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Mechanisms of Antibacterial Action in the Respiratory System

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INTRODUCTION

Perhaps the most striking finding in relation to the antibacterial activity in the respiratory system is the sterility of the bronchopulmonary apparatus from the primary bronchi downward. That these structures are ordinarily sterile, despite the continuous entry of droplet nuclei containing bacteria, has been known for more than 50 years (3). The mechanism by which the bronchopulmonary tree retains its sterility under ordinary circumstances is just beginning to be understood.

Our attention was drawn to the problem by the considerations that if, in fact, the bronchopulmonary tree were normally sterile, and if in chronic bronchitis the lower bronchial secretions were ordinarily heavily populated with bacteria, there might be a basis for investigating the pathogenesis of chronic bronchopulmonary infections from the standpoint of breakdown of mechanism of local antibacterial activity. Initial investigations, therefore, were directed toward the study of the bacteriological flora of the bronchopulmonary secretions, with the use of extreme precautions to avoid contamination of the cultured material by bacteria coming from the upper respiratory passages. These studies (10) confirmed earlier observations (1, 11) that bacteria were rarely found in the bronchopulmonary secretions unless there was manifest inflammation or exudation. By the same token, when such exudation was found, the bacteria tended to be present in large numbers ($>10^6$ colonies per milliliter of secretion), and often the bacteria in the bronchial secretions were not adequately represented in cultures of the sputum.

The present review is not intended as a detailed discussion of the implications of these findings in terms of bacteriological interpretation of cultures arising from the bronchopulmonary apparatus. Instead, attention will be directed toward the implication that the failure to find bacteria could be explained primarily on the basis of continued activity of a potent local antibacterial mechanism. That such a mechanism exists has been indicated by numerous studies in the past. For example, Stillman (16), working with pneumococci that had been instilled into the bronchi of mice, observed that the majority of these organisms were made nonviable by the lung shortly after instillation. Lurie, in his classic experiments on the fate of aerosolized tubercle bacilli, observed that as many as 100 organisms needed to be inhaled to set up one tubercle, even in genetically highly susceptible rabbits, and one or two orders of magnitude more bacteria were required to be inhaled to produce a tubercle in genetically more resistant strains of rabbit (12). It was apparent, then, that the overwhelming majority of the inhaled bacteria were being killed in some manner after inhalation.

BACTERIAL CLEARANCE IN THE NORMAL LUNG

To study the phenomenon of local killing in the lung, an aerosol apparatus was constructed out of simple and relatively inexpensive materials (8). The apparatus delivered more than 85% of its particles in the form of nuclei between 1 and 3 μ in diameter, and, in detailed studies of the function of the apparatus, it was found that a set of stand-

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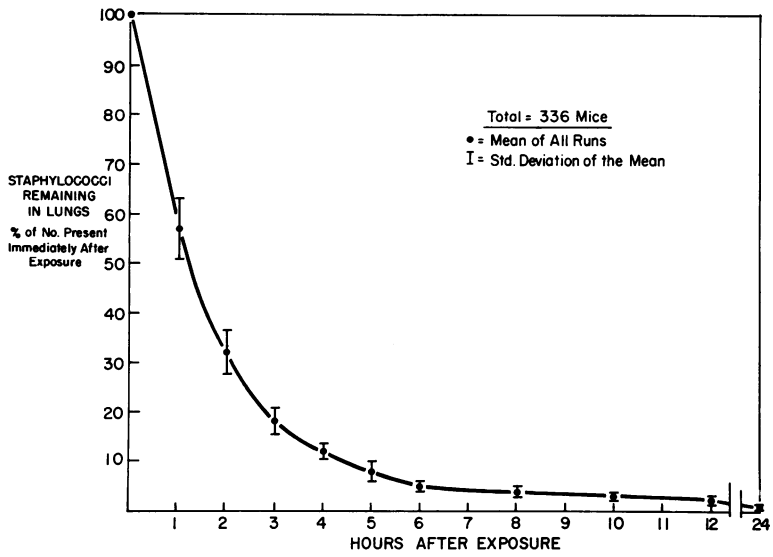

FIG. 1. *Disappearance of Staphylococcus aureus from the lungs of mice after administration by aerosol. The rapid disappearance and small standard deviations are especially noteworthy. (Reprinted from reference 8.)*

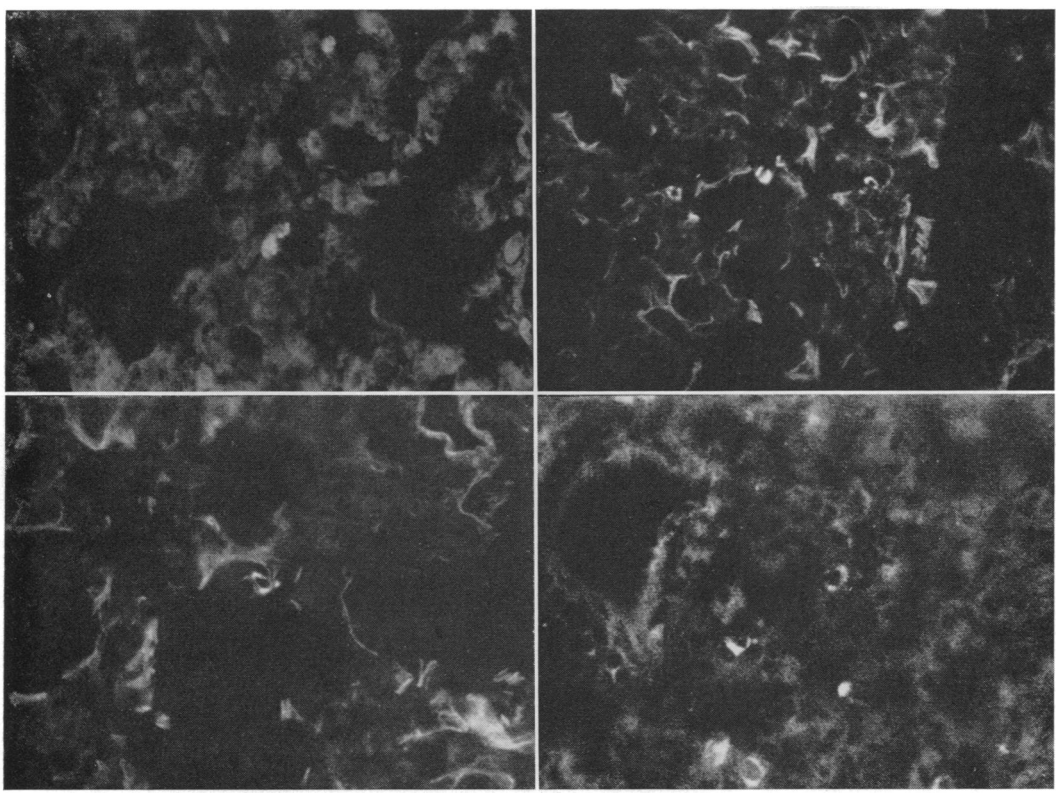

FIG. 2. *Lungs of mice taken 4 hr after exposure to aerosols containing Staphylococcus aureus. The sections have been stained with antibody to the Staphylococcus by use of fluorescein-labeled antibody, in accordance with methods given in reference 6. Note the intense staining of bacterial antigen in some cells lining the alveoli. In a few instances, discrete coccal bodies can also be seen.*

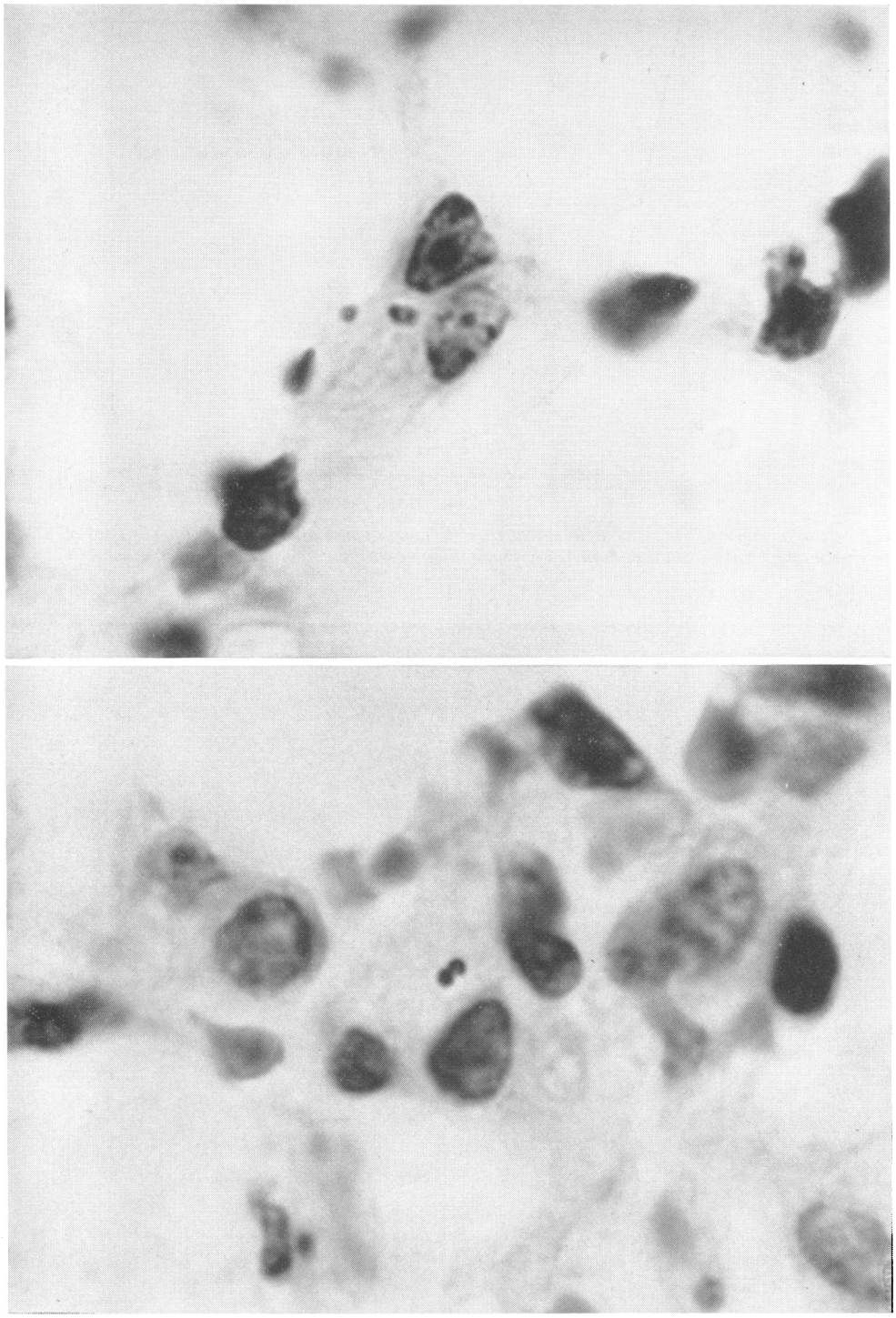

FIG. 3. *Section of mouse lung immediately after 30 min of exposure to aerosol of Staphylococcus aureus stained by Macallum-Goodpasture stain. × 2,500. In the upper photograph, staphylococci appear to have been ingested by an alveolar septal cell. In the lower, staphylococci may be in a mononuclear macrophage in the alveolar septum (see reference 6).*

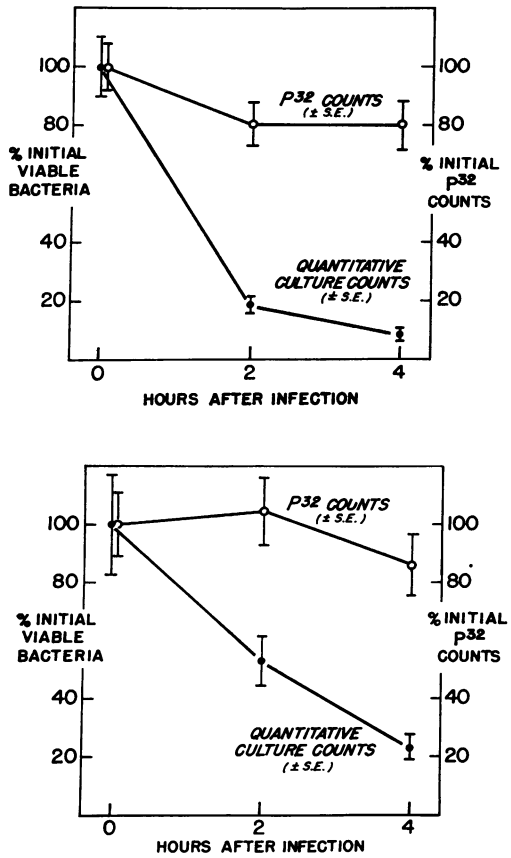

FIG. 4. *Clearance of P^{32}-labeled Staphylococcus aureus and Proteus mirabilis from murine lung. The change in number of viable organisms is compared with the change in radioactivity in the lung (6).*

ardized conditions could be obtained whereby a suspension of staphylococci could be delivered into the chamber in sufficient concentration that mice exposed to the aerosol for 30 min and sacrificed immediately thereafter were found to contain in their lungs approximately 50,000 viable units of staphylococci capable of producing colonies. When the lungs were cultured at regular intervals after exposure to the aerosol, the bacteria were found to have become nonviable in exponential fashion, so that within 4 hr approximately 85% of the bacteria could no longer be detected, and within 6 hr all but a few per cent had become nonviable. What was even more striking was the extraordinary reproducibility of the method, as seen by the small standard errors of the mean bacterial counts obtained at various time intervals after infection by aerosol (Fig. 1). The rates of disappearance of the bacterial particles were consistent with the anticipated rate of disappearance of any particles of this size, but as is well known, particles of this size tend not to impinge on the bronchial mucosa, and so the implication was clear that most of the disappearance of the bacteria was likely to be due to cellular systems or other antibacterial systems operating below the tertiary bronchi, beyond the level at which the mucociliary apparatus is active.

Clearance by the Alveolar Macrophage System

To test the implication that bacterial killing occurred primarily in the deeper portions of the lung, two experiments were conducted (6). In the first, fluorescein-labeled antibody was used to detect bacterial antigen in the lungs of mice that had been exposed to the aerosols, and invariably bacterial antigen was found in the epithelial cells lining the alveoli (Fig. 2). Occasionally, relatively intact bacteria could be found in these alveolar-lining cells (Fig. 3). When the bacteria were labeled with radioactive phosphorus and their fate was studied, it was found (Fig. 4) that, when approximately 85% of viability had disappeared, radioactivity had declined by only about 20%. Thus, the decline in viability was not due to transport of the bacteria away from the alveoli, as evidenced by the retention of radioactivity and the observation of bacterial antigen in the alveolar-lining cells. Only a small minority of the bacterial population could have been transported away during the time of maximal killing. The majority of the bacterial cells were destroyed in situ, and the alveolar macrophage system clearly seemed to be the principal agency for such removal.

Role of Bacterial Species

That the bacterial species is an important variable in the process of removal was demonstrated by subsequent studies (5) in which the rates of removal of a strain of *Proteus mirabilis*, one of *Staphylococcus aureus*, and one of *S. albus* were studied under comparable conditions (Fig. 5). *S. albus* was removed most rapidly, *S. aureus* somewhat more slowly, and the strain of *Proteus* still more slowly. More recently, observations have been made with a pathogenic strain of *Pasteurella* that frequently produces pneumonia in mice and, as might be expected, clearance of this organism in some, but not all, animals is slower than that of *Proteus*, and occasionally clearance is completely reversed and bacterial proliferation is observed.

The wide variation in clearance of aerosolized bacteria in relation to species suggests that simple mechanical factors, such as the action of the mucociliary apparatus, are an unlikely basis for the antibacterial action, since it seems unlikely

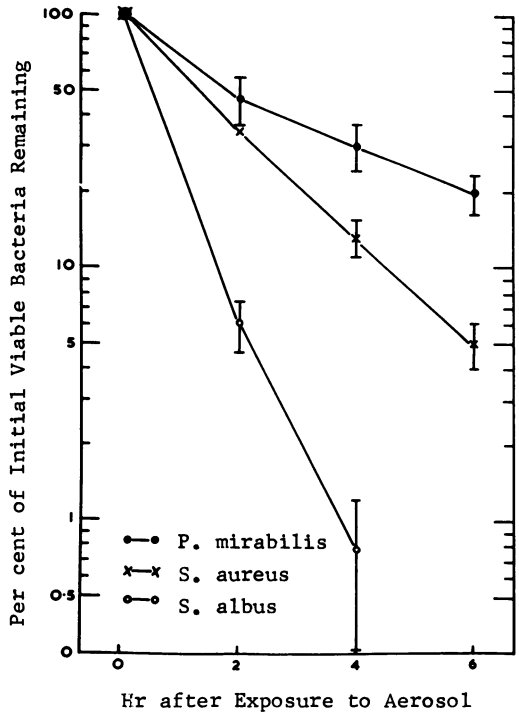

FIG. 5. *Clearance of Proteus mirabilis, Staphylococcus aureus, and S. albus by the normal mouse lung (5).*

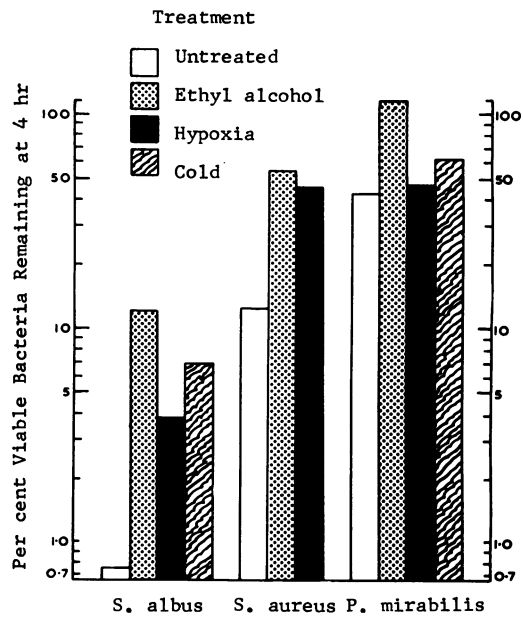

FIG. 6. *Relative effects of ethyl alcohol, hypoxia, and cold on the clearance of Staphylococcus albus, S. aureus, and P. mirabilis by the normal mouse lung. Although in general these circumstances delayed bacterial clearance, the effect of hypoxia in the case of Proteus was negligible. It is also noteworthy that, in the case of Proteus, ethyl alcohol sufficiently depressed bacterial clearance to allow bacterial multiplication to occur (5).*

that a mechanical system would show such a wide range of effectiveness against different species of similar size range. However, such differences in antibacterial activity against different bacterial species are well known in phagocytic systems (2, 14). The observations also indicate that, since the methods of clearance of different bacterial species may be different from one another in a given host, circumstances that alter the rate of clearance may permit one or another species to multiply instead of being cleared.

BACTERIAL CLEARANCE AND ENVIRONMENTAL AND METABOLIC DISTURBANCES

To investigate the relationship of a variety of metabolic events to the clearing mechanism, experimental animals were exposed to the aerosol of appropriate bacteria and then immediately exposed to a metabolic circumstance that might be expected to alter the rate of bacterial clearance (4). It was found (Fig. 6) that hypoxia equivalent to an altitude of 10,000 ft was sufficient to slow significantly the rate of bacterial clearing, and this effect could also be obtained with diminished oxygen tensions at sea level pressures. Ethyl alcohol inhibited bacterial clearing by the lung and did so in direct relationship to the dose of ethyl alcohol administered. Furthermore, the administration of oxygen to the intoxicated animals did not correct the defect. The latter experiment was performed because of the possibility that ethyl alcohol may have depressed respiration and thereby brought about depression of bacterial clearance. Acute starvation for 24 hr was associated with depressed clearance of bacteria by the lung, and once again the degree of depression of clearance was directly related to the amount of weight lost. In retrospect, however, the latter effect may not be entirely due to the weight loss itself, but may be related to such accompanying metabolic disturbances as acidosis (see below). Cortisol also depressed bacterial clearance significantly.

When the effects of the metabolic agents that inhibited bacterial clearance were tested in animals that had received different microorganisms in the aerosol, it was apparent that not all of the metabolically induced suppression of clearance was uniform regardless of species (5). For example, the clearance of staphylococci was only partly depressed by ethyl alcohol, but the clearance of *P. mirabilis* was completely inhibited and multiplication of the organism occurred. Thus, under conditions of ethyl alcohol intoxication,

with all three species of bacteria present in the lung, it might be expected that *Proteus* would emerge as the most likely organism to produce pulmonary infection. On the other hand, whereas hypoxia markedly inhibited the clearance of staphylococci, there was no depression of clearance of *Proteus* in the hypoxic animals. Presumably an oxygen-dependent system in the alveolar macrophage is operative against certain bacteria such as staphylococci, but not against other organisms, such as *Proteus*. Once again there are indications that specific environmental conditions in the presence of a mixed bacterial flora may favor the emergence of one or another bacterial species from the mixture. In vitro, phagocytosis by alveolar macrophages is depressed when oxygen tension is reduced (13).

Bacterial Clearance and Viral Infection

Another clinical circumstance in which pulmonary bacterial infection has been involved has been the presence of a precedent viral infection. Sellers and co-workers (15) demonstrated that in mice infected with influenza virus the intranasal insufflation of staphylococci was followed by virtually no clearance of the organisms by the lung, whereas in animals not infected with the influenza virus, the staphylococci were readily cleared by the murine lung. Detailed studies of this phenomenon have indicated (Fig. 7) that clearance of inhaled staphylococci is inhibited in the presence of a viral infection. However, quite unexpectedly it was found that the time of maximal inhibition of bacterial clearance by the virus-infected lung was toward the end of the 1st week after the induction of the viral infection. With relatively small inocula of virus, no inhibition of bacterial clearance was observed in the first 5 to 6 days after induction of the viral infection, even though the peak of viral multiplication had been reached by approximately 48 hr. On the other hand, distinct and striking inhibition lasting for 1 to 3 days was observed toward the end of the 1st week at a time when viral titers were falling rapidly. Precisely why this unusual time sequence should occur is a matter for future study. It is noteworthy, however, that, clinically, bacterial infections as superimposed complications of viral infections often appear about 1 week after the initial viral infection.

Previous bacterial infections with the same species seem not to inhibit function of the alveolar clearing mechanism very much, as might be expected from consideration of the relative numbers of bacteria inhaled in relation to the large numbers of alveolar cells available. Thus, when mice were exposed to an aerosol of staphylococci on succeeding days for 1 week, the rate of clearance was not different after seven successive exposures than after the initial exposure.

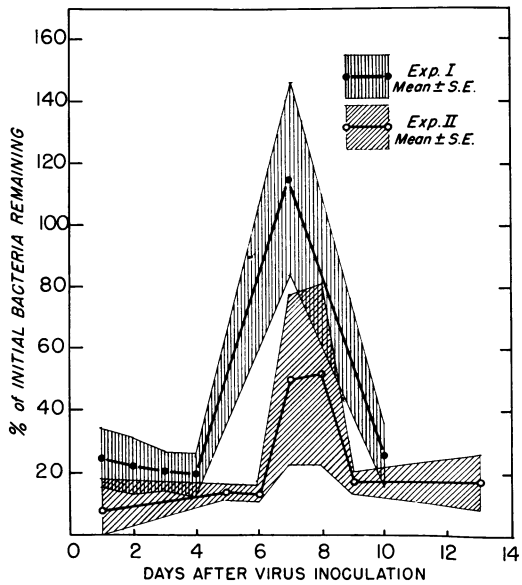

FIG. 7. *Effect of influenza virus infection on clearance of Staphylococcus aureus by the murine lung. The results of two separate experiments are given and plotted as the mean number of bacteria remaining after 4 hr, plotted as the per cent of the initial bacteria. The influenza virus infection was induced with 0.3* LD_{50} *dose.*

Bacterial Clearance and Pulmonary Injury

At first glance it might appear that any injury to the lung would be associated with diminished bacterial clearance. However, the widespread nature of the alveolar system might also suggest that focal anatomic lesions would not inactivate a sufficiently large percentage of available cells to inhibit measurably bacterial clearance, except as the anatomic lesions became overwhelmingly severe. The latter of these two points of view seems to be the correct one. Goldstein (*unpublished data*) has produced silicosis experimentally by the intratracheal administration of silica suspensions, and severe coalescent disease was produced in the lungs of the animals. There was remarkably little effect on bacterial clearance, except perhaps in the terminal stages of the silicotic disease, when a variety of other metabolic consequences of severe pulmonary disease also begin to operate.

Bacterial Clearance and Tobacco Smoke

Most recently, an additional effect on pulmonary bacterial clearance by a particle of major

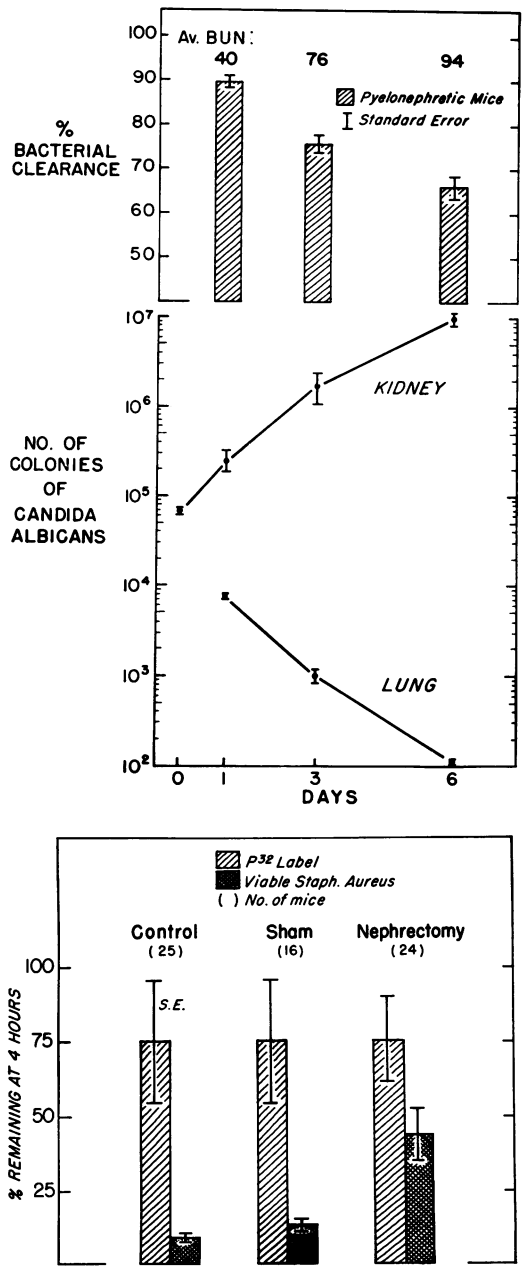

FIG. 8. *Upper portion of the figure indicates the numbers of colonies of Candida albicans in kidneys and lungs of mice after inoculation, and these are related to the levels of blood-urea nitrogen in these animals as well as the per cent clearance of Staphylococcus aureus observed 4 hr after completion of exposure to the aerosol. When numbers of Candida were highest in the lung but blood-urea nitrogen levels were relatively low, there was no significant effect on pulmonary bacterial clearance. As the renal lesion progressed and the numbers of organisms in the lung regressed, the blood-urea nitrogen rose, and there was a corresponding decrease in bacterial clearance. In the lower figure, the effect of nephrectomy on pulmonary bacterial clearance of S. aureus is demonstrated. Although the numbers of bacteria inhaled by each of the three groups is comparable, as evidenced by the comparable levels of P^{32} label in the bacteria, sham surgery slightly depressed bacterial clearance in 4 hr but nephrectomy markedly depressed bacterial clearance.*

public health importance has been observed. It was initially observed by Laurenzi et al. (9) that mice exposed to tobacco smoke suffered inhibition of pulmonary bacterial clearance. More recently, it has been observed by Green and Carolin (*unpublished data*) that the addition of tobacco smoke to cultures of pulmonary macrophages rapidly altered the capacity of the macrophages to cling to the culture flask and greatly diminished the killing power of these cells for added staphylococci. The nature of the agent in tobacco smoke that produces this effect is not yet clear, but the substance is water-soluble and affects macrophage function quantitatively.

A useful methodological innovation that has come from these studies is a consequence of the earlier demonstrations that radio-labeled bacteria may be killed quite rapidly by the lung, but most of the label is readily recovered from the lung after 4 hr, when most of the bacteria are nonviable. In consequence of this observation, it has been possible to study rates of clearance in individual animals rather than in groups of animals, and to do so with considerable precision. It is only necessary to expose animals to radiolabeled bacteria of known specific activity and, after a given period of time, to count the radioactivity and the viability in the homogenates of the lungs. The radioactivity will afford an approximation of the total number of bacteria deposited in the lung, and the direct bacterial counts will indicate residual viability. From these data, the degree of killing can be estimated.

The method has added substantially to the precision of study of the pulmonary antibacterial system, and has made the standard errors of respective points smaller still. Even more important, it has permitted the study of clearance in individual animals and thus has greatly increased the efficiency of the experimental work. Finally, the method offers some hope that it can be adapted to the study of clearance mechanisms in the human being.

Bacterial Clearance and Renal Failure and Acidosis

A recent insight into another major metabolic circumstance that has been clinically associated with apparently increased susceptibility to pulmonary infection has come from the observation that nephrectomized animals or animals whose kidney

function has been reduced in consequence of experimental candidiasis have decreased capacity to clear bacteria from their lungs (Fig. 8). It seems, from the present as yet incomplete analysis of the phenomenon, that the acidosis accompanying the uremic state in these animals is the primary source of the disturbed function of the macrophage system. The implication is clear that pulmonary macrophages harbor enzyme systems that are critical to phagocytosis or bacterial killing, and that are exceedingly sensitive to minute variations in *p*H. The search for such systems should be carried on forthwith.

SUMMARY

In summary, it is apparent that there is an in situ mechanism for clearing bacteria in the lung. This mechanism, which accounts for most of the antibacterial activity, appears to reside primarily in the pulmonary macrophage, and is relatively independent of the function of the mucociliary apparatus. Parenthetically, it has been observed that later in the course of events pulmonary macrophages laden with bacteria may become free and be carried upward by the mucociliary stream. The pulmonary macrophage system is peculiarly susceptible to a variety of metabolic situations, such as hypoxia, ethyl alcohol, acidosis, cortisol, tobacco smoke, and undoubtedly many others. The macrophage system responds differently to different bacterial species, and the metabolic circumstances that alter bacterial clearance do not affect clearance of each of the bacterial species in the same manner. Thus, a metabolic basis emerges whereby a single organism may emerge from a mixture of organisms as a pathogen under specific environmental circumstances. Viral infections inhibit the clearance of bacteria, but do so, strangely enough, after approximately 1 week of viral infection, and not at the time when viral replication is at its height. Multiple anatomic lesions, such as those accompanying diffuse silicosis, have relatively little effect on bacterial clearance, compared with the effects of the aforementioned metabolic states. Tobacco smoke has a water-soluble substance in it that inhibits the function of pulmonary macrophages.

How these observations relate to the genesis of chronic bacterial infection of the lung is only conjectural at present, but clearly the hypothesis can be stated that a variety of environmental circumstances may conspire to reduce slowly the capacity of the pulmonary macrophage to inhibit bacterial proliferation, and that then a chronic state of bacterial proliferation in the bronchial tree may result. It is conceivable that such a chronic state of bacterial habitation in the lung might be detected by appropriate methods long before manifest clinical pulmonary disease could be found, and in this sense the situation in which asymptomatic infections of the lung might be a precursor to chronic pulmonary infections could be analogous to a comparable situation in the urinary tract (7).

ACKNOWLEDGMENTS

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Discussion

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Dr. Kass has reported highly reproducible measurements of the rate of clearance of staphylococci and other bacteria from the lungs of mice after aerosol inoculation. The aerosol particles were 1 to 3 μ in diameter, and the dose, given in a 30-min inhalation, was sufficiently large to permit recovery of at least 50,000 colony-forming units. Studies of lung sections with fluorescein-labeled antibody and by conventional staining methods revealed staphylococcal antigen and some intact bacteria in alveolar lining cells.

With this model, the effect of hypoxia, alcohol, starvation, and other influences was studied. In addition, it was shown that influenza virus infection interfered with the clearance of *Staphylococcus aureus* from the lung.

At this point, it is perhaps of interest to consider briefly the relationship of clearance of staphylococci by alveolar macrophages, referred to by Dr. Kass, with other clearance mechanisms. It is well appreciated at this conference that particles of the size used by Dr. Kass largely escape trapping in the nasopharynx and are carried to the lung. Here a large percentage are deposited, and the remainder are exhaled. Sites available for deposition are the alveoli, the alveolar ducts, respiratory bronchioles, and more proximal airway structures. Although gas exchange occurs quite readily between the tidal air and the alveoli through the layer of residual air in the alveoli, this is effected chiefly by the process of molecular diffusion. In contrast, only 10 to 20% of aerosol in tidal air actually exchanges with residual air with each breath, and molecular diffusion is not a significant factor with particles of the size presently under discussion. It is suggested, therefore, that substantial alveolar penetration will require prolonged periods of breathing of aerosol, probably of the order of that used by Dr. Kass. With a few breaths, particles may be deposited in the lower respiratory tract proximal to the alveoli, and, with further breathing, the site of major deposition will progress peripherally, ultimately to the alveoli, as alveolar wash-in is completed. Parenthetically, I wonder if the slow movement of particles from tidal air to residual air may not be an important means of protection against toxic or infectious particulates in the environmental air.

Once deposited, particles may be removed from alveoli by alveolar macrophages and carried into pulmonary lymphatics. Some macrophages filled with particulates may also be discharged up the airway to the muco-ciliary blanket and then carried up the trachea. In the case of microorganisms which deposit in the respiratory bronchioles, the mode of disposition is not clear. Alveolar macrophages are apparently not available here, and the muco-ciliary blanket begins more proximally. Some studies, however, have described a hyperreactivity of respiratory bronchiolar lining cells which may be a special means of protection in this area. The small volume of lung airway represented by the tracheobronchial tree appears to be the best protected. Inhaled particles which deposit here are carried rapidly up to the posterior pharynx by the muco-ciliary mechanism, where they may be expelled or swallowed.

At present, I know of no studies which adequately describe relative degrees of deposition of small particles in peripheral lung areas in relation to the duration of exposure to small-particle aerosol. I believe the question to be of importance, since, if the foregoing concept is correct, it would be possible to deposit small-particle aerosol in