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# Effect of Exogenous Superinfection on Naturally Acquired Bovine Tuberculosis in Inbred Rabbits.*

MAX B. LURIE AND JOYCE BECKER.

*From the Henry Phipps Institute, University of Pennsylvania.*

Considerable evidence[1] suggests that continued exposure to exogenous contagion is an important factor in the origin of active tuberculosis in tuberculin-positive individuals. However, little is known of the effect of this continued exposure on an already existing active disease. The purpose of the present report is an attempt to answer the above question.

In a previous paper[2] it was shown that increasing concentrations of tubercle bacilli in the enviromental air of the highly inbred, genetically resistant rabbit family A, increases proportionally the incidence of the acquired disease, accelerates its onset, intensifies its dissemination and shortens its duration. In the inbred families C and F, of low resistance, an increase in the intensity of the contagion also resulted in an increase in the incidence of the acquired disease; however, its character was uniformly rapidly disseminating and of short duration whether acquired under conditions of high or low contagion. Since the disease originated as a single pulmonary focus under all conditions, the acceleration of the tuberculous process in the resistant family with increasing intensities of contagion could not be readily ascribed to a larger number of initially inhaled particles capable of producing pulmonary foci under higher intensities. Nor does it seem likely that the number of bacilli contained in the individual infectious particles in the air varies with the intensity of the contagion under which they arise. However, it is probable that after the disease had taken root, additional exogenous infective particles penetrate the lung more frequently under high than under low intensities of contagion. Therefore it seemed desirable to determine whether continued intense exposure to the inhalation of tubercle bacilli affects adversely the course of an already engrafted disease in animals of high and low resistance.

*Materials and Methods.* Accordingly, the following experiment was set up. Litter mate pairs of the families A and C were exposed to tuberculous rabbits, half of which were infected intravenously and half intrarenally[2] with highly virulent bovine tubercle bacilli (Ravenel). The artificially-infected rabbits were in runs on one side of a fine wire-mesh screen. The contacts were in individual cages, with wire-mesh walls in back and in front, on the other side of the screen, and 6 inches from it. The 3-tiered manifold previously described[3] containing both the sources of contagion and the contacts, was separated into 2 equal halves by a wooden partition.[4] To equalize the intensity of the contagion on both halves of the manifold the sources of contagion were interchanged daily between the 2 adjoining rooms. There were 8 sources of contagion and 10 contacts in each tier. As the sources of contagion died from tuberculosis they were replaced by similarly infected rabbits throughout the course of the experiment.

At biweekly intervals all the contacts were tested with tuberculin. Once a month roentgenograms of the chests of the contacts were taken. As soon as a clearly positive tuberculin reaction appeared in a certain contact it was immediately removed from the contagion and was placed in another room free from artificially-infected animals. Its litter mate, on the other hand, was left in contact with the sources of contagion in the manifold even after the appearance of a posi-

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1 Chadwick, H. D., and Pope, A. S., *The Modern Attack on Tuberculosis*, The Commonwealth Fund, New York, 1946.

2 Lurie, M. B., *J. Exp. Med.*, 1944, **79**, 573.

3 Lurie, M. B., *Am. Rev. Tubere.*, Suppl., 1941, **44**, 1-125.

4 Lurie, M. B., *J. Exp. Med.*, 1944, **79**, 559.

TABLE I.
Effect of Exogenous Superinfection on Course of Naturally Acquired Air-Borne Tuberculosis in the Highly Inbred Family C.

| Exposed to superinfection | | | | | | Not exposed to superinfection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit No. | Parents | Preallergic period in mos. | Max. inflammation to tuberculin in mm³ | Duration of disease in mos. | Presence of tuberculosis in testes | Rabbit No. | Parents | Preallergic period in mos. | Max. inflammation to tuberculin in mm³ | Duration of disease in mos. | Presence of tuberculosis in testes |
| C6-50 | C5-52 | 3.3 | 117 | 2.3 | 0 | C6-49 | C5-52 | 1.9 | 250 | 2.6 | 0 |
| | 40 | | | | | | 40 | | | | |
| 53 | 52 | 8.2 | 472 | 3.8 | 0 | 51 | 52 | 3.5 | 472 | 3.7 | 0 |
| | 40 | | | | | | 40 | | | | |
| 57 | 52 | 4.0 | 525 | 4.9 | 0 | 56 | 52 | 17.5† | None | None | — |
| | 40 | | | | | | 40 | | | | |
| 59 | 52 | 4.2 | 851 | 4.7 | 0 | 58 | 52 | 10.7 | 1116 | 3.8 | 0 |
| | 40 | | | | | | 40 | | | | |
| C7-15 | C6-11 | 4.9 | 873 | 2.6 | 0 | C7-14 | C6-11 | 2.3 | 990 | 3.6 | 0 |
| | **C5-40** | | | | | | C5-40 | | | | |
| 10 | C6-11 | 10.5† | None | None | — | 18 | C6-11 | 11.7 | 273 | 5.2 | 0 |
| | C5-40 | | | | | | C5-40 | | | | |
| 9 | C6-11 | 2.6 | 594 | 1.7* | 0 | 17 | C6-11 | ? | 256 | 4.9 | 0 |
| | C5-40 | | | | | | C5-40 | | | | |
| Avg | | | 572 | 3.6 | | | | | 571 | 3.9 | |

* Died of intercurrent disease and tuberculosis.
† Duration of exposure.
Incidence of disease, 85%.
Average preallergic period, 5.2 mos.
Average maximum tuberculin sensitivity, 565 mm³.

tive tuberculin reaction and remained in contact until its death. The isolated contacts were protected from cross contagion from each other by ultraviolet lamps placed immediately in front and above their cages, as it has been clearly shown that ultraviolet radiation eliminates the contagion of tuberculosis.[4] Thus half of the rabbits were not exposed to exogenous superinfection, while the other half of the litter mates of the same genetic history for the past 6 to 9 inbred generations and of the same environmental experience over a period of 11 years were exposed to continuous, intense exogenous superinfection. All but a few of the litter mate pairs were of the same age and sex.

*Results.* In Table I, Columns 2 and 8 record the parents of both groups of rabbits of the C family, of low genetic resistance to the disease. It is evident that the genetic history of both was identical. In Columns 5 and 11 are recorded the duration of the disease in these genetically similar rabbits. It is clear that there was no difference in the rate of progression of the disease whether the contact remained in continuous exposure to the contagion or whether it was removed therefrom immediately after the acquisition of a positive tuberculin reaction. In both instances the disease pursued its inexorable, rapid course. Those exposed to superinfection died of tuberculosis within an average of 3.6 months with a survival range of 2.3 to 4.9 months. Those not exposed to superinfection died within an average of 3.9 months with a survival range of 2.6 to 5.2 months. While those removed from contagion survived a little longer, the difference is clearly not significant. That the contagion to which the rabbits were exposed was very high is seen in the fact that 12 of the 14 contacts died with tuberculosis, an incidence of 85%. The rapidity of attack by tuberculosis, or the average interval elapsing between the beginning of exposure and the development of tuberculin sensitivity, was 5.2 months. It is noteworthy that the maximum average sensitivity to tuberculin developed by both groups of rabbits was the same. Continuous exposure to exogenous superinfection did not increase the allergic sensitivity developed during the course of the disease. Columns 6 and 12 show that in none of the 12 rabbits of this genetically susceptible family did tuberculosis of the testicle develop. This is significant since testicular disease nearly always results from tubular spread of medullary renal tuberculosis and is frequently associated with a chronic slowly progressive disease. Thus, whether the contacts were or were not exposed to superinfection, no case of chronic tuberculosis occurred.

In Table II are given the corresponding data for the rabbits of the genetically-resistant family A. Columns 2 and 8 show that the genetic history of the rabbits exposed to superinfection and those not exposed to superinfection was identical in most instances. It is clear from Columns 5 and 11 that the disease in the rabbits of this family was, in general, more chronic than that in the contacts of the C family. The difference, however, is not great. As demonstrated in a previous study[2] a high intensity of contagion tends to overwhelm the natural resistance of this family. Nevertheless, the incidence of the acquired disease was distinctly lower among the A rabbits as compared with those of the C family, an incidence of 73% instead of 85% in the latter. Likewise, the duration of the disease of the A rabbits, both those exposed and those not exposed to superinfection, was longer, an average of 4.6 and 5.4 months as compared with 3.6 and 3.9 months respectively, for the rabbits of low resistance. It is noteworthy that A rabbits tended to acquire tuberculin sensitivity before the C rabbits. The average preallergic period was 4.2 months for the former and 5.2 for the latter. This is in agreement with previous observations, where evidence was presented that the more resistant A rabbits acquire tuberculosis after a shorter interval of natural exposure than the less resistant C family.[2] Again, the intensity of allergic sensitivity of the A rabbits tended to be considerably less than that of the C rabbits, the maximum average sensitivity of the former being 321 $mm^3$ as compared with 565 $mm^3$ for the C rabbits. This also confirms previous ob-

TABLE II.

Effect of Exogenous Superinfection on Course of Naturally Acquired Air-Borne Tuberculosis in the Highly Inbred Family A.

| Exposed to Superinfection | | | | | | Not exposed to superinfection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit No. | Parents | Preallergic period in mos. | Max. inflammation to tuberculin in mm³ | Duration of disease in mos. | Presence of tuberculosis in testes | Rabbit No. | Parents | Preallergic period in mos. | Max. inflammation to tuberculin in mm³ | Duration of disease in mos. | Presence of tuberculosis in testes |
| A8-82 | A7-3<br>A6-27 | 1.4 | 64 | 3.4 | 0 | A8-83 | A7-3<br>A6-27 | 10.7 | 405 | 5.0 | + |
| | | | | | | 81 | A7-3<br>A6-27 | 5.6 | 64 | 1.0* | 0 |
| | | | | | | A7-3 | A5-30<br>A6-2 | 2.0 | 75 | 3.7 | 0 |
| A9-21 | A8-61<br>60 | 4.0 | 460 | 4.4 | 0 | A9-20 | A8-61<br>60 | 2.3 | 375 | 3.0 | + |
| 23 | 61<br>60 | 3.3 | 492 | 3.5 | 0 | 22 | 61<br>60 | 4.9 | 500 | 3.6 | + |
| 24 | 61<br>60 | 0.9 | 343 | 4.4 | 0 | 25 | 61<br>60 | 4.3† | None | None | — |
| 35 | 61<br>62 | 6.0 | 546 | 7.3 | + | 33 | 61<br>62 | 5.1 | 208 | 12.0 | + |
| | | | | | | 39 | 66<br>62 | 13.8† | None | None | — |
| 48 | 59<br>64 | 12.2† | None | None | — | 47 | 59<br>64 | 12.2† | ,, | ,, | |
| Avg | | | | 4.6 | | | | | | 5.4 | |

*Rabbit had a single primary pulmonary focus with slight caseation in homolateral hilum node. Died of intercurrent disease.
† Duration of exposure.
Incidence of disease, 73%.
Average preallergic period, 4.2 mos.
Average maximum tuberculin sensitivity, 321 mm³.

servations.[3] Finally, in 5 of the 10 rabbits that died of tuberculosis of this family, there was tubular spread of the disease to the testicles, while none of 11 rabbits of the C family showed a tuberculous orchitis. All these observations show that, while the difference in survival between the A and C rabbits was not marked under this high intensity of contagion, the A rabbits still retained some of the essential responses to the disease characteristic of this family in the past where, under conditions of low contagion, the contrast between the chronicity of the disease in the A family and its fulminating course in the C family was marked.

As to the main question at issue, it is clear that under these conditions of intense contagion there was no significant difference in the course of the disease in the rabbits which remained in continuous exposure to contagion after the acquisition of the disease as compared with those who were removed from contagion after their acquisition of tuberculin sensitivity. Again, as with the C family, the A rabbits not exposed to exogenous reinfection survived somewhat longer, an average of 5.4 months, than those continuously exposed 4.6 months. However, the difference is hardly significant.

*Summary and Discussion.* Under conditions of natural intense exposure to virulent bovine bacilli, rabbits of high and low genetic resistance to tuberculosis were not benefited by cessation of the exposure after the onset of the disease. The disease pursued its characteristic course whether exogenous superinfection took place or not.

That the course of the disease in the rabbits of low genetic resistance was not affected by the cessation of exposure after the onset of the disease may have been anticipated from previous data[2] where it was shown that in the C family the disease pursued the same fulminating course whether acquired under conditions of high or low intensities of contagion. In the resistant family A interruption of the contagion after the onset of the disease tended to retard somewhat the progression of the disease. However, the difference was slight. It is clear therefore that exogenous superinfection after the acquisition of a positive tuberculin reaction does not explain the intensification of the dissemination of the disease and the shortening of the duration observed in this family, with increasing concentrations of bacilli in their environment. It is possible that the initial inhalation of larger numbers of bacilli which do not at once give rise to visible pulmonary foci under high intensities of contagion may account for the more rapid progression of the disease in this family under these conditions.

It is noteworthy that while the differences between the rabbits of high and low resistance to tuberculosis were greatly diminished by the intense contagion prevalent in these experiments, they were not completely wiped out. As in previous studies the A rabbits tended to acquire tuberculin sensitivity more rapidly than the C rabbits, their allergy was less developed during the course of their disease and the progression of the infection was slower and anatomically more chronic.

It must be emphasized that these data do not warrant the conclusion that in human tuberculosis exogenous superinfection plays no role in the course of an existing disease. In the first place, bovine tuberculosis in rabbits is uniformly fatal, while a large proportion of active human pulmonary disease is arrested. In the second place, the intensity of contagion under which the disease was acquired in this study was so high that 73 to 85% of the exposed rabbits acquired tuberculosis, an incidence which never occurs in human life. Therefore, these experiments are not strictly comparable to those obtaining in the human disease.