BACTERIAL INTERFERENCE

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This paper describes in outline some experiments on bacterial interference already reported by us (1) and presents supplementary evidence from later experiments. Several types of interference are analyzed in an attempt to find a working hypothesis which would account for the wide differences in recorded findings. No attempt is made to give a detailed review of the subject.

Our interest in this subject stemmed from studies on the pathogenesis of different types of respiratory infection, principally in the guinea pig and the mouse. With background knowledge of how various organisms went about their jobs in the two host species, it seemed of interest to examine the effect of these same pathogens acting together and to compare the results in the two species.

For present purposes attention is directed mainly to investigations with *Brucella suis*, *Bacillus anthracis*, *Pasteurella pestis*, and *Mycobacterium tuberculosis*. The technique for respiratory exposure has been already described (2).

EXPERIMENTS WITH GUINEA PIGS

It is important first to describe the course of *B. suis* infection in the guinea pig under our conditions of test. An estimated respiratory retention of about 50 single cells of *B. suis* gives about a 90 per cent infection rate. The organisms grow quickly first in alveolar macrophages and infection of bronchial glands can be detected as early as the 4th day. By 8 to 10 days practically all animals that are to become infected yield brucellae from the bronchial glands and a few give positive spleen cultures. By the 3rd week there is well marked generalized infection reaching its peak at about 28 days. Cellular response in the lung is primarily in lymphoid and lymphatic tissue and the substance of the lung remains unaffected at least until very late in the disease. The infection progresses steadily until about the 5th week during which time there is a great increase in lymphoid tissue but there is no evidence of necrosis (figure 1). The bronchial lymph nodes undergo similar changes but these are followed by necrosis in the form of abscess formation with peripheral necrosis. By the 16th week the whole lymphatic system is rapidly returning to normal and few, if any, organisms are found in the spleen. A few abscesses in lung tissue now develop and there is an accompanying sharp rise in the bacterial content of the lung tissue.

Guinea pigs with this type of infection can be shown to possess either a high degree of nonspecific resistance to secondary infection or none, according to the organism used for secondary challenge or the route of entry to the host. A good example of resistance is given when brucella-infected animals are challenged by the respiratory route with anthrax spores.

The pathogenesis of infection in the guinea pig with *B. anthracis* is closely similar to that with *B. suis*. There is no invasion of lung tissue. The spores are transferred by wandering macrophages to nearby lymphatics where they germinate, multiply, and well over to produce septicemia and death (3).

In table 1 combined data from three experiments are shown in which animals infected with brucellae by the respiratory route were later exposed to a respiratory infection with anthrax spores. First it will be noted that the animals were challenged 3 weeks after infection with *B. suis*. Second, arrangements were made (as they were in all other experiments) not to give overwhelming doses of either the primary or secondary invader. Thus the dose in each instance was calculated to infect about 90 per cent of control animals. It is seen that only about 17 per cent of animals with *B. suis* died of anthrax while 95 per cent controls died of the disease. The survivors were autopsied 24 days after anthrax challenge and none showed evidence of infection with *B. anthracis* on culturing of spleen, liver, and cervical or bronchial lymph glands.

It is pertinent at this point to note that we believe this type of resistance can properly be classed as nonspecific, for there is no evidence to suggest that there is any antigenic relationship between the two organisms: even the most sensitive type of serological test, as for example the agar diffusion precipitation technique of Ouch-

terlony, fails to reveal any cross reactions. Further, we tested serum from animals at the height of brucella infection for power to protect normal animals against respiratory anthrax. It does not do so.

The route of primary or secondary infection can be shown either to alter or leave unchanged the course of events described above. An example of unchanged resistance is seen when animals are first infected with *B. suis* by the subcutaneous route and challenged later with anthrax spores by the respiratory route. Thus, in a typical experiment, 85 per cent of anthrax controls died but only 15 per cent of animals with brucellosis died of anthrax. On the other hand, the reverse is true when anthrax is superimposed by the intracutaneous route on animals with brucellosis. No trace of resistance is found and the course of the secondary infection with anthrax is apparently the same as in the controls. The strain of *B. anthracis* we used was highly virulent; two or three spores given intracutaneously were enough to kill. The challenge dose, therefore, was kept as low as practicable, *i.e.*, about 10 spores.

TABLE 1

Inhibitory action of brucellosis on respiratory infection with Bacillus anthracis

Exposed to:	No. of Guinea Pigs		Autopsy of Survivors
	Tested	Died	
B*	30	0	27 +B
B and A	60	10 +B+A 5 +A−B 1 +B−A	42 +B 2 −B
A	60	54 +A	Not examined

Animals exposed to anthrax spores 21 days after respiratory infection with *Brucella suis*.

* B = *B. suis*; A = *B. anthracis*; + = positive culture; − = negative culture.

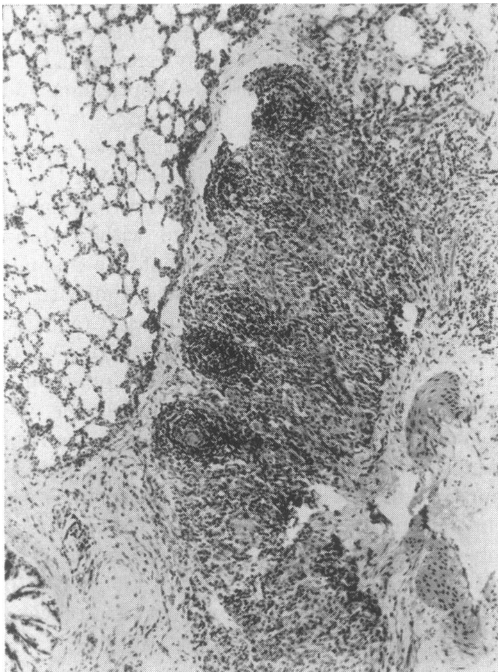

Figure 1. Lung of guinea pig 5 weeks after respiratory infection with *Brucella suis*, showing massive peribronchial lymphoid hyperplasia. There is no evidence of alveolar abscesses or invasion of lung parenchyma. Hematoxylin-eosin stain, ×71.

The reasons for these findings are probably basically simple enough. First, all the evidence points to the fact that *B. suis* introduced by any route produces a generalized infection with lymphatic hyperplasia showing marked monocyte and lymphocyte infiltration. *B. anthracis* given by the the respiratory route invades by the lymphatic pathway and when secondarily imposed on brucellosis is nonspecifically blocked. On the other hand, anthrax spores given intracutaneously find a nidus for continued multiplication at the site —*à la* Besredka. Under these circumstances this continued multiplication seems eventually to overcome the nonspecific resistance of the local lymphatic system. In this connection it may be useful to record that we measured the degree of resistance established against respiratory anthrax by raising the challenge dose and found that to get a 50 per cent death rate from anthrax in animals with brucellosis approximately 5 times the LD_{90} dose (estimated retention 4×10^5 spores) was necessary.

It was interesting to examine the state of immunity to anthrax in animals with brucellosis after surviving exposure to anthrax spores. An experiment was made in which anthrax was superimposed (a) intracutaneously and (b) by the respiratory route on animals with brucellosis of 3 weeks' duration. Here we had confirmation of the earlier findings, namely no demonstrable protection against intracutaneous anthrax but 95 per cent resistance against a respiratory challenge. Five weeks later (*i.e.*, 8 weeks after the initiation

of brucellosis) animals surviving the respiratory anthrax challenge were first bled from the heart to obtain serum for test for anthrax antibody. Next day they were challenged intracutaneously with anthrax spores. The result strongly indicated that at least some animals had by now developed specific immunity. Thus, whereas 17 of 20 control animals (with brucellosis of 8 weeks' duration) died of cutaneous anthrax, only 8 of 19 in the test group so died. Further, on examination of the sera for specific antibody by Thorne and Belton's agar diffusion technique about 90 per cent of them proved positive in varying degree.

Turning now to *P. pestis* as a secondary invader in guinea pigs with brucellosis, we find a picture not dissimilar to that obtaining with anthrax. Earlier work (4) had shown that two forms of plague, both originating in the respiratory tract, can develop according to the size of particle presented to the host. Small particles initiate a bronchopneumonia which leads to septicemia and death. Large particles establish a septicemia via the upper lymphatics and death results more quickly without trace of pneumonia.

In a typical experiment guinea pigs with brucellosis of 3 weeks' duration were exposed in two groups to *P. pestis*. One received a cloud of single organisms, the other a cloud of 12 μ particles (table 2). It is seen that there was about a 90 per cent infection in brucella controls and 90 to 100 per cent deaths in the 12 μ and single cell cloud *P. pestis* controls. No significant protection was afforded by brucellosis against single cell *P. pestis* but 85 per cent of animals were protected against the 12 μ challenge. At autopsy of survivors 21 days after the *P. pestis* challenge, no animal was found harboring the organism. In effect this result says, as in the case of anthrax, that if the invader is forced to enter the general circulation via a prepared lymphatic system, nonspecific resistance is manifested. The same type of result is obtained when brucellosis is established by parenteral injection. It may be noted here that we found by examination *in vitro* an interesting inhibitory effect of plague-infected tissue on the growth of *B. suis*. At autopsy, cut surfaces of infected tissue were rubbed on agar media (a highly selective medium having been developed for each organism). It was observed first that whereas the lung from uncomplicated cases of brucellosis gave a densely confluent growth, that from animals dying from superimposed *P. pestis* was never confluent, frequently very scanty and confined to discrete colonies at the periphery of the smear. Some further simple experiments showed (a) that tissue from animals dying of uncomplicated plague had strongly inhibitory action on the growth of *B. suis* from sources either *in vivo* or *in vitro*; (b) that *P. pestis* grown *in vitro* had no such inhibitory action; (c) that *B. suis* grown *in vitro* had no inhibitory action on *P. pestis* from sources *in vivo* or *in vitro*. From this action of *P. pestis* grown *in vivo* one might speculate that but for the unfortunate fact that an animal with brucellosis died of secondary plague it might have been cured of brucellosis, supporting, as it were, the old claim that "one ill cureth another." At least the finding draws attention to the manifold factors probably at work in combined infections.

The time of onset of resistance has not been closely followed. Experiments were made on the

TABLE 2

Effect of brucellosis by respiratory route on infection with Pasteurella pestis

Exposed to:	No. of Guinea Pigs		Autopsy of Survivors after *P. pestis* Challenge
	Tested	Died	
B*	17	0	15 +B
B and P (single cell particles)	30	26 +P+B 3 +P−B	1 +B−P
B and P (12 μ diameter particles)	30	5 +P+B 2 +P−B	21 +B 2 −B−P
P (single cell particles)	25	25 +P	
P (12 μ diameter particles)	25	23 +P	2 −P

Animals exposed to *P. pestis* 21 days after infection with *Brucella suis* (single cell particles).
* B = *B. suis*; P = *P. pestis*; + = positive culture; − = negative culture.

TABLE 3

Duration of resistance to Pasteurella pestis infection in guinea pigs with brucellosis

Time	Exposed to:	No. of Guinea Pigs		Survival	Autopsy of Survivors
		Tested	Survived		
wk				%	
3	B*	20	20	100	17 +B
	B and P	40	27	67	25 +B
	P	20	0	—	—
6	B	20	20	100	18 +B
	B and P	40	25	62	25 +B
	P	20	1	5	—
15	B	17	17	100	9 +B
	B and P	60	13†	21	8 +B
	P	20	2	10	—

Pasteurella pestis given as cloud of 12 μ particles 21 days after *Brucella suis*.

* B = *B. suis*; P = *P. pestis*; + = positive culture; − = negative culture.

† Deaths: 24 +B+P; 23 −B+P.

8th day after infection with *B. suis*, the time at which practically all animals to become infected will have done so but there was no evidence of nonspecific resistance. All we know is that it is firmly established by the 21st day. It remained to explore how long this resistance lasted. An experiment was made in which brucella-infected animals were challenged with *P. pestis* at 3, 6, and 15 weeks after primary infection (table 3). At 3 weeks the pattern is similar to that given in the previous experiment. There is an 85 per cent infection rate in *B. suis* controls. All of the *P. pestis* controls died but 67 per cent survived in the combined group. This latter figure, although clearly significant, is lower than expected by about 20 per cent for reasons unknown. At 6 weeks the same general pattern obtained. It may be recalled that about this time the maximum degree of lymphoid and lymphatic hyperplasia had developed. This does not seem to have effected a change in the degree of resistance. As to the results at 15 weeks, it should be recalled that by now the whole respiratory lymphatic system in brucella animals is rapidly returning to normal and systemic infection is also disappearing. This latter point is borne out by the finding that only 9 of 17 brucella controls were positive. The degree of resistance to *P. pestis* has practically vanished, but here again it should be noted that of the 47 animals dying, only 24 showed positive brucella infection. So one might reasonably conclude that once the lymphatic system is reconditioned, so to speak, an animal returns to its original state of susceptibility. We have not done this type of experiment with *B. anthracis* as secondary invader but it is not unreasonable to predict a similar result.

We carried out some experiments with brucellosis and tuberculosis as combined infections. We used the bovine strain of *M. tuberculosis* (Vallée) which was highly invasive and lethal for both guinea pigs and mice. It was selected largely because we were able to get suspensions of single units of *M. tuberuclosis* of about 1 μ diameter and clouds of even-sized particles were readily obtained; 1 to 10 units formed an infective dose. Macroscopic tubercles were observed at 10 to 14 days after infection. They developed at the sites of primary infection in the alveolar wall and associated lymphoid tissue. Secondary spread was via the peribronchial lymphatics to the bronchial lymph nodes.

When *B. suis* was the primary invader, all we could observe was a delay in the initiation and devlopment of infection by *M. tuberculosis*. Where *M. tuberculosis* was the primary invader quite a different picture resulted, as table 4 shows. Animals were exposed to *B. suis* 21 days after infection with *M. tuberculosis* and groups were autopsied and cultured at 2 to 5 weeks thereafter. All tubercle bacillus controls were positive throughout the period and the dose of *B. suis* used infected 85 per cent of controls. It is seen, however, that there is a very marked inhibition of brucellosis. Over the whole period only about 10 per cent of the animals developed the infection.

Established tuberculosis had a similarly marked effect on the suppression of anthrax superimposed by the respiratory route. A typical experiment showed that 90 per cent anthrax controls died whereas 85 per cent of tuberculous animals survived the secondary assault.

A range of tests was made in which survivors from *P. pestis* or *B. anthracis* infection experiments were reinfected with the other organism or with *B. suis* or *M. tuberculosis* but we failed to show anything other than the normal reaction to the secondary invader. We have also tried si-

TABLE 4

Effect of pulmonary tuberculosis on development of brucellosis

Exposed to:	Guinea Pigs Examined: Weeks after Exposure to *Brucella suis*:			
	2	3	4	5
TB*	10/10 +TB	10/10 +TB	10/10 +TB	10/10 +TB
TB and B	10/10 +TB (1/10 +B)	10/10 +TB (1/10 +B)	10/10 +TB (1/10 +B)	10/10 +TB (2/10 +B)
B	Nil	Nil	Nil	17/20

Animals exposed to *B. suis* 21 days after infection with *Mycobacterium tuberculosis*.

* TB = *M. tuberculosis*; B = *B. suis*; + = positive culture.

multaneous infections with pairs of organisms but have observed nothing other than an additive effect. There was certainly no evidence of inhibition of the one by the other or of synergic action.

The experiments with guinea pigs might be summarized as follows. There is no evidence of enhanced pathogenicity as a result of combined infection. There is some trace of evidence to support an old belief that a pre-existing infection could be suppressed by another superimposed. The only strongly positive findings that emerged were that in certain circumstances a pre-existing infection can induce a high degree of nonspecific resistance to a second and wholly unrelated one, whereas under other conditions using the same organisms no trace of resistance is observed. Perhaps a third finding is worthy of more attention than we have given it. This is the development of specific immunity to anthrax when superimposed on a primary infection with a totally unrelated organism. No such immunity develops in control animals surviving exposure to a cloud of anthrax spores. Clearly then an attempt at invasion, including germination and some multiplication, must have taken place which was sufficient to stimulate antibody production with the type of antigen we now know to be associated with the metabolism of the vegetative form of *B. anthracis*.

EXPERIMENTS WITH MICE

It is obvious that the findings outlined might have been determined solely by the particular characteristics of host species selected for test. This possibility led us to experiments with the mouse. This is a difficult species to handle satisfactorily in experimental cloud studies and we had first to develop a suitable technique for exposure. Each mouse is placed with its nose through a rubber diaphragm in a tube along which the cloud travels in a manner similar to that used for guinea pigs (2). Ten mice are exposed at a time and a standard exposure time of 5 min is used. Experiments with clouds of single bacterial cells rendered radioactive showed that, although the retention of particles varied from mouse to mouse by 2- to 3-fold, average retention per minute was about 7 ml of cloud. Surprisingly consistent results were obtained in experiments designed to gauge dose-response relationship with organisms such as *B. anthracis*, *P. pestis*, and *B. suis*. Groups of 20 to 30 mice per dose were used and after exposure animals were housed singly in 10-compartment boxes.

The first clear finding in this series of experiments was that the pathogensis of respiratory infection in the mouse with *M. tuberculosis* and *P. pestis* follows closely that observed in the guinea pig. On the other hand this species reacts entirely differently to infection with *B. suis* and *B. anthracis*.

B. suis proves to be about as infective by the respiratory route for the mouse as for the guinea pig. A retained dose of 80 single cells produces about 90 per cent of infection. When given by the subcutaneous route the ID_{50} is of the order of 10^6 to 10^7 cells but in the guinea pig the dose is around 10 cells. In the mouse, multiplication of the organisms commences quickly in phagocytic cells in the alveolar spaces and by the 4th day of infection small abscesses are already well developed (figure 2). Inflammatory cells are mainly monocytes and lymphocytes. This cellular change reaches a peak around the 10th to 14th day and by 6 weeks all that remain are small fibrotic foci, although viable organisms are still recoverable. Peribronchial or perivascular lymphoid hyper-

plasia is minimal or absent. Nevertheless a generalized infection is set up and positive spleen cultures are obtained as early as the 5th day after infection. Figure 3 shows graphically the course of infection in lung and spleen. Each assessment is based on tissues from 5 mice after grinding and pooling before counting. By the 3rd day, the lung count had risen about 1000-fold and by the 5th day positive spleen cultures were obtained. The highest lung counts here appeared around the 7th to 10th day. Spleen counts rose about as high as lung counts but infection, which is clearly a prolonged event, appeared more persistent in spleens. Under conditions of test, death from infection was very rare.

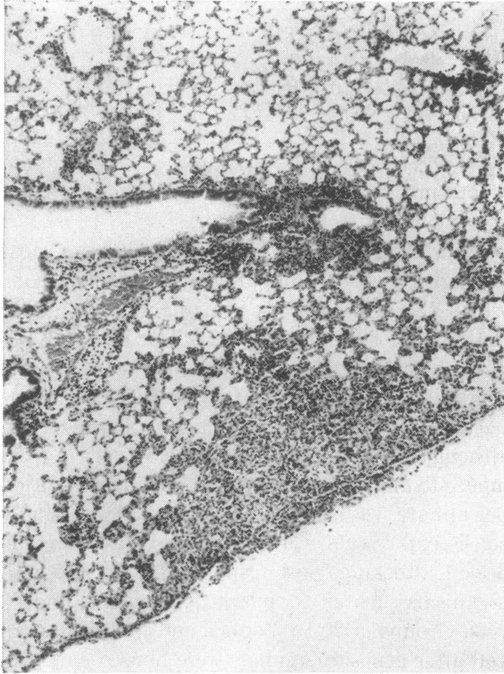

Figure 2. Lung of mouse 4 days after respiratory infection with *Brucella suis*. Abscess formation in lung parenchyma is commencing in alveolar spaces. Hematoxylin-eosin stain, ×71.

The LD_{50} dose of *B. anthracis* necessary to infect the mouse by the respiratory route is about 10^5 spores, whereas by the intracutaneous or intraperitoneal route the dose is 10 to 100 spores. Dosage-wise, therefore, it is, as it were, brucellosis in reverse. The first evidence of infection of the mouse lung with *B. anthracis* is found about 24 hr after exposure to spores. Vegetative bacilli in chains of 2 or 3 organisms are found in alveolar spaces. Here again no evidence of invasion of lymphoid or lymphatic tissue could be obtained. The peak of deaths occurs on the 2nd to 3rd day after infection, although sporadic deaths can occur up to about 3 weeks. At death lung capillaries are packed with bacilli but no other obvious lung changes are seen (figure 4).

The essential difference, therefore, in infection with both organisms in the guinea pig and the mouse is that in the former species lymphoid and lymphatic invasion is dominant; in the latter it is minimal or absent.

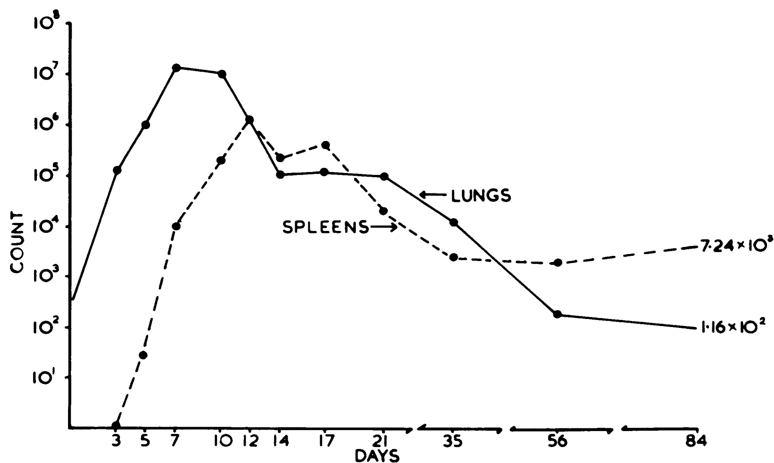

Figure 3. Course of *Brucella suis* infection administered to mice by the respiratory route. Retained dose, 617 (+25 per cent to −28 per cent) per lung. Each count is the pooled average from 5 mice.

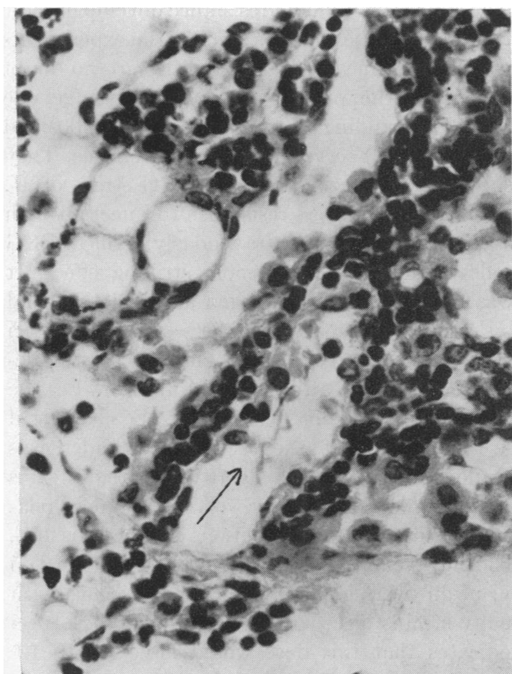

Figure 4. Lung of mouse dead of anthrax, showing a bronchial lymph node with no evidence of bacterial invasion. Vegetative organisms indicated by arrow are confined to blood capillaries. Hematoxylin-eosin stain, ×590.

A series of experiments with dual infections was made on exactly the same lines as with guinea pigs. Dosages of primary and secondary invaders were moderate, that is, they gave infection rates in control animals of 80 to 90 per cent. Where *B. suis* was the primary invader in the mouse, we obtained no trace of evidence of the development of nonspecific immunity to invasion with *B. anthracis*, *P. pestis*, or *M. tuberculosis*. The result was similar when *M. tuberculosis* was the primary invader. These findings did not vary throughout the course of the primary infection. There was neither any evidence of synergic action between any two invaders, nor of increased susceptibility to a secondary invader superimposed almost simultaneously or at very early stages after primary infection. In fact, the secondary infection either ran a normal course or if, say *B. anthracis* and *P. pestis* acted together, then all that was obtained was an additive effect.

How do these findings fit with the mass of recorded data on interference?

DISCUSSION

Ledingham, writing in 1931 (5) on the stimulation of nonspecific resistance, noted that "this subject which has now reached vast dimensions takes its origin from the classical experiments of Pfeiffer and Issaeff (1894)." He went on to say that from "what may be regarded as the better documented evidence. . . it seems that, if an animal has the power to deal successfully with small numbers of an organism which only in large doses and introduced at a particular site is capable of producing a lethal effect, there is no doubt that the sum total of the local and general defences can be definitely enhanced by [these] artificial stimuli." Three decades later we see this literature still further expanded. The question is: to what effect? If the ultimate objectives are to gain intimate understanding of nonspecific processes involved in the suppression or aggravation of naturally occurring diseases with a view to practical control, the harvest remains limited. However, major advances have been made in some areas. For example, in the very year that Ledingham was writing, Shope (6) published the first of his classical researches on the pathogenesis of swine influenza, a magnificent example of bacterial interference in precisely the opposite sense to that implied in the title of this symposium. In the field of nonspecific resistance it seems that so far the only area showing evidence of possible practical advance is in viral interference. The experiments described here contribute nothing of an evident practical nature, although they may have been of some help in giving a better insight into the disease processes *per se*. They have helped to confirm the earlier work of others such as Pullinger (7) and Mika and his colleagues (8) on the influence of brucellosis in preventing or reducing the severity of some types of secondary infections and *M. tuberculosis* has been shown to act similarly. However, to observe such phenomena it has proved essential to choose the test animal carefully in the sense that whereas the guinea pig responds the mouse does not. Nevertheless, in view of the fact that respiratory infection with, say, *B. suis* and *M. tuberculosis* leads to generalized infection, including in both species extensive invasion of the spleen, it is interesting to speculate why nonspecific resistance did not develop in the mouse, for this has been the species most widely used to demonstrate bacterial interference. The answer surely

can only be found in the radically different techniques from our own that other workers have employed. The author is aware of only one report, by Nyka (9), where the claim is made that primary brucella infection in the mouse induces a degree of nonspecific resistance to secondary infection with *M. tuberculosis*. However, the evidence that Nyka presents would not exactly satisfy the statistician. For example he describes, in all, three experiments: one with virulent *Brucella abortus* and two with an avirulent strain. In the first, mice had been given large doses of brucellae, enough to have killed 25 to 35 per cent of them. *M. tuberculosis* was later superimposed on 18 of the survivors and 12 mice received *M. tuberculosis* alone. As a result, he observes that the difference in survival time "is not very significant (19 days compared with 17 days for the control mice)." The author claims, however, that the experiment is remarkable for the fact that 11 of the 18 test animals failed to develop tubercles in the lung. He also observes that repeated doses of avirulent brucellae seem to be an important factor in the induction of resistance but concludes that "the final answer to the question must be provided by further experiments." Our own experiments with large groups of mice certainly do not substantiate his findings; however, let us remember the difference in technique.

There is, of course, a large body of evidence of nonspecific resistance in the mouse where inoculation of primary material by one route is followed by an infective agent presented by another route. The issue is why should resistance develop in these instances and be wholly absent in the experiments we have outlined? The possibilities are no doubt legion. In the present state of knowledge one seems outstanding. A recent report of Dubos and Schaedler (10) serves as a typical example. They showed that vaccination with BCG strain of *M. tuberculosis* increased resistance to infection with staphylococci given intravenously. Killed BCG cells produced an even more striking effect. It was manifested by prolonged survival time and by recovery of smaller numbers of staphylococci from infected organs. The effect was still detectable 10 weeks after primary injection. For the purpose of discussion, the point to be challenged is the claim that the secondary invader was virulent for the mouse. Relative to other strains of staphylococci this may well be so, but in relation to other mouse infections surely it is not. The intravenous dose of staphylococci was 0.05 ml of an overnight culture. Our experience with many strains of coagulase-positive staphylococci indicates that this dose would have 10^7 to 10^8 organisms. In the majority of papers on this subject it seems that closely similar conditions are described, which lead one back to Ledingham's observation that nonspecific resistance is only observed by such techniques when organisms of a low order of virulence are used as secondary invaders. Here it may be argued that in our experiments with *B. anthracis* given by the respiratory route the organisms could not be considered virulent in that the LD_{50} dose contained from

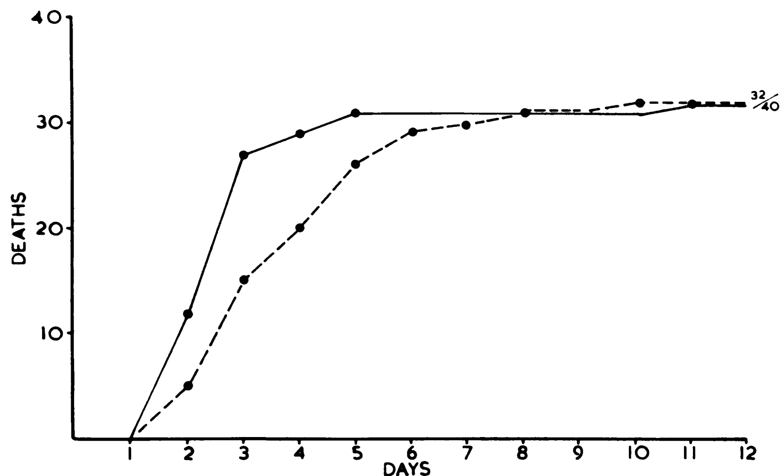

Figure 5. Influence of pertussis vaccine on respiratory anthrax in mice. Each animal received intraperitoneally 6 × 10^8 killed pertussis cells in 0.2 ml 24 hr before challenge.

10^4 to 10^5 spores. Our reasoning is as follows. First, all evidence points to the fact that in the guinea pig the anthrax spore is incapable of multiplying on the surfaces of the lung. Each spore must be phagocytized by a wandering macrophage and transported to lymphatic tissue, where it proceeds to germinate and multiply. This we believe accounts for the difference in the LD_{50} dose by the respiratory route between, say, *B. anthracis* and *B. suis*. The latter is clearly able to multiply rapidly before it reaches the lymphatics. The situation in the mouse is quite different, however, for germination and multiplication of *B. anthracis* can take place first in alveolar spaces, although in fact few spores seem successful in this effort. We could then say that the organism by this route in the mouse is of low virulence, but as it happens we have not been able by any means as yet to stimulate nonspecific resistance to prevent infection. Had we succeeded, we could with much more certainty than is possible at present express the opinion that the type of nonspecific resistance we have shown in guinea pigs is not only of a different order of effectiveness but is also qualitatively distinct from the successful experiments with mice reported by others. However, the point is in part at least strengthened by a few experiments we have made with mice, using the well tried pertussis vaccine as stimulator of resistance. We gave the vaccine intraperitoneally in a dose recommended by Dubos and Schaedler (11), namely 6×10^8 cells in 0.2 ml. Animals were challenged by the respiratory route 24 hr later. The results were disappointing. Figure 5 shows the result with anthrax. The delay in death time in the test group is of doubtful significance and it is clearly of no final importance. In the experiment with *B. suis*, a respiratory dose similar to that used in earlier work was given 24 hr after the pertussis vaccine. Eleven days later, when as we know lung infection in controls is near its height, lungs and spleen were removed and counts made. We examined 40 test and 40 control mice. There was the usual scatter in counts but when the data were examined statistically for differences there was a probability of 3.7 per cent that the lungs from the vaccine-treated mice had a slightly lower count and a probability of 7.8 per cent that their spleens gave a higher count. In other words there was no significant difference between the groups.

Finally, among the many unanswered questions there is one we wish especially could be answered. If infectious agents could be found that would choose the respiratory lymphatic pathway as the portal of entry, would nonspecific resistance manifest itself in the mouse as it did in the guinea pig?

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REFERENCES

1. Henderson, D. W., Lancaster, M. C., Packman, L., and Peacock, S. 1956 The influence of a pre-existing respiratory infection on the course of another superimposed by the respiratory route. Brit. J. Exptl. Pathol., **37**, 597–611.
2. Henderson, D. W. 1952 An apparatus for the study of airborne infection. J. Hyg., **50**, 53–68.
3. Ross, J. M. 1957 The pathogenesis of anthrax following the administration of spores by the respiratory route. J. Pathol. Bacteriol., **73**, 485–494.
4. Druett, H. A., Robinson, J. M., Henderson, D. W., Packman, L., and Peacock, S. 1956 The influence of aerosol particle size on infection of the guinea pig with *Pasteurella pestis*. J. Hyg., **54**, 37–48.
5. Ledingham, J. C. G. 1931 Natural immunity. In *A system of bacteriology in relation to medicine*, vol. 6, pp. 31–76. H. M. Stationery Office, London.
6. Shope, R. E. 1931 Swine influenza. J. Exptl. Med., **54**, 349–385.
7. Pullinger, E. J. 1938 Induced tissue resistance to *Brucella abortus* infection. J. Pathol. Bacteriol., **47**, 413–422.
8. Mika, L. A., Goodlow, R. J., Victor, J., and Braun, W. 1954 Studies on mixed infections. I. Brucellosis and Q fever. Proc. Soc. Exptl. Biol. Med., **87**, 500–507.
9. Nyka, W. 1956 Enhancement of resistance to tuberculosis in mice experimentally infected with *Brucella abortus*. Am. Rev. Tuberc. Pulmonary Diseases, **73**, 251–265.
10. Dubos, R. J. and Schaedler, R. W. 1957 Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infection. J. Exptl. Med., **106**, 703–717.

11. Dubos, R. J. and Schaedler, R. W. 1956 Reversible changes in the susceptibility of mice to bacterial infection. J. Exptl. Med., **104**, 53–65.

DISCUSSION

Use of the mouse for studies on resistance or susceptibility to infection is complicated by the variability in response of this experimental animal. In commenting on this problem, Dubos (New York) referred to recent investigations in his laboratory on the effect of the "normal" bacterial and ectoparasite flora of the "ordinary" laboratory mouse upon response of the animal to induced infection. Nelson at the Rockefeller Institute, New York, started a colony of Swiss mice obtained by cesarean section from the parent and reared in a "clean" environment by foster mothers which had been selected on the basis of being free of pathogenic intestinal bacteria, ectoparasites, and intestinal worms. The mice reared in the "clean" environment were not "germfree" but did not harbor the pathogens so frequently evident in these animals under ordinary laboratory conditions. These "clean" mice behaved quite differently than ordinary laboratory mice. Their response to nutritional deficiency was entirely different and, therefore, necessitates a repetition of studies carried out on ordinary mice. Furthermore, the pathogen-free mice were much more susceptible to infection by tubercle bacilli, Friedländer's bacilli, and staphylococci. Interpretation of data on resistance or susceptibility to infection in mice, at least, will require further characterization in animals rendered free of common pathogens, and, perhaps, preferably in "germfree" animals.