

EXPERIMENTAL EPIDEMIOLOGY OF TUBERCULOSIS
AIR-BORNE CONTAGION OF TUBERCULOSIS IN AN ANIMAL ROOM

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From the time of Koch (1) it has been known that normal guinea pigs confined in the same room, though not in the same cage, with tuberculous animals may acquire tuberculosis. Since then, many confirmatory reports have appeared, which have recently been reviewed by Perla (2). He also observed instances of tuberculosis so acquired and concluded that the route of infection in such cases is the respiratory tract. Many investigators, however, are still sceptical of the occurrence of tuberculosis under these conditions (3). Lydia Rabinowitsch-Kempner has recently published a questionnaire (4) on the subject. Amongst those who replied, Theobald Smith, Calmette and Uhlenhuth had never seen spontaneous tuberculosis, and Krause, Bruno Lange and Griffith had met with it, though extremely rarely. She concludes (5) from this as well as from her own work that although tuberculosis may be acquired naturally by rabbits and guinea pigs, the latter acquire tuberculosis but rarely when living together with tuberculous animals. Some writers (6) stress the hygienic conditions of the laboratory and the nutrition of the animals as determining factors. The following experiment was undertaken to obtain further information.

The main animal room of The Henry Phipps Institute, which measures 29 feet in length, 25 feet in width, and 16 feet in height—a space of 11,600 cubic feet—is accommodated with up to 270 metal cages, measuring 14 x 14 x 15 inches, with wire-mesh doors. These usually house about 2 animals per cage or a total of 540 animals. The cages are arranged on 4 metal stands of 3 tiers each. Upon each tier there are 2 rows of cages facing in opposite directions. Two normal guinea pigs, obtained from The Rockefeller Institute at Princeton, were placed in each of 27

of the cages and left undisturbed. These cages, painted black to make them conspicuous, were distributed evenly about the room and remained in the positions indicated in the figure (Fig. 1). The animals in all the other cages were variously treated during the different experiments in progress.

It is difficult to determine with exactitude what the actual, constantly changing tuberculous population in the animal room and its distribution in time and space has been throughout the period of these experiments—2 years and 8 months—

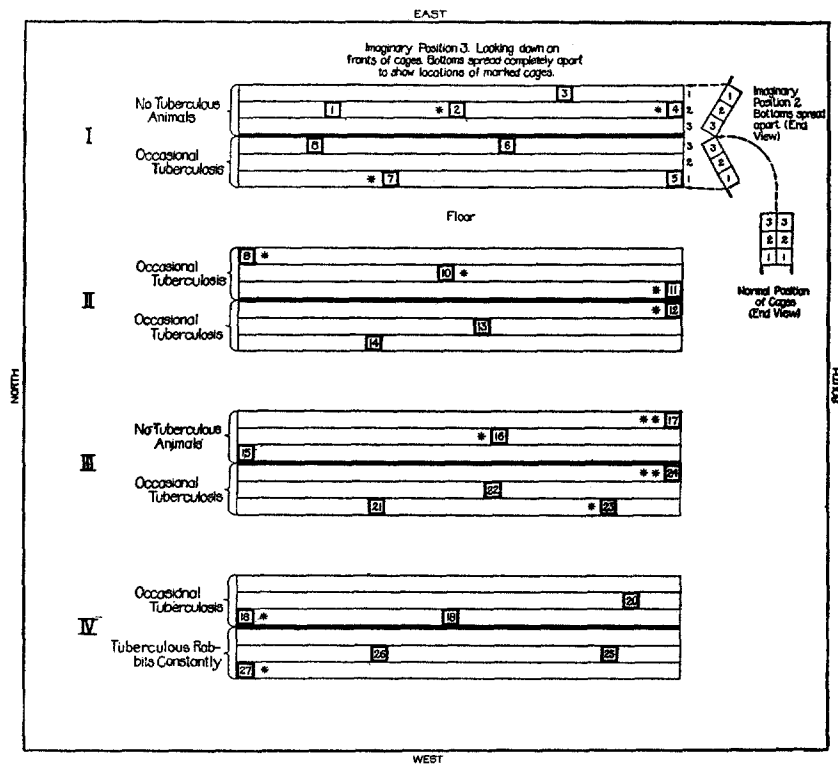


FIG. 1. Each star represents a case of contact tuberculosis

except that in the 3 tiers of cages adjoining and facing the west wall of the room there has been constantly a changing population of tuberculous rabbits. The 3 tiers of cages facing the east wall housed no tuberculous animals at any time; likewise the 3 tiers of Stand III facing the east; in the remaining cages there were often tuberculous animals.

The population of the 27 cages was maintained constant throughout this period by replacing the dying animals with normal guinea pigs. The general care and feeding of these animals has been described in a previous paper (7).

During the entire period 103 guinea pigs have been exposed in these cages. Of these, 58 died of other diseases some time during the 32 months; 15 died with tuberculosis; 21 were killed and showed no tuberculosis, and 9, which had survived the entire period and had shown repeatedly negative tuberculin reactions, were reserved for further experimentation.

The presence of tuberculosis was established on the basis of characteristic, unequivocal tuberculous lesions with characteristic acid-fast bacilli in the smears of these lesions, and, in the case of the slightest doubt, the conclusion was tested by animal inoculation.

In Table I are given the detailed observations in the 15 cases of tuberculosis. Eleven of these animals died of tuberculosis without any complicating cause. Their average survival was 551 days, ranging between 238 and 948 days of exposure. The disease was usually chronic in nature, characterized by a massive tuberculosis of the lungs, often (in 7 guinea pigs) with cavity formation. There was a massive fibrocaseous tuberculosis of the tracheobronchial lymph nodes with little or no involvement of the mesenteric and cervical nodes. Tuberculosis in the liver and spleen was usually only moderate in extent and often had healed in these organs, especially in the liver. In some cases there were lesions closely resembling human tuberculosis of the childhood type, one or several small nodules in the lung being accompanied by massive, caseated tracheobronchial lymph nodes. In others, however, pulmonary lesions occurred without any gross evidence of tuberculosis in the draining lymph nodes, or *vice versa*.

The experimental basis for the judgment as to the route of infection in these guinea pigs is presented in the last paper of this series.

The route of infection in the 3 guinea pigs in which the tuberculous lesions were limited to the tracheobronchial lymph nodes, the lungs or both, is obviously the respiratory tract. Five showed extensive pulmonary and tracheobronchial lesions as well as tuberculosis in other organs, but no affection of the mesenteric and cervical lymph nodes. The tuberculosis in these is therefore also definitely respiratory in origin. In 4 guinea pigs the route of infection is probably respiratory in the main, for although the mesenteric and cervical nodes are tuberculous the affection in the tracheobronchial lymph nodes is far greater. In Guinea pig 8 there was massive fibrous pul-

TABLE I
Tuberculosis in Normal Guinea Pigs Exposed in a Room Where Tuberculous Animals Are Housed

Cage No.	Guinea pig No.	Duration of exposure in days	Cause of death	Pathological observations	Route of infection
2	1	502	Tuberculosis	Extensive nodular pulmonary tbc.; extensive fibrocaseous tbc. of tracheobronchial lymph nodes; no tbc. of cervical and mesenteric lymph nodes; extensive tbc. of spleen and liver.	Respiratory tract
4	2	409	Tuberculosis	Extensive pulmonary tbc. with excavation; tracheobronchial lymph nodes slightly enlarged and caseous; single minute focus in mesenteric nodes; no tbc. in cervical lymph nodes; fibrosis of liver; no tbc. in spleen.	Respiratory tract
7	3	649	Tuberculosis	Extensive pulmonary tbc.; extensive fibrocaseous tbc. of tracheobronchial lymph nodes; no tbc. of cervical lymph nodes; slight tbc. of liver and spleen.	Respiratory tract ?
9	4	608	Tuberculosis and pleurisy	Moderate nodular pulmonary tbc. with excavation; extensive fibrocaseous tbc. of tracheobronchial lymph nodes; slight tbc. of mesenteric lymph nodes; tbc. of spleen; slight tbc. of liver with fibrosis.	Respiratory tract ?
10	5	624	Tuberculosis	Moderate pulmonary tbc.; extensive fibrocaseous tbc. of tracheobronchial lymph nodes; extensive tbc. of spleen and liver.	?†
11	6	538	Tuberculosis	Extensive pulmonary tbc.; extensive fibrocaseous tbc. of tracheobronchial lymph nodes; moderate tbc. of liver and spleen.	Respiratory tract ?
12	7	622	Tuberculosis	Slight pulmonary tbc.; all lymph nodes enlarged and caseous; extensive tbc. of spleen; moderate tbc. of liver.	?†
16	8	948	Tuberculosis	Massive fibrous pulmonary tbc. with excavation; tracheobronchial, mesenteric and cervical lymph nodes fibrous; fibrosis of liver; slight tbc. in enlarged spleen.	Respiratory tract ?

† These animals died in the summer in the absence of the writer and no note was made in regard to the involvement of the mesenteric nodes.

TABLE I—*Concluded*

Cage No.	Guinea pig No.	Duration of exposure in days	Cause of death	Pathological observations	Route of infection
17	9	615	Tuberculosis	Moderate fibrous pulmonary tbc. with excavation; massive fibrocaseous tbc. of tracheobronchial lymph nodes; no tbc. of mesenteric and cervical lymph nodes; slight tbc. with fibrosis in liver; moderate tbc. of spleen.	Respiratory tract
	10	513	Jaundice and tbc.*	Fibrous and fibrocaseous tbc. of tracheobronchial lymph nodes; no tbc. anywhere else in the body.	Respiratory tract
18	11	216	Tbc. and broncho-pneumonia	Moderate pulmonary tuberculosis; massive fibrocaseous tbc. of tracheobronchial lymph nodes; no tbc. in mesenteric; single focus in cervical lymph node; moderate tbc. of liver and spleen.	
23	12	238	Tuberculosis	Massive pulmonary tbc. with excavation; massive caseous encapsulated tracheobronchial lymph nodes; moderate caseous and fibrous tbc. of cervical, mesenteric and remaining lymph nodes; massive tbc. of liver and spleen.	Respiratory tract
24	13	257	Tuberculosis	Extensive pulmonary tbc. with excavation; massive fibrocaseous tbc. of tracheobronchial lymph nodes; no tbc. in cervical and mesenteric lymph nodes; no tbc. in liver; moderate tbc. of spleen.	Respiratory tract
	14	661	Tuberculosis	Chronic pulmonary tbc. with excavation; tracheobronchial lymph nodes enlarged and fibrous; no tbc. of mesenteric nodes; no tbc. in liver; moderate tbc. of spleen.	Respiratory tract
27	15	502	Pneumonia and tbc.*	Isolated pulmonary nodular tbc.; massive fibrocaseous tbc. of tracheobronchial lymph nodes; no evident tbc. anywhere else in the body.	Respiratory tract

* Virulent tubercle bacilli demonstrated by animal inoculation in addition to characteristic acid-fast bacilli found in all of the 15 guinea pigs.

monary tuberculosis with excavations, healing in the liver and spleen and fibrosis of the lymphatic system. Here again the infection probably entered by way of the air passages. The remaining 2 guinea pigs died in the summer in the absence of the writer and no note was made in regard to the involvement of the mesenteric nodes; hence in these the route of infection could not be determined. Thus in most and perhaps all of the animals the route of infection was the respiratory tract.

Although the incidence of naturally acquired tuberculosis amongst the 103 guinea pigs is only 14.5 per cent, only 20 of the animals survived exposure for over 2 years. If the duration of exposure is considered in relation to the incidence of the disease, it becomes apparent

TABLE II
Incidence of Tuberculosis in Normal Guinea Pigs Grouped According to Duration of Exposure in a Room Containing Tuberculous Animals

	Duration of exposure				
	3 weeks to 6 months	6 months to 1 year	1 year to 1½ years	1½ years to 2 years	2 years to 32 months
No. exposed.....	15	33	18	17	20
No. developing tuberculosis.....	0	3	5	6	1
Percentage incidence of tuberculosis.....	0	9.1	27.7	35.3	5

that a much greater percentage would have died with tuberculosis had they all survived the entire experimental period.

In Table II it will be seen that none of the 15 guinea pigs exposed for a period of 3 weeks to 6 months developed tuberculosis. Of the 33 animals that were exposed from 6 months to 1 year 3 or 9.1 per cent developed tuberculosis. Two of these died of tuberculosis, one after 238 days and one after 257 days of exposure. The other died 216 days after the beginning of exposure with tuberculosis complicated by bronchopneumonia. Thus a considerable proportion of guinea pigs exposed to tuberculous animals under conditions that are common in many laboratories where investigations in tuberculosis are carried on, have developed an extensive tuberculosis after 7 or 8 months in such an environment.

Of 18 guinea pigs that survived an exposure of 1 to 1½ years, 5 or 27.7 per cent have developed tuberculosis. Of 17 guinea pigs that survived an exposure of 1½ to 2 years, 6 or 35.3 per cent developed tuberculosis. Thus the incidence of tuberculosis increases with the duration of exposure up to this point. But of 20 guinea pigs that have survived an exposure of 2 years to 2 years and 8 months, only 1 or 5 per cent developed tuberculosis. Thus it appears that after a certain length of time a further extension of exposure to the tubercle bacillus is followed by a decrease instead of a still greater increase in incidence of the disease. This striking fact will be considered presently.

The distribution of mortality and morbidity from tuberculosis has been more or less even over the entire room, as is indicated by the stars in Fig. 1. There has been no evidence that guinea pigs exposed in cages close to cages containing tuberculous animals have developed tuberculosis more often than those at a distance from them. Thus in the first 4 cages placed amongst animals none of which had been inoculated with tuberculosis, there were two cases of tuberculosis, whereas in Cages 25, 26 and 27, which were constantly surrounded by tuberculous rabbits, there was only one case of tuberculosis. Apparently the organism is more or less equally distributed over the entire room.

DISCUSSION

Under controlled conditions, then, guinea pigs confined in cages in a room where tuberculous animals are quartered have developed unquestioned tuberculosis, as seen at autopsy and confirmed by animal inoculation. In a subsequent paper the character of their disease will be compared with that acquired by guinea pigs living in the same cage with tuberculous animals. The incidence of the infection acquired by air-borne contagion was increased by the duration of the exposure up to a certain point. It rose from none in the first 6 months to 9.1 per cent in the first year, to 27.7 per cent in 18 months and to a maximum of 35.3 per cent in 2 years. Thereafter, however, only 1 out of 20, or 5 per cent, was affected. This marked decrease in incidence, following a still further increase in duration of exposure, can be explained by one or more of the following possibilities.

The 19 guinea pigs may be individuals that by some chance have escaped the effective disease-producing dosage of tubercle bacilli;

they may be individuals of such great innate resistance to tuberculosis that they are able to overcome quantities of bacilli that in other animals would cause disease; they may be those animals in which the equilibrium between contagion and resistance was such that by virtue of exposure to certain doses of bacilli suitably spaced in time an acquired resistance to the disease was produced.

That the first is not a likely explanation of these results is indicated by the fact that 6 guinea pigs exposed for some time during the same period in the same cages with these 19 guinea pigs have developed tuberculosis. Furthermore, the lungs and tracheobronchial lymph nodes of these resisting animals showed a moderate to an extensive anthracosis. It was shown by Augustine (8) that although the air of rooms where cases of open human pulmonary tuberculosis are confined rarely contained living tubercle bacilli, the dust of 25 per cent of such rooms contained virulent tubercle bacilli. She found no tubercle bacilli in samples of air in our animal room tested during the progress of these experiments. Unfortunately the dust was not examined, but in view of the comparatively high incidence of tuberculosis of air-borne character among animals confined in this room, it is reasonable to assume that here too, as in human dwellings, the organism is present in the dust. Yet in spite of the considerable penetration of this dust, laden, presumably, with tubercle bacilli, into the lungs and tracheobronchial lymph nodes of these 19 animals, as is shown by their anthracosis, they have failed to develop tuberculosis.

The other two possibilities seem to be more likely and work is now in progress with the aim of determining which of these factors, the innate or the acquired resistance, is responsible for the reduction in the incidence of tuberculosis in guinea pigs exposed for such long periods of time. It is for this purpose that the 9 animals referred to above, along with other animals derived from other sources, have been reserved.

The relatively high incidence of tuberculosis in guinea pigs confined in the same room with tuberculous animals for 7 or 8 months is pertinent to the present widespread discussion as to the existence of a filterable form of the tubercle bacillus. No inoculation chancre and no satellite lymph node involvement occur after the injection of filtrates of tuberculous material. However enlarged lymph nodes, usually tra-

cheobronchial nodes, containing acid-fast bacilli are found, and sometimes, though rarely, tuberculous lesions, usually limited to the lungs and tracheobronchial lymph nodes, have been seen (3, 9). It is evident that before a conclusion can be drawn from such cases the possibility of air-borne contagion must be ruled out.

CONCLUSIONS

1. Guinea pigs living in the same room but not in the same cage with tuberculous animals acquire tuberculosis, characterized by a chronic course, a marked involvement of the lungs, often with cavity formation and a massive tuberculosis of the tracheobronchial nodes; the mesenteric and cervical nodes are slightly or not at all affected.

2. The route of infection in these guinea pigs is almost always the respiratory tract.

3. Of 103 guinea pigs exposed for a period of up to 32 months 15 or 14.5 per cent developed tuberculosis. The shortest period of exposure leading to fatal tuberculosis was 8 months.

4. The incidence of this tuberculosis acquired by air-borne contagion increases with the duration and intensity of the exposure up to a certain point.

5. A large percentage of the guinea pigs weathered a continuous exposure to the tubercle bacillus for 32 months without becoming tuberculous. This may be due to an innate natural resistance against tuberculosis, or to an acquired immunity resulting from the continuous exposure to the contagion.

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