KLOSSIELLA INFECTION OF THE GUINEA PIG.

By LOUISE PEARCE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 63 TO 70.

(Received for publication, January 31, 1916.)

In studying the lesions produced by arsenical compounds in the kidneys of different species of animals, certain parasites were observed in the renal tubules of guinea pigs, which strikingly resembled the coccidium, *Klossiella muris*, observed and described by Smith and Johnson[1,2] in the kidneys of the mouse. As we believed the guinea pig to be relatively free from spontaneous renal lesions, a search through the literature was made to ascertain if renal parasites of the guinea pig had been previously described. At the same time the study of a large number of guinea pig kidneys was undertaken, in order to determine the relative frequency of this parasite as well as to obtain information concerning the lesions which tend to be associated with it.

Seidelin[3] in 1914 describes a parasite which occurred in the kidneys of two guinea pigs from Nigeria, and which bears such strong resemblance to *Klossiella muris* in some of its stages that he considers that both parasites "must be regarded as belonging to one and the same genus, whilst the question of their specific identity or otherwise cannot at the present time be finally decided." He further states that the parasite does not appear to be a common one in West African guinea pigs, as he found only one case among about twenty guinea pigs of the Yaba series. Later he found identical parasites in sections of a kidney of another guinea pig from the same locality. He found no similar instance in guinea pigs procured in Liverpool.

[1] Smith, T., *Jour. Comp. Med. and Surg.*, 1889, x, 211.

[2] Smith, T., and Johnson, H. P., *Jour. Exper. Med.*, 1914, vi, 303.

[3] Seidelin, H., *Ann. Trop. Med. and Parasit.*, 1914–15, viii, 553.

Seidelin describes some of the stages of the parasite detected by him and suggests a probable life cycle. There is undoubtedly a close resemblance between this parasite and *Klossiella muris*, and again between both these parasites and the one about to be described. On the other hand, the question of the identity of Seidelin's parasite and the one we have observed will be discussed later on in this paper. There are certainly points of similarity between the two, as well as some points of difference.

It may be stated here that we have found the infection to be by no means uncommon in kidneys of guinea pigs. In sixty guinea pigs examined for renal parasites, twelve were found to be infected. In other words, in our experiments with guinea pigs we found that we must reckon upon at least 20 per cent of the animals from our sources being infected with this renal parasite.

The material examined consisted of kidney sections of sixty adult guinea pigs from two sources, one in Philadelphia, the other in New Brunswick, New Jersey. These pigs were either perfectly normal animals or were animals which had been used for toxicological experiments with various arsenical compounds and had survived the use of the drug not more than 24 hours. This arbitrary time limit was set in the choice of material in order that any chronic pathological process observed might not be confused with the drug action, since within this time (24 hours) no extensive proliferative change in the kidney can be attributed to the action of these compounds. The kidney tissue was fixed in Zenker's fluid and imbedded in paraffin. Sections were stained with hematoxylin and eosin, methylene blue and eosin, and Giemsa's stain. In addition, frozen sections were made from tissue fixed in 10 per cent formalin and stained with hematoxylin and eosin.

Description of the Parasite.

Various stages of the parasite have been seen in the kidneys of guinea pigs and in order to facilitate an interpretation of its probable life cycle, a certain sequence of description will be observed, beginning with the stage or form most frequently encountered.

Sporoblast Cycle.—The form most frequently seen is a small round or ovoid body measuring 7 to 8 μ in diameter. It stains a very light bluish pink with hematoxylin and eosin, and contains numerous small dark blue masses of chromatin scattered through the cytoplasm in no definite arrangement (Figs. 1 and 2). The cytoplasm is somewhat granular and slightly, if at all refractile. The chromatin is of two sizes, larger irregular masses and tiny pin points, both taking a dark blue stain with hematoxylin and methylene blue. Usually several of these parasites are found free in the lumen of the renal tubules and sometimes the lumen appears to be completely blocked with them. They are most frequently seen in the straight tubules of the inner half of the cortex, but are also found somewhat less numerously in the convoluted tubules and in the tubules of the upper medulla. Occasionally one or two may be found in the capsular space of the glomerulus. This stage of the parasite is apparently very similar to the so called "daughter sporoblast" stage of *Klossiella muris* described by Smith and Johnson.

In certain sections it has been possible to make out a confining cell membrane surrounding several of these bodies, as in Fig. 2. The membrane is all that remains of the epithelial cell that originally contained the developing parasites. As they grow and increase in size the host cell must necessarily enlarge until it becomes flask-shaped and bulges out into the lumen of the tubule. The parasites occupy the broad distal portion of the containing cell and the nucleus is usually crowded inwards and often to one corner of the cell. It frequently appears flattened, compressed, or shrunken, and eventually it disappears, apparently just preceding or about the time of rupture of the cell. The cytoplasm of the host cell gradually becomes more and more scanty and granular and finally all that remains of the cell is its enormously distended cell wall, which is ruptured by the escaping parasites. It is somewhat difficult to determine just how many parasites develop in the epithelial cell, as we have had no opportunity of examining any material except cross sections of kidney tissue. However, there are probably at least ten to twelve.

Each of these parasites next undergoes a division or segmentation into eight to twelve bodies (Fig. 3). These bodies are falciform or banana-shaped and are extremely small, measuring 1 by 4 to 5 μ, and are surrounded by a distinct membrane. Their cytoplasm is clear and non-granular, pink (hematoxylin and eosin, and Giemsa's stain), and each body contains a dark blue dot of chromatin. We believe that these tiny fusiform bodies are similar to the form designated as the sporozoites of *Klossiella muris*. The further history of these bodies is entirely conjectural and will be referred to later in the discussion.

Not uncommonly one will find in heavily infected kidneys a form (Fig. 4) which is evidently the precursor of the two stages just described. This form is comparatively large, and may measure 17 by 22 μ; it has the same appearance and staining characteristics as the smaller more usual forms first described (Figs. 1 and 2). A breaking up of this large mass into several constituent smaller bodies results in a picture similar to Fig. 2. In studying various infected kidneys, we have seen several microscopic fields in which there were all gradations of this division (Fig. 6), so that we are inclined to regard this large parasitic mass as corresponding to the so called "mother sporoblast" stage of *Klossiella muris*.

Ring Form.—In several of the sections of heavily infected kidneys we have seen a stage of the parasite which we have called the "ring," or "annular" form (Figs. 7 and 8). It is always within an epithelial cell and the cell itself is enlarged and may protrude into the lumen of the tubule to the extent of nearly occluding it, as in Fig. 8. This form measures 18 to 20 μ in diameter and consists of a series of twelve to eighteen definite divisions or segments arranged in a circle or ring. Each division is extremely small, ovoid in shape, and measures 3 by 5 to 7 μ. They are smaller than the more frequently encountered forms seen in Figs. 1, 2, 3, and 4 (the daughter sporoblasts), and in appearance are totally different. The cytoplasm is refractile and stains practically not at all, or at most a very pale pink with hematoxylin and eosin, and Giemsa's stain. Each segment contains one or two chromatin dots. In some instances the two chromatin dots appear to be fusing together; in others they are distinctly separate from one another. In other instances the one chromatin dot is elliptical in shape, and it is possible that the finding of two chromatin dots is due to the level of the section. In the center of the ring form is a small amount of a granular refractile pale pink staining material which is apparently a residuum of the cytoplasm of the surrounding ring segments (Figs. 7 and 8).

In Fig. 8 there is a typical ring body and another intracellular form which we think is also a ring body seen from the outside. In other words, a ring body is a section through such a hollow sphere as is seen in Figs. 9 and 10. Here there are eighteen small segments or divisions, each of which is similar in appearance and staining reactions to the segments of the ring form.

The ring segments are not only considerably larger than the final divisions of the sporoblast cycle, the sporozoites (Fig. 5), but are also of a totally different shape and appearance. Moreover, the immediate precursor stage, the ring form, is still enclosed in an epithelial cell, while the precursor stage of the falciform bodies shown in Fig. 5 is not necessarily intracellular. Indeed, when the division into the tiny falciform bodies or sporozoites occurs, the daughter sporoblasts are probably always extracellular and free in the lumen of the tubule. In addition, the sporozoites are contained in a very distinct spore, having a definite membrane, while there is no such structure surrounding the segments of the ring form. When the ring divides, the resulting segments are all apparently set free in the remains of the epithelial host cell and eventually in the lumen of the tubule when this cell ruptures. The ring segments seem to be identical with still another stage of the parasite about to be described and may represent its earliest and youngest form.

Hyaline Forms.—Figs. 11 to 16 illustrate a single stage of the parasite which from its appearance and staining character is evidently one of the segments of the ring form. It is very small, measuring from 5 to 8 μ in length and 3 to 5 μ in breadth, and is oval or ovoid in shape. The cytoplasm is non-granular, extremely refractile, hyaline, and stains pink with hematoxylin and eosin, or Giemsa's stain. It contains a relatively large clear-cut mass of dark blue staining chromatin placed towards one end of the parasite. At the opposite pole a small clear non-staining area may be seen, as in Fig. 12. In the section from which Fig. 11 was taken this clear area is visible, but it is very small and barely shows in the photograph. This stage measures 3 by 5.5 to 7 μ. It may be extra- or intracellular, as shown in the illustrations. In Fig. 12 it apparently is about to enter an epithelial cell of a renal tubule. In Fig. 11 it is clearly intracellular and surrounded by a vacuole. The nucleus of the host cell is immediately below the parasite. The epithelial cell opposite the parasite on the other side of the renal tubule contains an ovoid inclusion which is probably a similar parasitic body, although the mass of chromatin is not seen in the section. The cytoplasm of the two cellular inclusions is identical.

In Fig. 13 another intracellular parasite is seen and here the chromatin has divided into two distinct parts. The parasite itself measures 5 by 7 μ. The host cell has become enormously enlarged and protrudes into the lumen of the tubule. Fig. 14 shows a further division of the chromatin into four distinct segments. This parasite is also intracellular, but in order to bring the chromatin into focus for the photograph, the epithelial cell outlines are not seen. Just below this parasite is another smaller intracellular form similar to those shown in Figs. 11 and 12, with only one chromatin mass.

Schizogonic Cycle.—Fig. 17 illustrates a spherical form which we are inclined to believe represents the schizogony of the parasite. It it comparatively large—measuring 22 μ in diameter, and is composed of a large number (thirty to forty) of tiny fusiform bodies or merozoites enclosed in a rather indistinct and apparently very thin membrane. It does not appear to be enclosed in an epithelial cell, but is extracellular and free in the lumen of the renal tubule. The merozoites in longitudinal section measure 1.5 by 7 to 8 μ. Their cytoplasm stains pink with hematoxylin and eosin, and each contains a tiny dot of chromatin. These small falciform bodies are extremely similar to the sporozoites described above. The general appearance of this large form suggests its similarity, if not identity with the glomerular body of *Klossiella muris*, which Smith and Johnson interpret as the schizogony of the mouse parasite. We have not found this form in the capsular space of the glomeruli but in the convoluted tubules of the first order. It is not a common stage and we have found it in only one kidney.

Pathological Changes in the Kidneys of Guinea Pigs Associated with the Parasitic Infection.

The kidneys of guinea pigs infected with the parasite show certain pathological changes of a chronic nature which we are inclined to attribute to the presence of the organism. They have been found in all cases in which the parasite has been seen, and in several instances where these lesions were observed the infection was very light, and the parasite was found only after a thorough search.

The lesions which we think are caused by the parasite consist in an irregular accumulation of fibroblasts and small round cells about the base of some of the glomeruli. Both the distribution and arrangement of this infiltration are very irregular (Figs. 18 to 21). In a single microscopic field one or two glomeruli may be affected in this manner, while the adjacent glomeruli are normal in appearance. Moreover, the extent of the infiltration varies considerably. Some glomeruli have only a slight accumulation of round cells about their base, others are almost obliterated, as in Figs. 18, 20, and 21. Usually there seem to be relatively more cells of the small round mononuclear type than fibroblasts. The fibroblasts themselves are apparently not young cells.

These cellular accumulations seem to be fairly well confined to the immediate vicinity of the glomeruli. In certain instances, however, the round cells and fibroblasts extend outward to a limited degree into the labyrinthine tissue and along the medullary rays between the tubules, but in these cases the connection between this extension and the accumulation of similar cells about the neighboring glomeruli can be easily traced.

There seems to be no reaction of the kidney tissue in the immediate vicinity of the parasite itself, that is, in the inner half of the cortex where we have found the parasite to be most numerous. Here one may see half a dozen consecutive tubules filled with parasites and eight or ten epithelial cells containing ring forms, yet there is apparently no abnormality in the immediate interstitial connective tissue. The glomeruli, however, just above these infected tubules show a more or less extensive infiltration of round cells and fibroblasts. The portion of medulla just below the infected tubule shows no appreciable change. No gross changes in the kidneys referable to the renal parasite have been noted.

DISCUSSION.

The description of the various stages of the parasite found in the kidney of the guinea pig has been arranged so as to relate, tentatively at least, those forms which seem to belong to the same cycles of development. Certainly two different cycles of development have been observed. The exact interpretation of the cycles must be more or less hypothetical, since there is an obvious lack of knowledge of all the stages in the evolution of the parasite.

The first cycle, described under the sporozoites is similar to the so called sporoblast stage of *Klossiella muris* and is evidently similar to the stage described by Seidelin. However, we have never seen more than twelve daughter sporoblasts, and usually only eight to ten resulting from the first division of the mother or pan sporoblast form, while Seidelin gives sixteen to twenty as the probable number. This discrepancy, if the two parasites are identical, can probably only be settled by the study of fresh material or an extensive series of sections. In the second division, into sporozoites, we have never observed more than twelve, each sporozoite measuring 1 by 4 to 5 μ. Seidelin describes thirty sporozoites, each measuring 1.5 by 8 μ. If this difference in the number of sporozoites continues to hold after the study of fresh tissue, we shall be inclined to believe that the two parasites belong to different species.

That this cycle of the parasitic development represents the sporoblast phase is strongly suggested by the fact that the sporozoites are confined in what is apparently a typical spore. We have never seen these spore-like bodies breaking up in the lumen of the kidney tubules, and it is probable that the spores containing the sporozoites are excreted in the urine, which is afterwards swallowed by the same or other guinea pigs, and that the spore membrane is digested away by the gastric juice, thus freeing the sporozoites. The extremely small size and fusiform shape of these tiny bodies would doubtless enable them to pass through the gastric or intestinal mucosa into the blood stream and so into the kidney. On the other hand, we have never seen any sporozoites in the glomerular tuft or capsular space.

The ring forms are not numerous, but they are easily found in heavily infected kidneys. They differ markedly from any stages of the sporoblast cycle and do not appear to be an integral part of it. It is easy to differentiate between a mother sporoblast, for instance, and a ring form seen from the outside, or cut on a tangent, so that the annular appearance is not seen. Moreover, the resulting division or segments of the two forms are apparently very dissimilar. The sporozoites are slender fusiform bodies, 1 by 4 μ, with a tiny dot of chromatin; the ring segments are oval or ovoid, 3 by 5 to 7 μ, with a relatively large mass of chromatin. The ring segments are apparently identical with the small hyaline forms seen free in the lumen of the renal tubules or in the epithelial cells, and there is nothing in the nature of a spore membrane about the dividing ring to prevent their escape into the tubules when the ring completely segments. The final interpretation is, of course, one of conjecture only, but the cycle is strongly suggestive of a sexual phase. The comparatively large number of ring segments or hyaline forms suggests further that they may be the microgametes, and that the ring form may be the microgametocyte. We have not seen any bodies which we could interpret as macrogametes, or any process of fertilization or conjugation, unless Fig. 16 represents this phase.

Smith and Johnson describe in the sporoblast stage of *Klossiella muris* a budding process in which the chromatin occupies the periphery of the budding masses. One might think that the ring form is simply a cross section of some of these buds of the mother sporoblast. But the ring form possesses not only a different type of protoplasm from the mother or daughter sporoblast, but in addition its segments are totally unlike either the daughter sporoblasts on the one hand or the sporozoites on the other.

The asexual or schizogonic cycle is apparently represented by the large extracellular segmenting form seen in Fig. 17. It resembles the glomerular body described by Smith and Johnson and interpreted by them as the schizogonic form. We have not seen it in the glomerular space, but only in the convoluted tubules and its scarcity may be tentatively explained on the assumption that such a stage is present only in early and light infections. Later, apparently, the sporoblast cycle may supersede the schizogony, for this cycle only has been detected in our specimens of heavily infected kidneys. Its extremely large size and the great number of its segments or divisions preclude its belonging to either the sporoblast or ring cycles. Moreover, its situation free in the convoluted tubules is one of the locations where one would expect to find the development of such a stage, if, as we have already suggested, the infecting sporozoites find their way into the kidney by the blood stream. The merozoites or segments of this large body are similar to the sporozoites, the final divisions of the sporoblast cycle. The conspicuous difference between the two stages of segmentation, aside from the difference in their number, is that the sporozoites are enclosed in a definite spore membrane, while the existence of a membrane surrounding the merozoite is problematical. In the specimens we have seen there is a very indistinct membrane, which, as in Fig. 17, is apparently ruptured, allowing the escape of the merozoites into the renal tubules.

Seidelin found no glomerular bodies as described by Smith and Johnson for *Klossiella muris*, but he considers that some of his tubular forms appear identical with the glomerular forms depicted by them. Unfortunately, Seidelin gives no illustration of this particular tubular form, so that we are unable to compare satisfactorily the two apparently corresponding stages. Seidelin is inclined to the opinion that these tubular forms represent the schizogony. Smith and Johnson think that their glomerular body is the schizogony for *Klosiella muris* and that the tubular forms are stages in the sporoblast cycle.

The pathological changes in the kidney of guinea pigs which we associate with the presence of this parasite are slight but definite and consist of an irregular accumulation of round cells and fibroblasts about some of the glomeruli. There is but slight involvement of the labyrinthine tissue adjacent to the glomeruli and apparently none at all in the lower or inner half of the cortex where the majority of the parasites are found. This may be due to the fact that the infecting sporozoites enter the kidney by way of the glomerular capillaries and that here the most serious injury to the kidney occurs, with a subsequent infiltration of round cells and fibroblasts.

SUMMARY.

We have found in the kidneys of twelve supposedly normal guinea pigs, coming from Pennsylvania and New Jersey, a parasite that closely resembles in some of its phases *Klossiella muris*, described by Smith and Johnson, and the renal parasite of two West African guinea pigs, described by Seidelin.

The forms most commonly found by us and described as the sporoblast cycle, are evidently similar to those described by Smith

and Johnson and by Seidelin. There are certain discrepancies of measurement between the parasite described by Seidelin and the one here described, but the most important difference between the two is the different number of sporozoites resulting from a final division of the daughter sporoblasts. Seidelin has found thirty sporozoites; we have found from eight to twelve, while the usual number is eight. Further, we have found a ring form which is unlike any of the stages in either the sporoblast or schizogonic cycle, and which we interpret tentatively as the male element or microgamete. In addition, we have found a tubular form which resembles the glomerular body of *Klossiella muris* and which we think is the schizogonic phase of this parasite.

EXPLANATION OF PLATES.

The illustrations are all from untouched microphotographs except Fig. 8, which is a drawing of an actual microscopic field. All the specimens except Fig. 17 are from Zenker fixed tissue.

PLATE 63.

FIG. 1. Renal tubules showing a heavy parasitic infection in different stages, the majority being daughter sporoblasts. Hematoxylin and eosin. × 675.

FIG. 2. Eight daughter sporoblasts enclosed in an epithelial cell of a convoluted tubule. Hematoxylin and eosin. × 1,000.

FIG. 3. Three spores containing seven to nine sporozoites. Note the distinct spore membrane. Hematoxylin and eosin. × 1,000.

PLATE 64.

FIG. 4. Mother sporoblast enclosed in an epithelial cell. The cell outline is not distinct in the photograph. Giemsa's stain. × 1,000.

FIG. 5. Glomerulus containing a single parasite, probably a young mother sporoblast in the capsular space. Hematoxylin and eosin. × 1,000.

FIG. 6. Three greatly enlarged epithelial cells, two containing mother sporoblasts, the third in the center containing eleven daughter sporoblasts. Hematoxylin and eosin. × 1,000.

PLATE 65.

FIG. 7. Two ring forms in adjoining tubules, each enclosed in an enlarged epithelial cell. The ring to the right shows ten divisions. Hematoxylin and eosin. × 1,000.

FIG. 8. Two ring forms in distended epithelial cells; the lower form is seen from the outside. Hematoxylin and eosin. × 1,000.

PLATE 66.

FIG. 9. Two ring bodies and several daughter sporoblasts. The ring body on the left is a tangential section through the edge of a hollow sphere. Methylene blue and eosin. × 1,000.

FIG. 10. The same field as in Fig. 9, but at a lower level, showing that the so called ring body is a section through a hollow sphere. Methylene blue and eosin. × 1,000.

PLATE 67.

FIG. 11. Two intracellular hyaline forms, the one to the right showing chromatin. Hematoxylin and eosin. × 1,000.

FIG. 12. An extracellular spindle-shaped hyaline body showing the chromatin mass at one pole and a clear non-staining area at the opposite pole. One extremity of this hyaline form is in the protoplasm of an epithelial cell. Hematoxylin and eosin. × 1,000.

FIG. 13. An intracellular hyaline form in an enlarged epithelial cell. The chromatin has divided into two masses. The parasite is in a vacuole in the host cell. Hematoxylin and eosin. × 1,000.

FIG. 14. Two intracellular hyaline forms; the chromatin of the upper parasite has divided into four masses. Giemsa's stain. × 1,000.

PLATE 68.

FIG. 15. Two small intracellular hyaline forms. Giemsa's stain. × 1,000.

FIG. 16. Two intracellular hyaline bodies. The larger one shows two elongated chromatin masses. The smaller parasite to the left is at a level which shows no chromatin. Giemsa's stain. × 1,000.

FIG. 17. A schizogonic form showing thirty to forty merozoites. This body is free in the tubule and has no definite membrane surrounding it. Taken from a frozen section fixed in 10 per cent formalin. Hematoxylin and eosin. × 1,000.

PLATE 69.

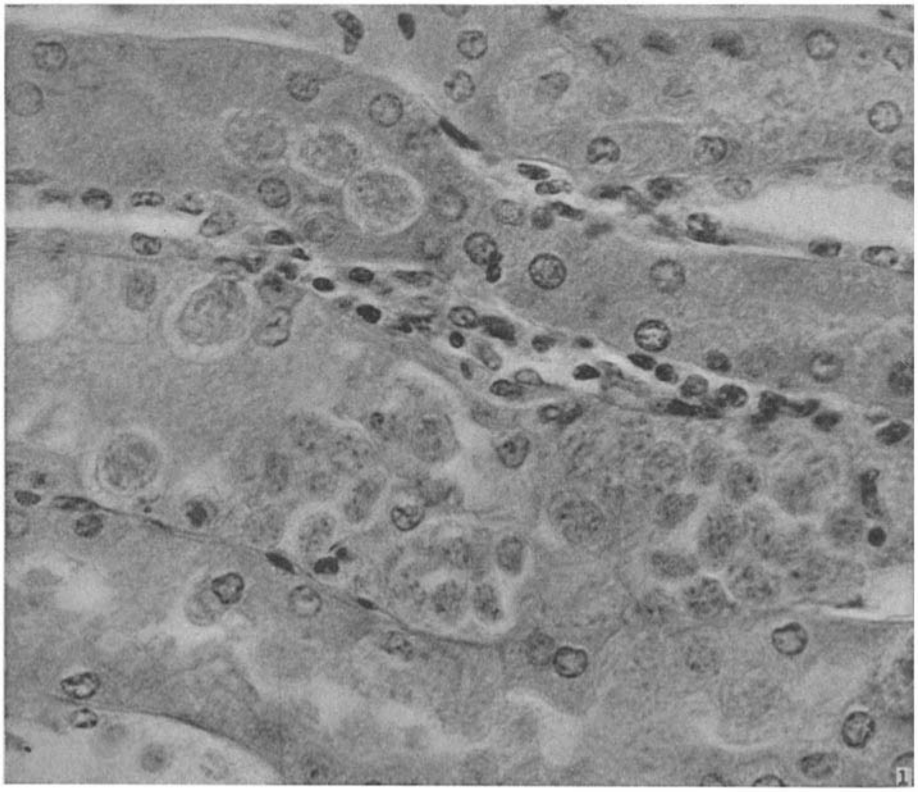
FIG. 18. Irregular cellular infiltrations, especially about the base of some of the glomeruli. Hematoxylin and eosin. × 125.

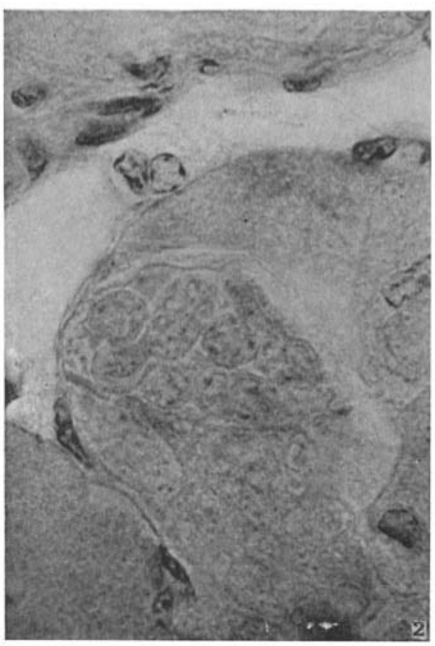
FIG. 19. Three glomeruli showing very slight cellular infiltration about the base. Two glomeruli show no such infiltration. Hematoxylin and eosin. × 210.

PLATE 70.

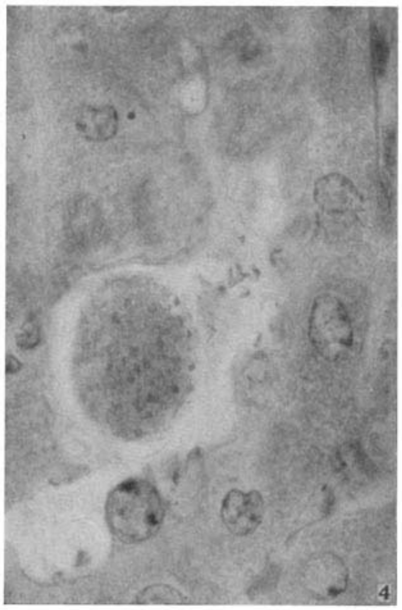
FIG. 20. The two lower glomeruli show a slight irregular accumulation of round cells and fibroblasts. The upper glomeruli are normal. Hematoxylin and eosin. × 210.

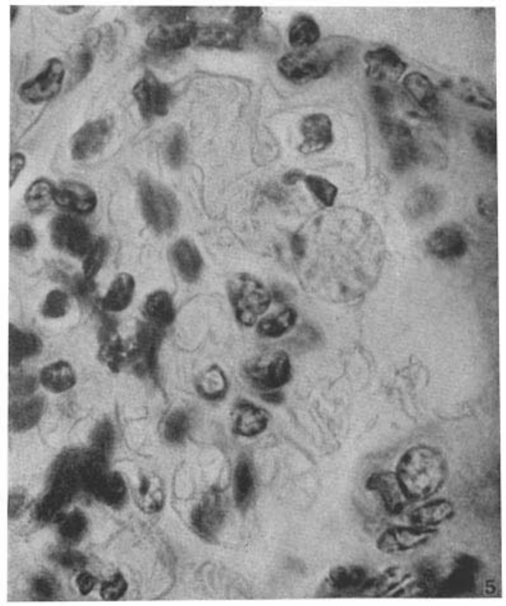
FIG. 21. The two glomeruli to the right show a fairly extensive accumulation of round cells and fibroblasts with an irregular extension into the labyrinth. The glomeruli to the left are practically normal. Hematoxylin and eosin. × 210.

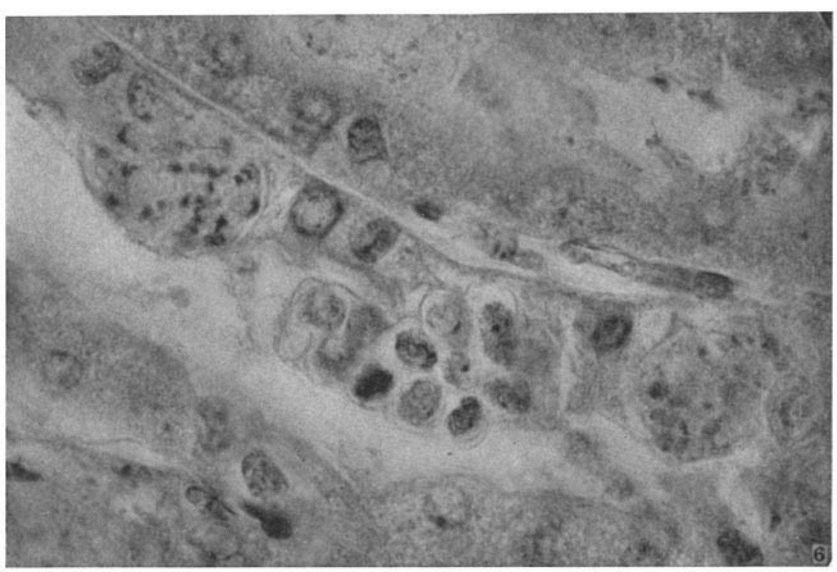




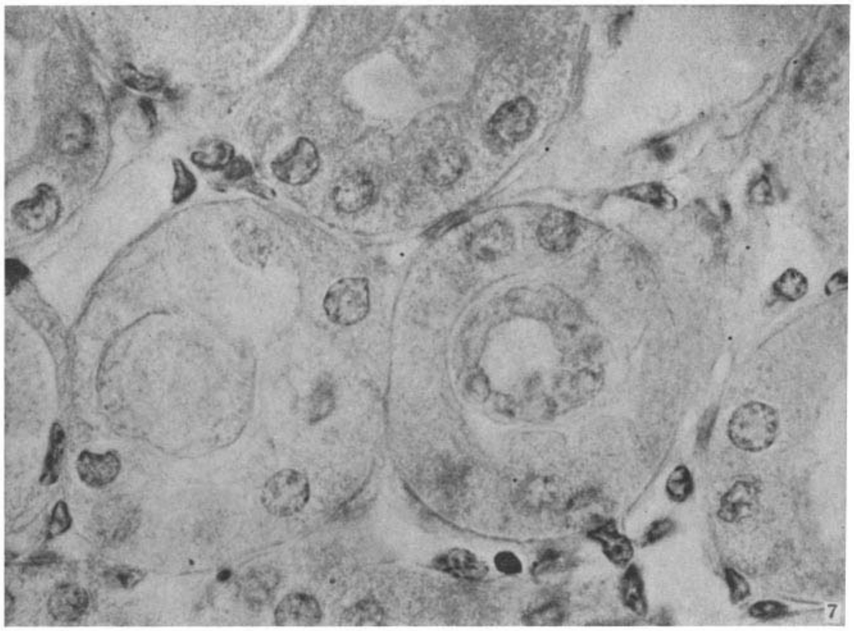
(Pearce: Klosiella Infection of the Guinea Pig.)



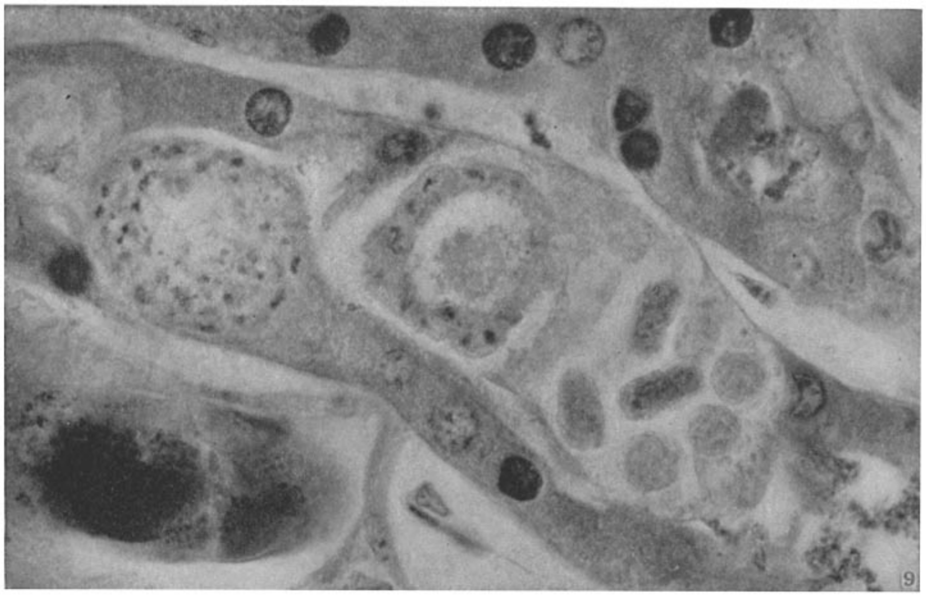




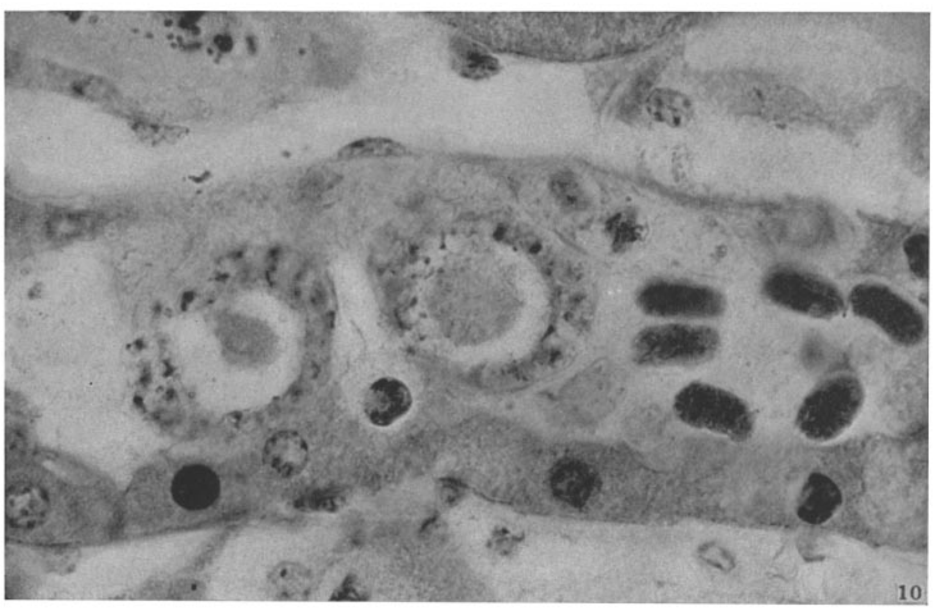
(Pearce: Klosiella Infection of the Guinea Pig.)



(Pearce: Klosiella Infection of the Guinea Pig.)

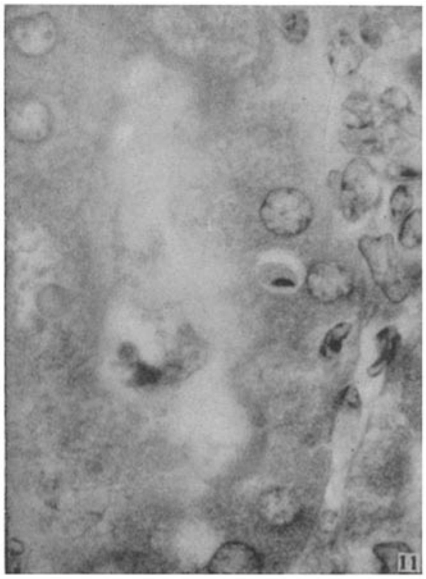


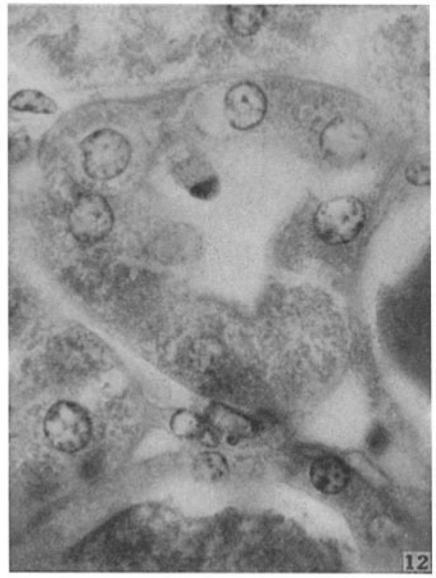
9

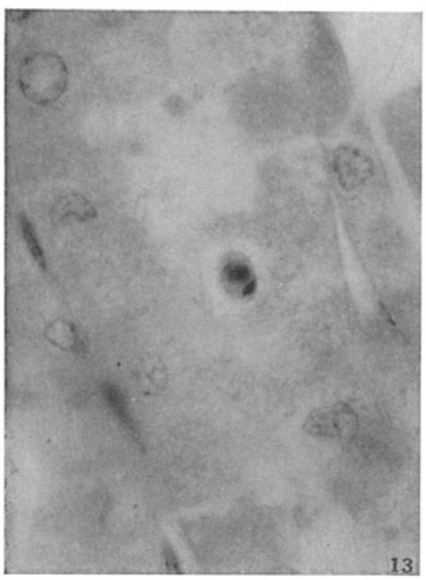


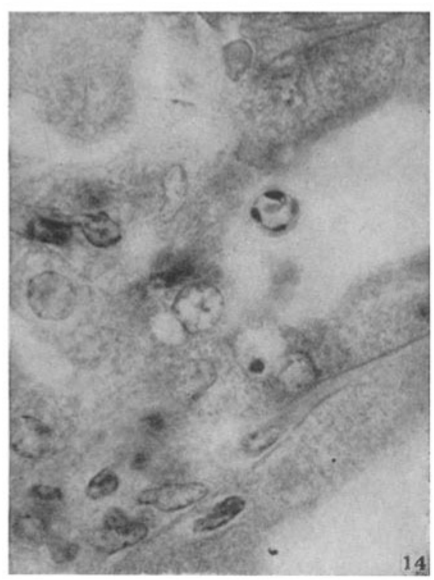
10

(Pearce: Klosiella Infection of the Guinea Pig.)

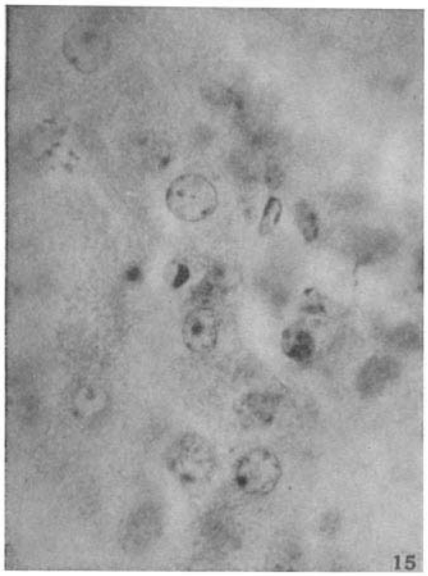


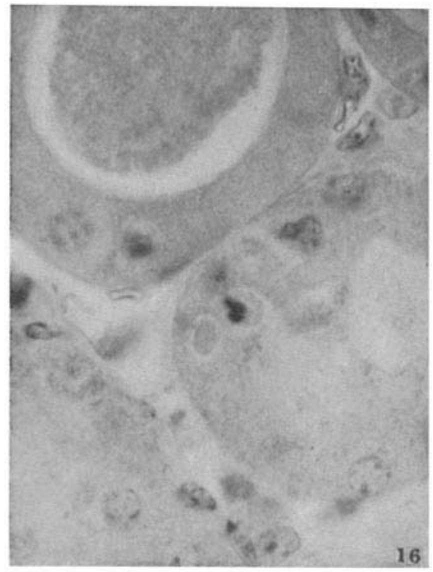


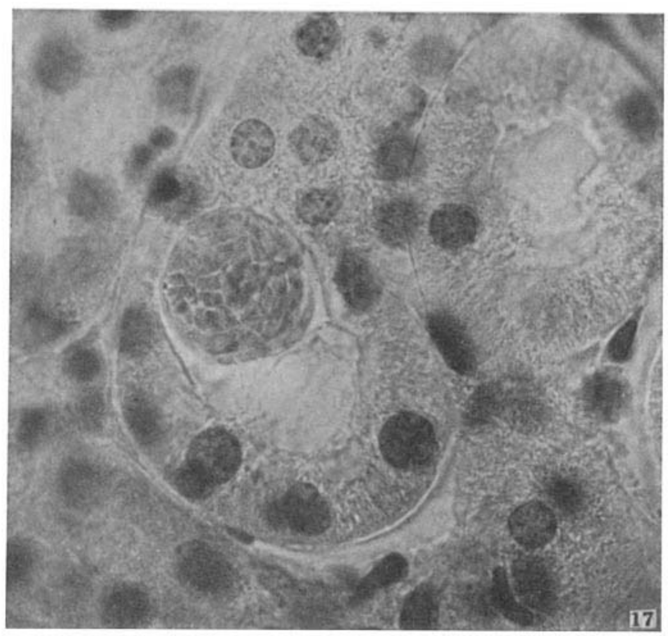




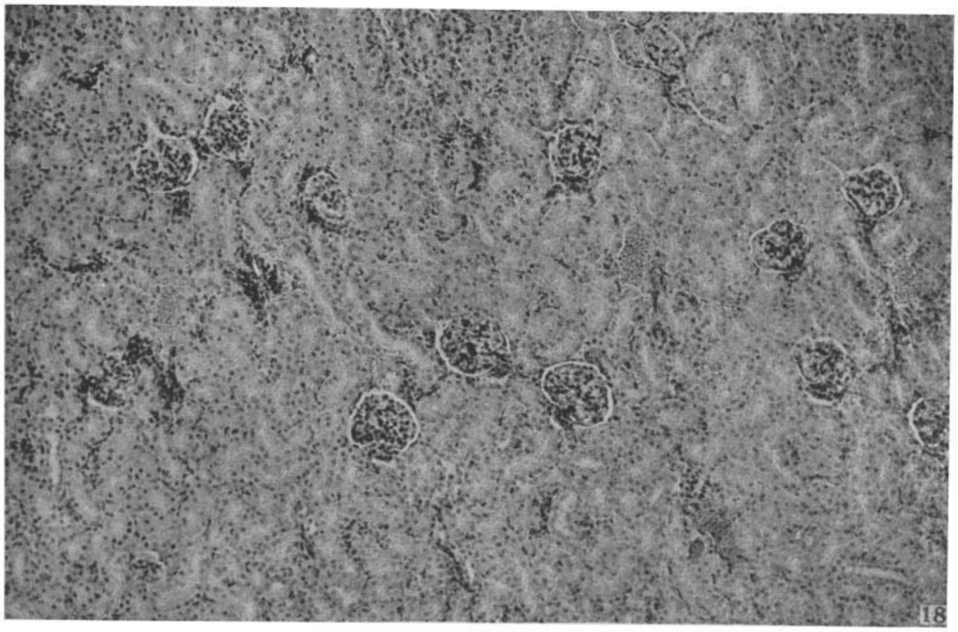
(Pearce: Klosiella Infection of the Guinea Pig.)



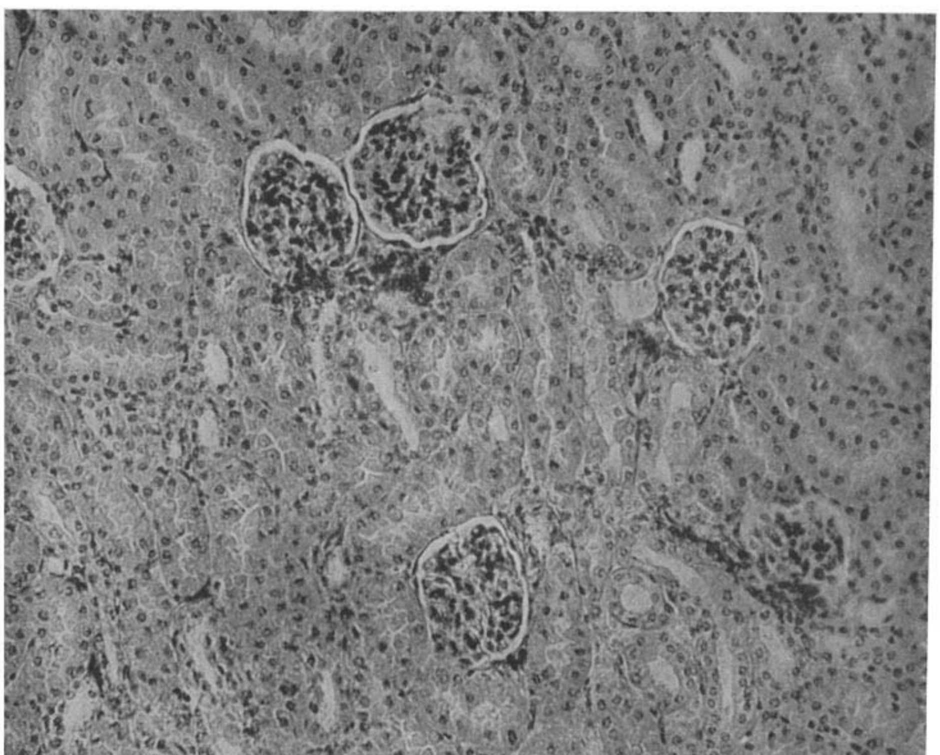




(Pearce: Klosiella Infection of the Guinea Pig.)

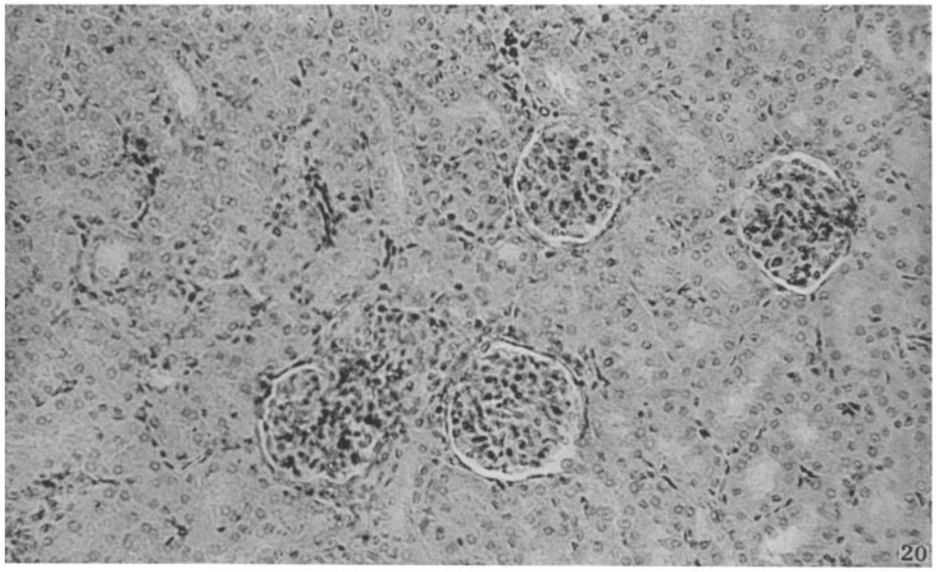


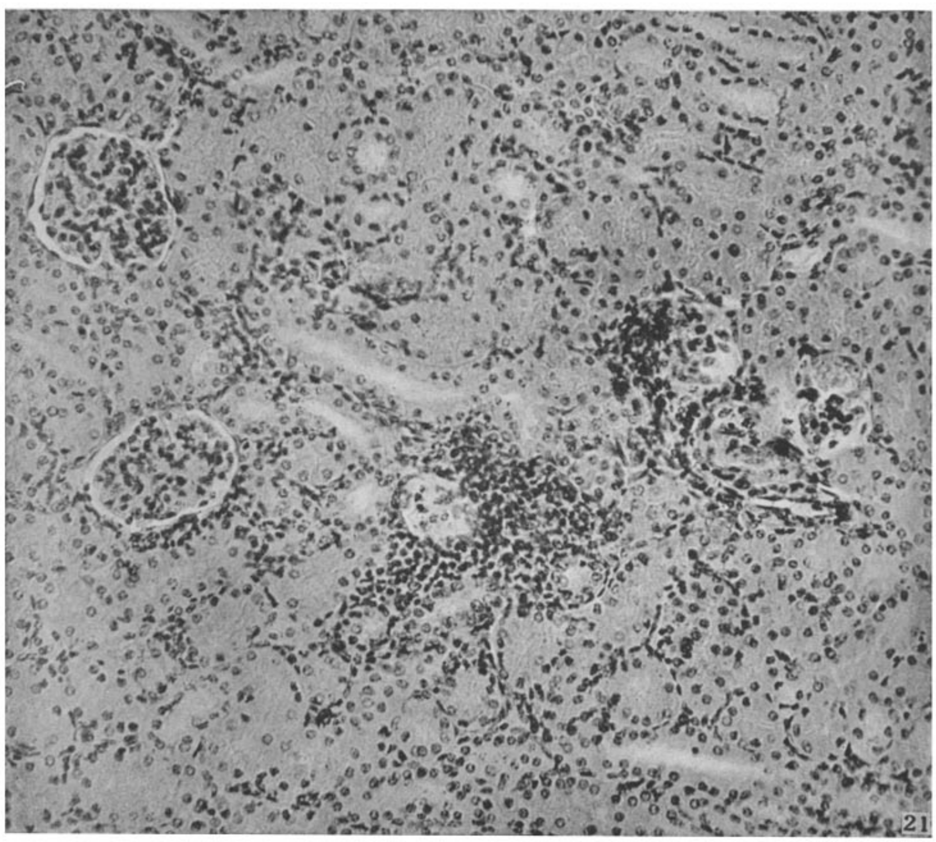
18



19

(Pearce: Klosiella Infection of the Guinea Pig.)





(Pearce: Klosiella Infection of the Guinea Pig.)