ISOLATION FROM NORMAL MICE OF A PNEUMOTROPIC VIRUS WHICH FORMS ELEMENTARY BODIES*

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PLATE 8

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In the course of attempts to isolate viruses by direct inoculation of albino Swiss mice with throat washings from clinical cases of influenza, non-influenzal pneumonias were frequently encountered in the passage mice. The pneumonias observed were of two types. One type was indistinguishable in the gross from that produced by influenza virus and the etiological agent of this type was found to be a filterable virus which was subsequently identified, on the basis of serological reactions, with that described by Horsfall and Hahn (1). Dochez, Mills, and Mulliken (2) and Gordon, Freeman, and Clampit (3) have described similar pneumonia-producing viruses isolated from apparently normal mice. The second type of pneumonia encountered was likewise found to be due to a filterable virus, but the microscopic demonstration of elementary bodies, the characteristic appearance of the early lesions, the greater virulence, and the serological reactions definitely distinguish this virus from the former. A brief description of it has been published (4). It will be dealt with in detail here.

Isolation of Virus

Albino Swiss mice from 4 to 5 weeks old were used throughout the course of this study. The first strain (*a*) of this virus was isolated in March 1939, a second strain (*b*) was isolated in April, and a third (*c*) in May of the same year. These three strains were derived from mice in the course of serial passages of throat washings from three different cases of clinical influenza. The passages were initiated by the intranasal inoculation of three or four mice with 0.05 cc. each of untreated throat washings from the respective patients. Although neither the mice nor the lungs showed gross signs of infection within 5 to 6 days after inoculation of the throat washings, the pooled lungs from each strain were ground with alundum and sufficient broth to give approximately 10 per cent

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suspensions. The lightly centrifuged supernates of these suspensions were inoculated intranasally into three or four mice and blind passages were continued in this manner until lesions appeared.

The first lesions in strain (*a*) appeared in the sixth passage. The lungs of three of the mice in this passage were normal in appearance but those of the fourth mouse were studded with small grey lesions when they were killed on the 5th day after inoculation (*cf.* Figs. 1 and 2). Only those lungs which showed lesions were used for the seventh passage into three mice, one of which died 2 days later with complete dark red pulmonary consolidation. The remaining two mice were killed 2 days after inoculation, one showing almost complete and similar consolidation while the other showed consolidation of about half of the lung tissue. In subsequent passages mice inoculated with 10 per cent lung suspensions were found dead or moribund 1 day after inoculation.

In strain (*b*) small greyish red pulmonary lesions had appeared in one of three mice of the second passage at sacrifice on the 5th day after inoculation. Similar lesions were observed in some mice of the next four passages at the time of transfer which was 5 or 6 days after inoculation. All of these mice were quite normal in appearance. Complete pulmonary consolidation was first noted in the sixth passage in one mouse which was moribund on the 5th day after inoculation. Not until the eleventh passage did mice die within 24 hours.

The first lesions of strain (*c*) appeared in the fifth passage, one mouse showing two small areas of greyish pulmonary consolidation and another complete greyish pulmonary consolidation when they were killed 5 days after inoculation. The three mice of the sixth passage were all dead 2 days after inoculation, the lungs showing complete greyish consolidation. Thirty-five mice were inoculated for the seventh passage, most of which were moribund on the following day and at autopsy showed complete or almost complete pulmonary consolidation.

All mouse passages subsequent to the first appearance of the lesions characteristic of this virus were made in mice from another source which was apparently free of the virus described.

Since convalescent sera from the patients from whom the respective throat washings were obtained failed to exert any neutralizing effect on these viruses, the probability of their mouse origin was considered. Mice from two different breeders (A and B) had been used in the course of the above passages. All of the mice from both breeders were derived from the initial breeding stock of breeder A. No mice from other sources were introduced into either colony.

To determine whether the new virus was carried by the mice of either or both breeders, suspensions from the lungs of uninoculated mice were passed serially in the manner described in mice from the same breeder. Following this procedure, the virus was isolated in two different series from the mice of breeder A, lesions first appearing in the seventeenth and fifth serial passages respectively.

In addition to the five strains mentioned above, all of which were maintained indefinitely in the laboratory, characteristic lesions were subsequently encountered occasionally in the mice of breeder A for a period of over a year in the course of influenzal and related studies, but no attempt was made to isolate further strains for study.

Although similar serial passages were made for over a year with the mice from breeder B, this virus was never isolated from his stock. This circumstance is the more noteworthy inasmuch as breeder B had obtained all of his breeding stock from breeder A, the last lot just 2 months prior to the first observed occurrence of the virus in the stock of breeder A. This suggested that the virus appeared suddenly in the colony of breeder A, possibly from a human source, such as the breeder himself. However, attempts to demonstrate neutralizing antibodies for this virus in the serum of this breeder, as well as attempts to isolate virus from his throat washings were entirely unsuccessful.

Pathogenicity for Mice

Mice could be killed quite regularly within 24 hours following intranasal inoculation of 0.05 cc. of a 10 per cent suspension of fully infective lung tissue, *i.e.* lung tissue obtained from mice showing marked characteristic symptoms within 24 to 48 hours after inoculation. This degree of pathogenicity could be developed for all the strains isolated by making the successive passages as soon as characteristic symptoms appeared. Death could be delayed as long as 20 days by decreasing the infecting dose. While 0.05 cc. of a 10^{-4} dilution of fully infective mouse lung usually constituted 1 M.L.D., the same quantity of 10^{-7} or 10^{-8} dilutions constituted minimal infective doses.

Mice inoculated with large doses rapidly developed signs of infection consisting of ruffled fur, hunched posture, indisposition to move about even when prodded, and increasingly labored respirations, with death after about 24 hours. Mice inoculated with smaller doses developed the same signs more slowly and in addition showed progressive emaciation, wet clicking respirations, and cyanosis of ears and tail, the latter presenting a beaded appearance. These last two signs in delayed infection were regularly prominent before the respirations became notably labored. The mice died 1 to 20 days following inoculation depending on the dose. Those which survived the smaller doses for 20 days usually recovered with gradual disappearance of all symptoms.

Pathology in Mice

When small infective doses were inoculated intranasally, the earliest lesions observed in the lungs were scattered focal, pin point, slightly elevated, greyish lesions in one or more lobes, the individual lesions increasing in size with progression of the infection until they coalesced to produce complete pulmonary consolidation. As long as the individual lesions were discreet, they remained grey and slightly elevated, with the surrounding pulmonary tissue presenting a

quite normal appearance (Figs. 1 and 2). With progressive consolidation, the lesions became darker red although the characteristic "bumpy" aspect was still discernible providing the infecting dose had been relatively small, permitting the development of the focal lesions described. With infective doses large enough to produce death in 24 hours, the consolidation progressed so rapidly as to obscure initial focal lesions (*cf.* Fig. 4). The lungs of such mice showed dark red pulmonary consolidation indistinguishable in the gross from that in the terminal stages of influenzal infection in mice.

General Properties of the Etiological Agent

The etiological agent was found to be a filterable virus. This was determined by passing 10 per cent suspensions of infected mouse lung prepared with broth or 20 per cent horse serum saline through Berkefeld N filters. The first portion of filtrate, amounting to at least half of the total filtrate, was discarded, while the second portion was inoculated in 0.05 cc. amounts intranasally into mice. All such filtrates induced typical, although delayed, infection in mice.

Impression smears prepared from cut sections of lungs in the first stages of infection and stained with Giemsa stain showed minute granules which varied from homogeneously stained spherical bodies to considerably larger forms somewhat less regular in morphology and exhibiting a more variable staining reaction (Fig. 5). With this stain the granules are reddish purple. With the Castaneda stain they take the methylene blue color and with Macchiavello stain the basic fuchsin color. The bodies stain more homogeneously with the latter two stains than with the Giemsa stain. On the basis of their size, regular contour, tinctorial characteristics, and the fact that they occur intracellularly it would seem justifiable to interpret them as representing elementary bodies.

Elementary bodies were found only within large mononuclear cells in the very earliest stages of the infective process. Virus multiplication presumably took place in these cells. In the later stages, they occurred in considerable abundance extracellularly and also within polymorphonuclear leucocytes. The latter observation would seem to represent phagocytic activity. In morphological and tinctorial characteristics, these bodies resembled the elementary bodies of the viruses of lymphogranuloma venereum, psittacosis, and other members of the psittacosis group. Their abundance was definitely related to the stage of the infection, in that they were more numerous in the advanced than in the early stages of infection.

The virus withstands desiccation and freezing for considerable periods of time, if not indefinitely. A 20 per cent mouse lung suspension in 25 per cent horse serum saline, desiccated from the frozen state and rehydrated to the original volume with sterile distilled water, was found to produce typical pulmonary infection in mice when tested more than 2 years later. Similarly 10

per cent and 20 per cent mouse lung suspensions prepared in 20 per cent horse serum broth and stored at −75°C. for at least 10 months produced typical infection in mice. Whole lungs preserved for many months at −75°C. likewise produced typical infection in mice.

Cultivation of the Virus

Numerous attempts to cultivate the virus from mouse lungs on the chorio-allantoic membrane of the developing chick embryo by the Burnet technique gave irregular results. Most of the attempts were unsuccessful. In a few instances such cultures were infectious for mice for the first few passages only. One culture strain was maintained through as many as eight membrane passages. Allantoic fluid cultures prepared as described for influenza virus by Nigg, Wilson, and Crowley (5) likewise gave irregular results in that such cultures were infectious for mice for only the first few serial passages. On the other hand, yolk sac cultures prepared by the Cox method were readily initiated and showed large numbers of elementary bodies in Giemsa-stained impression preparations made from the infected yolk sac (Figs. 6 and 7). 1 cc. of 10^{-3} or 10^{-4} yolk sac suspensions killed chick embryos, inoculated on the 6th day of development, within 6 or 7 days quite regularly.

Tropisms of the Virus in Mice

The tropisms of this virus were determined by studying its distribution following inoculation by various routes (see Chart 1):—

Intracerebral Inoculation.—Virus was inoculated intracerebrally with a threefold purpose; *viz.*, (1) to determine whether virus was still demonstrable in the brain several days after intracerebral inoculation and (2) if so, whether it could be passed serially from brain to brain, (3) whether virus inoculated intracerebrally was subsequently demonstrable in the lungs.

This series of experiments (Chart 1, *a*, *b*, *c*) showed (1) that virus inoculated intracerebrally was demonstrable in the brain 5 days later, (2) that virus was not passed serially from brain to brain, and (3) that virus inoculated intracerebrally was transferred in small amounts to the lungs within 5 days' time. These experiments, which failed to demonstrate neurotropism, demonstrated definite pneumotropism.

Intranasal Inoculation.—These experiments were designed to determine whether virus inoculated intranasally was demonstrable (1) in the brain and (2) in the spleen.

Two mice inoculated intranasally with the same 20 per cent mouse lung suspension used above for intracerebral inoculation were killed on the following day. Both showed extensive pulmonary consolidation. The presence of virus in the brains and spleens of these mice was tested by intranasal inoculation of suspensions prepared from the respective tissues.

The results (Chart 1, *d*, *e*) showed (1) that virus inoculated intranasally is apparently not transferred to the brain (indicating again the lack of neurotropism) and

CHART 1

Tropisms of Mouse Pneumonitis Virus

25th mouse passage.
20 per cent lung suspension.

- Inoculated i.c. into 4 mice; K_5. No symptoms
  - Brains normal, pooled
    - Inoculated i.n. into 2 mice; K_6. Lungs ++, +++ (*a*)
    - Inoculated i.c. into 4 mice; K_6. Brains pooled
      - Inoculated i.c. into 4 mice; K_5. Brains pooled
        - Inoculated i.n. into 2 mice; K_6. Lungs normal, pooled
          - Inoculated i.n. into 3 mice; K_{10}. Lungs normal (*b*)
  - Lungs normal, pooled
    - Inoculated i.n. into 2 mice; K_6. Lungs normal, pooled
      - Inoculated i.n. into 3 mice; K_5. Lungs showed typical focal lesions (*c*)
- Inoculated i.n. into 2 mice; K_1. Lungs ++, +++
  - Brains normal, pooled
    - Inoculated i.n. into 3 mice; K_5. Lungs normal, pooled
      - Inoculated i.n. into 3 mice; K_5. Lungs normal, pooled
        - Inoculated i.n. into 3 mice; K_5. Lungs normal (*d*)
  - Spleens pooled
    - Inoculated i.n. into 2 mice; K_5. Scattered focal lesions in lungs. Lungs pooled
      - Inoculated i.n. into 3 mice; D_4 ++++, K_4 ++++, K_4 ++++ (*e*)
- Inoculated i.p. into 2 mice; K_5
  - Spleens normal, pooled
    - Inoculated i.p. into 3 mice; K_5. Spleens normal, pooled
      - Inoculated i.n. into 3 mice; K_6. Lungs normal, pooled
        - Inoculated i.n. into 2 mice; K_5. Lungs normal (*f*)
  - Lungs normal, pooled
    - Inoculated i.n. into 3 mice; K_6. Lungs normal, pooled
      - Inoculated i.n. into 3 mice; K_5. Lungs normal, pooled
        - Inoculated i.n. into 2 mice; K_6. Lungs normal, pooled
          - Inoculated i.n. into 2 mice; K_5. Lungs normal (*g*)

i.c., intracerebrally; i.n., intranasally; i.p., intraperitoneally; K_1, K_5, etc., killed 1 and 5 days later respectively; D_4, dead 4 days later. ++, +++, ++++, extent of pulmonary consolidation.

Summary

(*a*) Results show that virus inoculated i.c. was still present in the brain when the mice were killed 5 days later.
(*b*) Results show that virus was no longer demonstrable in the brain after 2 brain-to-brain passages.
(*c*) Results show that virus inoculated i.c. was demonstrable in the lungs 5 days later.
(*d*) Results show that virus inoculated i.n. was not demonstrable in the brain at a time when pulmonary lesions were advanced.
(*e*) Results show that virus inoculated i.n. was demonstrable within 24 hours in the spleen
(*f*) and (*g*) Results show that virus inoculated i.p. was not demonstrable 5 days later either in the lungs or spleen.

(2) that virus inoculated intranasally is transferred in less than 24 hours to the spleen, indicating systemic infection and viscerotropism.

Intraperitoneal Inoculation.—These experiments were designed to determine whether virus inoculated intraperitoneally was demonstrable (1) in spleen and (2) in the lungs.

Two mice inoculated intraperitoneally with the same 20 per cent mouse lung suspension used above for intracerebral and intranasal inoculation were killed 5 days later. The presence of virus in the spleens and lungs of the mice was tested by intranasal inoculation of suspensions prepared from the respective tissues.

The results (Chart 1, *f, g*) showed that virus inoculated intraperitoneally was not demonstrable either in the spleen or in the lungs of the same animals.

Pathogenicity for Ferrets, Rabbits, and Hamsters

Attempts were made to establish infection in ferrets with this virus by instilling large amounts of infected mouse lung suspension intranasally.

One ferret, from distemper-free stock, was inoculated with material of the twenty-second mouse passage of the fourth strain of this virus. The temperature was taken twice daily for a period of 10 days. On the 2nd day after inoculation, the morning temperature was 104.2°F. and on the 7th day the morning temperature was 104.5°F. Otherwise the temperature was within normal limits and there were no other significant signs of infection. Another ferret, also from distemper-free stock, was inoculated with material of the twenty-sixth mouse passage of the same strain of this virus. This ferret remained afebrile for 10 days, although the respirations were increased for the first 4 days and the animal ate nothing on the 2nd day after inoculation, Otherwise, there were no signs of infection. At autopsy, 10 days after inoculation scattered focal hemorrhagic lesions were observed throughout the lungs and in addition areas of red healing consolidation at the hilum of several lobes. The microscopic examination for the presence of elementary bodies in these lesions was unfortunately not made and the significance of these lesions therefore remains a question. Results of experiments in other ferrets were obscured by an intercurrent epidemic of distemper among the stock.

Rabbits tolerated without signs of infection very large intraperitoneal doses (6 to 8 cc.) of 20 per cent lung suspensions. Since these animals received multiple injections over a period of many weeks in attempts to prepare specific immune sera no autopsies were performed.

Eleven hamsters (*Cricetus auratus*) were inoculated intranasally with 0.25 cc. of a 10 per cent lung suspension of the mouse pneumonitis virus.

Eight of the eleven animals developed labored respirations, ruffled fur, and died 4 to 10 days after inoculation. Several hours before death the animals were comatose, lay on their sides or backs gasping, and usually showed a terminal hemorrhage from the nose and mouth. Autopsy revealed a patchy, mottled deep red and greyish pink lobular type of pulmonary consolidation and a moderate amount of watery pleural exudate. Elementary bodies similar to those seen in mice were demonstrated in

impression smears of the lungs stained by Macchiavello's method. Four hamsters inoculated intranasally with 0.5 per cent mouse lung suspension survived with only slight signs of illness. Lung lesions in animals killed 2 to 3 weeks after inoculation were patchy grey and fibrotic. No attempt was made to pass the virus in hamsters.

Cross-Immunity Tests

Mice and hamsters were given repeated intranasal inoculations of sublethal amounts of the following viruses propagated in mouse lungs: The mouse pneumonia virus described above was used at dilutions of 10^{-5} to 10^{-6} in broth for inoculation of mice and 10^{-3} for inoculation of hamsters (6). The meningopneumonitis virus, strain MP-F97, sent us by Dr. Thomas Francis, Jr., was

TABLE I

Intranasal Cross-Immunity Tests in Mice and Hamsters with the Mouse Pneumonitis Viruses and Other Agents Which Produce Elementary Bodies

Immunized with virus of:	Results of intranasal tests with virus of:			
	Mouse pneumonitis		Human pneumonitis (strain S-F) in mice	Lymphogranuloma venereum in mice
	In mice	In hamsters		
Mouse pneumonitis	1/17	0/2	7/7	5/11
Meningopneumonitis	7/9	2/2	4/12	12/36*
Lymphogranuloma venereum	17/21	0/2	—	5/26*
Hamster virus 12XN	9/10	—	8/9	—
Nil (control)	35/35	2/2	14/17	31/31

In the four columns to the right, numerator is the number of animals dying; denominator, the number of animals tested.

* Reported in previous publication (9).

inoculated at dilutions of 10^{-7} into mice and 10^{-3} into hamsters. The virus of lymphogranuloma venereum (strain ST) received from Dr. Marion Howard of the Yale University School of Medicine was inoculated at dilutions of 10^{-2} into mice and hamsters. A pneumonia virus recently isolated from hamsters by Dr. R. M. Taylor (7) in Buenos Aires was also used to immunize mice at a dilution of 10^{-6}. The latter agent produced lung lesions in mice and formed elementary bodies similar to those of the mouse pneumonia virus. Animals were first demonstrated to be immune to intranasal inoculation of at least 10 M.L.D. of the homologous strains of virus and were then tested by intranasal inoculation with heterologous strains using the smallest dose which would kill all of the controls. These tests were done with the mouse pneumonitis virus, the virus of human pneumonitis (18), and the virus of lymphogranuloma venereum.

The results presented in Table I give a questionable indication of cross-immunity between the viruses of lymphogranuloma venereum and mouse pneu-

monitis, but there is no evidence of cross-immunity in mice with the virus of human pneumonitis or the hamster pneumonia virus.

Mice immunized by intracerebral, intraperitoneal, or intranasal inoculation of the mouse pneumonitis virus were not protected against death from inoculation of the meningopneumonitis virus by the same three routes. However, three of four hamsters, after recovery from pulmonary infection with the mouse pneumonitis virus, survived the intranasal inoculation of a dose of meningopneumonitis virus which killed five of six control animals. It will be noted in the table that hamsters immune to the meningopneumonitis virus did not resist infection with the mouse pneumonitis virus.

These results indicate only a doubtful cross-immunization at most in mice and hamsters between the mouse pneumonitis virus and other agents which are similar to it. The difficulties of immunization against intranasal inoculation with the latter viruses has already been noted (8, 9), repeated intraperitoneal inoculation of active virus failing to induce appreciable resistance to intranasal infection. It seemed that evidence of antigenic relationship, demonstrable by the experiments just described, was less definite than that found between the viruses of meningopneumonitis and lymphogranuloma venereum (9).

Complement Fixation Tests

In view of the fact that the morphologic and pathogenic characteristics of the mouse pneumonitis virus, as described in previous sections of this paper, resembled in many respects comparable properties of the viruses of human pneumonitis (8), lymphogranuloma venereum (12), and meningopneumonitis (6, 8), it was considered of some interest to determine whether or not antigens of the mouse pneumonitis virus would fix complement with sera of human beings and animals immune to the latter agents.

Antigens for complement fixation were prepared from yolk sac cultures of the mouse pneumonitis virus and the virus of lymphogranuloma venereum. The virus of meningopneumonitis was propagated in the allantoic sac of chick embryos. The techniques of preparing antigens, immune sera, and of performing the complement fixation test have been described previously (9). The results of the tests are presented in Table II.

Sera from cases of pneumonitis known to be caused by the elementary body virus described by Eaton, Beck, and Pearson (8), and from cases of lymphogranuloma venereum gave moderate to strong complement fixation with the mouse pneumonitis virus. Normal human sera gave weak or negative reactions. In addition to the results shown in Table II, tests on many other specimens of human serum indicated a parallelism between the reactions of these sera with antigens prepared from the viruses of lymphogranuloma venereum, meningopneumonitis, and mouse pneumonitis. There was no evidence of cross-reaction with other viruses as *e.g.* influenza and lymphocytic choriomeningitis.

The sera of animals immunized with the viruses of meningopneumonitis and lymphogranuloma venereum also gave complement fixation with the mouse pneumonitis antigen as indicated in the lower half of Table II.

Attempts to produce complement-fixing antibodies in mice, rabbits, guinea pigs, and hamsters by immunization with the mouse pneumonitis virus were only partially successful. Many of the animals (particularly guinea pigs)

TABLE II

Complement Fixation Reactions of Mouse Pneumonitis Antigen with Human and Animal Sera from Individuals Immune to the Meningopneumonitis-Lymphogranuloma Venereum Group of Viruses

Serum	Immune to:	Serum dilutions with:					Serum control
		Yolk sac antigen mouse pneumonitis				Normal yolk sac	
		1:4	1:8	1:16	1:32	1:4	1:4
Human A.....	Pneumonitis	++++	++++	++++	++++	±	0
Human B.....	Pneumonitis	++++	++++	++++	+++	0	0
Human C.....	Pneumonitis	+++	+++	++	+	0	0
Human G.....	Lymphogranuloma venereum	++++	+++	+++	±	0	0
Human H.....	Lymphogranuloma venereum	++++	+++	++	+	0	0
Human L.....	Normal	±	0	0	0		
Human M.....	Normal	+	+	0	0	±	0
Human N.....	Normal	±	0	0	0	0	0
Mouse 120....	Meningopneumonitis	++++	++++	++++	+++	±	±
Mouse 115....	Meningopneumonitis	++++	+++	+++	+++	0	0
G.P. 177......	Meningopneumonitis	+++	+++	++	++	0	0
Rat 12........	Lymphogranuloma venereum	++++	++++	+++	++	0	0
G.P. 117......	Lymphogranuloma venereum	++	0	0	0	0	0
Hamster 593..	Lymphogranuloma venereum	++	++	±	0	±	±

showed little or no reaction even with the homologous strain after repeated intraperitoneal inoculations. The results suggested that the mouse pneumonitis virus is a poorer antigen than the viruses of meningopneumonitis and lymphogranuloma venereum. Also, in the complement fixation test, it was necessary to use higher concentrations of the mouse pneumonitis antigen to obtain fixation comparable with that obtained with the other two viruses.

Complement fixation reactions of animal sera with the three viruses are presented in Table III. It will be noted that cross-reactions were rather irregular, some animals showing more fixation with the meningopneumonitis virus than

with the homologous strain. Mouse serum 129 was obtained from a group of mice immunized with the elementary body virus isolated from hamsters by Dr. Taylor. This agent, although incapable of producing active immunity against the strain isolated from mice, apparently contains a group-reactive antigen detectable by complement fixation.

TABLE III

Complement Fixation with Antigens Prepared from Mouse Pneumonitis, Meningopneumonitis, and Lymphogranuloma Venereum, and the Sera of Animals Immune to Mouse Pneumonitis Virus

Anti-mouse pneumonitis serum	Titer with antigens from:			
	Mouse pneumonitis	Meningo-pneumonitis	Lympho-granuloma venereum	Normal tissue
Mouse 114	16	0	8	0
G.P. 179	0	8	2	0
Hamster 581	8	16	8	0
Rabbit 44	32	8	16	8
Mouse 129*	16	32	32	0

* Immunized with pneumonia virus isolated from hamsters by Dr. Taylor.

DISCUSSION

The comparative data which have been summarized, show that although the virus under study has certain properties in common with other pneumonia-producing viruses, it is so sharply differentiated in certain other respects as to establish it as a virus hitherto undescribed. The characteristic pulmonary lesions alone would serve to differentiate it from all the other viruses with which it has been compared except that of Eaton, Beck, and Pearson. From this it is differentiated by its greater virulence and its complete lack of neurotropic properties.

On the basis of present knowledge, certain of the rodent-pneumonia viruses which apparently produce latent infections are classifiable into two groups:—

1. The mouse pneumonia virus described by Horsfall and Hahn (1), and the antigenically related hamster pneumonia virus isolated by Pearson and Eaton (10). These two agents produce lesions in mice which closely resemble those of the influenza virus. They are approximately the same size as the influenza virus. Like the latter, they are readily neutralized by homologous antiserum and produce solid immunity in experimental animals by subcutaneous or intraperitoneal injection. Horsfall and Hahn (1) have presented observations which indicate that their mouse pneumonia virus may possess a minor antigenic component in common with type A influenza virus.

2. The elementary-body group is antigenically distinct from the above described

group, produces focal or patchy grey lung lesions in mice, kills more rapidly after intranasal inoculation than the influenza-like viruses, and tends to produce systemic infection and carrier states. These properties, together with the formation of elementary bodies which show differential staining by the Castaneda or Macchiavello methods, serve to distinguish the mouse pneumonitis virus described in this paper and the hamster pneumonia virus isolated by Dr. Taylor from the influenza-like group of viruses. The rodent pneumonia viruses which form elementary bodies are not easily neutralized by immune serum and do not produce immunity on intraperitoneal inoculation.

The mouse bronchopneumonia virus recently described by Gönnert (11) probably belongs in group 2 on the basis of morphological similarity and cross-reactions with lymphogranuloma venereum in Frei tests. Karr (12) has recently described a pneumotropic virus which is very similar to if not identical with that described in this paper. It is not possible, from the information available, to say whether the mouse pneumonia viruses described by Dochez, Mills, and Mulliken (2) and by Gordon, Freeman, and Clampit (3) fall into either of the groups just described or represent still other agents as yet unclassified.

By their lack of neurotropism, the rodent pneumonia viruses classified in group 2 above are distinguishable from agents such as the viruses of lymphogranuloma venereum, meningopneumonitis, and psittacosis which form similar elementary bodies (*cf.* Rake, Eaton, and Shaffer (13)). However a relationship to the latter group of viruses is demonstrable by cross-reactions in the complement fixation test and by many other resemblances (*cf.* Rake, Jones, and Nigg (14)). It seems justifiable, therefore, to include all of these agents in one group despite the fact that the antigenic relationship of the mouse pneumonitis virus to other members of the group has not been conclusively demonstrated by active immunity tests in experimental animals.

Mention should be made of a virus, recently isolated by Baker (15) from a pneumonia of cats, which may be related to the etiological agent of human atypical pneumonias. The properties of this cat virus are similar in certain respects to those of the mouse pneumonitis virus described in this paper. The antigenic relationship between these viruses has recently been studied by Thomas and Kolb (16).

Other agents which produce lung lesions in rodents, namely the coccobacillary bodies of mouse and rat catarrh (Nelson (17)) and certain of the pleuropneumonia-like group (Edward (18)) are distinguishable from the elementary-body viruses just described by morphological, tinctorial, and cultural characteristics.

SUMMARY

1. A pneumotropic virus which forms elementary bodies has been isolated from apparently normal albino Swiss mice.

2. The antigenic relationship of this virus to those of meningopneumonitis, lymphogranuloma venereum, hamster pneumonia (7), and human pneumonitis (8) was established either by cross-immunity or complement fixation or both.

3. In spite of a relationship to other viruses, the virus could be differentiated from all the others studied by certain of its properties.

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EXPLANATION OF PLATE 8

These photographs were made by Mr. Julius Weber.

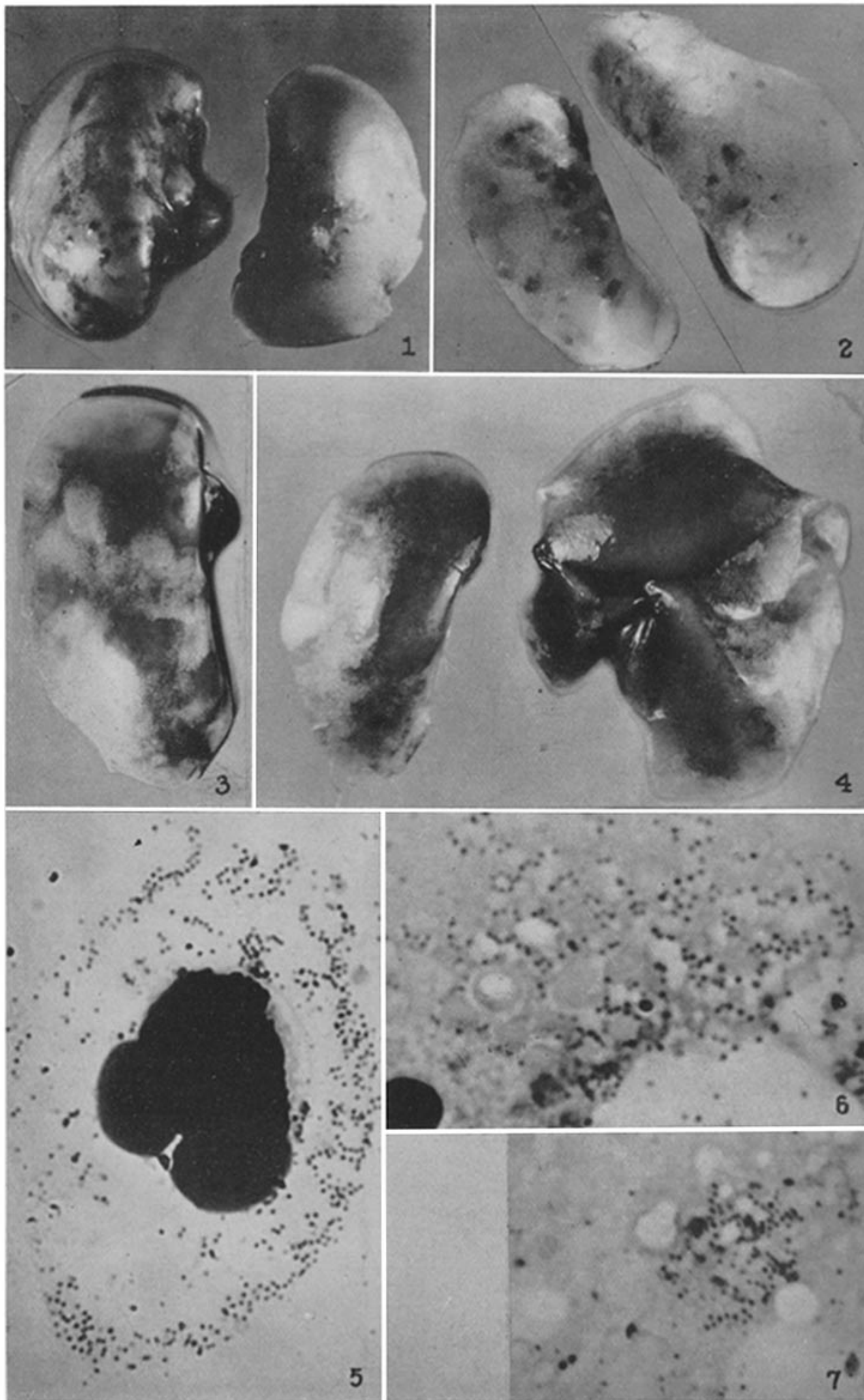
FIGS. 1 and 2. Focal pulmonary lesions in the left lobes of lungs from mice inoculated 7 days previously with 10^{-7} dilution of infected mouse lung. × 3.

FIG. 3. Areas of pulmonary consolidation in a mouse inoculated 4 days previously with 10^{-4} dilution of infected mouse lung. ×3.

FIG. 4. Pulmonary consolidation in a mouse inoculated 3 days previously with 10^{-3} dilution of infected mouse lung. ×3.

FIG. 5. Virus particles in the cytoplasm of a large mononuclear cell (the nucleus partially covers two erythrocytes) in an impression preparation from a cut section of infected mouse lung in the very early stage of infection. Giemsa stain. ×2500.

FIGS. 6 and 7. Virus particles in yolk sac cultures. Impression preparations. Giemsa stain. ×2500.



(Nigg and Eaton: Isolation from mice of pneumotropic virus)