

THE ENHANCING EFFECT OF CONCURRENT INFECTION
WITH PNEUMOTROPIC VIRUSES ON PULMONARY
TUBERCULOSIS IN MICE

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PLATES 21 AND 22

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It is well established that healthy mice may harbor in their lungs one or more viruses in a latent state and it has been shown that various non-specific stimuli may serve to provoke or activate these agents with the result that infection develops (1). As a consequence, a number of experimental manipulations, especially those in which native material of animal origin foreign to the host are injected, may incur the risk of inadvertent provocation of a latent virus. Since infection of mice with mammalian tubercle bacilli can result in chronic disease, it appeared possible that the course of tuberculous infection in these animals might be influenced by the concurrent development of infection with a latent virus. It would be important to know, therefore, whether viral infection of the lungs of mice, if superimposed upon infection with tubercle bacilli, can alter the course and outcome of the infection caused by the bacterium alone.

It is the purpose of this paper to present the results of experiments in which mice given tubercle bacilli intraperitoneally were also given small amounts of either pneumonia virus of mice (PVM) or influenza A virus (PR8) intranasally. It will be shown that, following infection with either of these pneumotropic viruses, tuberculous lesions in the lungs of mice develop more rapidly and become more extensive than in control animals infected only with tubercle bacilli.

Methods

Tubercle Bacillus.—One mammalian strain of tubercle bacillus, H37RV, was used. It was maintained in liquid culture in Tween-albumin medium and frequently was passed through mice (2, 3). The virulence for mice of H37RV maintained in this manner has been found to be constant (3). Throughout this study 7-day-old cultures in Tween-albumin liquid medium were employed; they contained approximately 10^8 to 10^9 living microorganisms per cc. As routine, the bacteria were injected by the intraperitoneal route in a total volume of 0.20 to 0.25 cc. Undiluted culture or culture diluted to varying degrees in sterile Tween-albumin medium was employed as indicated in the text. The quantity of tubercle bacilli injected is indicated in the tables in terms of the amount of culture present in the inoculum.

Viruses.—Pneumonia virus of mice (PVM), strain 15 (4), and the PR8 strain (5) of influenza A virus were employed. Each virus was maintained by occasional lung passage in Swiss mice and suspensions of infected mouse lungs were stored at -70°C . As routine, the desired virus was diluted in broth containing 10 per cent normal horse serum and given by the intranasal route in a volume of 0.05 cc. to each mouse under light ether anesthesia. The quantity of virus inoculated was calculated from the 50 per cent maximum score end point (*i.e.*, M.S.50) (6) which, prior to the experiment, was determined in mice of the strain employed. In certain experiments an amount of virus equal to 1 M.S.50 dose was used; in others, still smaller amounts were employed and they are indicated in the tables as fractional parts of 1 M.S.50.

Mice.—Two strains of albino mice were used. One, the so called Rockefeller Institute strain, was employed in Experiments 1 and 2; the other, a strain of Swiss mice obtained from a commercial breeder,¹ was employed in Experiments 3 to 7. The age of mice at the time of inoculation was 4 to 5 weeks. During the experimental period the mice were kept in glass jars, 5 to 6 animals in each, and were fed a diet of white bread and milk. It is important to emphasize that the composition of the diet has a marked influence on the course and outcome of tuberculous infection (7). The susceptibility of Rockefeller Institute and Swiss strains of mice to infection with tubercle bacilli, H37RV, was similar. The two strains of mice showed, however, different susceptibilities to infection with PVM. The M.S.50 end point with PVM was, therefore, determined by separate titrations in each strain of mice and the amount of virus given was calculated from the results obtained in mice of the same strain. In Experiments 1 to 3 an observation period of 3 weeks was employed; in Experiments 4 to 7 the observation period was extended to 6 weeks.

Pulmonary Lesions.—Following an observation period of desired duration, all surviving mice were killed and their lungs examined for the presence of tuberculous lesions. Mice which died within 2 weeks following inoculation with either virus were discarded. Mice which died later than 2 weeks after inoculation and showed extensive tuberculous lesions in their lungs were included in the experimental results. The lungs from numerous mice in both the experimental and control groups were fixed, sectioned, and studied under the microscope. Pulmonary lesions induced either by PVM or PR8 are very different from those caused by tubercle bacilli and the latter can be distinguished readily on gross examination. In the present study interest centered entirely on the tuberculous lesions in the lungs of mice. To facilitate the presentation of results and to express the extent of the tuberculous lesions observed, the following notation was adopted: 0 = lungs which showed no gross tuberculous lesions; \pm = lungs which showed small reddish spots or tiny hemorrhagic areas which could not definitely be considered to be of tuberculous origin on gross examination; + = lungs which showed one or two readily visible tuberculous lesions; ++ to ++++ = lungs which showed in increasing number and extent distinct tuberculous lesions ranging from large circumscribed lesions to innumerable smaller lesions which filled almost the entire lung field; D++++ = lungs of mice which died with extensive tuberculous lesions more than 2 weeks after inoculation. For the purpose of this study all lungs which showed tuberculous lesions that were assigned a value of + or more were included as positive in the analysis of the results.

To express in a single figure all of the results obtained in a group of mice the lung lesion score ratio was determined in a manner analogous to that employed previously for lesions induced with pneumotropic viruses (6). For this purpose results ranging from + to D++++ were given corresponding numerical values from 1 to 5. The sum of the numerical scores obtained for a group of animals was divided by the maximum possible score (*i.e.*, the

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number of mice in the group, multiplied by 5). The result is given in the tables as the lung lesion score ratio. This ratio possesses the advantage that it expresses not only the frequency but also the extent of tuberculous pulmonary lesions observed in a given group of animals. For example, in a group of mice which showed no lesions the score ratio would be 0.0, whereas in a group all of which died with ++++ lesions, the score ratio would be 1.0.

EXPERIMENTAL

Simultaneous Infection with Tubercle Bacilli and PVM.—Experiments were carried out to determine whether the course of tuberculous infection in mice would be altered by a superimposed infection with PVM when the bacterium and the virus were given almost simultaneously. Pierce, Dubos, and Middlebrook (3) showed previously that albino mice infected intraperitoneally with 0.20 to 0.25 cc. of undiluted culture of tubercle bacilli in Tween-albumin medium showed very few pulmonary lesions during an observation period of 3 weeks. However, extensive pulmonary lesions and often death of the mice resulted from the injection of the same quantity of tubercle bacilli when egg yolk was added to the inoculum.

Three different experiments (Nos. 1, 2, and 3) were carried out during a period of 4 months. In Experiments 1 and 2 the Rockefeller Institute strain of mice was employed; in Experiment 3 Swiss mice were employed. Each mouse was infected intraperitoneally with 0.20 or 0.25 cc. of undiluted culture of tubercle bacilli; in addition, half of the mice in each experiment were inoculated intranasally with PVM. Virus and bacteria were given on the same day. The amount of virus given was equivalent to 1 M.S.50 dose, an amount which is sufficient to cause, on the average, consolidation of approximately 50 per cent of the lung in each mouse. The observation period was 3 weeks. At the end of that time all surviving mice were killed and their lungs examined for the presence of gross tuberculous lesions.

The results are shown in Table I. It was found that in mice which received tubercle bacilli alone, 17 per cent showed pulmonary lesions (score ratio = 0.061) clearly demonstrable in the gross, whereas in mice which received tubercle bacilli and PVM (1 M.S.50) 73 per cent showed definite tuberculous lung lesions with a score ratio of 0.329. These findings indicate clearly that, under the experimental conditions employed, a superimposed infection with PVM led to a striking increase in pulmonary lesions associated with tubercle bacilli. It should be emphasized that the chief effect of concomitant infection with PVM appeared to be in accelerating the rate of the tuberculous infection in the lung. If mice in similarly infected control groups were observed for 6 weeks, the percentage which showed gross tuberculous pulmonary lesions was considerably increased.

The amount of PVM given in the experiments described above led to the development of relatively extensive viral pneumonia. It was of interest to determine whether amounts of virus sufficiently small to induce little or no pneumonia also would show a similar effect on the tuberculous process in the lungs.

Experiments were carried out both in Rockefeller Institute and Swiss strains of mice. The cultures of tubercle bacilli and the amounts given intraperitoneally were identical to those described above. The experiments (*i.e.*, 2a, 3a, and 3b) were carried out simultaneously with Experiments 2 and 3. One group of mice were given, simultaneously with the injection of tubercle bacilli, an amount of PVM equivalent to 0.1 M.S.50 dose; two other groups were given an amount of PVM equivalent to 0.01 M.S.50 dose. 0.1 M.S.50 dose of PVM induces

TABLE I
The Effect of Simultaneous Inoculation with Tubercle Bacilli and Either PVM or PR8 on the Development of Tuberculous Pulmonary Lesions in Mice

Ex- peri- ment No.	Amount of tubercle bacillus culture I.P.*	Intranasal inoculation 0.05 cc.	No. of mice	No. of mice with indicated pulmonary lesions 3 weeks after infection with tubercle bacilli							Per cent of mice with tuber- culous pulmonary lesions, + or greater	Lesion score ratio
				0	±	+	++	+++	++++	D++++		
1	0.25	0	8	7					1			
2	0.20	0	15	4	8	2	1					
3	0.25	0	29	20	4	3	1	1				
Total (1, 2, 3)			52	24	19	5	2	1	1		17	0.061
1	0.25	PVM 1 M.S.50	11	1		3	1	6				
2	0.20	" "	12	3	1	4	3	1				
3	0.25	" "	25	8		2	8	4	2	1		
Total (1, 2, 3)			48	12	1	9	12	11	2	1	73	0.329
3a	0.25	PVM 0.1 M.S.50	23	3	4	2	5	3	3	3	69	0.417
2a	0.20	PVM 0.01 M.S.50	13	8		2	2	1				
3b	0.25	" "	24	10	3	3	3	4		1		
Total (2a, 3b)			37	18	3	5	5	5		1	43	0.189
2b	0.20	Horse serum broth	15	9	2	4					27	0.053
3c	0.25	Human serum	12	1	1	4	4	1		1	83	0.334
3d	0.25	PR8 1 M.S.50	22	12		4	4	4		2	45	0.273

* In the tables I.P. = intraperitoneal inoculation.

only small lesions in the lungs of mice but is capable of causing some evidence of pneumonia in the large majority of inoculated animals. 0.01 M.S.50 dose of PVM induces very tiny lesions and, moreover, only a small percentage of inoculated animals show any evidence of pneumonia (8). The observation period in these experiments was 3 weeks. Animals which survived were then killed and their lungs examined as described above.

The results are summarized in Table I. It will be seen that 69 per cent of mice which received 0.1 M.S.50 dose of PVM showed gross tuberculous pulmonary lesions (score ratio = 0.417) and that 43 per cent of mice which received 0.01 M.S.50 dose of PVM showed similar lesions with a score ratio of 0.189. When these results are compared with those obtained in mice which

were given tubercle bacilli but no virus, it appears evident that concomitant infection with very small amounts of PVM led to a definite increase in tuberculous lesions in the lungs. Comparison with the results obtained in mice which were given 1 M.S.50 dose of virus shows that a tenfold decrease in the amount of virus inoculated did not decrease the effects of the viral infection on the course of the tuberculous infection. A decrease of 100-fold in the amount of virus inoculated caused some decrease in the effect but even this very small amount of virus was capable of causing a significant increase in the incidence of tuberculous lesions in the lung.

The Effect of Intranasal Instillation of Serum.—The virus employed in the experiments described above was diluted in broth containing normal horse serum which had been heated at 56°C. for 30 minutes. To determine whether intranasal inoculation with this diluent itself had any effect on the tuberculous process, a further experiment was carried out.

In Experiment 2b mice received 0.2 cc. of culture of tubercle bacilli intraperitoneally and also were given, by the intranasal route, 0.05 cc. of 10 per cent heated normal horse serum in broth. The animals were killed 3 weeks later and their lungs examined.

The results are shown in Table I. It was found that 27 per cent of the animals showed gross tuberculous pulmonary lesions with a score ratio of 0.053, a result which is not significantly different from that obtained in control animals given tubercle bacilli alone and no further inoculation.

In addition, the effect of the intranasal instillation of fresh human serum was determined. This was done because recent work in this laboratory has shown that fresh human serum is effective as a stimulus for the provocation of PVM latent in mouse lungs.

Simultaneously with the intraperitoneal injection of 0.25 cc. of undiluted culture of tubercle bacilli, mice in Experiment 3c were given intranasally 0.05 cc. of fresh human serum diluted 1:2 in saline. They were observed for 3 weeks, following which their lungs were examined.

The results are presented in Table I. It will be noted that 83 per cent of mice showed gross tuberculous pulmonary lesions with a score ratio of 0.334. Because of the length of the observation period and the fact that PVM is not demonstrable in mouse lungs later than 12 days after infection (9), it was not possible to prove directly in this experiment that latent PVM actually had been provoked in the mice infected with tubercle bacilli. There is, however, little reason to doubt that this occurred inasmuch as it was shown that the serum used was effective as a provoking stimulus for latent PVM in the strain of Swiss mice employed in the experiment. It seems probable, therefore, that the activation of PVM latent in the mouse lung results in an effect upon the tuberculous process which is similar to that induced by intranasal inoculation of the virus.

The Effect of Intranasal Inoculation with Influenza A Virus.—Because concomitant infection with PVM alters the course of pulmonary infection with tubercle bacilli in mice, it became of interest to determine whether infection with a different pneumotropic virus would cause a similar effect. For these experiments the PR8 strain of influenza A virus was employed.

In Experiment 3d mice were given 0.25 cc. intraperitoneally of undiluted culture of tubercle bacilli. In addition, one group of mice received intranasally an amount of PR8 equivalent to 1 M.S.50 dose. The observation period was 3 weeks.

The results are shown in Table I. It will be observed that 45 per cent of the mice which received both PR8 and tubercle bacilli showed gross tuberculous pulmonary lesions with a score ratio of 0.273. This result indicated that a superimposed infection with PR8 was capable of enhancing pulmonary tuberculosis in mice in a manner analogous to infection induced with PVM.

Infection with Tubercle Bacilli Followed by Infection with Either PVM or PR8.—In the experiments described above the inoculations were almost simultaneous whenever both tubercle bacilli and a pneumotropic virus were given. Further experiments were performed to determine whether infection with either of the viruses would influence the tuberculous pulmonary lesions even when initiated several weeks after injection of tubercle bacilli.

As has been shown, when 0.20 or 0.25 cc. of undiluted culture of tubercle bacilli was given intraperitoneally, a small percentage of mice developed gross pulmonary lesions during a period of 3 weeks. However, when animals infected in this manner were held for 6 weeks, the incidence of gross tuberculous lesions was increased considerably. In the present experiments it appeared desirable to extend the observation period to 6 weeks and, as a consequence, it was necessary to reduce markedly the quantity of tubercle bacilli injected. Preliminary experiments indicated that with the strain of mice used an inoculum equivalent to 0.005 cc. or less of culture would be suitable for the purpose of this study.

Two experiments (Nos. 4 and 5) were carried out in Swiss mice during a period of 5 months. In both experiments all mice were given 0.005 cc. of culture of tubercle bacilli intraperitoneally. Three weeks later the animals were divided into groups of approximately equal size. One group was given intranasally an amount of PR8 equivalent to 1 M.S.50 dose, another group was given intranasally an amount of PVM equivalent to 0.1 M.S.50 dose, and two groups received no second inoculation. Three weeks after inoculation with either virus, *i.e.* 6 weeks after inoculation with tubercle bacilli, the mice were killed and their lungs examined as in previous experiments.

The results are shown in Table II. It will be seen that among control mice given tubercle bacilli but not inoculated with either virus, 22 per cent showed gross tuberculous pulmonary lesions with a score ratio of 0.094. Among mice which received PR8 3 weeks after inoculation with tubercle bacilli, 61

per cent showed similar pulmonary lesions (score ratio = 0.238), whereas among mice given PVM under analogous conditions, 73 per cent showed gross pulmonary tuberculosis (score ratio = 0.572).

TABLE II
The Effect of Inoculation with Either PVM or PR8 Virus Following Infection with Tubercle Bacilli on the Development of Tuberculous Pulmonary Lesions in Mice

Ex- per- iment No.	Amount of tubercle bacillus culture I.P.	In- terval be- tween inoc- ula- tions	Intranasal inoculation 0.05 cc.	No. of mice	No. of mice with indicated pulmonary lesions 6 weeks after infection with tubercle bacilli							Per cent of mice with tuber- culous pulmonary lesions, + or greater	Le- sion score ratio	
					0	±	+	++	+++	++++	D++++			
4	0.005		0	30	5	22	3							
5	0.005		0	25	3	13	2	4	1		2			
Total (4, 5).....				55	8	35	5	4	1		2	22	0.094	
4	0.005	3	PR8 1 M.S.50	26	1	9	5	7	4			61	0.238	
5	0.005	3	PVM 0.1 M.S.50	22	2	4	1	3	1	2	9	73	0.572	
Total (4, 5).....				48	3	13	6	10	5	2	9	67	0.392	
6	0.000,5		0	23	4	15	2	1			1	17	0.078	
6a	0.000,5	3	PR8 1 M.S.50	18	1	7	3	2	1	2	2	55	0.311	
6b	0.000,5	3	PVM 0.1 M.S.50	18	1	5	4	3	2		3	67	0.345	
Total (6a, 6b).....				36	2	12	7	5	3	2	5	61	0.328	
7	0.000,05		0	16	5	9	1				1	12	0.067	
7a	0.000,05	3	PR8 1 M.S.50	24		20	2				2	17	0.120	
7b	0.000,05	3	PVM 0.1 M.S.50	16		13	2	1				19	0.062	
Total (7a, 7b).....				40		33	4	1			2	17	0.080	

Further experiments were carried out with even smaller amounts of tubercle bacilli. In Experiments 6 and 7 Swiss mice were given intraperitoneally 0.000,5 and 0.000,05 cc., respectively, of culture of tubercle bacilli. Three weeks later the animals in each experiment were divided into three groups. One group served as controls and did not receive a second inoculation. Another group was inoculated intranasally with PR8 equivalent to 1 M.S.50 dose, and the last group received PVM in a dose of 0.1 M.S.50. Three weeks after the virus inoculation, *i.e.* 6 weeks after inoculation with tubercle bacilli, all mice were killed and their lungs examined.

The results are shown also in Table II. It was found that among control mice which received 0.000,5 or 0.000,05 cc. of culture of tubercle bacilli alone, 17 and 12 per cent, respectively, (score ratios = 0.078 and 0.067) showed gross tuberculous pulmonary lesions at the end of the 6 weeks observation

period. Among animals given 0.000,5 cc. of tubercle bacilli and PR8 or PVM 3 weeks later, 55 and 67 per cent, respectively (score ratios = 0.311 and 0.345) showed similar lesions. However, among mice which received only 0.000,05 cc. of tubercle bacilli and were inoculated with either pneumotropic virus 3 weeks later, the incidence of tuberculous pulmonary lesions was not significantly different from that found in control animals. These results indicate that pulmonary infection with either PVM or PR8, when superimposed upon tuberculous infection previously initiated by relatively small inocula, increases both the incidence and the extent of gross tuberculous lesions in the lungs of mice. When, however, the inoculum of tubercle bacilli was sufficiently small (*i.e.*, 0.000,05 cc.), neither virus caused a definite alteration in the course of the tuberculous process during the relatively short observation period employed.

Histology of Pulmonary Lesions.—Pierce, Dubos, and Middlebrook (3) have shown that the intraperitoneal inoculation of mice with a sufficient amount of tubercle bacilli causes pulmonary lesions, enlargement of the spleen, and of the abdominal and thoracic lymph nodes. In the present study, as was stated earlier, it was the tuberculous pulmonary lesions which were of chief interest.

Lungs obtained from mice given tubercle bacilli alone, and from animals also inoculated with either PVM or PR8, were studied histologically. Lungs which showed, in the gross, lesions of varying extent were selected from different groups of mice in several experiments. They were fixed promptly either in Zenker's fluid or in formalin. Numerous sections were prepared for microscopic examination; some were stained with hematoxylin and eosin and other parallel sections were stained by the Ziehl-Neelsen technique.

Microscopic examination showed that all mice given tubercle bacilli intraperitoneally developed evidence of some tuberculous infection of the lungs. This was found to be the case irrespective of the amount of tubercle bacilli inoculated. No evidence of significant qualitative differences in the tuberculous lesions was found in the lungs of mice which received tubercle bacilli and either PVM or PR8 as compared with those which received tubercle bacilli alone. However, in agreement with the gross findings, striking quantitative differences were observed. The lungs of animals infected both with tubercle bacilli and a pneumotropic virus showed, in general, more numerous and more extensive tuberculous lesions than did control animals infected only with tubercle bacilli. It was found that the histological findings in different lungs, which on gross examination had been assigned similar lesion scores (*i.e.*, from 0 to ++++) according to the notation described above, corresponded reasonably well one with another. Moreover, it appeared that the extent of the lesions visible in the gross reflected fairly closely the degree of pathological alteration demonstrable on microscopic examination.

In lungs which showed no gross tuberculous lesions (*i.e.*, = 0) only occasional submiliary tubercles were seen. These lesions were composed largely of

epithelioid cells and were situated interstitially, usually in perivascular or peribronchial areas. Infiltration with lymphocytes and plasma cells was minimal. (Fig. 1.) The number of tubercles present varied somewhat but usually 2 to 6 were found in a section of such a lung. In lungs which showed \pm lesions in the gross, the microscopic picture was but little different. The tubercles were a little larger and usually infiltration with lymphocytes was more pronounced. In lungs which showed + to +++++ lesions on gross examination, definite miliary tubercles were present. These were usually composed of a number of small confluent tubercles which were often situated in peribronchial or perivascular areas. Epithelioid cells were predominant and there was some infiltration with lymphocytes and a few plasma cells. (Fig. 2.) At the periphery of such lesions and in the adjacent lung tissue a caseous pneumonic process was often present. The alveoli in these areas were filled with exudate which contained desquamated and degenerating cells. If the tubercle was large, true necrosis was found at its center. (Fig. 3.) One or more bronchi were often invaded by the process. In such instances tuberculous bronchitis was present and the bronchial lumen was filled with exudate, desquamated and necrotic cells. When tubercles were present near the surface of the lung, the pleura was invaded frequently and localized pleuritis occurred.

In sections stained to show acid-fast microorganisms no tubercle bacilli could be demonstrated in the small submiliary tubercles. However, in the large tubercles, when necrosis was present, tubercle bacilli were visualized and sometimes were present in tremendous numbers. (Fig. 4.) Cultures of the lungs were made in certain instances and tubercle bacilli were obtained regularly even from lungs which showed no gross lesions. It should be mentioned that in no instance was it possible to find typical multinuclear giant cells in the tuberculous lesions. The histological findings demonstrated clearly that the lesions observed on gross examination of the lungs were tuberculous in nature. (Figs. 5 and 6.)

DISCUSSION

The evidence obtained in this study indicates that the course of experimental infection with tubercle bacilli in mice is altered by concurrent or superimposed infection with either of two pneumotropic viruses. The chief effect of the viral infections appears to be an acceleration of the tuberculous pulmonary process. In control animals which were given tubercle bacilli but were not inoculated with either virus, gross evidence of pulmonary tuberculosis developed in a small but appreciable proportion of instances. If mice were inoculated with very small amounts of a pneumotropic virus (*i.e.*, PVM or PR8) in addition to the injection of tubercle bacilli, the frequency of the occurrence of gross pulmonary tuberculosis was increased strikingly.

Microscopic study of the lesions present in the lungs confirmed the gross observations. In all lungs examined some evidence of an active tuberculous infection was found. The differences observed were quantitative, not qualitative, in character; in the lungs of control animals, as a rule, only a few small tubercles were present, whereas in the lungs of animals with a superimposed viral infection there were, in general, more and larger tubercles.

It appears of considerable interest that infection with viruses, as different one from another as are PVM and PR8, should have similar accelerating effects upon the course of pulmonary tuberculosis in mice. Not only are PVM and PR8 wholly unrelated immunologically but also they are of very different size; present evidence indicates that the dimensions of the virus particles of PR8 are of the order of 2.5 times greater than those of PVM (10, 11). Moreover, the rates of multiplication of the two viruses in the mouse lung are dissimilar; with PR8 multiplication is very rapid, and maximal titers usually are reached within 24 hours or less of inoculation (12); with PVM the multiplication rate is slow, and maximal titers usually are not reached until 6 days or more after inoculation (9). However, the pathological alterations which develop in the lungs of mice infected with PVM or PR8 are in many respects closely similar, and it is very doubtful whether lesions induced by one virus can be differentiated from those induced by the other on the basis of histological findings.

It seems probable that the observed effects on the course of pulmonary tuberculosis in mice of superimposed infection with either of the pneumotopic viruses studied are to be attributed to alterations induced in the lung as a result of viral activity and not to any hypothetical action on the tubercle bacillus itself. Even when extremely small quantities of PVM (*i.e.*, 0.01 M.S.50 dose) are given, it can be shown that infection with and multiplication of the virus occur despite the fact that no demonstrable pulmonary lesions or only occasional very minute lesions develop (4). Thus it appears that such small virus inocula may lead to the development of alterations in the normal physiology of the lung and that one expression of the abnormal status so induced may be a relatively increased susceptibility, or decreased resistance, to local invasion by tubercle bacilli. The available evidence suggests that tubercle bacilli find, in the lung infected with small amounts of either PVM or PR8, conditions which are more favorable for growth and for the rapid production of gross pulmonary lesions than are present in the normal lung.

The results obtained indicate that the tuberculous process in the mouse lung can be augmented significantly when a minimal viral infection is initiated even as late as 3 weeks after the injection of relatively small amounts of tubercle bacilli. In this manner it is possible, by experimental means, to convert a mild and but slowly progressing tuberculous infection, which would lead to the development of gross pulmonary lesions in only a small proportion of animals

under the conditions employed, into a distinctly more serious and rapidly progressive disease which results in the appearance of gross pulmonary lesions in a large proportion of animals.

SUMMARY

The course of pulmonary tuberculosis in the mouse appears to be accelerated as a result of concurrent infection of the lung with either of two pneumotropic viruses. This effect is obtained with virus inocula sufficiently small as to induce little or no definite viral pneumonia.

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EXPLANATION OF PLATES

The photographs were all made by Mr. Joseph B. Haulenbeek.

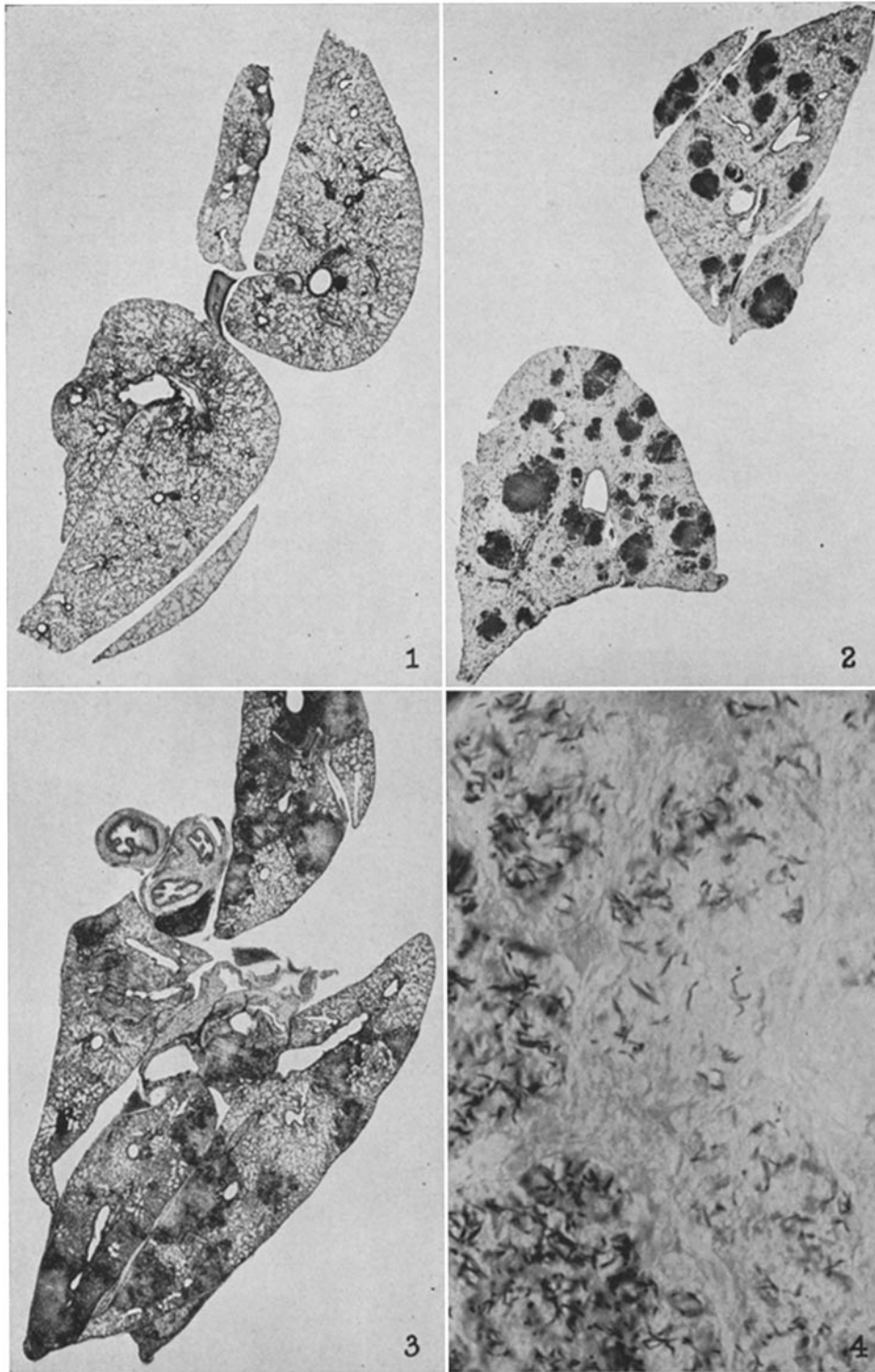
PLATE 21

FIG. 1. Section of the lung of a mouse which was given tubercle bacilli intraperitoneally 6 weeks previously. Hematoxylin and eosin stain. Note absence of gross tuberculous lesions (score = 0). $\times 9$.

FIG. 2. Section of the lung of a mouse of the same strain which was given tubercle bacilli intraperitoneally and PVM intranasally 3 weeks previously. Hematoxylin and eosin stain. Note numerous large tuberculous lesions (score = +++). $\times 9$.

FIG. 3. Section of the lung of a mouse which was given tubercle bacilli intraperitoneally 6 weeks previously and PVM intranasally 3 weeks later. Hematoxylin and eosin stain. Note large tuberculous lesions (score = +++). $\times 9$.

FIG. 4. Section of a tubercle in a mouse similar to that shown in Fig. 2. Ziehl-Neelsen stain. Note large number of acid-fast bacilli. $\times 1000$.

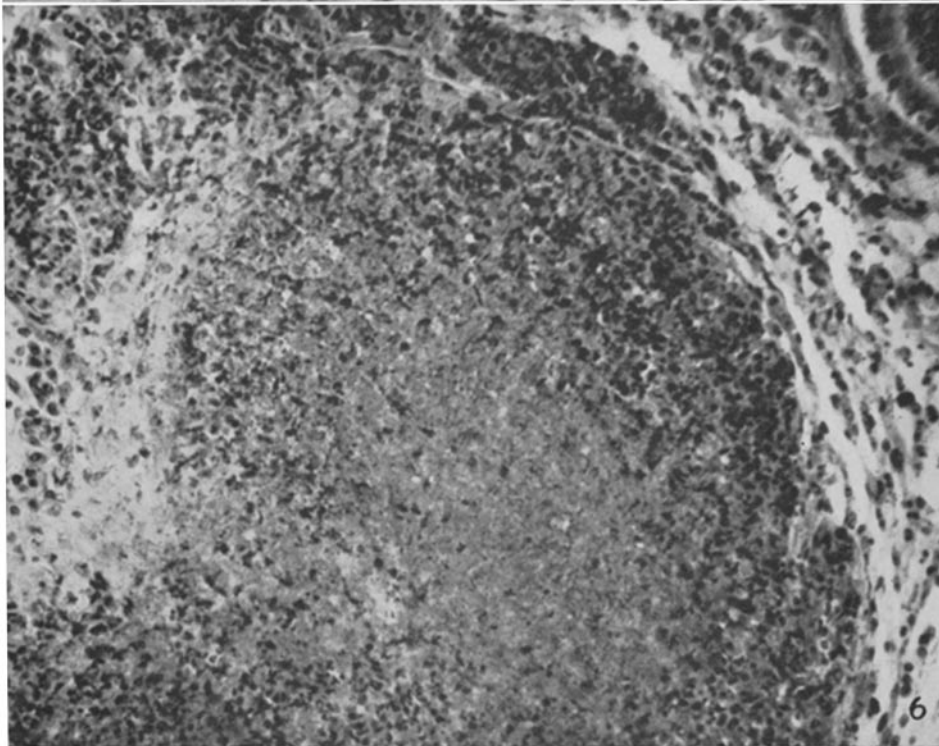
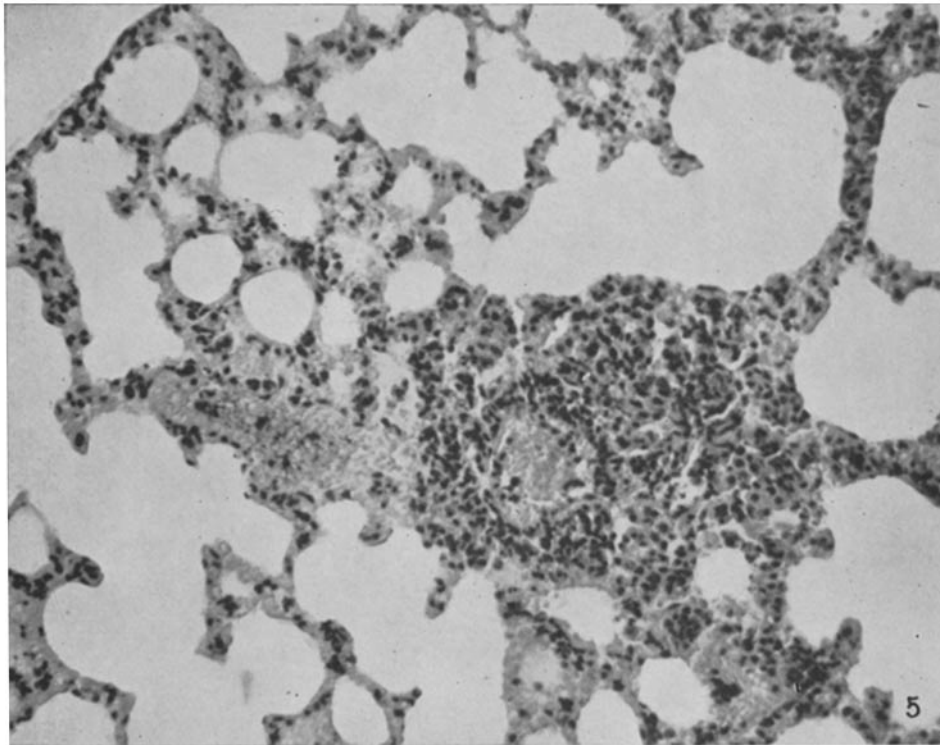


(Volkert *et al.*: Concurrent infection in mice)

PLATE 22

FIG. 5. Section of a submiliary tubercle in the lung of a mouse which was given tubercle bacilli intraperitoneally 6 weeks previously. Hematoxylin and eosin stain. $\times 275$.

FIG. 6. Section of a tubercle in the lung of a mouse which was given tubercle bacilli intraperitoneally and PVM intranasally 3 weeks previously. Hematoxylin and eosin stain. Note central necrosis. $\times 275$.



(Volkert *et al.*: Concurrent infection in mice)