# MYOCARDITIS AND PULMONARY ARTERITIS IN MICE ASSOCIATED WITH THE PRESENCE OF RICKETTSIA-LIKE BODIES IN POLYMORPHONUCLEAR LEUCOCYTES*

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Plates 59 to 64

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The disease described in this paper was discovered accidentally when brain suspension of a moribund mouse was inoculated intracerebrally into a litter of 3-day-old mice. Examination of the dead mouse disclosed no significant lesions in the half-brain examined, in viscera, or tissues; nor were any lesions found in one of the inoculated mice, which was killed for study on the 13th day. At this time a blind passage was made by intracerebral inoculation of brain suspension into a litter of 3-day-old mice. Only two of these survived the initial trauma; they were small, rough-coated, and hunched when they were sacrificed on the 17th day, but they were not paralyzed. It was in these two mice that the disease under consideration was first noted. On histologic examination, remarkable lesions of the heart and lungs were found. The disease has proved a difficult one to study, since many individual mice appear to be refractory on inoculation of presumably infective material, and we have not succeeded in transmitting it with regularity. Furthermore, there is great variation in the intensity of the lesions and, correspondingly, in the signs of illness manifested during life. Indeed, in many of the inoculated mice, it was only by careful histologic study that one could establish the presence of the disease.

The lesions were consistently associated with the presence of minute, coccobacillary bodies within the cytoplasm of polymorphonuclear leucocytes. We will describe in detail our efforts to identify these structures, though we have not as yet succeeded. In spite of this and other unsolved problems in connection with the disease, the unusual character of the lesions described warrants a report.

## *Pathology*

Our study of the pathology is based on the examination of approximately 200 mice, the majority inoculated during the early suckling period with material suspected of containing

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the active agent. The suspensions used for injection included brain, heart and lungs, chick embryo yolk sac, chorioallantoic membranes, and chick tissue culture supernatant. The routes of inoculation were intracerebral, intraperitoneal, intrathoracic, and intranasal. A small group of 4-week-old mice was injected intravenously. Some animals were inoculated simultaneously by more than one route.

As controls, there were available heart and lung sections from a much larger number of mice of comparable age and stock, which had been infected with one of many known or suspected viruses, as well as mice inoculated with normal mouse tissue or chick membranes. It can be said here that in no instance has there been found myocarditis of the type to be described, and in only two mice have we noted slight pulmonary arteritis. This would seem to exclude the possibility that we are dealing with a spontaneously occurring disease, uninfluenced by our experimental procedures.

After having established to our satisfaction the absence of lesions in other tissues than heart and lung, these organs only were taken as routine for section. The stains used, after fixation in Bouin's or Zenker's fluid, were hematoxylin-eosin, Giemsa, Gram, phosphotungstic acid hematoxylin, Laidlaw's stain for inclusion bodies, Nyka's and Noble's stain for rickettsiae. Impression preparations from lung and egg material were stained by Macchiavello's method.

*Gross Lesions:*

Lesions of the heart were noted in only a few of the many mice examined. They consisted of circumscribed greyish patches, single or multiple, of varying size and shapes seen on the surface of the ventricular muscle; in a few cases it was observed that the auricles had a milky appearance. Usually no macroscopic alterations were noted in the lungs, but in some mice inoculated intranasally patches of greyish consolidation, most often in the upper lobe, were observed. The spleen and the liver were not enlarged, and there were no other gross alterations.

*Microscopic Lesions:*

The essential lesion in the heart was an interstitial myocarditis, affecting both ventricles and auricles and varying greatly in severity as regards the distribution of the cellular infiltrates. In some instances the process seemed to be limited to the auricles, but in the majority both ventricles and auricles were affected. In the absence of serial sections, the relation of the cellular infiltrates to the coronary vessels could not be determined with accuracy, but so far as one could judge, the lesions were not primarily perivascular. This was obvious in the auricles where the inflammatory cells were often piled up beneath pericardium or endocardium.

The cellular exudate consisted of two types of cells, which were present in varying proportions. Polymorphonuclear leucocytes were always found and often in great numbers. The other cellular component was a mononuclear cell with large vesicular nucleus and a nucleolus, often quite large, sometimes surrounded by a clear zone and staining reddish with the Laidlaw acid fuchsin–orange G method. The cytoplasm of these cells was sometimes indistinct and

not strongly basophilic. It was difficult to decide whether these cells were histocytes, young fibroblasts, or possibly young regenerating myoblasts, although this last hypothesis seems unlikely. Small lymphocytes were not abundant in the lesions, and no typical plasma cells were seen (Figs. 1 and 2).

As has been stated, the myocarditis was essentially an interstitial one, and degeneration or necrosis of the muscle fibers in the inflamed areas was rarely noted. This was in striking contrast to the cardiac lesions which have been found in mice infected with various viruses of the Coxsackie group (Powers, Matulaitis, McCarthy, Conn. 5 and Ohio R), in which the initial change appeared to be a patchy necrosis of the muscle fibers, accompanied, it is true, by a cellular inflammatory reaction but nevertheless constituting an obvious and essential part of the picture (1). Indeed in animals which survived for 5 days or longer the necrotic fibers became calcified. We have never observed calcification in the myocarditic lesions with which we are now concerned, although most of the animals were not sacrificed until 15 days or more after inoculation.

We have not found any cellular infiltrates or thrombus formation on the valves, which were frequently included in routine sections. In this respect as in others, these cardiac lesions are unlike those reported by Moore *et al.* (2) and by Gray (3).

A most interesting and apparently distinctive feature of this disease is the involvement of many of the intrapulmonary branches of the pulmonary arteries. It will be recalled that the pulmonary arteries of mice are peculiar in that they are invested with a thick layer of striated muscle fibers of the cardiac type, external to the elastic lamina. These muscle fibers appear, indeed, to be a direct extension of the cardiac muscle.

In a large proportion of our injected mice, inflammatory lesions were present in and about the branches of the pulmonary arteries. When the artery was cut in longitudinal section, it could be seen that the lesions were focal, being limited to a small segment of the vessel and not extending diffusely along its course.

About the artery was a more or less nodular accumulation of inflammatory cells, predominantly lymphocytic, but invariably including a proportion of polymorphonuclear leucocytes, which were especially numerous near the arterial musculature. Indeed they often penetrated the arterial wall, between muscle fibers or occasionally actually invaded them. Necrosis of the fibers, however, was rarely if ever produced and the internal elastic lamella remained intact. There was no thrombus formation, and the arteritis seemed to produce no noteworthy alteration in the parenchyma (Figs. 5 to 7).

Whereas after intracerebral and intraperitoneal inoculations the lesions were restricted to the pulmonary arteries and their vicinity, a different pathologic picture was sometimes produced when infectious material was adminis-

tered intranasally. Many of these mice developed patches of pneumonia, visible in the gross as firm, smooth, greyish areas sharply demarcated from the pink aerated lung tissue. Microscopically, the bronchi and adjacent alveoli were found to contain purulent exudate. In a few cases there was suppurative bronchiectasis. In other areas there was marked septal thickening with proliferation and swelling of alveolar cells and often atelectasis. In the absence of pneumonic lesions one often found only the arteries affected.

*Rickettsia-Like Bodies:*

A clue to the etiology of these cardiac and pulmonary lesions was given by finding within the cytoplasm of the polymorphonuclears, innumerable minute microorganisms of coccobacillary form. These were revealed in sections stained with Giemsa, using citric acid and phosphate buffers in proportions recommended by Lillie (4) after fixation in Bouin's or Zenker's fluid. In our preparations, these intracellular bodies took a reddish color, which we did not succeed in changing to blue or purple by varying the proportions of acid and alkaline buffer in the stain (Figs. 3 and 4).

In Gram preparations, using aqueous safranin as counterstain, the bodies, though poorly stained, were definitely Gram-negative. In sections from Zenker-fixed material, stained for 24 hours with Mallory's phosphotungstic acid-hematoxylin, the bodies were sharply stained a dark bluish color.

As regards their distribution, they were found only in the polymorphonuclear leucocytes, and it could not be convincingly demonstrated that they occurred in other cell types—endothelial, mesothelial, or histiocytic.

Impression smears from some of the pneumonic lesions showed brilliant red intracellular bodies when stained by Macchiavello's method (Fig. 8). They were distinctly more bacillary than those seen in sections. In the absence of pneumonic lesions Macchiavello-stained impression smears were negative or doubtful.

At the present time we do not know whether or not these Macchiavello-staining bacillary bodies are identical with the coccobacillary bodies seen in sections.

## *Transmission Experiments*

As routine, 3- or 4-day-old mice have been preferred; a small number of animals was inoculated at 9 days and 3 and 4 weeks of age. When several materials were used for injection on the same day, litters were pooled and redistributed to avoid possible variation in litter susceptibility. The following tissues were used for inoculation:—

1. Brain suspension.
2. Heart and lung suspension (first intranasal passage, initiated from 4th yolk sac passage).
3. Yolk sac or chorioallantoic membranes.
4. Supernatant from chick-embryo amnion roller tube tissue cultures. The tissues were ground with alundum to 10 per cent or 20 per cent concentration by wet weight, using sterile

phosphate buffer, distilled water, or isotonic sucrose saline. Antibiotics were avoided because of their unknown effect upon the microorganisms under study. Materials were cultured for bacteria on blood agar and thioglycollate broth. No ordinary bacteria were found except in the lung suspensions, from which Gram-positive cocci and Gram-negative bacilli were recovered. Since none of the mice showed bacterial pneumonitis on histologic examination, the bacteria were regarded as inconsequential in the pathogenesis of the lesions.

The intracerebral route of inoculation was chosen in the early part of the study; later we used the intraperitoneal, intrathoracic, intranasal, and intravenous routes, either separately or in combination.

The diagnosis was based upon the presence of characteristic histologic lesions in either myocardium or lungs, or in both organs. Most of the mice showed no evidence of illness during life; and when they did appear ill, the signs were too indeterminate to serve as criteria of disease.

*Incidence of Lesions in Inoculated Mice:*

Without regard to route of inoculation, passage material used, or age of mice, the over-all incidence of heart lesions was 18 out of 203 examined (9 per cent); of lung lesions, 67 out of 162 (41 per cent). That these figures are minimal is very probable, since serial sections were not examined. A further analysis of these data is presented in the following Table I.

With our present knowledge, or lack of it, one cannot explain the variability in the results of these experiments. That the discrepancies are not due wholly to technical factors is suggested by the observation that litter mates inoculated with equal volumes of material, intracerebrally or intraperitoneally, are unpredictable in their reaction. Some mice show marked lesions, others none. For this reason titration and filtration experiments and neutralization tests have not been attempted. At this stage, a quantitative comparison with regard to type of passage material or route of inoculation would have little value.

The influence of genetic differences in susceptibility naturally comes to mind. The following observations suggest that this is worthy of further study. A litter of 8 mice of the Webster strain[1] proved completely refractory, whereas 7 of 17 of the Harvard stock developed pulmonary arteritis after inoculation of identical material by intraperitoneal and intracerebral routes.

We have considered the possibility that the agent responsible for the lesion may be harbored in inapparent form in the stock colony from which the original mouse was obtained and that the individuals are born with a variable degree of resistance to the agent. With this possibility in mind, excreta were collected from normal adults of the breeding colony, ground to 10 per cent suspension, filtered through a Boerner (centrifugal Seitz) filter which rendered it bacteria-free. The clear fluid was inoculated intranasally, intraperitoneally, or intracerebrally into 3 to 5-day old mice of the same stock. The results are shown in Table II.

[1] We are greatly indebted to Dr. C. A. Slanetz of Columbia University for sending breeders of the Webster strain from his laboratory stock.

There was thus in this small series an incidence of lesions at least as high as that in our most successful passage experiments. Coccobacillary bodies

TABLE I

*Lesions Produced by Passage Material in 3- to 9-Day-Old Mice*

| Inoculum | Route of inoculation | Myocarditis | Pulmonary arteritis |
|---|---|---|---|
| Original mouse brain | I.C. | 3/11* | 1/9 |
| 1st mouse brain passage | I.C. | 5/8 | 4/7 |
| 2nd mouse brain passage | I.C. or I.C. and cardiac | 2/19 | 3/10 |
| 3rd mouse brain passage (*a*) | I.P., I.C. | 4/32 | 11/33 |
| 3rd mouse brain passage (*b*) | I.P., I.C. or intrathoracic | 1/13 | 6/13 |
| 4th mouse brain passage | I.C. or I.N. | 0/11 | 1/11 |
| Heart suspension | I.P. | 0/6 | 1/1 |
| 1st passage mouse heart and lungs | I.P. or I.N. | 0/1 | 7/11 |
| 2nd mouse, heart, and lungs | I.N. | 0/7 | 6/7 |
| 3rd mouse, heart, and lungs | I.N. | 0/5 | 3/5 |
| 1st yolk sac passage | I.C. | 1/5 | 3/4 |
| 3rd yolk sac passage | I.C., I.P. or I.N. | 0/6 | 4/6 |
| 4th yolk sac passage | I.N. | 0/1 | 0/1 |
| 4th yolk sac passage (Egg 9) | I.C., I.N. | 0/9 | 4/9 |
| Chorioallantoic membrane | I.C., I.N. | 0/9 | 3/9 |
| Tissue culture supernatant | I.C. | 0/4 | 1/4 |
| Total‡..... | | 18/171 | 62/150 |

I.C., intracerebral.
I.P., intraperitoneal.
I.N., intranasal.
* Numerator, number with lesions.
Denominator, total number inoculated.
‡ Omitted from the Table are 3 and 4-week old mice, and mice from Webster and Schwentker stock.

TABLE II

*3- to 5-Day-Old Mice Inoculated with Fecal Filtrate**

| No. of mice | Myocarditis | Pulmonary arteritis |
|---|---|---|
| 7 | 2 | 4* |

* 1 mouse received unfiltered suspension of another sample of feces to which penicillin and streptomycin had been added.

identical with those described were present in the lesions. These observations support the thesis that the infection may be present in latent form in the Harvard stock mice, and they indicate further studies along this line (Fig. 9).

*Attempts to Identify the Infective Agent*

The regular occurrence of coccobacillary bodies in the polymorphonuclears in the lesions has already been mentioned, as well as our inability to reach a final conclusion as to their nature. As regards size and morphology, they conform closely enough to known rickettsial forms but their staining reactions are by no means typical. They constantly take a bright red color after prolonged Giemsa staining, whether the tissue be fixed in Bouin's or Zenker's fluid. Known rickettsiae characteristically stain purplish blue. The bodies could also be demonstrated by prolonged staining with Mallory's phosphotungstic acid–hematoxylin. We have not been able to demonstrate the bodies with the Nyka methyl violet–metanil yellow stain (5), although this admirably stains in tissue *R. prowazeki* and other rickettsiae. Nor have we had much success with Noble's basic fuchsin–orange G–methyl green method. On the other hand, we have obtained brilliant preparations in impression smears from several lungs with pneumonic lesions in which coccobacillary bodies were stained bright red with Macchiavello's stain (Fig. 8), but as has been stated, their identity with the Giemsa-stained bodies in sections is in doubt. Impression smears from lungs showing only the periarterial lesions were negative or inconclusive by this method.

We have emphasized the fact that the bodies appear to occur exclusively in the polymorphonuclear leucocytes. Only in Macchiavello smears from pneumonic lungs were bodies seen also within large mononuclears.

While this localization and apparent growth within the polymorphonuclears is certainly not characteristic of rickettsial infection in general, it is equally true that the rickettsiae of murine and epidemic typhus may be taken up in great numbers by polymorphonuclear leucocytes, especially after intranasal or intratracheal infection in experimental animals. One cannot therefore exclude their rickettsial nature on this basis.

In an effort to obtain further evidence, recourse was had to yolk sac inoculation, starting with 3rd mouse brain passage material. It was hoped that the bodies could be detected in numbers in Macchiavello's preparations or Giemsa-stained sections of the yolk sacs. But they could not even after 5 serial passages. However, as shown in Table I, inoculation of infant mice with yolk sac suspensions provoked characteristic lesions in 18 out of 34 animals—an incidence considerably greater than that obtained with mouse brain passage. Since 4 out of 9 mice receiving 4th yolk sac passage material yielded positive findings, it seems fair to conclude that some multiplication of the agent had occurred.

Although the yolk sac experiments do not rule out the rickettsial nature of the bodies, they have not yielded positive evidence in favor of it.

When 4th yolk sac passage material was dropped upon the chorioallantoic membrane of 12-day chick embryos, there were found after 5 days edema and greyish nodular thickenings at the site of inoculation and extending along the

blood vessels. Control membranes inoculated with normal yolk sac were thin and translucent. On further passage, these gross lesions were not invariably present.

Microscopically, the infected membranes showed epithelial proliferation, often with extensive necrosis and dense inflammatory infiltration and edema of underlying mesenchyma (Fig. 10). The inflammatory reaction in response to normal tissue was relatively trivial, and was not accompanied by epithelial necrosis.

Efforts to demonstrate the rickettsia-like bodies in Giemsa-stained sections of the lesions were confused by the well known pleomorphism of the chick polymorphonuclear leucocytes. These contain not only spherical or discoid bodies, but rods of various sizes and shapes, some of which might easily be mistaken for the rickettsia-like bodies under discussion. In sections stained with phosphotungstic acid–hematoxylin, only a few of the leucocytes contained minute bluish rods and granules; these may have been the rickettsia-like organisms (Fig. 11).

That the agent was present in these lesions is indicated by finding typical pulmonary arteritis in 3 out of 9 mice inoculated with ground suspensions of the chorioallantoic membrane. 11 control mice inoculated with suspension of normal membranes were free of lesions.

*Guinea Pig Inoculation:*

To obtain further evidence favoring the rickettsial nature of the bodies, 4 young male guinea pigs were injected intraperitoneally with 2nd passage mouse heart and lung suspension. No febrile nor scrotal reaction occurred. The guinea pigs were bled prior to inoculation, and 3 weeks and 4 weeks thereafter. Dr. Edward S. Murray of the Harvard School of Public Health very kindly carried out complement fixation tests with these sera against representative strains of typhus, Rocky Mountain spotted fever and Q fever rickettsiae. None reacted. In our own laboratory, "antigens" were prepared from infected yolk sac, chorioallantoic membrane, and mouse lung, and tested against the same sera. The mouse lung antigen reacted non-specifically with the preinoculation as well as the late guinea pig sera. No reaction occurred with yolk sac or chorioallantoic membrane antigens.

Because of the known susceptibility of the cotton rat to infection with typhus rickettsiae (6), a few were inoculated intranasally with 3rd mouse heart-lung passage material. They showed no signs of illness when killed on the 10th day. No gross lesions were apparent, but microscopic sections of the lungs showed in all 4 animals periarterial lesions (Fig. 12) identical with those of the mice and rickettsia-like bodies were demonstrable in the leucocytes. There was no myocarditis.

It would seem from these limited observations that the cotton rat is susceptible.

In summary, it can be said that the only basis for interpreting the coccobacillary bodies as rickettsial is their morphology and the demonstration of Macchiavello-staining minute rods in impressions from a few cases showing pneumonic lesions. This is far from conclusive.

Another possibility which had to be considered was an identity of the rickettsia-like structures with the coccobacillary bodies found by Nelson in the nasal passages and lungs of mice. In his most recent paper, Nelson classifies these with the pleuropneumonia group (7).

Cultures for pleuropneumonia-like organisms were made from a preparation of yolk sac and one of mouse brain which had been shown to cause characteristic lesions in mice, and from a 2nd passage chorioallantoic membrane, not tested in mice.

The medium used was heart infusion peptone broth containing 0.5 per cent glucose and 20 per cent filtered horse serum. Blind passages were made at 3 or 4 day intervals, and the 3rd passage was plated on the basic medium containing 1.6 per cent agar. After 5 days' incubation, agar blocks were cut out and rubbed over the surface of fresh medium. Both sets of plates, sealed to prevent evaporation, were incubated for 8 days, when agar blocks were transferred to the liquid medium. Subcultures in liquid medium were made 6 days later. Giemsa stains of broth sediment and impression smears from agar blocks revealed no morphologic elements resembling pleuropneumonia-like organisms. No suspicious colonies were detected on microscopic examination of plates.

These observations would appear to exclude the pleuropneumonia group from consideration.

## DISCUSSION

We may consider first the question whether the disease described is identical with the spontaneous myocarditis found in mice by other workers, and will briefly discuss the reports bearing on this subject.

Moore, Ridge, Huntington, Hall, Griffith, and Knowles (2) in their attempt to produce acute rheumatic-like lesions in mice, gave repeated intraperitoneal injections of egg white and found in the myocardium, focal lesions which they believed to be comparable to those of rheumatic fever in human beings. Untreated mice, however, were found to have similar lesions though less frequently and of lesser intensity. These were interpreted as probably due to a spontaneous exposure from time to time to foreign proteins. There was no relation between the severity of the anaphylactic reaction produced by the egg white injection and the severity of the pathologic changes. The authors did not consider the probability of latent infection, nor carry out transmission experiments.

Gray (3) in a similar attempt to produce experimental carditis, injected 100 mice intraperitoneally with egg white. 45 mice of corresponding age were used as controls. There was no significant difference in incidence or severity of cardiac lesions in the two groups

The lesions found differed from those in the disease which we have studied in that polymorphonuclears were infrequent, and the valves were often involved. The author suggests the possibility of latent infection in his stock, but no experiments in trans-

missions to other healthy mice were undertaken. Whether bodies similar to those found in our preparations were present cannot be said; they were not observed.

Lenke and Loewe (8) attempted to produce rheumatic lesions by intracerebral inoculation of 40- to 50-day-old mice with material from hearts of patients who had succumbed to acute rheumatic fever. The incidence of cardiac lesions in 200 inoculated mice and in 64 controls was approximately the same—about 30 per cent.

The lesions are described as resembling those of acute rheumatic fever in human beings—nodular perivascular foci resembling Aschoff bodies and verrucous lesions in the valves. Cultures were negative for bacteria, and no microorganisms were demonstrable in the lesions. Transmission experiments were not performed.

Laruelle and Reumont (9) report finding acute interstitial myocarditis in 65.6 per cent of "Souris Neuves" (*i.e.* uninoculated) mice as compared with 64.8 per cent in mice inoculated with Lansing, M.M., Columbia S.K., Theiler, Coxsackie, and ornithosis viruses. Myocarditis was not seen in the new-born, nor in mice infected with a Dalldorf strain of Coxsackie virus. No attempt was made to maintain the disease by passage in healthy mice of other stocks.

In this laboratory, lesions of the myocardium, often of great severity and extent, have been found in a fair proportion of mice inoculated with the Powers and Conn. 5 strains of Coxsackie virus, and very exceptionally with the DeMole pleurodynia strain (1). Interstitial myocarditis is also a feature of the disease caused by the EMC, Mengo, M.M., and Columbia S.K. strains.

The myocardial lesions produced by this group of agents differ from those in the disease under discussion in that there is extensive necrosis of muscle fibers often going on to calcification. The fuchsinophilic granules which have been described in the cardiac lesions produced by the Conn. 5 virus and other members of the Coxsackie group (10), do not resemble the rickettsia-like bodies found in this disease.

None of the authors referred to have described lesions of the pulmonary arteries, nor have we encountered such lesions in studies of the pathologic changes caused by various Coxsackie strains. It would seem, therefore, that we are dealing with a new disease or one that has previously escaped recognition.

It is obvious that there are still many unsolved problems in connection with this disease. The variability in the susceptibility of individual mice, and in the intensity of the lesions, where they are present; the possibility of latent infection in the laboratory stock and, most important, the nature of the infecting agent—to none of these questions has a final answer been given.

## CONCLUSIONS

A previously undescribed disease of mice, characterized by acute interstitial myocarditis, and by periarterial pulmonary lesions, is reported.

The transmissibility of this disease has been shown, but a large proportion of inoculated mice appears to be resistant.

Microorganisms, rickettsial in form but not positively identified as such, are consistently present in the lesions. They are found predominantly in the cytoplasm of polymorphonuclear leucocytes.

Filtrates of fecal material from normal mice of the Harvard breeding stock may produce lesions in myocardium and pulmonary arteries indistinguishable from those occurring in this disease. Rickettsia-like bodies are also found in these lesions.

We are grateful to Professor John C. Snyder of the Harvard School of Public Health for his advice and encouragement and to Dr. Edward S. Murray for performing complement fixation tests against various rickettsial antigens. We wish also to thank Mr. John Caribetses of the Children's Medical Center for the photomicrographs.

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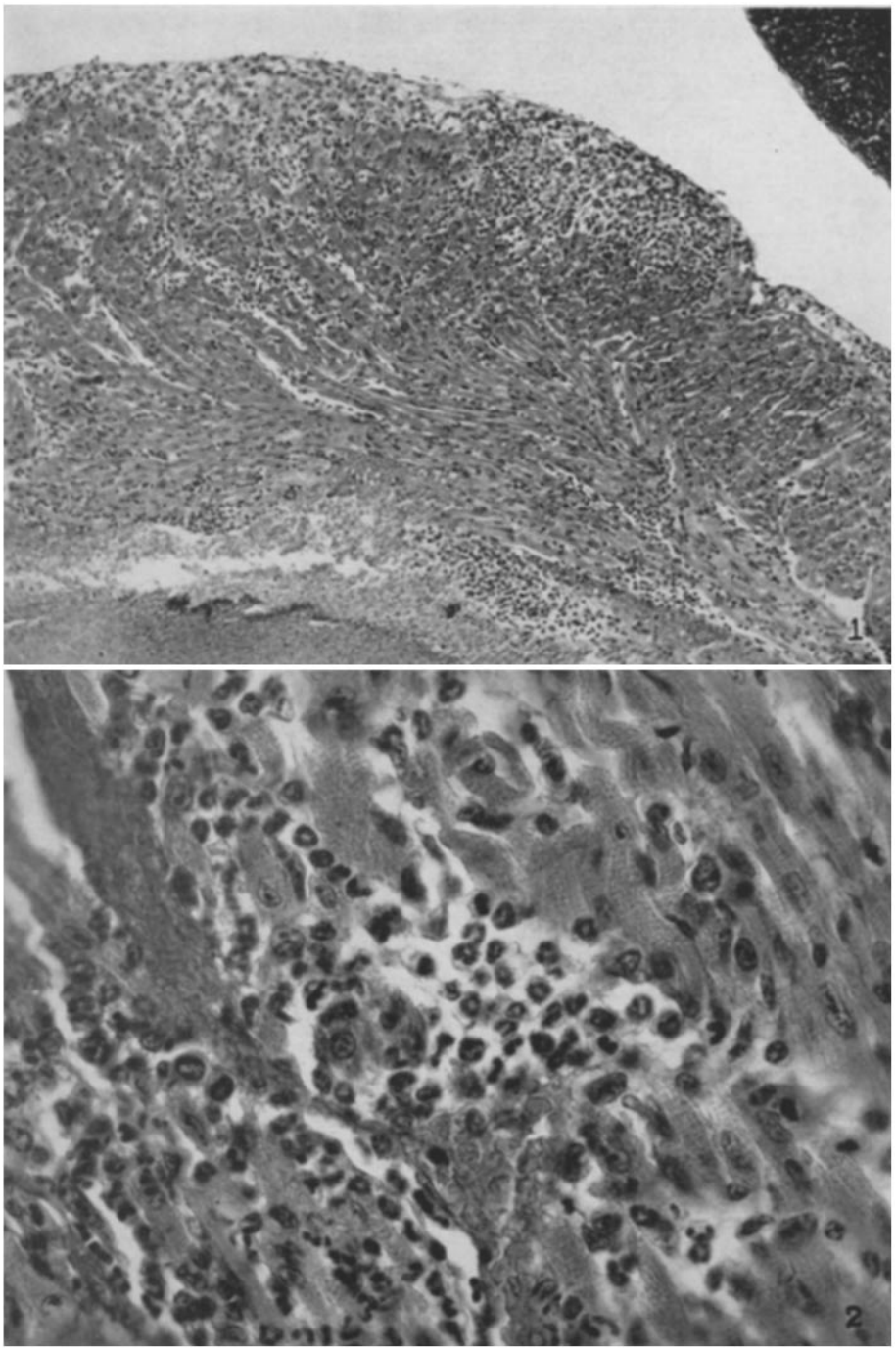
## EXPLANATION OF PLATES

### PLATE 59

FIG. 1. Mouse 5740. Inoculated intracerebrally at 3 days of age with $10^{-2}$ suspension of 2nd passage mouse brain. Killed 20 days later. Myocarditis of right ventricle. Hematoxylin and eosin. ×103.

FIG. 2. Mouse 5740. High power showing predominance of polymorphonuclear leucocytes in inflammatory reaction. Note absence of necrosis in muscle fibers. Hematoxylin and eosin. ×394.

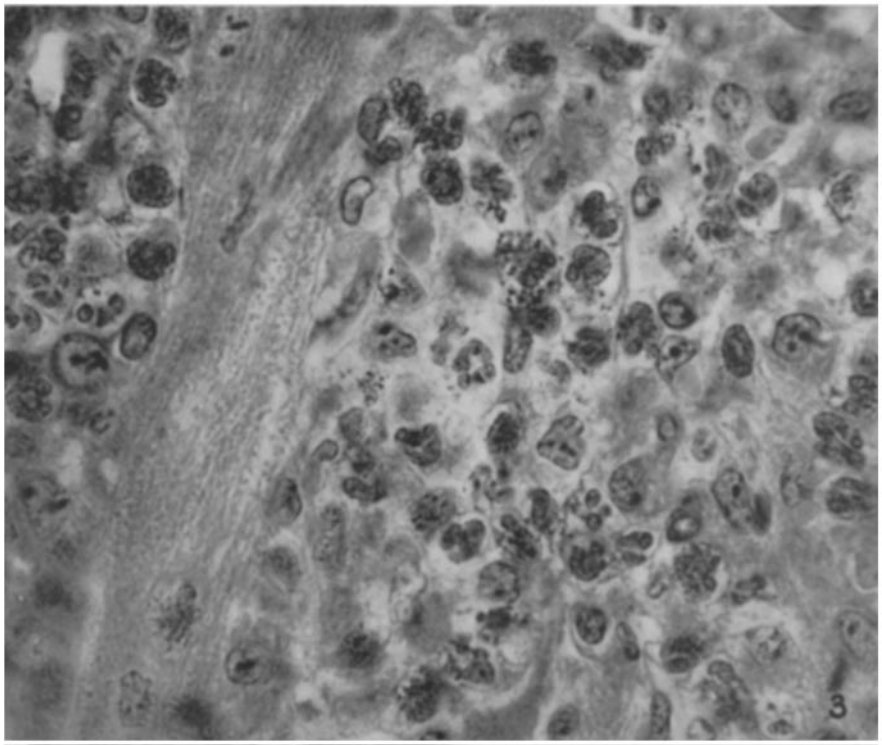


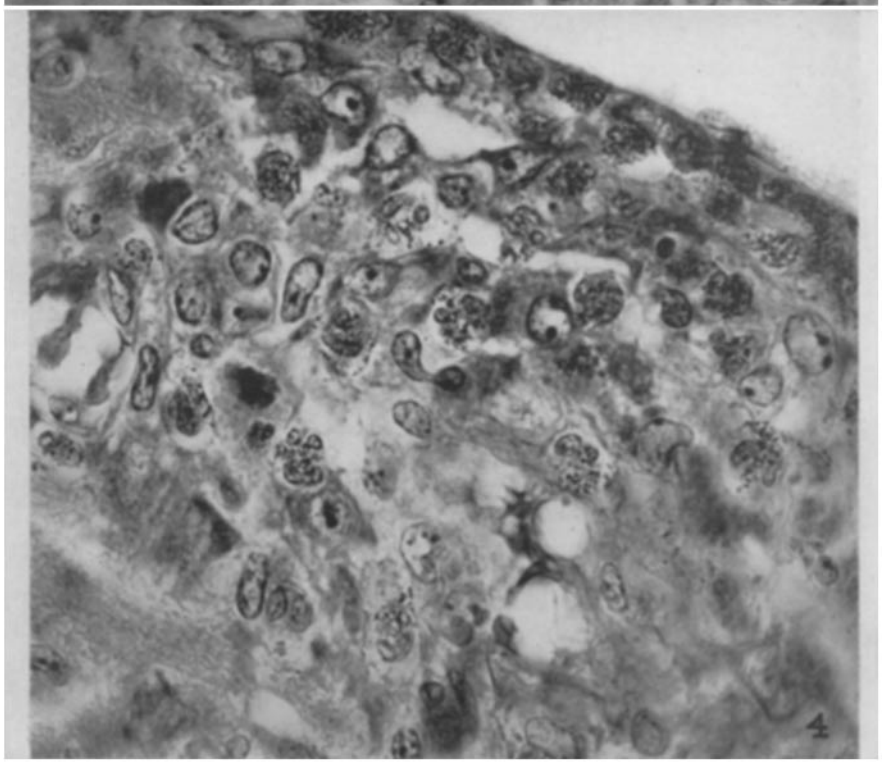
(Pappenheimer and Daniels: Myocarditis and pulmonary arteritis of mice)

PLATE 60

FIG. 3. Mouse 5628. Inoculated intracerebrally, at 3 days of age, with first passage brain suspension. Killed 17 days later. Heart. Rickettsia-like bodies in cytoplasm of polymorphonuclear leucocytes. Giemsa. ×1020.

FIG. 4. Mouse 5864. Inoculated intracerebrally and intraperitoneally with 3rd mouse passage brain suspension. Killed 15 days later. Rickettsia-like coccoid and bacillary bodies in cytoplasm of polymorphonuclear leucocytes. Giemsa. × 1020.



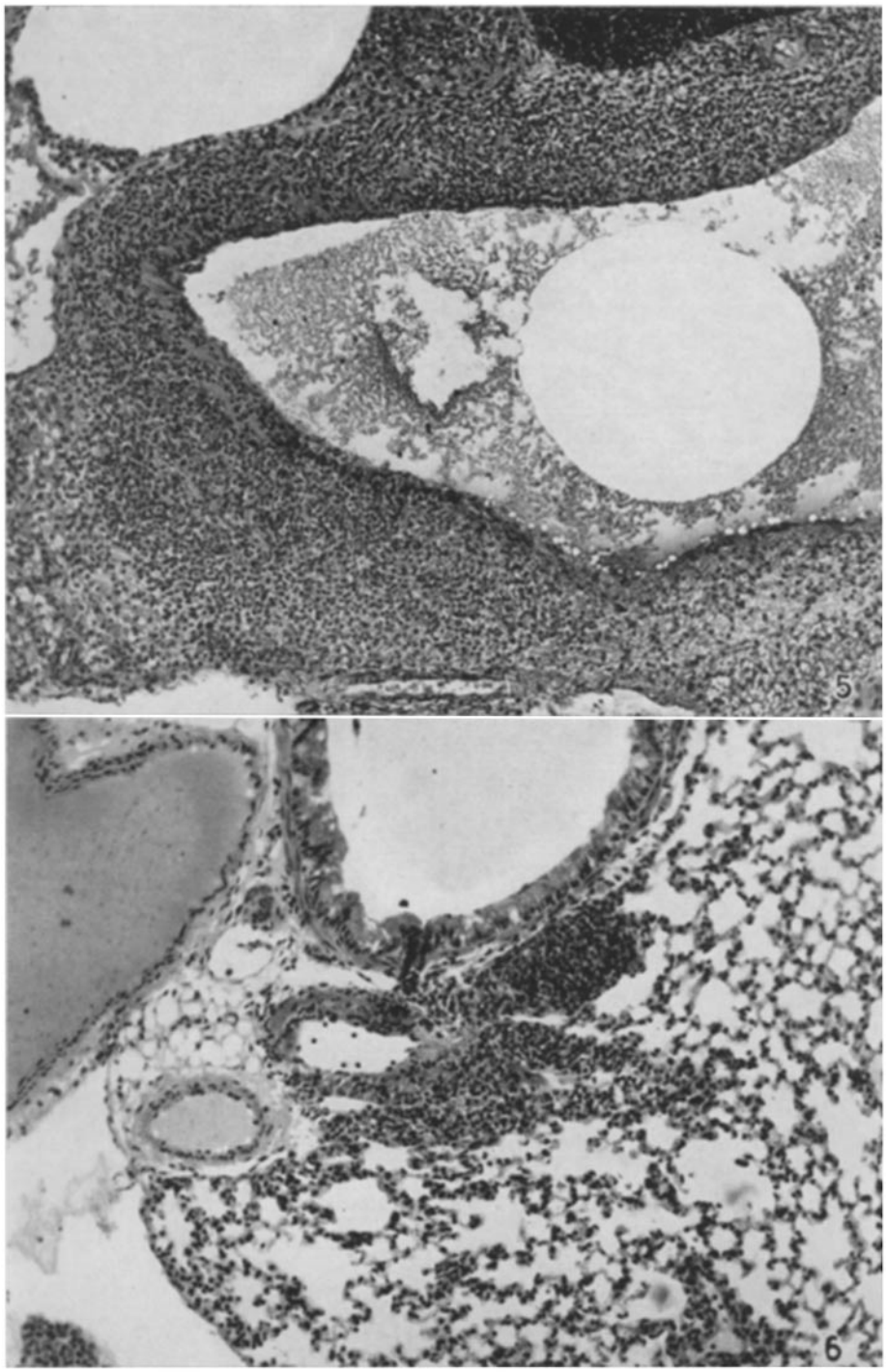




(Pappenheimer and Daniels: Myocarditis and pulmonary arteritis of mice)

PLATE 61

FIG. 5. Mouse 5740. Acute arteritis of main pulmonary artery. Hematoxylin and eosin. ×108.

FIG. 6. Mouse 5872. Inoculated at 3 days of age, intracerebrally and intraperitoneally with 3rd passage mouse brain suspension. Killed on 20th day after inoculation. A small pulmonary artery shows invasion of the muscular coat with polymorphonuclear leucocytes. About the vessels is a dense aggregation of lymphocytes and polymorphonuclears. Hematoxylin and eosin. ×182.

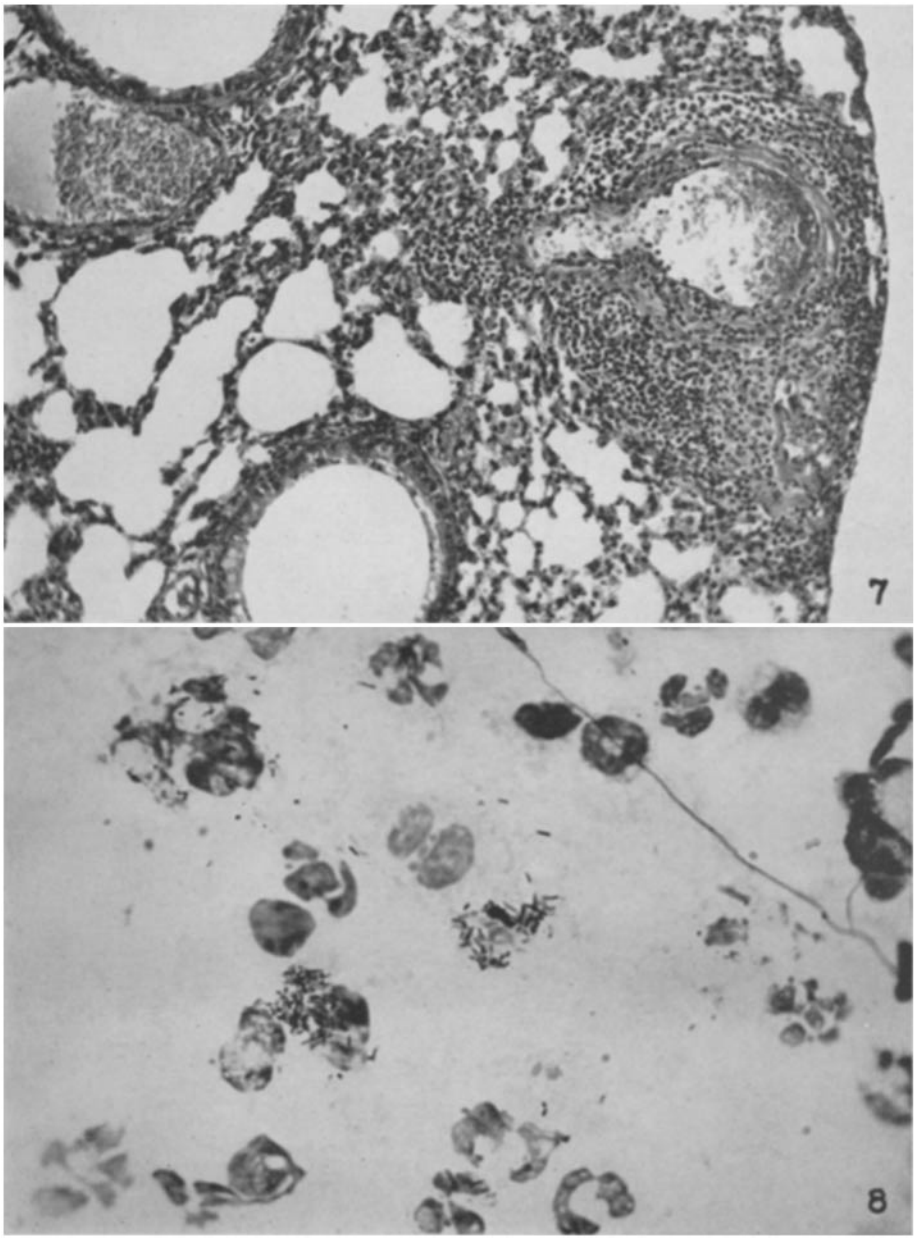



(Pappenheimer and Daniels: Myocarditis and pulmonary arteritis of mice)

## PLATE 62

FIG. 7. Mouse 5740. Acute arteritis and periarteritis of an arterial branch beneath the pleura. Hematoxylin and eosin. ×190.

FIG. 8. Mouse 6001. 4-day-old mouse inoculated intranasally with heart-lung suspension of mouse which had received 3rd passage yolk sac suspension. Impression smear from pneumonic patch, shows large numbers of red-stained rickettsia-like microorganisms, chiefly within polymorphonuclear leucocytes. Macchiavello stain. ×1036.

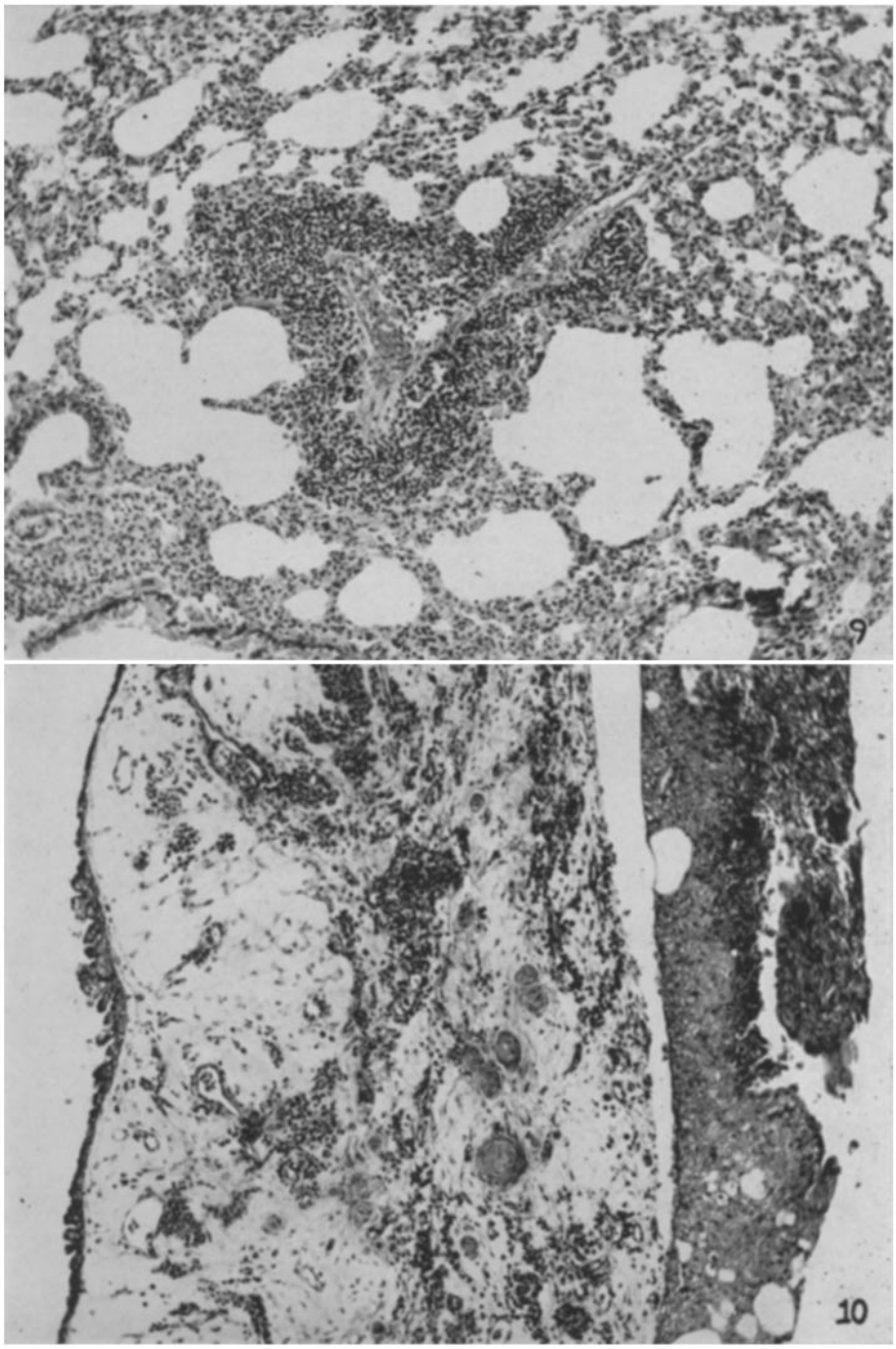



(Pappenheimer and Daniels: Myocarditis and pulmonary arteritis of mice)

PLATE 63

FIG. 9. Mouse 6080. Inoculated intracerebrally with bacteria-free filtrate of normal mouse feces. Killed 41 days after inoculation. Pulmonary artery surrounded by thick collar of lymphocytes. Amongst the cells are scattered polymorphonuclear leucocytes. Hematoxylin and eosin. ×190.

FIG. 10. Chick embryo 6096. Chorioallantoic membrane inoculated with 2nd passage chorioallantoic membrane suspension. Examined after 5 days. There is thickening and necrosis of surface epithelium, and inflammatory infiltration of the mesenchymal tissue. Hematoxylin and eosin. ×119.

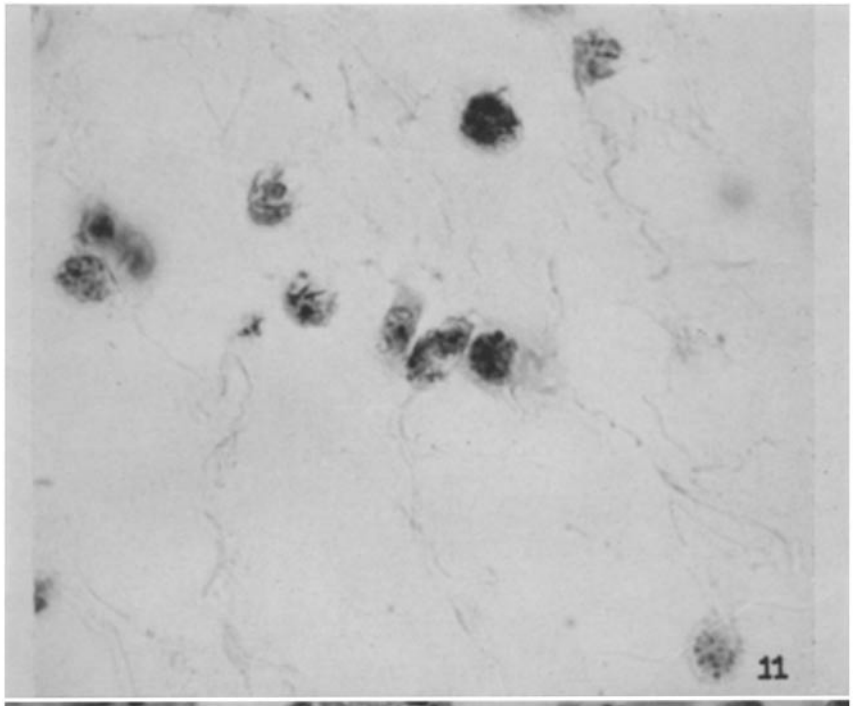


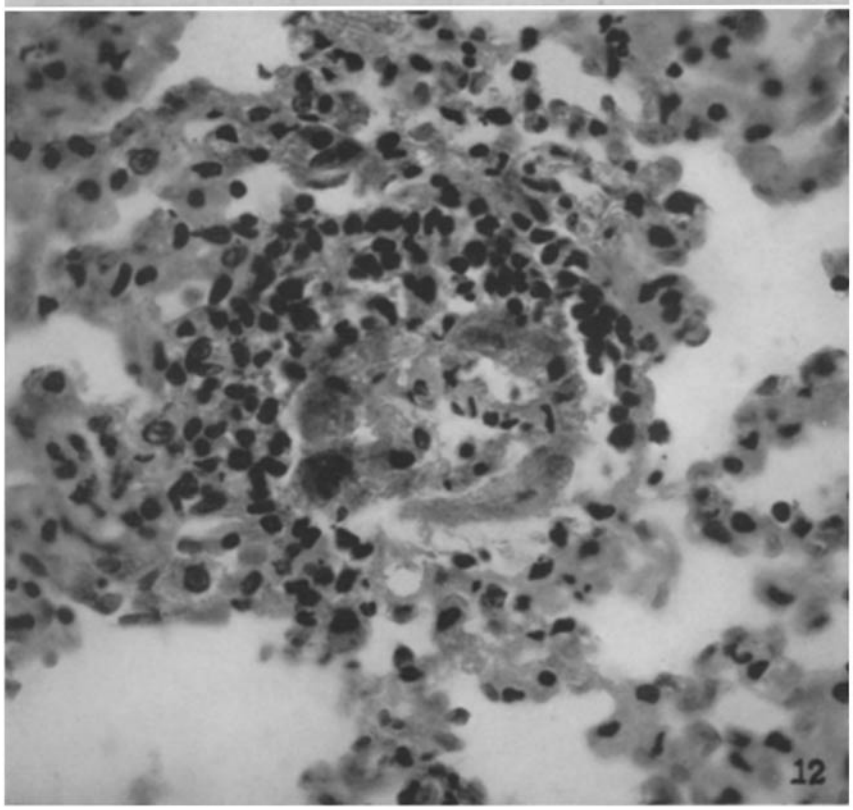

(Pappenheimer and Daniels: Myocarditis and pulmonary arteritis of mice)

## PLATE 64

FIG. 11. Chick embryo 6069. Chorioallantoic membrane, inoculated with 1st passage chorioallantoic membrane. Leucocytes containing minute coccobacillary bodies stained blue with phosphotungstic acid–hematoxylin. ×1500.

FIG. 12. Cotton rat 6065. Inoculated intranasally with 3rd passage mouse brain. Killed 10 days later. Pulmonary arteritis and periarteritis. Hematoxylin and eosin. ×600.

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