PSITTACOSIS

IV. Experimentally Induced Infections in Monkeys

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Plates 12 to 17

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In the three papers immediately preceding this one, psittacosis experimentally induced in parrots, rabbits, guinea pigs, and mice was described. None of the animals employed in the work detailed, however, evidenced pulmonary signs or lesions of any significance. Inasmuch as psittacosis in man manifests itself chiefly by pathological changes in the lungs, and since no one had employed monkeys for the experimental study of the malady, we decided to determine whether it is possible to produce in certain lower primates pulmonary lesions similar to those found in human beings infected with the virus of psittacosis. In this study, 6 experiments were performed in which 12 monkeys were inoculated one or more times.

Methods and Materials

Virus.—The psittacosis virus was obtained from the livers and spleens of Wenz C mice (see second paper of this series) carrying a human strain of the active agent. The emulsions containing the virus for each experiment were shown to be free from ordinary aerobic and anaerobic bacteria.

Animals.—Healthy medium sized Indian monkeys (*Macacus rhesus*) proved to be satisfactory for the work. Only animals whose lungs were shown by X-ray examination to be normal were used.

*Inoculations.**—The majority of monkeys was infected by intratracheal inoculations (1–4 cc.) accomplished by thrusting a small needle into the trachea below the larynx and then injecting the emulsion with a syringe. 2 monkeys were inoculated intracerebrally (1 cc.) through a small trephined opening in the skull. 2 monkeys received the infectious agent by intranasal instillations (1–2 cc.) of organ emulsions.

* All operations were performed under ether anesthesia.

EXPERIMENTAL

The objects of the first experiment were to determine whether monkeys are susceptible to the virus of psittacosis injected intratracheally or intracerebrally, and to ascertain whether the virus can be propagated by serial passages in these animals.

Experiment I

Monkey A, May 20: Temp. before inoculation not taken. Received intratracheally 2.5 cc. of liver and spleen emulsion from mice WC_{17}. *May 22*, temp. 103.8°, appears normal. *May 23*, temp. 105.4°, condition same. *May 24*, temp. 104.8°. *May 25*, temp. 104°. *May 26*, temp. 105°, seems sick and eating poorly. X-ray of chest shows a shadow extending to left of heart and mottling of both lower lobes behind the diaphragm. *May 27*, temp. 103.4°, still appears sick. *May 28*, temp. 102.6°, condition improved. X-ray of chest reveals an extension of the shadows noted on the 26th. Animal killed with chloroform. *Autopsy*: Heart, pericardium, liver, spleen, and kidneys appear normal. Lungs: No pleurisy. Both lower lobes are partially consolidated and have taken on a lilac-pink color. The other lobes on the right are involved in a patchy manner. The consolidated lobes on section appear homogeneous, smooth, free of edema. The bronchi are not raised above the cut surface and contain no exudate. The hilar lymph glands are enlarged and contain a few small hemorrhages. Smears from the lungs showed no ordinary bacteria, and none of the "minute bodies" found in parrots and mice infected with psittacosis virus. Cultures, aerobic and anaerobic, of the lungs and liver remained sterile. Pieces of involved lung were emulsified and injected into 4 mice intraperitoneally and into Monkey E intratracheally. 2 of the mice died 6 days after inoculation and showed typical psittacosis lesions in the liver and spleen. Numerous "minute bodies" were found in smears from these mouse organs. The other 2 mice were sick for a number of days and finally died of psittacosis.

Monkey E, May 29: Temp. before inoculation, 101.8°. X-ray of chest negative. Received intratracheally 4 cc. of lung emulsion from Monkey A. *May 30*, temp. 102°, animal well. *May 31*, temp. 104°, seems sick. *June 1*, temp. 105°, sick. *June 2*, temp. 103.6°, sick and has diarrhea. X-ray of chest shows no obvious lesions. *June 3*, temp. 103°. *June 4*, temp. 105.8°, diarrhea persists. X-ray of chest negative. *June 5*, temp. 104.4°. *June 6*, temp. 102.5°, animal still has diarrhea. X-ray of chest negative. Killed with chloroform and autopsied immediately. Pericardium contains 1 cc. of sticky greyish exudate; no "minute bodies" or bacteria found in smears; cultures sterile. Right lung bound down by fresh fibrinous adhesions. 3 upper lobes normal. Right lower lobe shows scattered subpleural hemorrhages. On section several small greyish nodules noted in the parenchyma. Hilar lymph nodes enlarged and hemorrhagic. Liver has a small yellowish area near the point where the round ligament emerges. Cultures from

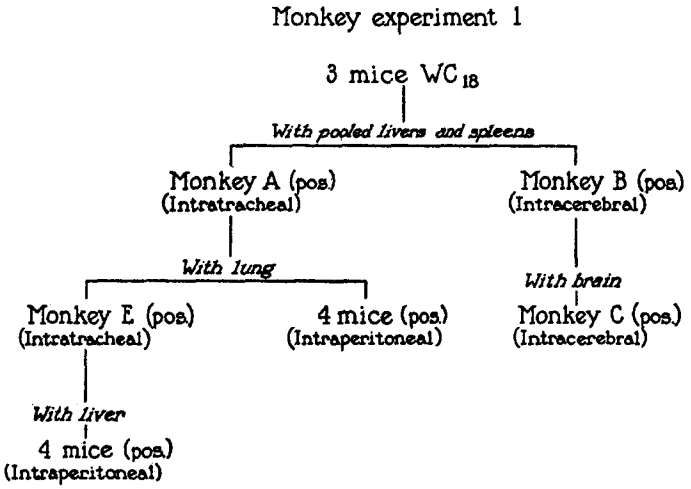
the lungs and liver sterile; no "minute bodies" found in smears from these organs. An emulsion from the liver was injected into 4 mice, 2 of which died 9 days later. The other 2 died 17 and 30 days respectively after inoculation. All the mice had psittacosis.

Monkey B, May 20: Received in the brain 0.75 cc. of an emulsion similar to that given Monkey A. *May 22*, temp. 104°, animal sick, weak, and has diarrhea. Was observed during a convulsion. *May 23*, temp. 104.5°, weak, ataxic; severe diarrhea. *May 24*, temp. 105°, condition worse. *May 25*, temp. 103.8°. Animal weaker and more ataxic. *May 26*, temp. 99°. Unconscious and having repeated convulsive seizures. Killed with chloroform and autopsied immediately. Lungs, liver, and spleen are negative. Brain injected and edematous. Cultures from brain and other organs sterile. Smears negative for the "minute bodies." Stained sections of the brain showed a mild encephalitis with some degeneration of nerve cells. The predominant lesion was a meningeal reaction characterized by mononuclear infiltration.

Monkey C, May 28: Received intracerebrally 0.75 cc. of a brain emulsion from Monkey B. *May 29*, temp. 102.8°. Animal well. *May 30*, temp. 102.6°. *May 31*, temp. 103°. *June 1*, temp. 102.6°. Animal still seems well. *June 2*, temp. 105°. *June 3–7*, temp. 104° or above. Animal definitely sick. X-ray of chest taken on June 4 negative. *June 8–14*, temp. 102–102.6°. Monkey appears normal again.

The results of Experiment I detailed in the protocols above and portrayed in Text-fig. 1 indicate that macaques are susceptible to the virus of psittacosis and that the virus can be passed from monkey to monkey by intratracheal or by intracerebral inoculations. There are several points of interest, however, that should be noted. The reactions in the first monkeys of the 2 series were much more severe than were those in the second lot of animals. Moreover, mice inoculated with emulsions of Monkey A's lung and Monkey E's liver developed psittacosis, but they died more slowly than did mice inoculated with mouse passage virus. These facts suggest the possibility that passage of the virus through monkeys alters it in such a manner that transfers from monkey to monkey become relatively difficult. Similar conditions may hold for human beings who seem to be quite susceptible to the virus emanating from parrots, but relatively non-susceptible when exposed to the disease in man. Attention should also be directed to the fact that monkeys receiving the virus intracerebrally developed no pulmonary lesions. Thus, it appears that the portal of entry of the virus profoundly influences its localization and the pathological changes

caused by it in these animals. This fact lends evidence in favor of the idea that the active agent enters man, in whom involvement of the lungs usually occurs, through the upper respiratory tract. Finally, the "minute bodies" found in parrots and mice infected with psittacosis virus were not seen in the lungs and brains of monkeys attacked by the same active agent. Yet emulsions of these organs produced psittacosis in mice, and smears from their livers and spleens showed the small bodies. Failure to find these structures in monkeys, however, does not necessarily mean that none were present.



TEXT-FIG. 1. Diagrammatic representation of Experiment I

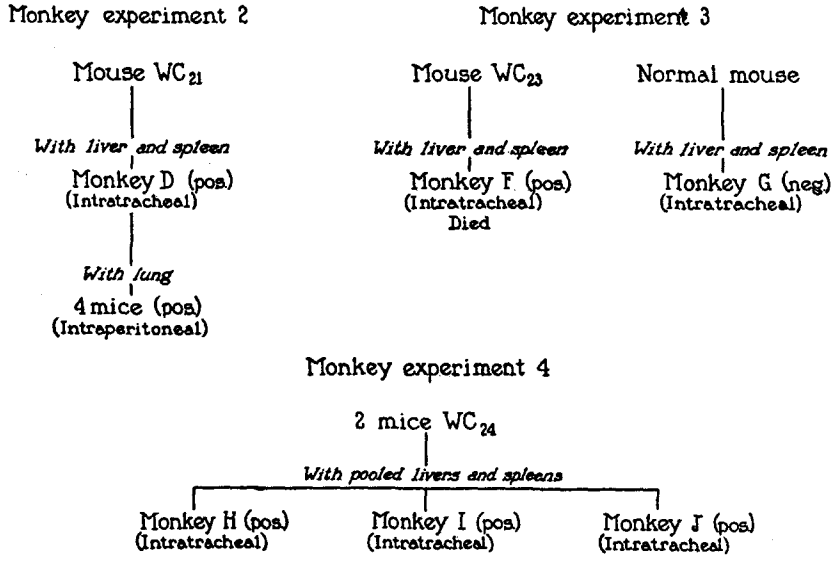
Before proceeding it seemed advisable to repeat part of the first experiment for confirmatory purposes.

Experiment II

Monkey D, May 28: Temp. 102.4°. X-ray of chest negative (Fig. 11). *May 29*, animal received intratracheally 4 cc. of pooled liver and spleen emulsion from mice WC_{21}. *May 30*, temp. 103.8°, seems well. *May 31*, temp. 105°, eating poorly. *June 1*, temp. 104.5°, sick. *June 2*, temp. 104.8°, slight cough. X-ray of chest reveals involvement of a large part of the right lung and of the left lower lobe (Fig. 12). Killed with chloroform and autopsied immediately. No evidence of pleurisy. The 4 lobes of the right lung and the two lower lobes of the left reveal a hemorrhagic, purplish consolidation spreading out from the hilum. On section, the surface is smooth and dry, similar to the cut surface of meat. No exudate in

bronchi. Free, straw-colored fluid in pericardium. Liver, spleen, intestines, adrenals, and kidneys appear normal. In smears from the lungs and pericardial fluid no "minute bodies" were found. Cultures of lungs and liver sterile. 4 mice were inoculated (0.5 cc. each) intraperitoneally with a lung emulsion from Monkey D. All died of psittacosis; 1 on the 3rd, 1 on the 6th, 1 on the 15th, and 1 on the 18th day respectively after inoculation.

The results of Experiment II, shown in Text-fig. 2, confirm those obtained in the first experiment. Having determined that liver and spleen emulsions from mice infected with psittacosis cause a consolida-



TEXT-FIG. 2. Diagrammatic representation of Experiments II, III, and IV

tion of the lungs, we decided to see what effect similar emulsions from normal mice would have. This was undertaken in Experiment III.

Experiment III

Monkey F received intratracheally 2.5 cc. of an emulsion of pooled livers and spleens from psittacosis mice WC_{23}, while Monkey G received in a similar manner 2.5 cc. of an emulsion of pooled livers and spleens from normal mice.

Monkey F, June 2: Temp. 101.6°. X-ray of chest negative. *June 3*, date of inoculation. *June 4*, temp. 106.2°, seems well. *June 5*, temp. 105.2°, stools loose. *June 6*, temp. 104.6°, sick, has cough. X-ray of chest shows extensive shadows in both lower lobes behind the dome of the diaphragm and also in the

right middle and upper lobes. *June 7*, temp. 104°, considerable difficulty in breathing. X-ray indicates that the middle lobe on the left side is also involved. *June 8*, temp. 104.8°, very sick, does not eat, has difficulty in breathing, stools loose. *June 9*, temp. 102.2°. X-ray shows large shadows throughout the right side with extension of the pneumonia in left lower and middle lobes; the left upper only remains clear. Shortly after the X-ray was taken the monkey died and was autopsied immediately. Lungs: No pleurisy; many subpleural hemorrhages. Right: The upper lobe with the exception of the edges is completely consolidated. The two middle lobes are less completely involved, the edges being quite free. The lower lobe is almost completely consolidated. Left: Upper and middle lobes are only slightly involved. Lower lobe is almost completely consolidated, edges alone remaining free. On section the consolidated lobes do not have the appearance usually seen in ordinary lobar pneumonia. They are not granular, have a meaty appearance with slight peribronchial areas of pallor. No exudate is noted in the bronchi. Heart: 3 or 4 cc. of clear straw-colored fluid in pericardium. Liver: Enlarged, edges rounded. Near where the round ligament emerges there appears to be a small infarct. The liver has a peculiar appearance suggesting widespread fatty degeneration with areas of necrosis. Spleen: Pulp very soft. Adrenals and kidneys appear normal. Cultures of the lungs, liver, and pericardial fluid sterile. Smears for the "minute bodies" negative.

Monkey G was treated in a manner similar to that in which F was handled, with the exception that an emulsion of normal spleens and livers was used as an inoculum. The animal evidenced no signs of illness, never had any fever, and 4 X-rays of the chest revealed no areas of consolidation.

The results of the above experiment (Text-fig. 2) indicate that an emulsion of livers and spleens from normal mice introduced intratracheally does not produce a pneumonia in monkeys. In the next experiment, No. IV, 3 monkeys received intratracheally psittacosis virus in an emulsion (2 cc. to each animal) of pooled livers and spleens from mice WC_{24}. The animals, having been watched and examined frequently by means of the X-ray were killed 2, 6, and 13 days respectively after inoculation. In this way lungs were obtained during different stages of the disease in order to study the evolution of the pathological process.

Experiment IV

Monkey H, *June 3*: Temp. 103.5°. X-ray of chest negative. *June 5*, temp. 103.2°. *June 6*, temp. 102.4°. *June 7*, date of intratracheal inoculation. *June 8*, temp. 105.3°, diarrhea. *June 9*, temp. 104.8°, diarrhea persists. X-ray of chest shows extensive mottling of right lower lobe with smaller shadows in the left lower. There is evidence of spreading of the infection along the bronchial tree into the

upper and middle lobes (Fig. 1). Animal killed with chloroform and autopsied immediately. Pericardium, heart, spleen, kidneys, and adrenals seem to be normal. A small whitish area in the edge of one lobe of liver near the round ligament. Lungs show lesions spreading out from the hilum along the bronchial tree into all the lobes. The involved portions are purplish pink, only partially consolidated, but sharply demarcated from the surrounding normal lung tissue. Cultures of the lungs sterile.

Monkey I, June 3: Temp. 102.5°. X-ray of chest negative (Fig. 5). *June 5*, temp. 103°. *June 6*, temp. 102.4°. *June 7*, date of intratracheal inoculation. *June 8*, temp. 105°, eating poorly. *June 9*, temp. 103.5°, sick and weak. X-ray shows small shadows in both lower lobes near the hilum (Fig. 6). *June 10*, temp. 103.2°, condition unchanged. X-ray: Shadows in both lower lobes have extended toward the periphery. The 2 middle lobes and possibly the upper on the right side are consolidated near the hilum. *June 11*, temp. 104.4°, condition worse. X-ray: The areas of consolidation have increased in size and the shadows are more intense. *June 12*, temp. 104.8°, condition worse. X-ray: Shadows indicate that the areas of consolidation have almost reached the periphery (Fig. 7). *June 13*, temp. 103.5°. X-ray shows no change. *June 14*, temp. 105°, still quite sick and has lost weight. *June 15*, temp. 104.2°. *June 16*, temp. 103.4°, better and eating a small amount of food. X-ray: Some clearing of the shadows at the periphery of the lesions (Fig. 8). *June 18*, temp. 100.8°, much better. X-ray: Marked clearing of the shadows (Fig. 9). *June 20*, temp. 102.4°, animal seems almost normal again. X-ray: A few irregular shadows persist near the hilum (Fig. 10). Monkey killed and autopsied immediately. Heart and pericardium normal. Both pleural cavities clear. Lungs: The upper and 2 middle lobes on the right bound to each other by loose adhesions. Only slight changes near the hilum are noted in the upper and one of the middle lobes, while in the other middle and lower lobes quite firm areas are still detected near the hilum around the vessels and bronchi. On the left side, the upper lobe is normal, while near the hilum areas of consolidation in the middle and lower lobes still exist. These are less extensive than are those in the right lower. On section the involved lobes exhibit air-containing alveoli around the periphery, while in the central portions along the bronchi and vessels a yellowish gray semiconsolidated tissue, dry and relatively smooth appearing, is found. Hilar lymph glands not greatly enlarged. Liver shows fatty degeneration. Kidneys and spleen normal in appearance. Hemorrhage in medulla of right adrenal gland. Cultures of lungs: One remained sterile, the other showed a few indifferent streptococci.

Monkey J, June 5: Temp. 104.3°. X-ray of chest negative. *June 7*, date of intratracheal inoculation. *June 8*, temp. 105°, seems well. *June 9*, temp. 104.5°, condition unchanged. X-ray: Shadows in both lower lobes near the hilum. *June 10*, temp. 104.2°, sick, weak, not eating. X-ray: All lobes on the right seem to be involved near the hilum; extension of shadow in left lower lobe. *June 11*, temp. 105.6°, worse. X-ray: Increase of shadows on both sides, but left upper and

middle lobes still relatively clear. *June 12*, temp. 104.6°, condition same. X-ray: No change in shadows. *June 13*, temp. 103.2°, animal still very sick and has difficulty in breathing. X-ray: The right lung is almost completely consolidated, the left upper and middle lobes remain relatively clear (Fig. 4). Animal killed with chloroform and autopsied immediately. The lobes on the right side, bound to each other and to the chest wall by a sticky mucoid fibrinous exudate, are completely consolidated with the exception of the peripheral portions. Slight amount of pleurisy on the left side with extensive consolidation of the lower lobe; the other 2 lobes relatively free. On section the color and consistency of the lungs similar to that described in other monkeys. Hilar lymph glands enlarged. Heart enlarged. In the pericardial sac are 1 or 2 cc. of sticky, whitish exudate. Liver shows a small area of necrosis near the round ligament. The intestines, spleen, kidneys, adrenal glands, and brain appear normal. In the different exudate and organs, no bacteria or "minute bodies" were found, and cultures from them remained sterile.

In Experiment IV, by means of X-ray examinations that were confirmed by autopsy findings, it was possible to follow the spread of the consolidation from the hilum towards the periphery and to see it regress in the reverse order. The course of events, shown by this set of photographs to occur in monkeys infected with psittacosis virus, parallels that observed in the lungs of man infected with the same active agent (1). Having demonstrated conclusively that a pneumonia in monkeys regularly follows the intratracheal inoculation of psittacosis virus, we considered it essential to ascertain, Experiment V, whether a pulmonary infection occurs when the inciting agent is instilled intranasally.

Experiment V

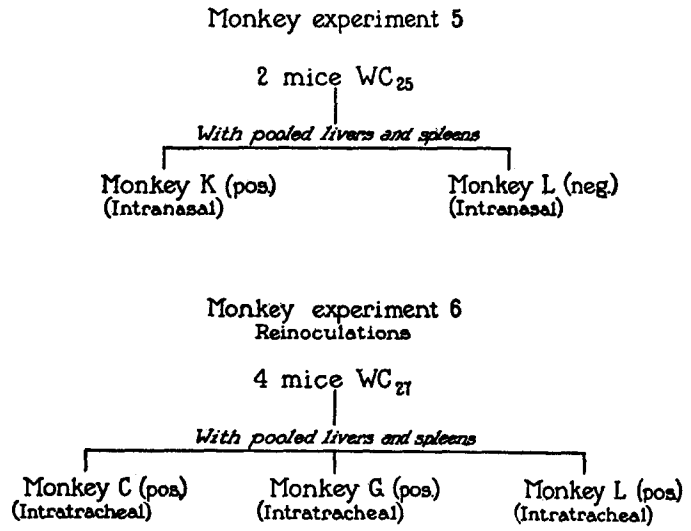
Into the noses of Monkeys K and L respectively, were instilled 1.5 cc. of an emulsion of pooled livers and spleens from psittacosis mice WC_{25}.

Monkey K, *June 10*: Temp. 101.6°. X-ray of chest negative (Fig. 2). *June 11*, date of intranasal inoculation. *June 12*, temp. 102.8°. *June 13*, temp. 105.4°, animal well. X-ray: Chest still clear. *June 14*, temp. 105°. X-ray: Beginning shadow at hilum on the right side, and slight mottling of right lower lobe. *June 15*, temp. 105.6°, eating poorly, slight diarrhea. *June 16*, temp. 105°, less diarrhea. X-ray: Shadow at hilum has increased and is spreading in a manner similar to that observed in man (Fig. 3). *June 17*, temp. 104.2°, animal does not appear very sick. X-ray shadows more intense. *June 18*, temp. 104.6°, condition same. X-ray shadows unchanged. Animal killed with chloroform and autopsied immediately. All organs appear normal with the exception of the right lung. In the lower portion of the upper lobe and at the top of the lower lobe are areas of

consolidation surrounding the main bronchi. On section, the surface of the consolidated tissue is dry, gray, and smooth. Cultures sterile.

Monkey L received an inoculum similar to that of Monkey K, but at no time did it have fever, or pulmonary consolidation as evidenced by repeated X-ray photographs.

Of the 2 monkeys receiving intranasal inoculations of psittacosis virus as described in the above experiment, 1 developed a typical pneumonia with fever while the other showed no signs of infection. The fact that only 1 animal became sick after this type of inoculation



TEXT-FIG. 3. Diagrammatic representation of Experiments V and VI

indicates that intranasal inoculations with psittacosis virus will not infect monkeys as regularly as do intratracheal injections. Nevertheless, it shows that, when the virus does enter in such a manner a pneumonia similar to that seen in man occurs. Thus, experimental evidence has been adduced in favor of the idea that man usually becomes infected through the upper respiratory tract.

The last experiment (No. VI, see Text-fig. 3), in which monkeys were used, consisted of reinoculations of Monkeys C, G, and L. Monkey C had recovered from an intracerebral inoculation administered 26 days previously; Monkey G had been given an intratracheal injection

of an emulsion of livers and spleens from normal mice; Monkey L had had virus instilled in the nose 12 days previously without any evidence of infection. Each animal was reinoculated intratracheally with 1 cc. of mouse virus, and all responded with a moderately severe pneumonia that was verified by X-ray examinations and autopsies. Monkey C was the only animal in the group that had previously evidenced signs of infection due to psittacosis virus. Consequently, it was the only one that might have been expected to show an immunity to reinfection. It did not, however. Whether this lack of immunity was due to the type of the first infection (intracerebral), whether 26 days between the first and second inoculations was too short a period of time for a demonstrable immunity to appear, or whether monkeys do not develop a solid immunity against psittacosis virus are questions that cannot be answered at present.

Microscopic Pathology

In the three preceding papers the pathological changes induced by psittacosis virus in parrots, mice, rabbits, and guinea pigs were described. Significant lesions, however, were not found in the lungs of these animals. Moreover, pulmonary lesions experimentally induced by the action of the virus in such hosts have been described by only a few workers, and in each instance the picture presented leaves one in doubt as to whether or not the morbid changes were caused by the active agent under discussion. In the present communication, the clinical and gross pathological pictures of psittacosis pneumonia in monkeys have been recorded. It now remains to describe the microscopic pathology of the pulmonary changes. Inasmuch as the animals were sacrificed after different intervals of time (2–13 days) had elapsed following inoculation, an excellent opportunity for a study of all stages of the morbid conditions in the lungs was presented. Therefore, the monkeys will be discussed in the order in which they were killed. There is, however, at least one fallacy in this mode of presentation, *viz.*, after an infection in the lungs has been under way for several days, early and advanced lesions may be found in the same animal. This fact must be borne in mind.

Two Days after Inoculation.—Monkey H. Examination of sections of whole lobes reveals that the consolidation first appears around the vessels and bronchi

near the hilum. Extending from the small areas of consolidation the walls of the alveoli (Fig. 15) are thickened and edematous, and show evidence of cellular infiltration. Study of many such sections leads one to suspect that the infection spreads along the alveolar walls. In many areas these structures are engorged with blood and contain a few polymorphonuclear cells (Fig. 16). In other places they are thickened by the swelling of alveolar cells and the infiltration of mononuclear elements (Fig. 17). Serous exudate (Fig. 16), fibrin, and desquamated cells are seen in many alveoli, while in definitely consolidated areas some of the alveolar spaces are filled with cells predominantly of the polymorphonuclear type (Fig. 18).

Four Days after Inoculation.—Monkey D. Sections of entire lobes again show that the consolidation begins near the hilum and spreads peripherally. In Fig. 20, the alveoli near the pleura are distended with serous exudate in which are embedded a few cells. In more centrally located areas, the number of cells in the exudate increases, and the alveolar walls are thickened (Fig. 21). As the hilum is approached, completely consolidated lung is encountered, in which some of the alveolar walls appear necrotic and the alveolar spaces are filled with a mixture of polymorphonuclear and large mononuclear cells (Fig. 22), while other areas reveal thickened alveolar walls and alveoli distended entirely by mononuclear cells (Fig. 24). Small hemorrhages and masses of fibrin are scattered throughout the involved portions of the lung. The origin of the mononuclear cells—there seems to be more than one kind present (Fig. 23)—in the exudate is not definitely known.

Six Days after Inoculation.—Monkeys F and J. The pictures presented by these monkeys are similar to those found in sections of Monkey D. Some differences, however, may be noted, for example, the infrequency of polymorphonuclear cells, and the appearance in the alveolar walls in certain places of giant cells and mitotic figures.

Seven Days after Inoculation.—Monkeys K and L. At this stage of the infection, practically no polymorphonuclear cells are found, hemorrhages are rarely seen, fibrin and serous exudates are still present in places, and a distinct perivascular cuffing with mononuclear cells, many of which are distended with pigmented granules, has made its appearance. This phenomenon of cuffing is noted in earlier monkeys but not to such an extent as in F and J and the others to follow. Evidences of cellular proliferation in the alveolar walls are pronounced and in many places it is difficult to distinguish these structures from the exudate or cells in the alveolar spaces (Fig. 19—Monkey K inoculated intranasally).

Eight Days after Inoculation.—Monkey A. This animal was killed on the 8th day after inoculation, the 1st day that an improvement in its condition was noted. An attempt to describe the microscopic pathology observed in the lungs will not be made, since no description can equal the pictures presented in Figs. 25, 26, and 27, an examination of which will show the condition of the alveolar walls and the contents of the alveolar spaces, and justify the conclusion that the pathological changes present are not those usually encountered in lobar pneumonia of man and monkeys.

Ten and Twelve Days after Inoculation.—Monkeys C and G. Clinically these animals were recovering from their pneumonia. Examination of the sections reveal evidences of resolution that will be discussed in connection with the next animal (Monkey I).

Thirteen Days after Inoculation.—Monkey I. This animal was killed 13 days after inoculation, 4 days after it began to show clinical evidences of improvement, and at a time when the X-ray photographs had almost returned to normal (Figs. 5–10). Grossly the lungs reveal more evidences of consolidation than had been expected from the X-ray pictures. Examinations of stained sections of whole lobes confirm the macroscopic pathology. Areas of dense tissue (Fig. 28) in which it is difficult to distinguish alveolar walls from alveolar contents are seen. Such areas are separated from each other by large air-containing spaces or distended alveoli (Fig. 29). A superficial examination of sections stained with eosin methylene blue suggests that a great deal of organization is taking place; but by the use of Mallory's aniline blue and orange G stain (Figs. 29 and 30) it becomes evident that only a slight amount of connective tissue is being laid down and that the alveoli are filled with large mononuclear cells. It is also obvious that resolution is occurring from the periphery towards the hilum. Consequently, the most marked involvement of the tissues is still found around the large bronchi and vessels near the hilum. Perivascular cuffing, in which lymphocytes are numerous, is conspicuous.

"*Minute Bodies.*"—In stained sections of the consolidated lungs from monkeys, the "minute bodies" found in livers, spleens, etc., of parrots and mice infected with the virus of psittacosis were searched for diligently. None were seen. Failure to find them, however, does not necessarily indicate that they were not present in small numbers. Many cells in the aveoli, alveolar walls, and perivascular cuffs contained granules of different sizes and nature. None of these however, resembled the "minute bodies."

Psittacosis Pneumonia in Rabbits

After we recognized the fact that intracerebral inoculations of the virus do not induce pneumonia in monkeys, while intratracheal and intranasal injections of the same agent do, we decided, in spite of our previous negative results with intracerebral methods, to infect some rabbits intratracheally in order to ascertain whether a pneumonia can be produced in these animals by such a procedure.

2 groups of rabbits were used. One set of animals received intratracheally 1 cc. each of an emulsion of livers and spleens from mice infected with psittacosis virus, the rabbits of the other group were similarly inoculated with an emulsion of livers and spleens from normal mice. The animals that received the infectious material developed fever. Some of them died, while others were sacrificed at different intervals for bacteriological and pathological studies. Cultures of the lungs for the

presence of aerobic and anaerobic bacteria remained sterile. Macroscopically and microscopically the lungs showed a pneumonia similar to that observed in monkeys 2–4 days after intratracheal injections of the virus. The rabbits that received the emulsions of normal livers and spleens had no fever and, when they were sacrificed for examination of their lungs, revealed no pneumonia.

From the results of the above experiment it is obvious that rabbits develop pulmonary lesions following intratracheal injections of psittacosis virus. Experiments reported in the third paper of this series, however, show conclusively that such lesions do not occur, or only rarely, when the infectious agent is placed intracerebrally.

DISCUSSION

The evidence—clinical findings, X-ray photographs, gross and microscopic pathology, and bacteriological observations—justifies the conclusion that emulsions of livers and spleens from psittacosis-infected mice injected intratracheally or instilled intranasally in monkeys are capable of producing a pneumonia unassociated with ordinary bacteria. Moreover, such a pneumonia is similar to, if not entirely identical with that observed in man (1–4) infected with the virus of psittacosis. Furthermore, the experimental study of the disease in monkeys offers opportunities for observation that cannot be made in man, because the animals can be sacrificed at any time during the course of the malady and in them pictures uncomplicated by secondary bacterial invasion are the rule rather than the exception.

The pneumonia occurring in monkeys under experimental conditions begins around the large bronchi and vessels near the hilum and apparently spreads towards the periphery along the alveolar walls. Resolution occurs in the reverse order. The pleura is rarely involved. Many of the evidences of pulmonary involvement—vascular engorgement, cellular infiltration, necrosis of alveolar walls, hemorrhage, serous exudation, fibrin deposition, desquamation of alveolar epithelium, distention of alveoli by polymorphonuclear and mononuclear cells—observed in the psittacosis-infected lungs have been described in other infectious processes of this organ. Yet the peculiar combination of these pathological processes in lungs infected with psittacosis virus immediately distinguishes the reaction from ordinary pneumonias and from ordinary bacterial infections of the lungs.

From the evidence presented in the three preceding papers it is obvious (1) that parrots, regardless of the portal of entry of the inciting agent, rarely, if ever, develop a pneumonia as a result of an infection with the virus of psittacosis, (2) that rabbits and guinea pigs do not have an involvement of the lungs when inoculated intracerebrally, intradermally, or intraperitoneally, (3) that mice inoculated intracerebrally or intraperitoneally remain free from pulmonary lesions. Furthermore, in the present paper it has been shown that monkeys receiving the virus in the brain, although they become infected, develop no pneumonia. Opposed to such observations are those whereby it has been demonstrated that rabbits and monkeys, the former inoculated intratracheally, the latter infected either intratracheally or intranasally, exhibit pulmonary lesions. From these observations one is justified in surmising that psittacosis pneumonia in man probably results from the entry of the virus through the upper respiratory tract. Moreover, the results of the work reported in the first and second papers of this series indicate that the source of the virus in parrots and in man is the nasal and oral secretions and feces of the former and sputum of the latter. Thus in the case of man, a fair conception concerning the source of the infection and the portal of entry of the virus has been obtained. Consequently the precautions necessary for prevention of the infection are obvious. Despite them it is difficult, nevertheless, to prevent the spread of the disease from parrots to human beings, as witnessed by laboratory infections occurring under good conditions. Spread of the malady from man to man occurs infrequently, however. Certain possible reasons, such as amount of inoculum encountered or alteration in the virulence of the virus in the human host, may account for the apparent difference in the contagiousness of the disease.

CONCLUSIONS

The virus of psittacosis inoculated intratracheally or intranasally in monkeys produces a pneumonia similar to that caused by the same active agent in man.

Intracerebral inoculation of the virus induces a meningo-encephalitis characterized principally by a mononuclear reaction in the meninges.

Indirect evidence has been adduced to show that the portal of entry of the virus in man is the upper respiratory tract.

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EXPLANATION OF PLATES

PLATE 12

FIG. 1. Monkey H, 2 days postinoculation. Roentgenogram of chest showing extensive mottling of right lower lobe with smaller shadows in the left lower. There is also evidence of spreading of the infection along the bronchial tree into the upper and middle lobes.

FIG. 2. Monkey K, roentgenogram of chest before inoculation.

FIG. 3. Monkey K, 5 days postinoculation. Two shadows at hilum on right side.

FIG. 4. Monkey J, 6 days postinoculation. Roentgenogram of chest: The right lung is almost completely involved, the left lower seems to be extensively consolidated, the left upper and middle lobes are relatively clear.

FIG. 5. Monkey I, roentgenogram before inoculation.

FIGS. 6–10. Monkey I, roentgenograms showing the progression and regression of the consolidation. The pictures were taken 2, 5, 9, 11, and 13 days respectively after inoculation. Note rapid clearing within 48 hours, as evidenced by Figs. 9 and 10.

FIG. 11. Monkey D, roentgenogram before inoculation.

FIG. 12. Monkey D, 4 days postinoculation. Roentgenogram of chest showing involvement of a large part of the right lung and of the left lower lobe.

PLATE 13

FIG. 13. Monkey H was sacrificed 2 days after inoculation. The painting shows a beginning pneumonia near the hilum. × 1.

FIG. 14. Monkey F died 6 days after inoculation. The painting reveals extensive pneumonia. Yet the edges of the involved lobes are not completely consolidated. Note the peculiar lilac-pink color. × 1.

PLATE 14

FIG. 15. Monkey H, 2 days postinoculation. Section of lung showing spread of the infection along the alveolar walls. × 125. Eosin and methylene blue.

FIG. 16. Monkey H, 2 days postinoculation. Engorgement of blood vessels, serous exudate in alveoli, and polymorphonuclear leucocytes. × 450. Eosin and methylene blue.

FIG. 17. Monkey H, 2 days postinoculation. In this part of the consolidated lung, polymorphonuclear leucocytes are absent. Compare with Fig. 18. × 450. Eosin and methylene blue.

FIG. 18. Monkey H, 2 days postinoculation. Polymorphonuclear leucocytes are abundant in this portion of the involved lung. Compare with Fig. 17. × 450. Eosin and methylene blue.

FIG. 19. Monkey K, 7 days postinoculation (intranasal). Note the thick alveolar walls and the large mononuclear cells in the alveoli. Polymorphonuclear cells are absent. × 450. Eosin and methylene blue.

PLATE 15

FIG. 20. Monkey D, 4 days postinoculation. Section reveals different stages in the process of consolidation. The portion of the tissue least involved is near the periphery of the lobe. × 125. Eosin and methylene blue.

FIG. 22. Monkey D, 4 days postinoculation. Alveolar walls are necrotic. Fibrin and many polymorphonuclear cells are present. × 450. Eosin and methylene blue.

FIGS. 21, 23, 24. Monkey D, 4 days postinoculation. The alveolar walls are thickened and the alveoli contain desquamated epithelial cells together with other types of mononuclear elements. Very few polymorphonuclear leucocytes are seen. × 450. Eosin and methylene blue.

PLATE 16

FIG. 25. Monkey A, 8 days postinoculation. The alveolar walls are greatly thickened by cellular proliferation and infiltration. The cells lining the alveoli are swollen and some are seen free in the sacs. × 450. Eosin and methylene blue.

FIG. 26. Monkey A, 8 days postinoculation. The alveolar spaces contain various kinds of mononuclear cells. × 450. Eosin and methylene blue.

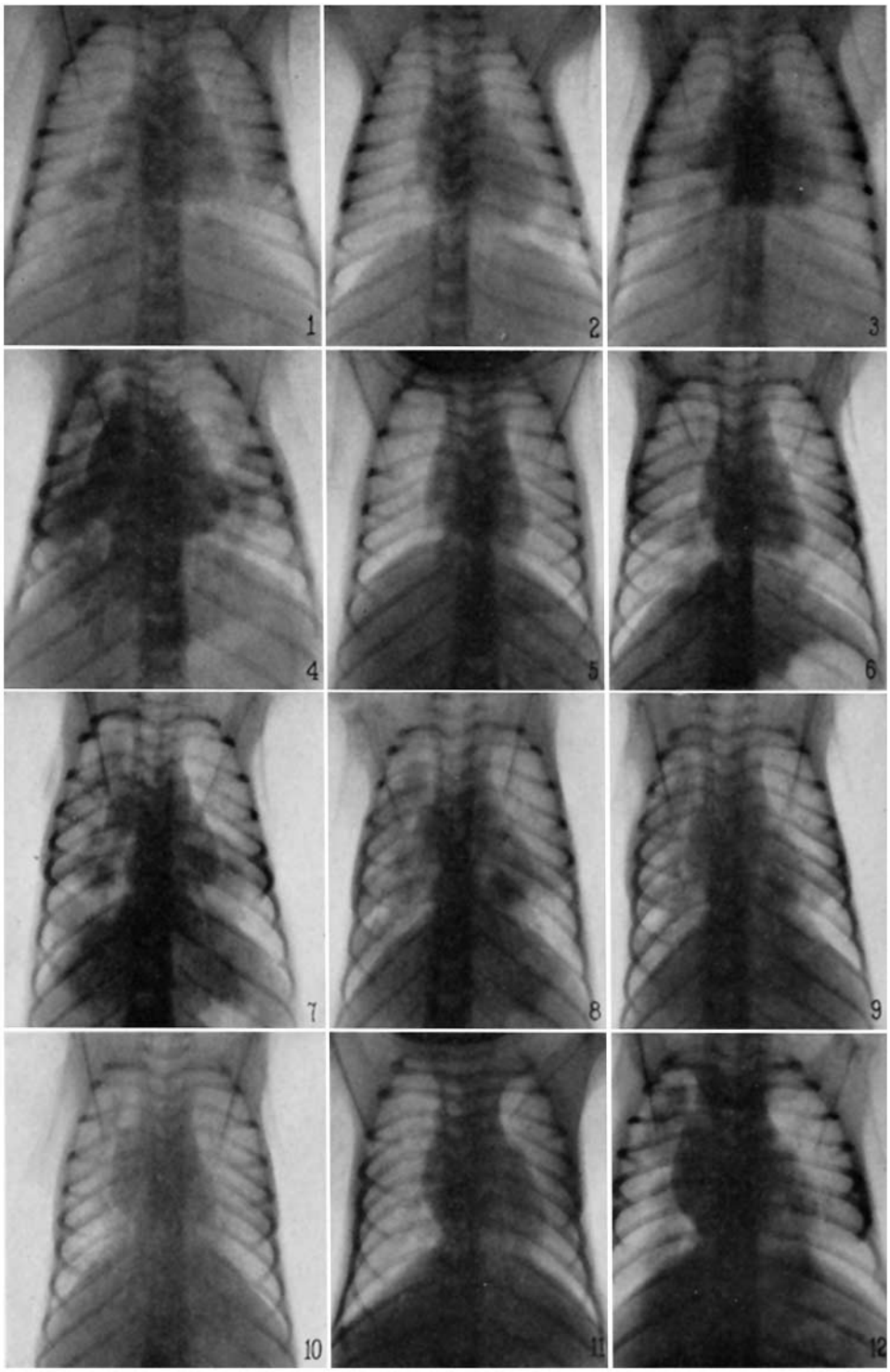
FIG. 27. Monkey A, 8 days postinoculation. It is difficult to distinguish the thickened alveolar walls from the cellular contents of the alveoli. × 450. Eosin and methylene blue.

PLATE 17

FIG. 28. Monkey I, 13 days postinoculation. At the time the monkey was sacrificed, resolution was progressing rapidly. Yet the section seems to indicate that organization had occurred. Compare with Figs. 29 and 30. × 450. Giemsa.

FIG. 29. Monkey I, 13 days postinoculation. Section showing resolving pneumonia. Many alveoli are greatly distended with air, while others still contain exudate. × 125. Aniline blue and orange G.

FIG. 30. Monkey I, 13 days postinoculation. The aniline blue and orange G stain clearly indicates that very little new connective tissue has been laid down. Compare with Figs. 28 and 29. × 450.



(Rivers and Berry: Psittacosis. IV)

13

14

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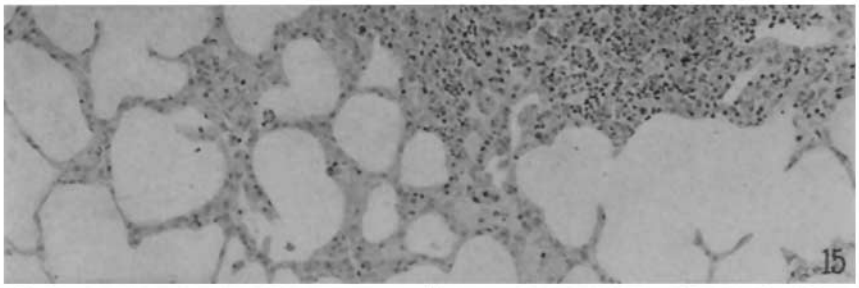
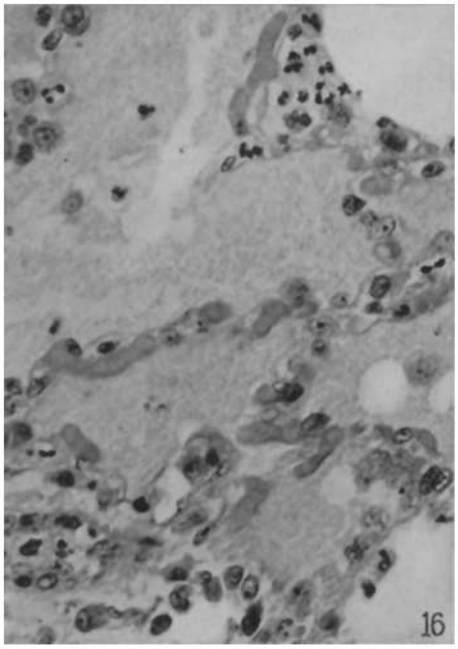
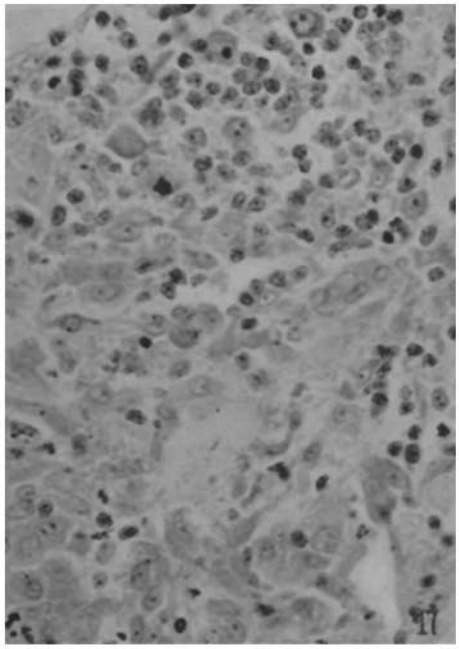
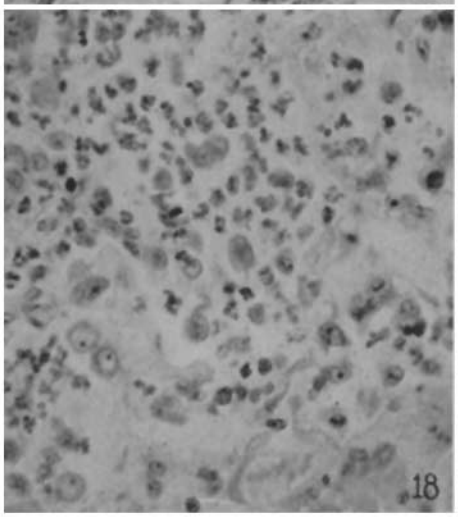
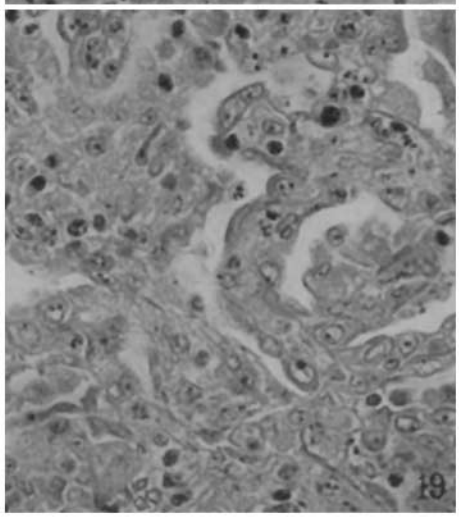
(Rivers and Berry: Psittacosis. IV)





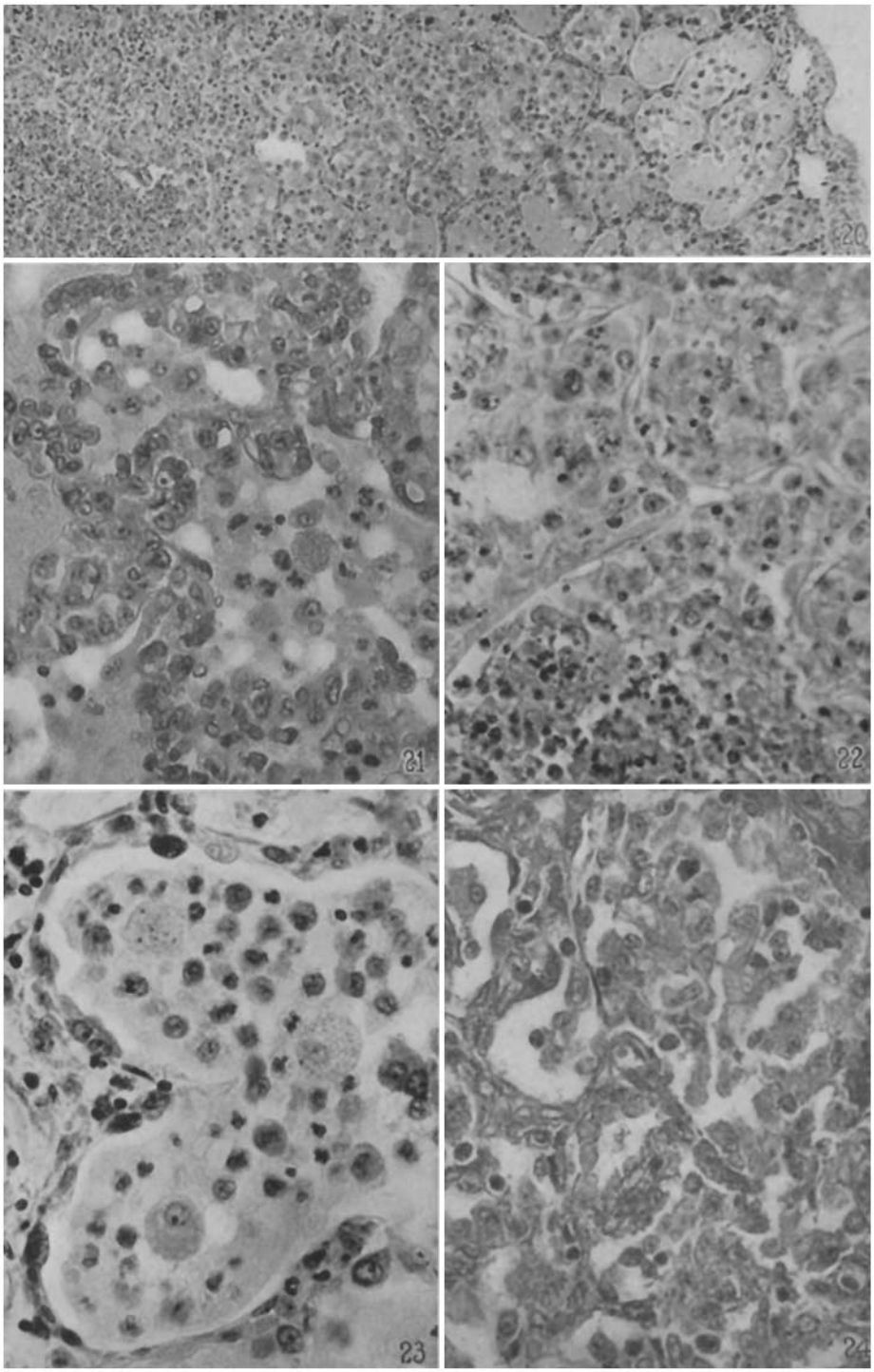






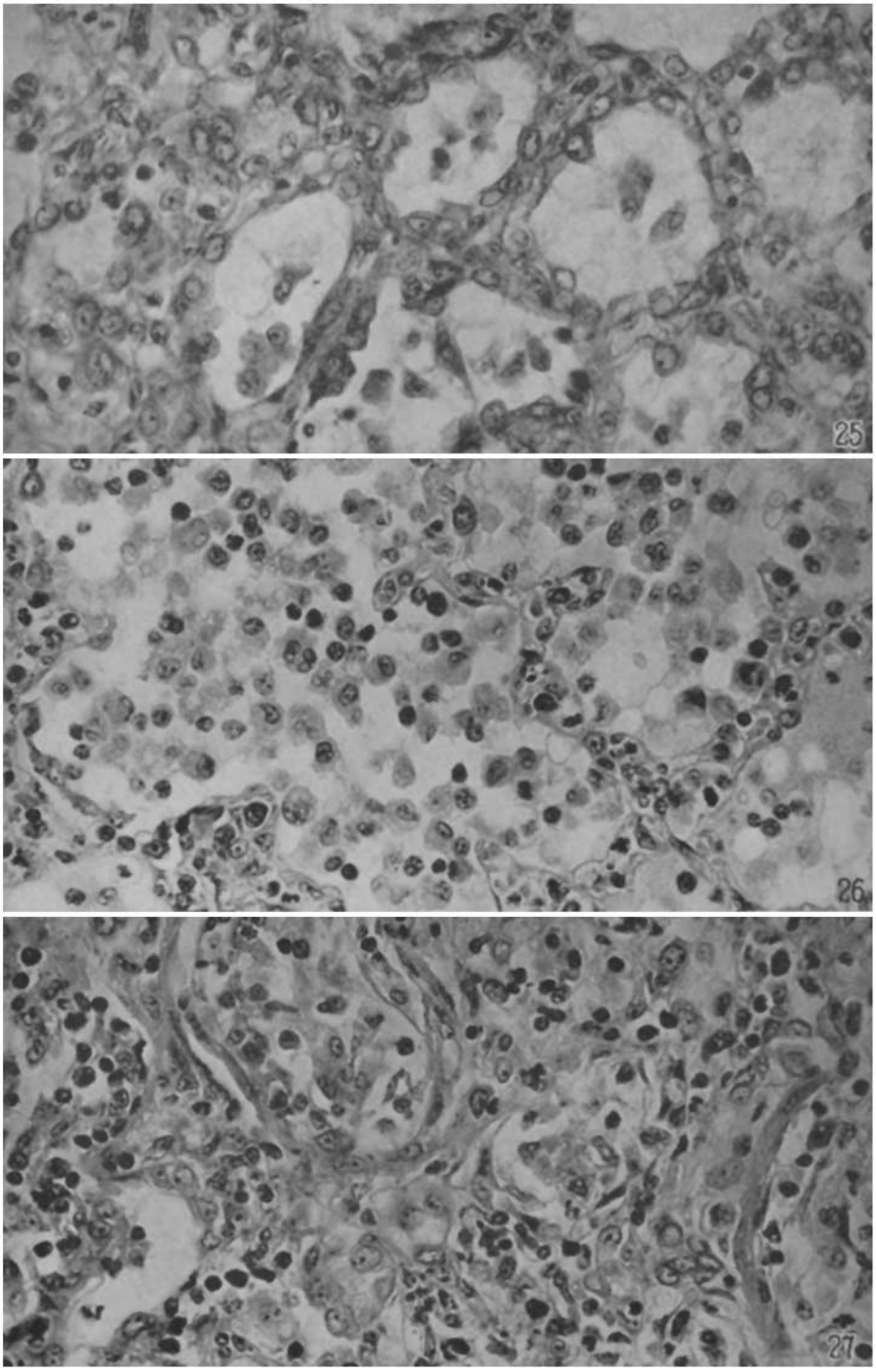
Photographed by Louis Schmidt

(Rivers and Berry: Psittacosis. IV)



Photographed by Louis Schmidt

(Rivers and Berry: Psittacosis. IV)



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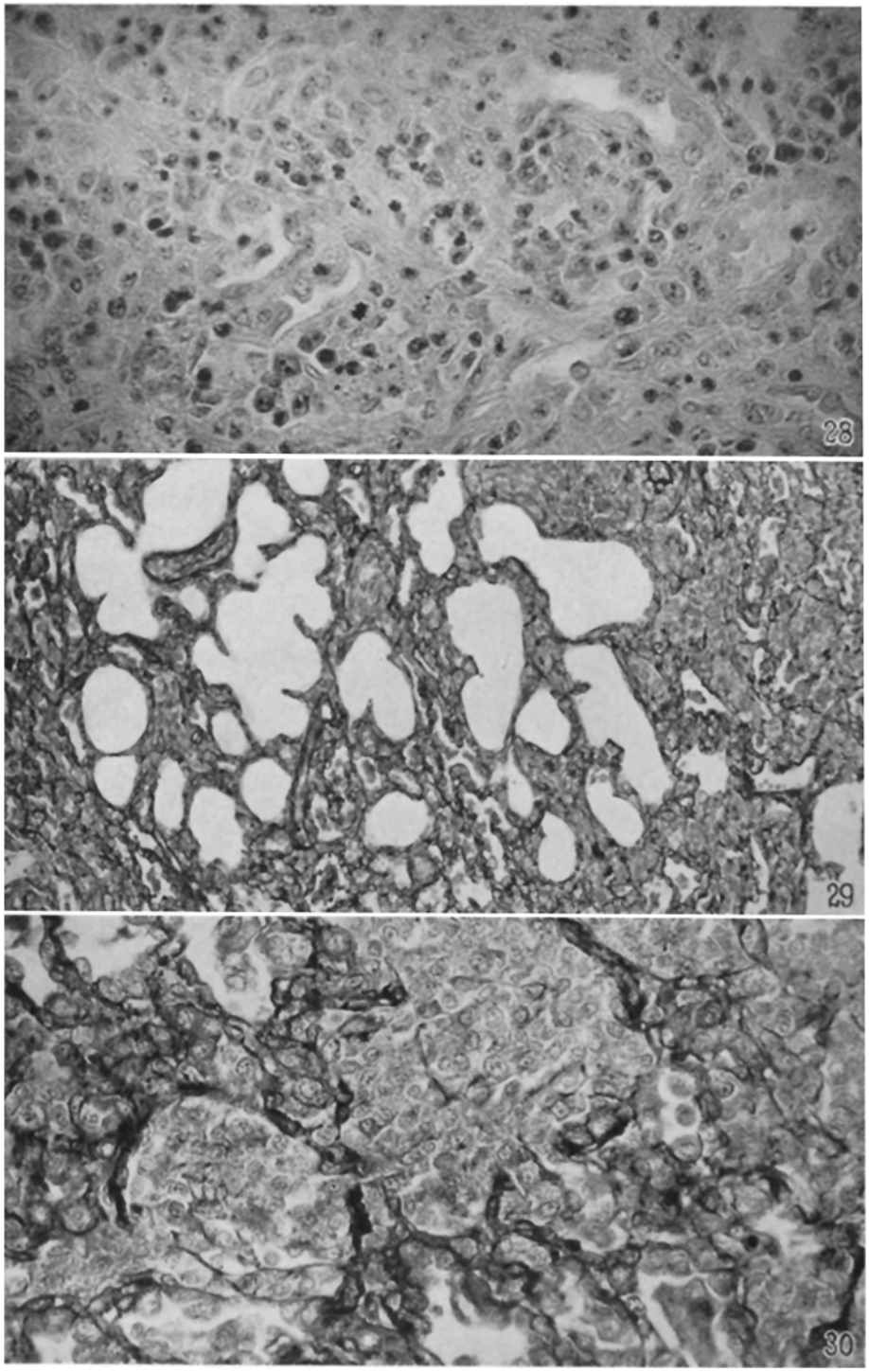

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