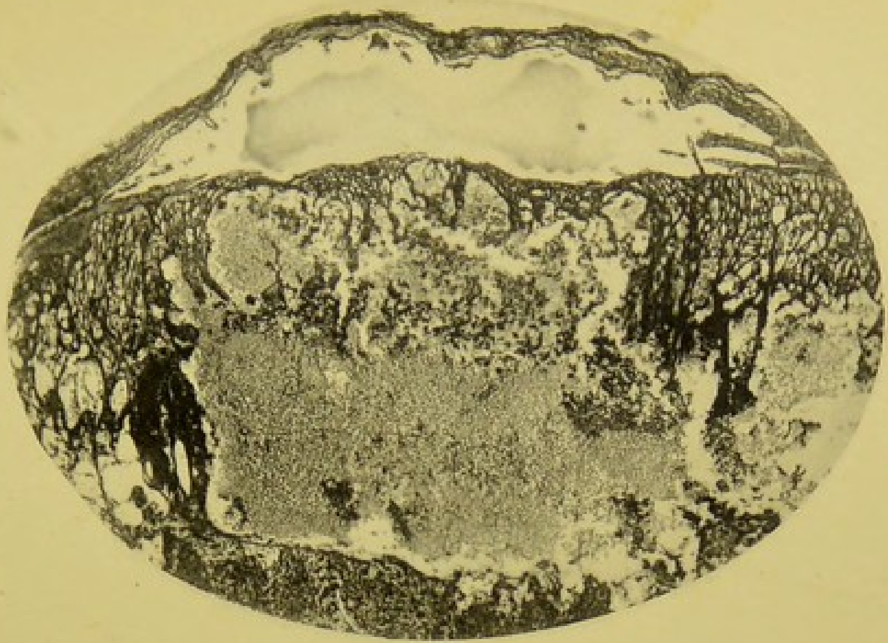

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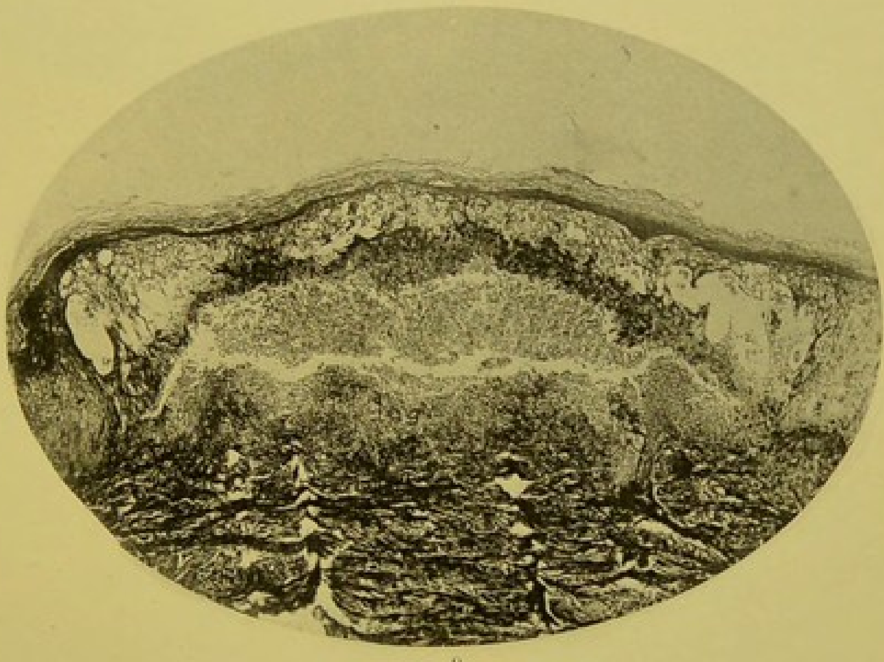

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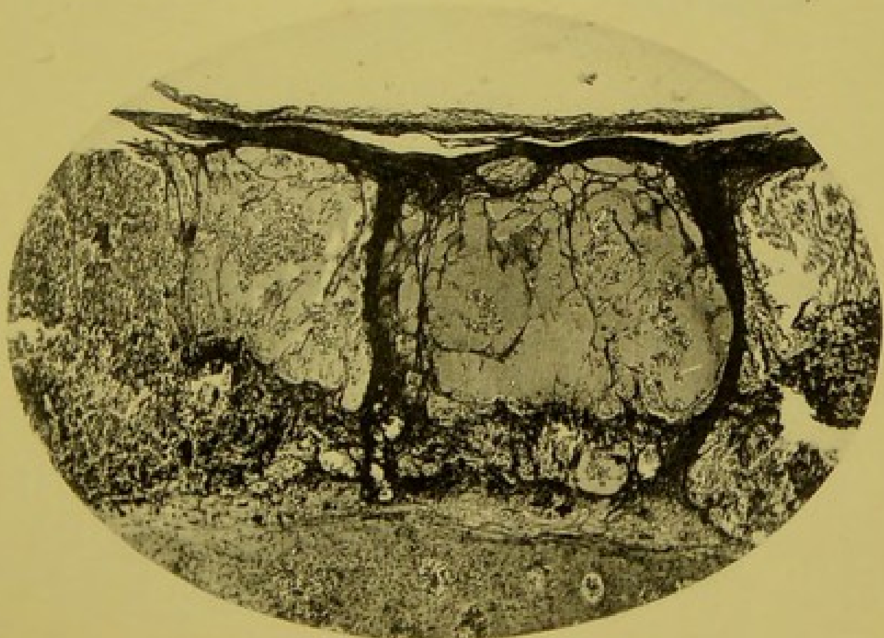

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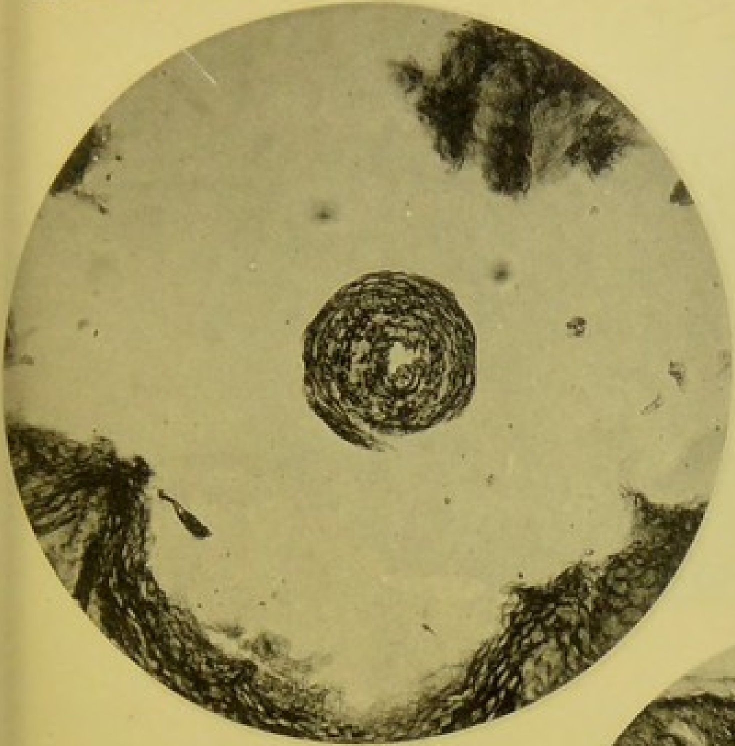

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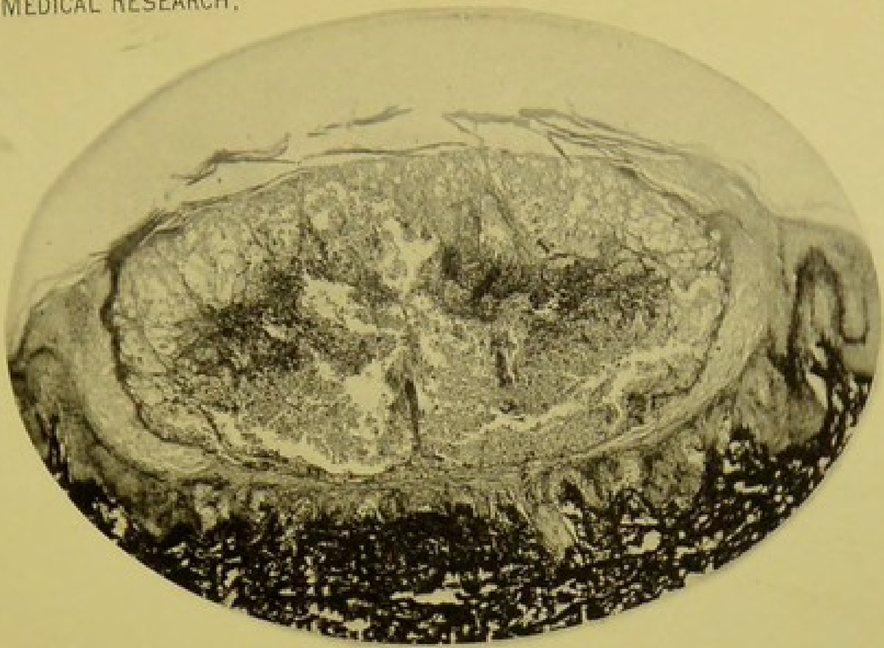

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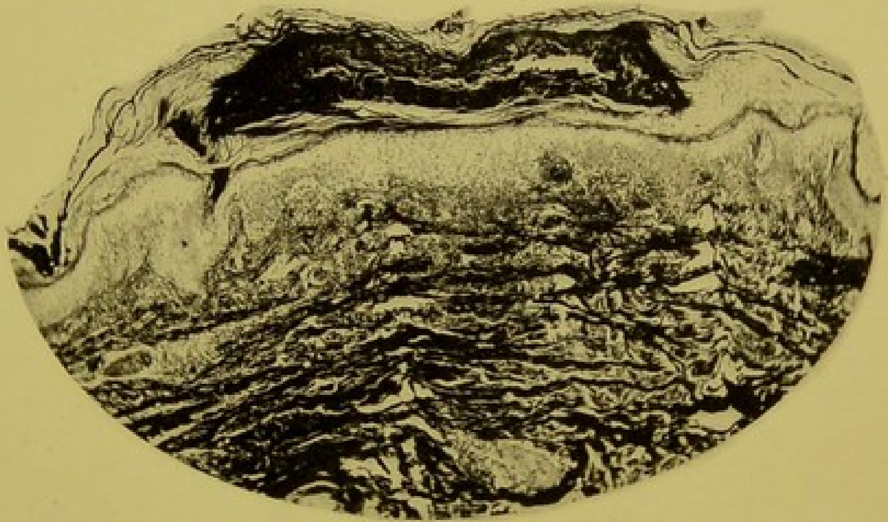

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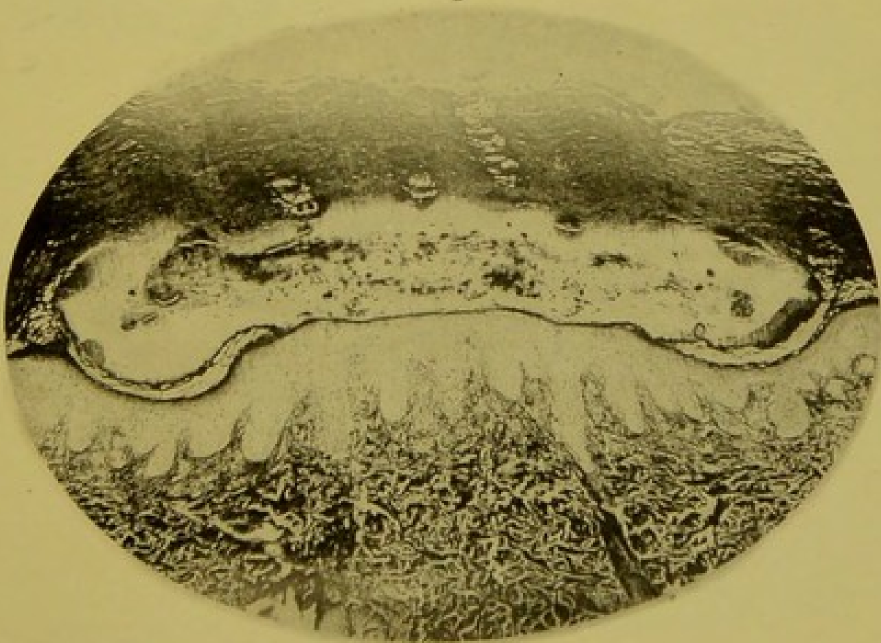

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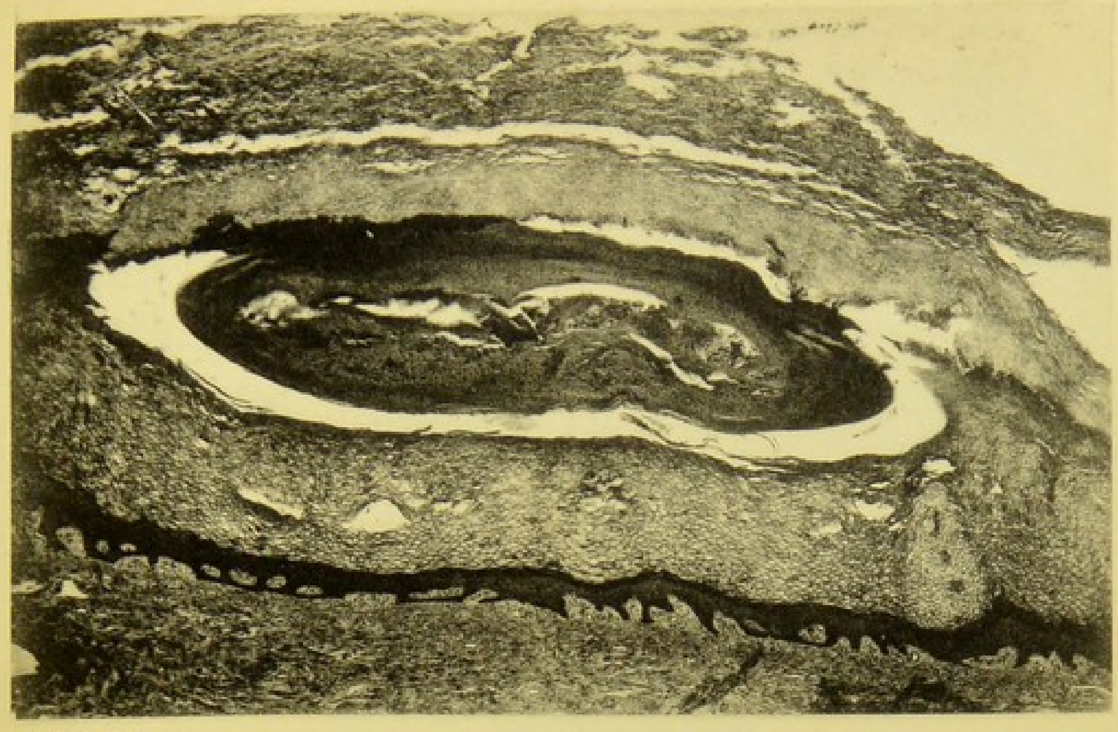

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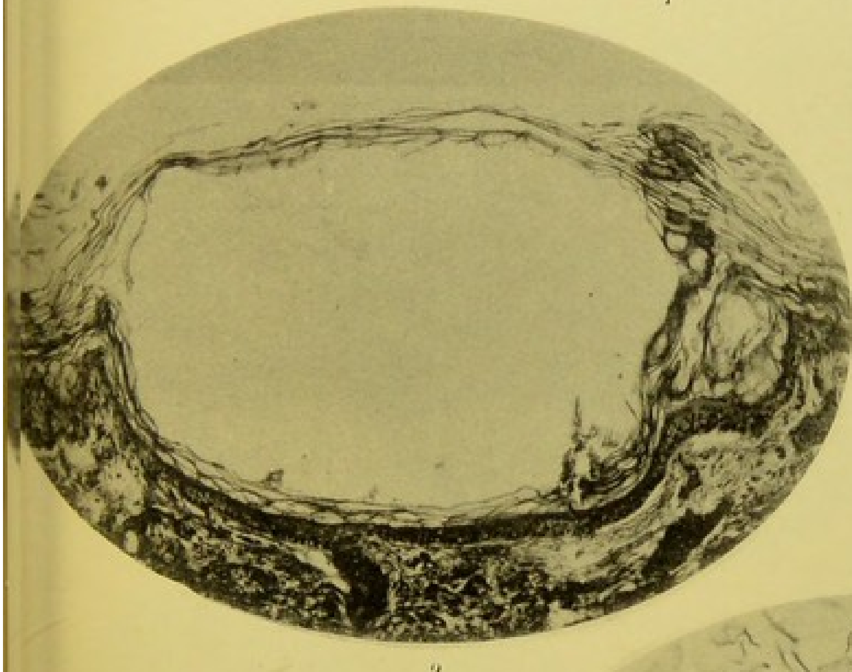

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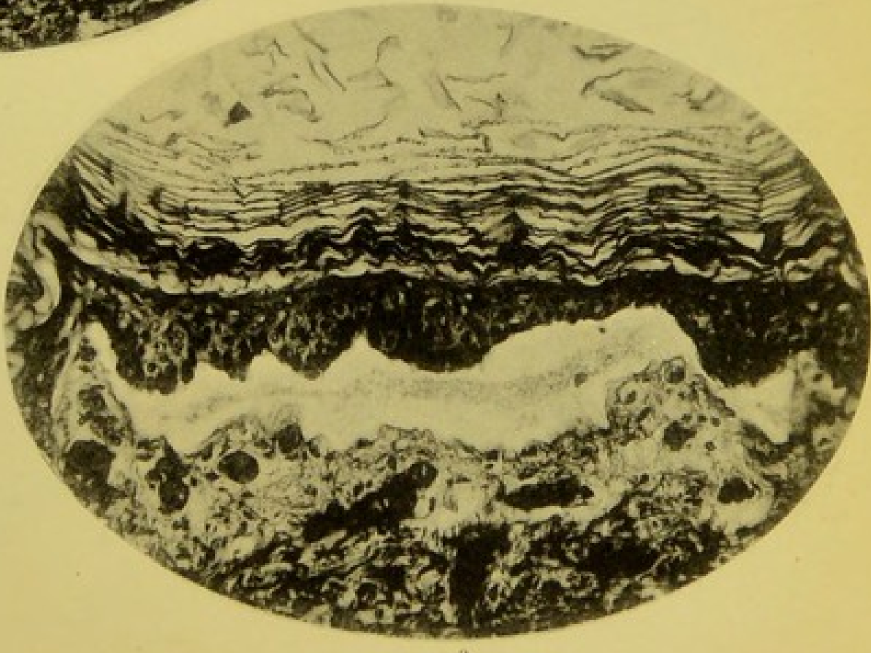

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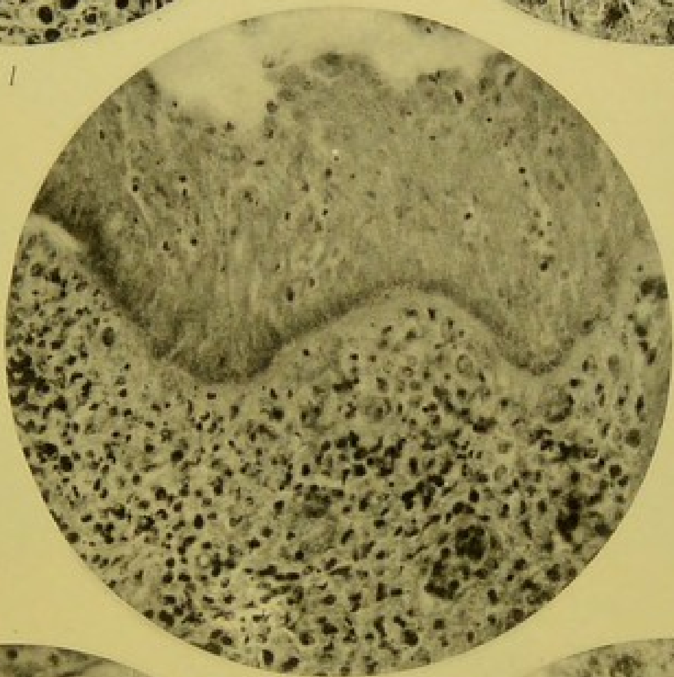

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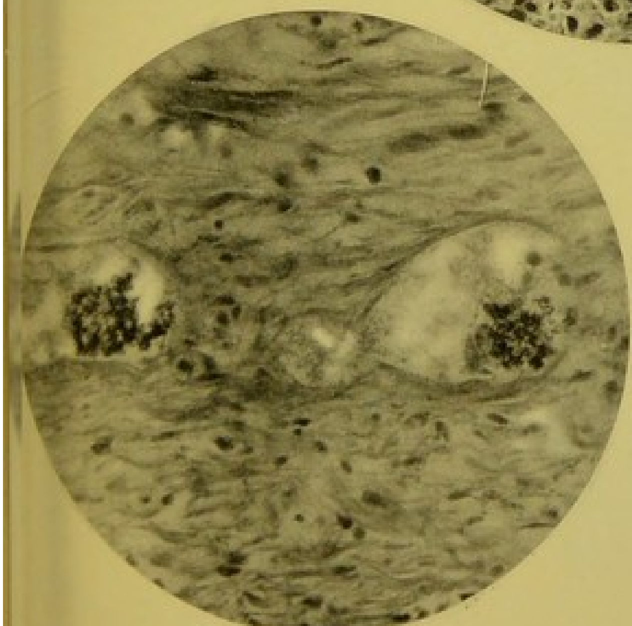

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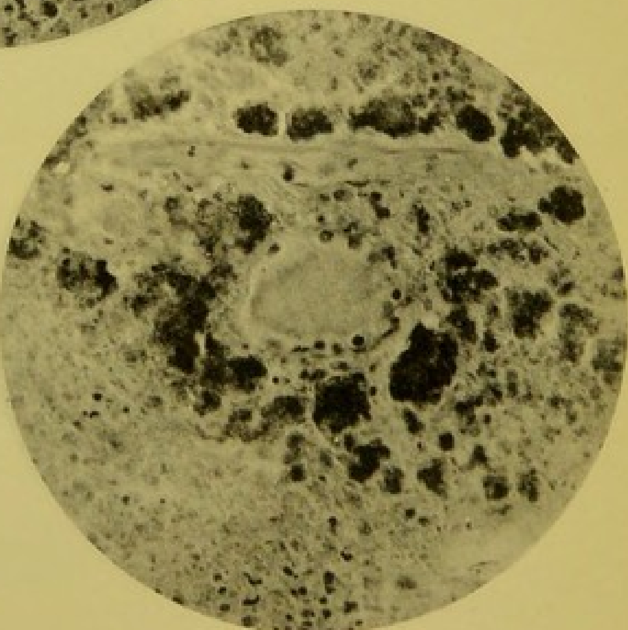

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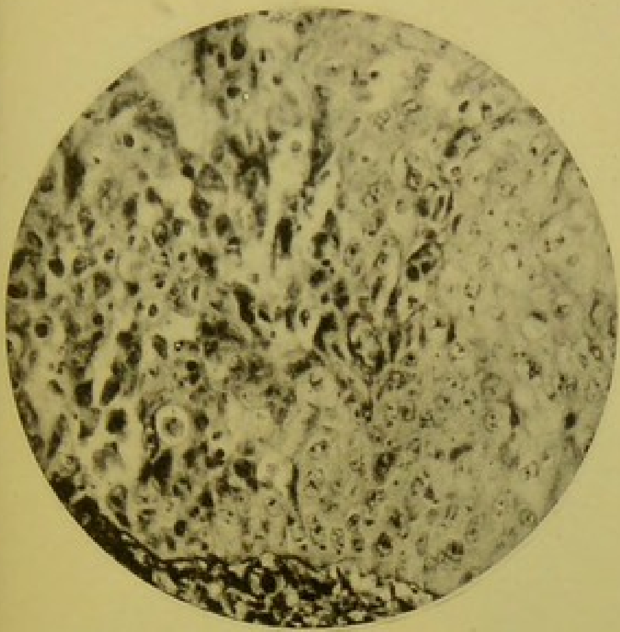

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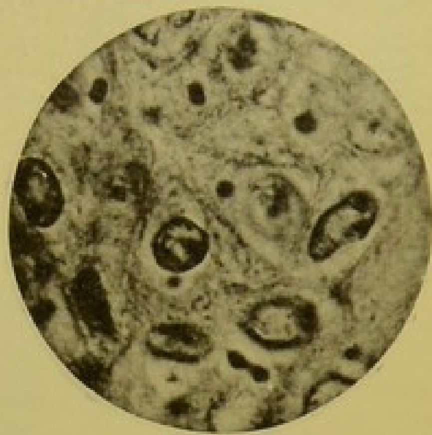

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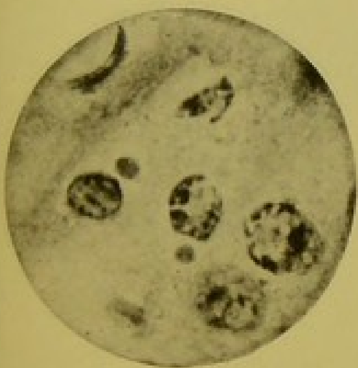

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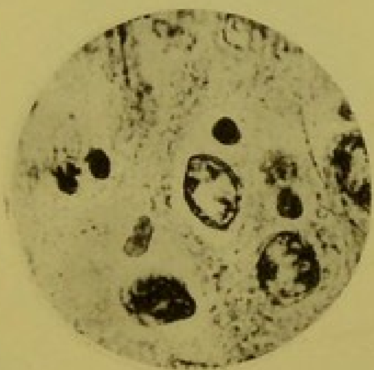

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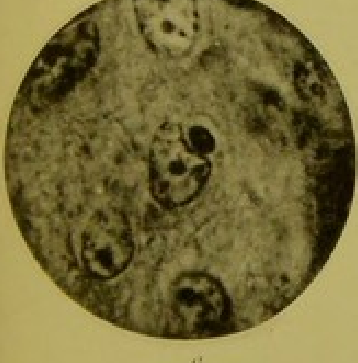

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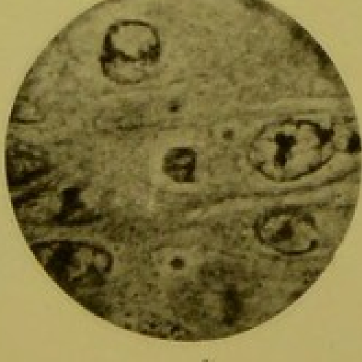

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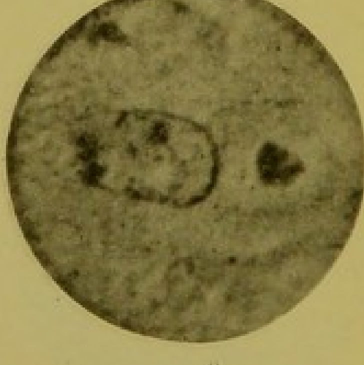

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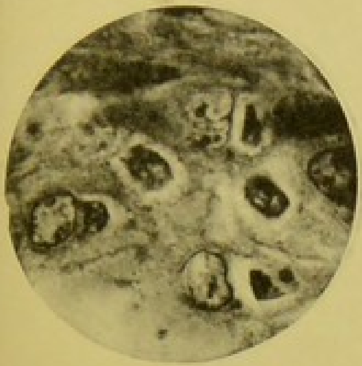

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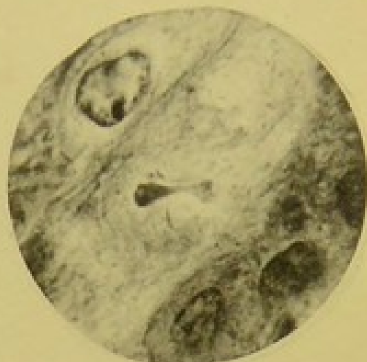

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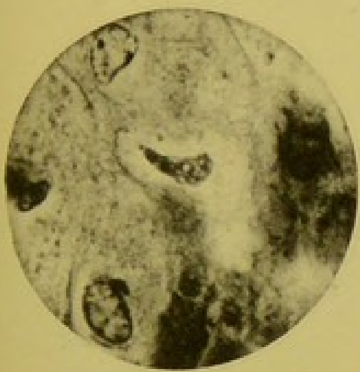

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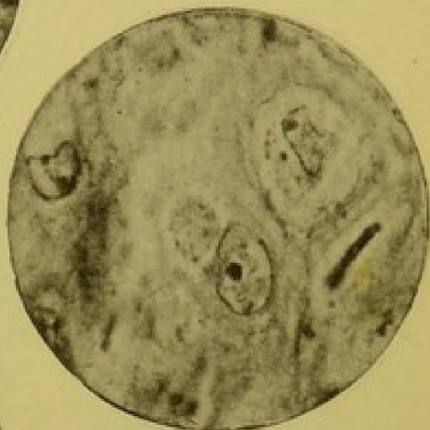

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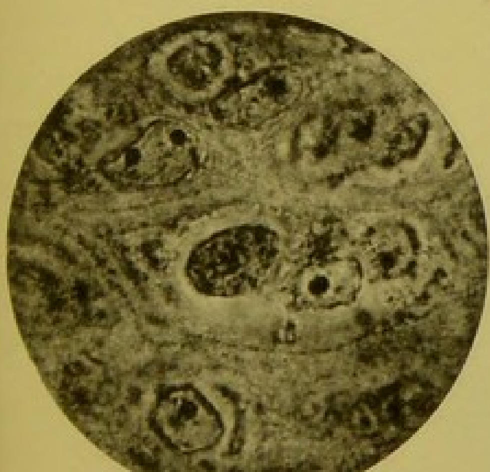

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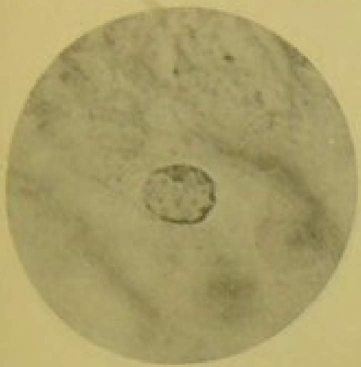

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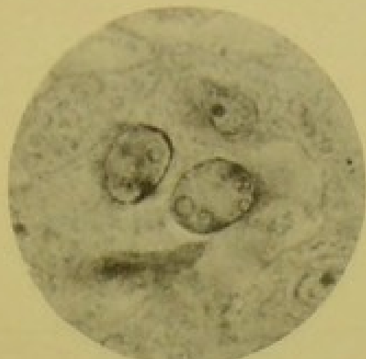

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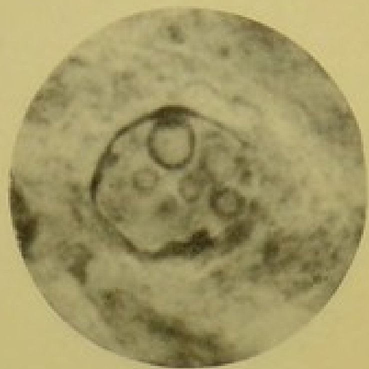

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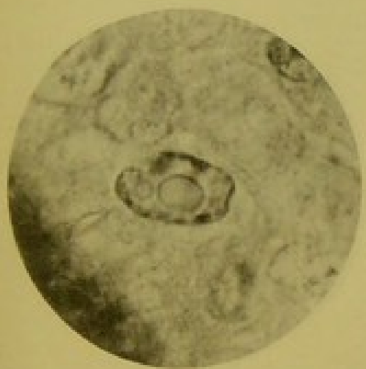

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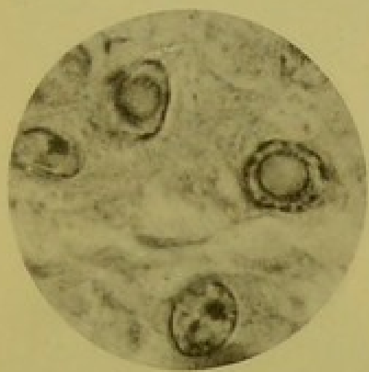

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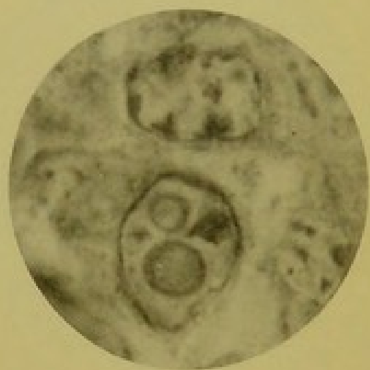

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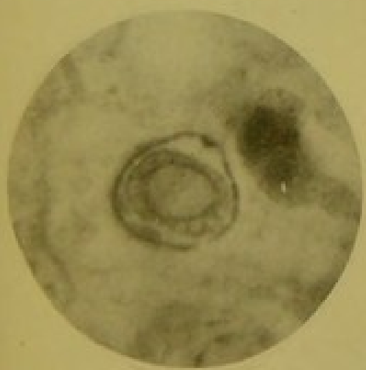

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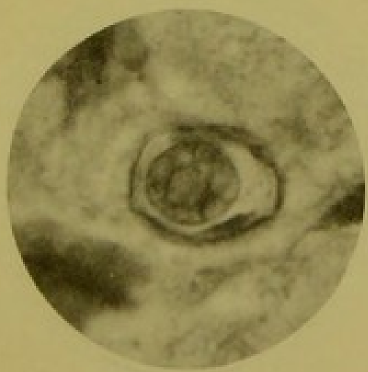

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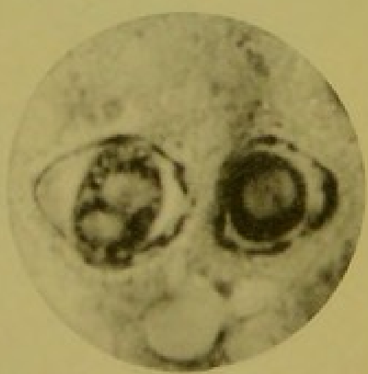

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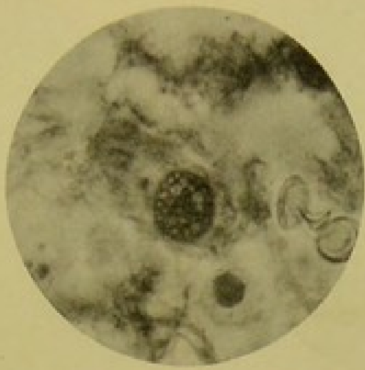

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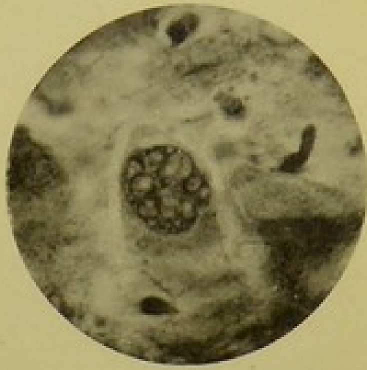

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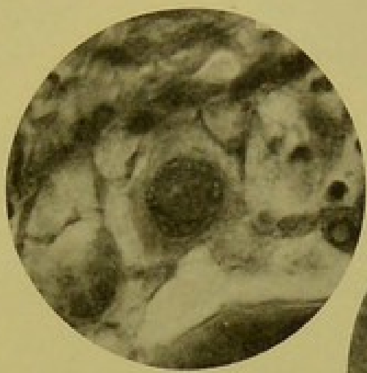

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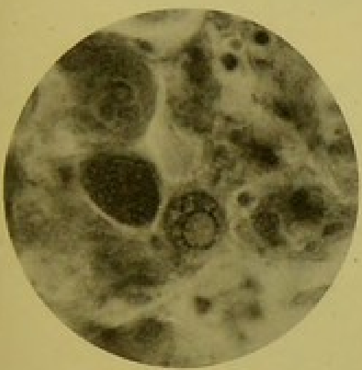

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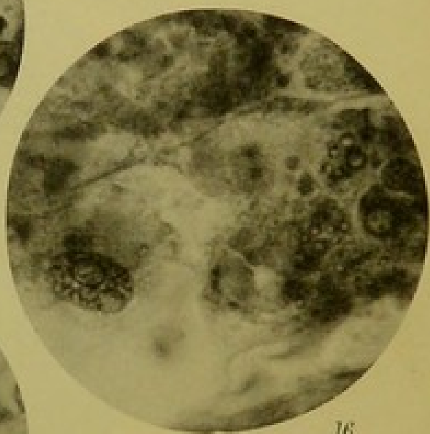

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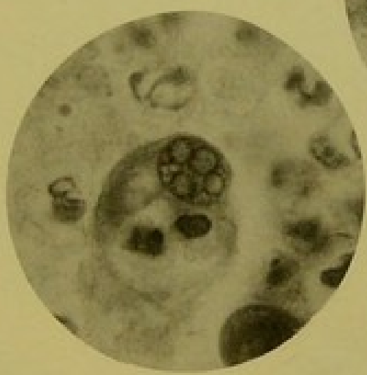

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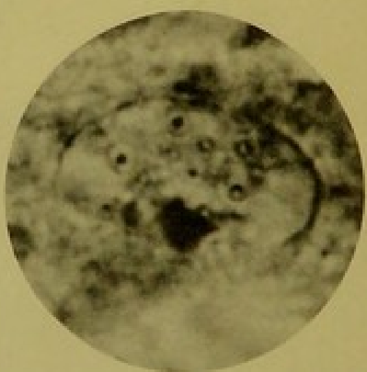

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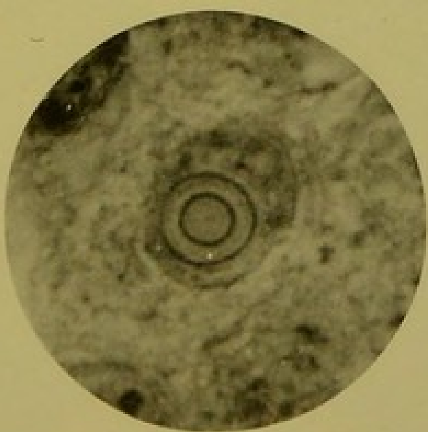

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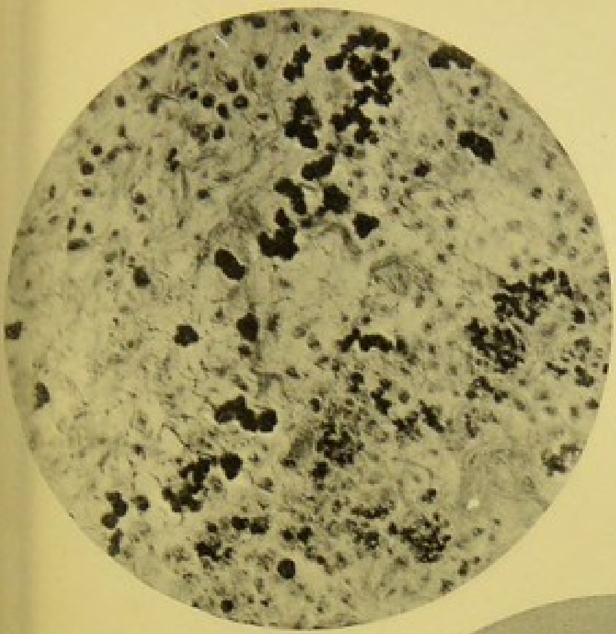

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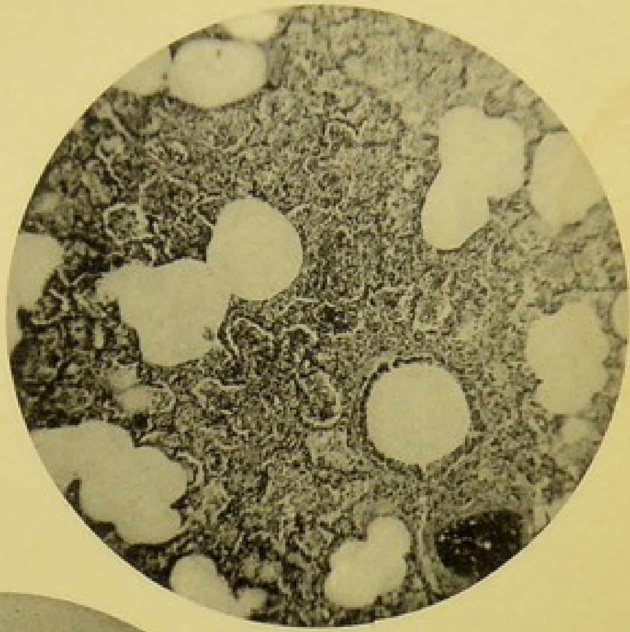

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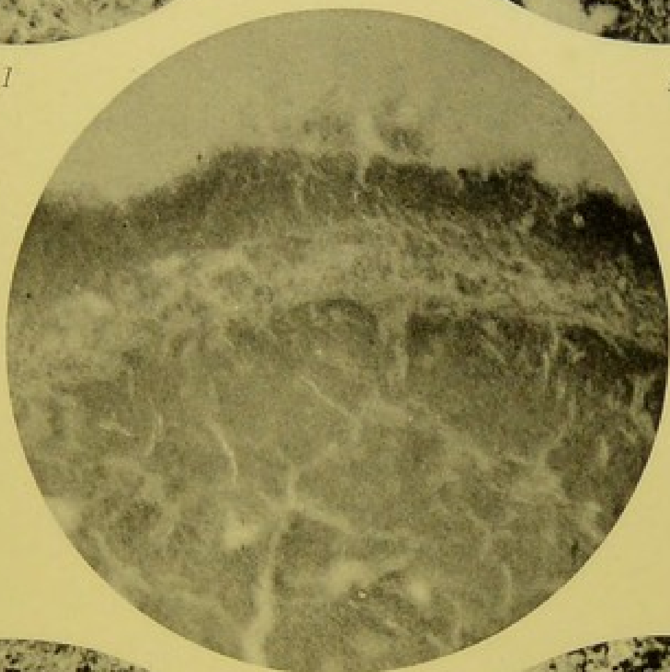

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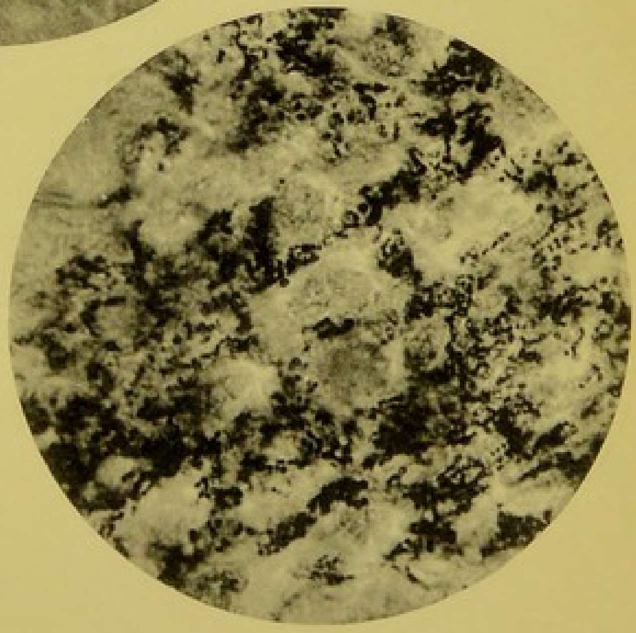

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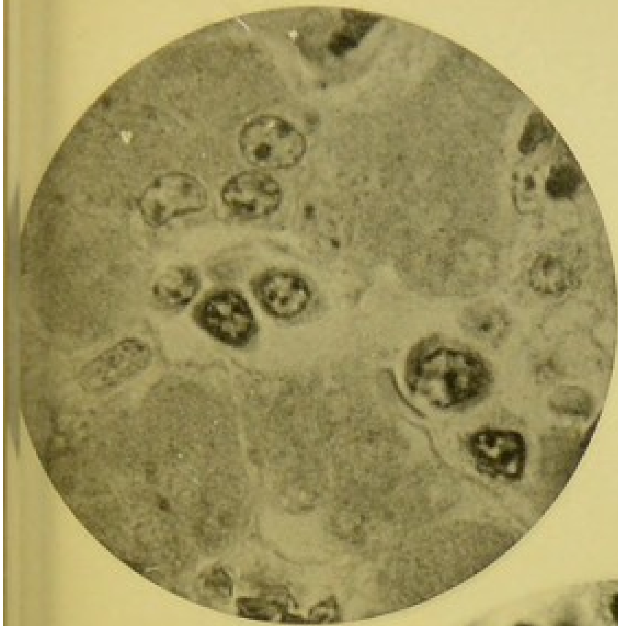

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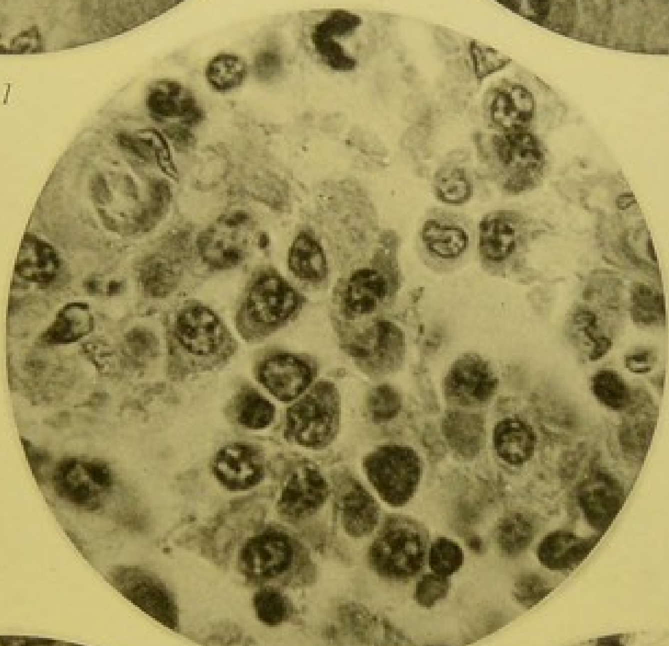

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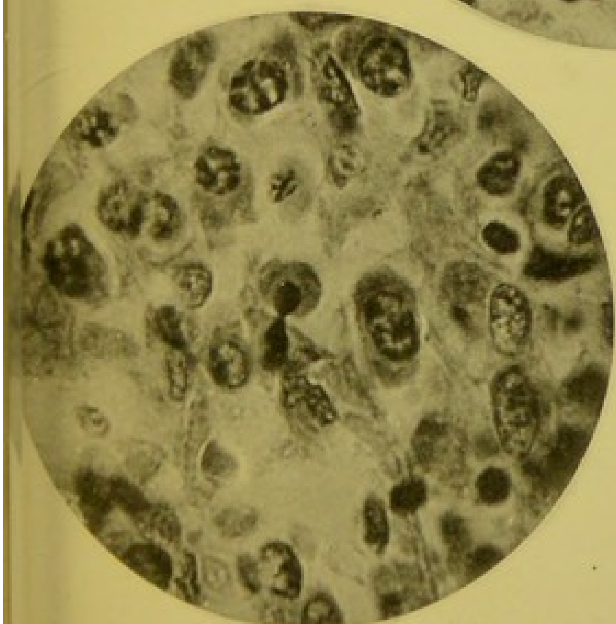

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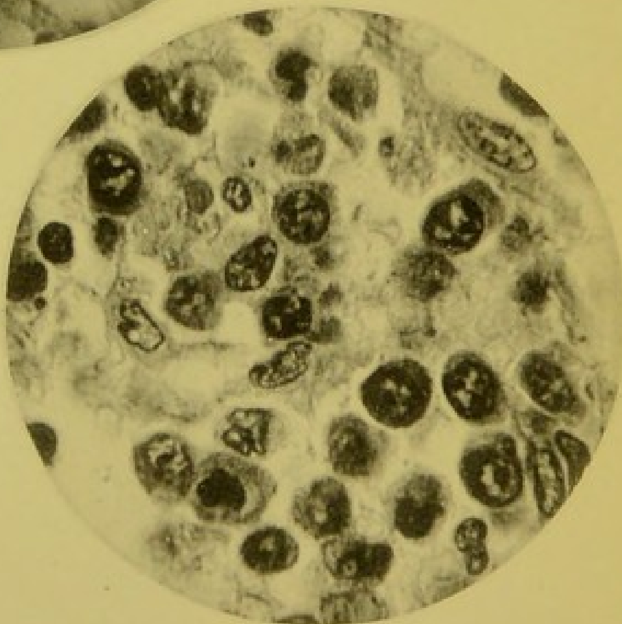

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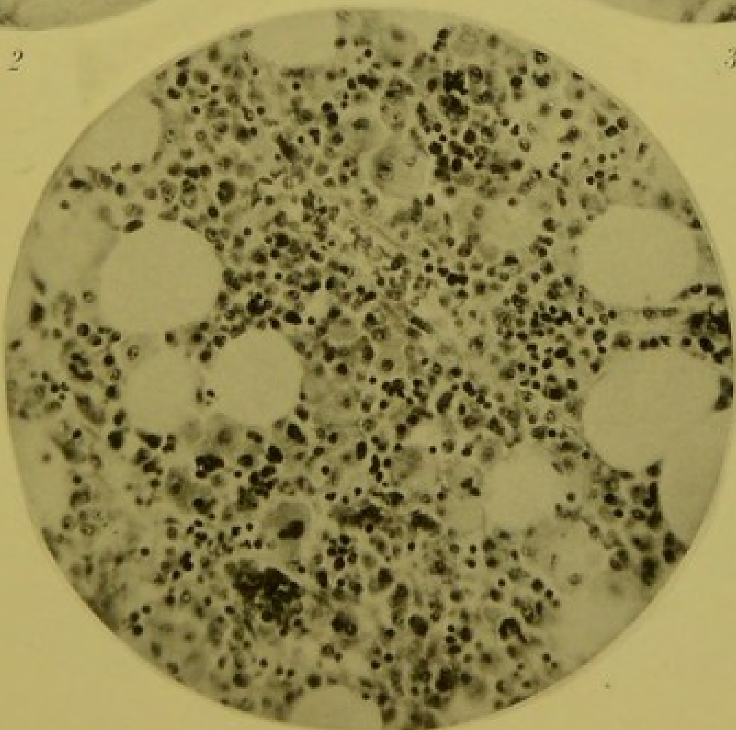

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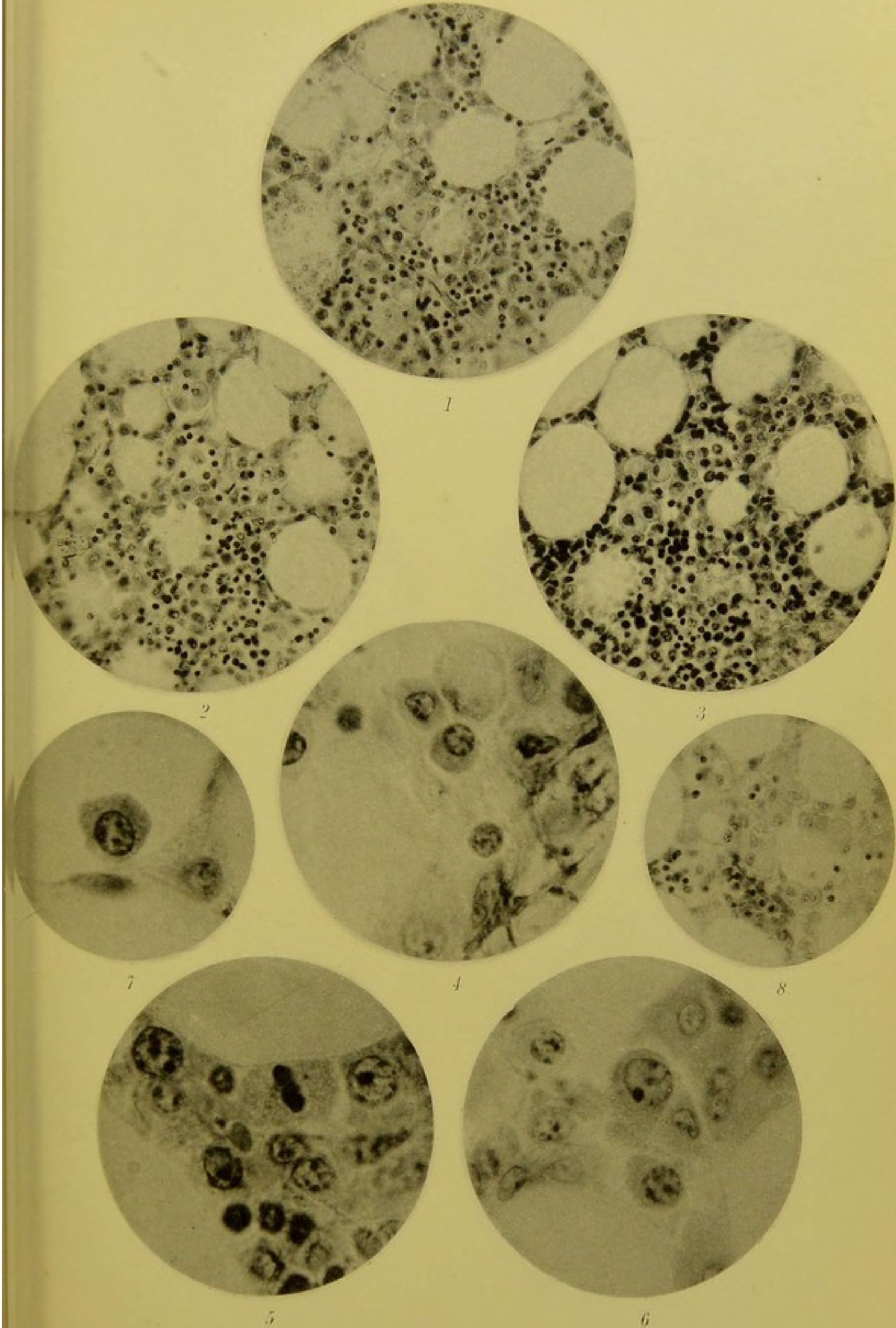

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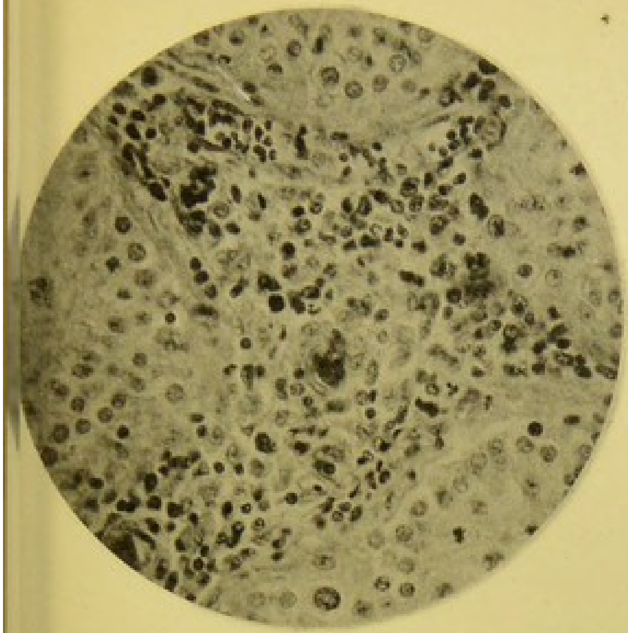

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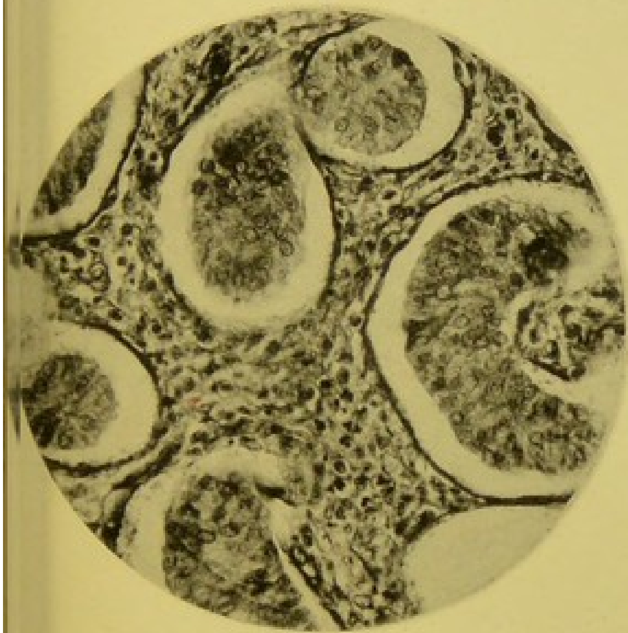

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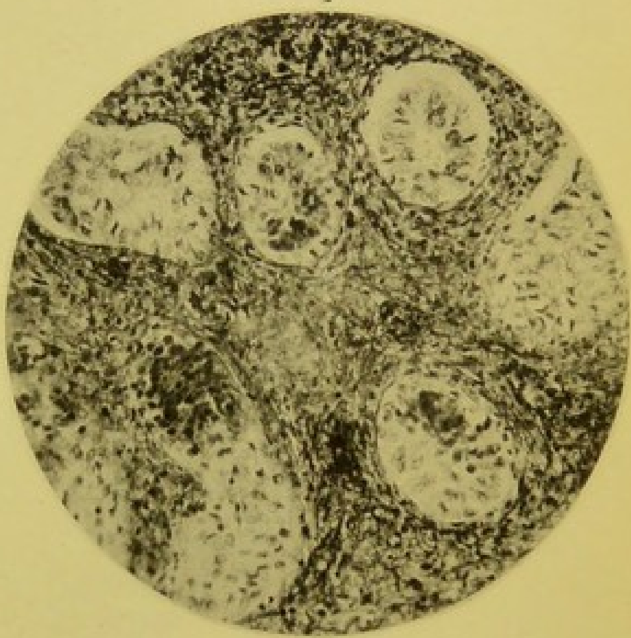

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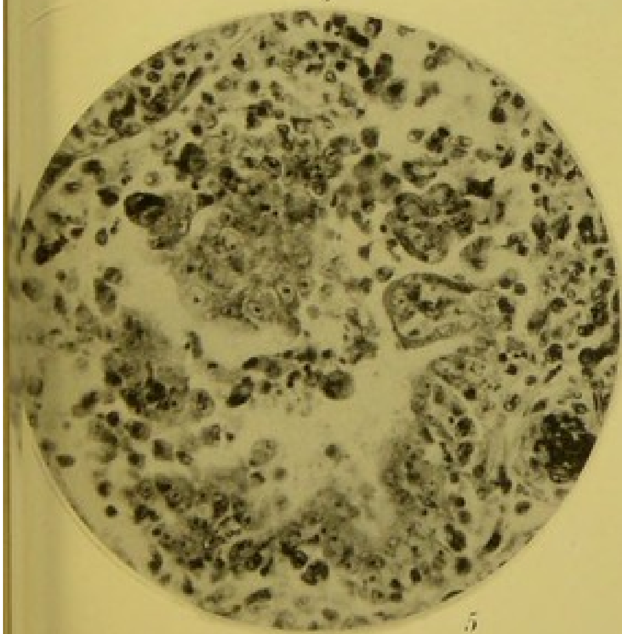

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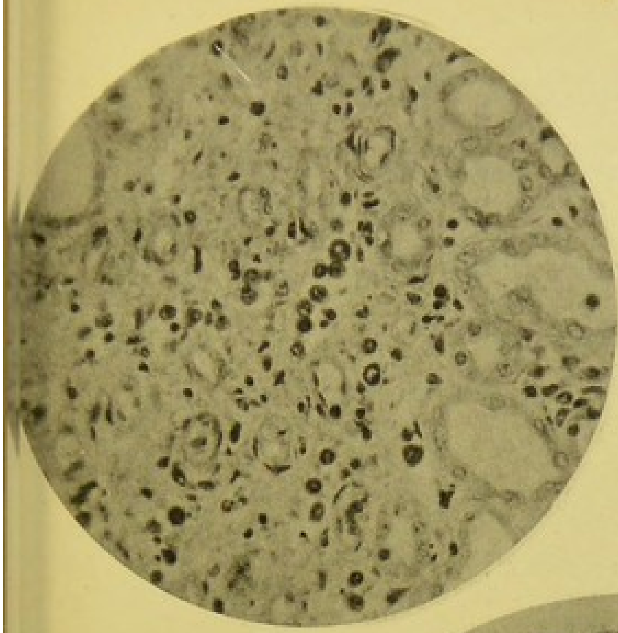

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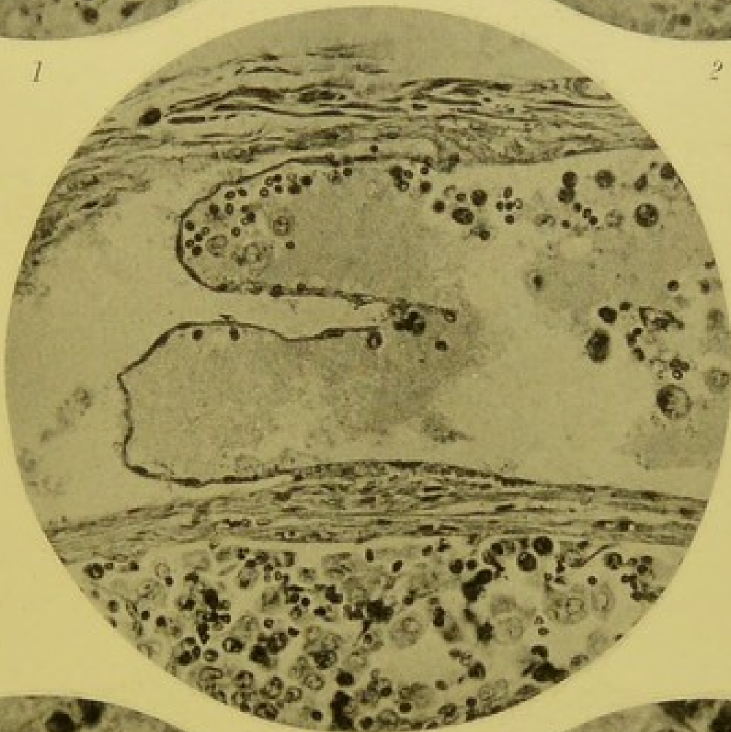

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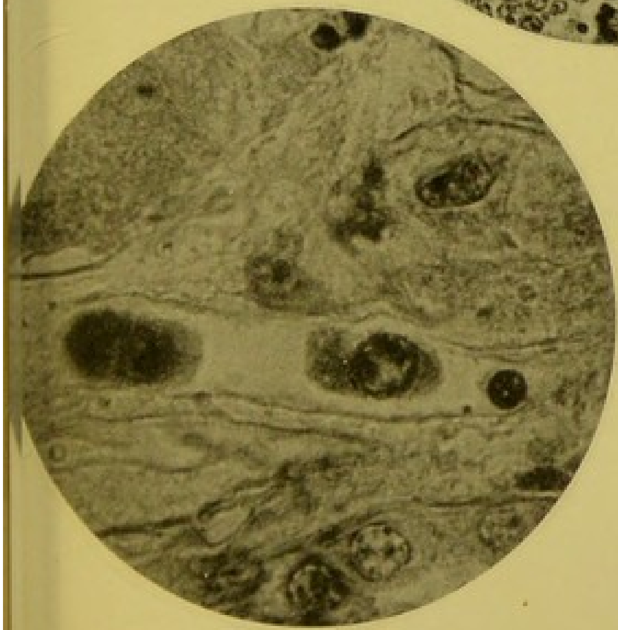

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FIG. 5. Nuclear figure in premyelocyte. X 1,000.
FIG. 6. Myelocytes. X 1,000.
FIG. 7. Large basophile cell of marrow. X 1,000.
FIG. 8. Group of phagocytic cells around a fat cell. X 200.

PLATE XIV.

FIG. 1. Small lesion in testicle adjoining tunica albugina. The interstitial tissue is dilated, and contains large lymphoid cells. No degeneration in tubules. X 200.

FIG. 2. Several small interstitial foci in testicle with more or less advanced degeneration of epithelium of tubules. X 25.

FIG. 3. From a child's testicle showing area of interstitial infiltration without degeneration of tubular epithelium. X 200.

FIG. 4. More extensive area with fibrin in interstitial tissue and foci of necrosis of the tubular epithelium marked by the darker areas. X 200.

FIG. 5. Cross-section of tubule of epididymis showing infiltration of the tissue with destruction of epithelium. In the lumen of the tubule there are lymphoid and phagocytic cells. X 200.

FIG. 6. Tubule of epididymis filled with large phagocytic cells. X 200.

PLATE XV.

FIG. 1. Section of pyramid of kidney showing cells of lymphoid type in vessels. X 200.

FIG. 2. Small focus of interstitial infiltration in pyramid of kidney. X 200.

FIG. 3. Longitudinal section of lymph node showing afferent lymphatic with valve. Within the lymphatic there are red blood corpuscles, lymphoid, and phagocytic cells. The lower portion of the section shows the peripheral lymph sinus containing phagocytic cells. X 200.

FIG. 4. Capillary in kidney showing two large basophile cells, one of them in mitosis; with typical lymphocyte on the right. X 1,000.

FIG. 5. Section of small vessel in pyramid of the kidney showing varieties of basophile cells. X 1,000.

THE LIFE HISTORY OF CYTORYCTES VARIOLÆ, GUARNIERI.

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There is scarcely a mollusc or an earthworm that fails to reveal one or more internal parasites belonging to the class of parasitic protozoa known as the sporozoa, while crustacea, insects, and lower vertebrates are commonly infested by them. They have been found in hosts belonging to protozoa, coelenterata, platyhelmia, nemathelmia, nemertina, chætognatha, rotifera, polychæta, oligochæta, hirudinea, gephyrea, polyzoa, crustacea, onychophora, myriapoda, hexapoda, arachnida, mollusca, echinodermata, enteropneusta, tunicata, cephalochorda, pisces, amphibia, reptilia, aves, and mammalia. With the apparent exception of the sponges, therefore, there is no phylum of the animal kingdom that is not liable to sporozoon infection, and, in view of this wide distribution, the surprising thing would be not that man should be infected, but that he should escape their ravages. With the growth of our knowledge concerning the sporozoa, it has been found that human organs and tissues, like those of all other animals, may indeed be the seat of certain types of these organisms. Thus Coccidium cuniculi (?) has been found in the human liver; C. perforans and C. bigeminum in the intestine; C. oviforme (?) in the brain; "Eimeria" hominis in the pleural cavities; Coccidioides immitis in the skin; Laverania malariæ, Plasmodium malariæ, and P. vivax in the blood, and Sarcocystis lindemanni and S. immitis in the muscles and liver. The specific nature of many of these has been contested, but some, *e.g.*, the malaria-causing organisms, have been established beyond question, and without Koch's final test of pure cultures and inoculations from them.

Among the contested forms must be included the so-called "vaccine bodies," which have been regarded by some observers as protozoan organisms, by others as degeneration structures or by-products of the cell, ever since Guarnieri, in 1892, giving them the specific name Cytoryctes vacciniæ, made the first experimental test to demonstrate their nature. Equal credit, however, should be given to von Wasielewski ('01), whose painstaking experiments and logical deductions left no reasonable doubt as to the nature of these inclusions in vaccinated tissues.

In every sporozoon of which the life history is known, there are definite reproductive phases characteristic, perhaps, of some phase of the disease, or of some peculiar environment of the parasite. Thus in the malaria-causing organisms the asexual reproduction, or schizogony, takes place in the blood corpuscles of man or birds, and the sexual cycle, or sporogony, takes place in the digestive tract of the mosquito. In all probability an analogous series of processes takes place in the parasite causing vaccinia and variola. The phase of the organism known as the "vaccine body," with which Guarnieri and Wasielewski experimented, is found in variola as one stage of the life cycle. A second and more important cycle takes place within the nuclei of the generative cells of the skin, and includes all of the processes of propagative reproduction. As the discovery of the mosquito phase in malaria gave the final proof in this disease, so here the discovery by Councilman, Magrath, and Brinckerhoff, of the intranuclear phase in Cytoryctes gave the key to the life history of this sporozoon.

It has been my great privilege to work with Dr. Councilman upon material prepared for his own use, and it is owing to his generosity in sharing this material with me that I am able to offer the following interpretation of the life-cycle of Cytoryctes variolæ.

Efforts to cultivate sporozoa on artificial media and thus to get pure cultures have never succeeded, and it is impossible, therefore, in our present state of knowledge, to apply Koch's test to prove that we have to do with an organism. Furthermore, owing to the nature of the subject, there is but a limited range of experiments possible, and the stages in the life-cycle must be worked out from morphology alone, and without the advantage of the living organism. Dependent thus upon fixed material, there are obviously many possible sources of error, and we cannot hope to fill in every gap, or to avoid errors of interpretation. In the first place, the material upon which the study is based could not be absolutely fresh when fixed, and the time which usually elapses between the death of the patient and the autopsy was much too long to justify the hope of obtaining perfectly normal protozoan structures. Only a student of these delicate unicellular organisms can appreciate the absolute necessity of obtaining protozoa directly from their normal environment, and I do not doubt that many of the bizarre structures which we have observed are due to this technical, but unavoidable, error. In the second place, Cytoryctes belongs in the group of microsporidia, and here, as in the allied myxosporidia, the complete life-history of no species is known, the sexual cycle being entirely unrecognized at the present time. Analogies for many phases of Cytoryctes must, therefore, be drawn from groups as distinctly related to it as the coccidiida and the gregarinida. In the third place, the morphological method involves the possibility of confusing artefacts due to technical methods, and degeneration products of the tissue cells, with stages of the organism. I have had this possibility of error constantly before me during my work, and freely confess that, owing to the extremely small size of certain stages and absence of differentiation, I cannot always distinguish them from artefacts. The spores, for example, even with their great refringency and conspicuous points (which are characteristic of all microsporidia spores), cannot always be distinguished from minute bits of matter of one kind or another. The difficulty vanishes only when these minute stages of the organism are accompanied by developmental phases which are always unmistakable. The one factor which, more than morphology or staining reaction, has guided me in my interpretation of the life-cycle, and has enabled me to distinguish between the minute phases and artefacts, is the fact that the growth stages are always present in sequence, a sequence which follows pari passu with the development of the skin lesions.

Although the microsporidia and the myxosporidia are so little known, and the published observations upon them so inadequate, they may serve as a basis for comparison with the complicated life-cycle of the small-pox organism.

The genus Thèlohania (Henneguy) as worked out by Thèlohan ('95), and more recently by Stempel ('02), begins as a minute uninucleate, spherical fragment (interpreted by Thèlohan as a "pansporoblast"). The nucleus forms a primitive mitotic figure, the cell thus dividing by indirect division; two daughter nuclei repeat the division; the four resulting nuclei again divide, and eight "sporoblasts" are finally formed. Each of these then changes into a single pyriform spore. Like the intranuclear phases of Cytoryctes, all stages of Thèlohania are vesicular.

In Plistophora (Gurley), which is also vesicular, the spheres are larger, and include a larger number of sporoblasts, each of which again forms a single spore.

The genus Nosema (Nägeli), because of its importance in the silk industry, is among the best known of the microsporidia. One species, N. anomalum, infests the stickleback; N. destruens, a marine fish, Calionymous lyra; another, N. ovoideum, the liver of Cepola rubescens and of Motella tricerata, which are also marine fishes; still another, N. bryozoides, infests the tissues of the bryozoön Alcyonella fungosa; N. lophii attacks the skate, and one other, finally, and the most important of all, Nosema bombyces, causes the famous pèbrine disease of silk-worms. In the latter case the parasites invade all of the tissues and organs of the caterpillars. The valves of the spores (which are taken in through the digestive tract) open and liberate an amœboid germ, which, like the zygote of the malaria organism, bores into or through the gut epithelium, and completes its development in the body cavity. It becomes multinuclear, each nucleus giving rise to a "pansporoblast," which, in turn, gives rise to many spores. Sex-differentiation and conjugation are absolutely unknown.

In Cytoryctes variolæ the life cycle is much more complicated. The first development of the germ in the host is unknown; it probably takes place in the seat of primary infection, forming an organism which reproduces by germs, probably similar to those which I have named "gemmules," the process being known as "multiplicative reproduction." The gemmules are probably carried in the blood to the skin, where the further development takes place. This early part of the development, therefore, is purely conjectural, but from this point the observations are fairly complete. The gemmules become intracellular (cytoplasmic), amœboid organisms which give rise to similar gemmules. This process, which Councilman has designated the "vaccine cycle," must continue for some time, for, in variola, the gemmules are distributed to all regions of the skin. Ultimately, the germs derived in this way give rise to forms which penetrate the nuclear membrane, and develop into gametocytes (?) of two types, one forming the supposed male gametes, the other the female. The gametes conjugate (?), the zygote thus formed develops into a comparatively large, amœboid organism, in which the pansporoblasts originate. These pansporoblasts give rise to primary sporoblasts, and the latter to multitudinous spores, the entire process taking place within the nucleus, and corresponding to the so-called "propagative reproduction" of other sporozoa. The spores thus formed may, in turn, infect fresh nuclei, and grow directly into new, secondary sporoblasts, which give rise to similar spores, a true "schizogony," and a second means of auto-infection, by which the organism spreads throughout the nuclei and cells of the skin, and possibly to many of the other organs of the body, like the allied Nosema bombyces, which infects every tissue and cavity of its insect host. These spores finally may transmit the disease to new hosts.

A. Methods Employed.

The material used in the study of Cytoryctes was all obtained from human skin taken at the time of the autopsy. It was fixed in Zenker's fluid, hardened and kept in alcohol, embedded in paraffine, and cut into as thin sections as the tough nature of the tissue would allow. The stains used were the iron hematoxylin of Heidenhain, Mallory's chloride of iron hematoxylin, the polychrome methylene blue-eosin combinations (Weigert and Romanowsky), and the Borrel stain.

The use of different stains on small-pox tissue shows that the organism is composed entirely of material which colors like the chromatin of tissue nuclei. The more delicate differential stains, such as the methylene blue-eosin compounds, or the Borrel, show, however, that, although it is like chromatin, there is differentiation, and two grades, differing apparently in the degree of nucleinic acid present, compose the bodies of these minute cells. The iron hematoxylin stain, either alone or with counter-stains, gives no such differentiation, and all stages stain as do the nuclei of the tissue cells, although some stain more intensely than does the chromatin of these nuclei. Iron hematoxylin, therefore, is not a satisfactory stain for tracing out the stages of the life-history.

The Romanowsky stain, used in a modified form for sections, gives beautiful results for certain phases. It is prepared from the directions given by Unna; the commercial polychrome methylene blue of Grübler is used full strength, and to this is added enough of a two-tenths per cent aqueous solution of eosin to form an insoluble precipitate on the surface, and after this is removed with filter paper the mixture is ready for use. The sections are run through successive grades of alcohol (the careless method of plunging directly from 95 per cent into water is to be avoided) to water, and when completely hydrated, they are immersed in the mixed stain and left for about thirty minutes. At the end of this period, they are thoroughly rinsed in water, and dehydrated by passing again through the successive grades of alcohol. Differentiation should take place in the higher grades (95 per cent or absolute alcohol), and the sections should be cleared in xylol and mounted in balsam. Preparations made in this way are not always successful, the result, frequently, being too pale, or the differentiation imperfect, but with care good results follow in the majority of cases.

When successful, the cytoplasmic phases of Cytoryctes are blue, with an occasional deeper blue central point, while those in the nucleus are either blue or pink. If in the earlier stages of sporulation, the entire organism is blue like the chromatin, and if in the later stages, only the spores and chromatin are blue, the residual bulk of the organism staining pink. This stain is very important for clearly defining the spores in the nuclei and for the early development of such spores. In the earlier stages of the pansporoblast, also, it has certain advantages, but is of no value in the later stages. It is also of great importance, when used in connection with the Borrel stain, in differentiating streptococcus.

The other combination of polychrome methylene blue and eosin (Weigert) is particularly good for the developmental stages of the primary and secondary sporoblasts, the early stages of spore-formation, and the differentiation of the cytoplasmic organism, but not so good for pansporoblast stages. The spores are also brought out very clearly by this stain.

The Borrel stain (see Borrel, '01) consists of indigo-carmine and picric acid, used after a preliminary staining with magenta in saturated aqueous solution. The picro-carmine combination is made by adding one part of a saturated aqueous solution of picric acid to two parts of saturated indigo-carmine solution. The sections are hydrated as for the Romanowsky stain and immersed for about twenty minutes in the magenta solution; they are then rinsed with water and immersed in the picro-carmine for about five minutes. Differentiation takes place in the alcohols. Borrel, in using this stain, obtained a brilliant red coloration of the tissue nuclei and of the cell inclusions (in carcinoma), but for variola material I find that the best results are obtained by extracting all of the red from the tissue nuclei, leaving the chromatin and cell bodies green, and the connective tissue blue, while the organism, in all its phases, stands out a brilliant red upon this striking background. The entire organism does not stain with the red, and many stages of its development indicate a differentiation shown by red and green colors. Thus the early cytoplasmic form stains a homogeneous red, but with growth, the green appears, and all stages of the further development are marked by the same differentiation. The nuclear form is usually red, but in the later period of sporulation the red spores lie in a green matrix, the same color as the chromatin of the tissue nuclei. The pansporoblast stages are also clearly demonstrated by the use of this stain. The developing sporoblasts are red, lying in the dense, homogeneous, green-colored plasm of the pansporoblast, which is distinguished from the cytoplasm of the cell by its form and its greater density. I cannot recommend this stain too highly for work of this nature, it has been my main reliance in working out the different stages of Cytoryctes, but it must be clearly understood that it is in no sense selective for the small-pox organism, and cannot always be depended upon to distinguish the parasite from chromatin.

B. Phases of the Life-cycle.

The forms to which the small-pox organism are most closely allied, and which are usually included under the name myxosporidia, cover a wide range in habitat, and have widely varied structures. As a rule they have certain definite points in organization and in development that bespeak a taxonomic unity and justify the grouping of many apparently diverse types under one heading. Of all sporozoa they bring about the most deleterious effects upon the cells and tissues of the invaded host, and, more than any other group of the protozoa, are the cause of the most virulent epidemics. Up to the present time no epidemics among men have been traced to them, but among the lower animals, especially the lower vertebrates and arthropoda, most destructive epidemics are caused by them. The organs attacked are many, and differ with the species. In recent classification-systems all of these malignant forms are grouped together under the name of neosporidia, and these are divided again into two orders, myxosporidia and microsporidia, which are distinguished, in a general way, by the fact that the former are intercellular parasites, the latter intracellular. This distinction, however, is somewhat arbitrary and has little value in taxonomy. Connective tissue and muscles are the more common seats of infection for all of these parasites, but they may occur in any tissue which is soft enough to be penetrated. Any specific form may attack a given tissue or organ, or it may spread over the entire host-organism, as in the pèbrine disease of silk-worms. In the concentrated types a cyst is usually formed, within which the bulk of the parasites are contained, but in the other type, which gives rise to the condition termed "diffuse infiltration" by Thélohan, the parasites are spread over comparatively large areas of the infected tissue. The cysts of the concentrated types may be seen by the unaided eye, but the diffuse types form no visible structures. It is the latter that cause the most severe epidemics in animals, and it is to this type that Cytoryctes, the cause of small-pox, belongs.

1. The early cytoplasmic stages. — *a*. The youngest forms. — Cytoryctes variola is first seen in the skin, in the form of a cytoplasmic inclusion of minute size. Like the inclusions in vaccinia, they are spherical, homogeneous bodies which gradually become differentiated as growth proceeds. The evidence of every stage in this cycle indicates that in vaccinia and variola we have to do with the same organism which, in vaccinia, has undergone some modification by reason of which the nuclear phase is inhibited. The cause of the functional difference, like the resulting immunity in the host, remains unexplained.

The early cytoplasmic form in variola is interpreted as a gemmule coming from the sporulation(?) ("multiplicative reproduction"?) of the organisms of the primary infection. The smallest that I have seen measures seven-tenths of a micron and in this earliest stage there is no evidence of structure, the minute spheres appearing perfectly homogeneous with every stain used. It may be alone in the cell or there may be several of them in different stages of development (Figs. 1, 2, 3, Plate XVI). All efforts to detect a central body which might be interpreted as a nucleus have been futile; like a spermatozoon within an egg, the organism is all nucleus, as it were, and differentiation into nuclear and cytoplasmic material comes later.

b. Growth and differentiation. — Growth of the parasite can be followed stage by stage from a center of development in the skin. Thus a field under the microscope, in which the cells contain the youngest germs, may be regarded as a center, while adjacent fields contain older growth stages arranged in regular sequence in respect to that center. A field next to that containing the youngest forms will not contain adult amœboid organisms, but a developing series of growing forms. Consequently, it is comparatively easy to trace the continuous development of the germ into the adult organism. One of the first indications of differentiation is vacuolization in the center and the occasional appearance of a minute central dot, which, with methylene blue, stains more intensely than the remainder of the body, but with the Borrel is not so clearly differentiated, appearing as a central aggregate of the same material as that forming the periphery. At this stage the organism measures about three microns. Differentiation in the peripheral portion is first indicated in the growing organism (Fig. 2, Plate XVI) by the appearance of minute, unstained spots. These become larger and then take the green stain of the Borrel combination, the rest of the periphery taking the red. From this point on the Borrel stain indicates a clearly-marked difference in the chemical make-up of the young organism. Like the microgametes of the malaria-organism (See text figure 4), comparatively large masses of the body stain red, while the general basis is green, a differentiation which is retained throughout. The red-staining material forms the substance of the gemmules which appear at a later stage, and is to be interpreted as the germ-forming material to which I shall give the provisional name *protogonoplasm.*[1]

3 microns

During these growth stages the form assumed varies

[1] πρωτος = first; Υόνος = seed; πλάσμα = anything formed.

widely; often it is spherical, but may be fusiform, pyriform, or amœboid, while pseudopodia are frequently caught in various degrees of extension (Figs. 4, 5, 6, 7, Plate XVI). Whatever the form assumed, the organism always lies in a vacuole which marks the limit of its range in the cell. A favorite position appears to be the immediate vicinity of the nucleus of the epithelial cells, and it is frequently found closely encircling the nuclear membrane (Fig. 8, Plate XVI). I have not enough evidence to warrant the belief that the organism, in the adult stage, can migrate from cell to cell, although in some cases where the cell boundaries are broken down by degeneration there is an indication that such migration may take place.

c. The adult amœboid organism and gemmule-formation. — In general structure the protoplasm of the cytoplasmic form is very simple. It is finely granular and apart from the chromatin-like granules of protogonoplasm is without inclusions. There are no vacuoles and there is no differentiation into ectoplasm and endoplasm. There is no nucleus, strictly speaking, the only approach to such an organoid is the occasional, more intensely staining dot in the early cytoplasmic form. No mitotic figure, like that described by Thélohan, in the genus which bears his name, is present at any period, and the only appearance of a division phase is an occasional elongate granule which has some resemblance to the "Hantelform" of the nucleus in some species of Nosema (Doflein, '98). The entire condition of the nucleus in Cytoryctes is identical with that in Lymphosporidium truttæ (cf. Calkins, '98), where the spores are likewise composed of similarly aggregated granules.

One prolific method of auto-infection in variola is by the "multiplicative" process of reproduction, by which the mature amœboid organisms give rise to great numbers of reproductive bodies which I have called the gemmules, to distinguish them from spores and sporozoites of other forms of sporozoa.

In the largest forms of the cytoplasmic parasite (measuring from ten to fourteen microns), the red-staining protogonoplasm is usually in some stage of gemmule-formation. In many cases it is distributed throughout the body of the organism in the form of minute spherical granules (Fig. 8, Plate XVI) which become more and more definite in the later stages of growth, until, when practically mature, each of them lies in a minute vesicle (Figs. 9, 10, 11, Plate XVI). These granules are demonstrated by almost every chromatin stain that has been used, and the later spore stages may be made out with the iron hematoxylin. The conditions in gemmule-formation were first clearly indicated by the Borrel preparations in which the gemmules stain red, while in addition there is a red framework or network which extends throughout the entire body. This is composed of very fine granules, and the accumulation of these into larger groups gives the appearance of masses similar to the gemmules. With the general diffuse, chromatin stains like iron hematoxylin, these are difficult to distinguish from the reproductive bodies, but with the more delicate stains the nature of the framework can be clearly made out (Figs. 12, 13, Plates XVI and XVII).

Nothing more definite can be stated in regard to the formation of the gemmules, although the process is probably similar to that which takes place in other enucleate organisms, as, for example, in Bacillus bütschlii (Schaudinn, '02 ; see text figure 3), or spore-formation in Lymphosporidium truttæ (Calkins, '98). The protogonoplasm in later adult stages is granular and diffused throughout the body, and the gemmules are formed by the accumulation of these granules, in the same way as in the parasite of the brook trout. However obscure the details of gemmule-formation are, there is no doubt whatever that the material composing the gemmules and that of the diffused protogonoplasm is one and the same substance. The gemmules in the mother-organism are, apparently, not always identical in size, measuring from seven-tenths to one micron, but they correspond to the smallest germs observed in the cytoplasmic cycle, and the differences are well within the range of individual variations of spores in general. In one instance (Fig. 10, Plate XVI) they were found emerging from the parent cell, some being outside, while others were within. The gemmules are liberated by the disintegration of the framework which remains as a residual structure in the host-cell (Fig 15, Plate XVII).

d. The cytoplasmic residuum. — This residual framework is of interest, for it persists in the cells of the skin for some time after the reproductive bodies are distributed, and may assume various fantastic forms. It is therefore of the nature of residual structures found in all forms of sporozoa, and described under different names (Sporenrest, Restkörperchen, Reliquat de différentiation, etc.). An apparently similar "stringy" residual structure is found in the sarcosporidia. "Between the pansporoblasts (?) there remain strings of protoplasm, which together form a network, or a system of chambers left over after the spores are gone" (Doflein, '01, p. 215). There are no figures in Doflein's work to illustrate this structure, and the staining reactions are not given, but the description applies very well to the residual structure of the cytoplasmic phase of Cytoryctes. Here there is always a more or less definite meshwork which becomes much distorted, until finally it becomes a mass of disarranged fibrils, still retaining, however, the characteristic staining reactions (Figs. 15 and 16, Plate XVII).

2. The early intranuclear phase. — (*a*) The gametocytes. — The germs formed by the multiplicative reproduction of the cytoplasmic, amœboid form of the parasite may develop into new cytoplasmic organisms, or, ultimately, may become germ cells within the nucleus. In the latter case they develop into structures which I regard as gametocytes, the products of which probably conjugate. The resulting zygote is the amœboid pansporoblast mother-organism. This hypothesis is based upon the following observations:

In regions of the skin where cytoplasmic reproduction occurs, some cells are flooded with young forms, apparently identical with the young cytoplasmic forms or gemmules (Fig. 16, Plate XVII). Some of these are seen in the nucleus and some on the nuclear membrane. Again, in regions of the skin where the beginning stages of the cytoplasmic organnisms are found, similar minute, clearly defined, homogeneous bodies are found within the nucleus (Figs. 18, 19, Plate XVII). These intra-nuclear structures are entirely different from the nucleolus-like chromatin masses and different also from the spores in the chromatin, for there is no vesicle and no refraction (see infra). Like the cytoplasmic form they stain uniformly, and a progressive series of sizes can be traced from the point of infection. We also find intranuclear, adult forms, apparently in the process of gemmule formation, but without the characteristic chambered structure of the cytoplasmic forms (Fig. 25, Plate XVI).

The gemmules formed within the nucleus are very definite in size and shape and are less numerous than those of the cytoplasmic organisms. I am unable conclusively to interpret this process, but think it highly probable that these intranuclear gemmules are connected with the sexual cycle and that the mother-organism may be the female gametocyte while the male gametocyte, in its latest phases at least, is quite different. Such a method of female gamete-formation is not without parallel in the sporozoa (see Nusbaum, '03). Unfortunately, however, there seems to be, as yet, no absolute way in which the supposedly younger nuclear forms of these gametocytes can be distinguished from chromatin of the tissue nuclei; for, although ordinarily green with the Borrel stain, it might be argued that we do not know what chemical changes the chromatin might undergo through the agency of the unknown toxines produced by the cytoplasmic parasites. The intra-nuclear amœboid form, like the cytoplasmic, becomes differentiated into red and green portions, but here again the objection might be raised that transitions between the green and red chromatin are to be expected in degenerating nuclei (Figs. 20–24, Plate XVII).

The direct entrance of gemmules into the nucleus from the cytoplasmic adult form, as mentioned above, is highly probable, for broken down residual structures are occasionally seen with partly developed gemmules, while bodies are seen in the adjacent nuclei which are similar in size and reaction to the gemmules. I am aware of the extremely meagre data upon which to base the assumption that these gemmules ultimately enter the nucleus. The above arguments are supported by the fact that the end of the regular growth sequence of the cytoplasmic form is the beginning of the intra-nuclear sequence, and this region is the place to look for young sporoblasts. The only conclusive proof for this assertion is to actually see the living gemmule enter the nucleus as Schaudinn has seen the sporozoite of Plasmodium vivax enter the human blood corpuscle. Since this is impossible, we must fall back upon the one incontrovertible fact that the pansporoblast mother-organism arises inside of the nucleus, and this being true, there must be some embryonic form in the same place.

In small-pox cases characterized by late cytoplasmic and early nuclear forms, there are frequent groups of spherical organisms in the cytoplasm, which appear like the young intranuclear forms. With methylene blue these stain intensely and appear to be cytoplasmic forms of the intranuclear spherical type. With the Borrel they sometimes stain red, but more often green, showing evidences of degeneration. In groups of gemmules which have remained in the region occupied by their mother-organism, similar "ring-forms" are found in stages varying in size from the gemmule to comparatively large forms (Fig. 16, Plate XVI). These stages are, I believe, to be interpreted as sexually mature gemmules which have failed to reach the definitive nuclear position where their further development is possible. From the fact that older stages are unknown, there is reason to believe that if they remain cytoplasmic they do not develop beyond the ring stage and ultimately change in color reaction from red to green. Within a nucleus, however, they retain their affinity for the red of the Borrel combination. Here they form what I regard as the male gametocytes, the female being formed, presumably, in the manner described above. They appear first as homogeneous granules or gemmules within the nucleus, but they early assume the spherical form with central red-staining masses (Figs. 26, 27, Plate XVII). As they grow the red-staining material remains in the center, while similar red-staining points derived, probably, from the central mass of protogonoplasm, develop at the periphery. In the early stages the entire sphere retains the red, but later the central portion and the points in the periphery alone retain the red, while the peripheral matrix stains green (Fig. 32). These bright red points are, as I believe, the almost sub-microscopical male reproductive elements. After their liberation the gametocyte framework persists as a residual structure analogous to that of the gemmule-forming cytoplasmic phase. It remains intranuclear, changes in color reaction from red to green, and ultimately degenerates (Figs. 74 and 75, Plate XIX). The latter process is indicated by the enlargement of the peripheral vesicles, and the change of the central mass into one or more great vacuoles. These frequently unite into a central spherical vesicle, thus forming residual bodies, consisting of one ring within another (Fig. 74).

The central bodies of the gametocytes are to be looked upon as "Restkörperchen" analogous to those of all other male gametocytes of the sporozoa. They, together with the central rings of the residual frameworks, serve to distinguish these structures from the sporoblasts.

In the entire sub-class to which Cytoryctes belongs — the neosporidia — no one has observed conjugation, and as Minchen ('03) observes, this fact makes it probable that conjugation takes place between minute forms. In the Cytoryctes-cycle no structure has been found which can be interpreted as a fertilization stage, although the pansporoblast phase, with its high potential of reproduction, would indicate that fertilization must take place.

In a footnote, Stempell ('02, p. 262) states that Schaudinn has seen the conjugation of two amœboid forms of Nosema bombyces which had just emerged from the spore. We await the publication of Schaudinn's result with interest, in the hope that it will throw light upon this obscure point in Cytoryctes.

b. The zygote. — Certain definite bodies are frequently found in many different cases which resemble the fertilized female gametocyte of Coccidium (Figs. 9, 34–40). These are more or less amœboid forms within the nucleus, characterized by a deeply staining body and a central nucleus-like mass of the protogonoplasm. Occasionally a similar amœboid organism is found in the cytoplasm, one which I observed being surrounded by a very definite capsule and clearly differentiated into cytoplasm and nuclear mass, while the remainder of the cell in which it lay had no trace of parasites. In the nucleus these bodies are spherical, and can always be distinguished from the usual nuclear inclusions by the host nucleus is pushed to one side (Fig. 39, Plate XVIII). of their definite form and dense structure, while the chromatin For the first time the organism now resembles a typical cell, differentiated into nucleus and cytoplasm, for the red-staining central portion of the protogonoplasm now appears as a nucleus, while the cytoplasm is dense and granular and stains uniformly green.

This zygote-like form becomes the pansporoblast and the mother cell of the primary sporoblasts, a process of such moment and requiring such a high potential of vitality that this fact alone justifies the a priori conclusion that fertilization has taken place. There is no visible egg membrane or other morphological structure to aid us in this interpretation. The nuclear body of the parasite at this stage is occasionally double or biscuit-formed, as though dividing (Fig. 38, Plate XVIII).

c. The pansporoblast. — If the nucleus of an ordinary Amœba should divide many times, and if a portion of the protoplasm containing a few of these nuclei, while still within the body of the parent Amœba, should be differentiated into a sporoblast-forming region, that limited portion would be a pansporoblast. Two, three, or more such limited portions of the cell body might be formed, and each would be a pansporoblast, the parent organism meantime continuing its individual existence. So far as known, this peculiar method of reproduction is met with nowhere but in the sub-class neosporidia of the sporozoa; and in Cytoryctes this stage forms one of the most important phases of the life-history, the pansporoblast being the seat of the vigorous "propagative" reproduction.

The adult intranuclear parasite of variola forms but one pansporoblast. At the outset this is a spherical body with very dense cytoplasm, easily distinguished from the nucleoplasm of the host cell, and with a small, spherical mass of protogonoplasm (Fig. 41, Plate XVIII). The protogonoplasm of this stage, which might here be called the nucleus, becomes distributed by fragmentation throughout the substance of the organism. The fragments are so minute at first that they are barely visible as red points within a green matrix (Borrel stain), and are smaller even than the mature spores (Figs. 41, 42, Plate XVIII). Stages in their growth can be followed, however, and with this growth the contour of the parent organism becomes more and more irregular. The nuclear membrane of the host cell ultimately disintegrates and liberates the parasite, which probably has the power of moving from cell to cell, since nests of ten or twelve are occasionally found (Figs. 48-49, Plate XVIII). It grows to a comparatively large size (ten microns to twelve microns) and from eight to twenty primary sporoblasts develop within it. In the older organism, vacuoles appear in the cytoplasm and this is the only differentiation.

One large portion of the protoplasm takes no part in the sporoblast formation (Figs. 46, 47, 50), but remains undeveloped, and, as a "Restkörperchen," gradually degenarates. It becomes irregular in form, and, with the Borrel stain, ultimately changes in color-reaction from red to green. Like the gemmules, the young sporoblasts are solid and homogeneous at first, but as they increase in size they become hollow, and in optical section appear as thickened rings (Figs. 47, 48, 52, Plate XVIII). They may be distinguished from the sporoblasts, which come directly from spores, by the uniform thickness of the ring. When these spheres have attained the diameter of about one and a half to two microns, the thickened periphery shows evidence of vacuolization, which becomes more definite with increase in size (Figs. 50, 51, 53, Plate XVIII).

There appear to be two types of primary sporoblasts, but I am inclined to think that the difference is due to imperfect fixation, or merely an optical effect due to flattening of the usually spherical body. In one, the spores develop in the peripheral vesicles, the central portion remaining hollow (Figs. 51, 54, Plate XVIII), while in the other the spores develop in all of the substance of the sporoblast (Figs. 67, 68, 69, Plate XIX). A characteristic residual body results from each of these types of sporoblasts, one a mere skeletal framework, the other apparently more solid. In both types the method of spore-formation is the same, the material of the spores, or protogonoplasm, accumulates about the minute vesicles, forming darker spheres lining the spaces, and later it segregates at one pole of each vesicle, forming a more deeply-staining body of the spore, while a characteristic vacuole, composing the bulk of the spore, is formed. The peripheral ring of spore-forming substance usually forms vesicles with but one spore each (Fig. 54, Plate XVIII), although vesicles may apparently run together to form larger vacuoles in which three or four spores may lie. Occasionally the sporoblast may be found with the spores immediately outside the broken walls of the vesicles (Fig. 66).

The sporoblasts, as in all other forms with pansporoblast-formation, are liberated from the body of the pansporoblast, apparently by the death and disintegration of the parent organism.

3. Secondary nuclear phases. — (*a.*) The Spore. — The following quotation, which I take from Minchin's recent work on the sporozoa, describes the conditions for any germ of the neosporidia. The minute size of the spore of Cytoryctes makes the description particularly pertinent in the present connection, and indicates the possible modes of spore-transportation. "From this point (liberation of the germ) the tiny parasite embarks upon migrations, in some cases very extensive, in order to reach the organ or tissue which is its final destination. It is not possible to state with any certainty how these migrations are either effected or

guided. In some cases the journey is perhaps performed on foot, as it were, the little amœboid germ pushing its way actively through the tissues, like a leucocyte. In other cases the parasite may be passively transported by means of the blood current. The latter method is probably the more usual, the little germ being carried along suspended in the blood plasma; at any rate, there is no evidence that it ever attacks the blood corpuscles. The one thing certain with regard to this stage of the life history is that the parasite is able to select and to seek out, in some mysterious fashion, the specific organ or tissue which it affects, and which may be situated at a considerable distance from the original seat of infection" (p. 290).[1]

The spore is extremely minute (.57μ) and is easily overlooked. Its distinguishing features are the presence of a vacuole, which occupies the greater part of its mass, and a thickened portion at one side. This gives to it a high refrangibility and it shines out with great brilliancy. It stains blue with methylene blue, and red with the Borrel combination, and may be thus distinguished from minute air-bubbles or possible bits of bichloride.

I could make out no trace of the filaments which are characteristic of the majority of neosporidia-spores, but it is not impossible that such structures exist, and that by the use of proper reagents they might be demonstrated. The vesicle, however, is characteristic of microsporidia-spores and it alone is enough to indicate the taxonomic position of Cytoryctes.

b. The secondary sporoblast and its sporulation. — The intranuclear spore, often embedded in chromatin (Figs. 55 and 56, Plate XVIII), develops into what may be called the secondary sporoblast to distinguish it from the primary sporoblast formed in the pansporoblast. This spore at germination might be described as a vesicle with a thickening at one side. As the spore grows, the thickened region becomes relatively large until, ultimately, it extends entirely around the vesicle.

[1] A Treatise on Zoology. E. Ray Lankester. Part I. Introduction and protozoa. 2d fascicle, 1903.

Minute vacuoles then appear within it, first in the original thickened portion, later throughout the entire periphery (Figs. 58, 59, 60, Plate XIX). Secondary vacuoles may then appear within the first set, that is, towards the center of the sphere, the entire sporoblast, finally, becoming a mass of vesicles, some large, others of minute size (Figs. 70, 71, 72, Plate XIX). These vesicles, both on the periphery and within, are the seat of the formation of spores which are apparently identical with those formed by the primary sporoblasts. The developing spores are at first tiny spheres lining the vesicles, but owing to their minute size, it is impossible to make out their origin. They are formed, apparently, from a substance in the vesicular meshwork, that is, the protogonoplasm which, as in gemmule-formation, again becomes metamorphosed into the reproductive bodies. The vesicles of the sporoblast thus correspond to the chambers of the cytoplasmic form.

In this phase there is, again, no trace of a morphological nucleus, beyond the fact of the more deeply-staining point and the gradual segregation of the protogonoplasm. Division figures were never seen.

With growth of the secondary sporoblast, the nuclear membrane of the host cell becomes more and more faint, until finally it entirely disappears and the parasite is free in the cytoplasm, which also degenerates, leaving the sporoblast in the more or less broken-down substance at the bottom of the pustule.

c. The sporoblast residuum. — After liberation of the spores from both primary and secondary sporoblasts, there is, as in gemmule-formation, a residual product, but in this case the form is quite definite and the appearance decidedly characteristic. A framework or meshwork would again be descriptive, but there is a far greater homogeneity than in the analogous structure resulting from gemmule-formation, and the general form is retained, apparently for a much longer period (Fig. 72, Plate XIX). Instead of becoming "stringy," the sporoblast residua eventually swell and become distorted; the periphery finally gives way and the vesicular mass breaks up.

An analogous residual structure is described by Stempell ('02) in the sporulation of Thélohania mülleri, but it is passed over without emphasis (see text-figure 1).

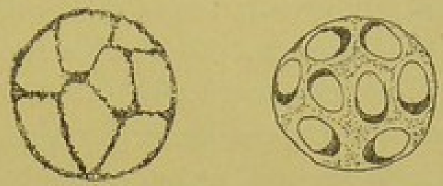

FIG. 1.

The following diagram gives a comprehensive view of the life-cycle of the small-pox organism as I have interpreted it. Every stage represented is from a camera drawing of the parasite, the tissue cells alone being schematized.

THE VARIOLA CYCLE OF CYTORYCTES VARIOLAE Guar.

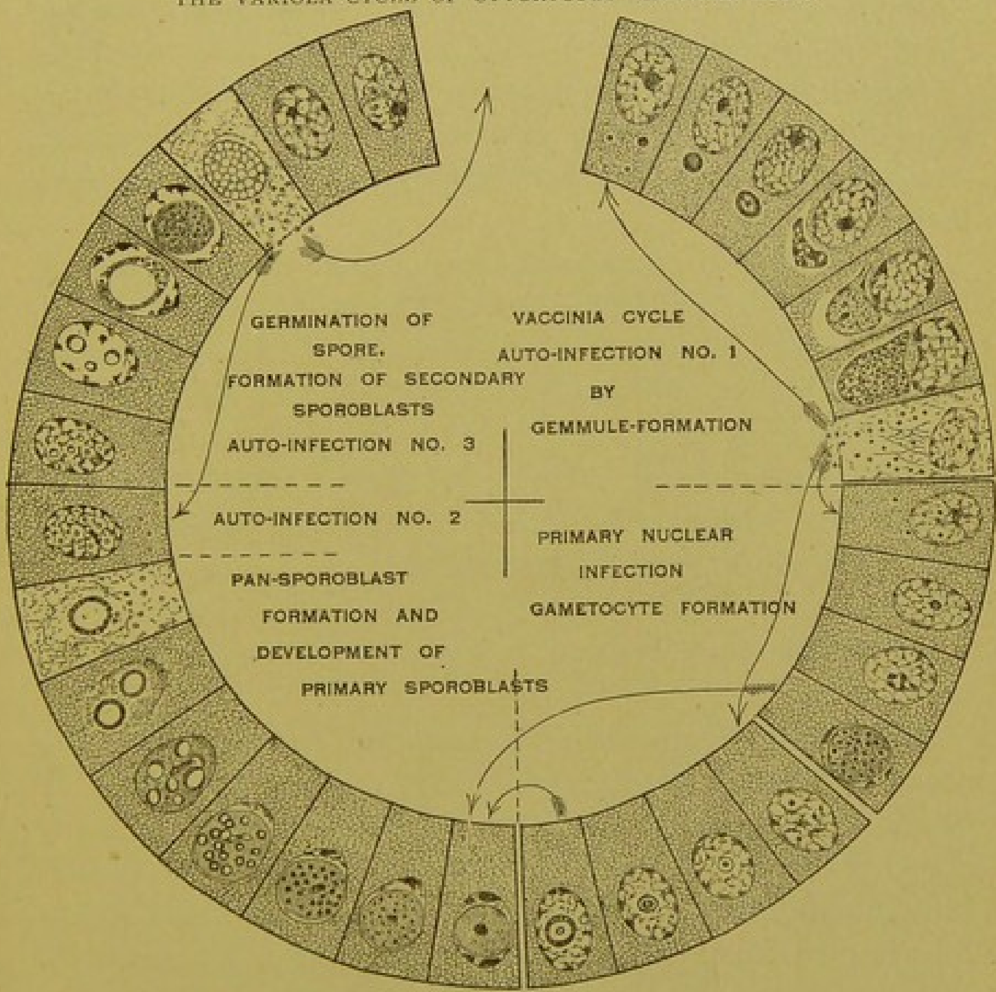

FIG. 2.

C. Biological Considerations.

1. Multiplicative reproduction. — Doflein ('98) has given to the endogenous reproduction which takes place in myxosporidia, and by which auto-infection in the host is brought about, the name " multiplicative reproduction," and to exogenous reproduction, by which the disease is spread to new hosts, the term "propagative reproduction." In a general way multiplicative reproduction is similar to schizogony of other forms of protozoa, but differs from this in certain minor points. Unlike the propagative reproduction, it is always asexual and takes place either by simple binary, or multiple division, which Doflein terms "plasmotomy," or else by fragmentation. In the first case the full-grown form with its many nuclei divides into two or more equal or unequal fragments, each of which is multinucleate. In the latter case the nuclear material of a young, immature form breaks up into many fragments by "multiple amitosis; " the cell-body then breaks up into many uninucleate fragments, which carry the disease into new regions of the tissue or to new organs.

This method of reproduction is accepted as very common, if not universal, among the tissue and cell-infecting forms of neosporidia. In Thélohania mülleri (L. Pfeiffer), according to Stempell ('02), a more involved method of multiplicative reproduction occurs, which Minchin considers intermediate between multiplicative increase by simple division, and by " multiple amitosis. The young forms of the parasite divide after direct division of the nucleus, but the daughter individuals remain connected and divide again without separation. Thus a chain of individuals is formed which breaks up only later.

Students of the protozoa will recognize the fact that in the process of gemmule-formation I have described a method of reproduction which so far as known is not duplicated in any other protozoön. Auto-infection in sporozoa is usually brought about by a process of sporulation and the spores thus formed, technically known as merozoites, are similar in form and size to the sporozoites, the essential difference between them being the mode of origin, which is asexual in the former, sexual in the latter. In the well-known forms there is thus an alternation of sexual and asexual generations — several of the latter (as in malaria) to one of the former. The process of gemmule-formation, however, belongs in the same category of reproductive phenomena as the multiplicative reproduction of myxosporidia and of other microsporidia.

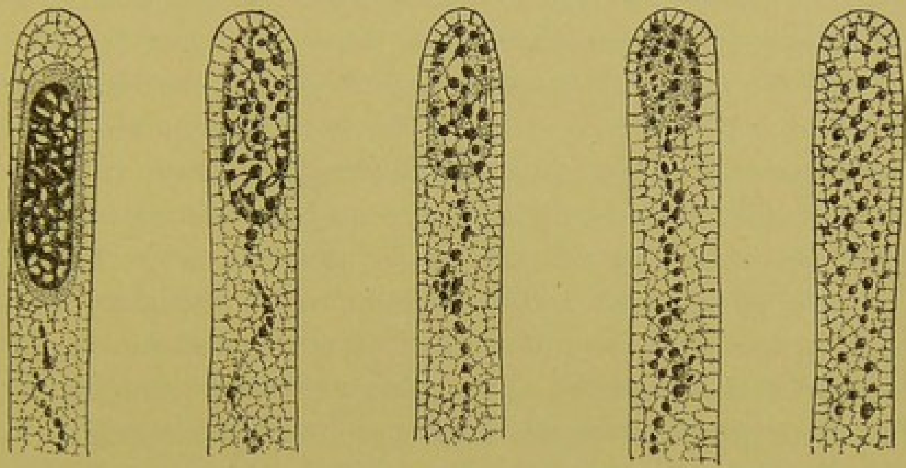

FIG. 3.

Thus Doflein ('98) observed the myxospore of Chloromyxum leidii forming daughter individuals by division, and recognized a similar mode of increase in Hoferellus cyprini, while Laveran and Mesnil ('02) observed the multiplication into many parts of Myxidium lieberkühni. It was early noticed by Cohn that this process occurred mainly during the winter months, and in this period the customary spore-formation was in abeyance. Although the students of the microsporidia (Thélohan, '95, Doflein, '98, Lühe, 1900) recognized the necessity of such a process to explain the enormous increase of the parasite while within the host, with the exception of Thélohania there have been no positive observations on this type of multiplication in forms allied to Cytoryctes. It is obvious, however, that some mode of reproduction, apart from schizogony and sporogony, might be expected in the small-pox organism.

The experiments of Guarnieri and of Wasielewski show conclusively that reproduction of the cytoplasmic phase must take place, and our observations on the structures of these forms in the late adult stages fully confirm the a priori conclusion. The method, however, appears to be almost unique in animal forms, although similar processes occur in bacteria. There is some evidence to justify the view that multiplicative reproduction in Cytoryctes may occur through simple division of the larger amœboid stages, and forms are frequently found of dumb-bell shape, or with thin strands of protoplasm connecting two larger masses. It is possible that the multiple infection, sometimes seen in a single cell, may be due to this method of increase, but I do not think that this is an efficient means of reproduction. Gemmule-formation is a much more prolific method, and satisfies the a priori requirements based upon the assured facts of distribution in the host and upon the potency of vaccine virus in which the cytoplasmic phase is the only organism found (see Councilman). The gemmules, therefore, are of the nature of bacterial spores, and like the latter are probably capable of withstanding unfavorable external conditions. The method of their formation, furthermore, is analogous to the formation of spores in bacteria. Here, according to Bütschli, Schaudinn, and other observers, there is no nucleus, but the cell contains scattered granules (protogonoplasm?) of what appears to be nuclear material identical, apparently, to the distributed granules in Cytoryctes. Schaudinn ('02) has clearly shown how this granular material collects at one end of Bacillus bütschlii to form the spore, and he calls attention to the fact that, when thus accumulated, the mass resembles a morphologically differentiated nucleus (see text-figure 3). The apparent nucleus, however, develops into a complete bacillus. So in the case of gemmule-formation in Cytoryctes variolæ, where the protogonoplasm collects in minute spherical masses, which, like the spore of Bacillus bütschlii, may develop into organisms like the parent, but, unlike the spore of the bacillus, they may in time develop into sexual organisms which are dissimilar to the immediate parent and have a definite reproductive function. Unlike Bacillus bütschlii, again, Cytoryctes forms not one but many of these reproductive bodies, and in this respect simulates the polysporous bacteria. In asexual reproduction, therefore, it may be stated that Cytoryctes approaches more closely to the bacteria than to the other forms of parasitic protozoa.

The amazingly wide-spread infection in variola, an infection which, within a comparatively short time after exposure to the disease, may involve nearly the entire surface of the human body, can be explained only by the assumption of both rapid and varied modes of increase of the parasite. Auto-infection by gemmule-formation is one such mode of reproduction. Another, and an even more potent method, is by schizogony or direct development of the spores into secondary sporoblasts. In some small-pox cases (*e.g.*, case 17) there is a notable absence of adult organisms in the epithelial cells. In many nuclei of these cells, however, there is at least one, while there may be three or four spores (Figs. 55, 56, Plate XVIII). In the protocols such cases are described as aberrant, and the virulence may be due to this wide-spread infection.[1] Here the definitive environment is not the cytoplasm, nor the general nucleoplasm, but the specific chromatin substance within the nucleus. It appears, then, that we have in this phenomenon another interesting biological fact, viz., "chemotactic selection" of chromatin, analogous to "chemotactic selection" of blood corpuscles by the malaria organisms, or selection of nucleoplasm by Cyclospora caryolytica Schaudinn, or similar selection by numbers of sporozoa of certain definite tissues of definite hosts.

How the spores get into the nucleus is a matter of speculation. We know that the effect is produced, and we find the definite spores in this position. The spores (0.57μ) are so minute that they approach well within the possibility of Roux's "invisible germs," and it requires no more stretch of the imagination to account for their wide distribution than

[1] Dr. Councilman and Dr. Brinckerhoff suggest the following interpretation of case 17. In the inoculation lesion of the ape, which corresponds to the hypothetical proto pustule in man, it has been shown that both cycles take place, the multiplicative cycle being followed by the propagative. It seems extremely probable that the skin infection

it does to account for an equal distribution of some toxin produced by an organism. We have described above the germination of such spores and the direct development of the secondary sporoblast, a most unusual phenomenon, whereby the primary sporoblasts, which are formed in the pansporoblast, are duplicated by direct development of spores. So far as I know, the only similacrum is the reproduction of trematode worms where larval rediæ give rise to similar rediæ. This process, together with that of gemmule-formation, is sufficient to account for the wide-spread infection which characterizes variola.

2. The distributed nucleus.— It is generally known that many unicellular organisms, *e.g.*, the bacteria, have no definite nucleus. This does not mean, however, that nuclear structures are absent or that Hæckel's group of enucleate organisms (monera) is justifiable. A morphological nucleus, which to many recent critics (*e.g.*, Foa, Doflein, etc.) appears to be the sine qua non of an intracellular parasite, is not only not necessary, but not to be expected in these lowest forms of sporozoan organisms. Critics who deny the appellation "organism" to certain cell inclusions, on the ground that there is no nuclear differentiation, are either ignorant of the facts or wilfully overlook them. As a matter of fact, it is generally conceded by cytologists at the present time that nuclear material, presumably chromatin, may be present in the cell in other forms than that of a morphologically differentiated nucleus. This occurs more and more frequently as we approach the lowest forms of living things until, in the cyanophyceæ and bacteria, it is the prevailing condition. Schewiakoff ('93) was among the first to show that such a condition is normal, and in the case of Chromatium he dem-

shown by the eruption usually takes place by means of gemmules, for the youngest forms in the cytoplasm of the epithelial cells correspond to these. Case 17 was one of delayed eruption. In such cases the eruption does not appear at the usual period after the chill, and when it appears the course is extremely rapid. In this case, also, there was almost complete absence of cytoplasmic forms. It is probable that the infection of the skin in this and similar cases takes place by spores entering the blood from the protopustule, and not by gemmules; the delay in the eruption is due to the longer time necessary for the spore-formation.

onstrated that the distributed granules can reproduce themselves by division. In the bacteria the granules are distributed throughout the cell body, a fact which led Bütschli ('90), at first, to regard the entire cell as a nucleus. In division of bacteria these granules are passively separated into two groups, but in spore formation they become aggregated to form the body of the spore (see Schaudinn, '02, and above).

An intermediate condition occurs in some flagellates; in Tetramitus, for example, the distributed granules collect prior to cell division and are divided into two equal portions while thus aggregated, while in Chilomonas they are permanently aggregated, although without a membrane. From this condition intermediate steps to the formation of definite morphological nuclei have been traced in the different types of protozoa.[1]

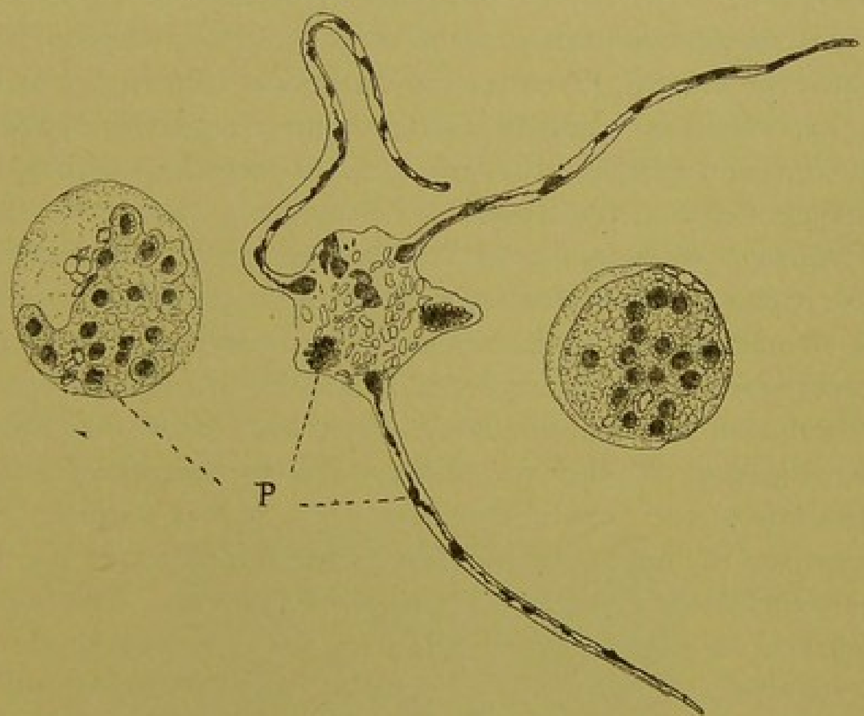

FIG. 4.

In the malaria organisms the nucleus cannot be regarded as a morphologically differentiated structure, for it frequently appears as a mass or masses of chromatin material (see text-

[1] See in this connection Calkins, '98, '01, '02.

figure 4). Finally, in Lymphosporidium truttæ, which caused a most destructive epidemic among the brook trout in a hatchery on Long island, a similar distributed nucleus occurs.

The term "distributed nucleus" was proposed by myself ('98), and interpreted as a primitive condition of nuclear substance before a definite morphological nucleus is differentiated. Hertwig ('02) proposed the term "Chromidien" for such distributed chromatin, and he, followed by Schaudinn, also regarded it as a primitive condition of the nuclear substance. As the term "chromidium" is already in use by the botanists to designate a very different structure, I have replaced it in this paper by the term "protogonoplasm."

3. Systematic position of Cytoryctes variolæ and description of *Caryorcytes cytoryctoides*, nov. gen. et spec. — So far as I am aware, there is but one other organism which is similar in structure to the intranuclear parasite of small-pox. This is an intranuclear parasite of the macronucleus of the ciliated protozoön, Paramœcium caudatum. In 1896 I made preparations from a mixed culture containing many of these infusoria, and noted at the time curious inclusions within the macronucleus. Little attention was given to them, and the preparations were laid aside. After seeing the intranuclear forms of Cytoryctes, I re-examined these preparations, and was struck by the close similarity between the two intranuclear forms. (Figs. 78–87, Plate XX). In none of the cultures of Paramœcium examined before or since have I found a like condition. The organisms of these preparations are beautifully fixed, every part is normal, and, except for the spaces in them in which the parasites lie, the macronuclei are characteristically homogeneous. The inclusions are no degeneration products, nor are they products of secretion. They are organisms, and protozoa of a definite kind, undoubtedly belonging to the same group as Cytoryctes variolæ. On account of the possible importance in comparison with the small-pox organism I shall name this genus Caryoryctes, and from its close resemblance to the latter, I shall give it the specific name "cytoryctoides."

The development of the spores of Caryoryctes may be traced in different specimens of Paramœcium, and shows a close agreement with spore development in Cytoryctes. The sphere enlarges, the collection of plasm at one side of the spore becomes vacuolated, and new spores are ultimately formed (Figs. 79, 80, Plate XX). Like spores in Cytoryctes, these lie in minute vacuoles on the periphery of the sporoblast (Fig. 81).

As in the latter, again, there are great residual structures with clear vacuoles, and skeleton frameworks which suggest Stempell's figure of the residum of Thélohania (text-figure 1, p. 157 and Figs. 77, 82, and 85, Plate XX). In one case (Fig. 84) a condition is apparent which may be analogous to gemmule-formation in Cytoryctes; a few of the vacuoles contain solid spherules, while the majority are empty. The scarcity of material makes it impossible to draw positive conclusions as to the complete life-cycle and to establish the complete harmony with the cycle of Cytoryctes.[1] There are enough analogous stages, however, to convince us that we have to do with an organism similar to that causing small-pox, and the nature of the single-cell host dispels any shadow of doubt that these structures are artefacts or degeneration products.

The known species of microsporidia are provided with more or less definite nuclei. This feature, therefore, separates the family nosematidæ (with which our organism seems to be most closely allied) from Cytoryctes caryoryctes and, propably, Lymphosporidium. With the exception of

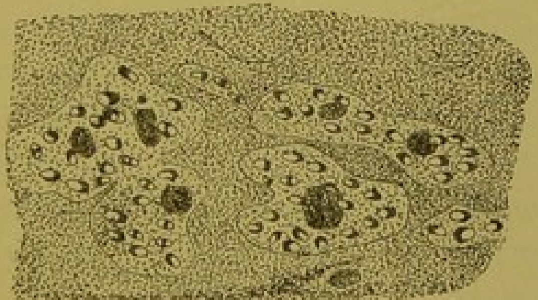

FIG. 5.

[1] The two preparations which I have from this particular culture contain about three hundred and seventy-five individuals, of which about eighty per cent are infected.

the last, the spores have the characteristic vacuole of the microsporidia (see text-figure 5), and I see no reason why these organisms should not be included in the tribe polysporogenea (Doflein). Under the family name of cytoryctidæ, I would place them next to the nosematidæ in Doflein's classification. Should other organisms be added to these, it is not unlikely that the characteristics will be sufficiently definite to warrant an ordinal grade, or possibly, a still higher grade in classification. Such a group, whatever it is, which includes these forms would be the lowest of the sporozoa, and would occupy a position between the parasitic bacteria, on the one hand, and the higher sporozoa on the other. For the present I do not think it advisable to go as far as this, and will place them provisionally as follows:

Class Sporozoa.

Sub-class myxosporidia (for sub-divisions see Doflein).

Sub-class neosporidia.

Order microsporidia.

Tribe polysporogenea:

a. Family nosematidæ. (The body continues to grow during sporulation. Many pansporoblasts, each without a membrane. Nuclei present.)

b. Family plistophoridæ. (Body completely used in the formation of the pansporoblast, with membrane. Nuclei present.)

c. Family cytoryctidæ. The organism forms one pansporoblast which is without a membrane. Nuclei absent.

1. Genus Cytoryctes, the cause of variola.
2. Genus Caryoryctes, the Paramœcium parasite.
3. Genus Lymphosporidium. Brook trout parasite.

In conclusion we may summarize our knowledge of Cytoryctes as follows:

We know:

1. The gemmule and its growth, leading to:

2. The cytoplasmic amœboid adult.

3. The process of gemmule-formation and auto-infection, No. 1.

4 The residual cytoplasmic structures.

5. The development of the pansporoblast.

6. The development of the primary sporoblast and auto-infection No. 2.

7. The spore and its germination.

8. The development of the secondary sporoblast and auto-infection No. 3.

9. The method of spore-formation.

10. Structures which appear like fertilized egg-cells.

11. Structures which appear like developing microgametes.

12. The residual structures of nuclear origin.

13. Similar organisms parasitic in nuclei of other animals.

We have evidence, but need more light upon:

1. The actual entrance of gemmules into the nucleus.

2. The significance of the intranuclear sporoblast following upon the adult cytoplasmic amœboid form.

3. The origin of the pansporoblast mother-organism. These may possibly all be summed up in:

4. The sexual phenomena.

We are ignorant in regard to:

1. The first stages of the organism in a new host.

2. The transportation of the infecting agents by the blood.

3. The method of cell and nuclear infection.

4. The significance of the inhibition of the nuclear phases in the vaccine-organism.

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DESCRIPTION OF PLATES.

All figures except 78 are drawn to the same scale and are equally magnified (about 1,500 diameters). Objective used was Zeiss 1.5 millimeters (N.A., 130); ocular Zeiss compensating 6; draw-tube 16. All figures are camera drawings from the object.

Abbreviations.

A. Amœboid form of Cytoryctes in cytoplasm.
AG. Amœboid form in gemmule-formation.
AN. Amœboid form in the nucleus.
C. Chromatin masses.
F. Filar substance.
G. Gemmules.
GN. Gemmules in the nucleus.
GV. Gastric vacuole.
M. Macronucleus.
m. Micronucleus.
MG. Microgametocyte.
N. Micronuclear niche in macronucleus.
P. Pansporoblast.
PM. Pansporoblast mother-organism.
S. Spore.
SB. Sporoblast.
SR. Sporoblast residuum.
X. Unidentified form, possibly microgametocyte.
YSB. Young sporoblast.

Plate XVI.

Fig. 1. A group of cells from the Malpighian layer infected with the youngest forms of Cytoryctes. The cytoplasmic gemmules are in different stages of development. Case 18.

Fig. 2. A similar group of cells containing older stages. Case 16.

Fig. 3. A cell with three young gemmules. Case 33.

Fig. 4. The cytoplasmic form in advanced amœboid phase. Differentiation is shown into matrix and protogonoplasm.

Figs. 5, 6, and 7. Similar stages with the protogonoplasm in clumps.

Fig. 8. Adult cytoplasmic forms with characteristic perinuclear amœboid forms. The protogonoplasm is fragmented into many small granules. In three of the organisms there is no indication of the filar plasm or network, but in the fourth it is distinct. Case 27.

Figs. 9, 10, 11, and 12. A group of cells with amœboid organisms in various phases of gemmule-formation. The filar meshwork is becoming evident on the periphery and each gemmule appears to be in a vacuole. In 10 the gemmules are breaking out from the parent organism. In the cell-nucleus of 12 are spheres shown in optical section which have developed from intranuclear gemmules. Case 27.

Plate XVII.

Fig. 13. A late stage in gemmule-formation. The filar substance (F) forms the framework of the chambers enclosing the gemmules. Case 27.

Fig. 14. A mass of gemmules forming a compact group in the cytoplasm of an epithelial cell. Case 27.

Fig. 15. The residual skeleton of the cytoplasmic form, after the disappearance of the gemmules and before distortion of the framework. Case 27.

Fig. 16. The remains of a cytoplasmic form with the resultant gemmules in different stages of development in situ. Some of these are within the nucleus and develop in the nuclear plasm. Case 18.

Figs. 17, 18, and 19. Three cells from the same field, one with neither nucleus nor cytoplasm infected (17), one with cytoplasm infected (18) and one with nucleus alone infected (19). Case 27.

Figs. 20, 21, 22, 23, and 24. Development of the supposed intra-nuclear form of the organism, showing differentiation similar to that of the cytoplasmic form. Case 27.

Fig. 25. A case of intra-nuclear gemmule-formation. This is probably the adult form of the intra-nuclear amœboid form. The chromatin is massed against the nuclear membrane. Case 28.

Fig. 26. An intra-nuclear gemmule with indications of the fragmentation of the protogonoplasm. The first stages are homogeneous as in Fig. 19. Case 2.

Figs. 27, 28, and 29. Later stages in the development of the intranuclear gemmule and formation of the possible microgametocyte.

Figs. 30, 31 and 32. Later stages in the formation of the microgametocyte. The protogonoplasm collects in minute granules on the periphery while a "Restkörperchen" is usually present. Cases 18, 28, and 33.

Plate XVIII.

Fig. 33. An unidentified form (x) in the nucleus. Possibly an early microgametocyte. Case 16.

Figs. 34–40. The parent cell of the pansporoblast. In each one there is a central mass of protogonoplasm which here appears to be the nucleus of a cell. In Fig. 40 there are two of these structures in the same nucleus. Cases 16, 18, 28, etc.

Fig. 41. The cell has become a pansporoblast. The material of the nucleus-like center is now fragmented and distributed throughout the cell. Each fragment will become a primary sporoblast. The residual central mass of protogonoplasm is a "Restkörperchen." Case 18.

Figs. 42–49. Stages in the growth and differentiation of the primary sporoblasts, and different conditions of the "Restkörperchen." In Fig. 47 the central vesicular condition is indicated. In Figs. 48 and 49 there is a multiple infection, due, probably, to the wandering of the pansporoblasts.

Figs. 50 and 51. Still later stages in sporoblast formation indicating the unequal growth of the different sporoblasts.

Figs. 52 and 53 show similar stages.

FIG. 54. A characteristic vesicular sporoblast with ripe spores. There is no trace of nucleus in the cell and the pansporoblast material is absent, the sporoblast has become free.

FIGS. 55 and 56. Two cells in which the nuclei are apparently normal except as to size, and within the nuclei are several spores, some free in the linin, others embedded in the chromatin. In this case the red of the magenta had been extracted from the tissue nuclei, leaving the spores alone colored. Case 17.

PLATE XIX.

FIG. 57. A large, degenerated cell with spores in the cytoplasm, some of them undergoing partial development outside of the nucleus. These, probably, do not mature. Other spores are indicated in the spaces surrounding the cell. Case 16.

FIGS. 58, 59, and 60. Early development of the intranuclear spores. The characteristic signet-ring form, which one obtains in optical section of the growing spore, is indicated in each case. An occasional spore is present in the cytoplasm. Cases 16 and 18.

FIG. 61. Secondary sporoblasts are here well developed; spores are also present in process of development. Case 18.

FIG. 62. Later developmental stages of the secondary sporoblast. Case 16.

FIGS. 63 and 64. Adult sporoblast with peripherally arranged spores. In 64 the spores are leaving the maternal organism which, like a seed-case, remains as a useless residuum. Case 16.

FIGS. 65 and 66. Late sporoblasts which, being free in the plasm, cannot be distinguished as primary or secondary forms.

FIGS. 67, 68, and 69. Sporoblasts, probably primary, in which the spores are developed throughout the entire substance and not merely in the peripheral portion. Cases 27 and 28.

FIGS. 70 and 71. Residual structures after departure of the spores from sporoblasts like those of Figs. 68 and 69.

FIG. 72. A residual structure with a few spores of the vesicular type of sporoblast. Spores probably derived from this sporoblast are found in abundance in the degenerated cytoplasm.

FIG. 73. A residual body derived, probably, from what we have interpreted as the microgametocyte. It and the chromatin in the host nucleus were red in this preparation, the magenta having been incompletely extracted. Ordinarily the structure represented here stains green, as in Figs. 74 and 75.

FIGS. 74 and 75. Two residual structures, the former a further stage in the breaking down of the microgametocyte remains, the latter, a residuum of the secondary sporoblast.

FIG. 76. Three cells and nuclei from normal skin fixed and stained in the same way as the small-pox material. They are drawn to the same scale as the other figures and show the comparative size of cells and nuclei in normal and in diseased skin.

Plate XX.

Developmental stages of *Caryoryctes cytoryctoides*, n. gen. n. sp.

Fig. 77. Macronucleus of Paramœcium with parasite in huge vacuole.

Fig. 78. *Paramœcium caudatum*, an holotrichous, ciliated infusorian. Low power. f = food vacuole.

Fig. 79. Macronucleus of Paramœcium with spores of Caryoryctes, cytoryctoides, n. gen. n. sp.

Fig. 80. Developing spores in the macronucleus of Paramœcium. The signet-ring character is well marked.

Fig. 81. Sporoblasts with spores in the same.

Figs. 82 and 85. Residual sporoblast structures in Paramœcium macronucleus.

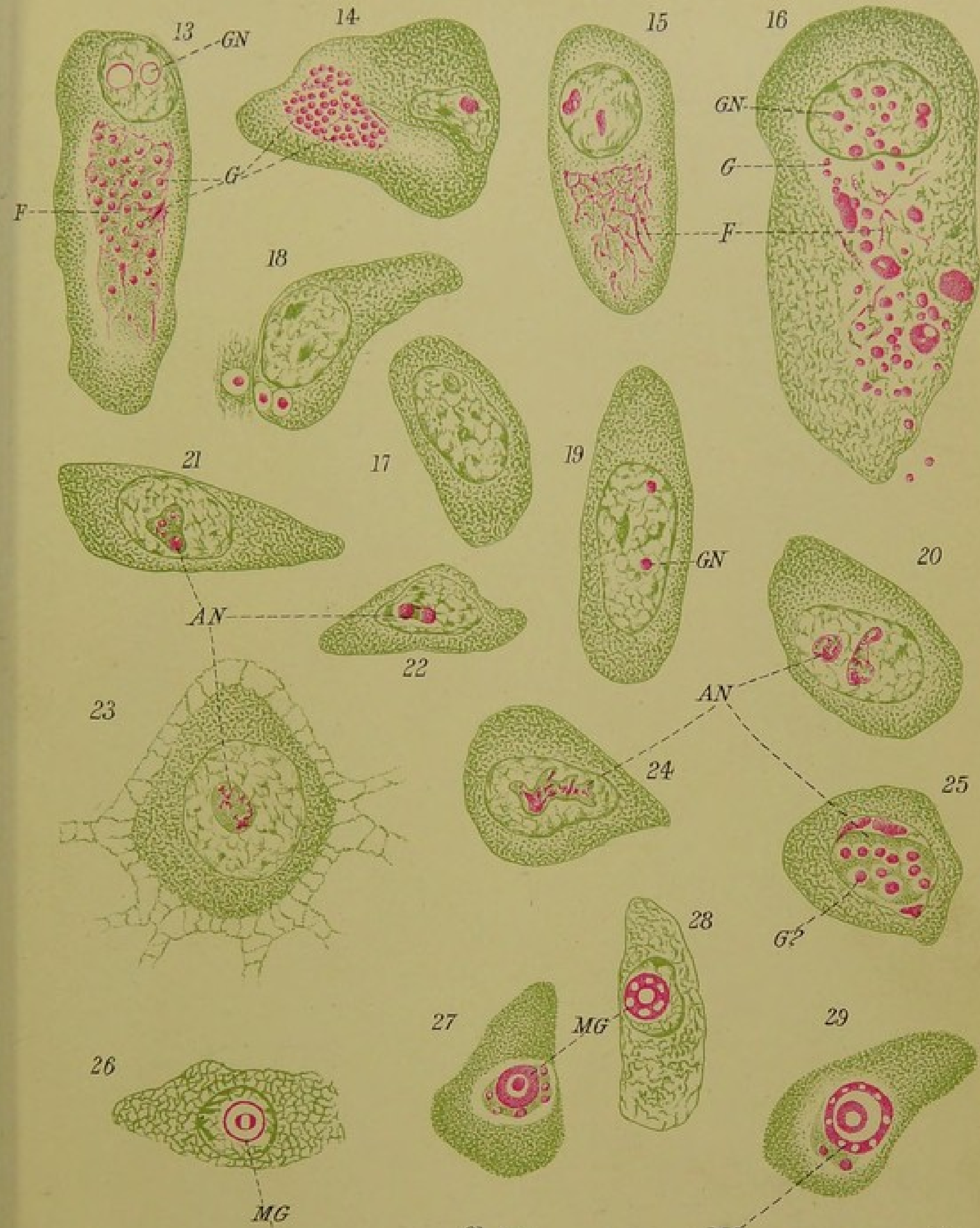
Fig. 83. Spores inside and outside of the sporoblast; compare with the similar structure represented in Fig. 72 of Cytoryctes variolæ.

Fig. 84. Macronucleus of Paramœcium with characteristic indentation from which the micronucleus (m) has migrated. In the macronucleus is a large vesicular structure similar to the sporoblasts, but contains gemmule-like structures.

Fig. 85. Entire macronucleus with large residual body of the parasite.

Calkins.

Cytoryctes variolae, Guarn.



Calkins. Cytoryctes variolae, Guarn.

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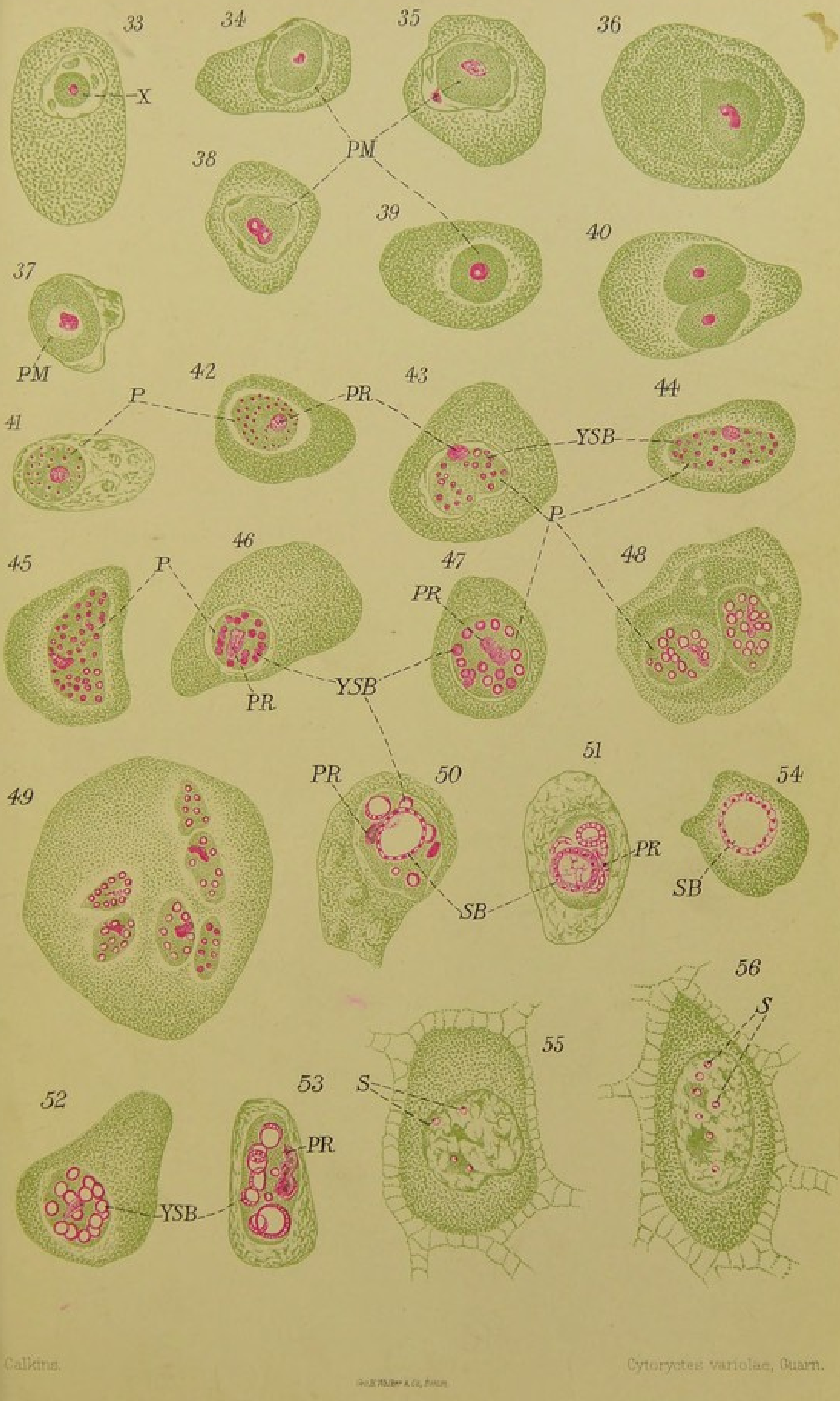

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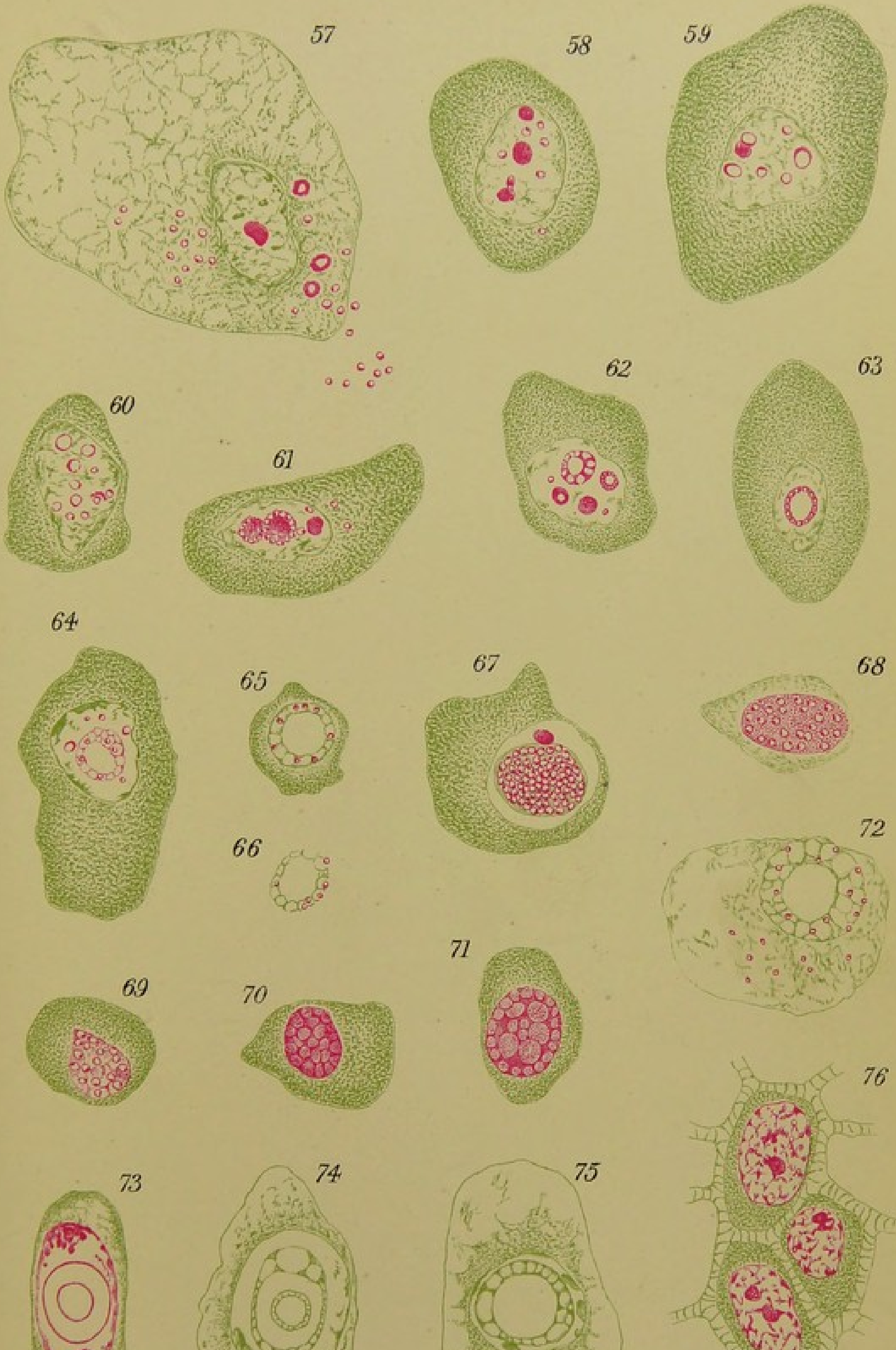
Cytoryctes variolae, Guarn.



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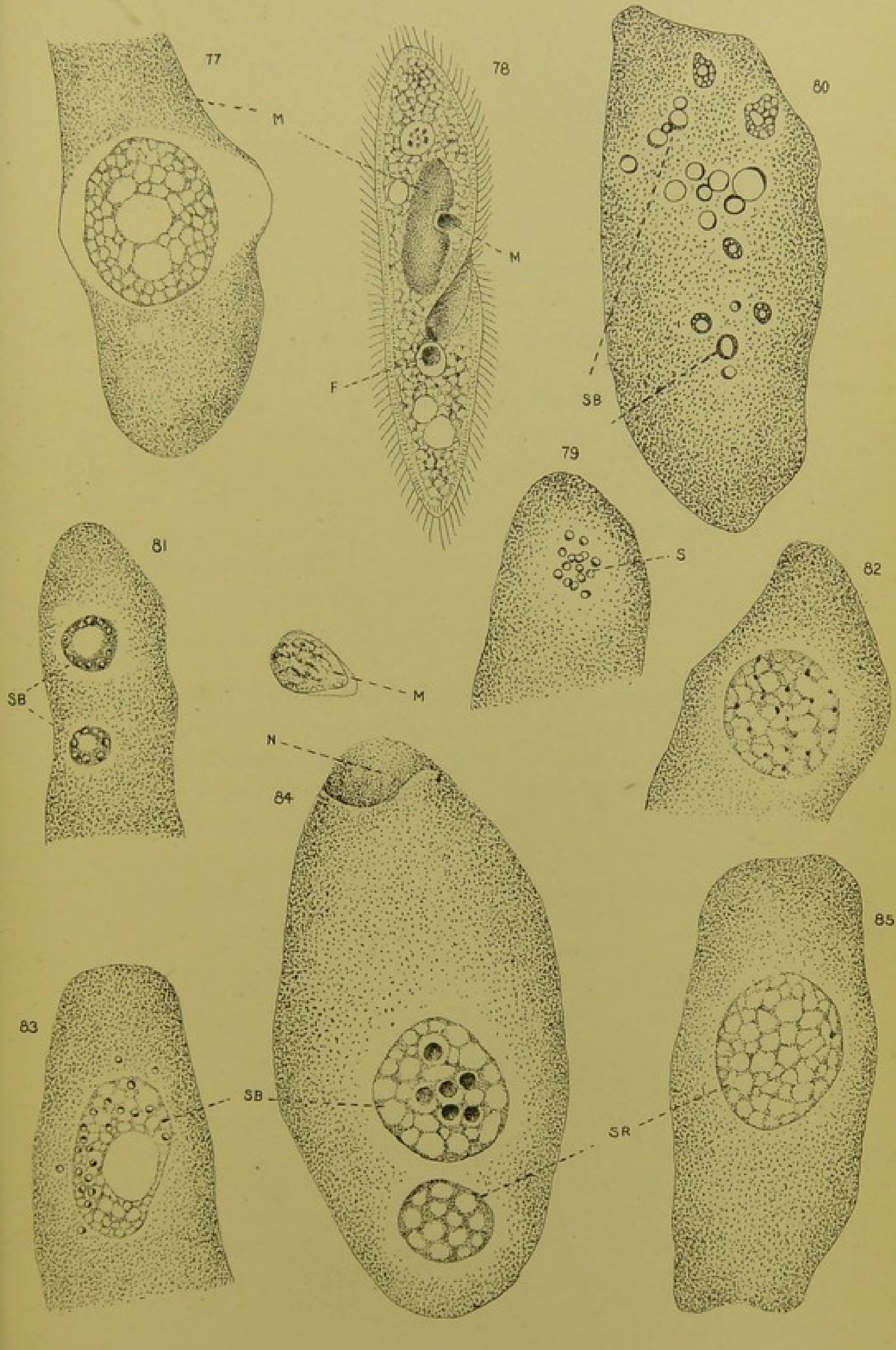


Calkins Small-pox

CARYORYCTES CYTORYCTOIDES

INFUSORIAN NUCLEI

ON THE OCCURRENCE OF CYTORYCTES VARIOLÆ, GUARNIERI), IN THE SKIN OF THE MONKEY INOCULATED WITH VARIOLA VIRUS.

G. B. MAGRATH AND W. R. BRINCKERHOFF.

Introduction — In the course of the investigation of variola, it became evident that a minute study of the lesions of experimental variola in the monkey was desirable for comparison with the lesions in the human skin. This portion of the general problem was assigned to the writers, and their results are here presented. We wish to express our obligation to Dr. W. T. Councilman for constant encouragement and guidance, and to Prof. G. N. Calkins, who placed freely at our disposal his knowledge of the protozoa in general and of the forms occurring in the lesions of variola in man.

So far as we are aware there is no literature bearing directly upon the subject of this paper.

Material.— Six rhœsus and Racacus Monkeys yielded the material. The lesions studied were twelve in number, nine being at the site of inoculation and three from animals in which a general exanthem developed. The duration of the lesions was as follows: primary inoculation, five days, one; six days, two; seven days, two; eight days, one; nine days, one; eleven days, one; exanthem, one day, one; two days, two.

Technic.— The lesions which develop at the site of inoculation vary according to the method of introducing the virus. For our purposes we have found that the introduction of the virus into superficial, single incisions yield the best results. It is important that the incision should not go deep enough to draw blood, and that the wounds should be sufficiently far apart to allow of the development of the pock without the separate lesions becoming confluent. If the inoculations are properly done, a section perpendicular to the axis of the incision will present a lesion comparable with that in the natural disease in man. In such a lesion the relative ages of different parts can be accurately determined.

The lesions were excised during life under anesthesia or immediately after death. The material was placed at once in Zenker's fluid. Paraffine sections were prepared and stained in a variety of ways. The following combinations were found useful: Mallory's chloride of iron-hematoxylin, followed by acid fuchsin and picric acid; the eosin-methylene blue stain; Borrel's magenta red, followed by indigo carmine and picric acid; and Stirling's aniline gentian violet, followed by Gram's iodine solution, and by indigo carmine and picric acid. This last stain was found of service in bringing out certain details of structure in the nucleus of the host cells.

The sections were studied with a Zeiss apochromatic optical series. We found that a powerful artificial light, such as a thirty-two candle power incandescent or an electric arc light, was of great assistance in, and in some cases almost indispensable to, the bringing out of fine details in the structure of the parasite.

Morphology of the parasite.— In our studies of the lesions of the monkey we have found certain bodies which are identical in morphology and in staining reaction with the structures arranged by Calkins in the life history of *Cytoryctes variolæ*. For purposes of description we distinguish: first, structures occurring within the cytoplasm; second, structures occurring within the nucleus of the epithelial cells of the skin. In addition to these, we find forms the orientation of which in the host cell is difficult, and forms which we interpret as degenerate parasites.

Cytoplasmic forms.— These bodies, which occur in the cytoplasm of the host cells, comprise a series of forms common to the cutaneous lesions of variola in man and of vaccinia in calves. They are of variable size and structure and are found in cells the nuclei and protoplasm of which, save for the presence of the parasite, are but little changed.

In the lesions of the monkey we have found these bodies present in all of the primary inoculations and in all of the exanthems, save in one primary lesion of eleven days' duration, in which repair was well advanced. These cytoplasmic forms have a definite relationship to the topography of the lesion. Thus they were found in the rete mucosum in what may be called the multiplicative zone of the lesion, namely, the part of the lesion in which cell multiplication, rather than degeneration, predominates. When the lesion is viewed from above, this zone corresponds to the elevated margin of the pock. In the vertical section of the lesion, the parasites are found in the cells of the hair follicles in the same relation to the cells, the deeper part of the follicle corresponding to the multiplicative area in the rete mucosum. In the older lesions, especially, this relation of the parasite to the topography of the lesion was well marked.

The cytoplasmic forms of the parasite constitute a developmental series leading through a structural differentiation of increasing complexity, from a small and relatively simple structure to a large form presenting evidence of "multiplicative reproduction." From this series certain forms may be selected as developmental types. We choose for description an early stage, a mid stage, and a multiplicative stage.[1]

Early stage. — The parasite appears as a small, structureless, red-staining sphere lying in the cytoplasm at a short distance from the nucleus, often surrounded by a vacuole. The minute size of the earliest of these forms has impressed us with the futility of attempting their demonstration, in the absence of a differential stain, before their entrance into the host cell. After these bodies have attained a certain size, differentiation of structure appears. A substance staining green is found along with that staining red. The earliest forms occur in greatest number at the outermost limits of the

1 The descriptions of the parasite are based upon the picture presented in the sections stained with Borrel's magenta red and indigo-carmine picric acid stain, used as directed by Calkins. Structural differentiation in the parasite is shown by varying power of retaining the magenta during differentiation in alcohol. As stated by Calkins, the stain is not selective and the picture depends upon the extent to which the basic dye is discharged.

multiplicative zone, and are found in all stages of the lesion up to repair (Plate XXII., Fig. 1).

Mid stage. — By insensible gradations we come to forms characterized by greater size, more distinct differentiation into red and green staining substance, and particularly by great variation in shape. The forms encountered suggest, as forcibly as fixed and stained bodies can, that they possess the property of ameboid motion.[1]

Multiplicative stage. — These forms link with the preceding through gradual changes in size and in structure. At this stage the parasite may exceed in size the nucleus of its host cell. It lies near the nucleus and often seems to crowd it to one side of the cell. The red-staining material of the parasite is in the form of small spheres of varying size which lie in the meshes of an irregular, red network (Plate XXII., Fig. 2). These "gemmule-bearing," ameboid forms are most numerous in the early lesions and occur principally in the inner part of the multiplicative zone. In the late lesions cells are frequently found with the red-staining network of the ameba, but without gemmules. In a few cells we have found the gemmules and the network of the ameba within the cytoplasm, and early nuclear forms within the nucleus (Plate XXII., Fig. 3).

Nuclear forms. — These occur within the nucleus of the host cell. They are most numerous just within the multiplicative zone of the lesion where the cells show a considerable degree of degeneration. The following forms which arrange themselves in a developmental series, as do the cytoplasmic forms, bear a rather definite relation in their occurrence to the duration of the lesion. The smaller forms are more numerous in the earlier lesions, while the larger and more complicated bodies are more numerous in the later lesions.

The nuclear forms are not found for as long a period in the lesions as are the cytoplasmic forms, being less and

[1] These various forms of the cytoplasmic parasite agree so perfectly with Professor Calkins' representation of the same forms in the lesions in man that we have not considered it necessary to fully describe and depict them.

less numerous after the sixth day of the evolution of the lesion.

The first appearance of the series of nuclear forms which we here describe is a small, red-staining body within the nucleus of an epithelial cell. This enlarges, forming first a sphere with clear contents. Small secondary spherules with like contents develop in one part of its wall, and it finally becomes a body so large as almost to fill the nucleus, and formed of a mass of small chambers, the walls of which stain green. The chambers are more or less spherical in shape, and their walls are relatively thin. In optical section the body may present a larger central chamber, with smaller ones grouped about it. In the large forms each chamber may contain a red dot, minute in size, and centrally placed. In other bodies the red dots may be in the septa between the chambers, usually at the point of junction of the walls (Plate XXII., Figs. 9, 10, 11). In certain of these bodies no red dots are demonstrable. During the development of this nuclear body the chromatin of the host nucleus becomes murally arranged, and the linin network disappears. A nucleus which appears in all other respects normal may harbor several of the early forms of the nuclear body.

Besides this series of intranuclear forms, which terminates in the formation of a sporoblast (Calkins), there are bodies in the nucleus which are of less definite structure. They consist in masses of homogeneous, green-staining material, which may appear structureless, or may reveal in the interior bodies similar to small sporoblasts. Some of these masses contain minute red-staining dots, while others take only the diffuse stain (Plate XXII., Fig. 8). These forms occur in the same regions and at the same stages of the lesion as the nuclear bodies above described. In the early stages of the primary lesions structures were frequently encountered which we identified with stages of Calkins' pansporoblast. These occurred as large bodies, often surrounded by the remains of the nuclear membrane, and consisted in a structureless, green-staining matrix, in which were embedded spherical, red-staining bodies of varying size, and also ring-like structures similar to those described in the early stages of sporoblast development. A residual mass was present in each of these (Plate XXII., Figs. 4, 8, 5).

In the early lesions a form was encountered which occurred either free or within the protoplasm of the host cell. This structure resembled somewhat a pansporoblast, consisting of a homogeneous matrix which stained green, and in which were embedded red-staining bodies, ranging in size from an almost submicroscopic point to a hollow red sphere, the red material being thicker at the poles (Plate XXII., Figs. 6 and 7).

In the lesions after the fifth day structures were often found in the epithelial cells in the position occupied by the larger cytoplasmic bodies, which, although resembling them in outline, were composed of finely granular material which stained with a diffuse stain. These bodies were often fragmented, and suggested degenerated cytoplasmic bodies.

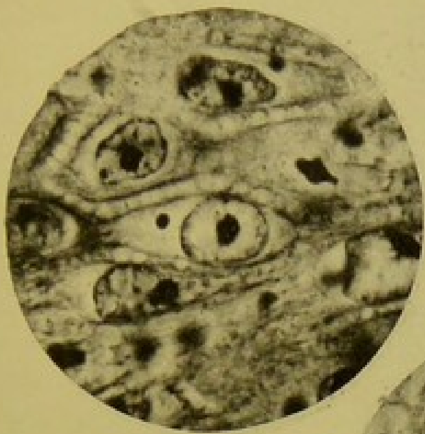
Summary. — In the lesions of experimental variola in the monkey we find within the epithelial cells certain structures which are identical in form and in staining reaction with those in the lesions of small-pox in man.

These forms fall readily into serial arrangements such as are described by Calkins in the lesions of variola in man.

These developmental series correspond with the evolution of the lesion.

The complexity of structure, the staining reactions, and the serial nature of these bodies, preclude the possibility of their being "products of degeneration."

The parallelism between the development of the parasites present in the lesions of variola inoculata in the monkey and the evolution of the lesion, together with the parallelism between the morphology and the staining reactions of these bodies and that of the bodies found in the lesions of variola in man, leads us to attribute to them an etiological rôle in variola.



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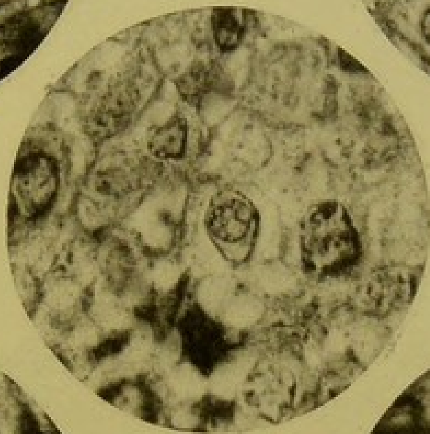

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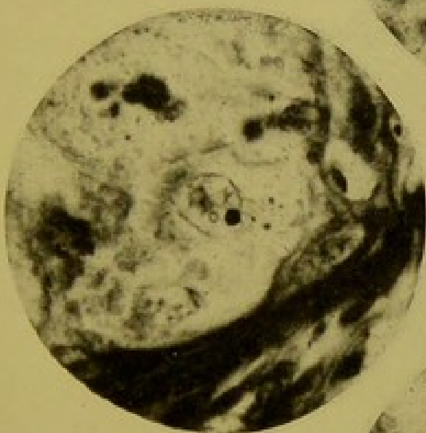

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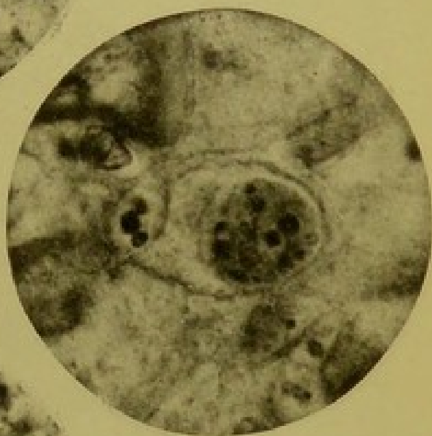

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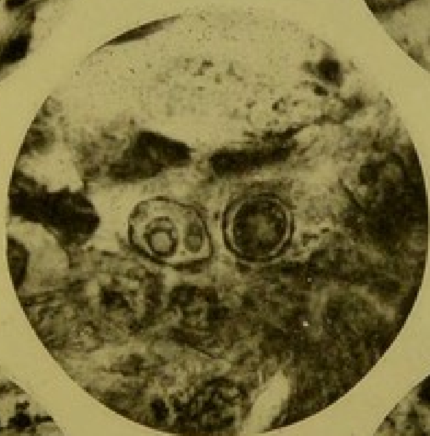

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Magrath and Brinckerhoff — Small-pox

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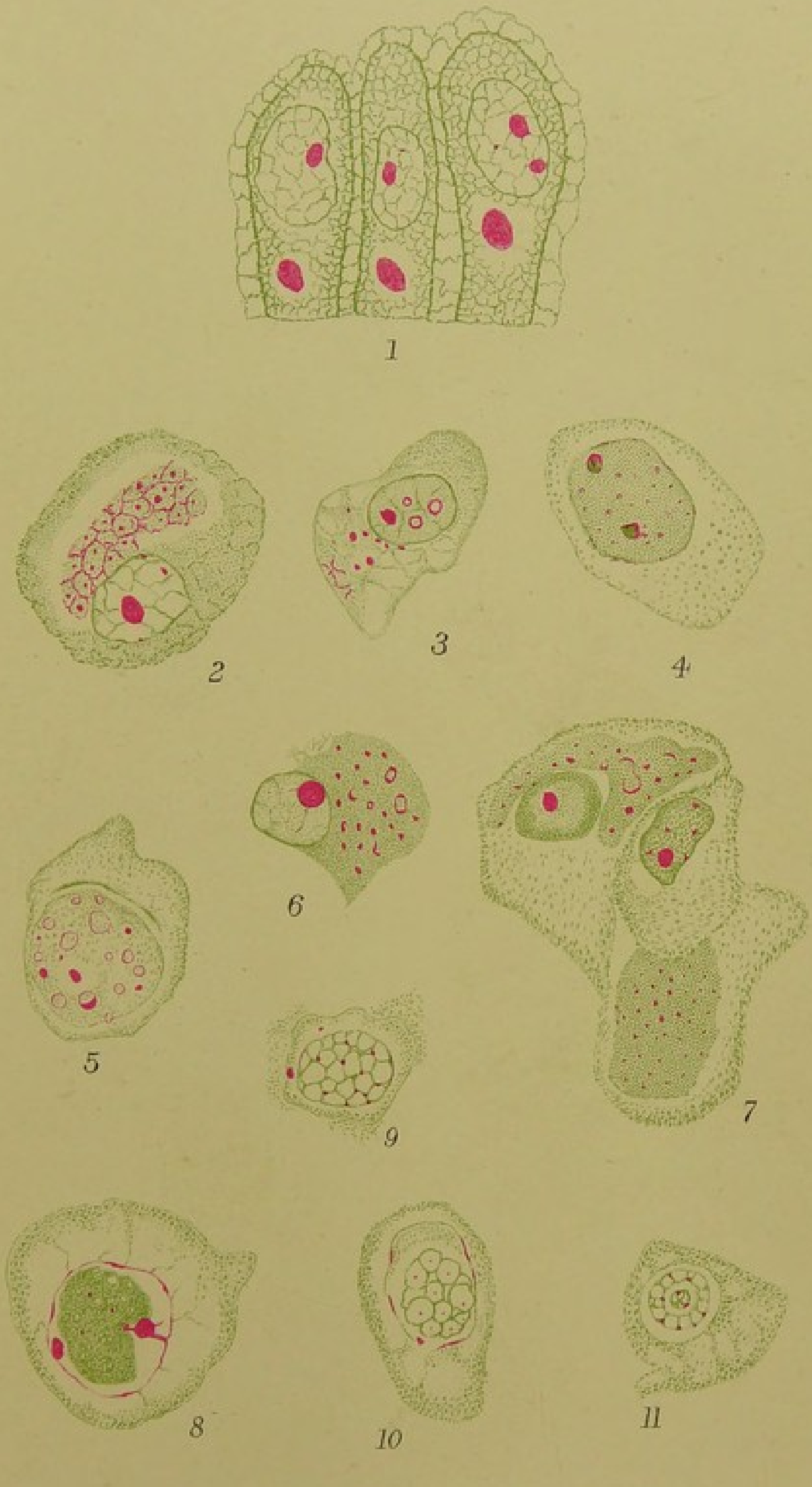

Magrath and Brinckerhoff. Cytoryctes variolae, Guarn.

DESCRIPTION OF PLATES.

PLATE XXI.

FIG. 1. Primary inoculation, duration five days. Early stage of cytoplasmic cycle.

FIG. 2. Primary inoculation, duration five days. Cytoplasmic cycle. Ameboid form in gemmule formation.

FIG. 3. Primary inoculation, duration five days. Gemmules in protoplasm, and early forms of nuclear cycle in nucleus.

FIG. 4. Primary inoculation, duration five days. Pansporoblast.

FIG. 5. Primary inoculation, duration six days. Nucleus containing three sporoblasts in different stages of development.

FIG. 6. Primary inoculation, duration five days. Sporoblast. Note minute point in chambers.

FIG. 7. Primary inoculation, duration six days. Sporoblast within nucleus.

FIG. 8. Primary inoculation, duration eight days. Nuclei containing sporoblasts.

All photographed at one thousand diameters.

PLATE XXII.

FIG. 1. Exanthem, duration two days. Epithelial cells containing cytoplasmic forms.

FIG. 2. Primary inoculation, duration five days. Cytoplasmic cycle, gemmule formation.

FIG. 3. Primary inoculation, duration five days. Drawing of cell represented in Plate XXI., Fig. 3.

FIG. 4. Primary inoculation, duration five days. Early stage of pansporoblast.

FIG. 5. Primary inoculation, duration five days. Pansporoblast. Drawn from same cell as that photographed for Plate XXI., Fig. 4.

FIGS. 6 and 7. Primary inoculation, duration five days. Bodies in cytoplasm similar to pansporoblasts.

FIG. 8. Primary inoculation, duration five days. Homogeneous body within nucleus, showing sporoblast-like structures.

FIGS. 9 and 11. Primary inoculation, duration six days. Intranuclear sporoblasts.

FIG. 10. Primary inoculation, duration five days. Intranuclear sporoblast. Note position of red dots with relation to septa.

THE ETIOLOGY AND PATHOLOGY OF VACCINIA.

E. E. Tyzzer.

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CONTENTS.

I. INTRODUCTION.

In the more recent investigations concerning the etiology of small-pox and vaccinia, a great deal of attention has been directed to the specific inclusions present in the epithelial cells in the lesions and commonly known as "vaccine bodies." Guarnieri was the first to claim that they were intracellular parasites, and he named them Cytoryctes variolæ or C. vacciniæ. Most investigators have confirmed Guarnieri's work and consider these bodies as organisms; others believe them to be cell degenerations specific to the disease; and still others deny their specificity and claim to produce bodies identical with them apart from the disease.

Among the most important monographs published since Guarnieri's first observations are those of Hückel and of Wasielewski. Hückel describes a number of cell inclusions found in the vaccination lesion of the rabbit's cornea, all of which he believes are derived from the substance of the epithelial cells. Wasielewski carried out a long series of experimental vaccinations, in which he finds intracellular bodies of a specific type constantly associated with the lesions. He finds strong evidence in support of the parasitic nature of these bodies and his observations agree in the main with those of Guarnieri. Inasmuch as these bodies are found both in variola and in vaccinia, and as the two forms of disease are closely related, a complete review of the literature concerning the etiology of both will be found in another chapter.

In the present investigation various animals were inoculated experimentally, in order to obtain lesions for study at various stages in the process. The purpose of these experiments was not only to make a careful study of the tissue changes following the inoculation of vaccine and variola lymphs, but also to obtain, if possible, further information concerning the infectious agent of the disease. In the study of the lesions the constant presence of the specific inclusions described by Guarnieri demanded special attention. As the result of an extended study of these bodies in the vaccine lesions of different species of animals and in various epithelial tissues in each species, I find that certain possibilities concerning their origin may be excluded, and that it is possible to demonstrate certain constant and characteristic morphological details in their structure.

II. MATERIAL.

Most of the experimental inoculations were made on rabbits' corneas, but other tissues and other animals were also used. Every stage of the lesions produced was obtained by killing the animals at various times after inoculation. The number and variety of the animals used, and the situations in which they were inoculated, are as follows:

1. Twenty-five rabbits successfully inoculated on the cornea with vaccine lymph.

2. Twenty rabbits successfully inoculated on the cornea with variola lymph.

3. Five rabbits inoculated on the cornea with fresh blood from small-pox patients. No characteristic lesions resulted.

4. Two rabbits inoculated in the testicles with active vaccine lymph. The resulting lesions were of an acute inflammatory nature and in no way specific, so that the testicular tissue was not considered favorable for further experimental inoculations.

5. Ten calves inoculated with vaccine lymph on both corneas. Five of the calves were also inoculated on the skin of the flank. The other five received multiple inoculations in places where the tissue was considered more favorable for histological purposes, such as the edge of the eyelid, the edge of the lip, the inner surface of the ear, and the udders. The mucous membrane of the vestibule of the nose was found to furnish especially valuable lesions.

6. One calf inoculated in the testicle with active vaccine virus; no characteristic lesion resulted.

7. Two adult cows inoculated in the skin with vaccine lymph.

8. Two sheep and several guinea-pigs inoculated with negative results.

The study of positive vaccine lesions constituted only a portion of this investigation. Control work was considered equally important. This consisted of the following and far exceeded the amount of vaccine material.

1. Corneas infected with the common varieties of pyogenic bacteria.

2. Corneas injured by various chemical substances. The material under both of these headings was furnished by the numerous experiments performed by Dr. Councilman in his study of the cells concerned in acute inflammation of the cornea.

3. Corneas inoculated with vaccine and variola lymphs known to be inactive.

4. Two corneas frozen several times with ethyl chloride.

5. Several corneas inoculated with diphtheria toxin.

6. Bovine skin containing infected hair follicles and pus tules produced by bacteria.

7. Lesions of foot and mouth disease in cattle.

8. Various forms of known intracellular parasites and the cell changes produced by them; namely, Coccidium oviforme in the liver and intestine of the rabbit; Klossiella muris in the kidney of the mouse; and a coccidium infecting the kidney of the guinea-pig.

III. TECHNIC.

1. The technic of inoculation is simple. In all cases the corneal inoculation was done either under local anesthesia by cocaine, or under general anesthesia by chloroform. A few drops of a two per cent cocaine solution are sufficient for the corneal inoculation in the rabbit, but complete anesthesia is advantageous for the operation in the calf. Either one or several shallow incisions were made on the surface of the cornea with any sharp pointed instrument sterilized by heat

before each inoculation. The best results were obtained with an instrument which is commonly used for corneal paracentesis. Care must be used in regulating the depth of the incision in order to avoid puncturing the anterior chamber of the eye. The virus was placed upon the surface of the cornea and gently rubbed into the incision.

In rabbits the skin of the inner surface of the ear was chosen most frequently, because here, by closing the ear with surgeon's adhesive plaster, the lesion may be protected from accidental trauma. There is also less hair in this situation than on other parts of the skin, so that less difficulty is experienced in cutting sections of the tissue for histological study. In all cases the ear was scrubbed with soap and water, rinsed with sterile water, afterwards with alcohol, and finally with ether and alcohol. As soon as the surface was thoroughly dry, scarification was done with a sterile instrument and the virus was rubbed in. In some cases a single incision was made instead of more extensive scarification.

In the inoculation of calves and cows areas of skin to be inoculated were not cleansed unless they were markedly dirty. In several calves the skin over the whole flank was shaved and afterwards vaccinated at several points. As it was found almost impossible to section this skin on account of the large amount of hair it contained, other sites were chosen in subsequent inoculations.

2. In the fixation of the tissues for histological study several methods were employed, but, as Zenker's fluid gave the best results, this was chiefly used. The tissues were embedded in paraffin and most of the sections were cut five microns in thickness. Much thinner sections ($2\ \mu$) could be obtained of the cornea. Sections cut across the inoculation incision in a plane vertical to the surface of the cornea afforded the best picture of the process, but sections made as nearly as possible in the plane of the surface furnished the greater number of vaccine bodies for comparative study.

A variety of staining methods was used, alum hematoxylin and eosin, eosin and methylene blue, Mallory's chloride of iron hematoxylin, carbol-fuchsin counterstained with blue, Mallory's connective tissue stain, phosphotungstic hematoxylin, Borrel's stain, and others. Although these different methods were valuable in determining the microchemical reactions of the specific inclusions, the eosin and methylene blue stain furnished by far the best results and brought out with great clearness all structural details. By the Borrel's stain the vaccine bodies were colored a deep red by the magenta and ordinarily appeared homogeneous. In sections stained by Weigert's modification of Gram's method the vaccine bodies retain the stain. They stain deeply with iron hematoxylin, and retain the stain when the nuclei of the tissue are almost completely decolorized.

IV. THE CORNEA.

1. *The Normal Histology of the Cornea.* — The epithelium of the cornea possesses certain properties which make it of great value for the experimental study of vaccinia. It is less liable to accidental bacterial infection than is the skin. The simplicity of its structure aids greatly in the study of the lesions. This fact is quite obvious, when its uniform epithelium is compared with the epidermis, with its topographical variations, its pigment, its many layers, and its special structures, such as the sweat glands, the hair follicles, and the like. Furthermore, the corneal epithelium is very delicate, being in the rabbit less than one-twentieth of a millimeter in thickness, so that it is fixed with great rapidity and lends itself to the exact technic so essential in the morphological study of the vaccine bodies.

Before taking up the inoculations it may be well to describe in brief those structures of the cornea which are more immediately concerned in this study.

The external surface of the cornea is clothed with an epithelium, but it consists chiefly of fibrous tissue which differs from that of the sclera in that it is more compact and quite transparent. Any departure from the normal condition produces opacity in some degree. A slight opacity may be instantly produced by pressure on the cornea with a needle point.

When the tension is removed, the opacity vanishes as quickly as produced.

The surface epithelium is of the stratified squamous variety; the deepest layer consists of short columnar cells. Outside this layer the cells are polygonal, and become more and more flattened as the surface is approached. Some authors divide this epithelium arbitrarily into four layers, but there does not appear to be sufficient grounds for such distinction.

The corneal epithelium in the rabbit is about thirty microns in thickness, but in the calf is much thicker. In the stained sections it is everywhere uniform except at the edge where it may be locally either thicker or thinner than elsewhere. It merges gradually into the epithelium of the conjunctiva, from which it differs in the following respects: The latter is much thinner, and contains cells which are distended with secretion and which become more numerous as the fornix is approached. The conjunctival epithelium is supported by a layer of loose connective tissue which allows for free movement of the eye. In the fixed specimens it is often thrown into folds.

On account of the importance of the specific cell inclusions which are found in the epithelial cells after the inoculation of vaccine lymph, it seems desirable to describe at length certain structural details found in the fixed and stained specimens of the cornea. Some of these structures are unquestionably due to artifact and others form a part of the normal structure.

A cement substance is demonstrable between the epithelial cells when treated with silver nitrate. Intercellular bridges are not conspicuous in the stained specimens except in edematous corneas where they stand out with great prominence. They are colored red in the eosin-stained sections and seem to be more closely related to the cell membrane than to the cytoplasm, which has a purplish tint. Epithelium examined in normal salt solution shows the protoplasmic bridges and the cell outlines with great clearness.

Some authors hold that there are minute branching spaces between the cells of the corneal epithelium. Others state to the contrary that this epithelium possesses no such spaces. Such spaces were found in practically all the corneas studied in this investigation. They apparently branch irregularly about cells and are regularly distributed in the deepest layer of the epithelium, where they indent adjacent cells and resemble not a little the bile capillaries in sections of the liver.

In the corneal epithelium of the calf the chromatin is generally collected into one or several irregular masses or karysomes. In the rabbit's cornea the karysomes are not so prominent. It is to be noted, however, that there is considerable variation in the arrangement of the chromatin in different individuals of the same species.

The nuclei of the corneal epithelial cells are often separated by a narrow space from the cytoplasm on all sides. This interval, sometimes designated the perinuclear space, is more apparent in the deeper layers than near the surface where the cells are flattened. It is not found under all conditions of technic, so that it is evident that it is produced by the unequal shrinkage of the nucleus and cytoplasm in the process of fixation and hardening. Delicate threads often extend from the nuclear membrane to the cytoplasm. In some cases they are so numerous and so regular in arrangement that a radiate appearance is produced.

The cytoplasm of the epithelial cells presents no distinctive characteristics. It is dense and stains a purplish tint when either hematoxylin and eosin or eosin and methylene blue are used. As the cells age they are pushed towards the surface and become flattened, the cell outlines are more distinct, and the cytoplasm becomes condensed and shows less structure. Cells near the surface in the calf's cornea are so extremely thin that their outlines are represented by lines running almost parallel, so that this part of the epithelium has a fibrillar appearance.

In the perinuclear space at opposite poles of the nucleus there are often found masses of irregular or triangular form more deeply stained than the cytoplasm and composed of an indefinite granular or reticular material. They are more marked in preparations which show the greatest degree of nuclear shrinkage, and appear most frequently in the cells of the innermost layer of the corneal epithelium. In these cells there appear two ill-defined, deeply stained, granular masses situated in the cytoplasm at either end of the nucleus, at times protruding into the perinuclear space. They are flattened on the side which is in apposition to the nuclear membrane. As a result of this flattening the mass which lies at the lower pole of the nucleus, and which is usually larger and somewhat elongated, often presents a triangular outline. This structural peculiarity is more marked in some corneas than in others, and was found in its greatest degree in two cases in which the tissue was not placed in the fixing fluid until several hours had elapsed post-mortem, so that it probably represents an artifact. The deeply stained masses above described agree closely with certain bodies described and pictured by Hückel under "Körperchen von dreieckiger, beziehungsweise pyramidenförmiger Gestalt." (Ziegler's Beiträge zur Pathologischen Anat. u. Path. Supplement, Vol. xxv, 1898, Taf. ii, Fig. 84.) Notwithstanding the fact that the method employed by Hückel was not used in this investigation, the triangular outline of the masses, their location at the ends of the nuclei, and their prominence in the deepest layers of the corneal epithelium seem sufficient to identify them with his bodies, which vary from "triangular to pyramidal form." Hückel endeavors to show that these triangular bodies bear a definite relation to the other series of bodies described by him, and, in his consideration of all the cell inclusions found in the vaccinated cornea, he places these with that group which are specific to vaccinia. Inasmuch as I have been unable to trace any forms intermediate between these "triangular forms" and those inclusions which are constantly associated with vaccinia, it does not appear to me that they are in any way related to the specific inclusions. As good examples were found in corneas which had not been vaccinated.

In every cornea examined small masses measuring from two to five microns in diameter were found in the epithelium. They were observed in the rabbit, the guinea-pig, the calf, and the sheep. The size varied somewhat with the species, but in a given animal it was quite constant. In sections stained in the ordinary way, the outline of these masses is generally wavy and indistinct, but they may be stained very deeply with iron hematoxylin. They then stand out with great prominence and are quite evenly distributed over the entire corneal epithelium, so that they need never be mistaken for the specific vaccine inclusions. They are usually situated between the epithelial cells, but they may indent the cells to such an extent in certain cases that they appear to be within the cytoplasm. They often occur in pairs and are rather more distinct in the deeper layers of the epithelium than near the surface. In the sheep these masses appear to consist of an aggregation of minute granules. In the calf they are relatively large and seem to be increased in size in some of the lesions produced experimentally. They are brought out with various stains, and the central portion is often colored more intensely. With Mallory's connective-tissue stain they are colored blue like certain of the intercellular substances. The significance of these bodies was not for some time understood. The thought suggested itself that they might in some way be related to the nerve endings of the cornea, and the special technic for the demonstration of nerve endings was applied.

By the gold chloride method delicate nerve fibers were found ramifying through the whole thickness of the corneal epithelium. There is a plexus of anastomosing fibers in the deep layer of the epithelium a short distance from the basement membrane, and from this more delicate fibers extend toward the surface. At intervals along the fibers there are bodies corresponding in size, shape, and distribution to the masses observed in sections stained by ordinary methods. Furthermore gold chloride sections stained with strong eosin presented no masses other than those along the nerve fibers. It seemed important to determine the exact nature of these bodies as they form a part of the microscopical picture of the corneal epithelium in both normal and pathological conditions. It is remarkable that these rather important structures should have been overlooked in the discussions as to nature of the vaccine inclusions. Perhaps their nature as normal constituents of the tissue was so obvious that description was deemed unnecessary. They have been investigated thus at length in order to determine if they stood in any possible relation to the specific vaccine bodies. Their distribution and character is such that there is no necessity of confusing them with the vaccine bodies, and they are not in any way related to the latter.

2. *Injury and Repair of the Cornea.* — Following a shallow incision of the cornea with a sterile instrument, repair is rapid. In the rabbit there is no increased injection of the circumcorneal vessels, and after the lapse of sixteen to twenty-four hours the corneal surface is smooth, so that it is often difficult to make out the line of injury. Sections of such a cornea show the defect of the corneal substance to be entirely filled with epithelium, which may be likened to a thin wedge filling the deficiency so that the corneal surface is smooth. In the healing of a wound of this sort there is at no time any undue proliferation, so that the epithelium never exceeds its normal thickness. Wasielewski calls attention to the fact that the defect is at first filled by the growing in of the epithelium from the area about the wound before sufficient time has elapsed for the multiplication of cells by division. That the defect is filled at the expense of the surrounding epithelium is seen in the thinning of the epithelium about the wound. After a relatively short time the epithelium regains its normal thickness through cell multiplication. A few leucocytes may be found at the site of such an injury, but they commonly occur in such small numbers that they may be entirely absent in given sections. Sections of a cornea a few days after such an incision show an increased number of corneal corpuscles immediately around the lesion. This increase is produced by a multiplication of the corneal corpuscles in the adjoining tissue. New fibrillæ of connective tissue are produced by these cells, so that there is formed a network of delicate crinkly fibers which extend mostly in a

direction parallel to the corneal surface. With the increase of these cells and their fibrillæ, the epithelial wedge which fills the incision gradually recedes, until it is finally replaced by the newly formed connective tissue.

In those instances in which either pyogenic bacteria or irritating substances have been introduced into the corneal tissue, we find a destructive process accompanied by infiltration with polynuclear leucocytes. Councilman has demonstrated the time occurrence of the various leucocytes in these inflammations of the cornea, as well as the course of their migration. It is quite clear that the larger number of leucocytes migrate from the conjunctival vessels into the conjunctival sac, and make their way thence into the injured portion of the cornea. Migration towards the injury by way of the corneal tissue means, in reality, a slower route, and it has been noted that leucocytes, making their way into the edge of the cornea, are commonly directed towards the corneal surface. In such inflammations of the cornea there is injection of the circumcorneal vessels, and usually a mucopurulent discharge. There may be marked edema, due in some cases to the character of the irritant, in other cases due to mechanical influences, such as a break in the membrane of Descemet, followed by an escape of the aqueous humor into the corneal tissues. The process of repair may be delayed for varying periods by active destruction of cells, accompanied by extensive infiltration with leucocytes.

V. THE INOCULATIONS OF RABBITS.

1. *Viruses.*

In the majority of the corneal vaccinations the commercial glycerinized virus was employed. As this is kept on sale for varying periods of time, and not always under the best of conditions, the lesions vary in degree much more than when a set of inoculations is done with fresh active virus. Corneas inoculated with fresh human lymph yielded lesions containing the specific vaccine inclusions in great numbers. Lesions produced by the inoculation of fresh lymph obtained from early small-pox vesicles were remarkable for their constancy of type. Variola lymph, obtained by shaving large numbers of the vesicles from a cadaver, was glycerinized and then used for inoculation. This, as well as smaller amounts of lymph obtained from the living patient and immediately glycerinized, gave constantly negative results. Great care was exercised in the preparation of this lymph, and equal parts of pure glycerine and sterile salt solution were used to dilute the fresh pulp. The mixture was kept in a cool place for a few days and used for inoculation. In all the corneas thus inoculated, the lesions appeared identical in all respects with those of aseptic wounds.

In several instances rabbits' corneas were inoculated with blood from cases of hemorrhagic small-pox. In one case the blood was drawn from the ear during life, in the other cases the heart's blood was taken a short time post-mortem. The corneas presented no process other than occurs in the repair of an aseptic wound. The corneas inoculated with small-pox blood, as well as those inoculated with glycernized variola lymph, gave in each case negative results. The corneal lesions, which are to be considered in greater detail, are those produced by the inoculation of vaccine lymph and those produced by the inoculation of fresh variola virus.

2. *The Lesions of the Cornea.*

A *Vaccination.*— Rabbits were killed at intervals of four, eight, sixteen, twenty-one, twenty-four, and twenty-seven hours, and on consecutive days (from two to eleven days) after vaccination of the cornea, and the lesions were studied in each case. As the process seemed most marked in the corneas at three, four, and five days after inoculation, a larger number of the lesions were taken at these intervals.

Immediately following the inoculation of the rabbit's cornea with vaccine lymph, the gross changes may be so slight that it is difficult to detect them. The incision or wound made by the needle is discernible to the unaided eye up to sixteen or twenty-four hours. Within thirty-six hours, however, the wound becomes entirely filled and its rough edges are obliterated, so that the surface of the cornea becomes normally smooth. All that can be seen at this stage is a faint, grayish line along the original inoculation. The incision at this stage has healed in practically the same manner as a sterile wound. Unless the cornea has become infected with bacteria, there is no injection of the circumcorneal vessels, and the scleral portion of the eye retains its normal color.

In corneas that have become infected with bacteria there is usually marked injection of the circumcorneal vessels, and this condition may be pronounced on the day following inoculation. The non-injection of the circumcorneal vessels on the day following inoculation is, therefore, a valuable sign that bacteria have been excluded from the inoculation. The line of the incision is marked by opacity, usually very slight, but dependent on the depth and character of the wound. If examined with great care, slight cloudiness of that part of the cornea adjoining the incision may be distinguished.

On the second day after a successful vaccination it is usually possible to make out slight unevenness, with elevation of the corneal surface at the site of the incision. A hand lens aids greatly in determining the finer changes affecting the corneal surface.

By the simple process of tearing up the fibrous tissue, it is possible to produce elevations of the surface of the cornea, which will not be eradicated in forty-eight hours, and which at that time may simulate closely the condition found in vaccination. If, on the other hand, care is taken to make uniform wounds, it is possible in most cases to make a positive diagnosis of vaccination from the gross appearances alone. There are, however, some cases in which the foci of the process are so small that they cannot be made out.

After the second day the uncomplicated vaccination is accompanied by a slight degree of circumcorneal congestion, but this never approaches the condition found in those cases in which bacterial infection is present. Wasielewski found that the epithelium of the affected area is separated more readily from the cornea than the normal. When the vaccinated cornea is scraped, in order to obtain virus with which to inoculate other animals, this property is quite evident. The normal epithelium seems tough, and is not removed by scraping, whereas the affected portion comes off with little effort, and forms a soft, juicy mass.

The histological study gives us more reliable data concerning the nature of the lesions. The changes following vaccination of the cornea with commercial glycerinized lymph do not always take place at a uniform rate. Some are as far advanced in two days as others are in three days after inoculation. Several factors may influence the development of the lesion, and the probable variation in the dose of virus actually inoculated is to be considered in this relation. A clear conception of the process is to be attained only through the study of a great many lesions. The stages of the process will be taken up consecutively in a description of the lesions obtained at various periods after inoculation.

The corneas of rabbits killed four hours and eight hours after vaccination show a break in the corneal tissue, which is not yet filled in with epithelium. In the eight-hour lesion the epithelium is found extending just over the edge of the incision. The corneal tissue about the injury is pale and glassy, and the fibers are swollen. Small numbers of leucocytes are present in the injury, and some have already migrated into the corneal tissue. The number of leucocytes is, however, so small that they may be absent in several consecutive sections. The lesions taken at four and eight hours after vaccination differ in no respect from simple injuries. There are but few mitoses present.

In the lesion taken sixteen hours after vaccination, the epithelium has filled in the defect in some cases partially, in some cases completely, in this respect resembling the simple incision. About the injury the epithelium is thinner than normal. The leucocytes are no more numerous than in the four and eight hour lesions. They are for the most part well preserved. In the epithelial cells filling the incision are a few of the cell inclusions, which may be regarded as specific to this disease. They are deeply stained bodies varying in size from one to five microns, and are usually situated near the nucleus. In describing the process it will be necessary to note the presence and distribution of these bodies in the lesion, but a full account of them will be given further on in this paper. From a study of the sixteen-hour lesion it is thus found that it differs from the simple incision in one respect, namely, the appearance of the specific cell inclusions.

In corneas fixed twenty-one and twenty-seven hours after inoculation a condition prevails similar to that which has been described in the sixteen-hour lesion (Plate XXIII., Fig. 4). The epithelium filling the incision is similar to that which fills an aseptic wound, except that the specific cell inclusions are present. These were more numerous in the twenty-seven-hour than in the earlier lesions.

Corneas fifty hours after inoculation present a process which may be considered typical. The most prominent features of the lesion are the large number of vaccine bodies and the proliferation of the epithelium (Plate XXIII., Figs. 1 and 2). The vaccine bodies are most numerous in the epithelial cells filling the incision, but are also quite numerous in the epithelium for some distance about the line of inoculation. They are rarely found in the corneal corpuscles and only in those in immediate relation to the lesion. The epithelial cells lying within the incision are larger than the normal epithelial cells of the cornea and have a glassy appearance, a condition which is more or less characteristic. They may fill the incision compactly or they may be dissassociated and lie free. It is noticeable here as well as in later lesions that the protoplasmic bridges between epithelial cells are more prominent than normal on account of the separation of the cells with fluid.

In several instances serial sections of the lesion demonstrated areas of epithelium which were degenerated and very thin, consisting of scarcely more than a single layer of cells, many of which contained vaccine bodies. Outside of this area the epithelium was several times its normal thickness. It seems questionable whether or not these limited areas of marked degeneration are due to the effect of the virus alone.

Although such areas were present in several vaccine lesions, others were met with in which they were entirely absent, so that it seems probable that some factor other than the direct action of the vaccine infection is concerned in their production. Such conditions were found in corneal injury apart from vaccination.

Epithelial cells are found here and there included within other epithelial cells. In a few instances epithelial cells are found which are markedly degenerated, with nucleus shrunken and irregular. Instances of direct nuclear division are met with. Portions of the protoplasm in some cells stain deeply by certain methods, so that a "flecked" appearance of the cytoplasm is not uncommon. This seems to be a definite change affecting scattered cells. Mitoses are numerous about the lesion and the epithelium is here two or three times the normal thickness. The leucocytes are not increased in number as compared with the previous stages and are often fragmented.

At the end of three days the proliferation of the epithelium about the incision is more marked and the vaccine bodies are found for a greater distance away from the incision.

In corneas five and six days after vaccination the epithelium is four or five times the normal thickness. The vaccine bodies are not so numerous as in the two or three day lesions. At this stage a great many epithelial cells are found included within other epithelial cells. Both the included and the including cell may appear normal, or one or both may be degenerated. The included cell may be represented by a pale hyaline mass, or this mass may contain deeply stained material at its center. Other included cells are so large that portions of their cytoplasm appear in a given section without any nucleus. When it is possible to orient such a cell, the remainder of the cell with its nucleus may be found in the next section of the series. Portions of such included cells often contain vaccine bodies and have been supposed by some to represent a large form of organism. Other types of inclusions are found. A normal cell may be surrounded by chromatic material derived from a fragmented nucleus, or the nucleus of the surrounding cell may have undergone direct division in degenerating, so that the included cell is surrounded by pale, irregular nuclei. The whole in turn may be included in a large cell. The inclusion of epithelial cells met with in the vaccinated cornea is to be accounted for by the activity of the epithelial proliferation, — the epithelial cells in the one case pushing into the less resistant cells and gradually filling the space occupied by them; in the other case surrounding cells which are probably more resistant.

The most constant change affecting the epithelial cells in corneal vaccinations is an increase in size. These swollen cells stain lightly and they present a variety of forms and are more rounded than the normal cells. This swelling of the cells is most marked in the inoculation incision where the specific cell inclusions are most numerous. It forms one of the characteristic features of the vaccine lesion of the cornea. Although scattered cells are found which are more markedly degenerated, the disintegration of cells does not form a prominent feature of the process at this stage in the rabbit's cornea. It is to be presumed that so great is the activity of the proliferation that individual cells becoming degenerated are, at this stage of the process, so quickly replaced by other cells that only scattered examples of them are found. The leucocytes are present in some preparations in considerable numbers, but are few in others. A large proportion of them are fragmented and degenerating. They take no part in the essential process, and their number is dependent on the amount and character of the injury brought about by the original wound of inoculation or on the occurrence of accidental bacterial infection.

Corneas seven days after vaccination differ from the six-day corneas only in the small number of vaccine bodies present. These inclusions are confined to small areas of the epithelium. In one seven-day lesion there was marked proliferation of the epithelium, but a great many sections were searched before vaccine bodies were found. It is observed that in the older lesions defects in the surface of the epithelium are found generally, but not always, at the site of the original wound. These defects may be so extensive as to amount to ulceration. They appear to be due to the degeneration and loosening of individual cells and are always associated with numbers of vaccine bodies.

In the older lesions taken on the ninth and eleventh days after vaccination, vaccine bodies are absent in some corneas, but are present in considerable numbers in small foci in others. The epithelial surface is modified in various ways. Defects in the surface, such as were just described in the seven-day lesion, are present. In some of these defects the epithelial cells are degenerated and disassociated, in others the proliferative changes are more marked and there are rounded or irregular processes of epithelium directed toward the surface. Several long, slender processes consisting of epithelium are found protruding from the surface of the cornea. One was situated near a focus of inflammation at the corneal edge entirely apart from the vaccine lesion. It consisted of two layers of epithelial cells and was infiltrated with leucocytes. It can be traced through a series of sections, so that it appeared to be of the nature of a thin membranous process. These epithelial processes are described by Wasielewski in connection with vaccine lesions of the cornea. The degeneration of the epithelium is much more marked in these far advanced lesions. The structure of the cytoplasm appears as a coarse reticulum, in the meshes of which are clear spaces. The chromatin of the nuclei is gathered into coarse meshes and the nuclear membrane is shrunken and irregular. This vacuolar degeneration of cells seems to bear a definite relation to the tissue-fluid, and is most marked in those cases where the cells are separated by fluid. The epithelium presents spaces in its substance where single cells or groups of cells have disappeared. Such spaces may be of considerable size and present a slight resemblance to vesicles. Empty vacuoles are also present within cells.

Summary.— The main points of the vaccination process in the rabbit's cornea are the following: The corneal injury at first heals in a manner comparable to an aseptic wound. The epithelium grows in from the sides, and fills the defect before there is time for any appreciable increase in the epithelium by growth and mitosis. Thus in the lesions up to twenty-four hours there is a thinning of the epithelium surrounding the wound. By forty-eight hours the epithelium in the vicinity of the inoculation is thickened. This thickening continues for several days, and the epithelium may be five times its normal thickness. The change is due, in part, to a numerical increase in the cells, and in part to an increase in their size. This proliferation, taken together with increase in the size of individual cells, presents a change which, so far as is known, is specific to vaccine inoculations. Wasielewski considers the increase in size as a phenomenon of parasitism, and believes that the host cell is stimulated to increased metabolic activity, in order to supply the needs of the parasite. When it is considered that the parasite must of necessity be of relatively small size, one finds it difficult to understand the extraordinary demands made upon the host cell for the sake of the nutrition of the parasite. In the case of many of the familiar intracellular parasites the expansion of the cell stands in definite relation to the size of the parasite. In the vaccine lesions the swelling of the epithelial cells may be found over such a wide area that it seems more probable that it is due to the action of some toxic substance rather than to the effect of an organism upon its harboring cell. The swelling of the cells does not stand in any constant relation to the amount of tissue-fluid, for edematous corneas were studied in which the epithelial cells were separated by fluid, and yet had undergone no such change. The instances of marked degeneration in these cells are so numerous, especially in the later stages of the lesion, that I have been led to consider the swelling of the cell as a degenerative change. The inclusion of cell within cell, as well as the proliferative changes, gives evidence of the disturbed equilibrium of cell growth. It is evident that the cells of the epithelium are not passing through their normal life changes. Cells are formed more rapidly than they are disposed of. Instead of being regenerated from below, and cast off at the surface, their growth is irregular. Swelling cells press upon the neighboring cells, and distort them. If a cell is somewhat degenerated, it then forms a weak spot in the epithelium, and is immediately invaded by another cell. Another manner in which cell inclusion takes place is through the growth of epithelial cells around cells which are degenerated, but which are too dense to be invaded.

The specific cell inclusions were found in a lesion sixteen hours after the inoculation. Wasielewski found several two and one-half hours after inoculation, and shows a photograph of the same. These inclusions increase, and become most numerous on the third or fourth day. They become less numerous in the older lesions, and are present in small numbers in some, and are absent in others. They were found in lesions eleven days after inoculation. According to Wasielewski, they may be present in small foci as late as thirty days after vaccination. Leucocytes are associated with the lesions in variable numbers, and are not essential to the process.

B. *Variolation.* — In ten rabbits whose corneas were inoculated with fresh variola lymph the typical process was found in one or both corneas of each (Plate XXIII., Figs. 6 and 7). The epithelium scraped from certain of these lesions was used to inoculate the corneas of four other rabbits. Of these, one gave no reaction in either cornea, another rabbit showed epithelial proliferation in one cornea, but no vaccine bodies in the sections studied, while the corneas of the two remaining rabbits showed both epithelial proliferation and vaccine bodies. This demonstrates that the virus obtained from a variolated cornea will produce a lesion identical with that produced by vaccine virus when inoculated upon another animal.

Virus was taken from a lesion of variola inoculata of an ape and inoculated upon a rabbit's cornea.[1] The development

[1] A more extended account of these experiments is to be found in Dr. Brinckerhoff's article.

of a lesion upon this cornea could be determined from the gross appearances. A portion of the lesion was taken to inoculate another rabbit's cornea, and a portion fixed and sectioned for study. In this manner eye to eye inoculations were made in a series of five rabbits with the virus derived from the ape.

A scraping from the cornea of the last rabbit of the series was used to inoculate another ape. This was successful, and virus from primary inoculation, as well as from vesicles appearing on the face of this animal, gave typical lesions when inoculated on rabbits' corneas. The juice of the axillary lymph node of the inoculated ape also produced a typical corneal lesion. A comparative study was made of the corneas in each rabbit of the above series. The type of the lesion is constant throughout the series. The process is identical with that found in corneas where vaccine lymph was inoculated. The proliferation, the swelling of the epithelial cells, the inclusion of cell within cell, and the tendency of the proliferated cells to degenerate were all in evidence. The specific cell inclusions were present in all the lesions, and after a careful study no characteristic was found by which they differed from those found in corneas inoculated with vaccine lymph. The constancy of the lesions is probably to be explained on the ground that here, in every case, the virus inoculated was taken fresh from the animal.

3. *The Lesions of the Skin.*

A. *Vaccination.* — The internal surface of the ear furnished the most favorable site for inoculation. Of fourteen rabbits thus vaccinated, positive results were obtained in ten. In the other four no reaction could be obtained, although they were repeatedly vaccinated. In the positive vaccinations the process, though slight, was, nevertheless, of definite character. After the vaccination the lymph became dry over the scarification and formed a thin crust. This condition persisted for a few days, and in case the inoculation failed the crust dropped off, leaving a healed surface. In those inoculations which were regarded as "takes," a change in the character of the skin appeared after a period varying from four to eight days. The first indication of this change was a slight reddening accompanied by elevation of the surface about the crust. This portion of the epidermis appeared more opaque than that of the adjacent normal skin. If this part was carefully pricked with a needle a drop of lymph could be expressed. From the gross appearances, however, the lesion could not be considered as vesicular in type. For one or two or three days this process gradually extended, covering an area several times that of the original scarification. That there was intense itching at this time was apparent by the rabbit's incessant scratching at the inoculated ear. In those cases in which the bandage remained in position, the crust extended over the surface of the lesion and in the course of a few days dropped off, leaving a surface completely healed. Thus a slight but characteristic process follows the inoculation of the rabbit's skin with vaccine lymph. In no case was a process developed of the extent found in man. Concerning the four rabbits in which no reaction was apparent it is impossible to state with certainty whether there was a process so slight as to escape notice or whether these animals possessed a natural immunity to vaccinia. No instance of natural immunity to the corneal vaccination was found.

Histologically, the skin of the ear lesion of three days' duration appears swollen to two or three times its normal thickness. The blood vessels and lymphatics are dilated. The tissue spaces are increased, and there are numerous polynuclear leucocytes and lymphoid cells scattered throughout. The endothelium of some of the smaller vessels is proliferated, and the connective-tissue cells are increased in number. In the central portion of the lesion there is slight hemorrhage into the tissues. The actual loss of substance is, however, very superficial, involving little more than the epidermis. The surface of the lesion is covered in part by a thin crust composed of polynuclear leucocytes imbedded in a hyaline material, evidently fibrin, in part by the horny layer of the epidermis, which has undergone a change and appears swollen and hyaline. Beneath this the epithelium presents a lesion distinctly vesicular in type. Vacuolar degeneration of epithelial cells is constantly present. Earlier stages of the process are found near the border of the lesion. In the older parts of the lesion these spaces are distended with fluid, and certain portions of the lesion are generally filled with leucocytes. Delicate anastomosing strands, continuous with the remnants of epithelial cells, extend across the vesicle. The epithelium just outside the vesicle contains many typical vaccine bodies which are confined chiefly to the deeper layers. The epidermis about the lesion is considerably thickened, in part due to the increased size of the cells, in part also to their numerical increase. Mitotic figures are more common here than in the adjoining normal epidermis. Thus the lesion which develops in the skin is found microscopically to be distinctly vesicular in type, in which point it differs radically from the lesion in the cornea, where vacuolar degeneration is very slight or absent, and where there is no attempt at vesicle formation.

B. *Variolation.* — The inoculation of the skin with small-pox lymph gave negative results in the few cases in which it was done. A number of rabbits were inoculated with virus collected on ivory points and in capillary tubes. In one rabbit there developed a deep ulceration extending to the cartilage of the ear. The skin at the edge of the ulcer became thickened and the ulcer healed very slowly. If any process was present it was masked by the suppuration which took place. In smears of pus from the ulcer, streptococci were demonstrated. The skin of all other rabbits inoculated in the above manner gave negative results. Variola pustules were transplanted from a cadaver to scarified areas in the ears of four rabbits. In all of these there was considerable loss of substance at the point inoculated, and deep, " gouged out " ulcers were produced extending into the cartilage of the ear. In section of these nothing was found typical of the vaccination process. As the cornea had already proved such an excellent tissue for experimentation, the skin inoculation with variola virus was abandoned. It was considered that the principal element interfering with successful results was the constant bacterial infection.

VI. THE VACCINATION OF CALVES.

Two series of vaccination experiments were made, one in 1902, the other a year later in 1903.

In the first series of experiments three calves were vaccinated upon the corneas and upon the skin. For this purpose two strains of commercial glycerinized lymph derived from different vaccine establishments were employed. The calf's flank was shaved, and the virus of one company was used to vaccinate one area and the virus of the other company another area. As the result of this double inoculation, it was impossible to demonstrate a difference in the action of the two lymphs. A process appeared two days earlier from the inoculation of one virus than from the inoculation of the other. The internal surface of the ear was also vaccinated, but the lesions differ somewhat from those of the skin. Vaccine lesions were obtained in the corneas of two of these calves.

A series of experimental vaccinations in calves, undertaken one year subsequent to the above, was complicated by the occurrence of foot and mouth disease, as the result of which several of the younger calves died. This disease, however, did not interfere with the development of the vaccine lesions, and it served to furnish lesions which were valuable for control study relative to vaccinia. Seven calves were vaccinated in these experiments. Inoculations were made in a great many places, and the corneas, the mucous membrane of the nose, the lip, the edge of the eyelid, the inner surface of the ears, and the skin at the bases of the teats and under the root of the tail were all vaccinated. A fresh active virus was used in these experiments. At the end of forty-eight hours changes were observed at the point of inoculation, and the process was more extensive than that observed in the experiments of the preceding year. Thus by the use of different samples of virus it is possible to demonstrate a variation in the degree of the process as well as in the time of reaction.

1. *Lesions of the Cornea.* — The corneal epithelium of the calf is much thicker than that of the rabbit. The cells are slightly larger, but the chief difference is found in the prominence of the chromatin masses in the nuclei of the calf's epithelium. The lesions were not all of a constant type. The process following the inoculation of commercial lymph in the first series differed in some respects from that following the second series of inoculations made one year later. The lesions in the first series could not be distinguished during life. As soon, however, as the eyes were placed in Zenker's fluid there immediately appeared several minute, rounded, opaque areas about the inoculation incision. None of these areas exceeded two millimeters in diameter, and there was a very superficial defect in the surface in several. Some of these lesions had developed at the edge of the incision. Others were situated at some distance from it. Three corneas were successfully vaccinated with this virus, and one was taken two days, the others five days, after inoculation. The lesions in all of these cases are minute and evidently represent an early stage in the process. Many of these lesions prove of value for the study of vaccinia, in that they furnish a process confined to small foci of the epithelium and are not complicated by the presence of leucocytes or foreign cells of any sort. That they represent distinct foci and have no connection with the main incision of inoculation was determined by serial sections. It is probable that the virus, when rubbed on the cornea, entered not only the main incision, but at various points where the injury to the epithelium was slight.

In the lesion taken two days after the inoculation there was little save the presence of numerous vaccine bodies in a small focus of the epithelium. The majority of the vaccine bodies were small and somewhat elongated (Plate XXVI., Fig. 10). Many of them were situated between cells. The amount of cell degeneration was slight. Large multinuclear cells, resulting from direct nuclear division in epithelial cells, were present. A small number of cells present a shrinkage and distortion of the nuclei with an arrangement of the chromatin in coarse masses.

The five-day lesions consist of small foci of degenerated epithelium in which are situated numerous vaccine bodies (Plate XXV., Figs. 1 and 2). A single type of degeneration is present in varying degree throughout the entire lesion. The cytoplasm of the epithelial cells is vacuolated throughout and the vacuoles are separated by a coarse reticulum. Vaccine bodies are situated in the larger spaces of the cytoplasm lying next to the nucleus, which they often indent (Plate XXV., Fig. 4). The nuclei, in some instances, are vacuolated or coarsely reticular and the chromatin is collected in coarse masses, either at the nuclear membrane or at some point of the reticulum. In certain cells the nucleus is disintegrated on the side next a vaccine body, and portions of the nuclear material are scattered throughout the cell. Certain nuclei are shrunken and irregular. The cell walls appear to be denser and are but little affected, so that where the process is marked the cells appear hollow, and the epithelium, as a whole, resembles a honeycomb in structure. At the periphery of the lesion only minute vaccine bodies were found, while in its central portion there were many of large size. The underlying connective tissue was not involved in this lesion and there were no leucocytes present. The epithelium about the lesion was not thickened, but the number of mitoses was increased.

In the corneal lesions of the second series of calves, where a more active lymph was used, elevation of an area of the surface about the incision was apparent during life. When placed in Zenker's fluid, this area became immediately more opaque than the rest of the cornea. On section this area was found to consist of thickened epithelium, a condition which prevails in every cornea inoculated with this virus. Lesions were taken four, five, six, seven, and thirteen days after inoculation. The vaccine bodies were distributed over a wider area than was the case in the preceding series. The most marked process was not always found at the inoculation incision, but in certain instances was at a distance from it (Plate XXV., Fig. 6). At certain points there are defects in the epithelial surface produced by the separation and desquamation of degenerated cells. Around these defects the vaccine bodies are most numerous, although they may be distributed about at a considerable distance from such a focus. It is possible to distinguish in a few instances vaccine bodies lying in such a defect which are not enclosed within cells. Multinucleated epithelial cells are occasionally met with in sections. Where the epithelium is heaped up at the site of the lesion it may be differentiated into two layers (Plate XXV., Figs. 6 and 7): an outer layer, apparently dense, which consists of deeply stained, squamous cells; an inner layer which consists of large quadrilateral or rounded cells which stain lightly and appear pale and transparent. The dimensions which these cells may attain is illustrated by the cell represented in Plate XXVI. (Fig. 11), which is drawn at the same magnification as the other cells of the plate. The change seems to be of the nature of an increase in the fluid constituents of the cell rather than an increase in its denser structure. The nuclei are affected as well as the protoplasm, but in a minor degree.

The nuclei are full and rounded in every instance and, except for the reticulum and the chromatin masses, appear transparent. This change in the character of the epithelial cells resembles that seen in the epithelial cells in the corneal vaccination of the rabbit. The process, however, is present in a greater degree and is extended over a larger area. A few of these cells contain vaccine bodies, but there are extensive areas of such epithelium which contain none. The cells containing vaccine bodies differ in no way, except for the bodies, from the cells which do not contain them. It is evident that the process in this instance represents the action of some agent (perhaps toxic) which affects uniformly a large number of cells, rather than the action of an agent affecting individual cells in a varying degree. Whatever way this change is to be interpreted, it is certain that such epithelium is less resistant, as is seen by the association with it of marked degeneration of cells.

In the corneal lesions up to seven days a few cells were present which were markedly degenerated, but there was no marked disintegration of cells. In the lesion of thirteen days the degeneration and disintegration of the epithelial cells is present in a marked degree, and the tissues in the vicinity of the lesion are infiltrated with leucocytes (Plate XXIV., Fig. 3). At this stage, when degeneration is most marked, the vaccine bodies are found in small numbers or are absent.

Summary. — Vaccine bodies occur in every vaccine lesion of the calf's cornea Enlargement of the epithelial cells and a variable amount of cell degeneration, which becomes more marked in the older lesions, are always present. Local thickening of the epithelium takes place, except in those instances in which the process is confined to minute foci.

2. *Lesions of the Nasal Mucous Membrane.* — The inoculation was made upon the septum of the nose just within the opening of the nostril. The epithelium of this part is thick and appears rather dense in stained sections. The various layers seen in the epidermis are not represented in this epithelium. It consists of cells of a uniform type throughout the greater part of its thickness, but near the surface the cells become flattened and there is a thin, laminated layer, presumably horny in character. Numerous racemose glands with wide-mouthed excretory ducts are present in this locality.

The point vaccinated becomes, in the course of two or three days, opaque and elevated. This area extends slowly from day to day, but nothing of the nature of a vesicle is apparent. The histological appearances of the lesions of five days are of interest. The epithelium is changed in character throughout its entire depth. There are large, irregular spaces within the affected epithelium, filled chiefly with serous exudate which may be considered as rude vesicles. Irregular branching trabeculæ of epithelial cells project into and extend through these spaces. The type of exudation varies considerably. There is a dense mass of leucocytes at the surface of the lesion and smaller masses are situated in various localities. In one portion there are large masses of fibrin, in other places a network of fibrin invests the individual epithelial cells. The escape of the glandular secretion is prevented in some instances by the changes about the mouth of the duct, and this material is to be seen in the spaces of the epithelium. All these changes consist of exudative phenomena which vary according to the structural composition of the epithelium.

The primary factor in the process is the degeneration of the epithelial cells, and it may be present with or independent of the secondary exudation. The type of cell degeneration is uniform throughout the affected epithelium, although it presents itself in varying degree in different localities. The earliest change is an enlargement of the epithelial cell. The increase is chiefly in the cytoplasm, although the nuclei are also somewhat larger. Whereas the normal cells appear dense and are stained purplish, these cells are lighter and stain blue. As the cells swell, the cytoplasm becomes coarsely reticular in structure, resembling somewhat that of cells in mitosis. The cytoplasm often appears to be filled with small vacuoles. The nuclei are full and hollow, and the chromatin is arranged in large, coarse masses. When the degeneration is more advanced, one or more clear spaces are present within the cytoplasm. During this process the cells have become rounded, have lost their bridges, and the periphery of the cell is represented by a dense, thick membrane. Such changes taking place in the outer portion of the epithelium result in a structure resembling somewhat a honeycomb. The framework consists of the adherent, dense cell membranes, each occupied by a clear space in which is situated a shrivelled nucleus. In the lower portion of the epithelium the cells are disassociated and do not tend to develop such shell-like cell membranes. Here various changes affect the nuclei. Some consist of large vesicles in which the chromatin is found next the nuclear membrane. In most of the cells the chromatin is arranged in large, irregular masses.

The linin may be present as a reticulum or it may be shrunken away from the nuclear wall and appear as a red-stained mass entirely distinct from the chromatin.

Besides degeneration there is irregular proliferation. Mitoses are numerous at the base and at the edges of the lesion. A large proportion of those at the base are atypical. Multinucleated cells resulting from direct nuclear division are numerous. In some of the excretory ducts the proliferation has resulted in irregular masses and processes of epithelium. In others the destruction of the cells has produced a thinning of the epithelium of the duct.

Vaccine bodies are present throughout the lesion. They accompany cell degeneration both in the surface epithelium and in the excretory ducts of the glands, but they are not found in every degenerated cell. Many are found here which consist of a great number of smaller bodies (Plate XXVI., Figs, 18, 19, and 20). Cells undergoing mitosis occasionally contain them.

In this lesion of the nasal mucous membrane is found a process which may be considered as intermediate between the vaccine process of the cornea and that of the skin. Spaces in the epithelium filled with exudate appear to be related to the vesicles of the skin to be described further on. The primary factor of the process is the hydropic degeneration of the epithelial cells associated with which there is irregular proliferation.

3. *Lesions of the Skin.* — In the vaccination of different sites upon the skin, the flank proved most unsatisfactory for histological purposes, as it is so thickly beset with hair follicles that it is almost impossible to make proper sections of it. In lesions of the hairy parts a crust forms early and extends over the larger part of the lesion. In fact, at the site of the primary inoculation there is little to be seen except a crust, which extends from day to day and around which the epidermis is slightly elevated and grayish. When the skin has been shaved, a number of small lesions may occur at some distance from the inoculation wound. These appear

one or two days after the process is evident in the primary lesion. They are at first very minute papules, and are more apparent to the touch than to the eye. Histologically they present a process similar to that found in the primary lesion. They did not occur in those cases which were not shaved, so that it is evident that these secondary lesions are accounted for by the inoculation of slight injuries of the epidermis.

The vaccination of the inner surface of the ear never produced a lesion which appeared distinctly vesicular in the gross. There was on the third day elevation at the site of scarification. A thin, yellowish crust then appeared and extended from day to day. At the edge of this the epidermis was elevated, but contained no appreciable fluid. About one week after the vaccination the lesion ceased to extend, and within a few days the crust could be pulled off, leaving the skin very nearly healed.

In the vaccination of the teats the lesions were distinctly vesicular in type (Plate XXIV., Fig. 6). Here, too, a crust usually formed at the site of the inoculation. Surrounding this was a ring-like vesicle containing fluid which was clear in the early lesion, but puriform in the late stages. In certain animals an areola was present about the vesicle.

The lesions of the eyelids were similar to those of the teats. The lesion extended on the external surface of the lid, but did not extend upon the conjunctival surface. The meibomian glands were involved in the lid lesions.

The most typical vesicles were produced by inoculating the skin beneath the root of the tail (Plate XXIV., Fig. 2). The skin here possesses practically no hair, is protected from accidental trauma, and is much cleaner than one would expect. Here a large vesicle develops which extends and finally acquires a crust over its central portion.

There is one type of cell degeneration common to the lesions of all these different localities upon the skin. It consists of the swelling of cells through the imbibition of fluid such as was found in the lesion of the nose. The variation in type in these lesions is evidently due to the peculiar structural characteristics of the part in which they develop. Certain factors which influence the process of vesicle formation are the amount and character of the exudate, and the type of structure which the epidermis presents as regards the retention or escape of this exudate. Thus, over portions of the skin where the hair follicles are set close together it is impossible for any considerable area of the skin to be lifted up. As the result of this arrangement, the leucocytes penetrate to the surface of the epidermis and the exudation dries upon the surface forming a crust. The structure of the skin lining the ear probably possesses mechanical characteristics which prevent the accumulation of any large amount of fluid. In places upon the skin where there is little hair and a moderately marked horny layer, the exudate is retained in fluid form, from which results the development of a vesicle. Such lesions agree very closely with skin lesions of variola in the human subject, so that the description of the latter applies to this lesion in the calf. Here, too, there are slender trabeculæ of epithelial cells extending through the cavity of the vesicle. The outlets of the sebaceous glands are usually involved and vaccine bodies are present within the gland cells.

Attending the hydropic degeneration of cells there are special changes affecting individual cells, most of which have been described in the lesion of the nasal mucous membrane. Multinucleated cells are very numerous. Cells containing from five to ten nuclei in a single section are common. Such nuclei may apparently survive the destruction of the cytoplasm and several may be found in a space in the epithelium. Fragmentation of the nuclei is not uncommon.

In all these lesions of the calf, as well as those of the rabbit, no nuclear inclusions, such as were found in human variola by Dr. Councilman, occurred. Notwithstanding the degeneration and disintegration of the nuclei, nothing occurred which resembled those inclusions in the least. On the other hand, the vaccine bodies were found constantly associated with the lesions. They are situated in the cytoplasm of epithelial cells of the various parts inoculated, in the corneal epithelium, the nasal epithelium, the ducts of serous glands, the epidermis, and the meibomian and sebaceous glands.

When one considers the vaccine lesions produced on various parts of the rabbit and the calf, it will be seen that all the lesions have certain characteristics in common. The vaccine bodies are present in all cases, and the swelling of cells, which in the nasal epithelium and the epidermis amounts to hydropic degeneration, is a constant factor in the process.

The relation of the lesions is apparent if one considers the nose vaccination as intermediate between that of the cornea and the skin. In the cornea there is swelling of cells and proliferation, but no vesicle formation. In the vaccination of the nasal epithelium there is hydropic degeneration, proliferation and a rude type of vesicle formation. In the skin, proliferation takes no part in the primary process, but there is hydropic degeneration of cells with the formation of well-marked vesicles.

VII. THE VACCINE BODY.

Since Guarnieri's first description of these cell inclusions there has been a diversity of opinion as regards their nature, which has persisted up to the present time. They have been confused with cell inclusions of various sorts, such as fragmented leucocytes, degenerated epithelial cells, and nuclear detritus. They have also been considered as degenerated red blood corpuscles, as extruded nuclear material, and as specific cell degenerations. That there is a superficial similarity in given cases to the inclusions above enumerated must of necessity be admitted. When studied, however, under the best of conditions and with careful technic the vaccine body will be found to bear no relation to them. The vaccine body possesses certain morphological characteristics which are constant, regardless of the tissue or of the species of animal in which it is found. Moreover, it is possible through the study of a large number of vaccine and other lesions produced in epithelial tissues to exclude certain hypotheses concerning the nature of the vaccine body. The objective description of the vaccine body will be followed by a consideration of these various hypotheses.

1. *Morphology of the Vaccine Body.* — The minute structural details of the vaccine body, as well as the finer cell changes with which it is associated, are best studied in the cornea. The vaccine bodies found in lesions of the mucous membrane and skin present structural characteristics identical with those in the cornea, but it is difficult with our present methods of technic to follow closely in these tissues the exact structural changes in the developmental series. The finer details are best made out in the rabbit's cornea, but the forms seen here can be duplicated in the skin lesions of this animal and in the lesions of the cornea, the mucous membrane, and the skin of the calf.

In the early corneal lesions, up to twenty-four hours after inoculation, the vaccine bodies are present in small numbers. They were found in a lesion sixteen hours after vaccination, but none could be found in a four or eight hour lesion. In these earliest lesions most of the vaccine bodies are of small size, and it is evident that an early stage in their development is here represented. They become numerous with the further development of the lesion, and this numerical increase is conspicuous while the process is rapidly extending. Their first appearance is at the site of the trauma of inoculation, but, as the lesion develops, they are found distributed over a correspondingly large area. In the central or older portions of the lesions bodies will be found which have attained their maximum size, but towards the periphery the smaller forms predominate.

Since the small forms are associated with the very early stages and the larger forms with the later stages of the process, it is evident that the vaccine body increases in size. Since the vaccine bodies occur in large numbers in the lesion, it is possible to follow the stages of their development step by step, taking growth as a general basis.

In the smallest forms it is impossible to distinguish any structure except under the most favorable conditions. They appear as small bodies situated anywhere within the cytoplasm of epithelial cells, and they also occur occasionally between cells. These smallest forms do not develop from mere points, but are of fairly constant size. As they occur in rounded form, they measure about one micron in diameter. Within the cells each body is situated in a definite space in the cytoplasm and stains more intensely than the latter. If it were not for these characteristics, the smallest forms might readily escape observation. No stain has been found as yet which has proved specific for the vaccine body. In general it may be said that they stain with but little difficulty, and when once stained resist decolorization longer than the tissue elements. In sections stained with carbol fuchsin and counterstained with methylene blue the vaccine bodies are stained red and all other elements blue. Although the vaccine bodies are readily distinguished by this stain it fails to bring out their structure so clearly as does the eosin and methylene blue method. They are often irregular or elongated, and their distribution is such as to be very suggestive of ameboid motion.

Associated with the smallest forms are bodies similar to them in most respects, but of slightly larger size. These represent a further development of the smallest forms, and they are found with few exceptions near the nucleus of the cell and generally within the perinuclear space. It would appear that the vaccine body takes this situation before increasing in size to any extent. With increase in size, structure becomes apparent. At first a deeply stained granule is distinguishable within the body. With still greater increase in size this granule becomes surrounded by an unstained area which makes it more easily visible. The space which surrounds the vaccine body is always relatively large as compared to the spaces about most other cell inclusions. It is commonly situated next to the nuclear membrane, so that it is continuous with the perinuclear space when the latter is in evidence. It is usually spherical in outline and may not at all conform with the outline of the vaccine body. In this situation next the nucleus the vaccine body increases in size, so that in the rabbit's cornea it attains a diameter of five to seven microns. In the skin and mucous membrane the vaccine body attains a greater size than in the cornea, reaching ten or eleven microns in diameter. In the corneal vaccination of the calf the bodies average smaller than in the rabbit's cornea, and are rarely found exceeding four microns. A great variety of outline is presented by the small forms. In some cases the body presents the form of two masses situated some distance from each other in the cytoplasm and connected by a delicate thread. Elongated forms are numerous and many have pseudopodia-like processes. In the corneal lesions as the bodies increase in size they tend less and less to assume irregular outlines, the large forms being usually rounded or reniform.

The internal structure of the vaccine body becomes more and more evident as it continues to increase in size. In large bodies the deeply stained granule is found within a definitely circumscribed clear aréa. This clear area with its central granule resembles the nucleus of stages of certain species of sporozoa, although no nuclear membrane is demonstrable. The central granule has the staining reaction of chromatin. The remainder of the vaccine body has a reticular structure and stains as cytoplasm. Although changes take place in the further development of the vaccine body, this general type of structure holds good throughout all the forms.

After the vaccine body has attained a relatively large size certain changes take place in the chromatin material. Instead of being present as a small dense granule, it becomes more abundant and irregular. It may appear as an irregular mass of granules. In the stage which follows this the granules are separated and distinct from one another. Some bodies contain a few distinct granules; others contain a great many. Whether a secondary division takes place in the chromatin granules, resulting from the first division just described, could not be ascertained by direct observation. In what may be considered a later stage the chromatin granules are minute and numerous and have taken a peripheral position in the vaccine body. Here they are found situated within small masses which project from the surface of the vaccine body. This stage resembles somewhat a mulberry and may be considered as a segmemting form. Owing to the minuteness of the small peripheral masses of these mulberry forms, their structure cannot be demonstrated in every specimen. Their chief characteristics are, however, clearly brought out by Weigert's modification of Gram's stain or by staining the sections intensely with eosin.

In many of the segmenting forms some small masses have become free. They may be situated in the space surrounding the main body or they may be distributed throughout the cytoplasm of the cell. They are identical with the smallest forms with which the series commenced. The entire mass of the vaccine body is not used up in the formation of small forms, but a shapeless, residual mass is left. Spaces are found in cells containing only these residual masses.

Thus it is possible to trace each stage of growth and differentiation from the smallest forms of vaccine bodies, in which structure is not apparent, to larger forms with definite structure, in which certain changes take place preparatory to segmentation. By segmentation small forms are produced identical with those with which the series commenced. These become separated, and are found distributed in the neighboring cytoplasm.

The segmenting forms were found in great numbers in a vaccine lesion of the nasal mucous membrane of the calf. They were much larger in this lesion than in the cornea, and the small masses produced were much more numerous (Plate XXV., Fig. 5, and Plate XXVI., Figs. 18, 19, and 20).

One of the constant cell changes which accompany the vaccine bodies is the formation of a relatively large space about the latter.

There is no evidence that this clear zone surrounding the vaccine body is other than a space in the cytoplasm of the cell. That it is a space or excavation in the cytoplasm, and not a capsule, is shown by the fact that these bodies may be displaced by mechanical violence to the mounted section, and, further, that it has not been possible to stain it. The space may contain blue-stained, granular material, lying in loose masses about the vaccine body, or delicate threads may extend in towards the vaccine bodies. The granular material is most prominent in those cells which are degenerated. Similar material is also found in small masses in the cytoplasm of other degenerated cells, or it may be collected at the poles of the nuclei representing the triangular or pyramidal masses described by Hückel. It is obvious that this material bears no relation to the vaccine body, but its association with the latter has led to confusion in certain instances. In deeply stained preparations this granular material investing a vaccine body may obscure its outline, but in sections which are properly differentiated the vaccine body will be found to possess a definite contour of its own. As regards the significance of the space in which the vaccine body is situated, Wasielewski regards it as the perinuclear space, and believes that it does not occur in life, but is an artefact due to the shrinkage of the cytoplasm away from the nucleus and the vaccine body. He thinks that the degree of shrinkage is probably increased by the action of the parasite upon the cell.

It is difficult to disprove this theory, but it should be noted (1) that a considerable space is found about vaccine bodies situated in the cytoplasm, apart from the nucleus, and (2) that in cells in which vaccine bodies and leucocyte fragments occur side by side, the space containing the leucocyte never attains the dimensions of that about the vaccine body. In studying the effects of certain of the coccidia upon cells, the parasites were not surrounded by a space of such relatively great dimensions as that enclosing the vaccine body. As the same methods had been employed in the fixation and staining of these preparations, it is reasonable to expect an equal degree of shrinkage. Most of the evidence would go to show that the large space which characterizes the vaccine body is not due wholly to shrinkage.

Another change met with in cells containing vaccine bodies is their marked increase in size. In the cornea the cells are large and glassy. In the skin and mucous membrane they at first increase in size and later become vacuolated or hollow. Swollen cells are found which contain no vaccine bodies, so that this change may evidently be brought about by some other agency than the direct action of the vaccine body on the cell. The nucleus is commonly indented by the vaccine body, and may present the various types of degeneration which have been given above in the description of the process. Most of the cells which contain the smallest vaccine bodies are normal in character, although these smallest forms may also be found in degenerated cells.

Summary. — The different types of vaccine bodies described above fall into a natural developmental series which bears a constant relation to the stages of a distinct pathological process, whether in the cornea, the mucous membrane, or the skin. The small forms are found in very early foci or at the periphery of older lesions. The larger forms are associated with the older portions of the lesion and apparently undergo segmentation. The small forms resulting from this segmentation are found attached to the main mass, free in surrounding space, and in the adjacent cytoplasm. They are identical in all respects to the small forms with which the series commenced. They increase rapidly in number while the process is extending. Structure is demonstrable in all but the smallest forms. The vaccine bodies contain a substance which reacts like chromatin with various micro-chemical reagents. The division of this substance into numerous small granules precedes the segmentation of the body as a whole. The other substance of the body resembles protoplasm both in its reaction to stains and in its structure.

2. *Hypotheses Concerning the Nature of the Vaccine Body.* — Before giving what seems to me to be the most reasonable and logical interpretation of the nature of the specific bodies found in the vaccine lesions, I shall discuss briefly the chief hypotheses which have thus far been advanced in regard to their origin.

A. *Red blood corpuscles.* — The hypothesis that these bodies are derived from red blood corpuscles has nothing in its support. Red blood corpuscles take no part in the process occurring in the cornea, and are not present in the lesions at any stage.

B. *Leucocytes.* — Polynuclear leucocytes are present in most of the lesions experimentally produced, and fragments of them are, at times, included within epithelial cells. The fragments may contain nuclear as well as cytoplasmic material. The nature of such fragments when well preserved is readily made out in the rabbit by one who has made a morphological study of these cells, on account of their granules. When degenerated their structure is of such a gross type that it is apparent that they bear no relation to the vaccine bodies. It is difficult to conceive of degenerated leucocyte fragments showing such delicate and constant structure as the vaccine bodies possess. The uniform distribution of the vaccine bodies throughout all the epithelial cells of a large portion of the vaccine lesion is difficult of explanation if the inclusions are to be considered as leucocyte fragments. The fact that minute corneal lesions containing large numbers of vaccine bodies occur, in which no leucocytes are demonstrable, furnishes definite proof that the vaccine body is not related to the polynuclear leucocyte.

C. *Centrosomes.* — The inclusions have occasionally a bi-polar arrangement relative to the nucleus, one inclusion appearing at either end and suggesting, by their position at least, centrosomes. This arrangement is, however, only a matter of chance and occurs where the inclusions are so numerous that single cells contain two. The occurrence of the vaccine body at the periphery of cells, as well as in cells at the surface of the cornea which probably possess but slight vital activity, is against this hypothesis. Small forms are also found between epithelial cells. The mitoses which occur in the cornea are in most cases typical and the centrosome consists of a mere point which is made out with difficulty. A vaccine body was found in one instance in a corneal corpuscle undergoing normal mitosis. In the lesion of the nose they are not uncommon in epithelial cells in the process of mitotic division.

Inasmuch as vaccine bodies occur in cells in which the centrosomes are accounted for this hypothesis needs no further consideration.

D. *Extruded nuclear material.* — In certain deeply stained preparations the vaccine bodies may simulate to a certain extent the chromatin masses in the nuclei. The appearance of vaccine bodies first in the peripheral protoplasm of cells which are distinctly normal does away with this explanation. In most rabbits' corneas the chromatin masses are not large, and no unquestioned instance of extrusion of chromatin from well-preserved nuclei was met with. The character of the detritus resulting from the disintegration of nuclei was in all cases obvious.

E. *Specific cell degeneration.*— It is difficult to conceive of cell degeneration showing such a constancy of reaction to stains, such delicate and constant structure, and such a variety of contour irrespective of possible external mechanical agencies. A study was made of cell degenerations accompanying other known processes. The material consisted of:

a. Various tissues infected with coccidia; *b*, a rabbit's cornea injured by freezing with ethyl chloride; *c*, rabbits' corneas inoculated with diphtheria toxine; *d*, lesions of foot and mouth disease.

(*a.*) A large number of lesions produced in the liver and intestine by Coccidium oviforme had been collected from previous work with that species. The most marked cell changes were found in the lesion of the intestine. The swelling of epithelial cells was marked even in portions of the lesion which contained only the smaller forms of the coccidium. The most striking change was found in the nuclei, which were greatly enlarged and appeared perfectly clear except for a single large, deeply stained mass of chromatin material. These changes, in consequence of which the epithelial cells presented a picture very unlike the normal, were widely spread throughout the lesion.

In the tubules of the mouse's kidney infected with Klossiella muris, there are, in the vicinity of the infection, groups of monster epithelial cells many times the dimensions of any of the normal cells. The cells which contain the parasites are large and pedunculated, so that they hang free in the lumen of the tubules.

The lesions produced by the coccidium which infects the kidneys of guinea-pigs were also studied.[1] Here, too, the cells are greatly increased in size. In all these species the only degeneration which might be regarded as specific is the enlargement of the epithelial cells. The relation of the vaccine body to the swelling of the epithelial cell bears a certain amount of analogy to similar changes due to the presence of known parasites.

(*b.*) An area upon the cornea of a rabbit was frozen several times and the animal was killed twenty-four hours afterward. Histologically the epithelium of the cornea was thinner than normal over its greater portion. The only conspicuous change in the epithelial cells was the presence in the nuclei of a large mass of chromatin.

(*c.*) The corneas of three rabbits were incised and a liberal amount of diphtheria toxine rubbed over the surface. One rabbit was killed twelve hours, one twenty-four hours, and one forty-eight hours after inoculation. A certain type of degeneration was found affecting both leucocytes and epithelial cells. The chromatin material in the nuclei appeared to be increased and was collected in one or several large masses. The remainder of the nucleus was represented by a red stained, reticulated structure enclosing this mass of chromatin. The protoplasmic portion of the cell appeared to be diminished and it usually took a deep eosin stain. The inclusions resulting from these degenerations are fully accounted for by the metamorphosis of component parts of the epithelial cell, and of included leucocytes. They may consist of nuclear and protoplasmic material, but they furnish but slight resemblance to vaccine bodies.

A large number of preparations of human tissue in diphtheria had been stored in the laboratory from the investigations by Drs. Councilman, Mallory, and Pearce upon this disease.[2] The mucous membrane of the nose affected by

[1] Certain stages of this organism were described by Pianese.

[2] Councilman, Mallory, and Pearce. A study of the bacteriology and pathology of two hundred and twenty fatal cases of diphtheria. J. Bost. Soc. of Med. Sci., 1900, Vol. v, No. 5, p. 18.

this disease furnishes cell inclusions which, however, bear no resemblance to the vaccine bodies.

(*d.*) In the lesions of foot and mouth disease affecting cattle extensive degenerative changes are met with in the epithelium, but no inclusions were found which in any way resembled vaccine bodies.

F. *Nerve endings.* — In this connection the possible relation of the vaccine body in the cornea to the small bodies found there attached to the nerve fibers should be mentioned. Coexisting as they do side by side in the same preparation, any intermediate stages could be readily determined. They are all larger than the greater number of vaccine bodies and possess different staining affinities. It is inconceivable that one or several of these should invade every epithelial cell of a given area. As found in the epithelium of the lesion these nerve bodies are often many times their normal size and are readily distinguished from vaccine bodies, although it seems probable that they figure among the various bodies described by Hückel.

3. *Discussion.* — The exclusion of the various hypotheses just considered strengthens the morphological evidence indicating the parasitic nature of the vaccine bodies. The adoption of any one of them to account for the vaccine bodies in the lesions of the various localities or even of a single locality is not possible. If, on the other hand, one considers the vaccine body an organism, its various morphological types constitute a natural series of developmental forms, which goes hand in hand with the various stages of the vaccine process. In the study of many of the intracellular parasites, one would gain but little insight concerning their nature from the observation of isolated forms. In order to understand their nature and significance, they must be studied as a series of related forms which represent transitional stages of development. In this way only can definite knowledge be acquired of their origin, the changes in their structural composition, and their ultimate fate.

Another form of evidence in the nature of ameboid motion has been sought by various observers. It has been neglected in the present investigation chiefly on account of the possibility of error in the examination of objects of such minute size in the fresh state. This matter is discussed at length by Wasielewski. Motion of the bodies within the epithelial cells has been observed by various investigators. Wasielewski found it possible to demonstrate motion by the change in the color refraction of the bodies which accompanied the change in its focal distance from the lens. He admits that ameboid motion has not been observed by a sufficient number of observers to establish the parasitic nature of the bodies on this phenomena alone. The form of the bodies in fixed sections is very suggestive of such activity.

VIII. GENERAL SUMMARY.

1. By a large number of experimental vaccinations made upon rabbits and calves it has been possible to demonstrate cell inclusions which are specific to vaccinia and which possess a definite and constant morphology.

2. By the inoculation of human variola virus upon the rabbit's cornea, lesions are produced which contain inclusions identical in every respect to these occurring in vaccinia.

3. The various types of vaccine bodies are closely related, and form a series which bears a definite relation to the development of the pathological process in which they are found.

4. No evidence has been obtained to show that the vaccine bodies represent a form of cell degeneration, and it is found impossible to duplicate them in any other process. They are simulated most closely by the youngest stages of certain intracellular parasites.

5. The process following vaccination varies with the site inoculated, and there is slight species variation.

6. The enlargement or hydropic degeneration of epithelial cells is constant throughout all lesions, and constitutes the primary change in the process. Epithelial proliferation is also constant in lesions of the cornea and nasal mucous membrane.

7. The exudation occurring in the process is variable, and its type and degree depend upon the character of the tissue and upon the occurrence of secondary infection with bacteria.

IX. CONCLUSIONS.

As the result of this investigation, I am fully convinced that the vaccine body is an organism, and represents the etiological agent in this disease.

This view is based on the following evidence:

1. The bodies consist of a substance which resembles protoplasm in structure, and which always contains masses having the staining reaction of chromatin.

2. The bodies show variations in form corresponding to a developmental cycle. They begin as small bodies of approximatively the same size in the early lesions, increase in size up to a certain point, and then undergo segmentation (both the body itself and the chromatin in it) to form the small bodies from which the series started.

3. All other hypotheses are insufficient to account for the presence of these bodies, their structure, and the changes met with in their growth and development.

The structural morphology of Cytoryctes, and the lesions with which it is associated, are not without analogy, for certain common forms of coccidia show a similar arrangement of chromatin and protoplasm, and produce lesions in which the increase in size and the proliferation of the epithelial cells form the most striking feature.

Although the morphology of Cytoryctes and the manner of its parasitism would place it among the sporozoa, the stages found in vaccinia do not furnish sufficient data for its exact classification at the present time. The interpreted cycle of its development in vaccinia may be outlined as follows:

Infection. — Epithelial cells are invaded by small forms in which it is difficult to distinguish structure. These small forms are found between cells and in various parts of the cytoplasm, but after their entrance into the cell they take a position near the nucleus.

Growth.— After becoming located near the nucleus they become larger, and with this growth the character of their structure becomes apparent. They then consist of a reticular protoplasm in which is a clear spot containing a mass of basic staining material. Although it is impossible to distinguish a nuclear membrane bounding this clear spot, it seems probable that this clear spot with the granule in it is the equivalent of a nucleus, and that the granule represents the chromatin of the organism. The organism is situated in a space in the cell, generally many times its own volume. This space is usually continuous with or is a part of the perinuclear space.

Division of the nuclear material.— Certain forms, in which the chromatin mass is irregular, precede those in which the chromatin is divided. In the latter, the chromatin granules may be few or numerous. The chromatin granules later take a peripheral position, where they then form the centers of minute masses which bulge from the surface.

Multiplication.— These small masses, becoming free, are found in the space occupied by the segmenting form and in the cytoplasm of the same cell. They constitute the small forms described as the first of the series. They now scatter and penetrate neighboring cells. The invasion of the surrounding normal cells by the small forms resulting from this multiplicative process constitutes auto-infection, and by it the process extends. The immediate effect of the parasite is to cause an increase in size of the epithelial cells. This increase in cell volume is accompanied in the corneal lesion by proliferation. The exudation which usually accompanies the lesions is secondary to the degeneration of the epithelium

DESCRIPTION OF PLATES.

Plate XXIII.

Fig. 1. A vertical section of a rabbit's cornea fifty hours after vaccination. The incision is filled with epithelial cells, and the epithelium on each side of the incision is twice its normal thickness. There is a defect in the surface of the cornea to the right of the incision.

Fig. 2. Same lesion photographed under higher power. Large numbers of vaccine bodies appearing as small, dark masses about the defect in the surface.

Fig. 3. A vaccine body of medium size situated in a space in an epithelial cell. In the vaccine body there is a clear spot, within which is a deeply-stained granule. From corneal vaccination of rabbit. × 1,000.

Fig. 4. Rabbit's cornea twenty-seven hours after vaccination. Epithelium fills incision, but is not otherwise thickened. Below this epithelial wedge is a small collection of leucocytes.

Fig. 5. A vaccine body somewhat kidney shaped. Structure identical with that of Fig. 3. From corneal vaccination of rabbit. × 1,000.

Fig. 6. Rabbit's cornea three days after inoculation with variola virus. Epithelium proliferated, and at least four times its normal thickness on both sides of the incision. It fills the incision, and extends into every space where the corneal tissue had been separated.

Fig. 7. The same lesion as seen in Fig. 6, but with greater magnification. Certain epithelial cells in central portion of lesions are vacuolated, and several near the surface contain vaccine bodies. Below is a large amount of cell detritus.

Plate XXIV.

Fig. 1. A large vaccine body in centre of the figure, in which the central structure, with its deeply-stained granule, is not in sharp focus. The remainder of the body is reticular, and appears to have a structure like protoplasm. × 1,000.

Fig. 2. A vaccine vesicle from the skin of a calf, four days after inoculation. The vesicle is filled with fluid in which leucocytes are numerous. Epithelium destroyed at base of vesicle. Trabeculæ of degenerated epithelium extend into certain portions of the vesicle.

Fig. 3. Calf's cornea thirteen days after vaccination. The epithelium presents various degrees of degeneration. Large numbers of leucocytes infiltrate the tissue. Vaccine bodies few in number and found only after a search through several sections.

Fig. 4. A large vaccine body containing two deeply stained granules. × 1,000.

Fig. 5. A segmentation form of the vaccine body. Several small masses are to be seen projecting from the surface of the main mass. × 1,000.

Fig. 6. A vaccine lesion of a calf's udder. Epithelial cells are hollow and distended with fluid. An early stage of vesicle formation present in the central portion of section.

Plate XXV.

Fig. 1. Cornea of calf five days after vaccination. Lesion confined to epithelium which at this point is no thicker than normal.

Fig. 2. A higher power of the same lesion as seen in Fig. 1. The epithelial cells show marked vacuolar degeneration and contain vaccine bodies. No leucocytes are to be found in this lesion.

Fig. 3. Four small vaccine bodies situated in the cytoplasm of epithelial cells situated at a distance from the nucleus. Gland duct of nasal mucous membrane of the calf. × 1,000.

Fig. 4. Two vaccine bodies indenting the nucleus of an epithelial cell. Vaccination of the calf's cornea. × 1,500.

Fig. 5. An epithelial cell in which a large vaccine body has given rise to numerous bodies of minute size. The whole mass is situated in a space within the cell and curves about the nucleus. Vaccinated nasal mucous membrane of the calf. × 1,000.

Fig. 6. Calf's cornea seven days after vaccination. A defect in the epithelium to the left and the incision of inoculation to the right of the centre. The deep layer of the epithelium appears much lighter than the outer layer which is dense and deeply stained.

Fig. 7. A portion of the same lesion, but with greater magnification. The cells of the deep layer are large and pale. The nuclei are larger than normal and the chromatin is arranged in small masses.

Plate XXVI.

Cumera lucida drawings with Zeiss comp. oc. 6 : obj. 2 mm., apert. 1.30. The cells represented in Figs. 1 and 9, inclusive, were taken from vaccination of the rabbit's cornea.

Fig. 1. Small vaccine bodies situated in vacuoles in the cytoplasm of an epithelial cell.

Fig. 2. Larger vaccine bodies. The one to the right lies next to the nucleus and shows definite structure. In its interior is a clear space which contains a dark granule. The epithelial cell is somewhat degenerated and the chromatin is collected in masses against the nuclear membrane.

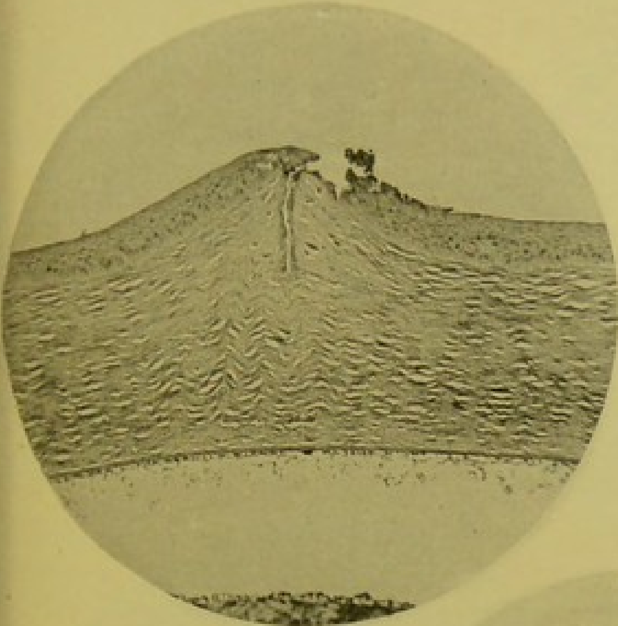
Fig. 3. A larger example of vaccine body having the same structure. It is situated within a large space which indents the nucleus of the cell. Towards the limit of this space there are granular masses which take a deeper stain than the cytoplasm of the cell.

Fig. 4. This vaccine body possesses several chromatin granules and has a process extending out from the main mass.

Figs. 5 and 6. Vaccine bodies, the chromatin substance of which is distributed irregularly in minute granules. Here, too, there is a considerable amount of blue stained, finely granular substance in the space about the vaccine body.

Fig. 7. Chromatin of vaccine body distributed regularly in the form of small granules occupying the interior of small rounded masses which project from the surface of the main body.

Figs. 8 and 9. Bodies similar to that seen in Fig. 7 stained deeply with



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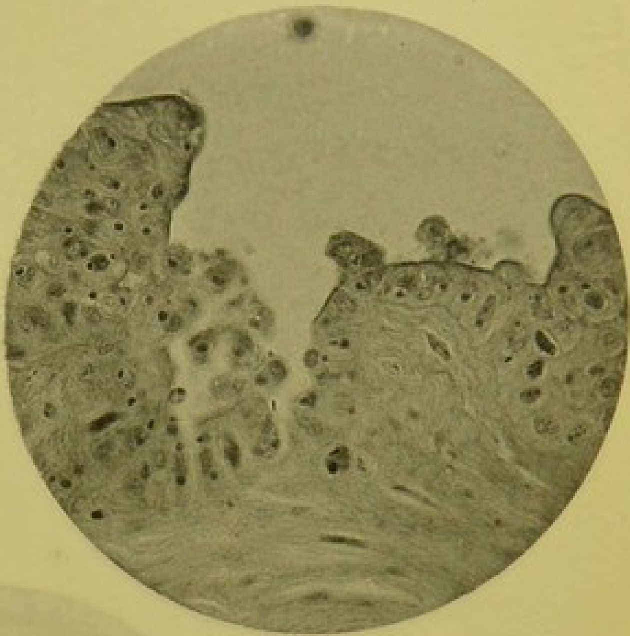

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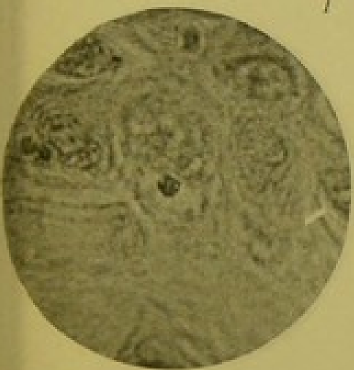

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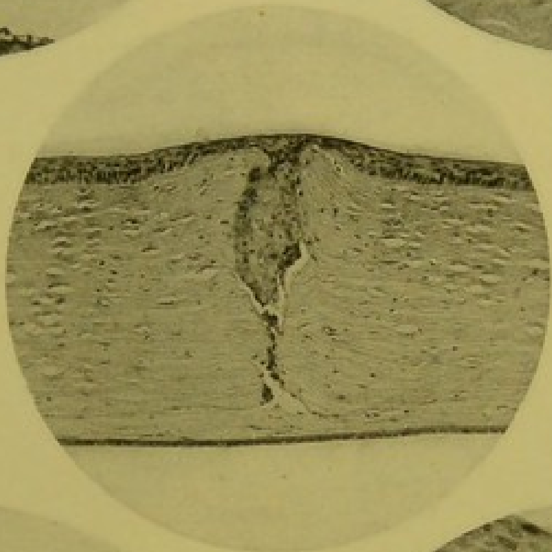

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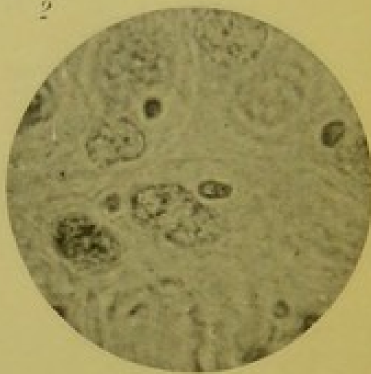

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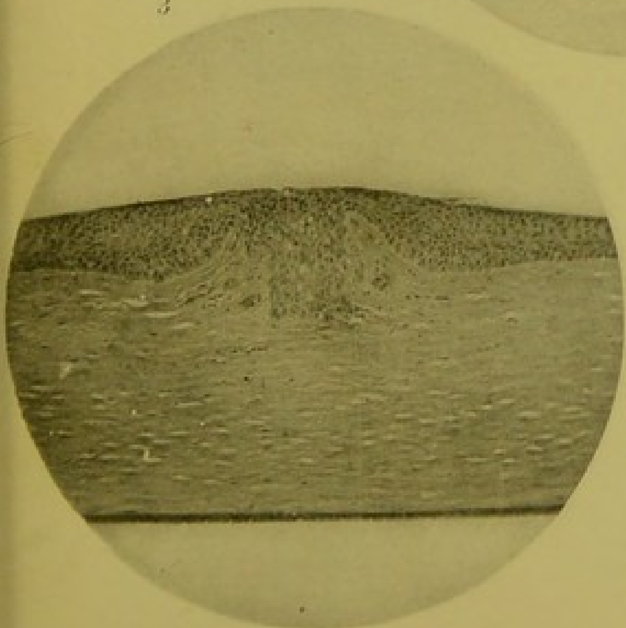

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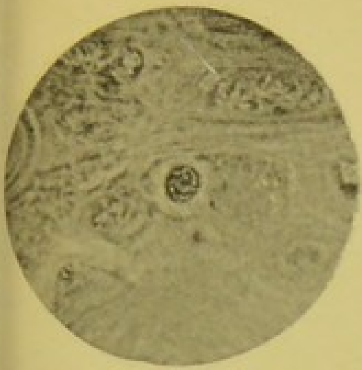
Tyzzer. Vaccinia

Heliotype Co. Boston.



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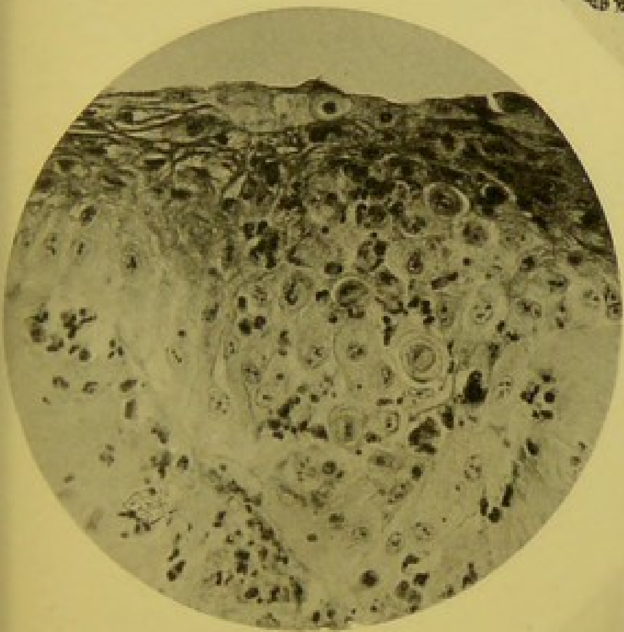

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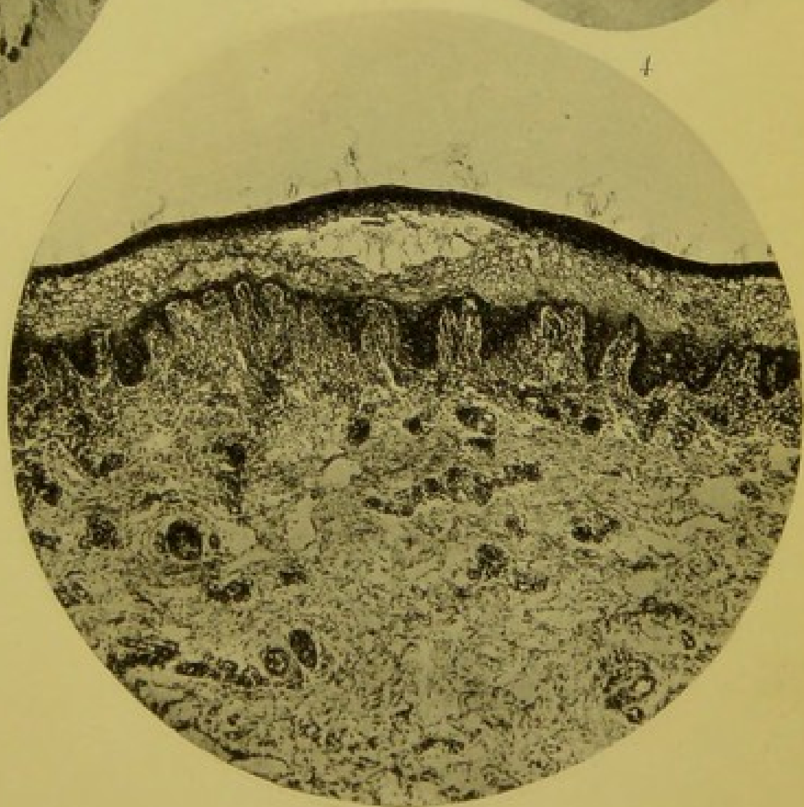

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Tyzzer.

Vaccinia

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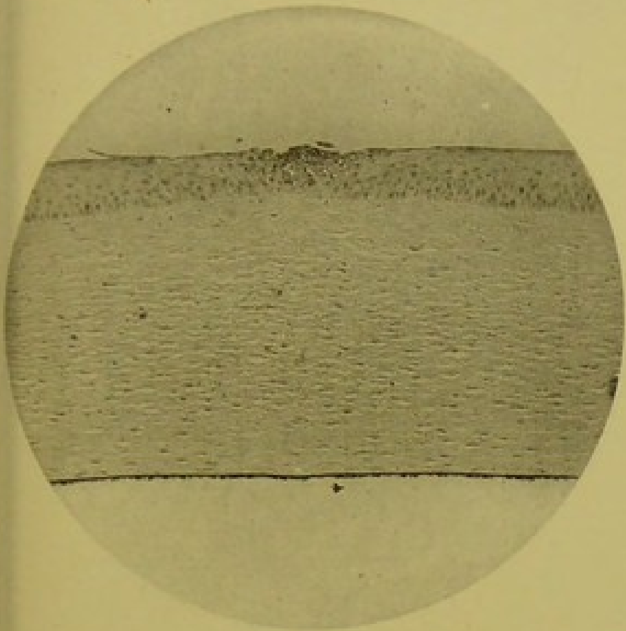

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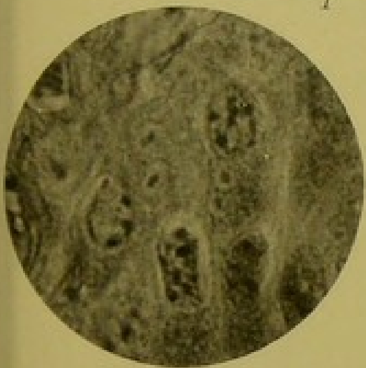

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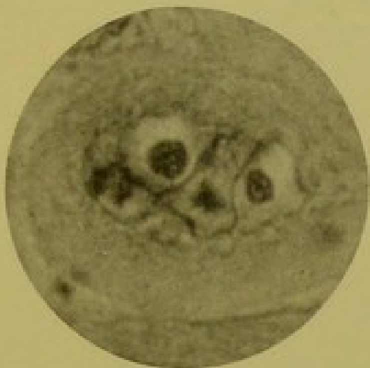

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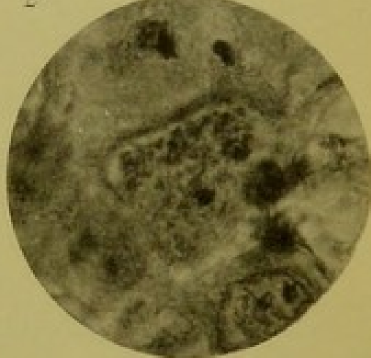

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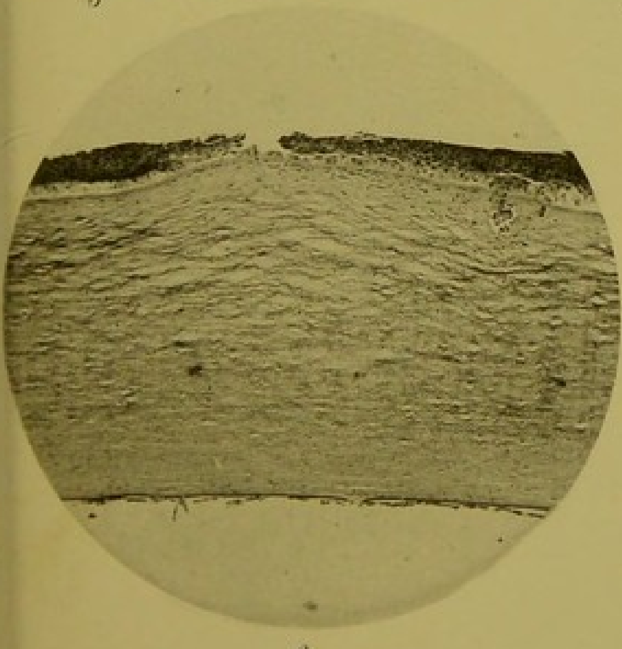

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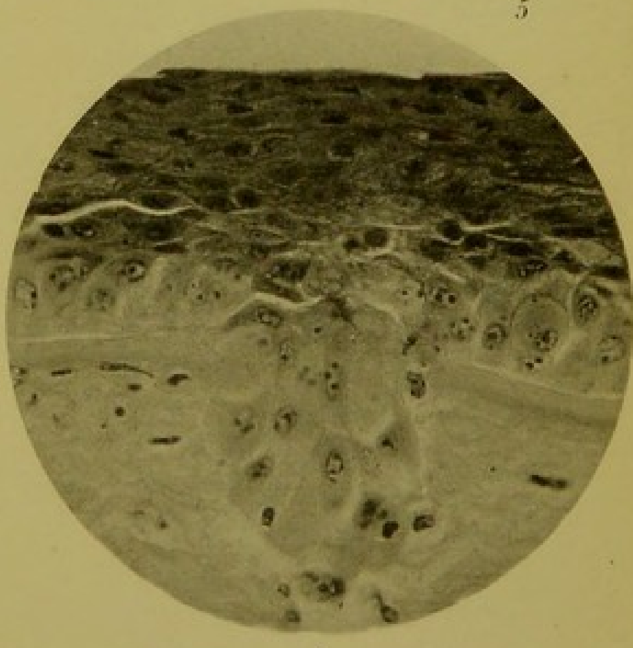

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Tyzzer.

Vaccinia

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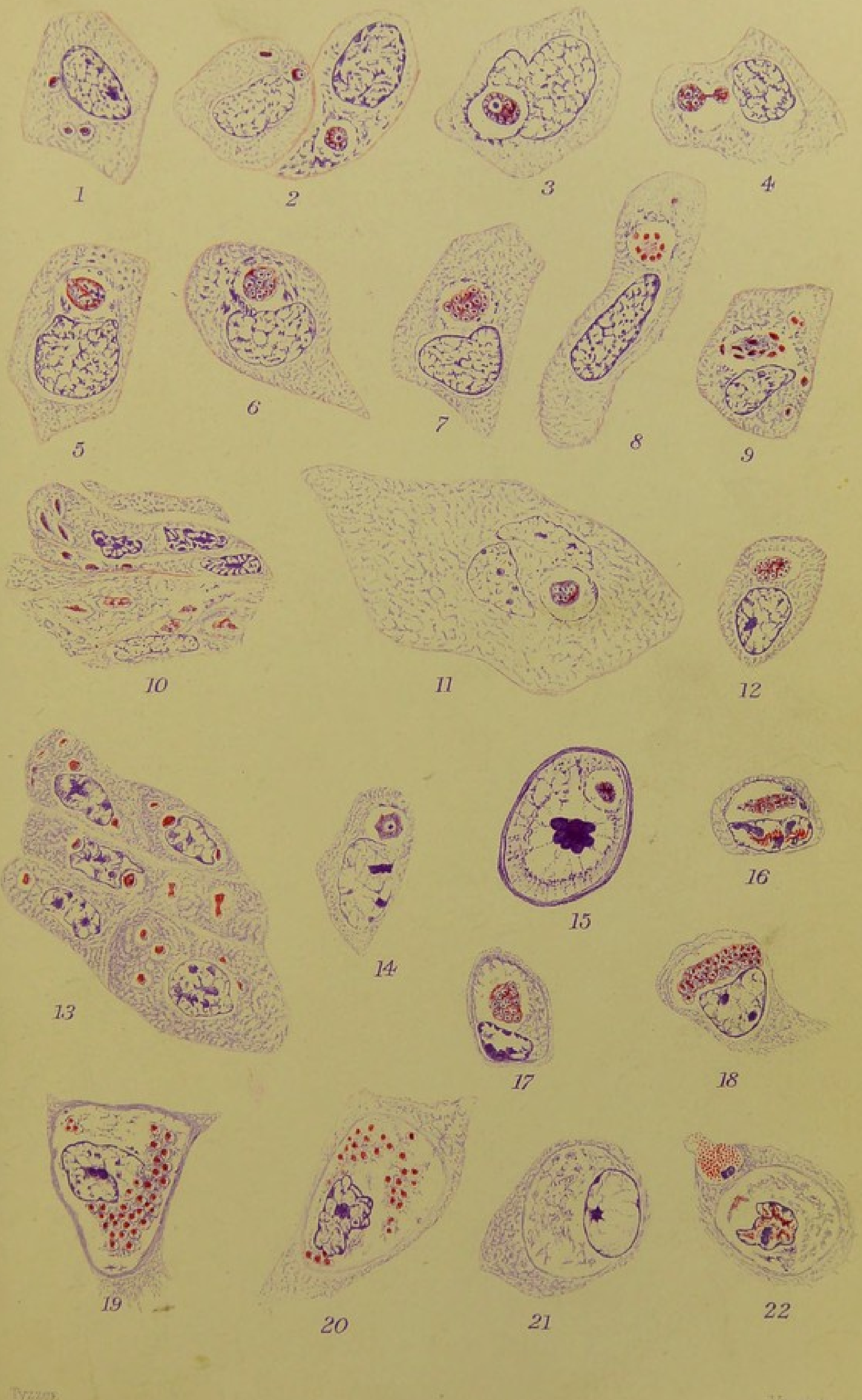

Tyzzer. Vaccinia.

Geo. H. Walker & Co., Boston.

eosin. In the right-hand figure small bodies are situated in the cytoplasm of the cell at some distance from the main body.

The cells represented in Figs. 10, 11, and 12 are from corneal vaccination of the calf.

Fig. 10. Several minute elongated forms of vaccine bodies situated in cells near the surface of the cornea. Larger forms situated below.

Fig. 11. A large epithelial cell, the nucleus of which has divided, containing a vaccine body.

Fig. 12. A form of vaccine body, the surface of which is studded with smaller bodies. Similar in most respects to Fig. 7.

The remaining figures represent cells from the vaccination of the nasal mucous membrane of a calf.

Fig. 13. Cells from gland duct. Vaccine bodies, small, some elongated, some irregular.

Fig. 14. Larger vaccine body with characteristic clear spot and granule.

Fig. 15. An atypical mitosis in a cell which contains a vaccine body.

Figs. 16 and 17. Bodies in which the chromatin is distributed in small granules.

Fig. 18. Small bodies apparent in the vaccine body.

Figs. 19 and 20. Small bodies becoming free from the main mass.

Fig. 21. An epithelial cell containing a vacuole empty except for some granular material. Nucleus large, vesicular, chromatin next the nuclear membrane.

Fig. 22. A cell in the border of which is a portion of a leucocyte. Spaces within cell empty except for some granular material. Nucleus shrivelled. Red stained material forms a mass quite separate from the chromatin.

ON EXPERIMENTAL VARIOLA IN THE MONKEY.

G. B. MAGRATH AND W. R. BRINCKERHOFF.

I. INTRODUCTION.

The studies forming the subject of this paper were undertaken in connection with a general investigation into the etiology of variola, at the suggestion of Dr. W. T. Councilman, whose constant guidance and advice in their prosecution we most gratefully acknowledge.

The research was conducted with the following general objects in view: (1) the observation of the course of variola inoculata in the monkey, with especial reference to the constitutional reaction, the specific lesions of the skin, and the disturbances in the leucocyte equilibrium incidental to the disease; (2) the gaining of additional knowledge in regard to the occurrence and the distribution of the etiological factor.

The work was carried on during the past year, for the most part in a small laboratory, temporarily established at the Boston Detention Hospital.

II. HISTORICAL REVIEW.

Experimental inoculation of monkeys has been practised by various investigators engaged in the study of variola, and it is a matter of knowledge that the monkey is an animal susceptible to the disease. Very little attention, however, seems to have been given in the course of such experiments to the histological characteristics of the induced lesions.

Zülzer (1874) variolated a Cercopithecus with the blood of a variola patient, producing constitutional symptoms and a general papular eruption. He was also able to produce the disease by exposing the animal to air containing finely divided particles of desiccated crusts wet with pustule contents. His efforts to inoculate through the alimentary tract were unsuccessful.

Buist (1887) found that inoculation of the ape with dry beer yeast produced the same reaction as that yielded by inoculation with vaccine and with variola lymph, and further that animals so treated displayed a diminished susceptibility to vaccinia and to variola.

Copeman (1894) demonstrated the susceptibility of Rhœsus alike to vaccinia and to variola, finding that in this animal the local reaction to both diseases first appears on the third day, that the vesicle formation is complete on the fifth, and that the maximum development of the lesion is attained upon the eighth day. He found the lesion to be less marked in vaccinia than in variola.

De Haan (1896) inoculated Macacus with the contents of variola pustules, obtaining on the seventh day local lesions in the form of papules surrounded by an areola. In one of a series of seven cases, he observed a few secondary papules upon the lips and upon the extremities.

Roger and Weil (1902) report a series of experiments upon Macacus. Inoculating with variola pus, they obtained pustules which dried up in fifteen days. Inoculations made by them beneath the skin produced no lesions. In a series of inoculations made with the blood of cases of hemorrhagic variola, they obtained pustule formation in an animal inoculated by scarification, and no lesions when inoculation was performed by injection of the blood into the animal. One animal subjected to the latter procedure died on the fifth day from streptococcus septicemia. These investigators also injected two monkeys with blood from a rabbit inoculated four days previously with variola pus. The animals showed typical changes in their leucocytes, but derived no immunity to vaccinia. These writers regard both vaccinia and variola as inoculable upon the monkey, but hold that neither confers perfect immunity.

Park (1902) found Java monkeys susceptible to variola and insusceptible to varicella.

Ewing, in the same year, reports that he produced typical variola lesions in Rhœsus and in an African monkey.

III. METHOD.

The animals used in these experiments were small monkeys, belonging to the Rhœsus and to the Macacus groups.

The experiments are presented in three series, in each of which the virus employed was from a different local epidemic. In the first series particular attention was paid to the constitutional reactions and the conditions of the blood, in the second and the third series attention was directed to the study of the evolution of the lesion.

Series I. — Prior to inoculation, each animal was subjected to a preliminary control observation, consisting in the determination of body temperature, the making of blood counts, and the microscopic examination of the freshly drawn blood. Temperature was taken per rectum. Blood was derived from small incisions made with aseptic precautions, either in the tail or within a shaved area upon the back. Microscopic examination of the fresh blood was conducted upon the warm stage, at body temperature, and by means of a Zeiss apochromatic optical series.

Series II. and III. — The general condition of the animal, the temperature, and the macroscopic appearance of the lesions were noted daily.

The method usually employed for introducing the virus into the animal was that of local inoculation of the skin. In one experiment intravenous injection was practised. The regions selected for inoculation were the upper arm, the abdomen, and the back between the scapulæ. Inoculation was performed by scarifying or by lightly incising the skin, previously shaved and rendered aseptic by washing with alcohol and rubbing in with a flamed knife blade the infective material.

Three strains of virus were used in these several series of experiments. Through the courtesy of the health authorities in Fall River, Mass., and in Philadelphia, Penn., we were able to obtain virus from cases occurring in local epidemics in these cities. The experiments are grouped according to the strain of virus inoculated. The samples of virus from the different individuals are indicated by a letter after the Roman numeral which designates the epidemic strain to which the virus belongs. Thus Virus I. *a* and Virus I. *b* are from the same epidemic, but from different cases in that epidemic. The strains are numbered as follows: Virus I., Boston epidemic; Virus II., Fall River epidemic; Virus III., Philadelphia epidemic. The virus was obtained by drawing the contents of a vesicle or pustule into a small sterile capillary tube. The material was used within twenty-four hours after collection. In certain experiments other infectious material was employed, such as powdered disks, or a paste made of the same. In many cases the material used for inoculation was tested on the rabbit's cornea.

Such animals as died or as were killed were promptly subjected to post-mortem examination, and their tissues preserved in appropriate fixatives. In certain cases corneal inoculations were practised with scrapings from the specific lesions or with material from certain organs of the animal in order to demonstrate the presence of the contagium. The tissues derived from autopsy were fixed in Zenker's fluid, embedded and cut in paraffine, and stained by a variety of methods. The most satisfactory results were obtained with the eosin-methylene blue stain. Bacteriological examinations at autopsy were made in appropriate cases.

SERIES I. — EXPERIMENT NO. I.

Animal a young male Macacus. — Preliminary injection with 0.002 gram Koch's tuberculin, followed by rise in temperature of about one degree during the subsequent forty-eight hours. On the second day following the tuberculin test, inoculation was made by rubbing into scarifications upon the shaved skin of the upper arm freshly-ground disk from a case of variola vera.

Temperature, already elevated about one degree above the normal for the animal, at 37° C., rose to 38°, remained at that point for one day, then fell to normal, rising again to 37° on the fifth day, and subsequently falling to an ante-mortem subnormal point of 34°.

The site of inoculation showed no inflammatory reaction, and the scarifications gradually healed, with no evidence of " take."

Constitutional symptoms of a specific character were wanting; the animal developed a dyspnea, and grew progressively weaker, dying upon the eleventh day after the inoculation.

Autopsy showed tuberculosis of the bronchial lymph nodes; these were greatly enlarged. and impinged upon and constricted the primary bronchi.

The red blood corpuscle count varied from about six and one-half millions per cubic millimeter at the beginning of the experiment to rather less thau five millions at the death of the animal.

The leucocytes showed an increase to 23,000 per cubic millimeter on the second day after inoculation, followed by a decrease to about 10,000 during the last week of life.

Histological examination of the skin at the site of inoculation showed slight evidence of repair, but no specific lesion.

Experiment negative.

SERIES I. — EXPERIMENT NO. 2.

Animal a young male Macacus. —Inoculated with vesicle contents from a case of variola vera, obtained in a capillary tube, and rubbed into a scarified area upon the arm. Under observation nineteen days (Virus I. *a.*).

Temperature rose a degree during the first twenty-four hours after inoculation, returning to normal upon the fourth day. On the fifth day the area of scarification showed well-marked induration and a faint, peripheral flush; under a hand lens distinct, transparent, nodular elevations were apparent. The temperature at this time showed a rise of two degrees to 38° C., and the animal displayed some restlessness and anorexia. During the next three or four days the local lesion gradually developed, showing on the ninth day well-marked vesicles, strongly elevated, confluent, and partially desiccated. At this time the temperature rose to 39°, three degrees above the normal. Following this was a gradual subsidence of the lesions, with desquamation of one of the crusts on the eleventh day. The temperature slowly returned to normal. On the sixteenth day the site of inoculation showed two dry, scaly crusts.

The red blood corpuscle count showed fluctuations between three and six millions per cubic millimeter from the fifth to the ninth days.

The white corpuscle count showed a leucocytosis of 26,000 per cubic millimeter on the third day after inoculation, and another of similar degree upon the tenth day persisting for three days. This was mainly a mononucleosis.

Nine days after the healing of the local lesion re-inoculation was attempted; material derived from scraping the base of a protopustule[1] of the animal, the subject of Exp. No. 3, was rubbed into scarifications upon the front of the chest and upon the back. Upon the third and the fourth days following this procedure the animal showed a slight rise of temperature, accompanied by a moderate polynucleosis; the site of inoculation showed a little induration, which soon subsided, and upon the sixth day was healed. Upon the twelfth day after the attempted re-inoculation the animal was vaccinated upon the back with points giving a positive reaction upon the rabbit's cornea. This vaccination failed to take. Animal allowed to survive.

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[1] The term "protopustule" (Pfeiffer) is used to designate the lesion developed a the site of inoculation.

Result of the experiment.—Production of variola inoculata (Virus I. *a*), with local lesion, the latter apparent on the fifth day, becoming fully developed on the ninth day, and gradually subsiding with final desquamation by crusts on the sixteenth day; fever from the fifth to the fourteenth day; leucocytosis on the third, and from the tenth to the nineteenth days. Immunity to re-inoculation (Virus I. *b*) and to vaccination.

SERIES I. — EXPERIMENT NO. 3.

Animal a young male Macacus. — Inoculated upon the belly with vesicle contents from a mild case of variola vera (Virus I. *b*). Temperature rose on the third day to 39.3° C. On the fourth day the site of inoculation showed evidence of reaction. At this time a second inoculation was performed with the same material as that employed in the first (Virus I. *b*). Temperature slowly fell during the third, fourth, and fifth days, rising again to 39.5° C. on the sixth day, forty-eight hours after the second inoculation, gradually returning to normal on the eleventh day. On the fourth day the animal showed marked constitutional reaction, with anorexia. This was again apparent upon the eighth day, the animal seeming weak and depressed. At this time the site of the first inoculation presented two areas one centimeter across and two to three millimeters in elevation, indurated, red, the center depressed and bearing a small, irregular crust; within one of these areas was a shotty papule. The second inoculation showed elevation and redness, with a few minute vesicles.

No observations upon the erythrocyte content of the blood were made during this experiment.

The white blood corpuscle count showed at the end of forty-eight hours after each inoculation a leucocytosis of about 30,000; differential count proved this to be essentially a polynucleosis, although throughout the experiment there was a gradual increase in the mononuclear elements of the blood.

Upon the eleventh day the animal was chloroformed. Autopsy showed nothing abnormal in the internal organs. Histological examination showed some cloudy swelling and fatty metamorphosis of the kidneys, proliferation of phagocytic cells in the axillary lymph nodes. The skin at the site of the first inoculation (protopustule eleven days after inoculation, seven days' development) showed crust formation, with superficial necrosis and degeneration of the epithelium beneath its middle portion; the epithelium contiguous to the edges of the crust showed some proliferation; no parasites were demonstrable in the newly-formed cells (Plate XVII., Fig. 2).

Result of the experiment. — Production of variola inoculata, two infections four days apart (Virus I. *b*); constitutional reaction; leucocytosis. No immunity from the first inoculation on the fourth day.

SERIES I. — EXPERIMENT NO. 4.

Animal a young female Macacus. — Inoculation upon two scarified areas on the abdomen with vesicle contents from a case of variola vera (Virus

I. *c*), subsequently proven to be of the abortive type. A second inoculation was made at the same time by linear incision upon the back, between the shoulders. The temperature rose in the following forty-eight hours two degrees to 39.5° C. On the fourth day the abdominal inoculation showed slight induration, peripheral flush, and a small central crust. On the fifth day both sites of inoculation showed well-marked reaction. The temperature was continuously elevated at 39° and 39.5°. Animal presented no constitutional symptoms. On the seventh day, numerous red, shotty papules appeared upon a shaved area of the back used for obtaining blood. Multiple lesions of the same general character were scattered over the trunk and the head. At this time curettings from one of the abdominal pocks were inoculated upon the rabbit's cornea with positive results.

White corpuscle count showed a very slight rise on the fourth day, followed by a somewhat more marked leucocytosis upon the seventh and the eighth days; differential count showed this increase to be shared in about equally by poly and by mononuclear elements.

Upon the eighth day after inoculation, and the fifth after the appearance of a local lesion, the animal was chloroformed.

Autopsy showed, in addition to the protopustules, about forty skin lesions in the form of nodular elevations, one to three millimeters in diameter, and distributed widely over the head, trunk, and extremities; all of these lesions were firm and shotty, the larger somewhat yellowish. The internal organs were normal.

Histological examination of the protopustule showed an extensive superficial crust, the tissue beneath which was infiltrated with leucocytes, the cells of the hair follicles at the edge of the lesion and those of the adjacent deep layers of the epidermis containing the variola parasite. The lesions of the general exanthem showed histologically a collection of leucocytes in and beneath the epidermis, together with necrosis, the cells of the included and contiguous hair follicles containing the parasite (Plate XXVII., Fig. 4).

Result of the experiment. — Production of variola inoculata, with protopustule and general exanthem (Virus I. *c*). Specificity proven by presence of organism.

SERIES I. — EXPERIMENT NO. 5.

Animal a full-grown female Rhœsus. — Inoculation by injection into the anterior jugular vein of a suspension in normal saline solution of about one cubic centimeter of the contents of a pustule from a case of variola vera (Virus I. *d*). Contents of the same pustule inoculated upon the rabbit's cornea gave a specific reaction. Upon the following day a large area of the back was closely shaved. Temperature at this time was subnormal; the animal seemed sick and refused to eat. Leucocytes 21,000 per cubic millimeter.

Upon the second day after inoculation the animal died. Autopsy showed between the muscles at the site of the incision in the neck thin, sanguineous fluid; smear from this exhibited many polynuclear leucocytes but no bacteria. The skin presented no eruption of any sort. Of the internal organs, the left lung showed a small patch of broncho-pneumonia; the liver, extensive central necrosis with hemorrhage; the kidneys, marked cloudy swelling and fatty metamorphosis. Bacteriological examination showed streptococcus in the heart's blood in pure culture, and in the wound of the neck a variety of other organisms. Histologically, the lung contained within the pneumonic area short chains of streptococci. No changes were present in the skin.

Result of the experiment. — Streptococcus septicemia; death in forty-five hours.

SERIES I. — EXPERIMENT NO. 6.

Animal a full-grown male Rhœsus. — Inoculation upon a scarified area of the back with contents of a pustule from a case of variola vera which gave a positive reaction upon the cornea of the rabbit (Virus I *d*). Temperature rose upon the following day one degree to 39° C., remaining at this point until the fifth day, when it increased to 40.3°. Upon the fourth day after inoculation the site of inoculation showed multiple areas somewhat elevated and indurated. No marked constitutional symptoms, the animal natural and eating well. Upon the sixth day small, secondary papules appeared in the immediate vicinity of the primary local lesions. Upon this day the animal was chloroformed.

White count showed a leucocytosis of 30,000 present before the beginning of the experiment, differential count showing this to be a polynucleosis; a fall in the leucocyte content occurred from the second to the fourth days, followed by a slight rise to about 15,000 on the fifth and sixth days, the rise mainly polynuclear.

Autopsy showed a normal condition of the internal organs. Cultures from the heart, liver, spleen, and kidney showed no growth.

Histological examination of the protopustule of the skin, sixth day after inoculation, the second of its development, showed a superficial crust, bordered on either hand by proliferated epithelium, and underlaid by an area of corium in which were hair follicles and glands in various stages of degeneration and diffuse infiltration with various migratory cells. The epithelial cells of the follicles under the center of the lesion, and those more towards the border of the lesion and in the adjacent epidermis, showed the variola organism.

Result of the experiment. — Production of variola inoculata with fever, but no apparent constitutional reaction (Virus I. *d*); specificity of inoculation proven by the presence of the variola organism in the protopustule.

SERIES II. — EXPERIMENT NO. 1.

Animal a young male Rhœsus. — Multiple, light incisions on the skin of the abdomen were inoculated with a paste made by grinding up with sterile salt solution a number of eight months old disks from a case of confluent variola vera. At the same time a rabbit was inoculated on both corneas with the material, which produced a typical variolous keratitis. The monkey showed a slight transient rise of temperature on the second day after the inoculation. No constitutional symptoms were noted. The inoculation sites showed no reaction save those incident to repair of the slight incisions. On the fifth day after this unsuccessful inoculation with disk paste a fresh area on the skin of the abdomen was inoculated with virus from a mild case of variola vera (Virus II. *a*). During the succeeding seven days the temperature ranged between 37.8° and 39° C. The inoculation sites showed, on the second day, slight elevation without redness. On the fourth day the lesion exhibited, in addition to the above, a peripheral flush. On the seventh day there was a well-developed central crust bordered by a pale elevation which merged peripherally into a narrow zone of redness. The axillary lymph nodes were distinctly enlarged during the active evolution of the lesion, and that on the right side was tender. No constitutional symptoms were observed. The lesions in this animal did not attain the size of those in the animals of the preceding experiments where Virus I. was used. On the seventh day after inoculation the animal was chloroformed and an autopsy done at once.

Autopsy. — Skin lesions as above. Considerable edema of the areola tissue beneath the inoculations. Axillary lymph nodes enlarged and red. On section the cut surface yields turbid blood-stained fluid. All internal organs appear normal.

Histological examination. — Inoculation sites. — The epidermis presents a lenticular cavity divided into smaller, irregular spaces by strands of compressed and degenerated epithelial cells. The vesicle is bounded above by cornified epithelium and inspissated exudate, below by the more or less degenerated cells of the rete, and at the sides by proliferated epithelial cells. Polynuclear leucocytes are present in large numbers in the vesicle, in the surrounding epithelium, and in the underlying tissue. The variola organism is present in the cells bordering on the vesicle.

Axillary lymph nodes. — The sinuses contain many lymphoid cells and phagocytes. The latter frequently include red corpuscles in their protoplasm. Inguinal and mesenteric lymph nodes normal. Lungs, liver, spleen, kidney, testicle, and bone marrow examined and found normal.

Result of the experiment. — Failure to produce a lesion by inoculation with "disk paste" which did produce a variolous keratitis in the rabbit. Subsequent production of a mild protopustule by inoculation with Virus II. *a*. Specificity of lesion proven by finding organism in the epithelia cells of the lesion.

SERIES II. — EXPERIMENT NO. 2.

Animal a young male Rhœsus. — Inoculated in six places on the skin of the abdomen with virus from a case of variola vera (Virus II. *b*). Lesions developed which were identical with those of the previous experiment. The animal was allowed to survive. Nine days after the first inoculation the procedure was repeated, this time with a new strain of virus (Virus III. *a*). This virus was clear vesicle contents from a case of mild variola vera. No reaction followed the second inoculation. The potency of the virus was shown by the results of Experiments III. 1 and III. 2, in which the same was used.

SERIES II. — EXPERIMENT NO. 3.

Animal a young male Rhœsus. — Inoculated at the same times and with the same materials as the previous animal (Experiment 2).

Result of the experiments. — Successful inoculation of both animals with Virus II. *b* with the production of a mild protopustule. Development on the ninth day after inoculation of an immunity to a virus of proved virulence. Specificity of lesion proven by immunity.

SERIES III. — EXPERIMENT NO. 1.

Animal a young male Rhœsus. — Inoculated on the abdomen with virus from a case of mild variola vera (Virus III. *a*). The temperature rose to 39° C. on the third day after inoculation, reaching 40° C. on the fourth and fifth days. On the second day the sites of the inoculations were elevated and indurated, but showed no change of color. The next day a pink flush was seen about the lesions, and the axillary lymph nodes were enlarged. On the two succeeding days the lesions became larger, the center exhibited a crust bordered by elevated epithelium, surrounded in turn by a red areola. On the fifth day the individual lesions measured eight millimeters across, and the axillary lymph nodes were enlarged and tender. The animal was chloroformed and an autopsy made at once.

Autopsy. — Skin lesions as above. The areolar tissue beneath the lesions edematous. Axillary lymph nodes enlarged and red. On section a turbid, blood-stained fluid flows readily from the cut surface. Inguinal lymph nodes normal. All internal organs appear normal.

Histological examination. — Skin lesions. — There is a narrow crust beneath which is a cavity containing finely granular material, in which are a moderate number of polymorphonuclear leucocytes and of degenerated epithelial cells, together with a loose network of fibrin. The vesicle is bounded laterally by proliferated epithelium, and below by the more or less degenerated cells of the rete. There is a moderate infiltration of the corium and of the epithelium adjacent to the vesicle, with polymorphonuclear leucocytes. Many stages of the variola organism are present, both protoplasmic and nuclear forms being represented (Plate XXVII., Fig. 1).

Lungs, spleen, liver, kidney, testicle, and bone marrow show no lesions. The sinuses of the axillary lymph nodes contain many endothelial cells which are frequently phagocytic. The inclusions are, in the main, red blood corpuscles.

Result of the experiment. — Production of protopustule by inoculation with Virus III. *a*. Specificity of the lesion proven by the finding of the organism.

SERIES III. — EXPERIMENT NO. 2.'

Animal a young female Rhœsus. — Inoculated on the skin of the abdomen with vesicle contents from a mild case of variola vera (Virus III. *a*). On the second day after the inoculation the temperature rose to 39° C., and ranged between that and 40.6° C., until the end of the experiment. On the second day after the inoculation the incisions showed a slight elevation with a faint, pink flush. On the succeeding days the lesions enlarged, showing a depressed crust on the fifth day, at which time the elevated margin and the red areola were most marked. After the eighth day the lesions did not increase in size. From the fourth day onward the axillary lymph nodes were enlarged and tender. On the eighth day two shotty nodules were noted, close together, and one centimeter from the primary lesion, in the direction of the left axilla. Two small vesicles were present on the anterior aspect of the pubes, one on the back, in the lumbar region, one on the inner aspect of the right arm, one on the inner aspect of the left fore-arm, and two on the lower jaw. In all, nine papules or vesicles were found. The animal was chloroformed the next day, the ninth after inoculation, and an autopsy made at once. There had been no further development of the exanthem, and the primary lesions had not enlarged.

Autopsy. — Skin lesions as above. Rabbit's cornea, inoculated from the primary lesions, developed a typical variolous keratitis. Axillary lymph nodes enlarged and red. On section, the surface yields turbid, blood-stained fluid. Rabbits' corneas inoculated from scrapings of the node showed no reaction. Inguinal lymph nodes normal. Lungs show advanced tuberculosis with cavity formation. Bronchial lymph nodes, spleen, and kidneys also the seat of tuberculosis. Rabbit's cornea inoculated with scrapings from the spleen pulp showed no reaction. Other organs appear normal.

Histological examination. — Primary lesion presents a thick crust lying upon the corium. On either side the epidermis is thickened, and in the cells are various stages of the variola parasite. The tissue of the corium is infiltrated with leucocytes, and the epithelial cells of the sheaths of the included hair follicles are more or less degenerated, and also contain the variola parasite.

Exanthem presents a small crust bordered by proliferated epithelium. Beneath the crust is a small mass of polymorphonuclear leucocytes, which cells are also infiltrating the adjacent corium. The variola parasite is present in the epithelial cells at the edge of the lesion.

Axillary lymph nodes. — Sinuses contain many phagocytic cells, which include both polymorphonuclear leucocytes and red blood corpuscles. Inguinal and mesenteric lymph nodes normal. Lungs, spleen, and kidneys show tuberculosis. Liver presents fatty infiltration at the periphery of the lobules. Bone marrow and testicle normal.

Result of the experiment. — Production of protopustule followed by a general eruption, as the result of inoculation with Virus III. *a*. Specificity of lesions shown by the presence of the variola parasite and by corneal inoculations. Failure to demonstrate the contagium in the axillary lymph nodes and in the spleen pulp.

SERIES III. — EXPERIMENT NO. 3.

In this experiment a virus was used having the following history: A rabbit's cornea was inoculated with scrapings from the primary lesion of the animal in Experiment 2 of this series. The inoculation was made on the ninth day of the evolution of the lesion. A typical variolous keratitis developed. After forty-eight hours part of the lesion was scraped from the cornea and divided into two portions. One portion was inoculated on the skin of a calf, but produced no lesion. The second portion was inoculated on the cornea of a second rabbit producing a typical variolous keratitis. After seventy-two hours a part of the lesion of this second rabbit was scraped off and transferred to the cornea of a third rabbit, producing there a typical variolous keratitis. After seventy-two hours this lesion on the cornea of the third rabbit was transferred to the cornea of a fourth rabbit, producing a typical variolous keratitis. After seventy-two hours the lesion on the cornea of this fourth rabbit was scraped off and divided into two portions. One portion was transferred to the cornea of a fifth rabbit, producing a typical variolous keratitis. The second portion was used to inoculate two small scarifications on the skin of the abdomen of a young male "hooded" monkey (Calcutta). Following this inoculation no rise of temperature was noted. The animal took nourishment well until the fifth day, when a slight anorexia was manifest. Coincident with this a profuse diarrhea developed. On the third day after inoculation the scarified areas were pink and slightly elevated. At this time the axillary lymph nodes were palpable. On the succeeding days the lesions enlarged, attaining on the seventh day a diameter of one centimeter, and presenting a depressed central crust bordered by an elevated margin which in turn merged into a red areola. With the development of the lesions, the axillary lymph nodes were notably enlarged and tender. The inguinal lymph nodes remained normal. On the seventh day after inoculation an eruption was observed consisting of five papules and vesicles. These lesions were situated as follows: one on anterior aspect of right thigh, one on right shoulder, one on right cheek, two on lower lip. Besides these cutaneous lesions the upper surface of the tongue presented three circular losses of substance about two millimeters in diameter. The animal was chloroformed and an autopsy done at once.

Autopsy. — Skin lesions as above. Material from primary lesion and from vesicle on the lip inoculated on rabbits' corneas. (In both animals a typical variolous keratitis developed.) On section the areola tissue beneath the primary inoculation edematous. Axillary lymph nodes enlarged and red; on section the surface yields turbid, blood-stained fluid. Scrapings from the surface of section of the node inoculated on a rabbit's cornea. A typical variolous keratitis developed. Inguinal lymph nodes normal. All internal organs appear normal.

Histological examination. — Primary lesion presents a crust bordered by thickened epithelium and underlaid by corium. Many polymorphonuclear leucocytes are invading the tissues about the lesion. The variola parasite is present in the epithelial cells at the edge of the crust. Exanthem. — There is a small cavity in the epidermis bounded above by the cornified epithelial cells, below and at the sides by the proliferated epithelium. The vesicle contains many polymorphonuclear leucocytes and much granular precipitate. The variola parasite is present in the epithelial cells about the lesion (Plate XXVII., Fig. 3). Tongue. — The cells of a small segment of the epithelium are more or less degenerated, and the tissue there and beneath the lesion is infiltrated with polymorphonuclear leucocytes. Cytoplasmic stages of the organism are present in the epithelial cells. Axillary lymph nodes. — The sinuses contain a number of polymorphonuclear leucocytes and of red blood corpuscles. These cells are frequently included in the protoplasm of phagocytic endothelial cells which are present in large numbers. Lungs, liver, and testicle normal.

Result of the experiment. — Production of a protopustule, followed by a general eruption on the seventh day, by inoculation with Virus III. *a* which had been transferred through one monkey and four rabbits. Specificity of lesion proven by corneal inoculation and by the presence of the variola parasite.

IV. DISCUSSION OF RESULTS.

From a review of these experiments it is apparent that positive results were obtained in ten out of twelve. The findings in these ten cases of successful inoculation may be grouped for discussion under the head of: (1) the course of the disease; and (2) the histology of the skin lesions.

1. The course of the disease offers for study the occurrence, the nature, and the degree of constitutional reaction; the character, the evolution, and the specificity of the skin lesions; and the disturbances of the leucocyte equilibrium.

Constitutional reaction was evident mainly by fever; some anorexia and restlessness were apparent in three of the experiments.

Fever began in one case on the first day, in one on the fifth day, and in three cases on the second day after inoculation; reached its maximum from the fourth to the eighth day; and, in those experiments sufficiently prolonged to permit of observation, declined by lysis, temperature becoming normal by the end of the second week. The extent of the temperature elevation averaged 2.3° C. The period of most marked fever coincided with that of the full development of the local lesion. Anorexia when present was most marked at about the fourth or fifth day.

The specific skin lesions consisted in a primary pock (protopustule), developed at the site of inoculation, and present in all ten of the cases; in secondary lesions, developed in the vicinity of the primary, present in two cases; and in a general papular eruption, in addition to the primary lesion, present in three cases.

The primary lesion was first evident upon the third day. In some animals no reaction was observed till the fifth day. Its full evolution was observed in four experiments. It is first evidenced by induration of the site of inoculation, the edges of the indurated region showing a faint pink color. The lesion gradually increases in extent and in elevation, reaching maximum development by about the eighth day, when it presents as a hemispherical, elevated, indurated area, from one to three centimeters in diameter, the center bearing the crust is surrounded by a pink areola. At a somewhat earlier stage, small vesicles containing clear fluid may be detected at the center of the lesion. The swelling gradually subsides, the vesicles dry up, and by the end of the second week desquamation of the primary crusts takes place.

The specificity of the skin lesions may be asserted upon the basis of a variety of data. Thus: Immunity to variola, to vaccinia, or to both conferred by inoculation; positive reactions when curettings from the lesions were inoculated on the rabbit's cornea; the presence of the variola organism on histological examination.

Disturbance in leucocyte equilibrium is manifest in the course of the disease. It seems probable that a transitory leucocytosis upon the second or third day after the inoculation, followed by a second leucocytosis at about the height of the development of the local lesion, is characteristic. These conditions prevailed in two experiments. There are good reasons for believing that in the second leucocytosis mononuclear elements play an important part, a condition, which, if true, may, perhaps, be interpreted as indicative of disturbance in cell differentiation in the bone marrow. We regard our data upon this point as inadequate for generalization.

2. The histological study of the specific lesions of the skin brought out points of difference, structurally, between the lesions of the exanthem in man and the analogous lesions in the monkey. The lesions of the monkey differ from those in man principally in that more polymorphonuclear leucocytes are present. The structure of the vesicle and of the pustule is developed at the site of inoculation practically the same as that of the lesions of the exanthem in man in variola vera. The lesions of the exanthem in the monkey resemble most closely the skin lesions in man seen in abortive variola. The process of healing differed in no wise from that observed in man. The minute study of the lesion, with respect to the presence and distribution of the etiological factor, forms the subject of another paper.

One animal was shown to be immune to both variola and vaccinia after successful inoculation with Virus I. Two animals were shown to be immune to variola (Virus III.) after inoculation with Virus II. These results are in keeping with those of other investigators.

The comparison of the lesions produced by inoculation with the different strains of virus from different epidemics is of especial interest, and seems to us to be suggestive. The mild lesions produced by inoculation with Virus II. contrasted sharply with the severe ones which followed inoculation with Virus I. or Virus III. The epidemic from which Virus II. was obtained was of a clinically mild type of the disease, while the epidemics from which Virus I. and Virus III. were obtained were severe.

A generalized eruption was observed three times in the eight animals who were successfully inoculated, and who were allowed to survive long enough to show the eruption. Of these eight animals three were inoculated with Virus II., and none showed a general eruption, while of the remaining five monkeys inoculated with Virus I. or Virus III., three developed the exanthem.

In two instances the monkeys failed to react to inoculation with the variola disk. In one of these cases the animal was subsequently shown to be susceptible to variola. The disk used was active when inoculated on the rabbit's cornea. We are unable to explain these results, and propose to make this the subject of further investigation.

The results obtained in the last experiment (Series III., No. 3), where a monkey was successfully inoculated with a virus that had been transferred from one rabbit's cornea to another for four generations, indicates a line of work that promises much. If the development of an exanthem in the monkey postulates variola rather than vaccinia, we have succeeded in passing variola through the tissues of the rabbit, a herbiverous animal, without its becoming vaccinia.

In one experiment, by corneal inoculation on the rabbit, the presence of the contagium was demonstrated in the axillary lymph node.

V. CONCLUSIONS.

1. Macacus and Rhœsus monkeys are susceptible to variola inoculata.

2. The disease in these animals pursues a typical course, with more or less constitutional reaction and with fever, and presents lesions of the skin consisting ordinarily of a primary pock at the site of inoculation, sometimes with local secondary lesions, and less commonly with an associated general exanthem.

3. The disease is accompanied by a disturbance in leucocyte equilibrium characterized by a polymorphonuclear, followed by a mononuclear leucocytosis, the full significance of which cannot be interpreted from the amount of data at our disposal.

4. The disease produced in the monkey by inoculation with variola virus is not identical with variola vera in man; it agrees with variola inoculata in man.

5. The lesions contain, within the epithelial cells of the epidermis and of the hair follicles, *Cytoryctes variolæ.*

6. Successful inoculation with variola confers immunity to subsequent inoculation with variola or with vaccinia.

7. Different strains of virus from different epidemics exhibit different degrees of virulence for the monkey. Virus from epidemics of clinically severe variola produces more severe lesions and is more likely to be followed by a general eruption. No differences were noted in the virulence of different strains of virus from cases of varying severity in the same epidemic.

8. Variola virus can be transferred from man to a monkey, from the monkey to the rabbit's cornea through four generations, and when then inoculated on the monkey can produce a protopustule followed by a general exanthem.

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DESCRIPTION OF PLATE.

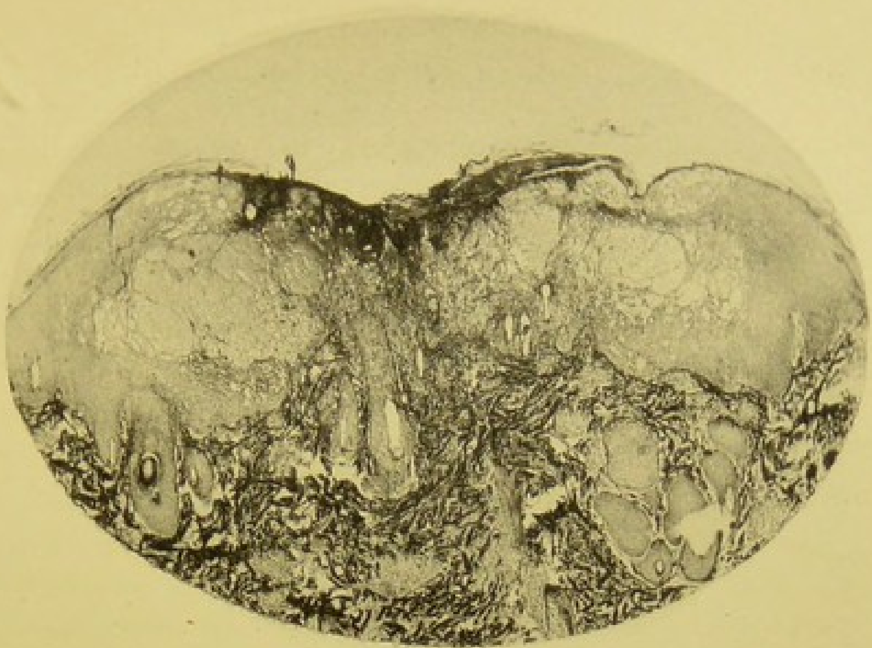
Explanation of Plate XXVII.

Fig. 1. Site of inoculation, fifth day of disease. Experiment No. 1, Series III.

Fig. 2. Site of inoculation, eleventh day of disease. The epidermis has grown under the crust. Experiment No. 3, Series I.

Fig. 3. Exanthem, first day of its development, seventh day of disease. Experiment No. 3, Series III.

Fig. 4. Exanthem, second day of its development, eighth day of the disease. Experiment No. 2, Series III.



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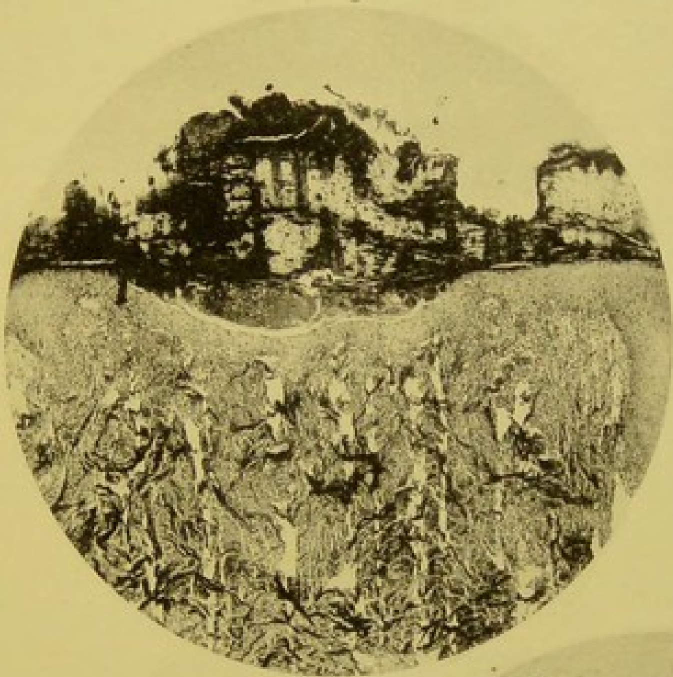

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THE LEUCOCYTE REACTION IN VARIOLA.

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The studies of the leucocyte reactions in variola which form the basis of this paper were made by the investigators in residence at the small-pox hospitals, in connection with their general study of the disease. The majority of the observations were made by Dr. Magrath, his work being supplemented by that of Drs. Bancroft and Brinckerhoff. We are indebted to Dr. Tyzzer for a number of leucocyte determinations and differential counts. Our general plan has been to analyze the work already published on this subject in the light of our own observations. For this reason it has seemed better to present a summary of our data than to give tabulations of all our leucocyte counts. In discussing the leucocyte reactions of each type of the disease we describe in detail a case which we consider a good example of the type.

GENERAL REVIEW OF PREVIOUS WORK.

A careful survey of the literature of variola has yielded us but twelve articles which deal with the leucocyte reactions in this disease. In only three of these (Courmont and Montagard, 1900; Weil, 1901; Ferguson, 1903) are the qualitative variations of the leucocytes considered.

Brouardel (1874) seems to have been the first to systematically investigate the variations of the leucocytes in variola. We regret that we have been unable to find a publication in which his actual leucocyte counts are given. He states that the leucocytes are increased during the febrile remission between the periods of eruption and of suppuration. He found them diminished during suppuration, an increase at this time or during desiccation signifying to him secondary pyogenic infection.

Verstraten (1875) examined the blood in eleven cases of variola, and concluded that the number of white corpuscles is increased in this disease and that the increase is proportional to the severity of the case. He found that leucocytosis was most marked in the hemorrhagic types of the disease, and that the leucocytosis of variola vera reached its maximum during the stage of suppuration and during desiccation, after which it returned to normal, providing that no complications intervened.

Halla (1883) reports a single leucocyte count of sixteen thousand five hundred in one case of variola vera on the fourth day of the disease. He remarks that there is a leucocytosis in the stage of suppuration. Although he gives no figures, he indicates that he observed this in eleven cases.

Pee (1890) reports a single leucocyte count of twenty thousand in a case of variola vera during the early part of the stage of eruption.

Sobotka (1893) had the good fortune to observe the leucocytes in three cases of variola, previous to the onset. His fourth case came under observation on the fourth day of the disease. He regards his data as insufficient for generalization, but points out certain relations of the leucocyte reactions in variola to those observed during the prodromal stage of certain other infectious diseases. He found a prodromal leucocytosis in variola, followed by a hypoleucocytosis which preceded or accompanied the initial fever.

Pick (1893) studied the leucocyte reactions in forty-two cases of variola. He concluded that infection with variola in itself caused no leucocytosis. He attributed the leucocytosis in the stage of suppuration to secondary pyogenic infection. He found leucocytosis absent in fatal cases.

Von Limbeck (1896) reports leucocyte counts from two cases of variola. He disagrees with Pick's conclusion that the leucocytosis is due to infection with pyogenic organisms.

Hayem (1899) found a leucocytosis beginning on the second or third day of the eruption; if the lesions are discrete the leucocytes may rise to from ten thousand to sixteen thousand per cubic millimeter on the third or fourth day and from twenty-eight thousand to thirty thousand during suppuration. He found the leucocytes much increased; in one case of the hemorrhagic type of the disease he observed a leucocytosis of thirty-two thousand.

Courmont and Montagard (1900) report their findings in twenty-nine cases of variola. They conclude that variola is accompanied by a leucocytosis, and from their qualitative studies they found this leucocytosis to be mononuclear in type, and believed it to be of diagnostic importance. In the same year these authors published a second communication based upon the study of fifteen new cases, their results being in accordance with those given in their first paper.

Weil (1901) has published the most exhaustive work upon this subject, based upon the study of twenty-four cases. He concluded that in variola there is an increase in the number of leucocytes which may be temporary and slightly marked in the varioloids, considerable in the suppurative types, insignificant or absent in the hemorrhagic cases. He found that the acme of the leucocytosis is at the time of the appearance of the eruption, or at vesiculation; that the typical leucocytosis is modified more or less by intercurrent infections. He considers the study of the leucocytes in variola to be of considerable diagnostic importance and of some value in prognosis. Weil also studied the hematopoietic organs in variola.

Ferguson (1903) presents results based upon sixteen cases of variola. He concludes that variola is always accompanied by a leucocytosis, which reaches its acme at about the ninth day of the disease, decreasing from that time onward unless complications set in. The leucocytosis is most marked in the severe suppurative cases. The qualitative changes consist in an increase in mononuclear elements, with a relative, though not absolute, decrease in the polymorphonuclear neutrophiles. He finds this characteristic leucocyte formula persisting for some time during convalescence.

Summary of literature.— All observers agree that variola is accompanied by an increase in the number of leucocytes The only points on which there is not entire accord have to do with the time or times at which the leucocytosis occurs and the cause to which it is due. Verstraten, Pick, Hayem, Weil, and Ferguson agree in saying that in variola vera the degree of the leucocytosis is, in general, proportionate to the severity of the case. All observers find the leucocytosis at its height at some time during the active stage of the eruption. The influence of secondary pyogenic infections upon the leucocyte count in causing still greater increase is noted by Pick, Weil, and Courmont and Montagard. The same authors record a terminal hypoleucocytosis in fatal cases. Pick stands alone in attributing the leucocytosis of variola to pyogenic infection, a view which has been controverted by all subsequent observers. Courmont and Montagard, Weil, and Ferguson agree that the leucocytosis of variola is characterized by an increase in the mononuclear cell types, and that many of these cells are members of the leucocyte series which do not normally occur in the circulation. Sobotka seems to have been the only one who has studied the leucocytes during the incubation period, finding then a hyperleucocytosis which was followed by a hypoleucocytosis accompanying the onset. Hayem's results supplement the latter statement.

In one type of the disease the findings of different observers are not in agreement. We find that Verstraten and Hayem state that the hemorrhagic cases are accompanied by a hyperleucocytosis, while Weil and Ferguson hold, on the contrary, that in this type of the disease the leucocytes are normal or diminished. We note that in the single hemorrhagic case cited by Courmont and Montagard, in which there was no eruption, the leucocytes were much increased in number.

METHOD AND RESULTS.

Technic.— In the course of this investigation the leucocytes were examined in fifty cases of variola. In only twelve of these, however, were serial observations made, *i.e.*, four or more consecutive counts. In the remaining thirty-eight cases the counts were scattering. The cases were distributed among the different types of the disease as follows:

Variola vera with recovery, thirty-one; variola vera fatal, thirteen; variola pustulosa hemorrhagica, two; purpura variolosa, four.

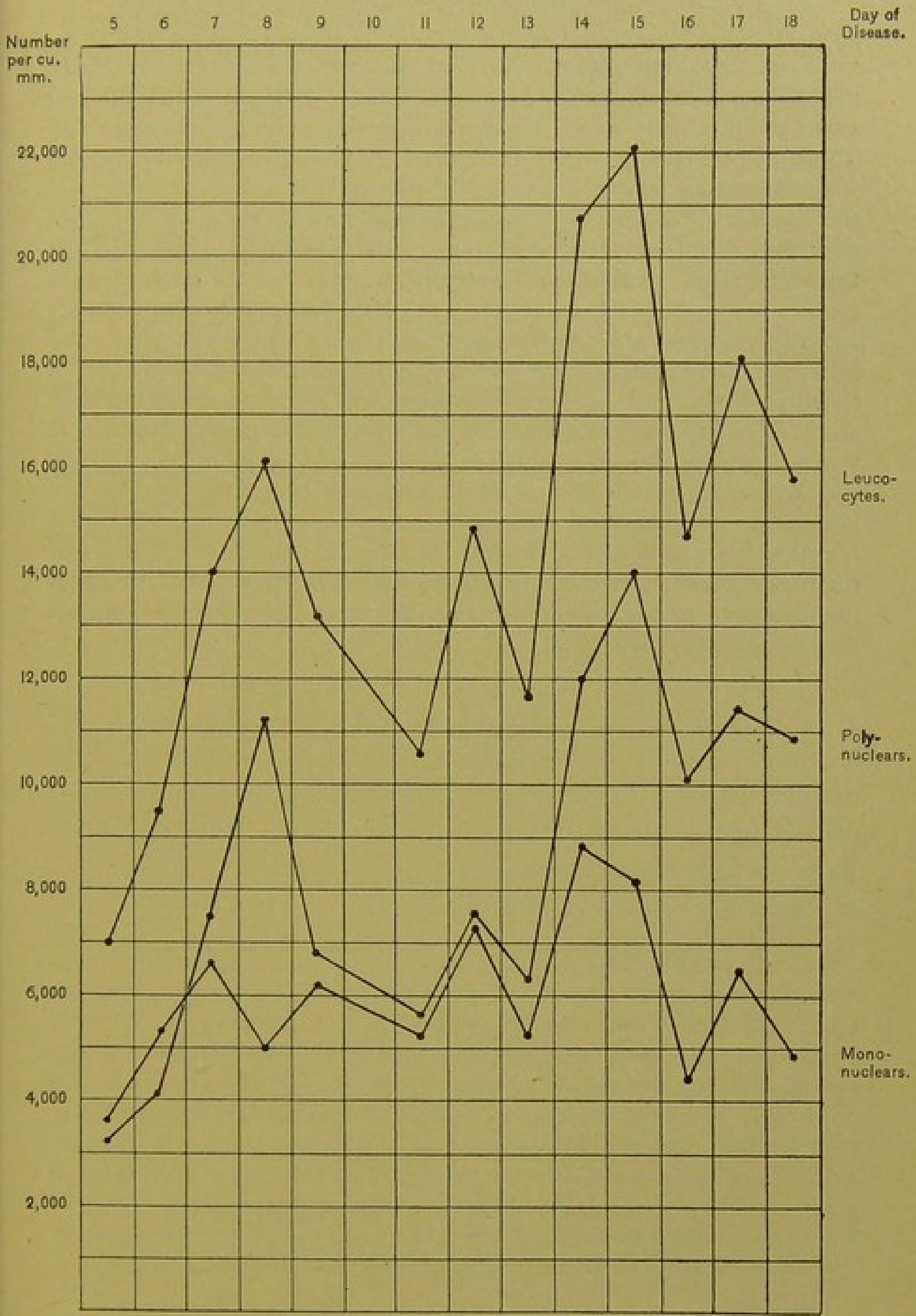
The blood was obtained from the lobe of the ear, and the leucocyte determinations were made with the Thoma-Zeiss apparatus. Differential counts were made from dried smears stained by Wright's method. For cell study, special blood stains were also used. For analyzing the qualitative changes in the leucocytes it has seemed best to adopt a comprehensive classification. In tabulating our results, the absolute number of mononuclear and of polynuclear leucocytes per cubic millimeter are given. These figures are computed from the number of leucocytes per cubic millimeter, and the percentage of mononuclear and polymorphonuclear cell types found by counting two hundred consecutive cells in a stained smear. The term "mononuclear leucocyte" includes both large and small lymphocytes, large mononuclear leucocytes, the various myelocytes, and the cells of Turk. The polymorphonuclear leucocytes include the neutrophiles, the eosinophiles, and the mast cells.

Findings.— Variola vera.— We shall consider here the results of our examination of the leucocytes in variola vera, treating separately the data derived from fatal and from non-fatal cases.

In a typical severe variola vera, in which the lesions become confluent on the face, and which runs its course to recovery without complications, the leucocyte curve shows, as a rule, a normal number during the febrile remission, followed by a rise reaching its acme on about the eighth day of the disease, then declining slightly, to rise again from the twelfth to the fourteenth day, finally returning to normal during convalescence. During the remission from eight to ten thousand leucocytes per cubic millimeter will be found; at the height of the eruption a leucocytosis of from twelve to sixteen thousand per cubic millimeter. During the early stages of desquamation the leucocytes may rise to from sixteen to twenty thousand per cubic millimeter. We have found the "mononucleosis" mentioned by Courmont and Montagard, Weil, and Ferguson to be present in our cases to a greater or less extent, but it has seemed to us that the variations in the leucocyte count were most actively influenced by absolute variations in the numbers of the polymorphonuclear neutrophiles. We have found the relative hypoleucocytosis during the remission of the fever to be due in large part to an absolute decrease in the polymorphonuclear neutrophile cells. The leucocytosis accompanying the development of the eruption is also dependent largely upon an increase in these cells. The same may be said of the leucocytosis observed during desquamation. We agree with previous workers in recognizing that the leucocytosis of variola is characterized by an increase in the mononuclear elements, but we find that in variola, as in other diseases accompanied by a leucocytosis, the variations in the total leucocyte count are brought about pre-eminently by fluctuations in the absolute number of polymorphonuclear neutrophiles. The following case will serve to illustrate these points:

CASE I. — Male, age thirty-seven. Severe variola vera, confluent on face. No clinical complications noted. Onset February 22. Eruption appeared February 24–25. First examination on fifth day of disease. Under observation until fifteenth day. The following table shows the results of the leucocyte determination:

Observation.	Day of disease.	Leucocytes per cu. mm.	Mononuclear per cu. mm.	Polymorphonuclear per cu. mm.
1	5	7,000	3,700	3,300
2	6	9,500	5,300	4,200
3	7	14,000	6,600	7,400
4	8	16,200	4,900	11,300
5	9	13,200	6,300	6,900
6	11	10,600	5,100	5,500
7	12	14,700	7,350	7,350
8	13	11,600	5,300	6,300
9	14	20,800	8,800	12,000
10	15	22,200	8,200	14,000
11	16	14,600	4,500	10,100
12	17	18,100	6,600	11,500
13	18	15,700	4,900	10,800


Day of Disease.
5 6 7 8 9 10 11 12 13 14 15 16 17 18
Number per cu. mm.
22,000
20,000
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
Leucocytes.
Polynuclears.
Mononuclears.

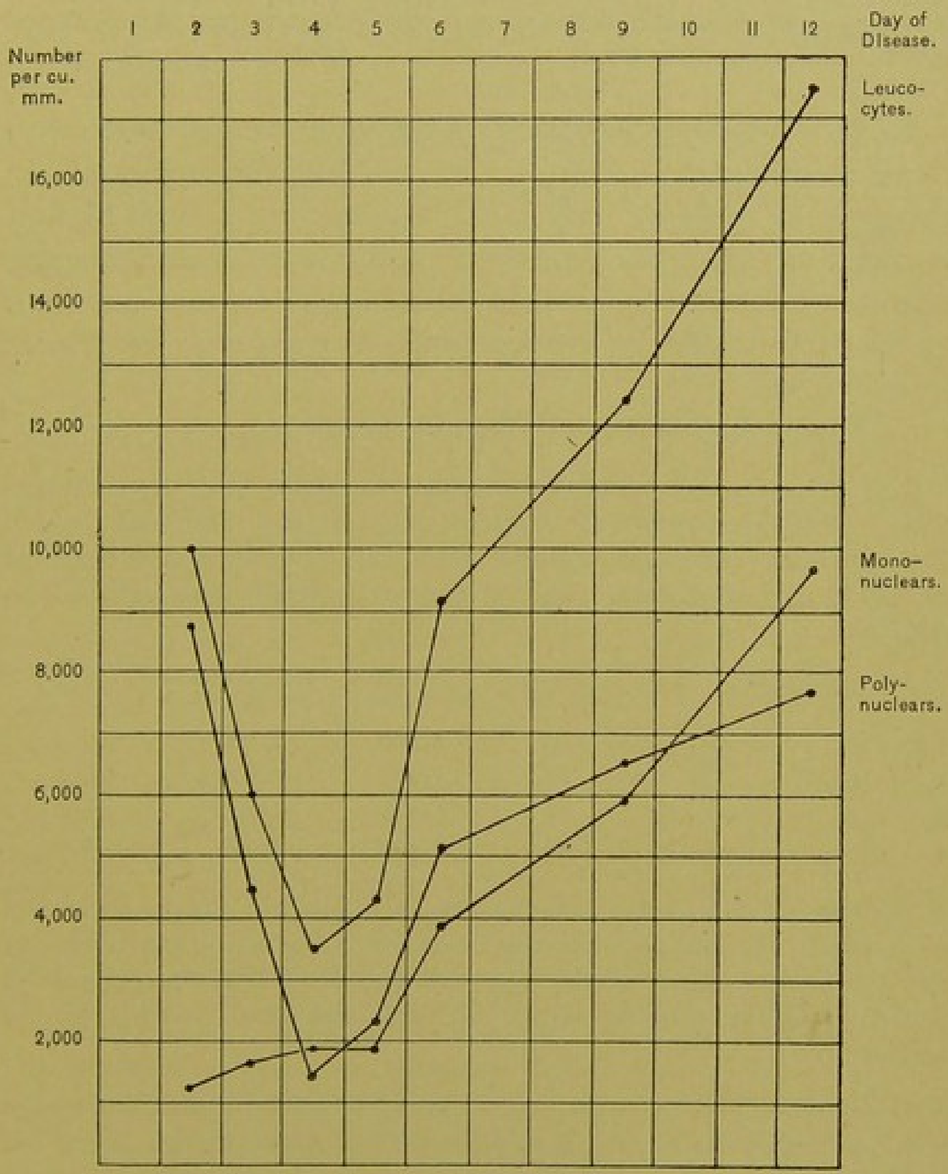
CASE I.

Summary.— The observations on the leucocytes begin on the fifth day, soon after the appearance of the eruption. From this time until convalescence was established, the total number of leucocytes per cubic millimeter remained above the normal. This leucocytosis is divisible into two periods, during which there was a marked increase above normal. These two periods of hyperleucocytosis are separated by an interval in which there is a notable decrease from the maximum, but not a return to the normal. The first hyperleucocytosis reaches its acme on the eighth day; the second, on the fifteenth day. The first rise is at its height at the beginning of pustulation, the second, at the end of the active stage of the eruption. An analysis of the leucocyte count shows that both the hyperleucocytoses are predominantly polymorphonuclear in type, the intervening diminution being due largely to a decrease in the number of the same type of cells. The mononuclear cells are increased throughout, and play an important rôle in the second hyperleucocytosis. This case is selected to show the disturbances in the leucocyte equilibrium in a severe case of variola without clinical manifestations of complications. We see in this case evidence of the characteristic "mononucleosis" of variola, but the picture is not so clear as in Case II., since it is complicated by quantitative disturbances of the polynuclear cells. We have here evidence of two polymorphonuclear leucocytoses each preceded by a polymorphonuclear hypoleucocytosis.

In very mild cases of variola vera we have found the leucocytes not to exceed normal limits. In cases of mild discrete variola the leucocytes may show only a gradual increase during the evolution of the eruption. In such cases mononuclear leucocytosis may be very marked. The following case is given as an example:

Case II. — Mild variola vera. Recovery without complications. Female, aged six years. Onset Dec. 18, 1902. Eruption appeared Dec. 21, 1902. Discharged well Dec. 31, 1902. Never vaccinated. Case under observation on the evening of the second day of the disease. Onset severe, with delirium and convulsions. Remission on the evening of the third day. From that time on no clinical symptoms were observed save the eruption. The skin lesions were few in number, about one hundred in all, and were principally on the face and the legs. Evolution of lesions typical of variola vera, the stage of suppuration, however, being of brief duration and mild in character. There were no suppurative sequelæ. The following table shows the results of the leucocyte determinations:

Observation.	Day of disease.	Leucocytes per cu. mm.	Mononuclear per cu. mm.	Polymorpho-nuclear per cu. mm.
1.	2	10,000	1,300	8,700
2.	3	6,100	1,600	4,500
3.	4	3,400	1,900	1,500
4.	5	4,200	1,900	2,300
5.	6	9,100	3,900	5,200
6.	9	12,500	5,900	6,600
7.	12	17,400	9,700	7,700



CASE II.

Summary.— The leucocytes show a marked diminution during the period of remission, followed by a gradual return to normal which passes into a hyperleucocytosis. The initial count on the second day of the disease must be regarded as within normal limits for the child. An analysis of the qualitative variations shows that the initial count is normal, or perhaps, for a child, tending slightly to a polynuclear leucocytosis. The hypoleucocytosis during the remission is due to an absolute diminution in the number of polymorphonuclear forms. The subsequent hyperleucocytosis is participated in by both the mononuclear and the polymorphonuclear cells, although the former take so large a part that one is warranted in calling it a mononuclear leucocytosis. We consider this as a typical example of the disturbance of the leucocyte equilibrium caused by the variola organism acting alone.

In fatal cases of variola vera our observations agree with those of others in showing a terminal hypoleucocytosis. In this type of cases we have found the leucocyte count highest at the time when the remission of the fever is due, ranging from ten thousand to fourteen thousand per cubic millimeters. Cases complicated by a pneumonia may show no coincident leucocytosis.

The following cases are given as examples:

Case III. — Severe variola vera. Fatal on twelfth day. No complications. Male, aged forty-five. Vaccinated in infancy. Poor scar. Onset Feb. 27, 1902. Eruption appeared March 1. Leucocytes first observed on ninth day of disease. The leucocyte determinations resulted as follows:

Observation.	Day of disease.	Leucocytes per cu. mm.	Mononuclear per cu mm.	Polymorphonuclear per cu. mm.
1.	9	5,200	2,500	2,700
2.	10	5,900	2,800	3,100
3.	11	6,100	2,800	3,300
4.	12	6,400	1,900	4,500

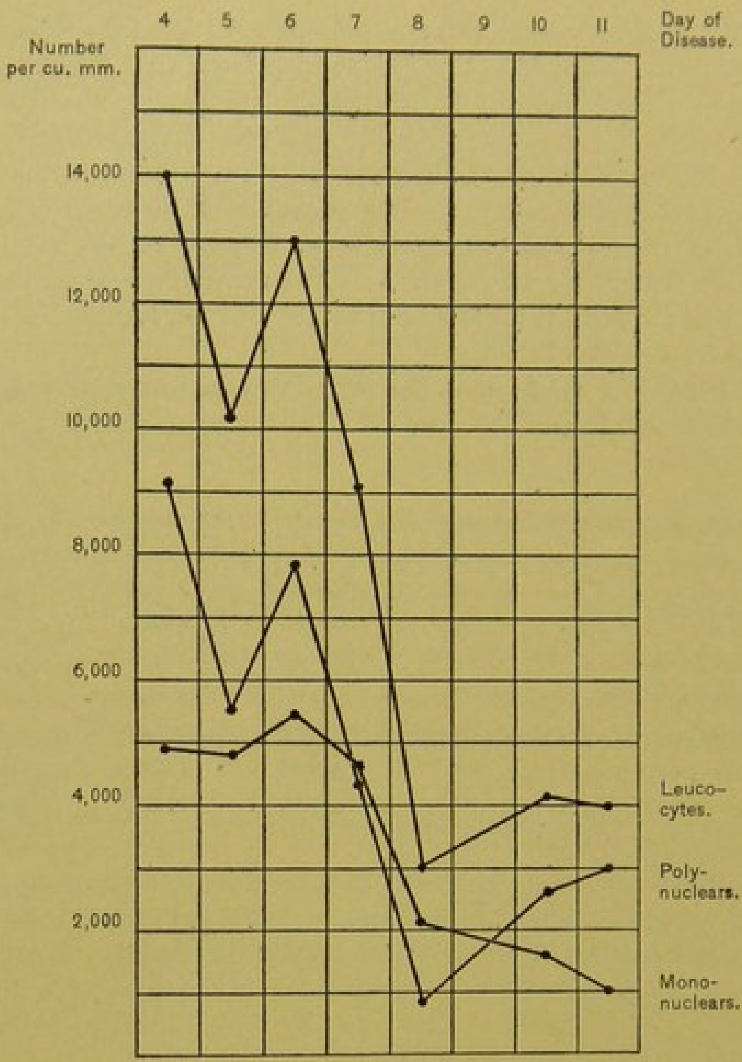
The case yielded the following anatomical diagnosis: Cutaneous lesions of variola in the late pustular stage, erosions of the mucous membrane of the soft palate, edema of the lungs, acute interstitial non-suppurative nephritis, cloudy swelling of the kidneys, and focal necrosis of the testicles.

Summary. — The leucocyte count shows a hypoleucocytosis during the four days before death. An analysis of the leucocytes shows a slight but continuous rise in the number of polymorphonuclear forms. This case presents the picture of fatal variola without complications in which leucocyte production is inhibited.

CASE IV. — Severe variola vera, pneumonia. Fatal on the eleventh day. Male, aged ten. Onset March 7. Eruption March 10. Patient more or less delirious while in hospital. During life the patient showed clinical signs of pneumonia. Anatomically the following diagnoses were made: Cutaneous lesions of variola in the pustular stage, erosions of the mucous membrane of the soft palate, lobar pneumonia of the right lung, focal pneumonia of the left lung, acute fibrinous pleuritis on the right, acute splenic tumor, cloudy swelling of the liver and the kidneys, acute degenerative lesions of the kidney, focal lesions of the testicle, focal lesions of the bone marrow, abscess of the leg.

Observations were made upon the leucocytes each day but one, beginning on the fourth day of the disease. The leucocyte determinations are tabulated below:

Observations.	Day of disease.	Leucocytes per cu. mm.	Mononuclear per cu. mm.	Polymorphonuclear per cu. mm.
1	4	14,000	4,900	9,100
2	5	10,300	4,800	5,500
3	6	13,100	5,400	7,700
4	7	9,100	4,600	4,500
5	8	3,000	2,100	900
6	10	4,200	1,600	2,600
7	11	4,000	1,000	3,000



CASE IV.

Summary.— The leucocytes show a practically continuous diminution in number. The first two counts in this case are in marked contrast with those in the two cases (Nos. I. and II.) of non-fatal variola just described. The differential counts show that the hypoleucocytosis is due to a diminution in both of the cell types considered. The typical mononuclear leucocytosis of variola is difficult to make out, the picture apparently being obscured by the coincident pulmonary infection. We select this case as an example of the disturbance of the leucocyte equilibrium brought about by a severe variola vera complicated by a fatal pneumonia. It will be noticed that the terminal hypoleucocytosis is in general similar to that of the case of fatal variola vera without complications just cited (Case III.).

Variola pustulosa hemorrhagica. — Two cases of this type of the disease were observed, and in each there was a marked increase in the number of leucocytes.

Purpura variolosa. — Four cases of this were observed and each showed a marked hyperleucocytosis. The lowest count recorded was sixteen thousand, the highest forty-six thousand two hundred. The mononuclear leucocytes were notably increased. Normoblasts were frequently encountered. The study of the stained smears in all types of the disease showed the more or less constant presence of cells belonging to the leucocyte series, but which were not normally found in circulating blood. The occurrence and significance of these cells will be considered later.

CASE V. — Purpura variolosa. — Fatal on fifth day. Female, age thirty-five; never vaccinated; onset March 26. Case ran course typical of purpura variolosa. The skin presented many punctate ecchymoses and petechiæ, and was overspread by a general terra-cotta flush. On the day of death small vesicles and papules appeared on the inner aspect of the thighs. Anatomically the case showed, besides the cutaneous lesions noted above, sub-serous punctate ecchymoses of the epicardium, pleura, and peritoneum, sub-mucous ecchymoses of the stomach, sub-epithelial hemorrhage in the pelvis of the kidneys and in the bladder, degenerative lesions of the parenchyma of the adrenal gland with foci of necrosis and cell infiltration, focal necrosis of the ovary, acute hemorrhagic splenitis with much cell detritus, acute interestitial non-suppurative nephritis, and masses of streptococci within the vessels in all of the organs.

Streptococcus pyogenes was isolated from the heart's blood. Sub-conjunctival hemorrhages and bleeding from all mucous membranes were noted during the last two days of life. Red corpuscles and leucocytes were counted on the fourth and fifth days. The results were as follows:

Observations.	Day of disease.	Leucocytes per cu. mm.	Mononuclear per cu. mm.	Polynuclear per cu. mm.	Erythrocytes per cu. mm.
1 . . .	4	46,200	20,600	25,600	4,384,000
2 . . .	5	33,000	15,000	18,000	4,160,000

In studying the stained smears, normoblasts were found in the proportion of two and one-half to every hundred leucocytes on the fourth day. Many of the polymorphonuclear leucocytes showed vacuolation of the protoplasm. A considerable number of cells were found which agreed with the description of Ehrlich's pseudo-neutrophile lymphocytes. The stained preparations taken on the day of death showed many polymorphonuclear neutrophiles whose nuclei stained homogeneously and were frequently distinctly fragmented. In the differential counts many of the cells which figure as mononuclear were neutrophile myelocytes.

Summary.— The leucocyte reaction in this case is a hyperleucocytosis brought about by an absolute increase in both the mononuclear and the polymorphonuclear cell types. There is a slight diminution in the number of red corpuscles and a notable number of normoblasts. This terminal hyperleucocytosis is in marked contrast with the hypoleucocytosis in the previous cases of fatal variola vera.

CASE VI. — Purpura variolosa. Fatal on fifth day. Male, age forty. The skin of the patient presented the punctate ecchymoses, petechiæ, and terra-cotta flush typical of the primary hemorrhagic type of the disease. No papules or vesicles developed.

Anatomically the case showed, besides the cutaneous lesions noted above, the sub-serous ecchymoses of the epicardium, pleura, and peritoneum, sub-mucous ecchymoses of the stomach, sub-capsular ecchymoses of the kidneys, and sub-epithelial hemorrhage of the pelvis of the kidney, early focal lesions of the testicles, hemorrhagic splenitis with much cell detritus, acute degenerative lesions of the kidney with acute interstitia, non-suppurative nephritis, masses of streptococci within the vessels of all of the internal organs.

Streptococcus pyogenes was isolated from the heart's blood.

The case was under observation for ten and three-quarters hours before death. During this time four leucocyte counts were made; stained preparations were studied from the first of these observations. The results are tabulated below.

Observations.	Day of disease.	Leucocytes per cu. mm.	Mononuclear per cu. mm.	Polymorphonuclear per cu. mm.
1	5	25,400	16,000	9,400
2	5	32,600		
3	5	35,000		
4	5	35,800		

The red corpuscles were counted at the time of the second observation and were found to number 5,450,000. In studying the stained smears from the first observation, the normoblasts were found in the proportion of ten and a half to every hundred leucocytes. The polymorphonuclear neutrophiles often showed vacuolation of their protoplasm and homogeneity of the chromatin of their nuclei, with fragmentation. As in the previous case a considerable number of the cells figuring as mononclears contained neutrophile granules in their protoplasm.

Summary.— The leucocyte reaction in this case is a hyperleucocytosis in which the mononuclear cell types predominate. The single observation on the red corpuscles shows them to be numerically within normal limits. Normoblasts are present in large numbers. As noted in the previous case, the terminal hyperleucocytosis contrasts sharply with the hypoleucocytosis of the fatal cases of variola vera.

GENERAL SUMMARY.

We find a greater or less degree of leucocytosis in all cases of variola. The typical case of severe variola vera which recovers without complications presents at the beginning of the eruption a normal or subnormal count, which increases with the development of the cutaneous lesions, then suffers a slight decline, rises again during the later stage of the eruption, and finally falls to normal during convalescence.

In fatal cases the leucocyte count may be high in the early stage of the eruption, but from then until death steadily falls.

In mild cases there may be no rise above the upper normal limits or a gradually increasing leucocytosis reaching its acme after the lesions of the skin have passed their active stage.

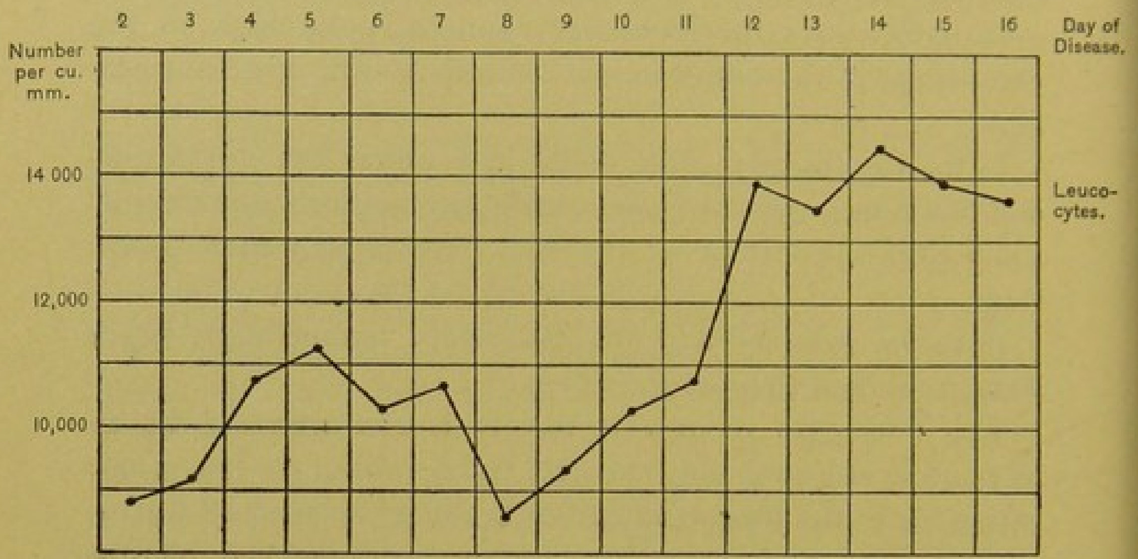
Both the primary and the secondary hemorrhagic types show a marked hyperleucocytosis.

The leucocyte picture of variola is characterized by an increase in the mononuclear cell types, although the minor variations in the leucocyte curves are dependent upon fluctuations in the absolute number of polymorphonuclear neutrophiles.

DISCUSSION.

As we have shown above, a typical case of variola vera presents marked quantitative changes in the leucocytes. We have thought it profitable to introduce here a chart which is plotted from the average of a large number of separate determinations derived from the literature, together with our own counts. In compiling the data for this composite curve, we have used only leucocyte determinations made on cases of variola vera which recovered without clinical complications.

The publications of Pick, Courmont and Montagard, Weil, and Ferguson have been freely used. Variola, above all other diseases, is characterized by a sharp onset, hence its leucocyte counts, for such a purpose as this, may readily be grouped by " days of disease." The figures used in plotting the curve represent the average of all recorded counts on the different days. Owing to the small number of counts made upon the first day of the disease, no attempt was made to begin the curve before the second day. For a like reason, no counts made after the sixteenth day were considered. This chart comprises the averages from four hundred and seventy-nine leucocyte determinations in seventy cases of uncomplicated variola vera with recovery.



Composite Curve. Seventy Cases of Uncomplicated Variola Vera.

On examining the above curve it will be noted that the leucocytes are at a high normal during the onset, rise with the evolution of the eruption, then fall slightly, and again rise during the time of desiccation of the lesions. If Sobotka's data were incorporated, and the curve could be extended into the period of incubation, we should have the typical leucocyte curve of variola vera presenting three eminences separated by two depressions. The formula would read as follows: Hyperleucocytosis during incubation; hypoleucocytosis, relative or absolute, during onset and febrile remission; hyperleucocytosis during formative evolution of eruption; relative hypoleucocytosis at height of pustulation; hyperleucocytosis during desiccation.

Unfortunately, no data are available to show the qualitative changes during the incubation stage, but the earliest differential counts that we have made, on the second and the third days of the disease, indicate that the characteristic mononuclear leucocytes have not yet appeared. The hypoleucocytosis which accompanies the febrile remission is due to an absolute reduction in the number of polymorphonuclear cell types. The two hyperleucocytoses of the stage of eruption consist of a polymorphonuclear leucocytosis superimposed upon a mononuclear leucocytosis.

When we consider the milder types of the disease, we find that the variations from the above curve are in part due to a diminished activity in the ebb and flow of the polymorphonuclear types.

In fatal variola vera the picture is one of hyperleucocytosis running into a hypoleucocytosis at the time when, in a favorable case, a hyperleucocytosis is to be expected. Qualitatively these terminal hypoleucocytoses indicate a failure in production of both types of cells.

In the hemorrhagic type of the disease the leucocyte reactions are marked and involve an absolute qualitative change in the cell picture. We have found mononuclear cells much increased in these cases.

The question as to whether or not there is a hyperleucocytosis in the hemorrhagic cases can from our data be answered in the affirmative only, but we recognize that our cases may represent an extreme type of the disease and that the study of the leucocytes in a series of cases of variola showing varying degrees of the hemorrhagic element might give all transitions between our cases with a terminal hyperleucocytosis and the cases with the typical terminal hypoleucocytosis of fatal variola vera.

The study of the qualitative variations in the leucocytes in variola brings up questions which can hardly be discussed without considering the conditions which were brought about by the disease in the hematopoietic organs. The minute study of these organs belongs to a field covered in another article in this series, but we may here indicate certain points of considerable theoretical interest which our data suggest. In discussing the significance of the variations in the leucocyte picture brought about by variola, we must of necessity repeat, in part, many generalizations already made by Ferguson, Weil, and Courmont and Montagard, for our objective findings are in accord with theirs. Concurring in general with the views of these workers, we seek here only to voice our interpretation of these variations.

The phenomenon of leucocytosis in general may be reduced to a matter of numerical cell equilibrium, which, when examined in detail, is seen to depend upon the numerical ebb and flow of certain definite cell types normally present in the circulating blood. The most mobile, numerically speaking, of these cells is the polymorphonuclear neutrophile, and to its variations are due the notable changes in the total number of leucocytes. These variations are dependent upon changes in the rate of supply and in the rate of destruction of the cells, and, under normal conditions, the interaction of the various factors concerned brings about the normal leucocyte equilibrium. The factors governing the supply of the cells to the blood stream may be arranged under three heads, viz.: those presiding over leucocyte motion, by which the adult cells are brought from the formative hematopoietic organs to the blood stream; those presiding over cell differentiation, by means of which the adult cells are formed from the ancestral cell types in the hematopoietic organs; and finally, those presiding over cell multiplication, whereby a sufficient number of cells is formed upon which the preceding factors may work.

In variola, as has been said, the leucocyte picture presents, at times, the phenomenon of a hyperleucocytosis in part made up of cells not normally present in the blood stream. These cells are not foreign to the adult body, but are foreign t the normal circulating blood. Without going into the details of leucocytogenesis and into the cytology of the hematopoietic organs, we can point out anew that these cells are mononuclear in type and are representative of the leucocyte series antecedent to the adult forms normal to the blood stream. From this it seems clear that the explanation of the peculiar mononuclear leucocytosis of variola is to be sought in disturbances in leucocytogenesis, and hence in accentuations or in inhibitions of one or all of the factors governing leucocyte supply. This being the case, we are forced to interpret the variations in the mobile polymorphonuclear leucocytes as primary and the mononuclear leucocytosis, due to the entrance of antecedent cells, as secondary and indicative of deficiency in the supply of the adult cell types called for. This deficiency is, in turn, dependent upon the failure of cell-differentiation and cell multiplication in the antecedent extravascular leucocyte series to keep up with the increase in the adult cell out-go incident to the disease. The analysis of the cell components of the leucocytoses indicates, at first sight, that not only cells of the polymorphonuclear neutrophile series are involved, but also those of the lymphocyte series. The large mononuclear eosinophiles and mast cells are not affected. We are inclined to think that that part of the mononuclear leucocytosis which might be attributed to the lymphocyte series is a vicarious phenomenon, and that the cells are essentially of the polymorphonuclear neutrophile series, a view in accordance with the finding of Dominici on the unity of the hematopoietic organs.

In short, the leucocytosis in the stage of eruption of variola owes its characteristic features to the failure of the hematopoietic organs to produce the adult cells called for. The findings in cases where there is an intercurrent secondary infection, a condition which ought to bring about a marked polynuclear leucocytosis, which latter in these cases is lacking, uphold this view.

The presence of evident degenerative phenomena in the polymorphonuclear neutrophiles in the hemorrhagic cases, as shown by vacuolization of the protoplasm, by condensation and fragmentation of the nucleus, and even by fragmentation of the whole cell (pseudoneutrophile lymphocytes), suggests that the variations in the leucocytes, in at least the severe types of variola, are in part dependent upon accentuation of the process of leucocyte destruction.

We have not considered the leucocyte examination to be of value in diagnosis, from the fact that by the time the characteristic changes in the differential counts are apparent, the evolution of the disease has progressed so far that diagnosis is easily made by other means. As to the prognosis, the leucocyte count has contributed little to the data required to form a reasonable estimate of the probable outcome of a case. We, however, do regard a high leucocyte count at the time of the febrile remission and a decreasing count during the formative evolution of the lesions, if the case is at all severe, as an unfavorable sign.

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THE INFECTIOUSNESS OF THE BLOOD IN VARIOLA.

G. B. Magrath and W. R. Brinckerhoff.

The work upon which this paper is based was carried on in connection with the general study of variola at the suggestion and under the direction of Dr. W. T. Councilman, to whom we here express our thanks.

Evidence of the infectiousness of the blood in variola, in other words, of the presence in the blood of the variola parasite, may be sought for in two ways: (1) by the direct method of microscopic examination; (2) by the indirect method of animal inoculation. Both of these methods have been employed by previous observers, and both were made use of in the course of the present work.

PREVIOUS WORK.

Zülzer (1874) states that by inoculating the monkey with the blood of a patient with hemorrhagic variola he produced a red papular rash, with pustules, which he regarded as variola.

Van der Loeff (1896) observed in the blood of variola patients, during the secondary fever, free-swimming bodies of ameboid form, provided with pseudopodia and often with flagella.

Doehle (1892) studied the blood in three cases of variola, and found it to contain bodies, some of which were small, from one-half to one micron in diameter, more or less refractive, and sometimes ciliated ; and others larger, two and one-half microns in diameter, contractile, containing irregularly arranged, refractive granules, and sometimes bearing a flagellum, in length from two to four times the diameter of the body. In addition to these, he found other bodies, darker, and with a less definite contour. These structures he found in the blood after the appearance of the exanthem, and continuing for several days.

L. Pfeiffer (1893) observed a body in the blood of variola patients and in that of vaccinated children and calves during the stage of fever. This body he describes as an ameboid cell, nucleated, one-fourth to one-half the size of a red blood corpuscle, provided with pseudopodia, and often with flagella taking Lœffler's stain, possessing ameboid motion, and occurring free in the blood.

Monti (1894) tested the infectiousness of the heart's blood and of various organs by means of inoculation on the rabbit's cornea, and found the virus constantly present in the skin, the pharynx, and the larynx ; occasionally present in the lungs, the testicle, and the spinal cord ; and absent in the heart's blood, the liver, the spleen, the kidney, and the brain.

E. Pfeiffer (1895) found in the blood of vaccinated calves ameboid bodies of about the size of a red blood corpuscle, round or pear-shaped, homogeneous, transparent, and containing a round, bright spot.

Weber (1896) saw structures of various sorts in the blood of variola, which he regarded as forms representative of developmental stages of a protozoon. He describes spherules, motile and non-motile, often bound together by threads, which he believed to originate from the division of larger ameboid bodies.

Reed (1897) confirmed the observation of L. Pfeiffer that small, granular, ameboid bodies occur in the blood in vaccinia and in variola. He found in the blood of the vaccinated monkey granular, ameboid bodies, one-third the diameter of a red blood corpuscle. Bodies of similar appearance, granulation, and size he observed occasionally in the blood of normal monkeys and children. He also found in the blood of variola patients and in that of the variolated monkey pale ameboid bodies containing dark, pigment-like granules. Bodies similar to these he also found in the blood of vaccinated children and monkeys.

Huguenin (1897), in the course of an extensive review of the subject of variola, discusses the infectiousness of the blood. In considering the question of whether the blood is infectious throughout the whole course of the disease or only in certain phases, he regards instances of fetal infection as significant of the presence of the virus in the blood of the mother during the period of incubation and also during the initial fever. He knew of no proof of the infectiousness of the blood during the period of remission, and cites Van der Loeff's observation of the presence of ameboid bodies in the blood as the only evidence of its infectiousness during the secondary fever, noting the lack of inoculation experiments covering that stage of the disease. Huguenin includes the blood during the secondary fever in an enumeration of the loci of the parasite of the disease.

Roger and Weil (1900) found small, round or oval bodies in the blood of variola, which were most numerous in severe cases. In the hemorrhagic types of the disease they found these bodies in the hemorrhages in the internal organs, notably in the spleen and in the bone marrow. They distinguished these structures from cell fragments by the fact that a clear zone surrounded a deeply-staining central part. The same observers (1901) found that the blood of rabbits inoculated with variola virus contained small refractive granules in cases where the blood was proven infectious by inoculation test, which granules were absent in cases where the blood was not infectious.

Dombrowski (1902) observed structures in the blood which he found to correspond with the smallest of the bodies present in pustule contents.

Roger (1902) states that bodies are found in the blood of variola, even in the mildest cases, and that they are most numerous in the severe and in the hemorrhagic forms of the disease. He describes them as of varying size, the smallest one micron, the larger three microns, in diameter, and finds them more characteristic than the bodies present in the vesicle. In the hemorrhagic types of variola, he states that these bodies are present in the hemorrhages and in the urine, and that at autopsy they may be found in the different organs, especially in the spleen and in the bone marrow. He found the bodies very numerous in the amniotic fluid of two pregnant women who died from variola vera. Here they showed motility, and occurred in a fluid practically free from leucocytes. In the rabbit inoculated with variola virus, Roger found similar bodies in the blood and in the internal organs. He discusses the possibilities that the bodies are parasites, nuclear debris, or special cells brought into existence by the variola virus. Mainly because of their susceptibility to cultivation outside of the body, and of their occurrence in the amniotic fluid, he was inclined to regard the bodies as parasites.

MICROSCOPIC EXAMINATION OF THE BLOOD.

A. *Of patients with variola.—Technic.*— The study of the blood microscopically was conducted mainly by means of film preparations of the blood in the fresh state. The blood was obtained by puncture of the lobe of the ear, the skin of the part having been previously cleaned with alcohol. The exuding drop was received on a clean cover-glass, which was immediately inverted and dropped upon a clean slide, the blood spreading out in a thin film between the opposed surfaces. The preparation was forthwith examined by means of a Zeiss apochromatic optical series, with oil immersion objective, either at room temperature or upon the warm stage (38° C.). Illumination was by daylight or by suitably corrected artificial light. A mechanical stage was usually employed. Experience showed that a maximum efficiency of eyesight was obtained by limiting the study of each preparation to a period of thirty minutes. Two or more preparations were usually examined at each observation.

The blood was studied by means of fixed and stained preparations in certain cases only. The methods employed will be noted in connection with reference to the examination of such material.

Method of procedure.— Cases were selected for study which were, as far as possible, representative of the various types and of the several stages of the disease. In many instances but a single observation was made; in others, examination was made from day to day, or the blood of the same case was studied at widely separated periods of the disease.

The blood was examined in twenty-two cases, of which sixteen were of variola vera, severe and in some instances fatal; five were of variola vera abortive or modified; and one was of purpura variolosa.

The earliest period in the disease studied was the second day; the latest the thirteenth day. In three cases the blood was examined on the day following that of the onset, and before the appearance of the eruption. The majority of the observations were made within the interval between the fourth and the seventh days.

In the examination of a preparation, any structure which could not be identified with one of the normal figured elements of the blood was measured, described, and often sketched, and its frequency noted.

Findings.— Morphology. — Bodies were found in the blood plasma which, according to their size and in some degree to their structure, present the following types:

Type I. — Bodies less than 1 μ in diameter. Granules, refractive, structureless, usually with a faint yellowish color, and with a vibrating or dancing motion. Many of these closely resembled the granules of leucocytes.

Type II.— Bodies from 1 to 3 μ, for the most part 2 μ, in diameter. Spherules, hyaline, refractive, sharply contoured, without structure, and colorless; with an oscillating, vibrating, or rotary motion, but without distinctly ameboid movements. Several of these bodies were sometimes united by fine threads, and in some instances they were clustered. Save in their lack of color, these bodies do not differ from detached portions of erythrocytes.

Type III.— Bodies from 3 to 5 μ, usually 4 μ, in diameter. Round or oval, generally sharply contoured, colorless, containing at the center a variable number, generally from six to eight, sharply refractive, faintly yellow granules. In some instances these bodies showed a small central vacuole. They presented some evidence of motility, changing form, and sometimes sending out and withdrawing short processes. The central granules of some showed a dancing or a gliding motion. These changes were most marked upon the warm stage. Bodies were seen of the same general character, but of somewhat greater size, from 7 to 8 μ in diameter; or of smaller size, from 1.5 to 2 μ in diameter. Others similar in every other respect were without granules.

Type IV. — Bodies oval or pear-shaped, with diameters from 1.5 to 2 μ, and from 2 to 3 μ, and bearing upon their pointed extremity a "flagellum" two or three times as long as the body. These bodies were fairly sharply contoured, colorless, and either hyaline or finely granular. They showed some degree of motility, rotating and changing position in a manner suggesting the action of a flagellum. Bodies approaching these in their general character, but possessing a tinge of hemoglobin color, were not infrequently seen and dismissed as erythrocyte fragments with an attached thread of fibrin. In one instance bodies similar to these were seen in a preparation fixed with alcohol and ether, and stained with Babes' aniline saffranin acid violet; they stained deeply with saffranin.

Type V.—Bodies averaging 5 μ in diameter. Round or oval, colorless, usually non-granular, and with a central opaque area in diameter about one-half that of the body. In some instances the peripheral part of the body contained dancing granules. These bodies were non-motile.

Occurrence. — The occurrence of these various bodies with relation to the different types of variola and to the several stages of the disease was as follows:

The smallest bodies, or granules (Type I.), appeared constantly; in but very few observations was there failure to note their presence.

The hyaline spherules (Type II.) were met with in all of the forms of variola studied, and in every stage of the disease; they were seen as early as the second and as late as the thirteenth day. In numbers these bodies presented no striking variability, although they seemed to occur somewhat more frequently in severe cases, and at the height of the disease, than under any other circumstances, and here usually in association with more or less deformity and fragmentation of the erythrocytes. In some instances these bodies seemed to be more numerous in a given preparation at the end than they were at the beginning of an observation, and near the edge of the film than elsewhere.

The granular, "ameboid" bodies (Type III.) occurred with the same frequency and distribution as the hyaline spherules, and like them were seen as early as on the second and as late as on the thirteenth days of the disease. Instances in which motility of the leucocytes was well-marked upon the warm stage were those in which these bodies were the most active. They were seen in largest numbers in the blood of a case of purpura variolosa in which there was a leucocytosis of fifty thousand and in which leucocytolysis was prominent.

Bodies conforming strictly to the pear-shaped, "flagellate" type (Type IV.) were comparatively rare. They were seen on the third day of variola vera in an infant, before the appearance of the eruption, and in association with the hyaline spherules; on the third and the fourth days of variola vera in a child, severe in onset, but subsequently abortive, here associated with hyaline spherules and with the granular ameboid bodies; in this case they were not found either on the second or on the fifth days of the disease. In another instance the body was seen at a somewhat later stage of variola vera, in connection with hyaline spherules. As has been already stated, structures in many respects similar to these appeared frequently in the blood, which were ruled out of consideration by reason of their color index.

The structures last to be described (Type V.), non-granular or containing but few granules, and presenting a central opaque area, were found only in the blood of the single case of purpura variolosa which came under our observation. In this they were numerous. It may be questioned whether or not these constitute adventitious elements. Preparations of the blood fixed by Scott's method and stained by Wright's modification of Leishman's stain showed numerous erythroblasts and blood-platelets. In the fresh preparation, however, it was not possible definitely to identify the several varieties of bodies found with either of these elements.

Control observations. — While recognizing the importance of observations directed to control those made upon the blood in variola, we found it impracticable in the course of the work to conduct any extensive series of such. The observers from time to time examined their own blood and found constantly refractive granules, occasionally hyaline spherules, and rarely granular, ameboid bodies comparable with those found in the blood in variola. In this connection, we make mention of a case of malignant endocarditis (streptococcus) admitted to the Detention Hospital upon suspicion of its being purpura variolosa, the patient dying on the fifth day of his illness. The nature of the disease was determined only by autopsy and by the histological examination of the skin. The blood in this case was examined by the routine method and was found to contain refractive granules, hyaline spherules, granular bodies with active ameboid motion, forming pseudopodia, and a pear-shaped body 2 by 3 μ, colorless, finely granular, with a tapering projection, but without any recognizable flagellum. The dimensions and the morphology of the bodies seen in this case for the most part coincide with those of the bodies found in the blood in variola.

Summary. — The blood of variola patients in the fresh state shows bodies of widely differing size and appearance, lying free in the plasma, incapable of identification with any intact normal elements, in general occurring in any type and at any stage of the disease, in the case of two (Types IV. and V.) infrequent, and limited in distribution. The majority of these bodies are most numerous under circumstances in which there are associated changes in the normal elements of the blood.

Bodies in most respects similar to these may be found occasionally in the blood of normal persons, and were seen in large numbers in a case of malignant endocarditis.

B. *Of the monkey inoculated with variola virus.— Technic.*— The microscopic examination of the blood of the variolated monkey was, in general, conducted in the same manner as the study of human blood in variola. The study of the blood formed a part of the general study of variola inoculata in the monkey, and material for such examination was obtained from a small incision made within a shaved and cleansed area of the skin of the back, coincidently with the taking of a sample of blood for the leucocyte count.

Procedure.— In each case the blood of the animal was examined microscopically on the day before inoculation, and again on the day of inoculation prior to this operation. Subsequent to inoculation, the blood was examined daily throughout the period during which the animal was under observation. Observations were conducted upon six animals of the Macacus and the Rhœsus varieties, five of which were inoculated upon the skin of the abdomen with vesicle contents from variola patients, and one by intravenous injection with the same material. Of these inoculations, one was negative; one was followed by death on the second day from streptococcus septicemia (intravenous inoculation); three resulted in variola inoculata, with local lesion only, developed on the fourth day; and one in variola inoculata with local lesion apparent on the fifth day, followed by general exanthem on the seventh day. The blood of the animal, in which inoculation was negative, was examined on eleven consecutive days; that of one of the animals with local lesion only, on fourteen consecutive days; that of the other animals during periods somewhat shorter and determined by the stage of the disease at which the animal was sacrificed. In the case of one animal which was allowed to recover from the local lesion, re-inoculation was attempted without success.

Findings.— *Morphology.*— The blood of the monkey inoculated with variola virus contains bodies which, according to size and to structure, conform to the following types:

Type I.— Bodies less than 1 μ in diameter and consisting of dancing specks or granules.

Type II.— Bodies from 1 to 3 μ in diameter. Spherules, round or oval, non-granular, hyaline, and colorless, some non-motile, others changing position with an oscillating or rotary motion, yet others changing shape and more or less ameboid. Some of these were seen to segment and to throw off fragments, which immediately assumed the form of the dancing specks (Type I.).

Type III.— Bodies from 2.5 to 5 μ in diameter. Round or oval, sharply contoured, colorless, containing a variable number of granules, highly refractive and sometimes oscillating or otherwise motile. One of the larger of these, a body 5 μ in diameter, itself immobile, was filled with granules showing an oscillating or a gliding motion; while under observation, this body broke to pieces, and the contained granules became dancing specks. Certain of the smaller (3 μ) of these bodies contained but very few granules. Many of these bodies showed upon the warm stage a certain degree of ameboid motion.

Type IV. — Bodies 5 μ in diameter; round, non-granular, sharply contoured, with a central opaque area 2 μ in diameter.

Occurrence. — These various bodies were found in the blood of the monkey with great constancy.

The small, refractive granules (Type I.) were invariably present, their occurrence being noted in every observation.

The hyaline spherules (Type II.) appeared in the blood of every animal either at the time of the preliminary observation on the day preceding that of the inoculation, or at the time of the observation made just prior to inoculation, and thereafter in almost every examination made. Their number did not vary noticeably in the course of any one experiment.

The granular, more or less ameboid bodies (Type III.) were found in one case in the preliminary observation, in one on the day before the development of the local pock (third day after inoculation), and in three during the development of the local lesion, a period of from two to three days in which the animals showed some symptoms of constitutional reaction. These bodies were seen also on the tenth and the twelfth days after successful inoculation, in a case in which there was no general eruption; and they occurred throughout a series of ten daily examinations of the blood of an animal in which the attempt at re-inoculation was unsuccessful. They were somewhat more numerous than at other times during the development of the local lesion.

The rather large bodies containing a central area of opacity (Type IV.) were seen but three times, on the eighth and the ninth days in the animal whose inoculation failed to take, and on the second day of local reaction in a case successfully inoculated.

Control observations. — The routine examination of the blood prior to inoculation, and the observations continued for ten days in the case of the animal whose inoculation failed to take, we regard as furnishing adequate normal control. As has been stated, in the course of such observations all of the forms of adventitious bodies described were seen.

Summary. — The blood of the monkey inoculated with variola virus contains bodies which in size and in general appearance resemble those found in the human blood in variola. Bodies identical with these occur also in the blood of the normal monkey. Certain of the forms seem to occur with greatest frequency during the period of local reaction at the site of inoculation; the same forms, however, may be seen in the blood prior to inoculation, and also several days after the subsidence of the local lesion.

INOCULATION TESTS.

By the method of animal inoculation, the infectiousness of the blood was tested in five cases of variola of from two to twelve days' duration, and in one of purpura variolosa. The animal used was the rabbit, and the site of inoculation was the cornea. The production of the specific corneal reaction was held to constitute proof of the presence in the blood of the parasite of variola.

Technic. — The blood for inoculation was obtained from the lobe of the ear by puncturing the skin, previously cleansed with alcohol, and catching the exuding drop in a sterile glass tube. Inoculation was practised by one or the other of two methods, both of which had proven satisfactory with the use of vesicle contents in control tests. The cornea was anesthetized by the instillation into the conjunctival sac of a few drops of two per cent solution of cocaine. The cornea then, according to the method employed, was either incised or pricked.

Following incision or pricking of the cornea, two loops-full of blood were thoroughly rubbed into the area of inoculation by means of a sterile platinum wire loop. The cornea of each eye was inoculated, and often the inside of the ear also. The sites of inoculation were examined daily with the naked eye and with a hand lens.

After a period of from forty-eight to seventy-two hours the animal was killed and the eyes were placed in Zenker's fluid. Segments of the cornea containing the inoculation sites were embedded and cut in paraffin, and sections stained with the eosin and methylene blue stain, or with Mallory's chloride of iron-hematoxylin followed by acid fuchsin and picric acid.

Findings. — *Summary.* — The blood of six cases of variola was examined, the several tests made in each instance yielding a total of twenty-two inoculations upon the cornea. Only one of these inoculations gave a positive result. This was made with blood from a case of purpura variolosa on the fourth day of the disease. Three other inoculations made at the same time as this and with the same sample of blood were negative.

GENERAL CONSIDERATIONS.

In the study of variola, one is forcibly impressed with the strength of the argument *a priori* for the existence in the circulating blood, certainly in the earlier stages of the disease, of some form or other of the specific parasite. In the light of present knowledge, to interpret the specific lesions of the skin and of the mucous membranes as the result of any other than a process of embolic distribution of specific organisms is practically impossible.

At the beginning of this paper we accepted as a postulate the proposition that evidence of the existence of parasites in the blood in variola lies in the direct observation of such bodies in the blood, or in such indirect proof of their presence as may be derived from animal inoculation.

It now remains to examine critically the quality of such evidence as we have at our disposal.

The bodies in the blood described by Doehle, L. Pfeiffer, E. Pfeiffer, Van der Loeff, Reed, Roger, and others, and by many of these observers unhesitatingly identified as parasites, present many characteristics that are common to all; they are similar in size, they are hyaline, they are granular, and they possess some degree of ameboid motion, while some of them are flagellate. The evidence accompanying the presentation of these bodies as parasites seems to us unsatisfactory. It is not apparent from the descriptions that the bodies, either by their intrinsic properties or by the careful exclusion of other possible interpretations of their nature, are susceptible of reasonably certain identification with parasitic forms of life. The difficulties in the way of such identification are admittedly great, but without good evidence to show that the unity of these bodies with blood corpuscle products is improbable, we believe that the assigning to them of the part of parasites is of doubtful propriety. The observation by Reed of bodies of this character in the blood of normal children and of normal monkeys seems to us to go far toward disproving alike their parasitic nature and their specificity either to variola or to vaccinia.

The inoculation tests practised by Zülzer, although positive, owing to the meagerness of his descriptions, are inconclusive. Those of Monti, who found the heart's blood non-infectious, seem to have been performed in a wholly acceptable manner.

Reviewing the evidence embodied in the results of the work upon which this paper is based, we believe that it is sufficient to enable us to place a proper valuation upon any data bearing upon infectiousness which may be derived by simple morphological examination of the blood. Although we regret that our opportunity to study the blood in the very earliest stages of variola was limited, we feel that enough observations were made to give a fair idea of the content of the blood in this disease. We also believe that the data obtained from the study of the variolated monkey increases the sum of the evidence.

In analyzing this evidence, we come at once into contact with the problem of the interpretation to be placed upon the bodies found in the blood. To identify them with living organisms demands means for recognition which, at present, we do not possess. No less difficult is it to exclude these bodies from the category of structures which we know to be derived from the fragmentation of both red corpuscles and leucocytes, or from the extrusion of portions of their nuclear or their cytoplasmic contents. We have repeatedly observed processes of this sort resulting in the formation of bodies in almost every respect identical with those described by previous observers, and seen by ourselves in variola. Many of them, we believe, should be classed with the blood-plates and with the bodies described by Arnold, rather than receive further attention as possible micro-organisms.

There are, however, certain positive objections which would seem to eliminate the possibility of the specificity of these bodies, as well as the likelihood of their being parasites. We have found them in normal individuals, in septicemia, and in normal monkeys. We believe that they will be found to occur not uncommonly in film preparations of the fresh blood, and suggest as an explanation for the fact that they have not been noted in the study of malaria, that in the case of that disease the attention of the microscopist is directed to the blood corpuscles, and these bodies are easily overlooked unless sought for.

It of course is not to be denied that some form of *Cytoryctes variolæ* may be represented by certain of these bodies, or may occur in the blood and have been overlooked. We contend only that if so present it is not capable of recognition. In this connection we would make mention of bodies found in preparations of the blood of the case of purpura variolosa fixed in methyl alcohol and stained with hematoxylin and carbol-fuchsin. Here were seen lying in the cytoplasm of the leucocytes round bodies, 2 μ in diameter, of about the size of the gemmule of *Cytoryctes variolæ*, and staining with fuchsin. They were very rare, only four being found.

The evidence obtained by corneal inoculation might seem to warrant the immediate conclusion that the blood of a patient with variola does not contain the contagium. Such a sweeping conclusion must, however, be modified when we consider the conditions under which the experimental data were collected on which such an assertion might be based. In arriving at a definite conclusion from the procedures adopted as to the presence of the contagium, many elements of uncertainty must enter. The first question that might arise is with relation to the specificity of the corneal reaction. This, for reasons discussed in other papers, can be answered in the affirmative. That is to say, a positive corneal reaction means that the contagium was introduced. Of equal importance in this connection is the question as to whether the presence of the contagium in the material introduced necessarily leads to a positive reaction. Here as definite an answer cannot be given, for it is reasonably certain that a quantitative relation is involved. It is by all means probable that a certain definite number of the organisms must gain entrance to produce the lesion. Bound up with this general question is that of the possibility that certain stages of the life cycle of the organism or certain degrees of virulence at any given stage may govern the reaction even so far as to inhibit its appearance altogether. It is necessary, then, to qualify the conclusions drawn from the results of corneal inoculations until these points have been settled. We are justified, however, in saying that a negative result following corneal inoculation indicates that the material introduced does not contain the contagium either as it occurs in the contents of vesicles or pustules or as it is found in the powder or the paste made from ground disks.

A second question, of equal importance, is that as to whether the blood used in the inoculation was a true sample of the blood of the patient. We must here determine first, whether the contagium, if present, could have been destroyed before the inoculations were made; and second, whether the contagium gained access to the sample from without. Inasmuch as unheated and undiluted blood was used for inoculations, the only way in which the organisms in the sample could have been destroyed would seem to be by some change in the blood itself after drawing. From the fact that the inoculations were made at intervals varying from a few minutes to many hours after the collecting of the blood, and that only one positive reaction was obtained, it seems hardly probable that the negative results can be so explained.

In one instance we have to consider the possibility of the contamination of the sample. In the instance of the single positive reaction, the blood was obtained from a case of purpura variolosa, and in view of the widespread infection of the epithelial cells in this type of the disease it is very probable that a few organisms gained entrance to the sample of the blood. As Zülzer does not describe the mode in which he collected the blood, his single positive inoculation of the monkey might have been so produced.

CONCLUSIONS.

(1.) Bodies widely diverse in size and somewhat different in structure occur in the blood of patients with variola and in that of the monkey inoculated with variola virus. They are somewhat more numerous during the secondary fever than at other times, and in severe than light forms of the disease.

(2.) Bodies of like sort occasionally are seen in the blood of healthy men, in that of the normal monkey, and were numerous in a case of malignant endocarditis.

(3.) These bodies do not admit of positive identification with any known form of *Cytoryctes variolæ*. They may be accounted for as derivatives or as degeneration products of blood corpuscles, as blood platelets, and in some instances as erythroblasts.

(4.) The blood of patients with variola, obtained by puncture of the ear, inoculated upon the cornea of the rabbit does not produce a variolous keratitis.

(5.) Blood so obtained from a case of purpura variolosa and so inoculated may produce a variolous keratitis, probably because of contamination of the sample by infected epithelium.

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ON THE INFECTIOUSNESS OF THE LATE STAGE OF THE SKIN LESION IN VARIOLA.

W. R. Brinckerhoff.

The following investigation was undertaken at the suggestion of Dr. W. T. Councilman, and I wish to express my indebtedness to him for his direction and encouragement in the prosecution of the work.

The problem was to demonstrate, if possible, the contagium in the final stage of the skin lesion of variola, the so-called "crust, seed, or disk," and to attempt its isolation and cultivation in vitro.

A review of the literature on variola furnishes scant data on the infectiousness of the late stage of the skin lesion. Certain observers, as Copeman[1] and Zülzer,[2] have used the dry crusts for infecting animals, but always used them in connection with other contagious material. No attempt was made by them to get the contagium from the disk and free from the associated bacteria. Weil[3] states that the variola crusts remain infectious for two years. Our knowledge of the infectiousness of the crust, then, seems to rest upon the tradition that the Chinese variolate by putting crusts in the nose of the subject[4] and the general impression of those who have to deal with small-pox that the disks are infectious.

Occasionally one finds reference to the use of crusts for inoculating calves, as, for instance, in the work of Ishigami,[4] but as a rule vesicle contents is chosen for such purposes.

As Ishigami claims to have cultivated the contagium from the variola crust, I will consider his publication at some length.

This author used variola crusts, variola lymph, and vaccine lymph, which he made bacterium free by treatment with one per cent phenol. As the details of his experiments were not given in full in any of his publications accessible to me,[5] I suppose that his "cultures" from the variola crusts were inoculated with fragments of disks so treated. He states that the cultural results were identical, no matter with which of the three sources he started. The material containing the contagium was planted in a special medium and incubated at 37°–38° C. Inoculations were practised on calves. Identical results were obtained with the three sorts of cultures (variola crusts, variola lymph, vaccine lymph). A decreasing infectiousness was noted in succeeding generations, the cultures being inactive after the third transplant. The only mention that I can find as to the nature of the special medium used is as follows: "A special medium containing the epithelial cells of non-vaccinated calf as the essential element." In order to prove that multiplication has taken place in the special medium, he made parallel inoculations in a glycerine bouillon and in the special medium. In the second series in the special medium he did not incubate, and made his inoculations at once from three dilutions. With the series in the special medium that had been incubated, the inoculations were "good" with the first and second generations, but "moderate" with the third. The series in glycerine bouillon gave "not" (good?) results in all generations. The series in the special medium without incubation was "not" (good?) in all dilutions. He explains the poor results in the glycerine bouillon series as due to unfavorable media. The good results in the series where incubation in the special media was practised he contrasts with the poor results in the series in the same media without incubation. From this he concludes that the contagium has multiplied in the special medium, and that the results are not due to simple dilution. He does not consider spontaneous vaccinia (vide Park [6]). No account is taken of the greater opportunity for dissemination of the virus in the incubated cultures over those simply diluted and inoculated at once. Further mention will be made of the claims of Ishigami in a later part of this paper.

Funk,[7] Dombrowski,[8] and Roger [9] each claim to have cultivated either the organism of variola or vaccinia. The work of all of these authors lacks confirmation. In the case of the work of Funk and of Dombrowski, the recent findings of Perkins and Pay [10] seems unfavorable to their claims. The latter found no evidence that the bodies described were organisms, but, on the contrary, they were able to convince themselves that the so-called parasites were products of fatty degeneration of epithelial cells.

Material. — The disk may be defined as the inspissated contents of the specific skin lesion of variola which remains between the layers of the epidermis after the healing of the lesion. Before the case can be discharged from the hospital all of the disks must be removed. Through the kindness of the nurses I was able to obtain a number of disks from a series of cases. As soon as the disks were removed they were put into pasteboard boxes bearing the name of the patient and the date of collection. This date was used in calculating the age of the disk. These boxes were sent to the laboratory and the contents transferred to small bottles and stored in a leather case.

Disks were studied from the following series of cases:

Very light variola vera	2
Variola vera	12
Variola confluens	2
Abortive variola *	6

Technic. — The procedures adopted fall naturally into two groups.

I. Tests of infectiousness of the disk.

II. Method for the isolation of the contagium.

I. Testing for contagium in the disk. — Ground dry. The dry disks were rubbed up to a fine powder in a sterile porcelain mortar. The resulting powder was used for inoculation on the rabbit's cornea.

* The term "abortive variola" is used to designate cases in which the evolution of the skin lesion is atypical, in that, at some time in the course of its development, the lesions, or a majority of them, dry up without going through a true pustulation. This phenomenon is usually observed in the early part of the vesicular stage. It occurs in cases with few or many lesions.

The two cases of "very light variola vera" were ones found by the health officer who visited houses in which groups of cases appeared simultaneously. Both patients gave a history of "grippe" two weeks before. The disks that I studied were found in their palms.

Disk paste. — The dry disks were ground up as before, but from time to time small amounts of fluid (sterile salt solution, 0.85 per cent, or fresh sterile rabbit serum) were added, so that the end product was a thick paste. This paste was used for corneal inoculations.

Fresh serum "cultures."—The fluid in the tube containing the disk was used for inoculation. The method of "culture" will be described later.

II. Method used for isolation of contagium.*— If the disk be planted on ordinary culture media a number of bacteria grow out vigorously. As many of these bacteria interfere with the corneal test for the presence of the specific contagium, and as the corneal inoculation is now the only test available for proving the presence of the contagium, it is important to adopt a technic that will eliminate bacteria.

In order to bring this about two things are required; namely, the use of a medium inhospitable to as many of the bacteria as possible, but which is not lethal for the contagium, and the examination of a large series of disks from selected cases, in order to get ones in which the organisms are absent which are not excluded by the special medium. The first of these requirements is fairly well met by the use of fresh sterile rabbit serum;† the second requires a proper selection of cases for study. The abortive variolas were found best for this purpose.

Cultures. — Disks were washed in three changes of sterile salt solution, and put into small culture tubes containing one cubic centimeter of fresh sterile rabbit serum. Cultures were incubated at 37.5° C. In examining cultures for bacteria, both stained smears and sub-cultures were used. The smears were fixed by heat or with methyl alcohol. Löffler's alkaline methylene blue, Borrel blue,[11] and carbo-fuchsin, followed by Löffler's blue, were the stains most used. Sub-cultures were made, as a rule, on Löffler's beef blood serum.

* The word "isolation" is used in the sense of separation from bacteria.

† Other animal sera were used (horse, cat, monkey), but, with the possible exception of horse serum, were found to offer no advantages over rabbit serum.

In some instances plain bouillon or agar agar was employed. The agar agar was kindly furnished by Dr. J. H. Wright, and was one per cent glucose, nine-tenths acidity, by the American Public Health Association standard. All the media used was freshly prepared. When anaerobic conditions were desired, Wright's method was employed.[12] No attempt was made fully to identify all the strains of bacteria that appeared in the cultures. The object of this part of the work was not to make a catalogue of the bacteria which could be isolated from the disks, but rather to determine when bacteria were absent from the "cultures." One strain of the pseudo-diphtheria bacillus, so frequently found in the disk cultures, was identified as the bacillus described by Nakanishi.[13] When the term "streptococcus" is used, it has only the force of a flattened coccus arranged in chains. No attempt was made to distinguish possible streptic pneumococci. When the words "bacterium free" occur, with reference to a disk culture, no more is intended than to state that I was unable to demonstrate bacteria with the procedures adopted.

The testing of the cultures for the presence of the contagium was done by corneal inoculations on the rabbit. The resulting lesions were classified as follows:

Positive (variolous keratitis). — Exuberant proliferation of the epithelium at the site of the inoculation, with the presence of stages in the epithelial cells of the intraprotoplasmic cycle of the variola organism.

Pyogenic. — Destruction of epithelium with more or less necrosis of corneal substance. Many polymorphonuclear leucocytes present in all stages of degeneration.

Repair. — Wound filled by new formed epithelium and corneal corpuscles, but without evidence of variolous keratitis or pyogenic reaction.

Diagnosis was based upon microscopic examination of paraffin sections stained with Unna's alkaline methylene blue and eosin, or with Mallory's chloride of iron hemotoxylin, followed by acid-fuchsin-picric acid. Zenker's fluid was used for fixation.

DETAILS OF EXPERIMENTS.

I. Inoculation of corneas with dry disk powder.— Material from one case of variola confluens, three cases of variola vera, and one case of abortive variola.

CASE 380. — Variola confluens. Disk one day old. Two corneas inoculated. After forty-four hours one shows repair, and the other shows variolous keratitis.

CASE 382. — Variola vera. Disk two days old. Two corneas inoculated. After seven days both show repair.

CASE 394. — Variola vera. Disk one month old. Two corneas inoculated. After ninety-six hours both show repair.

CASE 467. — Variola vera. Disk one day old. Two corneas inoculated. After seventy-two hours both show variolous keratitis.

CASE 396. — Abortive variola. Disk thirty-four days old. Two corneas inoculated. After forty-four hours both show variolous keratitis.

Disks from same case, but eighty-eight days old. Two corneas inoculated. After seventy-two hours both show variolous keratitis.

Inoculation of corneas with disk past. Material from one case of variola confluens.

CASE 380. — Disk twenty-two days old. Ground up with fresh sterile rabbit serum. Two corneas inoculated. After fifty-six hours one cornea shows repair and the other variolous keratitis.

Same case. Disk fifty-two days old. Ground up with sterile salt solution. Two corneas inoculated. After seventy-two hours both show variolous keratitis.

Tests for the infectiousness of the disk "cultures" will be detailed later.

II. Isolation of contagium from the disk. — The examination of the incubated tubes containing disks in rabbit serum yielded two sorts of data. The results can, therefore, be conveniently considered under the following heads:

I. Bacteria-containing disk-serum tubes.*

II. Bacterium-free disk-serum tubes.

I. Bacteria-containing disk-rabbit-serum tubes. — Disks from fifteen cases were studied. In all seventeen "cultures" were made. The cases selected were one of very light variola vera, eight of variola vera, one of variola confluens, and five of abortive variola.

Very light variola vera.

CASE 314. — Disk one day old. After twenty-four hours incubation tube showed a streptococcus and a pseudo-diphtheria bacillus.

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* Disks from four cases were planted in the water of condensation of Löffler's beef blood serum. On incubation all these showed a profuse growth of bacteria, in which a non-pigment producing staphylococcus was prominent.

Variola vera.

CASE 381. — Disk two days old. After thirty-six hours showed a streptococcus and a pseudo-diphtheria bacillus.

CASE 382. — Disk two days old. After thirty-six hours' incubation yielded a streptococcus and a bacillus.

A second disk from the same case, but thirty-six days old, on incubation yielded a streptococcus after twenty-four hours.

CASE 383b. — Disk two days old. After thirty hours' incubation showed a streptococcus and a pseudo-diphtheria bacillus.

CASE 386. — Disk seventeen days old. After twenty-four hours' incubation showed a streptococcus.

CASE 388. — Disk one day old. After twenty-four hours' incubation showed a streptococcus and a pseudo-diphtheria bacillus.

A second disk from the same case, but twenty-three days old, after twenty-four hours' incubation showed a streptococcus, a pseudo-diphtheria bacillus, and a large, square-ended bacillus.

CASE 392. — Disk one day old. Atfer twenty-four hours' incubation yielded a streptococcus and a pseudo-diphtheria bacillus. The latter organism was cultivated on other media and was found to agree with the bacillus isolated by Nakanishi from vaccine lymph and the lesions of vaccinia.

CASE 394. — Disk one day old. After forty-eight hours' incubation shows a streptococcus and a pseudo-diphtheria bacillus.

Variola confluens.

CASE 383a. — Disk two days old. After thirty-six hours' incubation shows a streptococcus and a pseudo-diphtheria bacillus.

Abortive variola.

CASE 384. — Disk two days old. No bacteria were demonstrated up to one hundred and seven hours. At that time a moderate growth of a pseudo-diphtheria bacillus was found by stained smear. Corneal inoculations after twenty-four and seventy-two hours' incubation showed only repair.

CASE 385. — Disk seventeen days old. No bacteria could be demonstrated up to one hundred and seven hours. A streptococcus was then found in stained smear. Corneal inoculation after twenty-four hours gave only repair.

CASE 387. — Disk five days old. After five days' incubation a streptococcus was demonstrated in stained smear.

CASE 389. — Disk two days old. After twenty-four hours' incubation showed a pseudo-diphtheria bacillus.

A second disk from the same case after forty-eight hours' incubation yielded a streptococcus.

CASE 391. — Disk five days old. After twenty-four hours' incubation a pseudo-diphtheria was found.

II. Bacterium-free disk-rabbit-serum tubes.

CASE 330. — A single disk was brought to me by a health officer, Dr. Irving R. Bancroft, which he had found in the palm of a boy living in a house where a number of cases of variola appeared at once. The disease had not been recognized in the boy and he had not previously been seen by a physician. The case was regarded as the probable source of infection for the group of cases observed in the house.

Disk two days old. Planted in one cubic centimeter of fresh sterile rabbit serum. No bacteria could be demonstrated in stained smear taken after twelve, twenty-four, thirty-six hours, four, five, seven, ten, and thirteen days. Sub-cultures on Löffler's beef blood serum after twenty-four hours, five and seven days showed no bacterial growth. A sub-culture on Löffler's beef blood serum taken after twelve hours showed, after five days incubation, a profuse growth of a rather large coccus. On the preceding days no bacteria were found in this tube by stained smear of the water of condensation, nor was there evidence of growth on the surface of the media. The coccus did not appear in any of the subsequent sub-cultures from the original rabbit serum tube containing the disk. The coccus was, therefore, regarded as a contamination of the sub-culture. Corneal inoculations with the organism gave negative results.

On the seventh day a sub-culture was made on Löffler's beef blood serum and the free oxygen removed by Wright's method. No bacteria developed on incubation.

On the ninth day a sub-culture was made in melted agar agar (glucose one per cent. 0.9 by Am. Pub. Health Standard) media at 40° C when inoculated. Two such tubes were inoculated with a large loopful each of the original disk-serum tube. No bacterial growth could be made out on incubation.

Corneal inoculations were practised with the disk-serum after twenty-four hours, five and thirteen days of incubation.

RABBIT 337. — Two oëse of the disk-serum, after twenty-four hours' incubation, were inoculated on each cornea of a rabit. After fifty-one hours microscopic examination showed repair.

RABBIT 342. — Two oëse of the disk-serum, after five days' incubation, was inoculated on each cornea of a rabbit. After fifty-one hours microscopic examination showed variolous keratitis in one cornea with repair in the other.

RABBIT 354. — Two oëse of the disk-serum, after thirteen days' incubation, was inoculated on each cornea of a rabbit. Care was taken to obtain the fluid from the top of the column in the tube. The tube had not been disturbed for twenty-four hours. Microscopic examination of the cornea, after seventy-four hours, showed repair.

RABBIT 356. — Both corneas inoculated with a drop of fluid obtained from the bottom of the tube. The fluid for inoculation was withdrawn with a sterile capillary pipette. The fluid so obtained contained particles of the disintegrated disk. After seven days microscopic examination showed repair in one cornea and variolous keratitis in the other.

Repeated examination of the fluid from the tube in hanging drop was negative for formed elements that could be recognized as organisms. Zeiss two millimeters apochromatic oil immersion with compensation oculars from four to eight were employed. The study of the stained smears likewise yielded no elements that could be interpreted as an organism.

CASE 396. — From a case that promised to be a severe variola vera, but which aborted. The lesions dried up in the vesicular stage without going through a true pustulation. The disks on which the following studies were made were collected from the dorsum of one hand. Care was taken to remove all the overlying epithelium with a spud, and to use for culture only the disk proper.

Disk three days old. Planted in one cubic centimeter of fresh sterile rabbit serum. On incubation no bacteria could be demonstrated in stained smear after twenty-four and forty-eight hours.

Disk fifty-one days old. Planted in one cubic centimeter of fresh sterile rabbit serum. On incubation no bacteria could be demonstrated in stained smear after twelve, twenty-four, forty-eight, and eighty-three hours. The softened disk was then ground up in the tube with a sterile glass rod. Twelve and seventy-two hours after no bacteria could be demonstrated in stained smear. Sub-cultures were made on Löffler's beef blood serum after twelve and twenty-four hours' incubation. On incubation of these tubes no bacterial growth was observed.

Disk twenty-five days old. Planted in one cubic centimeter of fresh sterile rabbit serum. No bacteria were demonstrable in stained smears after twelve, twenty-four, forty-nine, and ninety-seven hours, five, seven, eight, nine, and thirteen days.

Sub-cultures on Löffler's beef blood serum after twelve and twenty-four hours, five and six days, were negative for bacteria. Sub-cultures were also made on the same medium, and the free oxygen removed by Wright's method. No bacteria developed on incubation.

Sub-cultures were made on two tubes of melted agar agar, as in the previous case. No evidence of bacterial growth was seen on incubation. A sub-culture on fresh rabbit serum from the disk tube after eight days was also negative for bacteria.

Corneal inoculations were made from the disk serum after twenty-four hours, five, nine, and thirteen days.

RABBIT 336. — Both corneas were inoculated with fluid from the disk-serum tube after twenty-four hours. Microscopic examination of the corneas after sixty hours showed repair in one cornea with variolous keratitis in the other.

A second rabbit had both corneas incised, but not inoculated. This animal was put in the same basket and cage as rabbit 336, following twenty-four hours behind that animal. This second rabbit was killed after sixty hours, and on microscopic examination of the cornea showed repair. The second rabbit was used to rule out, as far as possible, the chance of spontaneous infection.*

Rabbits were inoculated from the disk-serum tube containing the disk after five, nine, and thirteen days. On the latter occasion two animals were used and top fluid and sediment were inoculated as in the preceding case (330). All these corneas on microscopic examination showed repair. No variolous keratitis or pyogenic reactions were observed.

Examination of the disk-serum cultures (bacteria free) in hanging drop and in stained smear was negative for forms that could be interpreted as parasites.

Various attempts were made to transfer the contagium from one tube of serum to another, but without result. Attempts were made to bring the contagium into a fluid in which it was hoped it would multiply. For this purpose the fluid contents of calves' eyes, together with as much of the corneal epithelium as could be scraped off, was ground up in a mortar and was passed through a small Berkefeld filter. The resulting fluid was shown to be bacterium-free. No evidence of presence, still less of multiplication, could be made out in this fluid after inoculation with material in which I had reason to believe that the contagium was present. As the calves' eyes were obtained from the abattoir, it was not possible to tell whether or no some of the eyes were from calves that had been vaccinated. Owing to the lack of specific directions in Ishigami's publications, accessible to me, I was unable to make up the special medium on which he claims to have cultivated the organism of variola.

Inoculations were frequently made from various bacteria-free fluids in which the contagium might be expected to be if it had stood transfer or had multiplied. All these inoculations were negative.

* Inoculated rabbits were kept in separate cages or baskets. During the time that cultures were being tested (a period of three months) no vaccinated rabbits, or indeed vaccine in any form, were used in the laboratory. The cages and baskets used for animal containers were new. The other fittings of the laboratory had not been used for work on vaccinia. A month before the "cultural" work was begun, a rabbit was inoculated with a commercial vaccine point, but without a take. Rabbits whose corneas were incised, but not inoculated, were, from time to time, put in the cages or baskets with the inoculated animals. In no case was there any evidence of a spontaneous infection.

Bodies similar to those described by Roger [9] were occasionally seen, but no relation was demonstrable between them and the occurrence of the positive corneal inoculations.

SUMMARY.

Infectiousness of the disk. — Three out of the five cases gave positive results when inoculated on the cornea in the dry state. Disks from two cases gave negative results.

The positive reactions were obtained from a case of variola confluens, a case of variola vera, and a case of abortive variola. The negative reactions were from two cases of variola vera.

Of the disks that gave positive reactions, two were one day old, one was thirty-four days old, and one was eighty-eight days old. The two disks that gave negative reactions were one and thirty days old respectively.

In short, seven out of twelve corneas inoculated with disk powder gave variolous keratitis comparable with that which follows the inoculation of vesicle contents.

Disk paste from a case of variola confluens (disks twenty-two and fifty-two days old) gave positive reactions in three out of four inoculations.

Bacterial content of the disks. — It is obvious that no definite conclusions can be drawn from the bacteriological findings in the disk-serum cultures. It is, however, of interest to note that a rapid growth of streptococci is not usually found where the disks are from the clinically mild types of the disease. It is not possible to say that the bacteria which grow out in these cultures are from in, rather than on, the disks, but the fact remains that with the above method of treatment fewer bacteria are found in the disk cultures of the abortive variolas than in those from the variola veras.

Isolation of contagium. — In two cases it was possible to demonstrate the presence of the contagium by corneal inoculations, but no bacteria could be found.

DISCUSSION.

Throughout this work the corneal reaction of the rabbit has been used for the test of the presence of the contagium. When working with an organism not susceptible to cultivation and for which a differential stain is not available, some indirect method of proving its presence must be adopted. It is not possible for me to go into the discussion of the nature of the bodies which are found in the cornea only after inoculation with vaccine or variola virus. They are in any case specific. The fact that a variolous keratitis follows the inoculation of a given material on a rabbit's cornea may be taken as evidence that the contagium is present in the material. A negative result following an inoculation does not, however, necessarily prove the absence of the contagium. Either the organism may be in a stage at which the corneal epithelium is inhospitable, or mechanical difficulties may prevent its entrance. I am inclined to refer to the latter cause the failure of some of the inoculations of the cornea with the dry disk powder.

Owing to the rapid destruction of the corneal epithelium by the streptococcus pyogenes, the presence of that organism in any numbers in the material inoculated prevents the development of the typical reaction of variolous keratitis. The other bacterium commonly found in the disk cultures, the pseudo-diphtheria bacillus, probably identical with that described by Nakanishi, causes only a slight pyogenic reaction. It is of interest to note that the cornea seems to be able to resist the number of streptococci which are introduced in inoculations with dry disk powder.

The general belief that the disk is contagious is fully sustained by the results stated above. The positive reactions obtained with a disk that was eighty-eight days old goes to confirm the current opinion that the disk remains a source of danger for long periods of time. I find that Ishigami obtained a characteristic lesion on a calf with variola crusts a year and a half old.

The results obtained by incubation of the disks immersed in rabbit serum show that a certain number of the bacteria present can be eliminated from the product. It seems reasonable to suppose that this result is brought about by the bactericidal power of the fresh serum. The infrequency of the bacteria not eliminated by the serum, in the milder types of the disease, permits of an occasional demonstration of the contagium in a fluid where bacteria are not demonstrable by the ordinary methods of culture or by stained smear. It is not possible to make a positive statement that bacteria are absent, for it is conceivable that bacteria might be present whose temperature and nutritional requirements had not been met by the conditions of the experiment.

The analysis of the results of corneal inoculations with the "cultures" shows that the contagium was often not demonstrable when it might reasonably be supposed, in view of its almost constant presence in the dry disk, to have been present.

From this I have been led to believe that the fresh serum is not without germicidal action on the contagium. This is not in accord with the results of Beclere,[14] who found that normal fresh rabbit serum had no antivirulent powers towards vaccine lymph. In this connection both Dr. Tyzzer and myself have noted that the variola virus does not resist glycerinating, as does vaccine lymph.

In two of the disk-serum "cultures" (330 and 396) the contagium had apparently outlived the bacteria, though it was present in very small amount. I have no evidence that the contagium ever multiplied in any of the fluids in which it was placed.

It must be remembered that if we are dealing with an organism of the nature of an animal parasite, with metamorphoses in its life history, it is possible that a multiplication may have taken place which was associated with a loss of the power to cause variolous keratitis when inoculated on the rabbit's cornea. As has been mentioned above, the failure to demonstrate presence, as well as multiplication, may be due to such a phenomenon. If this be the case, I can only establish a partial negative to the statement that the contagium lived and multiplied in the "cultures."

I regret that, owing to the absence of specific directions, I was unable to repeat the procedures of Ishigami by which he claimed to have cultivated the contagium.

CONCLUSIONS.

1. The contagium is demonstrable in the variola disk.

2. By appropriate treatment a product can be obtained from the variola disk in which the contagium is demonstrable, but in which bacteria are not found.

3. The contagium does not appear to multiply in fresh rabbit serum or in filtered eye fluid of calves.

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THE CENTRAL NERVOUS SYSTEM IN VARIOLA.

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Very little of importance has been made out concerning the nervous system in variola. The brain and cord escape gross injury. With a view to making out finer lesions, resort was had to the Nissl method, with Weigert's myelin sheath method and the Marchi method as auxiliaries. No constant types of cell injury were found by the Nissl method. The myelin undergoes no change demonstrable by Weigert's method. A negligible amount of blackening occurs in a few Marchi sections.

Pieces from a number of human brains and cords * and from a number of brains and cords of the ape in variola inoculata and in vaccinia† were examined. Preparations (as far as possible from adjacent areas) were stained,

1. by Nissl's method:

a. by the nearest obtainable imitation of Nissl's own procedure,

b. by a similar method with paraffin imbedding,

c. by a method of formalin fixation and frozen section with staining as in the Nissl method;

2. for myelin by Weigert's method;

3. for fatby the Marchi method.

Characteristic degenerations are lacking. The human material shows a certain variable amount of change confined, as a rule, to the cell bodies of the nerve-cells, which show irregular dissolution or dust-like transformation of the granules, with occasional vacuolation. The axone-hillock is rarely stained. There is occasional chromophilia of isolated cells. The nuclei are as a rule in place, occasionally swollen; their membranes are occasionally wrinkled. The nucleoli are rarely visibly affected. There is a suspiciously large accumulation of trabant cells about certain of the larger nerve-cells of the motor cortex. These changes, whatever their

* Cases 11, 12, 16, 34, 35, 37, 38, 461.

† Cases 352, 437, 448, 457, 458, 471, 472, 488.

significance, are without relation to the severity or course of the disease. Beside the cells showing such changes are other cells of normal appearance. It was found impossible to pick the variola sections out from sections of several other brains in other (usually acute febrile) disorders.

The ape material shows still fewer changes. These are not characteristic enough to admit picking out, for instance, variola sections from vaccinia or from normal ape sections.

Huguenin, 1897,[1] was able to collect twenty-six references upon the nervous system in small-pox, dating from 1864 to 1892. These references deal chiefly with the (somewhat characteristic) secondary infections and sequelæ of the disease, together with various phenomena, the result of intercurrent affection.[2]

The only finding in the nervous system which can be regarded as an essential part of variola is the hemorrhagic tendency[3] sometimes seen in the cortex and cord in variola hemorrhagica. The majority of cases of abscess,[4] meningitis,[5] otitis media with sinus-thrombosis,[6] disseminated myelitis,[7] can be safely attributed, in the light of present knowledge, to secondary infection with the streptococcus; but the pneumococcus is occasionally responsible.[8] There is a further series of phenomena, the causes of which are obscure: prominent here are aphasia[9] (usually of muscular origin), isolated muscular paralyses,[10] neuritis. The most constant nerve finding from the clinical aspect is some degree of delirium, which is, perhaps, best regarded as the delirium of exhaustion.[11] Recovery is here the rule. There seems to be no reported case in which the cortical state can be regarded as the cause of death. In the interpretation of terminal deliria the possible effects of the pushing of alcohol in the treatment must not be forgotten.[12]

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A CLINICAL AND EXPERIMENTAL STUDY OF THE BACTERIOLYTIC COMPLEMENT OF THE BLOOD SERUM IN VARIOLA.

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In studying the bacteriolytic complement content of the blood serum in variola, experimental work was done on rabbits previous and subsequent to vaccination and variolation, on apes who were successfully inoculated from cases of small-pox, and on patients during the various stages of the disease. It was at the suggestion and under the direction of Dr. W. T. Councilman that this work was done, and I wish to express to him my indebtedness and sincere thanks.

Rabbits were used for the greater part of the experimental work, as it was found that the blood serum of this animal corresponded very closely in germicidal power with human serum against the bacteria employed in the tests. Although rabbits suffer no clinical disturbance from variolation, nevertheless, certain definite reactions occur, *e.g.*, the corneal lesions, after eye inoculation, which show them susceptible to the disease. Apes were also used for experimental work, as in these animals a typical variola exanthem was obtained by Drs. Magrath and Brinckerhoff.

The clinical studies were made from patients at the Boston Detention Hospital, and represent fairly the various stages and varying types of variola.

BACTERIOLYTIC COMPLEMENT OF NORMAL, VACCINATED, AND VARIOLATED RABBITS.

Methods. — The blood for these experiments was obtained under aseptic precautions, either from the ear vein of the rabbit or from the vessels of the neck. If from the ear, the hair was clipped and the ear thickly smeared with a depilatory powder containing barium sulphide. The ear was washed subsequently with sterile salt solution to remove all traces of the powder, the vein distended by pressure from below, and an incision made into it with a sharp pointed pair of scissors. From this incision seven to ten cubic centimeters of blood was obtained. Control cultures were made, and, in all instances where the serum was used, were sterile. In no instance was the blood kept longer than twenty-four hours after being drawn, the animal being bled the day preceding the tests, and the blood allowed to separate in the ice-box over night. The remainder of the technic is essentially the same as that used by Longcope[1] in estimating human complement.

The first experiment was to determine the bacteriolytic power of the serum in normal rabbits and to see to what degree this varied. For this eight rabbits were used, and, as was expected, some variations occurred. It was found, except in two instances, that one cubic centimeter of unheated active serum gave complete bacteriolysis for the typhoid bacillus in six hours. Of these two, one, starting with an immediate growth of twenty-two thousand colonies, showed two hundred and eighty colonies in six hours, the other, starting with an immediate growth of nine thousand six hundred colonies, showed three colonies in the six-hour plate. Both gave sterile plates in the twenty-four-hour plants, so that bacteriolysis was complete in twenty-four hours in all cases for B. typhosus with one cubic centimeter of fresh serum.

When one cubic centimeter of heated serum was reactivated with one-twentieth cubic centimeter of fresh serum (complement) nearly similar results were obtained. The two sera that showed growth in the six-hour plates where one cubic centimeter of fresh serum was used also showed growth in the tubes where reactivation was attempted with one-twentieth cubic centimeter of the same serum. One other serum that gave sterile plates with one cubic centimeter in six hours showed six colonies in the one-twentieth cubic centimeter six-hour reactivated plate. Otherwise the results were parallel, showing that one-twentieth cubic centimeter of fresh serum was as efficient in destroying the bacteria, when supplied with sufficient amboceptors, as was one cubic centimeter of the whole serum.

For the colon bacillus a more considerable variation occurred: one cubic centimeter of serum destroying all the bacteria in the twenty-four-hour plates in only five instances; in three instances giving a growth of two hundred and sixty, twelve hundred and eighty, and sixty colonies respectively; immediate plants being seventeen thousand, seventeen thousand, and five thousand nine hundred colonies. In reactivating for B. coli, six-tenths cubic centimeter of fresh serum was used, and this gave reactivation in only three of the five instances where sterile plates were obtained in twenty-four hours with one cubic centimeter of the fresh (unheated) serum. This seems to show that the reaction of the blood serum of the rabbit to the colon bacillus is not so uniform as it is to the typhoid organism, and that the latter is the better in general for observations of this sort. With smaller amounts of the bacteria, however, a more constant result would probably be obtained.

It may be stated that the sera that showed the most marked diminution in complement for B. coli were the same that showed the diminution for B. typhosus.

As far as could be seen from subsequent observations, the rabbits that showed this diminution of complement were in all respects normal.

The results of these observations on the normal rabbit show a marked germicidal power of the blood serum, although attended with some fluctuation, for the typhoid bacillus, which corresponds closely with the germicidal power of human blood serum for this same organism. The results with the colon bacillus are likewise similar, but apparently suffer more considerable fluctuation.

The second series of observations was conducted on vaccinated rabbits. The rabbits were vaccinated on the inner surface of the ear in two places. Tests were made as above at different intervals and no variation in the bacteriolytic complement of the blood serum that could be considered due to the vaccination was found.

The third series of observations was made on variolated rabbits. The vesicle contents, pustule contents, and disks —

the inspissated contents of the dried lesion remaining between the layers of the skin in patients convalescing from small-pox — were used for inoculation. The vesicle and pustule vaccinations were made both on the corneas and ears, and where disks were used they were planted subcutaneously. As far as could be observed, the animals showed no clinical symptoms following these inoculations. No decrease in the bacteriolytic power of the blood serum was noted in these cases. The observations were made on four rabbits six days after variolation. With six rabbits, observations were made on the day of variolation, two days after variolation, and six days after variolation.

Observations on one rabbit were made to see if any immediate reaction occurred in the blood serum in the course of the absorption of the small-pox contagium. For the inoculation a number of disks from virulent cases of small-pox were ground in a mortar, suspended in normal salt solution, and the emulsion injected, part into the ear vein and part into the peritoneal cavity of the animal. The rabbit's blood was drawn immediately after inoculation and at intervals of thirty minutes, one hour, and three hours. The fresh defibrinated blood was used for these tests. The heated serum to which this blood was added for reactivation was from another rabbit, but previous experiment has shown us (as in the experiment of Longcope, loc. cit., with human serum) that the interactivating power of normal serum does not vary. The results in this case show that no bacteriolytic diminution of the blood occurs immediately after inoculation — sterile plates belng obtained in twenty-four hours in all cases for B. typhosus with one-twentieth cubic centimeter reactivation. There was a growth of a few colonies in the six-hour plates at the first and third bleeding.

The complement content of two rabbits was also determined two weeks after variolation, and as in the previous cases no diminution was to be noted.

During the course of this work it was not infrequent to lose rabbits from various diseases which are incident to animals in confinement. Several animals at various times were taken when they appeared at the point of death from certain laboratory or induced diseases, chloroformed, the blood withdrawn, and the animal subsequently killed and autopsied. The results from four rabbits so examined show a remarkable and uniform decrease in the bacteriolytic complement of the serum.

Of these rabbits, one was affected with snuffles, and autopsy showed abscesses of the right lung and liver. The second rabbit was used when at the point of death from marasmus. The third rabbit showed colon septicemia, and the fourth leptothrix infection.

All of these rabbits gave a growth of from twenty thousand to innumerable colonies in the twenty-four-hour plates, and a correspondingly large growth in the six-hour plates for B. typhosus with one cubic centimeter of fresh serum and with one-twentieth cubic centimeter reactivation.

These latter observations are of interest as coinciding with the decrease in bacteriolytic complement found in individuals in the course of disease.

To summarize briefly, we may say that the bacteriolytic complement of normal rabbit blood serum is closely allied to the bacteriolytic complement of normal human blood serum for the typhoid and colon bacillus.

Normal rabbits show some individual variation in complement for the typhoid bacillus, and more marked fluctuation for the colon bacillus.

Vaccination and variolation do not affect the bacteriolytic complement of rabbit's serum for the typhoid and colon bacillus.

The blood serum of rabbits in the last stages of certain fatal diseases shows a marked diminution of bacteriolytic complement.

THE BACTERIOLYTIC COMPLEMENT IN VARIOLATED APES.

For the study of the bacteriolytic complement of the ape I was enabled, through the kindness of Dr Magrath and Dr. Brinckerhoff, to observe the blood serum of two species of ape (rhœsus and macacus) during variolation. The influence of the complement on typhoid bacilli was studied under the same conditions as in the human and as in rabbits.

Observations on the bactericidal power of the blood of the ape are of interest, since the blood serum of this animal so nearly approaches the human in various respects, *e.g.*, its precipitin reaction, and since a true variola may be obtained in this animal by inoculation.

Experiments were first made with normal animals, and it was found that, as in the human, one-twentieth cubic centimeter of fresh (unheated) serum was sufficient to reactivate one cubic centimeter of heated serum so as to give sterile plates with the typhoid bacillus in from six to twenty-four hours. With this as a standard, the following observations were made. (The animals are catalogued in the same order as the experiments appear in the paper by Drs. Magrath and Brinckerhoff, and I am indebted to them for permission to quote from their observations as to the clinical conditions, temperature, blood counts, etc., which they made.)

Ape II. — Macacus. Showed vesicle formation five days after inoculation. Well-marked "take" four days later. Fever from fifth to fourteenth day. Marked leucocytosis from tenth to thirteenth day. Reinoculation after healing of lesion without a "take." Later vaccination attempted without result.

Observations were not made on this animal during the progress of the first inoculation. Beginning a week before the second inoculation, observations were made every few days until after the second inoculation and the vaccination (twenty-three days). Sterile plates were obtained in all instances from twenty-four-hour tubes. In one instance there was a growth of six colonies in one of the six-hour tubes with one-twentieth cubic centimeter reactivation. This was the day following inoculation.

Ape III. — Macacus. Double inoculation; the second four days after the first. Marked constitutional reaction, anorexia. Leucocytosis forty-eight hours after each inoculation. First inoculation showed crust formation; second inoculation elevation and redness and slight vesicle formation. Parasites not demonstrated histologically.

Five observations of the complement were made on this ape. The first the day before the inoculation; the second forty-eight hours after inoculation, when there was a leucocytosis of 30,000.

The first of these observations shows a growth of eight hundred and forty colonies in the twenty-four-hour plate. The second gave innumerable colonies in the twenty-four-hour plate. The subsequent examinations also showed a diminished complement, although on the day of autopsy a sterile twenty-four-hour plate was obtained.

The animal was in poor physical condition at the start, and showed more marked constitutional symptoms after inoculation than did any of the other animals. He refused food and appeared weak and sick. Autopsy, however, did not show any specific disease.

APE IV. — Macacus. Production of general exanthem eight days after inoculation. No constitutional reaction. Leucocytosis seventh and eighth days. Cytoplasmic forms of the parasite found histologically. Four observations were made on the complement, the first previous to inoculation, the second the fourth day, when there was a leucocytosis of 15,000, and the others with the appearance of and subsequent to the eruption. There was no diminution of complement observed at any time. Sterile plates being obtained in eighteen hours in all cases with one-twentieth cubic centimeter reactivation.

APE V. — Rhœsus. Inoculated with pustular contents into jugular vein. Died of streptococcus septicemia forty-five hours after inoculation.

Observation before inoculation showed normal complement content.

The second observation made twenty-four hours after inoculation, and the third made just before death, showed innumerable colonies in the twenty-four-hour plates, both *B. typhosus* and streptococci being present.

APE VI. — Rhœsus. Well-developed lesion six days after inoculation. No constitutional reaction (animal natural and eating well). One degree elevation of temperature. Leucocytosis of 30,000 before inoculation; 15,000 on fifth and sixth days. Parasite present in primary pustule. Observations continued throughout the experiment showed always a normal complement. Sterile plates being obtained in eighteen hours with one-twentieth cubic centimeter reactivation in all cases.

To sum up briefly, we have five apes, in four of which variola inoculata was obtained. Of these four, only one showed marked constitutional reaction, that is, decided weakness, depression, and anorexia, although there was an average elevation of temperature of two degrees Centigrade in the inoculated animals. There was a leucocytosis in all cases, which did not apparently affect the complement content. There was one case of streptococcus septicemia.

Observations on the blood serum show that the production of experimental variola causes no diminution of the bacteriolytic complement content in this animal.

The diminution of complement in apes III. and V. tend to confirm the experiments previously made, which show that lowered vitality and terminal infection cause a diminution in the germicidal power of the blood.

Ape.	Reactivation.	Immediate.	Six hours.	Twenty-four hours.
II. Variola inoculata.	1/20 cc.	16,000 to 32,000.	6 to sterile.	Sterile (all instances).
III. Variola inoculata, anorexia, weakness.	1/20 cc.	14,000 to 30,000.	3,000 to 200.	840 to innumerable. (One observation sterile with 1/10 cc. reactivation.)
IV. Variola inoculata.	1/20 cc.	6,000 to 12,000.	Sterile.	Sterile.
V. Streptococcus septicemia.	1/20 cc.	9,000 to 16,000.	14,000 to 20,000.	Innumerable.
VI. Variola inoculata.	1/20 cc.	12,000 to 16,000.	Sterile.	Sterile.

THE BACTERIOLYTIC COMPLEMENT IN VARIOLA.

In undertaking the study of the complement content in human variola, one of the first difficulties that presented itself was to obtain sufficient amounts of blood from each patient to carry out the number of observations that it seemed necessary to make in the different stages of the disease. In an infectious disease of this sort, where secondary infections are so common, it seemed hardly justifiable to open several times, or even once, one of the larger veins, and, moreover, the extensive edema and distribution of the skin lesions in many of the cases would render the opening of an arm vein a rather dangerous and delicate surgical operation. While there are methods for determining the complement content of the blood with small amounts of serum, notably those of A. E. Wright [2] and Ainsley Walker,[3] they seemed to us less satisfactory than the technic described by Longcope (loc. cit.) in his study of chronic disease. This latter, however, which seemed to be the most satisfactory in many ways, presented the difficulty of requiring considerable amounts of blood serum in the carrying out of its details.

A consideration of this method shows that the greater part of this serum is used after inactivation to supply sufficient amboceptor for the complement tests, and that in reality very minute amounts of the fresh patient's serum is necessary for the destruction of the B. typhosus, provided one has sufficient amboceptor. If, therefore, any means could be devised whereby an amboceptor, other than that of the patient himself, could be substituted that would link with the bacteria on the one hand, and with the complementary body on the other, a method would be obtained whereby extended observations could be carried on in the course of a given disease, because of the fact that only a very minute amount of the patient's serum would be required for the test.

Longcope (loc. cit.) showed that heated serum of different individuals could be substituted indifferently in reactivating a given complement, so that it made no difference whether a given complement was added to the heated blood serum of that same individual or to the heated blood serum of another individual in obtaining bacteriolysis. If, then, different human amboceptors would link with a given complement so as to give bacteriolysis, the thought suggested itself: Why might not certain animal sera contain an amboceptor that would fit the haptophore of a human complement, and accomplish the same result?

The previously described observations on the complement content of the rabbit showed a remarkable parallel between the reactivating power of rabbit and human sera. The experiments of Grünbaum and others on the precipitins show a close analogy between the blood of man and of monkey.

Therefore a series of experiments were undertaken to determine whether or not sera of other animals when deprived of complement could be substituted for heated human serum in supplying an amboceptor for human complement. The result of these preliminary experiments show conclusively that the amboceptors of at least three different blood sera, namely, those of the monkey, of the rabbit, and of the horse, will combine with the complement of human serum, giving bacteriolysis for the typhoid bacillus in identically the same manner as when the heated and unheated serum of the same person is used for the test. Therefore complement estimates can be made as often as desired without danger or disturbance to the patient, as a simple puncture of the ear yields enough serum for this test.

An understanding of this phenomenon may, perhaps, be aided by enlarging upon the simile of Fischer, who likens the cell or bacterium to a lock, the amboceptor to a key, and the complement to the hand that turns the key. Bordet showed that the heated serum (amboceptor) could be reactivated so as to produce hemolysis by the addition of complement from various sources. In other words, given the lock and key, almost any hand could turn the key. On the other hand, the fact that several different keys might fit the same lock had not been given consideration. But that such is the case our experiments show. The only thing being necessary is to have keys of very similar construction, and in the case of human and some of the animal sera, the similarity is so near that the lock may be turned by either of these keys, provided there is strength enough in the hand (complement) to manipulate the key. To complete this analogy, bringing in the importance of a deficiency of complement as a cause of disease, we may say that the hand is not strong enough to turn the key, and thereby the door remains unlocked. Such may be the case in certain individuals; there is a deficiency of complement, and, though we have the lock and key, immunity fails to close the door, and the individual suffers the results of the invasion of a pathogenic organism.

In following this analogy, however, it is well to remember that the importance of only one of the factors concerned in bacteriolysis is emphasized, and that in infectious processes we have many things other than the complement of the blood to consider. The complement content is, however, of marked importance, and a deficiency of the same, we may say in the light of our present knowledge, cannot exist without some danger to the individual.

Technic. — In the beginning of these experiments rabbit serum was used for reactivation, as it could be easily obtained, the blood of one large rabbit being sufficient for a considerable number of observations. Later on horse serum was substituted for the rabbit's serum, as it was found to answer the purpose, and saved the time necessary for obtaining rabbit's blood. Normal horse serum was kindly furnished me through the courtesy of Dr. Kinyoun.

This facilitated the work considerably. A point in regard to the horse serum is that it was found necessary to heat it for one and one-half hours at 57°–58° C. to completely destroy the complement.

Vedder[4] has noted that when a small amount of complement is added to normal salt solution (one-twentieth cubic centimeter of complement to one cubic centimeter NaCl) that some bacteriolysis takes place. That is to say, the salt solution plays to some extent the same part, apparently, as does the immune body in heated serum when complement is added to it, or the small amount of immune body present (for complement is not to be obtained pure) acts in some way in the presence of salt solution. The same phenomenon has been noted in my experiments, but in no instance has there been sufficient bacteriolysis where complement was added to salt solution to compare with the results where heated serum was reactivated. The term "complement" is, of course, at present only relative, as no satisfactory way to separate it wholly from the bacteriolytic amboceptors is at hand. The appearance of such a method will doubtless clear up many problems connected with work of this sort.

It might be stated that, in inactivating a large number of tubes of serum at the same time, they should not be closely packed, but should be so separated as to assure the full force of the heat reaching each tube immediately on being placed in the oven; otherwise tubes may be found which will give sterile plates without reactivation.

The steps of the process of the complement determination were as follows:

1. Obtaining rabbit's serum. — The animal is chloroformed and the carotid exposed under aseptic precautions. The artery is then ligatured above, a sterile capillary tube connected with a sterile flask inserted into the artery, the clip removed, and the blood allowed to flow into the flask. The blood is placed in a cool place until the serum has separated. When the separation is complete, the serum is transferred by means of a sterile pipette into small tubes, one cubic centimeter to each tube. The tubes are then placed in an incubator at 57° C. for one hour to destroy the complement. The serum is now ready for use, or it may be kept for weeks, if necessary, in a cool place.

2. Obtaining patient's blood and reactivating rabbit's serum. — The blood for the complement observation is obtained from the patient's ear in

the same manner as is generally used in procuring blood for a Widal test. Sufficient blood may be obtained by a single puncture, as one-fourth cubic centimeter is ample amount for a test. Tubes for this may be readily made from glass tubing. They should be sterile and plugged with cotton. The serum separates quickly, especially if a sterile platinum wire be run between the clot and the glass. When the serum has separated, the necessary amount (one-twentieth cubic centimeter) is transferred by means of a sterile capillary pipette into the tube containing one cubic centimeter of inactivated rabbit's serum. The serum thus obtained was sometimes used immediately on its separation from the clot (about one hour after withdrawal), or was withdrawn at night and used the next morning (twelve to eighteen hours after withdrawal). Walker (loc. cit.) has stated that a slight fluctuation in the complement is incident to the time the serum is used after withdrawal. These slight fluctuations, which he gets in the complement content from hour to hour, are very different from the marked changes observed in the course of investigations of disease, and would not be notable in an investigation of this sort, where changes when they occur are much more marked.

Controls were made between the serum used immediately after withdrawal and the serum which had stood for twelve to eighteen hours, and no differences that would affect the general results here given were observed.

3. Preparation of typhoid emulsion. — The typhoid emulsion was prepared by adding to a tube containing three cubic centimeters of sterile (.85 per cent) salt solution, a small amount of the growth from a twenty-four-hour streak culture of B. typhosus on agar or Lœffler's blood serum. The amount used is necessarily not always the same, but such an amount that one loopful of the mixture (salt solution + B. typhosus) will give a plate of from ten thousand to thirty thousand colonies can be uniformly obtained. A double loop was used in transferring this mixture to the reactivated rabbit serum, three double loopfuls (approximately one-fiftieth cubic centimeter) being added to the serum.

4. Plating. — Having now the preparation of amboceptor (heated rabbit serum) plus complement (patient's serum) plus B. typhosus emulsion, the mixture should be thoroughly shaken and two double loopfuls (or four single loopfuls) added to the agar and planted. The agar should be very thoroughly shaken before plating to insure a uniform growth of colonies. The tubes and plates are then placed in the incubator, 37.5° C., and new plates are made at the desired intervals.

5. Counting colonies. — The exact estimation of the colonies in the plate where there is a considerable growth is not of particular importance, as when the colonies run into the thousands a few hundred one way or another make no particular difference, but when there is a scant growth, showing that more or less bacteriolysis has taken place, they should be counted carefully. The colonies were estimated in the cases where there was considerable growth by counting several square centimeters at different parts of the plate, and from them making an average of the number of

colonies on the whole surface. Where there was a scant growth the entire surface of the plate was counted. A No. 3 ocular was used to facilitate the counting process.

Having in mind the idea previously outlined, the first observation was to see if bacteriolysis could be obtained for B. typhosus by reactivating one cubic centimeter of heated rabbit serum with one-twentieth to one-tenth cubic centimeter of fresh serum from a normal individual. Such was found to be the case, bacteriolysis being complete in six hours with both these reactivations on two healthy individuals from whom Widal amounts of blood were obtained for the test. While this was not conclusive evidence that the rabbit's serum furnished an amboceptor parallel to the human serum, nevertheless it pointed to such being the fact, and so the following test was performed:

R. T. Healthy male, bled from arm (twenty-five cubic centimeters). Serum separated from clot twelve hours after withdrawal and pipetted into tubes, one cubic centimeter in each tube. These tubes placed in the incubator at 57° C. for one hour. Tubes containing one cubic centimeter of freshly drawn rabbit's serum (A) and rabbit's serum (B) two weeks old that had been kept in a cool place were inactivated at the same time. These tubes were then reactivated with one-twentieth to one-tenth cubic centimeter fresh, unheated (R. T.) serum, and an equal quantity of suspension of B. typhosus added to each. The control plant of one loopful of this suspension gave a growth of thirty thousand colonies.

The results of this experiment show an accurate parallel between the tubes, whether heated human or rabbit serum was used for the intermediary body, as may be seen from the following:

(R. T.)	Immediate.	6 hours.	9 hours.	24 hours.
1 cc. heated human serum plus 1/20 fresh human plus B. typhi,	12,000	Sterile	Sterile	Sterile
1 cc. heated human serum plus 1/10 fresh human plus B. typhi,	9,600	"	"	"
1 cc. heated rabbit serum A plus 1/20 fresh human plus B. typhi,	13,200	"	"	"
1 cc. heated rabbit serum A plus 1/10 fresh human plus B. typhi,	8,400	"	"	"
1 cc. heated rabbit serum B plus 1/20 fresh human plus B. typhi,	6,000	"	"	"
1 cc. heated rabbit serum B plus 1/10 fresh human plus B. typhi,	12,000	"	"	"
1 cc. unheated human serum plus B. typhi	15,000	"	....	"
1 cc. unheated rabbit serum plus B. typhi	15,600	"	....	"
1 cc. heated human serum plus B. typhi	12,000	30,000	....	Innumerable
1 cc. heated rabbit serum plus B. typhi	14,000	32,000	....	Innumerable

The test was carried out in the same manner, substituting heated monkey serum (rhœsus) and heated horse serum in place of the heated rabbit serum. The results were the same as in the first experiment. Either of these latter sera serving to replace the heated serum of the individual tested, and giving sterile plates in six and twenty-four hours when reactivated with one-twentieth cubic centimeter of the normal human complement containing serum. That this destruction of the bacteria would not take place in the heated animal serum alone was shown by the fact that when plants were made from these tubes of heated serum before reactivating with fresh human serum there was always a growth of from thirty thousand to infinite colonies in the twenty-four-hour plates.

Having shown, then, that these heated sera could be used in quantitating the complement of an individual, a series of observations were made on apparently healthy individuals to see what variations might exist. Heated rabbit serum was used for the intermediary body. It has already been shown by Wright (loc. cit.) and by Longcope (loc. cit.) that there is a considerable variation in the complement content in normal people, depending, in their cases, especially upon overwork and anxiety These observations bear out these facts, and show that in individuals who may in no way be called diseased a considerable diminution in the complement content may occur, which can at times be accounted for, but sometimes not.

These observations were made on twelve healthy individuals about the hospital, including physicians, nurses, and laborers. Of these, seven showed a high complement content at each observation, several observations being made on each during a period extending over six weeks, all giving sterile plates in six hours and twenty-four hours, with one-twentieth cubic centimeter of serum.

In two other cases the observations were of interest. In the first of these, both one-twentieth cubic centimeter and one-tenth cubic centimeter inactivation failed to give sterile plates, even in the twenty-four-hour plants. The man on whom the observations were made had been doing exacting work for some time, with loss of sleep and loss of air and exercise. Two days after the observation was made, two small streptococcus infections appeared on this man's hand. With a relief from work and the healing of the infections, it was found that one-twentieth cubic centimeter of his serum gave sterile plates in six hours. The other of these cases had shown high complement on several observations, one-twentieth cubic centimeter giving sterile plates in six hours, but during the course of an attack of influenza the complement was found diminished, one-twentieth cubic centimeter of his serum failing to give sterile plates in twenty-four hours.

Of the three remaining cases of this group, one observation was made in two cases: the first, a girl, aged five years, who failed to give sterile plates in twenty-four hours, when either one-twentieth cubic centimeter or one-tenth cubic centimeter of her serum was used for reactivation. Though in good health at the time, she had had an attack of broncho-pneumonia several weeks previously. The second of these cases, a male, middle-aged, failed to give sterile plates with one-twentieth cubic centimeter or one-tenth cubic centimeter of his serum. The last of this group was a young, healthy Irish girl, on whom a number of observations were made, but in no case was such reactivation obtained, with one-twentieth or one-tenth cubic centimeter of her serum, as to give sterile plates in twenty-four hours. In this latter case, especially, no apparent reason presents itself for the diminution of the complement, and we must conclude, therefore, that in certain individuals this complement diminution exists. It makes it necessary, then, in studying any given disease, to take the possibility of such conditions into account before drawing too definite conclusions as to the disease being always the cause of the diminution of complement.

Assuming, then, from the above observations, that in the greater number of normal individuals one-twentieth cubic centimeter of their fresh serum would reactivate one cubic centimeter of heated rabbit serum so as to give sterile plates in from six to twenty-four hours, a series of observations were undertaken in patients afflicted with variola to see what changes, if any, might occur in the course of this disease.

It is to be understood that in studying a disease like variola, unless the epidemic be unusually severe, one cannot pick material, but must use whatever he is fortunate to find at his disposal at the time. In this investigation a fairly representative group of cases of the different forms and different degrees of severity of the disease came under observation. In all, fifteen cases were studied and may be grouped as follows:

1. Variola vera, abortive type. Majority of lesions not coming to pustulation, six.

2. Variola vera, mild, but disease running complete course, five.

3. Variola confluens, all with more or less secondary infection, three.

4. Purpura variolosa, one.

A complete account of these cases has previously been published[5] and here only a summary of the results will be given.

In all instances where an observation of the patient's blood was made early in the course of the disease a low complement content was found. The nearest approach to an exception to this rule is in one case where the first observation made on the fifth day of the disease showed a normal complement.

In the abortive cases and the cases running through the entire course of the disease, but unattended with secondary infection, there is a rapid return of the complement to the normal standard. This rise of complement content in the abortive cases is in coincidence with the beginning involution of the lesions. In the cases running through pustulation and unattended with secondary infection, this rise may be noted in the later pustular stage or during the early stage of dessication. One may, perhaps, say in some cases that an absence of secondary infection may be predicted when one gets a return of the complement to normal in the early pustular stage; but a prediction that secondary infection would occur could not be made at this time from a low complement, for in some of the mild cases complement may be low throughout the entire course of the disease, just as it may be low in certain normal individuals.

Two cases offer an interesting comparison as regards the complement content reduction being in inverse ratio to the severity of the disease. Two patients, a boy and a girl, both infected from the same case, entered the hospital at the same time. At the time of their entrance, and until the vesicular stage was well developed, there was practically no difference to be observed in the severity of the two cases. The girl, however, did not go on to the pustular stage. Her lesions aborted and she quickly returned to health. Her complement was normal throughout.

The boy, on the other hand, continued on through a well-marked pustular stage, though without secondary infection, his case being considerably more severe and more prolonged than that of the girl. His complement showed a reduction until the late pustular stage, when just before involution of the pustules it rose to normal and so remained.

In the cases attended with secondary infection (from which streptococci were isolated) there was always a low complement content — the diminution continuing throughout convalescence in all of the observed cases, and the patient being discharged while the complement content was still low. In one case there was some increase in complement content at the last observation, though not a complete return to our arbitrary normal standard.

That there should be rapid rise of complement in the uncomplicated cases of variola seems natural from the resistance that the individual offers to this disease. In variola the recuperation of the patient is remarkable. With the passing of the lesion the patient in most cases is well in all senses of the word, and that a normal complement should accompany this quick return to health is not remarkable.

That a low complement content should persist when secondary infection occurs is of considerable interest, in that it bears out the work previously done in showing that a low complement is coincident with secondary infection (Longcope, loc. cit.). In this connection the case of hemorrhagic smallpox is of value in establishing the conclusion that a low complement is coincident with terminal infection. This case suffered a progressive complement diminution from the time of her entrance to the hospital until her death.

Since the completion of this work, Perkins and Pay[6] have published observations of the bacteriolytic complement in variola. Their observations, for the most part, were made on fatal cases; the serum being taken after death. In all their severe cases attended with secondary (streptococcus) infection a diminution of complement was also noted.

To briefly summarize these observations, then, we find in variola:

1. A diminution of complement content in the early stage of the disease.

2. A rapid return of the complement content to normal in the cases unattended with secondary infection. This return is seen in the abortive cases with the beginning of the involution of the lesions; in the others, during the late pustular or early dessicating stage.

3. A failure of the complement content in its return to the normal in cases in which there is secondary infection.

4. A progressive continued diminution in the complement content when the case is complicated by terminal septicemia.

An interpretation of these results may be aided by a brief consideration of the disease, variola. In variola we have a clinical entity which involved two processes. (1.) Infection by a definite specific contagion. (2.) A secondary local or general pyogenic process, which may or may not be present, but which, if present, may offer the gravest consequences to the patient.

From our observations we must conclude: That infection with the specific contagion of variola causes a diminution of the bacteriolytic complement of the individual, and that this diminution is more or less dependent upon the severity of the attack. Also that a mild infection, in which there is very little clinical disturbance, might occur without such complement diminution being noticed. This should be inferred from the fact that in one of our mild cases no diminution was present at the time the patient entered the hospital.

Given an individual attacked by a definite specific contagion, it is reasonable to suppose that this individual will exert all possible resistance against the contagion. Provided this resistance is of avail, it is shown by a rapid return of the complement to the normal standard. Provided, however, the resistance of the individual is for some reason weak or the infection is unusually severe, this rise of complement to normal does not take place, and we have a patient entering the pustular stage of variola with a diminution of the bacteriolytic power of the blood serum.

Given, then, a lowered complement and a supply of pyogenic organisms (streptococci were isolated from the lesions of practically all of our cases), it is easy to see how variola may be complicated by a local or general pyogenic process; and from the fact that the complications due to pyogenic infections, and that death from streptococcus septicemia were, in the cases observed, always accompanied by a continued lowered complement content, we must conclude that this lowered complement content in variola is the cause of such infection taking place and is of danger to the individual.

The foregoing observations emphasize the fact that systematic studies of the bacteriolytic power of the blood serum carried out in various diseases would be of value. Especially does this seem to be true from a prognostic standpoint. The rapid advances that are now being made both in the technic and the understanding of the phenomenon of bacteriolysis should make such observations of practical importance.

SUMMARY.

1. Most normal individuals have a high bacteriolytic complement content. This complement content is, however, remarkably fluctuant, conditions of lowered vitality, due either to fatigue or infection, causing a diminution which may under proper conditions be promptly restored.

2. Certain individuals show on repeated examination a low complement content, the cause of which may not be evident.

3. The blood serum of rabbits and of apes shows under normal conditions a high bacteriolytic complement content similar to that in the human. The complement in these animals is not diminished by vaccination or variolation, but is diminished in certain fatal diseases, notably septicemia.

4. In individuals suffering from variola there is a diminution of complement in the early stage of the disease, with a return to normal in the cases in which there is no secondary infection. In cases in which there is secondary infection and terminal septicemia there is a continued diminution of complement.

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CLINICAL OBSERVATIONS ON VARIOLA.

I. R. BANCROFT.

This paper is based upon the personal experience of the writer as resident physician at the Boston Detention Hospital during the epidemic of small-pox in 1902–3, and presents the results of the study of about twelve hundred cases.

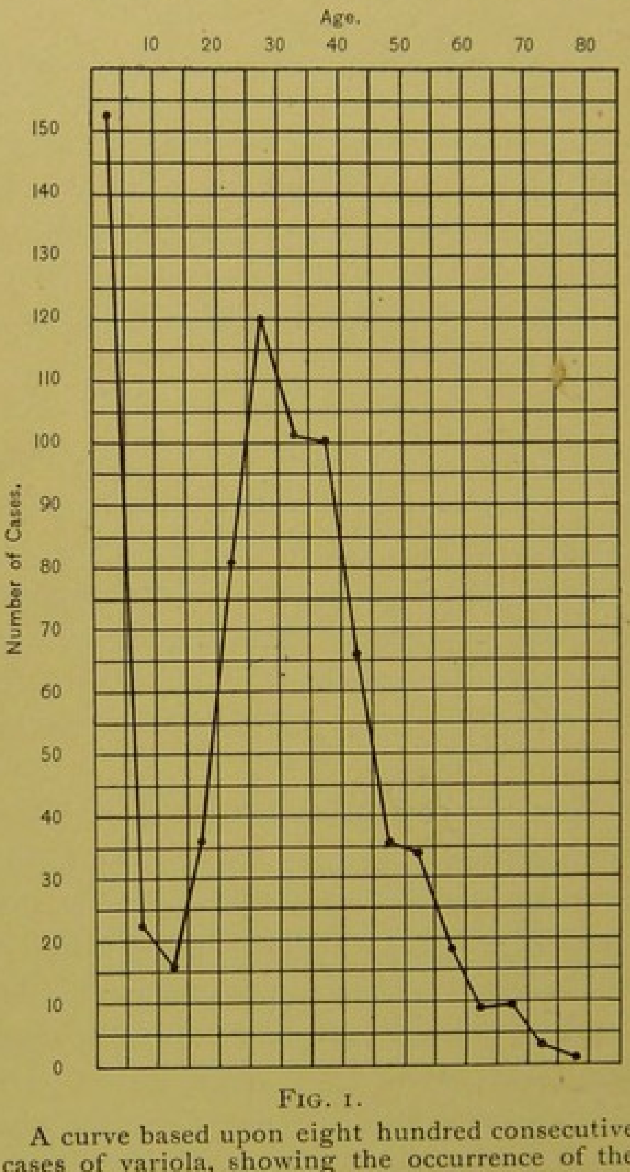

FIG. 1.

A curve based upon eight hundred consecutive cases of variola, showing the occurrence of the disease at different age periods. Note the infrequency of the disease in the period coinciding with that of the school age and of compulsory vaccination.

The epidemic referred to was of moderate extent and severity. About sixteen hundred cases of the disease were reported, and the general mortality was eleven and one-tenth per cent. A large proportion of the sick were of Canadian birth. Individuals of all ages were susceptible, although children between the ages of five and fifteen years showed a certain degree of immunity referable to primary vaccination upon entering school. The various types of the disease were well represented.

The following classification is adopted:[1]

1. Variola vera and its modifications.
2. Variola sine eruptione.
3. Variola hemorrhagica, primary and secondary.
   (*a.*) Variola pustulosa hemorrhagica.
   (*b.*) Purpura variolosa.

A. VARIOLA VERA.

The clinical course of the ordinary form of variola includes two periods: (1) the interval between the onset of the disease and the appearance of the eruption, usually about three days; and (2) that between the outbreak of the eruption and its final disappearance, usually about two weeks. These will be referred to as the initial stage and the stage of eruption.

Initial stage. — The onset of the disease was usually sudden and marked by definite symptoms, such as headache, backache, chill, and vomiting; occasionally it was gradual and accompanied by malaise, fugitive pains, and gastric disturbances. Headache, as an early symptom, was common, usually severe, and often sharp and agonizing; in some instances it was no more intense than the headache of any other febrile disease. The localization of the pain varied; in some cases it was diffuse; in others it was referred to the frontal and to the cervical regions. The next most common symptom was backache, often acute and usually localized in

[1] The writer is inclined to attribute the mildness of the disease in variola without eruption and in abortive variola vera to the absence of secondary infection.

the lumbar region; it was sometimes slight in degree, sometimes absent. It occurred either during the last days of the initial period or at the onset of the disease, and usually disappeared with the fall of the temperature. The pain in the back was often accompanied by pain in the thighs and the hips, and in the bones and the joints. Pain in the chest, in the epigastrium, and in the abdomen, either alone or in connection with the backache, was often present.

A chill, more or less definite in character, usually marked the beginning of fever. The temperature rose rapidly to about 104° F., and remained quite steadily at its height, with very slight morning remission, for about three days. In the lighter cases the temperature fell to normal at the end of the third day; in the more severe, the decline was gradual and the return to normal not completed before the fifth or sixth days of the disease. With the fall of the temperature appeared the eruption, the full development of which was reached after normal temperature was established. In a case of moderate severity, the temperature usually reached the normal point two days after the beginning of the eruption.

The pulse and the respiration were both raised in proportion to the degree of the fever.

Gastric symptoms were commonly observed during this period of the disease, at the onset in the form of nausea and vomiting, and later as anorexia and gastric irritability, often lasting for two or three days. The tongue was usually covered with a thick yellow coat, and the breath was offensive.

Cough and sore throat occurred in a small number of cases, and chiefly during the winter months. It is probable that in many instances the presence of these symptoms was a coincidence.

Nosebleed, in cases of moderate severity, was present only occasionally, but in the more severe forms of the disease it constituted a common early symptom.

Disturbances of the central nervous system were frequently manifest. Insomnia, in part due to pain, occurred even in mild forms of the disease. Somnolence, sometimes to the extent of unconsciousness, was present, especially in children. Convulsions occurred in the cases of several children, some of them suffering from only a mild form of variola, during the initial fever. One child, with constantly recurring convulsions, was in the status epilepticus for an entire day. Nearly all patients complained of terrifying dreams, and many even tried to keep awake because of the horrible nature of their dreams. Delirium was often present, even in cases which subsequently showed a very slight eruption. It was nearly always violent and of the type seen in inebriates. Patients sometimes refused food and drink. Occasionally the most violently delirious would suddenly become rational. Others were exhausted by the violence of their delirium, and died before the full development of the eruption. In two cases katatonia and sensory disturbances in the form of local anesthesia were apparent. Great weakness and vertigo were not rare, and occasionally were mistaken for alcoholic intoxication. Syncope, even in the most rugged, was a not infrequent symptom. Disturbances of speech, exclusive of those incidental to delirium, were observed in three cases: in one, consisting of a slight stammering; in two, of a transitory aphasia, lasting from the third to the fifth days.

Metrorrhagia occurred frequently during the initial stage, and in one case was the cause of death. Abortion occasionally took place in this period, but more often later in the disease.

The urine at this time usually contained albumin, and presented the characteristics common to febrile diseases.

The spleen was sometimes found to be enlarged, but the enlargement was neither constant nor persistent.

The length of the initial stage was usually three days. Variations from this interval were observed in two hundred and sixty-three out of five hundred and thirty cases. In seven the initial stage was absent; in twenty-six it lasted for one day; in ninety-one for four days; and in one hundred and thirty-nine for two days. Analysis of these cases shows that no relation exists between the length of the initial stage and the severity of the disease.

Stage of eruption.— With the subsidence of the temperature, at the end of the initial stage, the patient was usually free from symptoms, possessing a good appetite and a retentive stomach.

The eruption was often preceded by a diffuse erythema of the face, especially of the forehead. This erythema was distinct from the initial rash (vide. infra.).

The specific eruption varied widely in its characteristics according to the part of the body involved and the thickness of the skin. Patients ordinarily noticed it first on the forehead and on the wrists; close examination, however, usually showed a few macules on the abdomen before these regions were affected. As the most typical may be regarded the lesions occurring upon the anterior surface of the wrist, at the carpal fold, where the skin is of moderate thickness. The lesion first appears as a small red macule, one to three millimeters in diameter. It is sometimes preceded by an area of slight induration. Upon pressure the macule loses color, and there appears outside of it an area paler than the surrounding skin and resembling the blanched circle of a mosquito bite. Removal of pressure is quickly followed by return of the blood, during which may be seen in the macule a capillary pulse. The latter is more readily seen if but slight pressure be made. It is, however, often visible without manipulation. The phenomenon is demonstrable after the lesions have become well-marked vesicles.

The macule is not of uniform color, but may be divided into an inner area of hyperemia, surrounded by a zone of grayish color, beyond which is a second zone of hyperemia. The middle, gray zone contains fluid within the epidermis, confined in spaces and not removable by a single prick of a needle. With the evolution of the lesion, there appears at the middle of the central red area a small gray spot, which gradually becomes filled with fluid. The gray, vesicular zone increases in size, rising above the level of the inner part of the lesion, and forming a crater or umbilication. It later becomes connected with the gray area at the center by radial channels traversing the intervening red zone, which, for a time, shows disconnected remnants, but finally vanishes. The pock has now become a tense, hemispherical elevation, either umblicated or rounded, containing gray, transparent fluid, and surrounded by a red areola. The fluid gradually assumes a yellowish tinge, and by the eighth day of the disease the pock is usually filled with a distinctly puriform fluid.

From this climax of development a gradual retrogression, beginning usually on the tenth day, takes place. The lesion becomes less tense and less rounded, and umbilication, due to the collapse of the center, may appear. Its contents dry up, and it becomes converted into a solid, reddish-brown, gutta-percha-like substance. This, for a time, is closely adherent to the solid base, but later separates and falls off as a flat, hard, circular disk, of an average diameter of three millimeters.

The lesions on the face differ from the type pock above described, in that the concentric areas are in them seldom well marked and that they present a more pronounced initial induration. The early lesion of the face is a papule, often with a shallow depression at its apex. In this region the evolution of the lesion is more rapid than elsewhere, and the pustular stage more quickly reached, the climax of development occurring on the eighth or the ninth day. By the ninth day, in all but the lighter cases, an exudate appears upon the surface of the pock, consisting of a thick, yellowish material which soon dries into a gray or greenish-gray crust. When the lesions are close together, this crust may completely envelop the face and harden into a tough brown mask. When they are discrete, the exudate in drying forms a disk upon the apex of the pock. Upon the scalp the lesions appear later than they do upon the face, and may cause great pain. Marked edema of the scalp and of the face, leading to closure of the eyes, may appear just before pustulation.

Upon the arms many of the lesions show a region outside of the areola of lighter color than that of the skin in general, forming a white circle, the diameter of which is about twice that of the lesion. This circle by the fifth or sixth days disappears. The eruption on the hands usually is abundant, causing an edema which occasions great pain. The patient at this time assumes a characteristic attitude, elevating both hands and spreading the fingers as widely as possible. When the horny layer of the epidermis of the palms is thick, the pock may pass through all of its phases without distinctly elevating the surface. After the absorption of the vesicle contents, large disks remain beneath the horny layer which must be picked out before the patient is discharged from the hospital.

The eruption on the trunk is later in appearing than is that on the face or on the arms. It is usually less abundant on the abdomen and in the groins than elsewhere. It is profuse on the back, especially in its upper part, and here the lesions are generally accompanied by an extensive area of erythema surrounding the areola, forming irregularly rectangular areas, the long axis of which is parallel with the ribs. This erythema persists until the pustular stage is reached. The pocks on the body are often umbilicated, but lack the details of the concentric ring formation.

The lower extremities, in general, exhibit the eruption later than any other part of the body, although a small area of pock formation may appear very early upon the inner aspect of the thigh. The lower leg is often almost wholly free from eruption.

The drying of the lesions ordinarily begins before the decline of the temperature. Those on the face dry first, next those on the arms, later those on the trunk and the lower extremities. The crusts of the individual lesions fall off, and sometimes the epidermis of the palm or the sole is cast off in one piece, carrying upon its inner surface disks. When the lesions are deep, scars usually result. In some severe cases the nails fell off. In nearly all cases with secondary fever, the hair fell out during convalescence.

The eruption on the mucous membranes is typified by that on the soft palate. It begins here before the appearance of any lesions, upon the skin with the formation of a papule.

This papule develops into a vesicle which ruptures, forming a white, crater-like erosion. In this region, the eruption is most abundant upon the soft palate, although the entire mucous membrane of the fauces may be affected.

The pocks may pursue a course of development unlike that described as typical. One of the most common variations, seen in severe cases of the disease, is delay in the evolution of the eruption, whereby the pocks, which in lighter cases are well filled out and pustular by the eighth day, do not reach their full development before the twelfth day.

Another atypical condition is seen occasionally toward the fatal termination of the disease. Instead of developing into full, rounded pustules, the pocks may remain umbilicated or even flatten down, puckering in the center, and presenting a dull gray, instead of a glistening yellow color.

Not infrequently the center of the pock becomes dark red from hemorrhage. This hemorrhagic condition is seen mostly in the lesions of the forearms and of the legs.

These atypical pocks show less than the ordinary degree of peripheral inflammatory reaction.

An eruption which has shown atypical characteristics during the first week of the disease may later become typical; the reverse of this is also true.

The usual development of the pocks upon the trunk may further be modified during the vesicular stage by the formation of impetiginous lesions about a centimeter in diameter, which remain flat. In other cases the pocks are merged into superimposed bullæ, often of large size, which contain a fluid, nearly clear, and so continuing.

The pock contents may become mixed with blood, or the areola assume a purpuric character, conditions often seen in the eruption on the lower extremities.

At times, in the place of ordinary vesicles, the earlier lesions consist in purpuric macules, which may either develop into the usual pustule surrounded by a purpuric areola, or remain as small, bright red spots.

The temperature, normal at the beginning of the stage of eruption, usually so remained for three or four days. It then gradually rose, often without morning remission, reaching a maximum of 102° to 104° F. by the twelfth or thirteenth

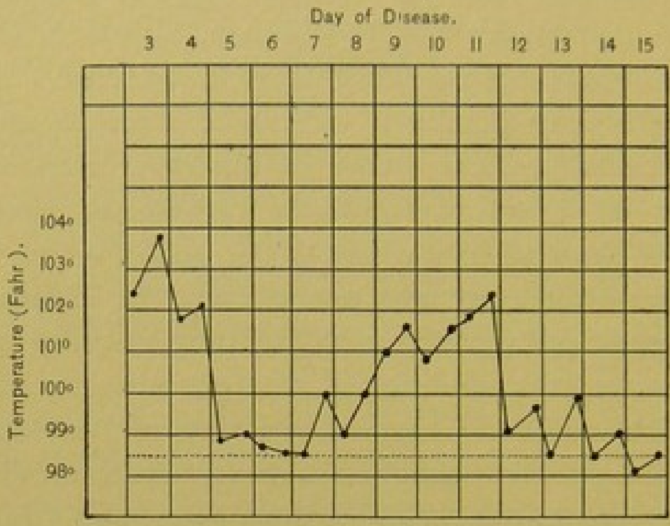

FIG. 2.
Variola vera, typical case

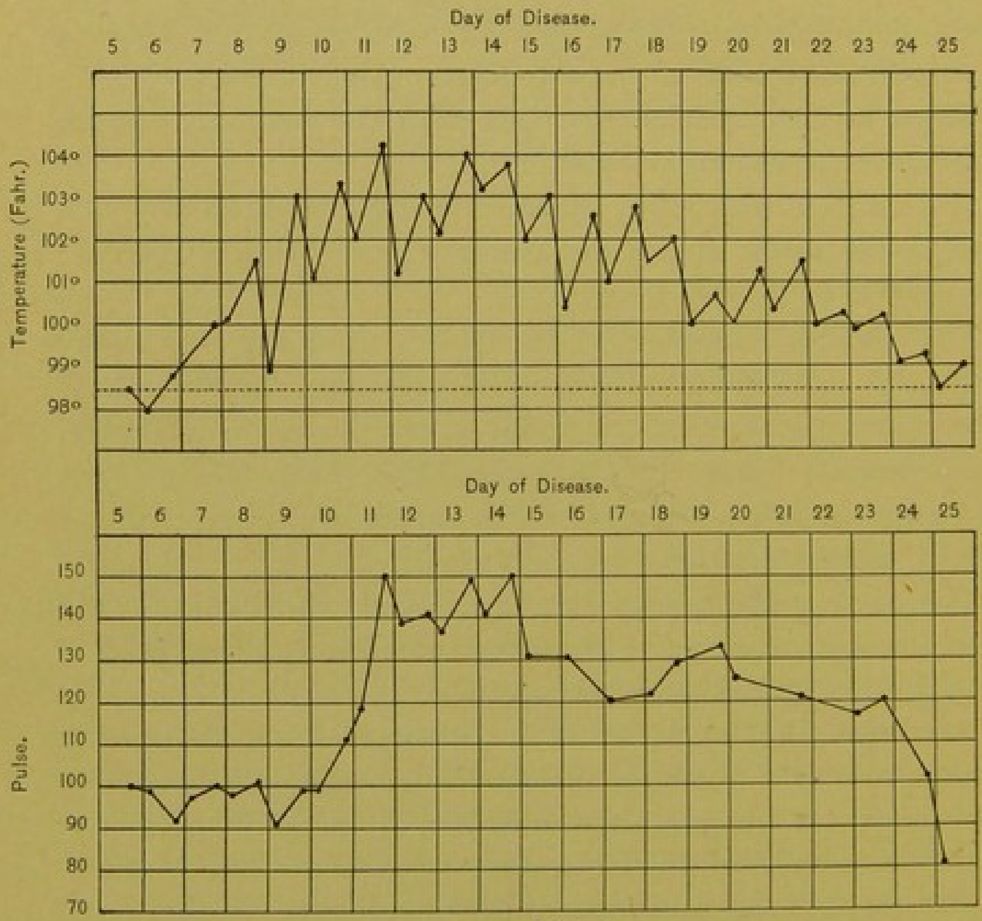

FIG 3.
Variola vera, severe.

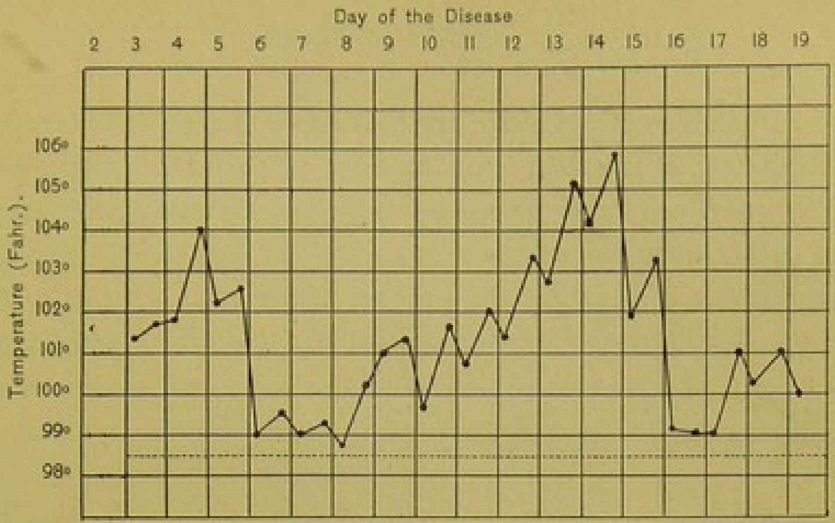

FIG. 4.

Variola vera, severe, with crisis on the fourteenth day of the disease.

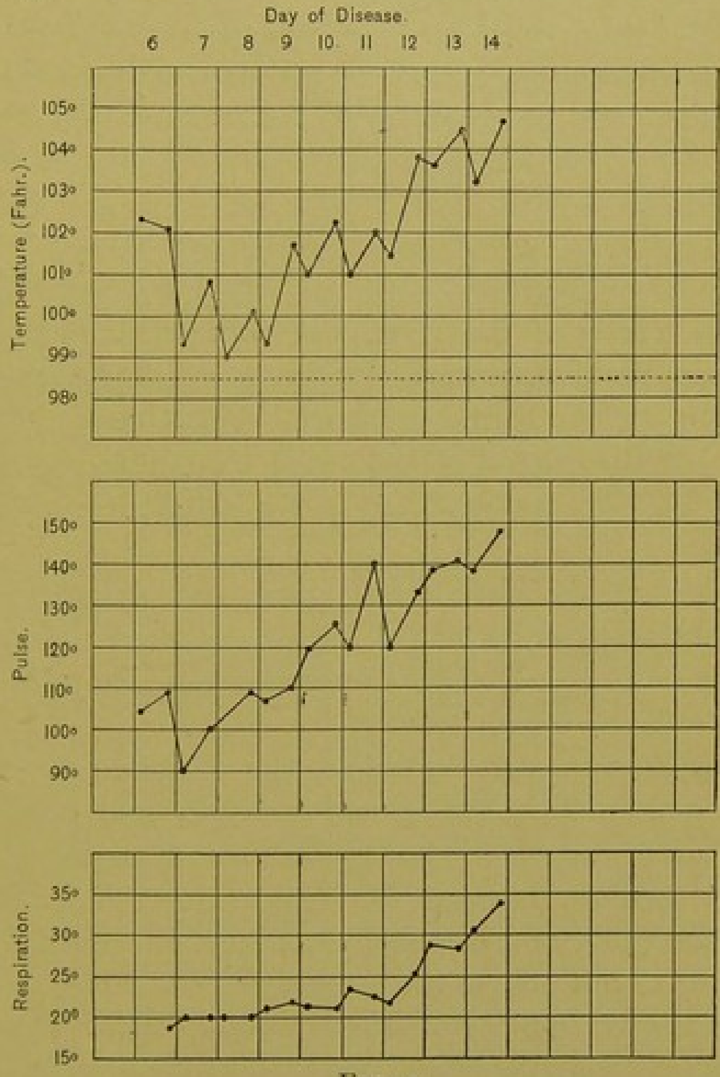

FIG. 5.

Variola vera, fatal on the fourteenth day of the disease.

day of the disease. In the more severe cases the temperature did not return to normal at the end of the initial stage, but declined only two or three degrees, rising again on the sixth or seventh day of the disease.

The following table shows the maximum temperature in two hundred cases of variola vera, one-half of which were fatal:

Temp. (Fahr.)	Number of cases. With recovery.	Fatal.
100°	2	1
101°	17	6
102°	37	16
103°	27	21
104°	16	25
105°	1	21
106°	. .	8
107°	. .	2
Total cases . . .	100	100

The character of the fever is of some value in prognosis. The earlier the temperature reaches its maximum, the lighter is likely to be the form of disease. Persistence of the fever throughout the period of the initial stage forecasts a severe form of the disease, while its early subsidence means the contrary.

The decline of the temperature was usually gradual. After continuing at its height for two or three days, in case of recovery it slowly fell, sometimes not returning to the normal until the fourth week of the disease.

The cases were rare in which the temperature dropped from its maximum to the normal in twenty-four hours.

The pulse rate, which during the afebrile period was often perfectly normal, followed the temperature in its rise, and sometimes, even in cases with recovery, exceeded a rate of one hundred and fifty to the minute.

Disorders of the respiratory system, other than those occurring as complications, were not common, but when present consisted of cough, dry, sometimes severe and persistent, and occasionally accompanied by bloody or rusty sputum. Obstruction of the larynx, necessitating intubation, occurred in three cases, all children under five years of age.

Disturbances of the digestive system were infrequent. Rarely there was slight nausea. Sufficient nourishment could generally be taken throughout the stage of eruption. Glossitis was seen in a few cases, in one of which it was so severe as to demand recourse to nasal feeding. Occasionally there was a profuse secretion of saliva.

Disorders of the central nervous system were seen at this stage of the disease rather more frequently than during the initial stage. Delirium, in inebriates often of violent character, appeared on the fourth or fifth day, and such individuals frequently died before the disease had reached its climax. Unconsciousness was common in severe cases during the last three or four days of life, or, in such as recovered, for an equal period prior to the climax of the disease. On the other hand, some patients having a severe and fatal form of the disease retained consciousness throughout.

Albuminuria was present in severe cases at the height of the secondary fever and occasionally during convalescence.

Nodules in the testicles, small and hard, were present in certain severe cases at the pustular stage of the eruption.

Complications.— The complications of variola vera are numerous and varied.

Toxemia, other than that incident to local secondary infection, was evidenced in certain cases by a rise of temperature on about the sixteenth day.

Secondary infections of the skin were frequent during the late stages of the disease in the form of furuncles, small abscesses, erysipelas, and cellulitis. These conditions occurred upon any part of the body. Purulent inflammation of the elbow-joint was seen in the third week of the disease in two cases. Acute non-suppurative arthritis was common.

Conjunctivitis was nearly always present. Pock formation leading to ulceration was seen upon the conjunctiva, but save in one case of pannus was not observed upon the cornea.[1] Lesions at the limbus often extended to the cornea, and gave rise to ulcerative keratitis. Ulceration of the center of the cornea, without previous lesions, were seen late in the disease.

Disorders of the respiratory system were common complications. Broncho-pneumonia was frequent in fatal cases; lobar pneumonia was rare, but occasionally developed after convalescence seemed to be established.

Pleurisy and empyema were uncommon. Bronchitis was so frequent as to justify regarding it as a sympton rather than as a complication. Edema of the larynx was occasionally present, and in many cases there was aphonia.

Inflammation of the salivary glands was frequent, and occurred usually in the third week. The parotid gland was affected most often. In the absence of suppuration there was a profuse secretion of saliva.

Suppurative parotiditis was a frequent and sometimes fatal complication. Noma and local gangrene following this condition were seen in three cases.

Inflammation of the middle ear was a rare complication, present chiefly in children.

Endocarditis was uncommon. Impairment of the action of the heart was usually referable to degenerative myocarditis or to anemia.

Disorders of the genito-urinary system were infrequent. Acute nephritis was rare. Suppurative orchitis was not observed.

Abortive types.— Under this head are included forms of small-pox in which the course of the disease, both as to constitutional symptoms and as to eruption, is of much less than ordinary severity, and which are generally designated as "varioloid."

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[1] The case here referred to was that of a woman who gave a history of definite exposure to variola on October 20, from three until ten P.M., and of onset of the disease on October 30, at noon. The eruption appeared November 1. Old keratitis, with pannus, was present in both eyes, more marked in the left. Upon the vascularized portion of the left cornea occurred a typical papule, which underwent the evolution characteristic of the specific lesion upon a mucous membrane.

The onset in such cases may be marked by very indefinite symptoms, such as slight headache or pain in the back, initial fever being unnoticeable or absent. In some instances an initial fever of the most severe type was present, accompanied by delirium and unconsciousness, and continuing for four or five days. More commonly the initial fever was of short duration.

The eruption in cases of this sort was of varied character. In many, the lesions at the onset were numerous, closely-set, but finer and more superficial than usual. The vesicle developed on the apex of the papule, never completely replacing it, and on the fifth or the sixth day, instead of becoming a full, tense pustule, collapsed and dried up, forming a thin brown crust which was soon desquamated. This modification of the ordinary evolution was seldom manifested by all of the pocks upon the body; more commonly certain lesions continued to develop into fully-formed pustules, so that scattered among the abortive pocks were occasional typical pustules. The lesions of the lower extremities showed the least tendency to modification. The eruption in certain cases was rendered yet further atypical by the development of purpuric areolæ about the lesions, or by the appearance of purpuric macules among the pocks.

Another modification of the eruption consisted in sparseness of the pocks, which, notwithstanding their small numbers, presented a natural evolution. In certain cases of this type the individual lesions were of very large size. In such, the course of the disease was sometimes fairly severe, although unaccompanied by secondary fever.

In a few instances the pocks were larger than the ordinary and presented a solid base, the latter persisting as warty protuberances long after the disappearance of other evidences of variola.

A further modification of the eruption was characterized by the appearance of successive crops of lesions. In cases showing this modification, a sparse eruption, becoming pustular by the fifth day of the disease, developed first upon the face, and was followed by the appearance of lesions upon the other parts of the body on the third, the fourth, and the fifth days. This resulted in the co-existence of pocks at all stages of development, the earliest lesions being dry by the time the later had become pustules. The vesicles in such cases were very superficial, and those upon the arms could easily be broken by the pressure of the finger In this form of the disease no symptoms were present after the subsidence of the initial fever, and complications were rare.

B. VARIOLA SINE ERUPTIONE.

Illness of indefinite character, appearing on the twelfth day after exposure, was seen in hospital attendants and in others exposed to infection. The symptoms, lasting for two or three days, consisted of headache, pain in the back, fever, and nausea. After the subsidence of the fever, a few definite pocks occasionally appeared. These conditions were observed in four physicians and in eight attendants.

Incidents in the course of the epidemic warrant the belief that variola without eruption and unrecognized was not infrequent. Inquiry at the time of the removal to hospital of a patient with well-marked small-pox often brought to light the fact that about two weeks previously another member of the household had been sick, who, upon examination, frequently showed a few healed pocks. In one such case, the crust of the "pimple" from the suspected individual gave the characteristic corneal reaction in the rabbit. One patient, who had a well-marked initial fever accompanied by a typical initial rash, subsequently presented no eruption whatever. Another, a pregnant woman, remembered having a slight headache after exposure to the disease, but was not otherwise ill. Her child showed a typical eruption when two days old. Cases of this sort were doubtless the cause of outbreaks of the disease in hospitals and in other institutions. A group of three cases in one of the large hospitals in Boston, the onset in whom was nearly simultaneous, was traced to a ward tender who had had an attack of what was supposed to be the "grip."

C. VARIOLA HEMORRHAGICA.

The hemorrhagic varieties of small-pox differ widely from the other forms of the disease. They are classified according to the relation of the purpura to the specific eruption. The form in which the purpura precedes the specific eruption is known as purpura variolosa, or primary hemorrhagic small-pox; that in which the purpura follows a pre-existing specific eruption is designated as variola pustulosa hemorrhagica, or secondary hemorrhagic small-pox.

(*a.*) *Variola pustulosa hemorrhagica.* — In the course of the epidemic, this form of the disease was frequently observed. It occurred usually in young adults, and was seen in no one under twenty years of age. Its occurrence bore no determinate relation either to sex or to habits.

The onset, which in some cases was preceded by malaise and by indefinite pain, was usually marked by severe symptoms. The initial fever was high, but the course of the disease in the initial stage was not essentially peculiar, save that at its end in severe cases there was no fall of temperature. In such the purpuric conditions were first evidenced by hemorrhage into the areolæ of the pocks. In some instances the hemorrhagic tendency was first indicated by persistent nose-bleed, and by hemorrhage from the conjunctivæ, and from the gums; and in yet others by hematuria and by metrorrhagia. A condition was seen simulating hemophilia, in which slight accidental wounds bled persistently.

In less severe cases the hemorrhagic condition appeared in the vesicular stage of an eruption developed in the usual position after a severe and protracted initial fever. The hemorrhage showed first on the lower extremities in the centers of the pocks, fillng them with bloody fluid, a condition often coincident with hemorrhages from the mucons membranes.

Patients usually lived for several days following the appearance of the hemorrhages, which allowed for the development of pustules. Although some lesions showed an admixture of blood and pus, the exudate was never sufficient to cause complete filling out of the pocks.

In cases with recovery the pustules of the lower extremities sometimes contained a little blood, and occasionally there was nosebleed. Patients showing well-marked hemorrhagic tendencies invariably died, others in whom this tendency appeared late in the disease, and was of slight degree, recovered.

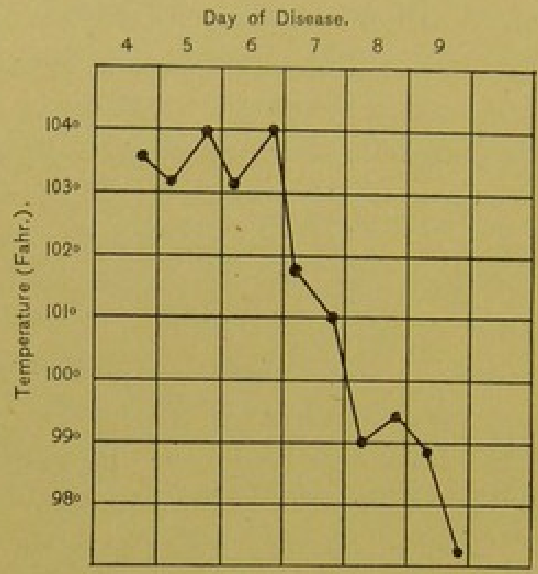


FIG. 6.
Variola pustulosa hemorrhagica.

(*b.*) *Purpura variolosa.* — In this form of variola a general erythema precedes any other outward sign of the disease; this and other characteristics distinguish it sharply from variola vera, and from other types of small-pox.

The occurrence of purpura variolosa varies widely in different epidemics. In that of 1870 it was frequent; in the recent outbreak it was present in one per cent of twelve hundred cases.

The relation of this type to other forms of the disease was wholly indeterminate. In two instances it followed exposure to mild forms of variola vera. In no instance did one case of the purpuric form give rise to another. Cases of variola traceable to purpura variolosa as a source of infection showed no uniformity in type.

Relative to vaccination, analysis of the twelve cases showed that recent vaccination had been practised in none, although in two unsuccessful attempts had been made within the two weeks preceding the onset of the disease; that primary vaccination in childhood had been attempted in seven, of which only three, men of an average age of twenty-seven years, showed typical foreated scars; and that three were unvaccicinated.

As to sex, ten of the cases were men, and two women; neither of the latter was pregnant.

The age limits were twenty-four and fifty-one, with an average of thirty-four years.

Alcoholism as a factor in predisposition was of doubtful importance. Five individuals used liquor to some extent; of the three of these who showed typical scars of vaccination in childhood, two were markedly intemperate.

The period of incubation could in no case be exactly determined.

The onset, preceded by prodromal symptoms of indefinite character, differed but little from that of variola vera. The temperature was not as high as in the latter form of the disease, and their history showed that patients had in several cases worked during the first two days of the initial stage, in two instances at hard labor.

On the second day, an erythema, superficial, bright red, and resembling that of the initial rash, appeared on the chest or on the arms, which within twenty-four hours overspread nearly the whole body, and was accompanied by marked increase in the severity of the constitutional symptoms. Pain, severe, deep-seated, and agonizing was present in many parts of the body, including the bones and the joints, but was most intense in the præcordium and in the epigastrium. Pain in the back was not a prominent symptom.

The temperature was rarely high, usually less than 103° F. The pulse was rapid, reaching 160 to the minute. The respiration was rapid and labored without evidence of organic disturbances in the lungs. Cough was present and persistent, accompanied by the expectoration of fluid consisting almost wholly of blood. The tongue became swollen and covered with dark crusts of dried blood and mucus. Nausea and vomiting were persistent, the vomitus often consisting almost wholly of blood.

The erythema, first apparent on the second day of the disease, on the third day changed from bright red to a bluish terra cotta color. This change was most marked on the face and on the extremities. At the same time the skin became mottled with petechiæ. These were of two sorts: one, a small, round, or irregularly-shaped area, usually less than two millimeters in diameter, bright red in color, superficial, and widely distributed, although most numerous on the face, on the extremities, and on the abdomen; the other, somewhat larger and more deeply seated than the above, from two to fifteen millimeters in diameter, of irregular contour, plum colored, and most numerous on the eyelids and on the alæ nasi.

In addition to the erythema, and to the petechiæ, many of the cases presented a few, small superficial gray vesicles, containing a small amount of clear fluid, and most frequently seen on the face and on the body. In some instances, a dozen or less of these rudimentary vesicles were the only evidence of the specific eruption. In others even these were absent.

Individuals who lived beyond the fourth day of the disease usually showed the beginning of a typical small-pox eruption, with lesions on the palms and on the soles, but rarely elsewhere. At this time the petechiæ increased in number, and the erythema grew yet darker. With the progress of the disease, hemorrhage from the mucous membranes became more and more marked. Bleeding from the stomach, the intestines, the kidneys, and the bladder was common. All of the women suffered from severe metrorrhagia. Dark crusts of dried blood formed in the nares and in the mouth, from beneath which blood slowly oozed. The gums became red and soft, and bled easily. The breath was extremely fetid, and the tongue was swollen, dry, and crusted.

The urine was scanty, high-colored, and bloody, and contained blood casts in small numbers.

The sensorium was surprisingly clear; the patient was at times drowsy, but could always be awakened to complete consciousness, and was able to converse intelligently up to the time of his death.

Death always took place within a week from the onset; the average duration of the disease was five days.

Initial rashes. — During the initial stadium there may appear upon the skin an erythematous or a petechial rash, lasting from a few hours to several days, and bearing no resemblance to the specific eruption. In many instances the two forms of rash were associated.

The erythema was observed less frequently in this epidemic than it has been in others. Of sixteen cases of initial rash, the pure erythematous type occurred in but six, and in these the variola was of mild form, without secondary fever. The rash usually appeared on the second day of the disease, reached its full development on the third day, and gradually faded, finally disappearing before the development of the specific eruption. It was sometimes prolonged into the stadium of eruption for a few hours, or even several days, but in such cases usually did not persist beyond the late vesicular stage of pock formation.

The distribution of the rash varied from a mere flushing of the face, frequently seen during the last day of the initial fever, to a general erythema, a condition seen more rarely, in which the skin of the entire body was of a bright red color. The erythema of the lower extremities was often faint, and, even when elsewhere intense, was here patchy and coarsely mottled with areas of normal skin. In one instance the rash was composed of fine macules; this bore no relation to the specific eruption, and lasted for twenty-four hours.

The coincidence of the erythema with the eruption gave rise to yet other conditions, in general characterized by the presence of large areas of erythema surrounding or in the vicinity of pocks, and developed by the extension of the areolæ. These areas, often four or five centimeters in diameter, were irregularly oval, and were most numerous upon the back, parallel with the ribs. In children large areas of this sort, eight to ten centimeters in diameter, and widely distributed, occurred without relation to pocks.

The petechial form of rash was seen in ten cases. It seldom occurred unassociated with erythema, and even when well-marked often appeared as a diffuse, dark red erythema, which only upon pressure disclosed petechiæ, bright red, round or irregularly oval, and from one to two millimeters in diameter.

When not general, this rash was most commonly present upon the abdomen, within an area below the umbilicus, shaped like the wings of a butterfly, a region usually comparatively free from pocks. This fact has suggested the possibility of a relation between the presence of the initial rash and the absence of the specific eruption. In one instance in this series of cases initial rash in the region designated was followed by an abundant eruption of pocks.

In the less marked cases the rash occurred in detached patches situated usually in the folds of the skin at the axilla, the groin, and, less commonly, at the elbow and the knee. These areas were most extensive when the petechial rash was accompanied by erythema than when it occurred alone.

The petechiæ were numerous in scar tissue, notably in the striæ albicantiæ of the abdomen.

Of ten cases in which this form of rash was well-marked, four exhibited a mild form, two a severe form, and two a fatal form of the disease.

The duration of the petechial rash was somewhat larger than that of the erythema. Its appearance changed rapidly after the third day, when the associated erythema disappeared, and the petechiæ gradually became yellowish-brown, and at the end of the week generally disappeared.

This rash may closely resemble that of purpura variolosa; both appear at the same stage of the disease, and at first look very much alike. Later, the erythema of purpura variolosa assumes a characteristic, dark, terra-cotta red color, and is often further differentiated by the presence of ecchymoses in the deeper layers of the skin and beneath the mucous membranes.

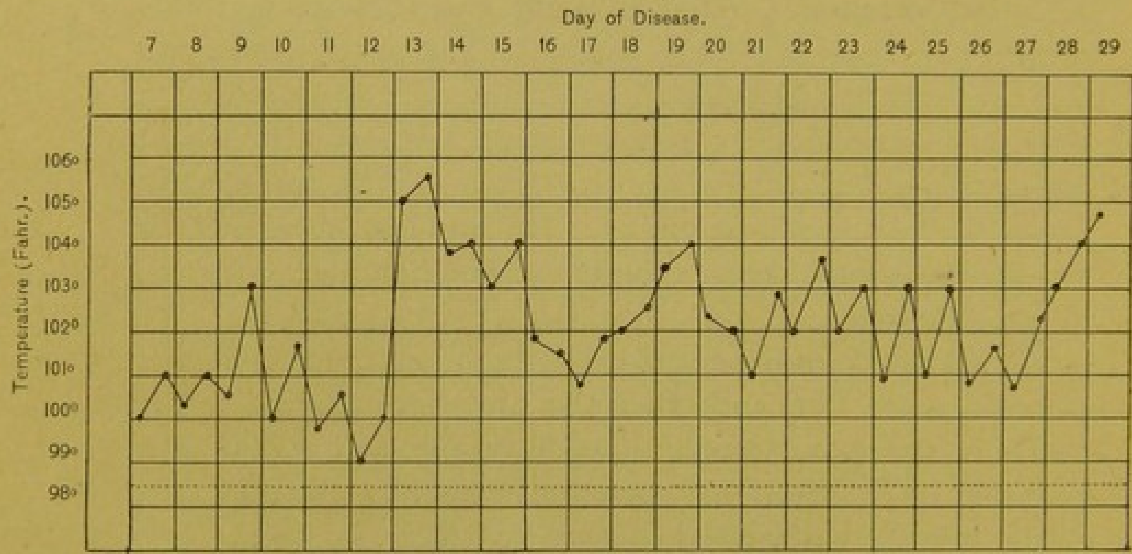
Secondary rashes. — Another type of rash, developed late in the disease, within or following the period of crust formation, was observed in a small number of cases, less than one per cent of the whole series, and with one exception occurring within a space of three months, near the close of the epidemic.

This secondary rash exhibited two forms: (*a*), a fine, red maculo-papular eruption; and (*b*), a mottled erythema. The first mentioned followed light cases of variola, was present chiefly on the body, rarely on the arms, hands, and face, and was unaccompanied by itching. It appeared soon after the climax of the secondary fever, and lasted for about ten days. Coincidentally with it occurred fever, and usually headache, nausea, and vomiting. In some instances the rash was present without these symptoms; in others they occurred without the rash.

The second form of secondary rash, the mottled erythema, was seen more frequently than the first. It occurred in the course of the more severe types of variola, and was always preceded by constitutional symptoms which usually appeared on the thirteenth day, and consisted of fever, in seven cases amounting to 104° F., headache, nausea, and vomiting. The eruption commonly appeared either with the rise of temperature or on the day following, and closely resembled that of scarlet fever. In the more severe cases the erythema was general, in the lighter it was localized on the abdomen, on the flexor surfaces of the arms and on the legs, and in the folds of the axillæ and of the groins. Its duration was ordinarily from two to three days, and it was followed by desquamation. In several of the cases there was subsequent rise of temperature, occurring repeatedly, and with some suggestion of periodicity. In two cases there was an associated suppurative cellulitis in the region of the elbow. One case died on the twenty-ninth day of the disease (See Fig. No. 7).

The condition was first regarded as scarlet fever, and patients suffering from it were isolated. One such, who exhibited a rash on the ninth day of the disease, accompanied by fever on the ninth, the twelfth, and the fifteenth days, was discharged from the hospital on the twenty-fifth day with desquamation incomplete. Eight days later, a member of his family was admitted to the scarlet fever wards of the Boston City Hospital, with no known source of contagion. On the other hand, the writer, who was constantly exposed to cases of this sort, six months later, after the conclusion of his services at the hospital, acquired scarlet fever.

FIG. 7.



Variola vera, light case, with secondary rash; death on the twenty-ninth day of the disease.

DESCRIPTION OF PLATE.

PLATE XXVIII.

FIG. 1. Variola vera. Duration ten days. Note characteristic attitude of hand due to tension of skin caused by pustules. Form and arrangement of lesions typical.

FIG. 2. Severe variola vera. Duration fourteen days. Soles of the feet to show the disks.

1

2

Bancroft

Small-pox

Heliotype Co. Boston

EPICRISIS.

W. T. Councilman.

In the early stage of the specific lesions of the skin and mucous membranes in small-pox, bodies are found which vary in form, structure, and size. We regard these bodies as the parasites causing the disease. They occur within the epithelial cells, within the nuclei, and free. The forms within the nuclei are subsequent to those which develop within the cytoplasm. They are present in the greatest numbers in cases of the greatest severity and rapidity of course. They do not occur as isolated structures, but one form follows another by gradual transitions, forming a cycle which corresponds with the cycle of development of living things. In the different cases the same forms are found at the same period of the disease. The bodies increase rapidly in the lesions, and the lesion increases in extent by continuous infection of adjoining epithelial cells. The same forms are found in corresponding situations in the lesions in different cases.

Bodies simulating those which occur in small-pox may be found in other diseases. In all of these the bodies represent an accidental product. There is no complexity of structure such as is found in most of the small-pox bodies. There is no sequence representing growth development. These accidental cell inclusions simulate most closely the early forms of the cytoplasmic bodies before structural differentiation is apparent. Such inclusions are the product of a number of conditions.

Red blood corpuscles may be carried into the epithelium of mucous membranes and are found both between and within the cells. We have found this, however, only in cases where there was abundant hemorrhage and edema in the underlying tissue, and where the cells were separated, allowing the exudate to freely pass through. The most striking example of this was found in the soft palate in a case of diphtheria. The red blood corpuscles retain their characteristics, though single corpuscles may break up into two or more pieces, and should not be confounded with the parasites. There is more chance of confounding leucocytes or their products with the parasites, but any one with the usual knowledge of forms of degenerating leucocytes should be able to recognize them. These sources of error can also be dismissed, because red corpuscles are not carried into the intact epidermis by exudation streams, and leucocytes are absent in the earliest lesions in the skin where the cytoplasmic forms are found in the greatest numbers. The possibility of confounding certain artefacts which are formed in the red blood corpuscles with intranuclear forms of the parasites should be considered. By hardening red blood corpuscles in Zenker's fluid or sublimate, staining and imperfectly dehydrating them, an appearance of small refractive vacuoles, many of which contain a minute dot, is produced in them. We have rarely found red blood corpuscles in a section presenting this appearance, but it is easily produced in a cover-slip preparation of the blood. There might be a possibility of confounding certain forms of the parasites with the nuclear detritus from lymphoid cells, but these cells are rarely found in the epidermis, and nuclear detritus from them occurs only in the pustule contents.

Various conditions simulating the parasites may also be seen in vaccinia. In describing the vaccine bodies in the cornea, Dr. Tyzzer calls attention to the swellings on the terminal nerves which might be confounded with the parasites. Occasionally in the epithelial cells of the cornea a space is formed around the nucleus by shrinkage, and this space may contain a coagulum. The protoplasm of the epithelial cells may stain more sharply immediately around the nucleus, and in various ways suggestions of the specific inclusions may be given. We have never seen such formations in the skin lesions of small-pox.

The morphological products of cytoplasmic degeneration do not simulate the parasites. Such products are usually multiple, devoid of internal structure, and have no fixed relations with the cytoplasm, such as a vacuole around them. Chromatin particles arising from nuclear fragmentation and enclosed in the cytoplasm cannot enter into consideration, for the nuclei of epidermis cells do not fragment in the same way as the more solid nuclei of the mesenchymatous tissues. Moreover the cells in which the youngest forms of parasites are found have morphologically normal nuclei and cytoplasm. We have in another place called attention to the fact that the earliest degeneration is found in the more superficial cells of the epidermis, while the parasites are in the lower. Specific products of epithelial cells such as Kerato-hyaline granules are too numerous, have no definite size, and are formed in parts of the epidermis where parasities are rarely found. The parasites in both variola and vaccinia have been attributed to degeneration of the centrosome. No proof has even been given that the centrosome does degenerate. If the centrosome could by any process of degeneration give rise to such a product as any one of the cytoplasmic parasites it would have to increase enormously in size and change all its characteristics. The parasites are also found between cells and within cells in mitosis, in which latter it may be assumed that the centrosome is normal.

The young cytoplasmic parasites may be closely simulated by certain products of nuclear degeneration. We have spoken of the clumping of chromatin in the nucleus as a common form of degeneration in various epithelial organs. It takes place in some situations more readily than in others. The mass collects in the center of the nucleus and may be round or irregular in form. In the eosin and methylene blue stain it frequently changes its staining reaction, becoming more refractive and taking a pale lilac which gives place to a bright eosin stain. Such masses may pass from the nucleus without evident injury of the nuclear membrane. In the cytoplasm they have the size, the refraction, and the staining reaction of the young undifferentiated cytoplasmic parasites. We have never been able to make out the passing of the chromatin from the nucleus in any of the small-pox lesions, though it probably does do so. In diphtheria in the degenerated epithelial cells beneath and at the edge of the diphtheritic membrane this process undoubtedly does take place. We have specimens in which this is shown as clearly as is the migration of leucocytes. The chromatin when it passes into the cytoplasm lies near the nucleus and not in a vacuole.

The larger of the cytoplasmic parasites are more characteristic than the smaller forms, though it is possible to confound these with products of degeneration. In advanced degeneration of the epithelial cells found at the edge and at the bottom of vesicles, masses of separate cytoplasm, often intermingled with shreds of fibrin, may be found which offer a remote similarity to the large forms. There is no doubt that single objects formed in various ways may be found in the cell cytoplasm in small-pox and in other diseases which may simulate to a greater or less extent any of the forms of the cytoplasmic parasites. We have often found such single objects in small-pox and been unable to say whether they were or were not parasites. We think it possible that some of these represent abnormal or degeneration forms of the parasite. Every one who has had any experience with the study of unicellular organisms in tissues knows that it is often impossible to say whether or not a single object belongs to the host cells or is a parasite. Not only must form and size and structure of the single individuals be studied, but their relation to their surroundings and their developmental cycle must always be borne in mind.

Many points in structure which have been described are only to be distinguished after long study and in the best preparations. Careful staining by the methods which have been described is essential and requires considerable practice. We have found photography of enormous assistance and have often become aware of certain details of structure only after the study of negatives. Details can also be made out better by the aid of a properly screened arc light than by any other form of illumination. The differentiated structure in the cytoplasmic forms of from two to three microns in diameter was first seen in the negatives. There is no similarity in size, structure, and relation to the cell between the cytoplasmic parasites and the various forms of cell inclusions in tumors, and the study of small-pox has thrown no light on the question whether the inclusions in tumor cells are or are not parasites.

The intranuclear parasites are less apt to be confused with accidental products than are the cytoplasmic. They are found in nuclei in which there is no change in the chromatin, and they stand in no relation to the chromatin. Certain degenerations of chromatin may present some similarity to certain forms of the parasite. In the clumps of chromatin formed in the center of the nucleus small, clear, highly refractive vesicles may be found which suggest the chambers in the sporoblast. Pianese in his valuable studies of the histology of carcinoma has described and depicted various conditions within the nucleus which somewhat resemble certain of the intranuclear parasites. We have never found forms similar to those depicted by Pianese in the epithelial nuclei in variola or in any other infection of the skin. The vacuoles in the chromatin clumps are much more apt to be found in the mucous membranes, particularly in the esophagus, than in the skin. The best example of this formation was seen in the nuclei of degenerated muscle fibers in a case of fibrous myocarditis. It would be easy to say that the sporoblast could represent a more extensive formation of the same sort, that the vacuoles enlarge and form the chambers, and the remains of the chromatin represents the skeletal framework. A process very similar to this does take place in the testicle. We have already spoken of the peculiar bodies which are found in the testicle and which arise in connection with aspermatogenesis and from degeneration of the chromatin of the spermatids. Many of these bodies closely resemble the younger forms of sporoblasts, but they are formed from chromatin and stain like this. They represent merely a skeletal framework of chromatin enclosing vesicles. They are formed of the nucleus, while the intranuclear parasites grow within the nucleus and have no connection with its constituent parts. No structures resembling the completed sporoblast are so formed. Each form of the intranuclear parasite must be studied not as an isolated object, but as a stage of a cycle and by comparison with the forms which precede and follow it.

In vaccinia we find the same forms of parasites as in the cytoplasmic cycle in variola. None of the intranuclear forms have ever been found in vaccinia in any animal used for inoculation. We have never had an opportunity to examine the developing vaccine vesicle in man, but we have no reason to suppose that the lesion in man would differ from that in the monkey. In vaccinia there is some difference in the size and in the rapidity of development of the parasites in the different tissues and in the different animals. Their development seems to be most typical in the skin and mucous membrane of the calf. In the cornea their development is less perfect. They correspond in size, structure, and manner of multiplication with the cytoplasmic forms of the variola parasites in man and in the monkey. There are minor differences easily accounted for by differences in the soil in which they grow and their better preservation in the tissues of animals. The differences are not greater than will be shown by the same flowers in different gardens.

We believe that these bodies in vaccinia and in variola are living things. We see no possibility of another conclusion. Otherwise we must assume that they are degenerations of a specific character occurring under no other conditions, and that the products of degeneration undergo a development similar to that of a living thing, increasing in size and complexity of structure, and finally breaking up into a number of forms similar to those met with in the beginning, and which undergo the same development. Moreover, the manner of development constantly repeats itself, and similar stages are found at similar time periods. We know of no such degenerations, and all that we know of pathological processes is against such an interpretation. For the view that they are living things we have the analogy of structure and development with other things which are generally conceded to be living. The supposed parasites of variola and vaccinia appear first as bodies of small size, but not as microsopic points. They increase in size, and, with growth, details of structure appear which are always repeated, and for which the time relation, as far as can be determined, is the same. At the end of growth a form of multiplication takes place. The absolute proof that an object is living is very difficult to produce, for there is no single criterion which suffices. Ameboid motion has been made out in the vaccine parasites by so competent an observer as Wassielewski, but although believing that the bodies are parasites, he very properly does not regard this as proof.

Assuming these structures to be parasites, are they to be regarded as the cause of the disease? For this, also, it is impossible to produce actual proof. It is impossible to prove that the bacteria which are found associated with lesions of certain disease are their cause. Certainly every one believes that the pneumococcus has a direct causal relation with lobar pnemonia, but the proof is absent. The same is true of the leprosy bacillus and of the typhoid bacillus. The view that the Cytoryctes variolæ is the cause of variola must for the present rest on the fact that it is always associated with the lesions of the disease, develops further as the lesions develop, and is found under no other conditions. Every analogy which can be drawn from the study of other infectious diseases, both those in which it is possible to fulfil Koch's law, and those in which this is not possible, supports the view of its causal relation. It is not too much to say that an organism which has the power of invading and destroying living tissue, and which is constantly found in relation to the developing lesions of a certain disease, and only here, and unaccompanied by other living organisms, must be regarded as the cause of the disease. We have constantly found the organism in connection with the developing lesions of the disease. After the lesions have reached their full development, after local immunity is established, and the host cells are no longer capable of affording a suitable place for development, we do not find the organism. It is an obligate parasite, it develops within the living cells of a tissue, and is only found in connection with these. Those which are found free in the tissue are in the bottom of the vesicle or pustule, and have become free by the complete destruction of the cell in which they developed. These free forms represent the skeleton framework of sporoblasts in which spores have developed. In the contents of vesicles and pustules examined by smears and by sections, and by every technical method we could apply, we have not certainly found the organisms, though we know that the material examined is infectious. It has been equally impossible to say that the organisms were absent. To show them present we must be able to differentiate bodies of extremely minute size, from 0.37 μ to one micron, which have no characteristic staining reaction, from the mass of small objects, such as bacteria, cell and nuclear detritus, and granular precipitates from the exudate, which the pustule contains.

Bacteria are also usually found in the lesions. All the varieties found have been cultivated and the impossibility of their causal relation shown. Their presence is not constant. Wassermann first showed by cultures that pustules could be free from bacteria, and we have been able to confirm this in several instances. The histological study of the pustules shows bacteria to be not constantly present and usually in very small numbers. They give the impression from their location in the lesions of being only accidental. Perkins has found the tissues free from bacteria in a case of purpura variolosa in a child. We have further shown that variola can be produced by material in which bacteria were demonstrated to be absent by carefully carried out cultural methods.

We believe that variola and vaccinia are due to the same organism. It has been repeatedly demonstrated that vaccinia can be produced by inoculating cows with variola. As has been said, in vaccinia only the cytoplasmic forms of the parasite are met with, and these occur in the calf and rabbit with the same regularity, whether material from vaccinia or small-pox has been used. Vaccinia with only the cytoplasmic form of parasite is produced in the monkey when vaccinia is inoculated. When the monkey is inoculated with small-pox, both the cytoplasmic and intranuclear forms of parasites appear. In man we know that both forms appear in small-pox, and we have not had opportunity for the study of vaccinia in man. We believe that the cytoplasmic and intranuclear forms of the parasites represent each a distinct cycle. The cytoplasmic cycle is perfectly simple, and no forms are found in it which can be interpreted as sexual. The intranuclear cycle, found only in variola, follows the cytoplasmic, is infinitely more complicated, leads to a more active multiplication of the organism, and to the production of forms which can be air-borne and are capable of producing infection without intermediate agents. That this second cycle is sexual in character is extremely probable, though we have not positively identified the sexual forms. We have made no experiments as to the comparative resistance and durability of the vaccine and variola virus, nor do we know that any have been made. From analogy with other organisms it would seem probable that the products of the asexual cycle in vaccinia should be less resistant and in the absence of sexual rejuvenation should gradually decline in infecting power. There are some vague statements in literature that the efficiency of a strain of vaccine does decline in the course of years, but there is no positive information to this effect. We have repeatedly failed to get positive results on the cornea from inoculations with various sorts of commercial vaccine, but have never failed to get typical positive results with vesicle and pustule contents of small-pox lesions. The future experimental study of the diseases on an animal susceptible to both can alone decide these points.

Of the mode of infection in variola we are ignorant. The hypothesis which seems most plausible and which is most generally held is that infection takes place by the reception of the air-borne virus on the respiratory mucous membrane. The organism increases in the favorable soil and produces a local lesion, the so-called protopustule, from which the infection of the blood takes place. Blood infection is marked by a sharp onset and the skin eruption is embolic in character. No lesion in the respiratory mucous membrane which could be regarded as an infection-atrium, a protopustule, has even been seen. In all of our cases a careful search for such a lesion was made. There are many grounds against the assumption of such a protopustule. The period of incubation which should correspond to the development of the protopustule is, in the great majority of cases, entirely without symptoms. We know, however, that very considerable lesions in the lungs may not be accompanied by any symptoms. We see this particularly in tuberculosis. It is not impossible that the lesion may have gone through its development and healed without leaving a recognizable trace. It is extremely improbable that the protopustule could be formed in the lungs, for no specific lesions of the disease are found in them. There is every opportunity for their infection both from the surface and by the blood during the disease, and we cannot think that immunity limited to an organ was produced by the primary infection. In many cases we find losses of substance in the pharynx and other mucous membranes which might be the remains of an initial lesion, but they may be absent. Since the eruptive lesions in the mucous membranes have the same general character as those of the skin, analogy would lead us to believe that a primary lesion in a mucous membrane would correspond to the lesion of the skin which follows a primary inoculation. Judging from the character of the initial lesion produced on the monkey by inoculation, it would seem impossible that a primary lesion in the pharynx or on any other mucous membrane could run its course without symptoms. Cases have been known in which accidental infection of the skin and mucous membranes followed by the eruption have taken place. So far as we have been able to judge these cases from their reports, they have taken the course of variola inoculata and not that of true variola. An interesting case has been brought to our notice, in which a primary lesion of the hand, followed by a typical eruption, occurred after shaking hands with an individual who had taken care of a small-pox patient. In variola inoculata in man a primary lesion develops which is followed by the eruption. We see the same in the monkey. But variola inoculata has a different history from variola vera. The period of incubation is shorter and the disease incomparably milder. Variola inoculata in man has not been studied since the introduction of the thermometer and careful clinical methods. In man and in the monkey local reaction following inoculation is manifest on the fourth or fifth day. The constitutional reaction in man appears on the seventh or eighth day, but is shown in the monkey by rise of temperature on the third day and before the development of the local reaction. The exanthem appears in the monkey on the eighth day, and in man on the eleventh. The contents of early vesicles were used for inoculation in the production of variola inoculata and the possibility cannot be excluded that undeveloped organisms were used. Our experiments have not been sufficiently extensive to enable us to say that there is a difference in the course of the disease depending upon the stage of the lesion from which the virus is taken. It must be insisted upon that variola inoculata is not variola vera, and that we know nothing about the mode of infection in the latter.

From the contents of a small-pox vesicle of pustule three forms of the same disease can be produced; in the calf and in the rabbit vaccinia. This is to be regarded as a true variety of the disease, which, when established, always comes true from the seed. There seems to be some evidence that the variety is not established at once, but that the parent stock can be produced in suitable soil after the first generation. The virus is not air-borne. Inoculation of man and the monkey with the same material produces variola inoculata, a form of disease not so sharply marked as vaccinia, which cannot be established as a variety, and which is distinguished from variola vera by its shorter period of incubation and its milder course. It is only by rare accident that opportunity will be again given for the study of the disease in man; we know the disease only from the meager records of the past. Infection of man by natural and unknown means produces variola vera, the type of which is well characterized. There is no evidence that variola vera is ever produced in the monkey. The details of Zueler's case, in which he infected a monkey by placing in his cage small-pox material, are not sufficiently definite to enable us to say that the disease was not variola inoculata. Any one form of the disease produces immunity from all other forms.

It is generally assumed that the eruption is embolic in character and follows the blood infection. It is difficult to explain it in any other way, but it is equally difficult to understand the progress of the eruption by this assumption. The eruption appears first on the face and next on those parts of the skin habitually uncovered. An exception to this rule is the skin over the instep, where the eruption is abundant and early. It is possible that the skin infection is simultaneous and that certain parts, owing to increased vascularity or some unknown condition, allow a more abundant and earlier development. The vascularity of a part certainly seems to have an influence. Parts of the skin surface which have been rendered more vascular by the application of a mustard plaster or any slight trauma almost certainly have a more abundant eruption. The eruption may be greatly influenced in other ways. A young man of twenty in the period of incubation and one day before the onset was operated on in the City Hospital for bilateral hammer toe, and both feet and ankles put in a corrosive dressing. The eruption was abundant and took the usual course. The dressing was not removed until the fourth day of the eruption, when the skin beneath it was found normal. Parts subjected to friction of clothing or where occupation subjects the skin to friction, as the shoulder of a violin player, have a more abundant eruption. It does not seem probable that this can be merely a matter of increased vascularity which gives a greater opportunity for infection, possibly combined with more favorable conditions for the organism. Parts which are habitually subjected to friction, as the axillæ and crotch, are comparatively free from the eruption. It would be extremely interesting in this connection to know the distribution of the eruption in people habitually naked, but on this we have found no observations. In other eruptive diseases it is equally impossible to understand the distribution of the eruption. There is little evidence of the infectiousness of the blood derived from inoculations. The only time when this would seem most probable is at the end of the period of incubation and at the beginning of the onset. In two light cases inoculations of the blood on the rabbit's cornea were made during the period of onset and before the eruption, with negative results. In these cases it is possibie that the parasites may have been contained in the blood in such small numbers that the chances would have been against a positive result. The only positive result of corneal inoculation with blood was in a case of purpura variolosa in which the parasites were so abundant in the skin that the possibility of contamination of the blood must be considered. Support for the theory of blood infection at the period of onset is also given by fetal small-pox. In this the lesions of the fetus are described as twelve days later than those of the mother, the fetal infection being supposed to take place at the time of onset in the mother. The lesions in the case of fetal small-pox which was among our autopsies and those seen in Dr. Engleman's specimens were considered due, not to the parasites, but to toxic substances passing from the mother to the fetus. The lesions were atypical, and no organisms were found in them. Since then another case of fetal small-pox has been studied in which the lesions were typical, and in them all forms of parasites were found. The infection of the fetus must take place by means of the mother's blood, but in our case it was not possible to determine the period of fetal infection. Support is also given to the embolic theory of the skin eruption by the observation of the cornea. We have never seen at autopsy specific lesions on a cornea, nor have we found certain descriptions of specific lesions in the literature. Dr. Bancroft has, however, seen a typical lesion develop on a cornea which had become vascular from old disease.

Although bacteria can be excluded as a primary cause of small-pox, they play a very prominent part in the pathology of the disease. As the results of our study of the disease, both by cultures of lesions and organs, and by the microscopic examination of tissues, we are inclined to regard bacterial infection as a more important agent in bringing about a fatal termination than the specific parasite. It is impossible to say to what extent the lesions in internal organs are due to bacteria and their products. Some of them, notably the changes in the blood and blood-forming organs, and those in the testicle we are inclined to regard as specific and due to the action either of the specific parasite or toxins produced by it. We have never been able to demonstrate the parasites in the testicle, but this is by no means proof that they are not present. The degenerative lesions are very much the same as in other infectious diseases in which there is a combined bacterial infection. There are some differences, but apart from the condition of the blood vessels we do not think it would be possible to make a certain diagnosis of small-pox from the examination of the liver and kidney. There is also evidence of the absence of tissue inhibition to bacterial invasion and growth given in the abundance and character of the growth in the tissues, apart from the observations of a diminished complement. The bacteria are chiefly streptococci. There is analogy in respect to the importance of the part played by bacterial infection in the other exanthemata, notably in scarlet fever.[1]

It would be idle to discuss further the general pathology of the disease. In various articles in the series the general application of the knowledge obtained from investigation is given to some extent.

We hope that our work will lead to a greater interest in the disease, more investigation, and more knowledge. Small-pox is the most perfect type of an infectious disease which we have. It has features which are unknown in other diseases. It is not impossible that in the other exan-

[1]Pearce (Boston City Hospital Reports, 1899, p. 39).

themata immunity against the true disease will be found in the production of a mild variety which repeats itself. All that we can learn of small-pox may be of immense importance in combating other exanthemata. Opportunities for its study will be present in places where they can be taken full advantage of. There has been but little study of the disease since 1873, until the present century. The most important, if not all, forms of the disease can be studied experimentally, and experimental study must go hand in hand with study of the disease in man. The recognition of the specific cell and nuclear inclusions as parasites, and the primal cause of the disease, must come to any one from careful study. It would be too much to expect that a cycle of development of the parasite complete in all its details should be given at once. We can see no objections to the cycle given by Professor Calkins, but there is much uncertainty about the interpretation of many of the forms, particularly of the sexual forms. The most important and immediate practical result of the work should be the testing of the quality of vaccine virus by rabbit inoculation. As a means of diagnosis in obscure cases of variola, rabbit inoculation may be of great importance.

Small-pox can, but probably never will be, wholly eradicated. The chief obstacle which stands in the way of its eradication is an inability to recognize facts, and to make the proper deductions from them, which seems to be associated with certain orders of mind. The facts with regard to the production of small-pox immunity by vaccinia are perfectly established. The order of mind which leads to their denial will probably never disappear from the human race.

DESCRIPTION OF PLATES.

PLATE XXIX.

Showing various conditions simulating forms of Cytoryctes vacciniæ and variolæ.

Camera lucida drawings: Zeiss comp. oc. 6, obj. 2 mm., apert. 1.30.

FIG. 1. Normal corneal epithelium of the rabbit. The small eosin-stained bodies between epithelial cells, some of which they indent, are attached to nerve fibers distributed through this epithelium.

FIG. 2. Normal corneal epithelium of the calf. Deeply stained granular reticulated masses with indefinite outline situated at the poles of the nuclei, those at the lower pole being somewhat triangular in shape. This material extends in some cases into the perinuculear space, but in other cases is confined to the adjacent cytoplasm. It probably represents a coagulum of some sort, and is commonly met with in greater or less degree in most preparations of this tissue. These masses are identical with the triangular or pyramidal bodies of Hückel.

FIG. 3. Epithelium of rabbit's cornea twelve hours after inoculation with diphtheria toxin. It is impossible to state whether the shrinkage here presented is due to the effects of toxin, to changes brought about through technic, or to both these factors. The nucleus is finely reticular in structure, a definite nuclear membrane is not apparent, and at either or both poles there is apparently an extension of the chromatin. The nucleus lies in a space and except at the poles is separated from the cytoplasm of the cell. Similar nuclear changes are represented by Gorini in the vaccine lesion of the cornea.

FIG. 4. A swollen epithelial cell from an old vaccination lesion of the calf's cornea. It contains three leucocyte fragments each containing nuclear and protoplasmic substance.

FIGS. 5, 6, 7, and 8. The cells in these figures are drawn from a section of rabbit's cornea twelve hours after the inoculation of diphtheria toxin.

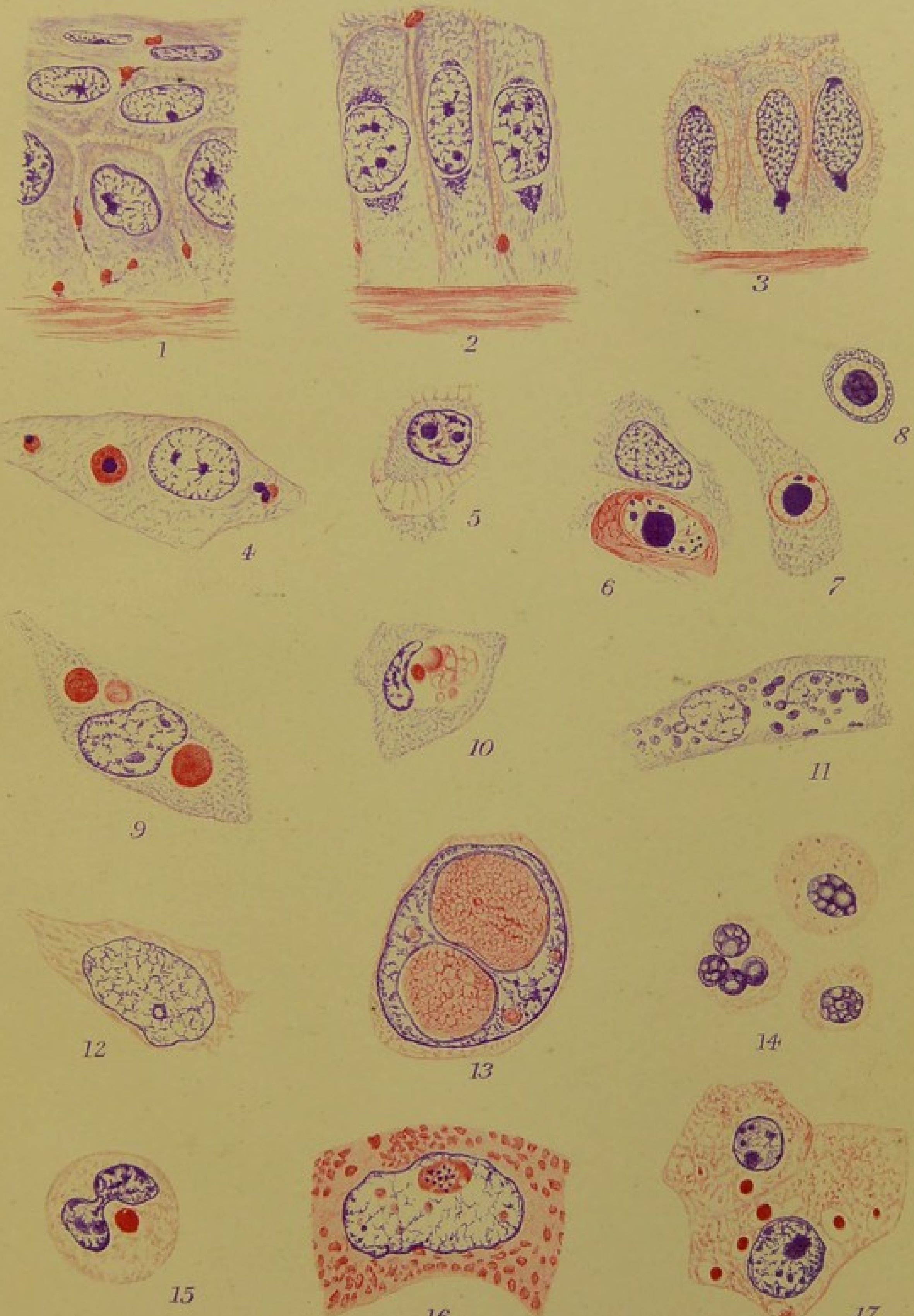
FIG. 5. An epithelial cell which has shrunken away from the surrounding cells. The nucleus contains two large masses of chromatin.

FIG. 6. The lower epithelial cell shows marked changes in both nucleus and cytoplasm. The nucleus contains some red stained reticular material and a large mass and several small masses stained blue. The protoplasm dense, and takes the eosin stain.

FIG. 7. An epithelial cell, the nucleus of which shows marked degeneration. There is a large, deeply stained, central mass from which fine lines radiate to the nuclear membrane which is stained red.

FIG. 8. Represents a cross section of Fig. 7.

FIGS. 9 and 10. From the mucous membrane of the soft palate in diphtheria showing red corpuscles in epithelial cells. In Fig. 9 there are two corpuscles in a large vacuole in the cell. The smaller bodies evidently represent the contents of corpuscles.



Councilman.

Degenerative changes simulating Small-pox.

FIG. 11. Mucous membrane of pharynx in diphtheria beneath diphtheritic membrane showing chromatolysis.

FIG. 12. Basal cell in small-pox testicle showing vacuolated refractive body.

FIG. 13. Nucleus and part of cytoplasm of large cell from a perithelial angio-sarcoma. Numerous nuclei contained one or several acidophilic bodies with smooth contours surrounded by a chromatin membrane. The bodies were composed of a substance either granular or faintly vesicular, evidently representing a coagulum.

FIG. 14. Small-pox testicle showing two degenerated spermatids and four spermatozoa with vacuolar degeneration of chromatin.

FIG. 15. Leucocyte with a body resembling early cytoplasmic parasite in dry blood smear from case of purpura variolosa, taken on day of death. Stained with hematoxylin and carbol fuchsin. Body stains with fuchsin. Products of nuclear fragmentation stain blue.

FIG. 16. Degenerated muscle cell from heart, sub-acute myocarditis, showing clumping of chromatin with vacuolar degeneration.

FIG. 17. Hyaline degeneration of liver cells from small-pox showing round hyaline masses in vacuole.

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