THE HISTOLOGICAL LESIONS OF EXPERIMENTAL GLANDERS.[1]

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Research into the histological lesions of experimental glanders has hitherto failed to yield comprehensive results. The literature of the subject is scanty and affords at best an inadequate description of the acute lesions. The views held by various observers regarding the histology of experimental glanders are conflicting and observations upon the tubercle of this infection are extremely contradictory.

Baumgarten[2] states that the histogenesis of the tubercle of experimental glanders is essentially the same as that of the miliary tubercle differing only in that the former undergoes necrosis more quickly than the latter. He holds that the primary effect of the glanders bacillus upon a tissue is the proliferation of the "fixed cells" with secondary degenerative change and not destruction of tissue and polynuclear invasion. Wright,[3] on the contrary, concludes that the experimental glanders tubercle and the miliary tubercle are not analogous in their histogenesis, and states that there is primary necrosis of the tissue followed by exudation, which is frequently of a suppurative character. Tedeschi,[4] probably working with cultures, in virulence intermediate between those used by Baumgarten and by Wright, concluded that the primary effect of the bacillus of glanders was necrosis of the tissue with leucocytic invasion.

These conflicting observations have led us to undertake the following experimental work in the hope of determining whether or not the histological changes in experimental glanders vary directly with the virulence of the organism employed.

[1] Read before the Association of American Physicians, May 8, 1907.

[2] Lehrbuch der path. Mykologie, 1885.

[3] *Jour. of Exper. Med.*, 1896, i, 577.

[4] *Zeigler's Beiträge*, 1893, xiii, 365.

METHODS.

The animals employed throughout this work were rabbits and guinea-pigs. The methods of inoculation were subcutaneous, intravenous, intraperitoneal, intra-orbital and corneal. The inoculations are grouped in series according to the mode of inoculation and the virulence of the culture employed.

Rabbits were the only animals employed for corneal inoculations. Two methods were used, (1) inoculation with parallel incisions, and (2) subepithelial injection. After etherization the eyeball was steadied with fixation forceps, care being taken not to injure the cornea. Under aseptic precautions three parallel incisions into the corneal substance were made about 2 mm. apart and well away from the sclero-corneal junction. The cornea thus prepared was then inoculated with the glanders culture by means of a platinum needle.

With the second method a suspension of bacilli was injected beneath the corneal epithelium by means of a sterile hyperdermic needle at a distance from the point of entrance. With both methods one eye was used as a control in every instance. The animals were afterwards killed at intervals varying from fifteen minutes to three weeks.

The same strain of bacillus Mallei was used throughout the work which extended over a period of two years. The animals were injected with varying amounts of a highly virulent culture, of a moderately virulent culture, of an attenuated culture, and of a culture killed by heat.

The attenuated culture was obtained by frequent transplantation during a period of eighteen months. It is interesting to note that the bacilli of lowered toxicity change remarkably in their morphological and cultural characteristics. They lose their short plump appearance and become thin, elongated rods with definite clear spaces at regular intervals along the body. On the ordinary culture media such as potato, agar-agar, blood serum, etc., the culture within twenty-four hours becomes extremely dry, tenacious and stringy; it is so tenacious that it is difficult to take off a part of the growth with a platinum loop, the whole surface growth coming away *en masse*. In our experience these peculiarities of the surface culture are characteristic of the period of non-virulence.

The highly virulent bacilli were obtained by frequent passage through a series of guinea-pigs. Devitalized bacilli were prepared by suspending in fifteen cubic centimeters of physiological saline solution a twenty-four hour growth of a virulent agar slant culture and heating the suspension for two hours at 56° C.

The bacilli were prepared for inoculation from a twenty-four hour growth upon one per cent. dextrose agar slant by washing with fifteen cubic centimeters of bouillon or salt solution and thoroughly shaking to ensure an even emulsion. The dosage varied with the virulence of the bacillus and the animal employed, whether rabbit or guinea-pig.

In general the dosage was as follows:

1. Of attenuated culture, 3 c.c. for guinea-pigs and 10 c.c. for rabbits.

2. Of moderately virulent culture, 1 c.c. for guinea-pigs and 5 c.c. for rabbits.

3. Of highly virulent culture 0.5 c.c. for guinea-pigs and 2 c.c. for rabbits.

For the corneal injection a very much smaller amount was employed. Here the exact quantity of unsuspended bacilli was not accurately determined, but approximately it amounted to an injection of 0.1 cubic centimeter in each case.

In animals partially immunized to the virulent glanders bacilli larger doses were finally employed. This partial immunization was effected by administering every three or four days during a period of four weeks, gradually increasing doses of the virulent organism.

As stated by one of us in an earlier publication, it is only occasionally that rabbits succumb to experimental inoculation with living virulent glanders bacilli and they are far less susceptible to this form of infection than are guinea-pigs. The rabbit stands large and repeated doses. Pregnant rabbits and mothers are much more susceptible. Though guinea-pigs are very prone to glanders infection it is noticeable how much less susceptible is the female than the male.

For histological study the tissues were fixed in Zenker's fluid, alcohol, or formalin and embedded in paraffin. Eosin-methylene-

blue was used in the routine staining of sections. Mallory's phosphotungstic-hæmotoxylin and connective tissue stains were employed for the special differentiation of tissues. Sharlach R. was employed to demonstrate the presence of fat. As a routine stain the eosin-methylene-blue (Mallory) was by far the most satisfactory.

The staining of the bacteria in the tissues was accomplished by means of a special method which is described elsewhere in the present number of this Journal.

HISTOLOGY.

In the following histological description of the lesions in experimental glanders the organs will be considered separately.

Cornea.—Owing to the absence of blood vessels and the peculiar histological structure of the cornea this organ was selected as being the most suitable for the study of the earliest tissue changes.

The cornea of the rabbit was used in all three sets of experiments owing to the greater resistance of this animal to glanders infection compared with the guinea-pig and each experiment was repeated in order to control the results.

In the first series, in which the highly virulent culture was used, the animals were killed at varying periods ranging from half an hour to forty-eight hours after inoculation. In the second series, in which the culture employed was of moderate degree of virulence the rabbits were killed at varying intervals extending over a period of five days. In the third series, inoculated with the attenuated culture, the animals were killed at intervals extending over a period of one month.

The lesions thus produced in the cornea fall readily into two distinct groups, viz., the acute exudative and the chronic proliferative, the determining factor being the virulence of the culture.

The corneal lesion resulting from the introduction of the highly virulent bacillus is to be described as inflammatory with marked disintegration of the tissues and of the invading leucocytes. The disintegration was often observed about the site of injury within half an hour of inoculation and before the migrating leucocytes from the vessels of the limbus had time to approach—a destruction so severe in some cases as to render unrecognizable the individual cells comprising the lesion.

The character of the acute inflammatory lesion varies to some extent with the time elapsing between the inoculation and the killing of the animal. The

vessels in the "danger zone" of rabbits killed in fifteen minutes after the corneal inoculation show no evidence of change. There is no apparent dilatation of either the vessels or the perivascular spaces and there is an absence of inflammatory cells migrating towards the site of injury. Nevertheless at the site of inoculation there is a marked disintegration of the corneal tissue into cell fragments and deep blue homogeneous chromatin-like masses of varying size, apparently the result of a direct action of the glanders toxin upon the tissues. These chromatin bodies have been described by various investigators as characteristic of the glanders nodule. The absence of polynuclear cells approaching the site of injury, and the fact that the vessels in the sclero-corneal zone are as yet not dilated together prove that the detritus and chromatin bodies are the product of nuclear elements derived from the disintegrated cells of the injured area. After a few hours the injured area becomes invaded with the inflammatory cells from the vessels of the limbus, which in turn suffer fragmentation and disintegration.

The cornea inoculated with the bacilli of moderate virulence affords an excellent opportunity to study the nature and origin of the cells comprising the acute exudative lesion. In this series of experiments the toxin was not of sufficient power primarily to destroy the tissue or the invading leucocytes.

By means of a special method we were able to determine the number and situation of the glanders bacilli and their relation to the various cells. The fact that the bacilli remain local with no tendency to spread through the tissues explains the focal character of the lesion.

The most interesting feature of the early lesion is the appearance of peculiar acidophilic cells, which collect in considerable numbers about the site of inoculation as early as fifteen minutes after the introduction of the bacilli. At this period the incised corneal epithelium is gaping and the corneal spaces contain numbers of cells with large deep-staining red granules. There is no evidence that these cells have come from the blood vessels. They are interesting in view of their nature and origin, and of the possible rôle played in inflammations produced by certain toxins and chemical agents. These leucocytes are generally polymorphonuclear though sometimes the nucleus is similar to that of the transitional leucocytes.

The nucleus is surrounded by twenty or thirty closely arranged coarse granules which stain intensely with eosin. These cells differ from the polymorphonuclear leucocytes of the rabbit, (1) by the size and staining reaction of their granules, (2) by their appearance in the corneal lesion long before the vessels in the limbus (from which the ordinary pus cell migrates) react, and (3) by the fact that they are not phagocytic for the invading bacilli.

These large acidophilic granule cells apparently come from the tissues in the neighborhood of the injury or enter through the corneal incision from the conjunctival sac. For in the earliest lesion there is an absence of any change in the nearest vessels and a wide clear field intervenes between the vessels and the outermost cells about the lesion. Following the early injury to the cornea these cells are the first to respond to the bacterial invasion and

practically constitute the histological lesion. (See Plate XIII, Fig. 2.)

Opie[5] states that the polynuclear eosinophile cell rarely, if ever, ingests bacteria. Kanthack and Hardy,[6] however, claim for the eosinophile cells a primary attack directed against the invading bacteria. There is every reason to believe that the large granule cells attracted early to the corneal injury are eosinophilic in nature; and their failure to take up bacteria would indicate the absence of defense against bacterial invasion.

The polynuclear leucocyte of the rabbit whose granules are smaller and less deeply stained can be traced from the vessels in the sclero-corneal junction in an unbroken path along the corneal spaces just beneath the epithelium, to the site of the injury where they become actively phagocytic for the bacteria.

The first vascular change noted in the sclero-corneal angle occurs about one hour after the inoculation of the cornea, and consists of a dilatation of the vessels and of the perivascular spaces, with an accumulation of polynuclear cells within the lumen and their migration into the surrounding tissues. Simultaneously with the migration of the leucocytes about the vessel collect numerous lymphoid and plasma cells, which apparently do not follow the polynuclear cells to the site of the injury but remain in the tissue about the vessel.

The control eye in each case shows no reaction about the incision for six hours or more. Where the incisions penetrate only a short distance into the stroma the repair is complete in less than twelve hours, as indicated by the congealed epithelial surfaces without the least sign of an inflammatory infiltration. Often the injury is recognized only by the mass of heaped up epithelial cells projecting below the limit of the normal corneal epithelium.

Following inoculation of the bacilli of low degree of virulence the proliferative type of lesion ensues. The best results were obtained by deep corneal injection of the bacilli by means of a fine needle without serious injury to the corneal epithelium. (See Plate XVI, Fig. 14.) The earliest change noted is swelling of the

[5] *Johns Hopkins Hospital Bulletin*, 1904, xv, 71.
[6] *Jour. of Physiol.*, 1894–1895, xvii, 81.

corneal corpuscles and accumulation of moderate numbers of polynuclear eosinophiles. Later the epithelioid cells collect and give rise to a more or less well defined focus. The microscopic picture in from five to seven days after inoculation is similar to that of the lesion produced by the tubercle bacillus. The lesion consists of numerous cells of an epithelioid nature and moderate numbers of lymphoid and polynuclear cells. Giant cells were not observed in the lesions of the cornea. The occurrence in the proliferative type of corneal lesion of epithelioid cells and the absence of giant cells, whereas in the other organs under similar conditions the two types of cells are associated, would indicate a connective tissue histogenesis for the epithelioid cell. On the other hand the lesions in the region of the limbus contain multinucleated cells associated with epithelioid cells.

Liver.—The lesions in this organ in rabbits and guinea-pigs differ in no essential feature. The extent and character of the lesion depend upon the virulence of the culture. In a large number of animals the liver shows well-defined changes, presenting microscopically the picture of miliary tuberculosis. In general the gross changes consist of discrete, glistening, grayish-white foci varying in size from minute points to areas one millimeter in diameter. These areas are invariably found in greater numbers just beneath the capsule of the liver but in no case where the areas are seen on the liver surface are they absent from the deeper parts of the organ. In a number of animals however no macroscopic changes were discernible although subsequent microscopic study revealed definite lesions.

Microscopically sections of liver tissue show a gradation in the lesions varying with the culture employed, the mode of inoculation and the susceptibility of the animal, the virulence of the culture playing the most important rôle. In general these lesions are of three types: (1) the acute inflammatory, (2) the degenerative, and (3) the proliferative.

Inoculation of the ear vein of a rabbit with the highly virulent culture produces acute miliary abscesses of the liver. The partially attenuated culture similarly injected at repeated intervals gives rise to focal lesions of a proliferative character. The acute inflammatory lesions in the liver consist of foci in which the cells have been destroyed and subsequently invaded by polymorphonuclear leucocytes. These leucocytes are often so closely packed that the fixed cells of the part are made out with difficulty. The lesion varies in structural character from a focus composed of fragmented and disintegrated nuclei and protoplasmic masses, to one in which the cell collection is well preserved.

The lesions of intermediate grades are met with in animals which have received repeated inoculations. In general it may be stated that the difference in severity noted in the acute inflammatory lesions depends upon the toxicity of the culture. The miliary abscesses are always sharply defined and do not differ (except possibly in the sharpness of definition) from acute miliary abscesses in this organ resulting from other infecting agents.

The more severe type of lesion presents the picture described by most observers as characteristic of the experimental lesion. The microscopic appearance consists of a collection of large and small deeply staining chromatin masses, scattered nuclei and protoplasmic fragments. Fibrin is present in variable amount. The glanders bacilli are readily distinguished by a special staining method as small rods arranged singly and in pairs in the center of the focus. Although the liver cells within the lesion cannot be distinguished, those at the periphery remain unchanged, that is, the necrotic zone is sharply defined by apparently normal cells.

The less severe form of the acute lesion is composed of polynuclear leucocytes in a good state of preservation. In this type of lesion parts of liver cells and occasionally whole liver cells, though necrotic, are readily distinguished. There is an absence of "chromatic globules" and of the macerated appearance so characteristic of the tissue changes in the more severe type of lesion. Occasionally an isolated necrotic liver cell within the focus contains several bacilli and threads of fibrin.

The miliary abscesses of the liver are found in no particular site; they occur either intralobular or interlobular. However the lesion within the lobule is smaller and more sharply defined. There is less tendency for the intralobular foci to spread and coalesce with neighboring foci. Many of the lesions are extremely small, consisting often of less than a dozen closely collected polynuclear cells. These early intralobular collections of leucocytes seem to form within the subsinusoidal space. More commonly the lesion begins as a collection of leucocytes within the capillary space, being preceded by an injury to the lining endothelium, indicated by the occurrence of fibrin about the swollen, deeply stained lining cells. In some of the early lesions the leucocytes are collected about large mitotic endothelial cells still attached to the capillary wall.

In the sections of liver tissue the bacilli occur in association with the lesion. In the intravenous series of animals killed forty-eight hours or more after inoculation there is a tendency for the bacilli to lodge along the vessel wall. From a careful study of the early acute lesions it is obvious that the bacilli lodging along the route of the portal circulation either by direct action or the action of their toxin injure or destroy (depending on the virulence) the lining endothelial cells and cause on the one hand a focal leucocytic collection, or on the other a proliferation of the endothelium. This proliferation of the endothelial cells of the capillaries plays an important rôle in the formation of the glanders tubercle, especially in connection with the origin of the giant cell.

The degenerative lesions of the liver will be considered under two headings: (1) focal necrosis, and (2) vascular changes.

Focal necrosis of the liver occurs regularly in the rabbit and guinea-pig which have been inoculated with the moderately virulent bacilli. The central and zonal types of necrosis rarely occur. The most extensive lesion develops

in the animals which have been partially immunized by repeated intravenous injections. As a rule the liver necrosis does not develop earlier than seven to ten days after the first inoculation.

In a rabbit that died of glanders infection fourteen days after the first inoculation all the lobules are affected, and in many the necrosis comprises more than half the lobule. (See Plate XVI, Fig. 15.) The occurrence of focal necrosis apparently depends upon the degree of virulence of the culture. The lesion never occurs in animals infected with the highly virulent culture except in those partially immunized and subsequently receiving a fatal dose. In guinea-pigs the lesion is less common than in rabbits and usually occurs after the repeated injection of the attenuated culture over a period of weeks.

The areas of necrosis vary from a single cell to areas composed of many cells. There is apparently no selection of special parts of the lobule. The same section may show focal areas of necrosis in the central, mid-zonal and peripheral parts of the same lobule. Not infrequently a single necrotic cell occurs between normal liver cells with which it is sharply contrasted as it stains more intensely with eosin. In some areas of necrosis the liver cells contain large and small vacuoles; and the failure of these to stain with Sharlach R, would indicate "vacuolar" and not fatty degeneration. Some of the vacuoles contain fibrin threads either single or arranged in crossed bundles. Occasionally the vacuoles contain an eosinophile cell or a polymorphonuclear leucocyte. The absence of an inflammatory reaction in connection with the focal areas of necrosis is remarkable; even in the older lesions where the liver cells have lost their nuclei and appear as deeply stained eosin masses which are only distinguished as liver cells by the shape and size, there is rarely any polynuclear invasion.

In addition to this form of focal necrosis, the result of the action of the strong toxin, there is another form of necrosis which undoubtedly results from an inflammatory exudate collecting about an area of liver cells. In a guinea-pig which received intraperitoneally a highly virulent culture and succumbed to the infection on the fourth day, the liver presented focal areas of necrotic cells surrounded by well defined zones of polymorphonuclear leucocytes. These areas contained twelve to thirty swollen, deeply stained liver cells, many of which were without nuclei, and the whole mass was encircled by a broad zone of acute inflammatory exudate. The central mass of degenerated liver cells remained remarkably free from leucocytes.

The eosin-methylene-blue stain is admirably suited for the demonstration of the focal lesions in the liver. The earliest change in the liver is indicated by the tendency of the cells to stain more intensely with eosin. At first the nuclei stain a deep purple blue in contrast to the light blue of the normal nucleus; then they stain less intensely until finally the nucleus is lost. Many of the liver cells though completely degenerated preserve their shape and size long after all trace of the nucleus has disappeared.

The protoplasm of the liver cells, the seat of necrosis, is often converted into curious products of degeneration. These products occur in three distinct variations. In one form the protoplasm of the liver cells undergoes a curious transformation into numbers of well defined and separated "chromatoid bodies" resembling in their structure the nucleus of a lymphoid cell; they differ, however, in that they are smaller and stain intensely with eosin. There is also a

resemblance to blood platelets though the latter are more angular in outline, whereas these protoplasmic bodies are spherical. These curious structures may number twenty or more in a cell, and are often arranged to form perfect geometrical figures. They occur only in the liver cells with the more advanced stage of necrosis.

The second group of protoplasmic bodies in necrotic liver cells appear as small and large globules (the smaller undoubtedly coalesce to form the larger). These globules stain intensely with eosin and are homogeneous throughout. This form of cell degeneration is more frequently met with than the form above described.

The third variety of degeneration product appear as finely granular masses filling the necrotic liver cell. This granular conversion of the protoplasm begins soon after the cell loses its nucleus and presents the earliest change in the cell protoplasm.

It would seem that focal necrosis of the liver in experimental glanders results chiefly from a plugging of the interlobular capillaries with fibrinous thrombi. In the vessels adjoining the necrotic areas of liver cells masses of fibrin either partially or completely occluding the lumen can be demonstrated.

Occasionally large phagocytic cells and giant cells are found in the capillaries connected with the focal areas of liver necrosis. Here the sinusoids are narrowed owing to the swelling of the parenchymal cells and the accumulation of serum in the subsinusoidal spaces.

Masses of fibrin also occur in the capillaries of these necrotic areas. Frequently the lining endothelial cells contain mytotic figures which would indicate some special action of the toxin on the vessel wall. The dividing cells later become detached and escape into the lumen where they assume a phagocytic rôle. It is reasonable to suppose that the greater number of phagocytic cells found free in the blood channels of the liver originate in the endothelium of the liver.

The bile radicles in the areas of necrosis are often dilated and show aneurysmal bulgings.

Vascular changes occur in the perivascular vessels and in the central veins of the lobules. Here the muscle fibres are often in an advanced stage of degeneration. However in some vessels only small groups of muscle fibres or single isolated fibres show alteration. The fibres in the earlier stages of degeneration are filled with fine particles which take the Sharlach R. stain for fat. The

vascular lesions are well marked in the lobules where the parenchymal cells are apparently normal.

The proliferative changes in the liver occur in animals inoculated with the attenuated culture and in animals receiving repeated inoculations. The transition from the acute inflammatory type of lesion to the chronic proliferative lesion is striking, and is the result of alteration in virulence of the culture. The liver offers an excellent opportunity to follow the various changes in the character of the lesion from the acute focal destructive type to the chronic focal proliferative type. Though lesions of these two types differ essentially in their cells they are alike in that they are both focal in character. The lesion produced with the highly virulent culture simulates an acute miliary abscess, while the lesion produced by the attenuated culture resembles the miliary tubercle.

The glanders tubercles in the liver occur as discrete miliary foci composed of epithelioid and lymphoid cells associated with giant cells. Just as the lesions of acute miliary tuberculosis vary in character for the same case, so also varies the glanders tubercle. Out of a series of twenty-four rabbits eighteen showed proliferative lesions of the miliary tubercle type. The animals were inoculated intravenously or intraperitoneally and killed twelve to twenty-four days after the first inoculation. In fourteen cases the lesions of the liver were visible to the naked eye as discrete grayish-white tubercles studded throughout the organ, but most marked just beneath the capsule. On section they were translucent and of firm consistence. Microscopically they are focal areas composed chiefly of epithelioid cells arranged more or less concentrically. Giant cells occur in the lesions as a rule, about the periphery of the tubercle. In some areas three or more of these multinucleated cells were present, and in one tubercle there were eight. These giant cells often contain twenty or more nuclei which usually occur in the center of the cell. The same section not infrequently would contain lesions of different ages, the older foci showing necrosis. This necrosis of the glanders focus in no way resembles the necrosis of the acute inflammatory lesion in which fragmentation of nuclei and chromatin globules are so characteristic, but resembles the necrosis of the tuberculous lesions.

Lungs.—The lungs are not as frequently a site of pathological change as the other organs of the rabbit and guinea-pig. However when lesions occur they are significant, especially the proliferative type, owing to analogy with the lesion of tuberculosis. Pulmonary lesions occur in the guinea-pig more frequently than in the rabbit. Here as in other organs the type of lesions depends upon the virulence of the culture and the susceptibility of the animal, though the former is by far the more important determinating factor. The manner of inoculation does not materially influence the character or frequency of occurrence of the pulmonary lesion.

The lesions arising in the lungs are exudative and proliferative. The proliferative lesions are in the form of miliary tubercles and are best developed in guinea-pigs killed two to three weeks after the first inoculation. In three cases the pigs had received more than two inoculations. The animals were partially immunized by injecting small doses of the attenuated bacilli and then given the fatal dose of a virulent culture.

The lesions of an exudative nature occur in the form of miliary abscesses scattered throughout the parenchyma and result from the injection of the highly virulent bacilli. The extent of destruction in the pulmonary tissues, as in other organs, depends upon the virulence of the bacilli. In general, bacilli of a low grade of virulence give rise to focal proliferation; bacilli of a moderate degree of virulence produce focal lesions of an acute exudative character in which the cells are well preserved; the bacilli of exalted virulence cause disintegration of the tissues, which occurs as areas filled with fragmented nuclei and degenerated protoplasm.

Often there is no appreciable change in the gross appearance of the lungs which on microscopic examination contain definite focal lesions.

The protocols are here given of animals which well represent the two types of lesions.

Guinea-pig VII.—Female, weight 550 grams, was inoculated intraperitoneally on October 18 with 0.5 c.c. of a twenty-four hour culture of attenuated virulence suspended in 2 c.c. of bouillon. Slight area of induration appeared about the site of inoculation at the end of the second week; animal was in good health. Second inoculation was given into the peritoneum October 30; the animal was killed November 2; the weight being 440 grams. Autopsy shows an area of

induration of the skin and subcutaneous tissues about the site of inoculation. A few Gram negative bacilli are found in the smears. The tunica vaginalis is swollen and œdematous and on section thickened and studded with discrete and conglomerate pin-head and smaller nodules. The omentum is curled upon itself, and on separating the folds the serous surface contains innumerable discrete, glistening grayish-white tubercles 1 mm. to 2 mm. in diameter. Beneath the capsule of the liver and spleen are similar grayish-white nodules; many of these areas are too small to be detected in the deeper parts of the organs. The peritoneum is studded with tubercles, though they are relatively few in number as compared with those in the omentum. The inguinal and mesenteric glands are enlarged. The heart, kidney and adrenals show no visible change. The lungs are voluminous and crepitant throughout, though the whole subpleural surface presents scattered, glistening grayish-white tubercles 1 mm. in diameter. These tubercles, though present in the deeper parts of the lungs, are not as readily distinguished as those under the pleura. The mediastinal nodes are enlarged and on section exhibit areas of necrosis. A pure culture of *B. mallei* was recovered from the heart's blood.

Guinea-pig XI.—Weight, 555 grams. Inoculated subcutaneously October 30 with 0.5 c.c. of a 1 c.c. bouillon suspension to which one loop of a twenty-four hour culture of moderate virulence had been added. The animal died November 9 of general septicæmia. The autopsy showed a large caseating area at the site of inoculation. The glands were not enlarged. The peritoneal cavity presented diffuse fibrinous peritonitis. The liver contained three small pin-head sized nodules. The spleen was somewhat enlarged and congested. The heart and kidneys were normal in appearance. The testes and tunic were studded with glanders nodules. Both lungs, though voluminous, dry and crepitant throughout, were thickly seeded with innumerable discrete grayish-white translucent tubercles 1 mm. in diameter. These areas were apparently more numerous just beneath the pleura, and gave to the surface a rough finely granular appearance. The adrenals showed similar lesions beneath the capsule and in the substance of the glands.

Guinea-pig VI.—Weight 555 grams. Inoculated subcutaneously on October 3 with 0.5 c.c. of a twenty-four hour culture of low degree of virulence. Two weeks after the first inoculation the animal was in good health. The second inoculation was given subcutaneously October 18 with double the amount of the first dose. The animal weighed 585 grams and was in good health one week after the second inoculation. A third dose was given intraperitoneally on October 25. The amount of the dose was 1 c.c. of a moderately virulent culture suspended in 5 c.c. of bouillon. Five days after the inoculation the animal developed the testicular lesion, and was killed seven days after its appearance.

The autopsy revealed a small area of induration at the recent site of inoculation. The inguinal and axillary glands were enlarged. The omentum contained a few discrete grayish-white tubercles; otherwise the peritoneal cavity showed nothing remarkable. The mesenteric and bronchial lymph nodes were slightly enlarged. Beneath the capsule of the spleen and liver there were numbers of discrete grayish-white tubercles 1 mm. in diameter. The adrenals and kidneys were negative. The testes and tunica vaginalis presented extensive induration and caseation. The lungs were voluminous and crepitant except for a slight amount of œdema in the dependent portions.

The focal distribution of the lung lesions is especially characteristic. It will be noted that they are focal in each animal and occur in the form of discrete tubercles. In general the gross appearance of the lungs in which the lesions result from the repeated dosage with bacilli of a low degree of virulence is identical with the lesion of acute miliary tuberculosis.

In Guinea-pigs VII and XI the lung tubercles on microscopic examination are composed of cells representing two distinct types of lesions. In one the tubercles are made up of polymorphonuclear leucocytes; in the other they are composed chiefly of epithelioid cells. In Guinea-pig XI the remarkable feature of the lesion is that there is not the slightest inflammatory reaction about the tubercles or elsewhere in the lung tissues.

Guinea-pig VI represents the exudative type of lesion. Here the lungs contain areas composed of polymorphonuclear leucocytes, fibrin and desquamated cells. These acute foci are often composed of broken and fragmented cells. The acute lesion of a less severe type presents the same focal character but differs in that the individual cells are well preserved. Thus miliary abscesses occur more commonly in the peribronchial tissues usually about a blood vessel. Others are found near the periphery of a lobe, involving there a limited number of the alveoli. The older foci occasionally show invasion with eosinophiles and lymphoid cells. There is little or no tendency for the surrounding tissue to proliferate even in the more advanced lesions. The tendency is to enlarge by constant accumulation of polymorphonuclear leucocytes and by means of fusion with neighboring foci.

These tubercles are scattered throughout the lung parenchyma. Some of them are just beneath the pleura while others are in the peribronchial tissue. They vary in size from a focus which involves less than half a dozen alveoli to those including many air sacs. (See Plate XVI, Fig. 13.) In no case was there detected a zone of inflammatory reaction. The tubercles, whether early or advanced, are composed of closely packed epithelioid cells filling and entirely obliterating the alveolar meshwork of a given area. In Guinea-pig VII the absence of necrosis associated with the lesions was remarkable, and was probably referable to the early stage of the process and partly to the low grade of virulence of the culture.

This picture of discrete tubercles without necrosis is not uncommon in guinea-pigs infested with tubercle bacilli of low virulence. The necrosis in tuberculosis seems to occur earlier with the more virulent culture. The picture presented in Guinea-pig VII was analogous to that obtained in tuberculosis with bacilli of moderate virulence. On the other hand highly virulent tubercle bacilli produce pulmonary lesions analogous to those produced by the glanders bacilli of moderate virulence. In both, the lesions contain necrosis without the characteristic fragmentation of cell nuclei and formation of chromatin masses.

Giant cells are frequently a part of the lung tubercles. The

lesions in the lungs of Guinea-pig XI did not contain giant cells, while in Guinea-pig VII giant cells were numerous.

The tubercles in Guinea-pig XI, though distinctly proliferative, are more acute than the lesions found in the lungs in Guinea-pig VII where giant cells are numerous. It would seem that the production of giant cells occurs only under certain conditions of specific stimulation. This occurrence of the giant cell after a certain attenuation of the bacilli was noted throughout the work. The culture for the first year after isolation failed to produce proliferation of the invaded tissues; the lesion was invariably inflammatory. However, as the culture generally lost in virulence the inflammatory type of lesion lessened in severity until finally the culture produced only the proliferative lesion. This gradual fall of virulence with a corresponding change in the character and nature of the lesion is of significance.

Testicles.—Here as in other organs the lesions are the acute exudative and the chronic proliferative. In either case the process begins in the tunica vaginalis as discrete foci and spreads towards the skin and the testicle.

The acute focus begins as a collection of polymorphonuclear cells which either undergo rapid disintegration or remain intact, depending upon the virulence of the culture.

The proliferative lesion following the injection of bacilli of low virulence is striking. The tunica propria is studded with discrete and conglomerate tubercles composed of closely packed epithelioid cells and two or more giant cells (Plate XVI, Fig. 16). In the tunic the tubercles are more numerous than in any other organ and tend to coalesce and form large irregular areas which later undergo necrosis. The young tubercles, however, show no evidence of necrosis (see Plate XVI, Fig. 17).

The periphery of the tubercle often shows numerous lymphoid cells. As a rule multinuclear cells occur in the outer zone though often they are situated at the center around which are grouped the epithelioid cells. Some of the giant cells contain thirty or more nuclei, arranged either about the periphery or heaped up in the center of the cell. The nuclei are ovoid and vesicular and stain light blue. In some sections as many as six giant cells can be distinguished lying together in close proximity to one another; often they are adjacent to a tubercle. In sections of the tunica vaginalis in Guinea-pig VII there are as many as nine separate tubercles in a single field under the low power of the microscope. It is not uncommon to find associated with one tubercle six or more giant cells (see Plate XVI, Fig. 17).

The giant cells in relation to the situation of the nuclei may be described in three groups: first, the cell with nuclei arranged centrally; second, the cell with

nuclei situated around the periphery, and third, the cell with nuclei at one or both poles. The giant cell of the glanders tubercle often contains bacilli.

It may be here stated that the glanders bacilli in the tissues differ somewhat in morphology from those of artificial growth. In stained sections the bacilli are more elongated and delicate and contain clear spaces along the sides.

The focal lesions in the tunica vaginalis occur usually in colonies, whether acute or chronic. Beyond the colony of tubercles the tissue is greatly thickened by a fibrous new growth rich in cells. This fibrous cell proliferation often forms a dense zone about the tubercles. In places the connective tissue extends in between the seminiferous tubercles and contains foci of lymphoid and plasma cells. Again the connective tissue invades the muscle fibers which in turn undergo degeneration. Usually there is an active proliferation occurring in the tissue far removed from the seat of the acute inflammation, which is due in part at least to the diffused glanders toxin.

Heart.—Lesions in the heart muscle rarely result from the glanders infection. In two animals where the living bacilli were injected important myocardial changes occurred. Both cases were those of guinea-pigs which had received repeated doses of the attenuated culture over a period of three weeks. The killed bacilli injected intravenously in small and repeated doses sometimes give rise to a marked fatty degeneration of the myocardial fibers in the rabbit.

The lesions are of two types: (1) a fatty change in the muscle fibers which at first is patchy and later becomes more or less diffuse in distribution, and (2) collections of lymphoid and plasma cells which later lead to a fibrous tissue new growth and give rise to definite areas of chronic fibrous myocarditis.

Sections of heart muscle fixed in formalin and stained for fat often show the muscle fibers filled with fat in the form of small globules and fine particles. In some areas the fatty change is diffuse, in others it is slight, while in certain fields only isolated fibers show this degeneration. In addition to the early degeneration of the muscle fibers there are small areas in which the muscle fibers have been replaced by fibrous tissue. Some of these areas are infiltrated with lymphoid and plasma cells, while other areas show only dense fibrous tissue.

Vessels.—The vascular lesions occurring in experimental glanders have been described in detail in a former contribution by one of us (Duval[7]). Babes[8] also mentions the occurrence of vascular changes in glanders in horses.

The lesions are of three general types, (1) the exudative, (2) the proliferative and (3) the degenerative. This grouping of the

[7] *Jour. of Exper. Med.*, 1907, ix, 241.
[8] *Zeit. f. Hyg.*, 1902, xxxix, 217.

lesions corresponds closely with the degree of virulence of the culture employed. In general it can be stated that the highly virulent culture produces vascular lesions of an acute inflammatory type, the culture of moderate virulence produces the proliferative changes, while the degenerative changes result from either the proliferative or exudative lesion.

The vascular changes are focal in distribution. As in other organs the acute lesion is an aggregation of polynuclear cells. They occur in the subendothelial layer of the intima especially in vessels of the peripheral system (Plate XV, Fig. 12). Thrombosis is a frequent lesion in the veins. The pericapillary "round cell" infiltration commonly occurs in the affected areas. Focal collections of eosinophiles in the vessel wall are occasionally met with.

Lymph Glands.—The lymphatic glands in the animals infected with the highly virulent culture are always enlarged and sometimes contain areas of suppuration, especially the lymphatic glands of the inguinal and axillary regions after subcutaneous inoculation, and those of the mesentery and the mediastinum after intraperitoneal injections. In the chronic cases the glands are enlarged and firm, and often contain well-defined areas of caseation.

The acute lesion consists essentially of collections of polynuclear leucocytes, masses of fibrin and disintegrated cells. Here, as in other organs, the more severe of the acute lesions consist of a focus of large and small globular masses of fragmented cells mingled with much indistinguishable material and a few well preserved polynuclear cells. The less acute lesion shows no disintegration of the cells but focal areas composed chiefly of polynuclear cells and fibrin. These acute areas occur in no especial situation. Sometimes they are within or bordering upon the follicles; at other times they occur in the large sinuses, especially in the efferent blood spaces.

The proliferative lesion does not differ in any essential feature from the chronic lesion described for other organs. It is largely composed of the so-called epithelioid cells which form well defined tubercles. Giant cells are found frequently in the proliferative areas of the lymph nodes and often contain the glanders bacilli.

In addition to the proliferative lesions the centers of the follicles contain areas of swollen and degenerated phagocytic cells. These cells often contain fat globules, red blood cells, blood pigment and cell nuclei. The areas of phagocytic cells stain intensely with Scharlach R. and appear in marked contrast to the surrounding deep blue stained cells of the follicle.

Though normally there are numbers of phagocytic cells scattered throughout the lymph nodes of the guinea-pig, in chronic glanders infection they are markedly increased and are especially numerous in the efferent blood sinuses.

Adrenals.—The adrenals are less often the seat of lesion than any other organ, even in animals dying of an acute infection. Only in seven out of forty cases infected with the highly virulent culture were there lesions in the adrenals.

The acute lesions occur both in the cortex and in the medulla and consist essentially of polynuclear collections in which the cells are either fragmented or well preserved. The parenchymal cells about the acute foci are swollen, vacuolated and granular. In some cells there can be demonstrated an occasional polynuclear cell and fibrin threads. The adrenal cells could not be distinguished in the more acute lesions as the degenerative products in the form of large irregular shaped chromatin masses were too abundant. However, in the less severe lesions with perfectly preserved polynuclear cells the necrotic adrenal cells were easily recognized. The adrenal cells within the lesion and those immediately bordering upon the lesion show extremely interesting changes. Many of the adrenal cells for a considerable distance away from the lesion contain mitotic figures. The attempt at division on the part of the parenchymal cells is more manifest and the dividing cells more numerous as the lesion becomes proliferative. Adrenal cells in mitosis are not observed in association with the acute inflammatory type of lesion.

It is interesting to note that there often occurs in the proliferative lesion a central island of adrenal cells enclosed by a well defined broad band of epithelioid cells (see Plate XV, Fig. 10). These "locked off" adrenal cells within the lesion stain intensely with eosin and are often without nuclei, though the general shape of the cell is well preserved. The cell is swollen and the protoplasm contains an excess of fat as demonstrated with the Sharlach R. stain. These areas of necrotic adrenal cells when stained with eosin-methylene-blue present the same general picture as the focal necrosis of the liver parenchyma. Mitotic figures do not appear in the adrenal cells of these areas. The cells immediately surrounding the lesion are also swollen and stained deeply with eosin but to a less degree than the central mass of cells within the lesion. Karyokinetic figures occur only in the adrenal cells neighboring upon the glanders tubercles, thus indicating an attempt at reproduction due to the action of the glanders toxin.

Omentum.—In the acute infections, the omentum is shrivelled up and bathed in a fibrino-purulent exudate.

Microscopically the acute lesions appear rather as definite foci in which the cells are broken and fragmented, or as closely packed collections of polynuclear cells in the meshes of a dense fibrin network.

In the chronic infections the omentum is curled, greatly thickened and contains nodules, many of which are caseous. The microscopic sections show a dense new growth of connective tissue in which are numerous tubercles. In places the fat cells have been entirely replaced by fibrous tissue. There are definite foci of epithelioid cells and areas of necrosis. Giant cells are numerous in the chronic lesions. In this type of lesion many of the vessels are obliterated by old and recent thrombi. There occur definite peri-vascular collections of lymphoid and plasma cells and eosinophiles.

The chronic type of lesion does not differ in any particular from that seen in certain forms of tuberculosis.

Pancreas.—This organ is very seldom the seat of lesion and then only by direct extension of the lesion from the mesentery or neighboring tissues. The lesions are acute and chronic. The acute changes occur in the stroma, only rarely involving the glandular tissue. The chronic lesions are represented by an increase in the connective tissue in scattered areas about the periphery of the lobules and by focal areas of epithelioid cells. The lesion of glanders here differs in no essential from that in other organs.

Intestines.—Lesions of the intestine occurred in only two animals of the whole series. In one the foci were in the large intestine and involved the serous and muscular coats. The lesion consisted of solitary tubercles of the proliferative type and undoubtedly extended directly from the peritoneum. In the other case the lesions consisted of ulcers with undermined edges. Microscopically these ulcerations involved the mucosa and the submucosa and contained necrotic areas about which were numbers of large phagocytic cells, epithelioid cells and polymorphonuclear leucocytes.

Spleen.—The spleen both in the rabbit and the guinea-pig is frequently the site of histological change. Discrete grayish-white areas result from the inoculation of the virulent culture. In this organ as in the others there are two distinct kinds of lesions, the acute inflammatory, resulting from the virulent culture and the chronic proliferative following the inoculation with a culture of low virulence.

The acute inflammatory lesions are of two general types. The first consists chiefly of circumscribed areas of fragmented polymorphonuclear cells and detritus characterized by masses staining dark blue. The second grade of lesion consists chiefly of polymorphonuclear leucocytes that show no tendency to break up and give rise to the characteristic chromatin masses above described. These lesions in addition contain large masses of fibrin which is arranged in a dense meshwork through the lesion.

The spleen sections of Guinea-pig XV of the intraorbital series of inoculations contain irregular masses of fibrin in the form of thrombi plugging the capillaries (see Plate XV, Fig. 11). The animal had recovered from the acute infection. The eyeball had been completely destroyed and the tissues of the orbit were greatly thickened presenting a large sloughing caseating mass.

The proliferative type of lesion occurs as well defined tubercles scattered throughout the section, though the site of predilection seems to be in connection

with the Malpighian bodies. Hence the lesion apparently begins just outside the follicle, gradually increases in size and invades by extension. These lesions are best developed in animals killed two or three weeks after inoculation with bacilli of a moderate degree of virulence.

Giant cells occur frequently and are either associated with the tubercles or are in their immediate neighborhood (see Plate XV, Fig. 9). Many of the tubercles are formed about the giant cells. These multinucleated elements contain from four to twenty nuclei arranged either centrally or peripherally within the cell protoplasm and are phagocytic for the glanders bacilli (see Plate XIV, Fig. 6).

The tubercles range from one third to one half the size of the Malpighian body and are composed chiefly of large cells having an ovoid lightly-stained vesicular nucleus. Lymphoid and plasma cells are found in the periphery of the tubercle. The contrast between the foci composed of these large proliferated cells and the cells of the Malpighian body with the eosin-methylene-blue is striking. The dark blue nuclei of the cells constituting the Malpighian body are in marked contrast to the light blue nuclei of the epithelioid cells, the nuclei being embedded in a protoplasmic matrix staining pink. These areas of epithelioid and giant cells are histologically similar to the miliary tubercle. They increase in size and coalesce with neighboring tubercles to form large conglomerate areas, which later undergo necrosis.

In addition to the glanders tubercle the blood sinuses contain large numbers of phagocytic cells containing pigment, fragmented nuclei and red blood cells. In the section of a spleen of a rabbit with subacute focal lesions the phagocytic cells often contain from three to six ovoid vesicular nuclei. Occasionally a Malpighian body contains multinucleated phagocytes undergoing fatty and hyaline change; other Malpighian bodies contain in well defined foci phagocytes in which the fat is admirably demonstrated with the Sharlach R. stain.

Giant cells and epithelioid cells are more numerous in the spleen of animals killed two or three weeks ofter inoculation. Here they occur in the blood channels, either free in the lumen or attached to the endothelium. The attached cells are swollen to many times their normal size, often causing partial or complete occlusion of the vessel (see Plate XV, Fig. 8). Not infrequently these swollen attached cells contain in addition to separate nuclei, well defined mitotic figures. Animals inoculated with bacilli of low grade of virulence afford an excellent opportunity to follow the development and function of these multinucleated elements.

Kidney.—This organ with the exception of the pancreas and adrenal is less commonly the seat of lesion than any other. In the cases of acute infection following the injection of highly virulent bacilli, albuminous and hyaline degeneration of the tubular epithelium usually occurs. The glanders nodules, so common in other organs, are rarely met with in the kidney. Occasionally miliary abscesses are encountered.

Intravenous injection sometimes gives rise to a peculiar type of

kidney lesion in which the organ presents the appearance of an acute hæmorrhagic nephritis. The following autopsy protocol of Rabbit XIX well illustrates the gross appearance of this type of lesion.

Rabbit XIX.—A full-grown female rabbit was inoculated intravenously with 1 c.c. of a twenty-four hour agar slant growth suspended in 6 c.c. of bouillon. The animal at the time of inoculation was nursing her young. Three other rabbits, one male, one pregnant, and one non-pregnant female, were inoculated at the same time with equal amounts of the culture under identical conditions. The animal with young developed a severe grade of acute glanders and was killed three days following the inoculation. The animal was very ill. A thick, purulent discharge ran from her nose and conjunctivæ. She was constantly wheezing and presented the typical appearance of snuffles. At autopsy the mucous membrane of the conjunctivæ and naso-pharynx was swollen, deeply congested and bathed in a sero-purulent exudate, which on examination was found to contain numerous bacilli of the morphology of *B. mallei.* A pure culture of this microörganism was recovered by plating the material from both the nose and pharynx. The veins of the ear about the site of inoculation are hard and in places nodular. On section they contain organized thrombi. The mammary glands are swollen, tense and on section markedly hæmorrhagic. The peritoneal cavity appears normal. The kidneys are twice their normal size and presented innumerable discrete pin-point and larger petechial hæmorrhages over the surface. These hæmorrhagic areas were all the more striking owing to the pale grayish-red color of the cortex. On stripping away the capsule, the cortical hæmorrhages remain attached to the cortex and project above the surface as firm coagulæ. On section these hæmorrhagic areas extend all through the organs, parallel to the surface and in more or less beaded streaks which apparently correspond to the general direction of the tubules.

The adrenals are swollen and deeply congested. The liver presents grayish-white irregular areas which are plainly visible beneath the capsule. Microscopically these areas are those of focal necrosis. The lymph nodes throughout the body are enlarged. The other organs are negative.

Sections of the kidney fixed in Zenker's fluid and stained with eosin-methylene-blue show the renal tubules filled with coagulated blood stained serum. The microscopic examination of sections under the low power show the tubules everywhere greatly dilated and the lumen filled with a light material staining pale pink in striking contrast to the deep distinct blue color of the tubular epithelium. The same material staining pink is also present in the capillary spaces of the glomeruli. On closer study, this material is found to be composed of altered blood cells and serum and occurs in at least three different forms: (1) finely granular reticulum, (2) homogeneous pink coagulum, and (3) large and small spherical globules. In the glomerular spaces and convoluted tubules, this material occurs as a structureless homogeneous mass; in the straight tubules it appears granular and finally as spherical globules.

The tubular epithelium, though compressed, is for the most part well stained and everywhere intact.

The deep red hæmorrhagic points projecting above the surface are firm coagula. In addition to the altered blood filling the tubules there is still another material, epithelial in origin, which is met with in three forms. (1) Complete separation of the intact lining epithelial cells forms casts which occupy the central parts of the lumen. These cells, from the absence of nuclei and their intense eosin staining, are unquestionably necrotic though their shape is well preserved and it would seem as though they were destroyed in situ and later fell away in columns from their basal attachment. (2) The tubules contain material composed of granular and globular degeneration products of epithelial protoplasm. (3) Tubular epithelium free in the lumen shows early granular degeneration with the nucleus still intact, and the cells are also seen converted into small and large globular masses staining a deep purple red.

In the collecting tubules the epithelium is often swollen to three times its normal size. Each cell, though markedly vacuolated, preserves its shape. Often the free edges meet and completely obliterate the lumen. The epithelium of the tubules of the pyramids, in addition to this swelling and vacuolation of the protoplasm, undergoes proliferation and in places the cells are heaped up six or eight deep and many contain mitotic figures.

The glomeruli are enormously swollen and filled with altered blood in the form of material staining pale pink. Many glomeruli show distinct masses of fibrin occluding the capillary space. Occasionally the lining cells of the glomerular capillaries are swollen and contain mitotic figures. In a few glomeruli the capillaries are filled with polynuclear cells.

The connective tissue stroma of the kidney, even in the acute infections, rarely shows change except that it contains occasionally foci of lymphoid cells and a few scattered eosinophiles.

The peculiar material described by Wright as filling up the kidney tubules of certain animals dying of acute glanders infection undoubtedly had its analogy in Rabbit XIX, although he does not describe the gross appearance of the kidneys. From the above description of the gross and microscopic appearance of the kidneys of Rabbit XIX it will be readily seen that this peculiar material is coagulated blood stained serum.

DISCUSSION.

From the foregoing description of the histological lesions of experimental glanders it will be seen that the glanders bacillus and its toxin produce three distinct types of change in the tissues, exudative, proliferative and degenerative, the determining factor being the degree of virulence of the culture. Highly virulent bacilli produce exudative changes; bacilli of lowered virulence produce proliferative changes. The exudative type includes acute lesions; the proliferative type includes chronic lesions, the degree

of chronicity varying directly with the attenuation of the culture. The degenerative type is always secondary either to the exudative or proliferative lesion.

The exudative lesions fall naturally into two groups, in accordance with the degree of virulence of the culture employed.

In the first group, produced by the most virulent cultures, the tissue cells and the rapidly invading polymorphonuclear leucocytes are broken up by the virulent toxin, and the broken down nuclei of these cells form the degenerative "chromatin masses." These chromatin masses were described by Wright as the chief characteristic of the glanders nodule. Wright's observations were limited to the effects of cultures of high virulence. He produced experimentally the lesions only of this first group, *i. e.,* lesions of the exudative type.

In the second group, produced by a less virulent culture, the lesion consists of closely packed collections of well preserved polynuclear leucocytes and fibrin. There is here an entire absence of the destructive action of the more virulent bacilli, which alone is evidence that the lesion varies with the degree of virulence of the culture.

The degenerative lesions occur for the most part in the liver, adrenals, heart and arteries. In the liver the degeneration occurs as a focal necrosis involving either single widely separated parenchymal cells, small groups of cells, or larger groups of cells comprising the greater part of a lobule. This cell necrosis is caused by the large multinuclear cells and by thrombi of fibrin. Mallory[9] states that thrombi of fibrin form in the capillaries under at least two conditions, viz., adjoining necrotic liver cells and around endothelial or other cells which have undergone necrosis within the capillaries.

The adrenal degeneration also occurs in the parenchyma either in single cells or in large and small groups of cells scattered throughout the cortex and medulla. It is caused partly by direct toxic action and partly by interference with nutrition resulting from the encircling epithelioid cells; the latter leads to the isolation of groups of adrenal cells.

[9] *Jour. of Exper. Med.,* 1895, iii, 611; *Jour. of Med. Research,* 1901, i, 264.

Degeneration of the heart muscle undoubtedly occurs as the result of repeated doses of the glanders poison. It is exhibited by the occurrence of fine droplets of fat within the muscle fibers. Some of these degenerated fibers are later replaced by newly formed connective tissue, giving rise to definite areas of chronic fibrous myocarditis.

The degenerative vascular lesions have been described by one of us in a former paper. Briefly there is a fatty change in the muscle fibers of the inner zone of the media secondary to primary intimal proliferation.

The main object of this series of experiments was to determine whether or not the glanders bacillus, under certain conditions, produced lesions resembling histologically those of tuberculosis and if such lesions existed, to determine the analogy between the glanders tubercle and the miliary tubercle, especially with regard to their histogenesis. The inoculations were made with glanders cultures of three degrees of virulence with the hope that the bacilli of moderate or low virulence might exert an irritative rather than the destructive action so characteristic of the highly virulent bacilli, and instead of causing exudation give rise to proliferation.

It became apparent that as the bacilli became less virulent the character of the lesion approached more closely that of tuberculosis. A gradation in severity of the lesion could be followed from the acute to the chronic changes, *i. e.,* from exudation to proliferation, until finally the type of lesion became analogous to that of tuberculosis.

The histogenesis of the tubercle of glanders is primarily a proliferation of the invaded tissues with the production of the so-called epithelioid cells, associated with giant cells (see Plate XIII, Fig. 1).

When Baumgarten stated that the primary effect of the glanders bacillus on the tissue was a production of epithelioid cells and that the glanders tubercle was essentially the same as the miliary tubercle, he was correct, but he failed to recognize the special conditions under which Bacillus mallei gives rise to the proliferative type of lesion and he did not observe that the organism produced more commonly the acute inflammatory lesion.

The glanders tubercle is best studied in the spleen, lungs, testes

and lymph nodes. It is composed almost entirely of closely packed epithelioid cells; its outer limits often show numerous invading lymphoid cells, and a few polynuclear leucocytes penetrating toward the center. The giant cells are usually arranged about the periphery, although not infrequently they occur in the central part of the tubercle (see Plate XV, Fig. 9).

The histology of the glanders tubercle is essentially that of the miliary tubercle and varies with the virulence of the infection; but the variation is the reverse of that which occurs with the lesion of tuberculosis. In the early tubercles of acute miliary tuberculosis produced by highly virulent tubercle bacilli there is an absence of giant cells and necrosis. In the early tubercles produced by the attenuated bacillus of glanders the giant cell is absent and there is only rarely necrosis. The lesion of the highly virulent tubercle bacillus corresponds with the lesion of the attenuated glanders bacillus.

The epithelioid cell marks the transition from the acute exudative to the chronic proliferative lesion in which it is the essential element. Even in the subacute lesion it occurs frequently in the inflammatory zone. It constitutes the entire formation of the early tubercle. In the lungs frequently occur discrete tubercles composed entirely of epithelioid aggregations without evidence of surounding inflammatory reaction. The origin of the epithelioid cell is still doubtful. It would seem to originate from the connective tissue cell. The fact, however, that it never becomes phagocytic either for bacteria or for cell products in the glanders tubercle is rather against its endothelial origin because the endothelial cell is phagocytic under the most varied conditions.

In general the epithelioid cells are large with pale staining ovoid vesicular nuclei. In morphology and staining reaction, and also in their focal arrangement they are identical with the epithelioid cells of the tuberculous lesion; indeed, under the microscope the glanders tubercle is indistinguishable from that of miliary tuberculosis.

The giant cells of glanders are found in the tubercles of the proliferative lesion, in the capillaries and in the lymph spaces at some distance from and entirely outside of the lesion. As in tuberculosis they are of both the tuberculous and " foreign-body "

type; indeed these two types are often associated in the same lesion. The giant cells are especially numerous in lesions of the testes, lungs, lymph nodes and spleen. The lesions in the spleen afford an excellent field in which to trace the evolution of the giant cell.

The first sign of a reaction is the swelling of isolated endothelial cells of the blood or lymph channels to twice or three times their Such a cell bulges far into the lumen although still attached to the vessel wall gradually becoming larger and encroaching upon the lumen. This increase in the size of the endothelial cell is due to the proliferation of the nucleus which first undergoes karyokinetic division. The nuclei resulting then divide directly, this process continuing until the cell becomes dilated to enormous proportions. Such a cell bulges for into the lumen although still attached to the vessel wall. The adjacent endothelial cells may not show the slightest change; this fact is remarkable as there seems to be no explanation for the peculiar activity in isolated cells. Either in this stage or in the later detached stage the cell may be so large as to occlude and even distend one portion of the lumen of the vessel while the adjacent portions maintain their normal size. The detachment of the endothelial cell occurs usually before the proliferation of the nuclei is complete, the distended cell containing twenty or more nuclei arranged in rouleaux about the periphery.

In one section it was possible to follow the endothelial cell through various transitional stages to the formation of large multinucleated elements. This section was cut from the spleen of a guinea-pig killed two weeks after inoculation with a glanders culture of low virulence. The bacilli were stained in the lesion and recovered from the organs.

In addition to the primary swelling of the attached endothelial cell it is not uncommon to find in the small blood vessels large cells from twenty to thirty microns in diameter whose limiting membrane is sharply outlined and whose nuclei present every evidence of direct division. Such cells have three or four separate nuclei arranged more or less in a row and two or more nuclear masses apparently undergoing direct division (see Plate XIV, Fig. 7). These nuclear masses may consist of two bulbous ends connected by a narrow bridge of nuclear substance. In one bulb which is twice

the size of the other, there is evidence of division into separate compartments by means of fine threads. In each compartment there is a deep staining granule. The other bulb is composed of one compartment only and this contains a single large granule; the whole mass appears as a distinct nucleus except for the bridge still holding it to the smaller mass. One can easily perceive that a section though a plane above or below this joining bridge would give the effect of separate nuclei (see Plate XIV, Fig. 5).

In the study of other giant cells in the same section the evidence of direct nuclear division is even more striking. In one cell the dividing nuclear masses are connected by narrow bands and the whole arranged in a semicircular manner about the periphery of the cell. One end of this nuclear chain consists of smaller masses more nearly perfected and separated than the masses of the outer end of the chain where they are larger and less distinctly divided off (see Plate XIV, Fig. 4). Still another stage in the development of the giant cell is one in which the nucleus is apparently beginning to divide. In such a cell the nuclear mass is situated centrally and is composed of three or more vesicular bulbous swellings, each having the internal structure of a separate nucleus and connected in a circular manner one with the other by means of narrow bands of nuclear substance (see Plate XIV, Fig. 3).

This manner of nuclear division continues until the cell is crowded with nuclei. Finally the cells undergo necrosis in a manner characteristic of giant cells. Occasionally these giant cells contain pigments or parts of other cells. In the glanders tubercle they frequently contain numbers of glanders bacilli (see Plate XIV, Fig. 6).

The study of the various lesions produced by B. Mallei suggests that stimulation of the endothelium by the weak toxin is the deciding factor in the production of the glanders giant cell. It is well recognized that the endothelial phagocyte is capable of undergoing a marked increase in cell extent whether the nucleus divides or not. Even when the cell has migrated and is no longer a part of the vessel wall its protoplasm is capable of enormous distension, an example of this condition is in the phagocytic endothelial cell of typhoid fever.

While there is no evidence of the fusion of cells in the formation of the giant cell of glanders there is a proof of its evolution from the endothelial cell through nuclear proliferation.

SUMMARY.

The bacillus of glanders may be so modified in virulence as to produce experimentally lesions differing widely in their histological features.

The highly virulent culture causes primary necrosis and disintegration of the tissue followed by the invasion of the injured area by polymorphonuclear leucocytes. The bacilli of moderate virulence give rise to a primary lesion of an acute inflammatory nature in which the cells show no evidence of necrosis or disintegration. The attenuated bacilli produce primary tissue proliferation with the formation of epithelioid and giant cells.

There is every grade of lesion between the acute exudative and the chronic proliferative depending upon the toxicity of the cultures.

The glanders lesion whether exudative or proliferative is focal in character.

The strong toxins of the glanders bacilli cause degeneration or necrosis of cells and exudation, while the dilute and weak toxins produce proliferation.

The giant cell of glanders undoubtedly originates from the endothelial cell of the blood and lymph channels and is formed by division of the nucleus of the endothelial cell and not by cell fusion.

Histologically the lesion of glanders resulting from the culture of a low degree of virulence is proliferative and is analogous to tuberculosis; the lesions are focal and bear an intimate relation to the glanders bacillus.

DESCRIPTION OF PLATES.

PLATE XIII.

FIG. 1. A glanders tubercle. The proliferative type of lesion which results from the invasion of the tissues by the attenuated bacilli.

FIG. 2. Section of the rabbit's cornea stained by a special method to demonstrate the glanders bacilli in the tissues. The section also shows the early collection of large acidophilic granule cells about the injured area.

PLATE XIV.

FIGS. 3, 4, 5. Represent the various stages in the nuclear division of the endothelial cell in the production of the glanders giant cell.

FIG. 6. Shows a typical giant cell containing glanders bacilli.

FIG. 7. Shows a large multinucleated cell free in the lumen of a capillary.

PLATE XV.

FIG. 8. Section of spleen of the guinea-pig showing (*a*) single swollen endothelial cell of the capillary, (*b*) endothelial cell enormously enlarged and containing many nuclei and still attached to the vessel wall, and (*c*) large giant cell in the lumen of capillary.

FIG. 9. Section of spleen of the guinea-pig showing glanders tubercle, which is composed of epithelioid cells grouped about a central giant cell.

FIG. 10. Section of the rabbit's adrenal showing an area of necrotic adrenal cells surrounded by a dense zone of epithelioid cells.

FIG. 11. Section of the guinea-pig's spleen under low magnification. Note the fibrin thrombi everywhere plugging the blood sinuses.

FIG. 12. Section of the guinea-pig's omentum containing a small artery. Note the focal collection of polynuclear cells between the intima and media.

PLATE XVI.

FIG. 13. Section of lung of rabbit showing a sharply defined "glanders tubercle" near the pleural surface. The tubercle is composed of epithelioid cells, giant cells and necrotic material; and histologically is analogous to the miliary tubercle.

FIG. 14. Early glanders tubercle in the rabbit's cornea. The lesion is focal and composed chiefly of epithelioid cells, which undoubtedly have their origin in the corneal connective tissue cells.

FIG. 15. Section of rabbit's liver showing extensive focal necrosis. Fibrinous thrombi are numerous in the sinusoidal spaces of the affected lobules.

FIG. 16. Section of the tunica vaginalis of the guinea-pig showing multiple glanders foci composed of epithelioid and giant cells.

FIG. 17. Section of the tunica vaginalis of the guinea-pig showing a solitary glanders tubercle which is composed of epithelioid and giant cells, and moderate numbers of polynuclear leucocytes.

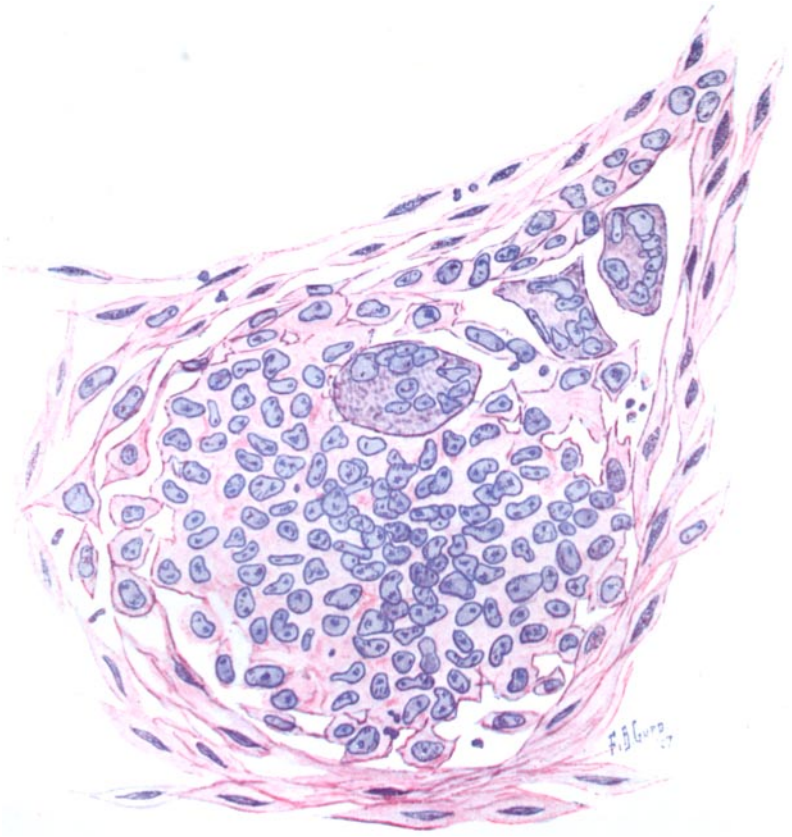


FIG. 1

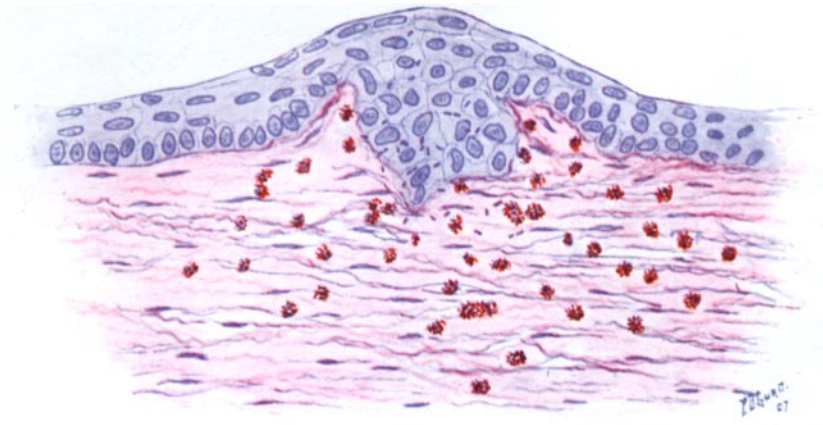


FIG 2.

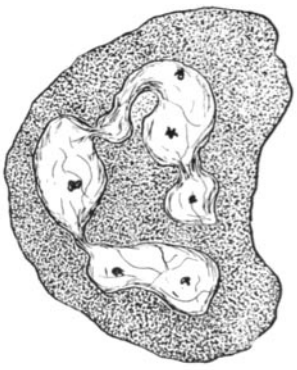


FIG. 3.

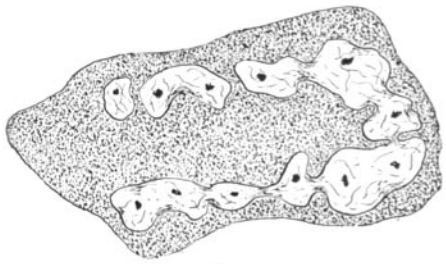


FIG. 4.

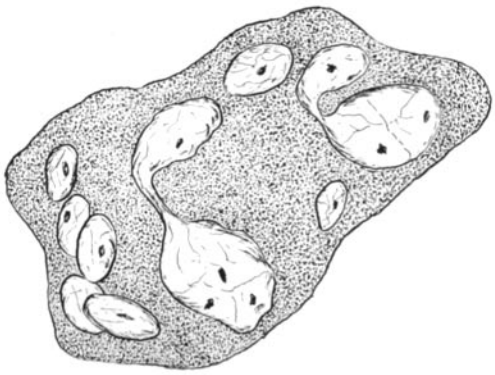


FIG. 5.

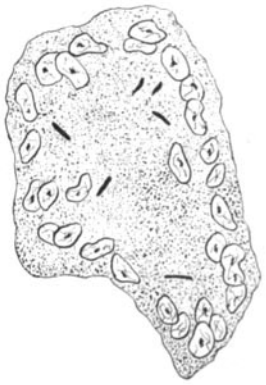
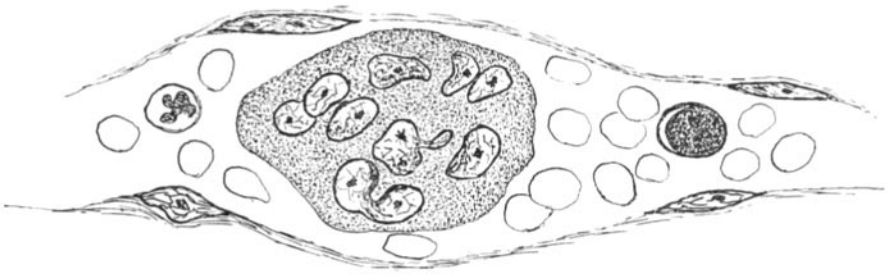


FIG. 6.



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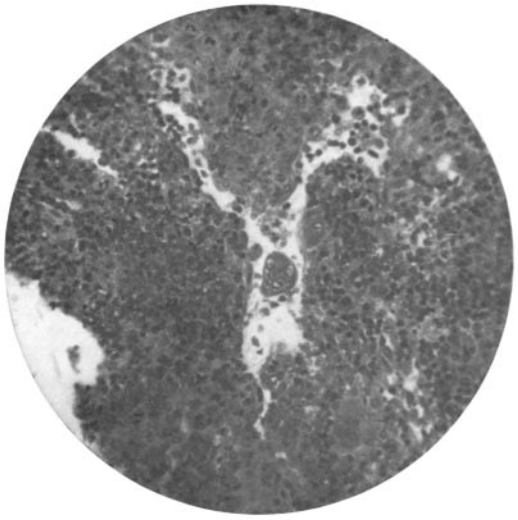

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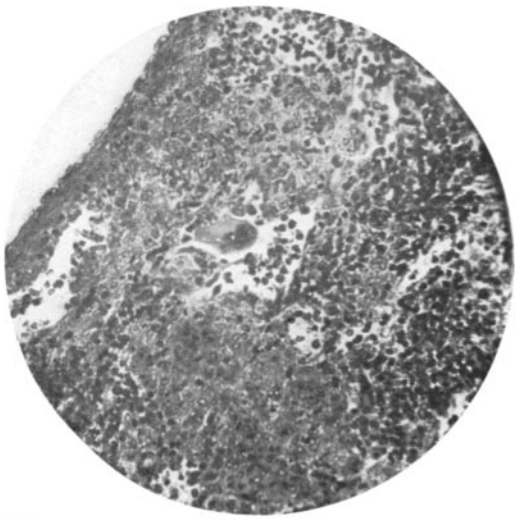

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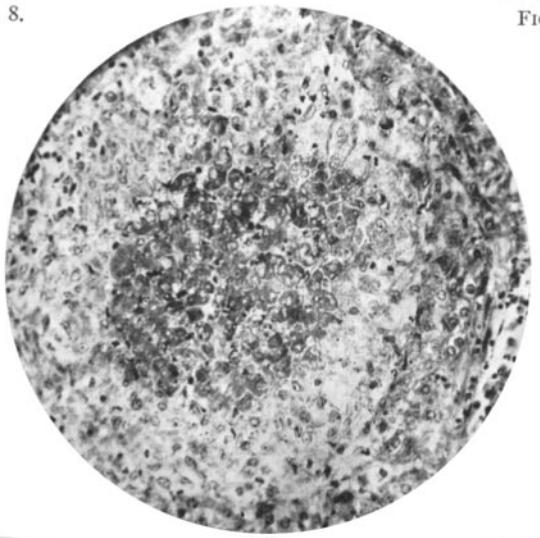


FIG. 10.

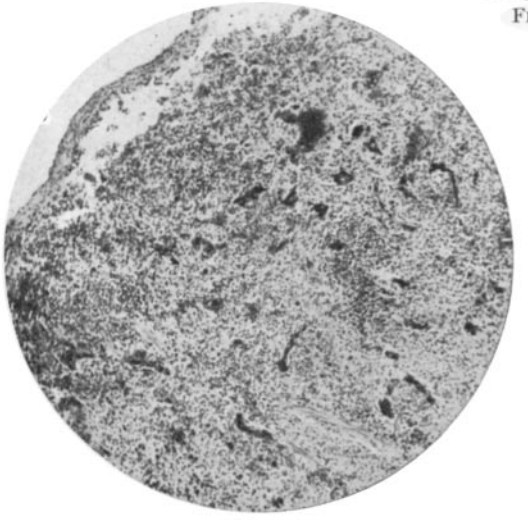


FIG. 11.

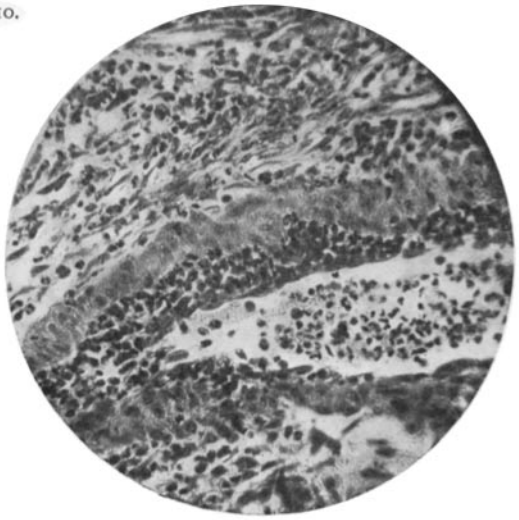


FIG. 12.

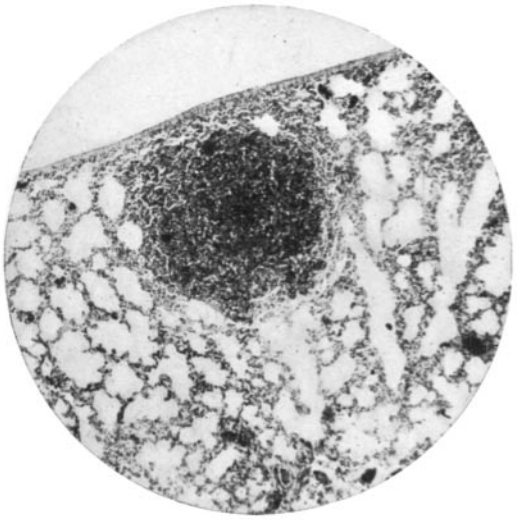


FIG. 13.

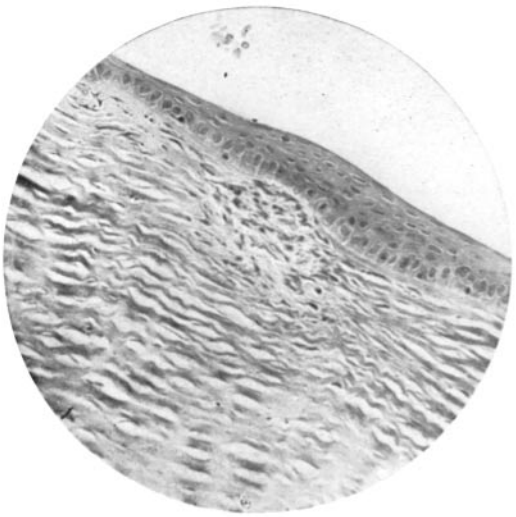
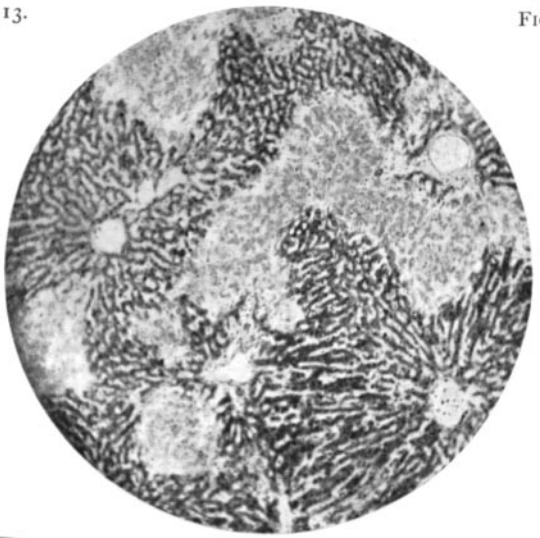


FIG. 14.



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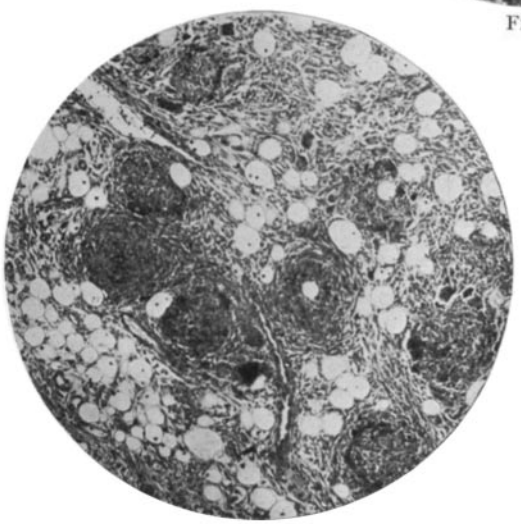

FIG. 16.

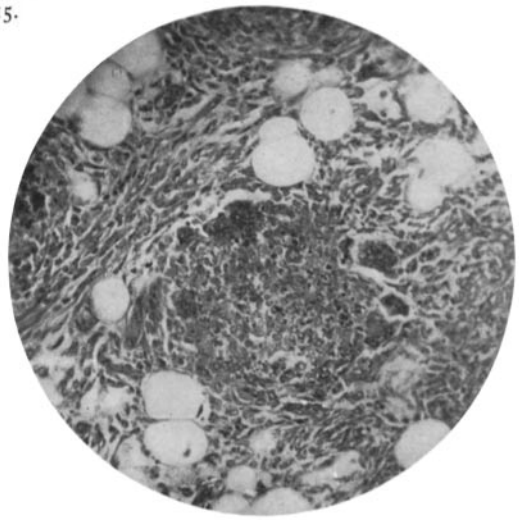

FIG. 17.