

## A VIRUS DISEASE OF PARROTS AND PARRAKEETS DIFFERING FROM PSITTACOSIS

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PLATES 32 TO 34

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Recent widespread epidemics of psittacosis have caused a more general interest in the diseases of cage birds. Special attention has been focused on maladies of the parrot family because these birds are the natural hosts of psittacosis from which man usually contracts that disease. Pacheco, Bier, and Meyer (1-7), while investigating pathological conditions of parrots in an attempt to obtain evidence of the presence of psittacosis in Brazil, discovered a morbid process which was subsequently shown to be induced by a transmissible agent capable of passing through Berkefeld N candles and of producing nuclear inclusions in affected host cells. Infected parrots presented a picture similar to that seen in birds sick of psittacosis. Attempts, however, to transmit the infection to guinea pigs, white mice, chickens, pigeons, and monkeys were unsuccessful. Furthermore, in spite of the fact that no precautionary measures were taken, none of the investigators became ill. Consequently, the conclusion that man is not susceptible seemed warrantable. The investigators speak of the disease as avian psittacosis and state that the strict adaptation of the Brazilian strain of virus to the Psittacidae probably accounts for the absence of human psittacosis from their country.

Dr. Pacheco sent us some of his infectious agent in order that its activities might be compared with those of a virus known to have caused psittacosis in human beings. This comparison has been made and a preliminary note about the results has already appeared (8). The purpose of the present communication is to record a detailed account of the work.

### *Methods and Materials*

*Virus.*—The virus was sent to us in infected parrot liver preserved in glycerol and kept on ice during transportation. Upon arrival the piece of liver was washed several times in sterile Locke's solution and macerated in a mortar. Sufficient Locke's solution was then added to make a 10 per cent emulsion which was used as an inoculum. Subsequent virus emulsions, 10 per cent, were prepared from brains, livers, or spleens of infected birds.

*Animals.*—For passages and for testing the presence or absence of active virus, grass parakeets (*Melopsittacus undulatus*) were employed. Rabbits, guinea pigs, white mice, canaries, chickens, and chick embryos were tested for susceptibility to the active agent.

*Inoculations.*—Inoculations of the birds were accomplished by means of intracerebral or intramuscular injections of the virus emulsions. 0.1 cc. and 0.5 cc. were the respective amounts used. Mice received 0.025 cc. intracerebrally or 0.5 cc. intraperitoneally. Rabbits and guinea pigs were given intracerebrally 0.25 cc. and 0.1 cc. respectively. All operative procedures were made under light ether anesthesia.

*Cultures.*—The presence or absence of ordinary bacteria in infected organs or emulsions was determined by means of aerobic and anaerobic cultures in broth or on blood agar plates.

*Filtration.*—New Berkefeld candles, V, N, and W, were used. All candles were cleaned and tested (air pressure) according to the methods described by Mudd (9). 1 or 2 per cent virus emulsions prepared with broth instead of Locke's solution were used for filtration. Records of the time and pressure required to obtain certain amounts of filtrate were kept. At the time filtrations were made proteus bacilli or diphtheroids were placed in the emulsions as test organisms. Sterility of the filtrate, 1–2 cc., was determined in each instance by means of cultures.

*Preservation of Virus.*—50 per cent glycerol in Locke's solution and freezing followed by desiccation (10) were the methods tested for preservation of the virus.

*Sections.*—Tissues were fixed in Zenker's fluid, sectioned, and stained according to Giemsa's method or by means of eosin and methylene blue.

## EXPERIMENTAL

### *Intramuscular Inoculations*

Upon receipt of the virus it was necessary for us to determine whether it was active and to acquaint ourselves with the clinical and pathological pictures produced by it in a natural host. To this end parakeets were inoculated intramuscularly with an emulsion of the liver sent by Dr. Pacheco and passages from bird to bird were made by means of intramuscular injections of liver emulsions (Text-fig. 1).



*Clinical Picture.*—For 3 or 4 days immediately following inoculation the parakeets appear normal. Then they gradually lose a certain spontaneity of action and prefer to sit quietly on their perch. The feathers are ruffled, the head is drawn down close to the body, and the eyes are closed. If the birds are undisturbed, this position is maintained for hours. In the early stages of the disease, however, they are easily aroused. As the disease progresses the signs of illness become more marked and the birds are aroused with difficulty. A progressive weakness is evident and towards the end of a fatal infection the birds prop themselves against the side of the cage and grasp the wires with their bills in order to remain on the perch. Finally, they lie huddled up on the floor of the cage and usually die in this position. At no time during the illness do the parakeets have a nasal discharge, nor is diarrhea a prominent part of the picture. The birds continue to eat and lose very little weight. As a rule death occurs 6–10 days after inoculation, and usually none of the parakeets that show signs of illness recover.

In individual cases it is difficult to distinguish the clinical picture of this disease from that of psittacosis. In general, however, it seems that nasal discharge, anorexia, diarrhea, and loss of weight are less pronounced in this malady than in psittacosis.

*Microscopic Pathology.*—At times the pectoral muscles near the site of inoculation are swollen, edematous, and hemorrhagic. This change becomes more obvious when the muscles are incised. Uninoculated muscles present no apparent abnormalities. When the body cavity is opened, the liver is seen to be paler than normal and to be studded with whitish spots, the picture of fatty degeneration with focal necrosis. The spleen is not usually enlarged. At times, however, it may be twice the normal size. It is pale and also studded with whitish spots. Macroscopically, the heart, lungs, intestines, kidneys, brain, and bone marrow present nothing of interest.

*Microscopic Pathology. Muscle.*—The pectoral muscles at the site of inoculation may show degenerative changes in the muscle fibers, an increase in the number of nuclei immediately surrounding the muscle fibers, and a marked infiltration of mononuclear cells (Fig. 5). Many of the nuclei contain typical acidophilic inclusions (Fig. 5). Uninoculated skeletal muscles and heart muscle show no obvious pathological changes.

*Liver.*—In many of the livers there is a marked fatty degeneration (Fig. 3), while in others this is not a prominent feature (Fig. 4). The livers of all infected birds contain areas of focal necrosis (Figs. 3 and 4). The amount of necrosis varies and the situation of such focal lesions appears to have no definite relation to the structure of the liver lobules. In and around the areas of necrosis, little or no infiltration of cells occurs. Many affected liver cells contain typical acidophilic intranuclear inclusions (Figs. 3 and 4, inserts).

*Spleen.*—The normal architecture of the spleen disappears (Fig. 1) and areas of necrosis are observed. In some instances the amount of necrosis is marked. Scattered throughout the spleen are numerous cells with acidophilic intranuclear inclusions (Fig. 2). At times it seems that nearly all of the mononuclear elements contain these peculiar bodies.

*Lungs.*—No obvious pathological changes are found in the lungs, yet a few typical nuclear inclusions are seen in cells of this organ. It is impossible to state the type of cell involved.

*Kidneys.*—The kidneys usually show a slight amount of cloudy swelling and occasionally there is an infiltration of mononuclear cells into the interstitial tissue. In cells within the infiltrated areas and in adjacent tubular cells acidophilic nuclear inclusions are seen.

*Bone Marrow.*—Apparently the bone marrow is not severely damaged. In spite of this fact, however, it is not unusual for typical nuclear inclusions to be seen in the mononuclear elements.

*Brain.*—No evidence of severe injury to the brain is observed. Meningitis and perivascular infiltrations are not present, and no nuclear inclusions are found.

By means of intramuscular inoculations the virus has been passed through a number of parrakeets. Eleven of the passages are charted in Text-fig. 1. The disease produced in this manner presents features of a septicemia with marked necrotic lesions in the liver and spleen, and in certain respects resembles yellow fever.

#### *Intracerebral Inoculations*

Having acquainted ourselves with the clinical and pathological pictures caused by intramuscular inoculations of the virus, it remained to determine the course of events following intracerebral injections of the active agent.

The pooled livers of Parrakeets 13 and 14 were macerated and mixed with Locke's solution. 0.1 cc. of the emulsion was injected into the brain of Parrakeet 15. The bird died, the brain was removed, and an emulsion was made of which 0.1 cc. was given intracerebrally to Parrakeet 18. In this manner the virus was passed serially through 5 parrakeets (Text-fig. 1).

Parrakeets inoculated intracerebrally sicken and die in a manner similar to that of birds receiving the virus intramuscularly. At no time do they evidence clinical signs of meningitis or encephalitis. At autopsy the brains macroscopically show nothing of interest. Microscopical examinations reveal a slight mononuclear infiltration into the meninges most marked near the site of inoculation, a slight proliferation of the ependymal cells, and no perivascular infiltrations. A few acidophilic intranuclear inclusions are seen in the meninges, ependyma, choroid

plexus, and the substance of the brain near the site of inoculation. The other organs macroscopically and microscopically present a picture identical with that which follows intramuscular injections of the virus.

The intracerebral inoculation of the virus appears to induce a septicemic form of the disease without definite signs referable to the brain. In this respect the disease-inciting agent also acts in a manner similar to that of yellow fever virus (11) before it has been adapted to mice.

#### *Susceptibility of Alien Hosts*

In the study of a virus the determination of its species specificity is essential, because a knowledge of the host range of an active agent is of assistance in its identification and in its comparison with other viruses. Consequently, attempts were made to infect mice, guinea pigs, rabbits, chickens, and canaries with the Brazilian virus.

*Mice.*—6 mice inoculated intraperitoneally with 0.5 cc. of a 10 per cent emulsion of the parrot liver received from Brazil remained well for a period of 40 days. 4 mice similarly inoculated with a liver and spleen emulsion from Parrakeet 1 also showed no evidence of infection. Finally, each of 6 mice received intracerebrally 0.025 cc. of a 10 per cent emulsion of the pooled livers of Parrakeets 13 and 14. 4 of the animals also received intraperitoneally 0.5 cc. of the same emulsion. None of the mice evidenced signs of illness. See Text-fig. 1.

*Rabbits.*—2 rabbits were inoculated intracerebrally with 0.25 cc. of a 10 per cent emulsion of the pooled livers of Parrakeets 13 and 14. After a slight elevation of temperature on the morning following the inoculation, both rabbits remained without fever and other signs of infection during 3 weeks of observation. See Text-fig. 1.

*Guinea Pigs.*—2 guinea pigs were injected intracerebrally with 0.1 cc. of a 10 per cent emulsion of the pooled livers of Parrakeets 13 and 14. There was no elevation of temperature other than a rise on the 1st day following inoculation. One of the pigs remained well for an observation period of 3 weeks. The other animal was sacrificed on the 9th day after injection. Nothing except a mass of caseous retroperitoneal lymph glands was found. See Text-fig. 1.

Each of 2 guinea pigs received intracerebrally 0.1 cc. of a 10 per cent emulsion of the pooled livers of Parrakeets 15 and 16. One of the animals remained well and afebrile for a period of 3 weeks. The other pig developed a fever of 104.6°F. on the 9th day after inoculation. It was sacrificed and a 30 per cent brain emulsion was prepared. Each of 2 guinea pigs received intracerebrally and intraperitoneally 0.1 cc. and 0.5 cc. respectively of the emulsion. One of the animals remained entirely afebrile and well for 3 weeks. The other, however, on the 2nd day following inoculation had a temperature of 106.4°F., and on the 3rd day, 105.3°F.

At this time the pig was killed and a 30 per cent brain emulsion was made. Each of 2 guinea pigs received intracerebrally and intraperitoneally 0.1 cc. and 5.0 cc. respectively of the emulsion. Both animals were afebrile and normal in appearance for an observation period of 28 days. Histological preparations of the brains and other organs of the 2 pigs that had fever and from which passages were made to other pigs revealed no evidence of infection due to the Brazilian virus. See Text-fig. 1.

*Canaries.*—Each of 2 canaries was inoculated intramuscularly with 0.5 cc. of a 10 per cent liver and brain emulsion from Parrakeet 30. Each of 2 canaries received intracerebrally and intramuscularly 0.25 cc. and 0.5 cc. respectively of a 10 per cent liver emulsion from Parrakeet 31. None of the birds showed any signs of infection during a long period of observation. See Text-fig. 1. Other canaries were inoculated intracerebrally and killed 4–5 days later. Histological examinations of their brains revealed no nuclear inclusions or other signs of infection.

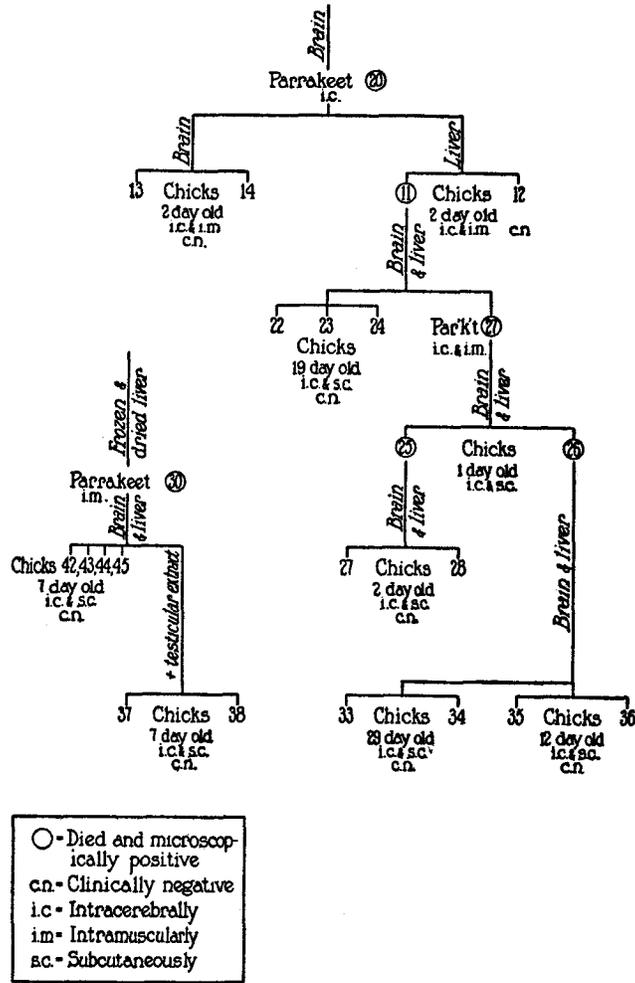
*Chickens.*—A number of attempts were made to infect young chickens. Rhode Island Red and White Leghorn chickens, 1–29 days old, were used. The birds were inoculated intracerebrally and intramuscularly or intracerebrally and subcutaneously with liver, brain, or liver and brain emulsions from parrakeets. Finally, an attempt was made to infect chickens by means of intracerebral and subcutaneous inoculations of a mixture of liver and brain emulsions from parrakeets plus a rabbit testicular extract (12). In most instances (Text-figs. 1 and 2) the chickens showed no evidences of infection and the experiments will not be described in detail.

In one set of experiments, however, evidence was obtained that young chickens may be slightly susceptible to the Brazilian virus, and the results of this part of the work are recorded in Text-fig. 2. Chick 11 was inoculated intramuscularly and intracerebrally with a 10 per cent liver emulsion from Parrakeet 20. It sickened and died 4 days after inoculation. An emulsion was made from its liver and brain with which 3 chickens (Nos. 22, 23, and 24) 19 days old and a parrakeet (No. 27) were inoculated. The chickens were negative clinically while the parrakeet died. A 10 per cent liver and brain emulsion was made from the parrakeet with which Chicks 25 and 26 were inoculated. Chick 25 died in 24 hours and Chick 26 died in 4 days. Chicks 27, 28, 34, 35, and 36, injected with liver and brain emulsions from these dead birds, showed no signs of illness (Text-fig. 2).

Macroscopically the brain, liver, and spleen of Chick 11 showed nothing of interest. Microscopically the liver and spleen were fairly normal, but the brain revealed a severe meningeal reaction with typical acidophilic intranuclear inclusions in the mononuclear cells of the exudate. Parrakeet 27, macroscopically and microscopically, presented a typical picture of the disease caused by the Brazilian virus. Chicks 25 and 26 showed nothing of particular interest except a decided meningeal reaction in which a number of typical inclusions were found.

The experiments described above seem to indicate that liver emulsions from parrakeets infected with the Brazilian virus are incapable of

inducing a disease in mice, rabbits, guinea pigs, and canaries. Such emulsions, however, appear to be capable at times of producing a fatal



TEXT-FIG. 2. Graphic representation of passages of the virus through young chickens.

malady in 1 or 2 day old chickens manifested chiefly by a meningitis. Serial passages in young chickens, however, were not accomplished. Further attempts should be made.

*Susceptibility of Chick Embryos*

The facts that young chickens appear to be slightly susceptible to the Brazilian virus and that, according to work of Rous and Murphy (13) and Goodpasture and Woodruff (14), chick embryos, especially the membranes, are more susceptible to certain diseases than are chickens themselves induced us to determine whether embryos are suitable hosts for serial passages of the agent under investigation. To this end the following experiment was performed.

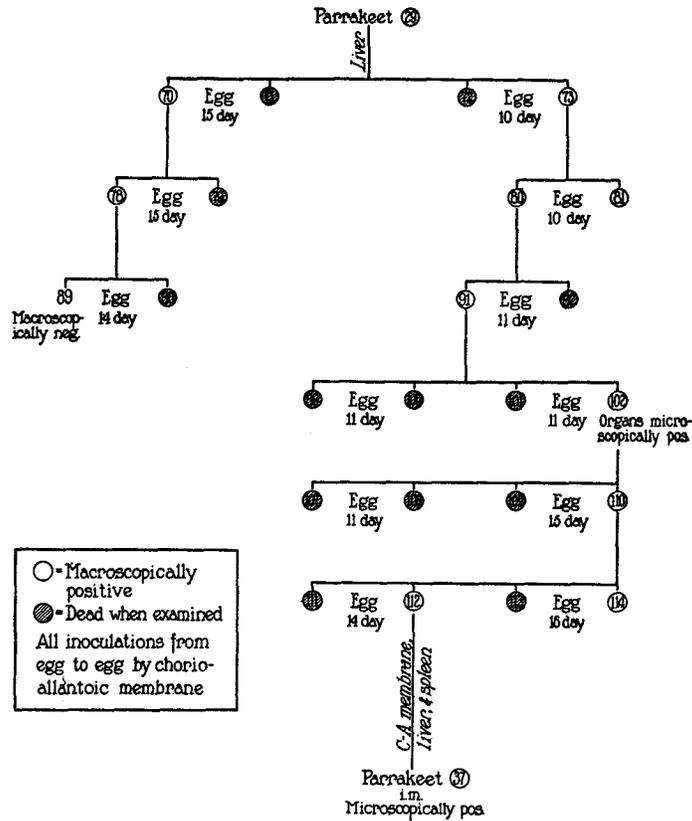
The chorio-allantoic membrane was used for serial passages of the Brazilian virus through chick embryos. After sterilization of the shell with alcohol, the egg was opened at the end over the air sac. This method of opening the egg enabled us to make the inoculations with the least amount of trauma to the embryonic tissue. It is adaptable, however, only to 10 day or older embryos, because in younger ones the chorio-allantoic membrane has not reached the air sac. After removal of the shell, a piece of the shell membrane was torn away and a bit of virus emulsion or tissue containing virus was placed on the chorio-allantoic membrane that had been pierced several times with a small sharp instrument. After completion of the inoculation a small amount of sterile vaseline was put around the opening in the shell which was then closed by means of a sterile cover-slip placed in contact with the vaseline.

Many of the infected embryos were dead by the 5th day after the inoculation. A few were dead by the 3rd day. Consequently, transfers were made every 3 days. The shell was sterilized with alcohol and then removed so that the membrane and embryo were well exposed. A portion of the infected membrane was removed and used for passage and histological studies. Then the embryo was examined and tissues were fixed for microscopic examination.

*Macroscopic Lesions.*—The macroscopic lesions in the chorio-allantoic membrane consisted of a whitish opaque area extending for several millimeters beyond the point of inoculation. Radiating from this central zone, numerous small isolated areas of similar appearance, usually situated along blood vessels, were seen. In some of the embryos macroscopic lesions, white spots, were found in the liver, spleen, and kidneys.

*Microscopic Lesions.*—Examinations of stained sections of the membranes showed hyperplasia and necrosis of the epithelium, cells of which contained typical acidophilic intranuclear inclusions. Within the connective tissue there was a marked infiltration of cells, and in these areas of infiltration nuclear inclusions were also found. The infected livers and spleens revealed areas of necrosis with nuclear inclusions in the involved cells. The kidneys at times showed cloudy swelling of the tubular cells and areas of necrosis involving the tubules and interstitial cells. There was also a marked infiltration of cells into the interstitial tissue (Fig. 6). Nuclear inclusions were found in the involved tubular and interstitial cells (Fig. 6, insert).

In the manner outlined above it has been possible without difficulty to pass the virus in series through 6 sets of chick embryos. From the last lot of embryos a parrakeet was inoculated and it developed the



TEXT-FIG. 3. Graphic representation of passages of the virus through a series of chorio-allantoic membranes of chick embryos.

typical clinical and histological picture of the Brazilian virus disease (Text-fig. 3). This experiment clearly indicates that the chick embryo and its chorio-allantoic membrane are more susceptible to the virus than are hatched chickens.

*Filterability of the Brazilian Virus*

Pacheco and Bier (7) reported that the virus passes through Berkefeld N candles. When opportunities arose, we filtered virus-containing emulsions through Berkefeld V, N, and W candles. A description of this work follows.

*Experiment 1.*—Approximately 5 cc. of a 2 per cent emulsion of the liver and spleen from Parrakeet 2 were filtered through a Berkefeld V candle that had been previously tested. No record of the pressure employed was made because the filtration was completed in a few seconds. Cultures of the filtrate on blood agar plates and in broth, aerobic and anaerobic, remained sterile. 0.5 cc. of the filtrate was injected intramuscularly into each of Parrakeets 3 and 4. Both birds died of the Brazilian virus disease. See Text-fig. 1.

*Experiment 2.*—A 2 per cent emulsion was made of the pooled livers from Parrakeets 3 and 4 and filtered through a Berkefeld V candle at a negative pressure of 50 cm. of mercury. The test organism, a small diphtheroid, was placed in the emulsion before filtration. 10 cc. of filtrate were collected in 3 minutes. Aerobic and anaerobic cultures of the filtrate remained sterile. 0.5 cc. of the filtrate was injected intramuscularly into each of Parrakeets 5 and 6. Both birds died of the Brazilian virus disease. See Text-fig. 1.

*Experiment 3.*—A 2 per cent emulsion was made from the pooled livers of Parrakeets 19 and 20. One portion of the preparation was set aside as a control. To the other portion proteus bacilli were added as test organisms. This part of the emulsion was then divided: one portion was filtered through a Berkefeld N candle at a negative pressure of 40 cm. of mercury—4 cc. of filtrate were collected in 30 seconds; the other portion was filtered through a Berkefeld W candle at a negative pressure of 48 cm. of mercury—6 cc. of filtrate were collected in 90 seconds. Aerobic and anaerobic cultures of both filtrates remained sterile. 0.5 cc. of the W filtrate, 0.5 cc. of the N filtrate, and 0.5 cc. of the control emulsion were injected intramuscularly into Parrakeets 21, 22, and 23 respectively. The birds that received the control emulsion and the N filtrate died of the Brazilian virus disease, while the one that was given the W filtrate remained well. See Text-fig. 1.

The results of the above experiments indicate that the Brazilian virus readily passes through Berkefeld V and N candles and that the infectious agent is still present in filtrates free from ordinary bacteria.

*Preservation of Virus*

Most viruses can be preserved in 50 per cent glycerol or by desiccation following freezing. In this respect the Brazilian virus adheres to

the rule. In Text-fig. 1 are shown the results of storing the active agent on ice in 50 per cent neutral glycerol for 23 and 25 days. Further work indicates that the virus can be preserved in this manner for at least 4 months. Also in Text-fig. 1 are charted the results of investigations regarding the effect of freezing and desiccation on the active agent. The methods of freezing and drying employed were those described by Sawyer and his coworkers (10). The Brazilian virus resists freezing and desiccation. Furthermore, tests have shown that it remains active in the dried state when stored on ice for at least 1 month, and there is no reason to suppose that it will not continue to be potent for much longer periods of time.

#### DISCUSSION

The results obtained by the Brazilian workers have been confirmed and extended. The active agent described by them undoubtedly belongs to the group of filterable viruses. Cells affected by the virus exhibit acidophilic intranuclear inclusions similar to those seen in varicella, herpes febrilis, and Virus III infections (15). The active agent readily passes through Berkefeld V and N candles and is fairly species-specific. In the experience of the Brazilian workers no hosts other than members of the parrot family were found susceptible. In this respect our experience has been similar to theirs with the exception that we were able to show that 1 or 2 day old chickens are partially susceptible, while chick embryos are suitable hosts for the serial passage of the virus. Neither group of workers took precautions to prevent the spread of the infection to individuals in the laboratory, and in no instance has an investigator or a laboratory helper contracted the disease from birds. Hence, man is not highly susceptible.

Although we have been able to confirm the results obtained by the Brazilian investigators, we do not believe that the disease described by them should be spoken of as avian psittacosis, nor do we agree with the conclusion that the strict adaptation of the virus to the Psittacidae accounts for the absence of human psittacosis from Brazil. We are of the opinion that a new virus disease of parrots and parrakeets, which is in no way related to avian and human psittacosis, has been discovered. It is true that the clinical pictures of the two diseases in parrots are somewhat similar and that the macroscopic lesions produced in the

liver by the two viruses are indistinguishable (16). Yet the host range (17-19) of the two agents is quite different—psittacosis virus attacks man, monkeys, rabbits, guinea pigs, and mice, while the activity of the Brazilian virus is almost wholly limited to the parrot family. Cells affected by the Brazilian virus contain acidophilic intranuclear inclusions, while in psittacotic infections minute basophilic coccoid bodies (16, 20) are found in the cytoplasm of parasitized cells. These differences in the host range and in the intracellular pathology are sufficient evidence to show that the Brazilian virus and the agent causing psittacosis are unrelated. It is important, therefore, that investigators studying psittacotic infections in parrots and parrakeets bear in mind the fact that at least one other filter-passing agent is indigenous to these hosts and that if accidentally encountered, it may lead to confusion or to erroneous conclusions.

#### CONCLUSIONS

The virus of parrots and parrakeets discovered by Pacheco, Bier, and Meyer is unrelated to the agent causing psittacosis either in birds or in man. The virus is fairly species-specific and manifests itself chiefly by the production of areas of focal necrosis in the liver and acidophilic intranuclear inclusions in affected cells.

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## EXPLANATION OF PLATES

## PLATE 32

FIG. 1. Section of spleen from an infected parrakeet. The normal architecture is destroyed. Giemsa.  $\times 150$ .

FIG. 2. Higher magnification of section shown in Fig. 1. Arrows indicate intranuclear inclusions. Giemsa.  $\times 950$ .

## PLATE 33

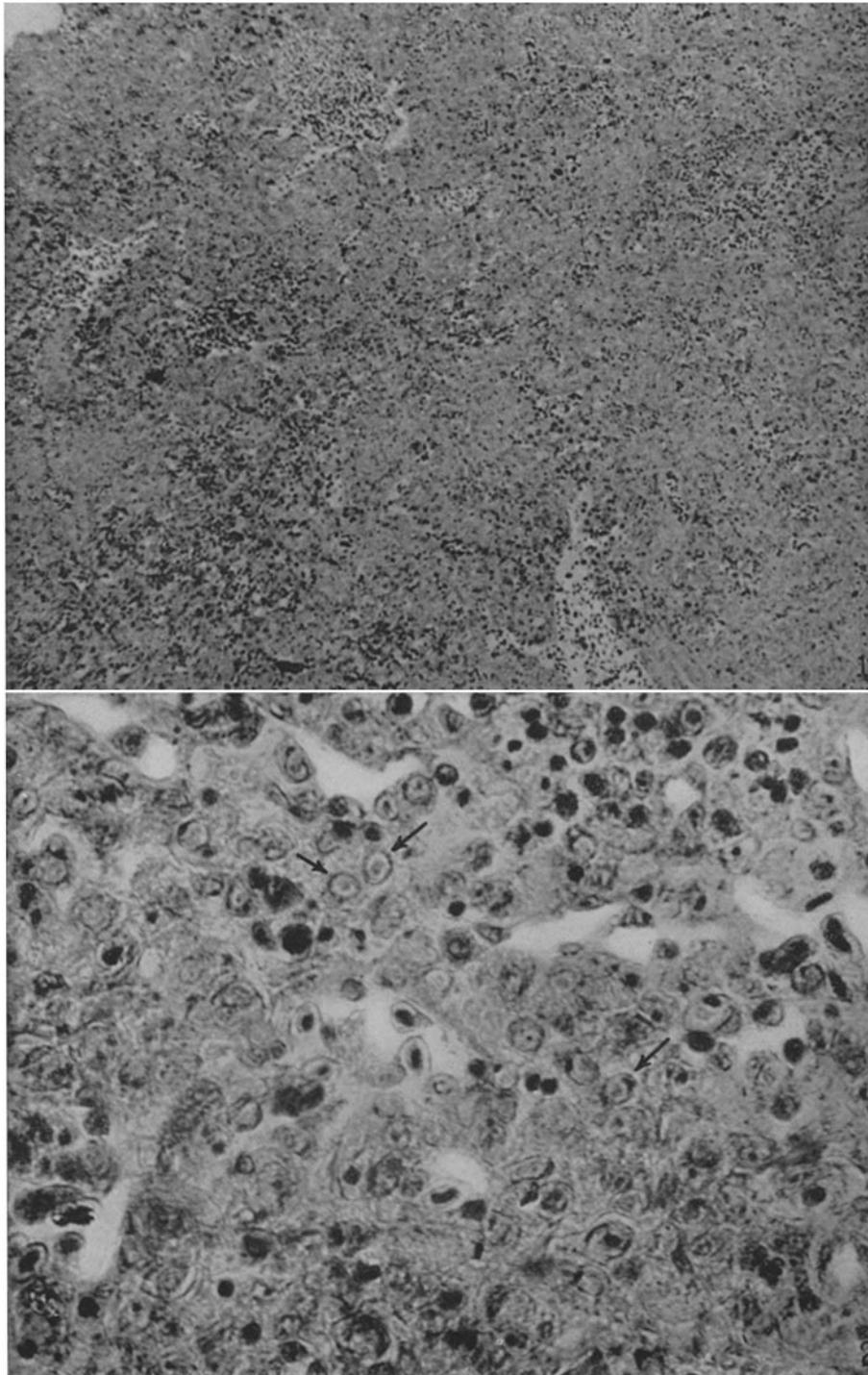
FIG. 3. Section of liver from an infected parrakeet showing fatty degeneration of the liver cells and areas of focal necrosis. Insert shows intranuclear inclusions. Giemsa.  $\times 150$  and 950.

FIG. 4. Section of liver from an infected parrakeet showing areas of focal necrosis without fatty degeneration of the liver cells. Insert shows intranuclear inclusions. Giemsa.  $\times 150$  and 950.

## PLATE 34

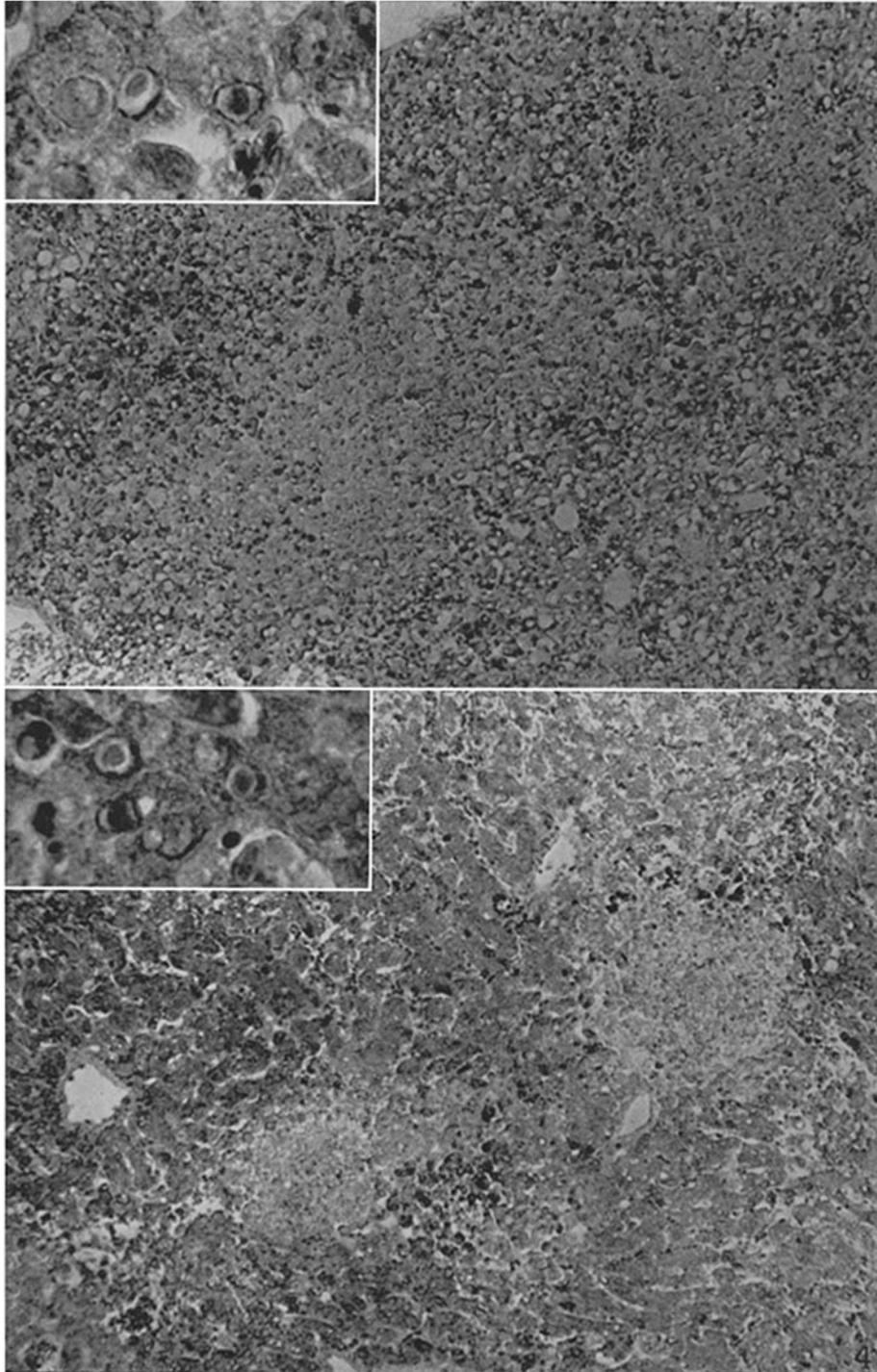
FIG. 5. Section of inoculated pectoral muscle of a parrakeet. Some of the muscle fibers are degenerated and there is a marked infiltration of mononuclear cells. Arrows indicate intranuclear inclusions. Giemsa.  $\times 950$ .

FIG. 6. Section of kidney from an infected chick embryo showing degeneration of tubules and infiltration of mononuclear cells into the interstitial tissue. Inserts show intranuclear inclusions in tubular cells and in cells of the interstitial tissue. Giemsa.  $\times 150$  and 950.



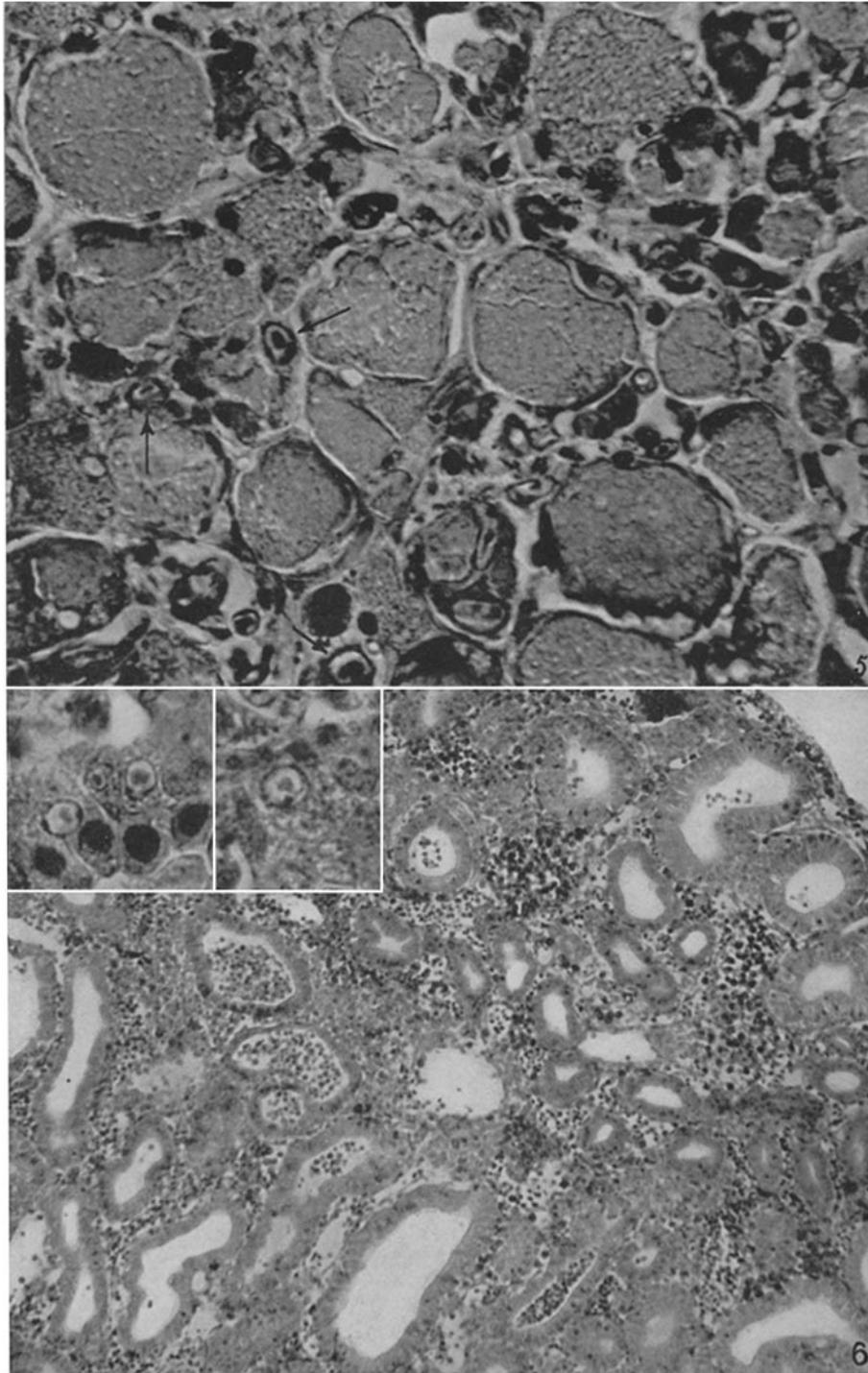
Photographed by Louis Schmidt

(Rivers and Schwenker: Parrot disease differing from psittacosis)



Photographed by Louis Schmidt

(Rivers and Schwentker: Parrot disease differing from psittacosis)



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(Rivers and Schwenker: Parrot disease differing from psittacosis)