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Measurement of Different Mechanisms for Elimination of Bacteria from the Lung

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INTRODUCTION

Several clinical and experimental studies have shown that the normal defense of the lung against bacteria might be affected by environmental agents, such as toxic gases (2), alcohol (7), and decreased temperature (3).

Although experimental studies of the disappearance rate of viable bacteria or of changes in mortality among bacteria-exposed animals are of great interest, a complete understanding of the recorded effects requires the study of the different elimination mechanisms separately. In turn, certain of the agents used to produce changes are able to affect one or more of these mechanisms.

This report presents a brief description of experimental methods used for the elucidation of the function of the different elimination mechanisms, and suggests an experimental set-up whereby the efficiency of the various mechanisms can be measured when the short-term elimination of bacteria from the lungs is being studied. In view of the bacteriological findings in chronic bronchitis (5), the present work has been performed with nonpathogenic bacteria, which were originally cultured from the mouths of the animals.

METHODS AND RESULTS

Guinea pigs were exposed for 10 min to a flow of radioactive bacteria in a monodisperse aerosol in a stainless-steel exposure chamber. The number of bacteria present at various sites in the lung was determined by use of bacteriological and autoradiographic (1) techniques. The number of bacteria at various times after the exposure was expressed as the percentage of the initial number remaining.

The number of viable bacteria in the whole lung was determined by use of a grinding technique similar to that described earlier (6). However, to ensure complete lysis of phagocytes, saponin was added to the grinding fluid in the present experiments. The number of viable bacteria was found to decrease fairly rapidly from these whole-lung preparations (Fig. 1). The results are generally in accordance with earlier reports. As the disappearance rate from the whole-lung preparations is a determinant of all the elimination mechanisms, the following experiments were performed to elucidate the individual functioning of the different mechanisms.

The airways were washed with sterile saline by use of a modification of the technique of LaBelle and Brieger (4). The lungs were flushed three times via the trachea with 10 ml of saline under aseptic conditions; then the number of bacteria in the fluid was determined. When *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, which all were present in the normal mouth flora, were used, it was found that the rate of decrease of viable bacteria in the fluid was more rapid than the decrease from whole-lung preparations (Fig. 1). If saponin was added to the wash-out fluid, a larger number of viable bacteria was found. If the autoradiographic technique was used, even more bacteria (*E. coli*) could be detected in the fluid (Fig. 2).

When the insides of isolated pieces of trachea, standardized with respect to length, were flushed with saline, the number of viable bacteria was found to be rather high during the first few hours after exposure but decreased later on. When saponin was added to the tracheal wash-out, a significant increase in the number of bacteria was only occasionally found (Table 1).

COMMENTS

The above findings suggest that, during the duration of these experiments, most of the *E. coli* that deposit on the mucus are carried upwards out of the lung without prior phagocytosis. The very rapid decrease in number of bacteria found

in the wash-out from the airways is apparently dependent upon the mucus transport. As viable bacteria and autoradiographically detectable bacteria could be expected to be removed by mucus at identical rates, the difference (Fig. 2) indicates that other mechanisms may be involved.

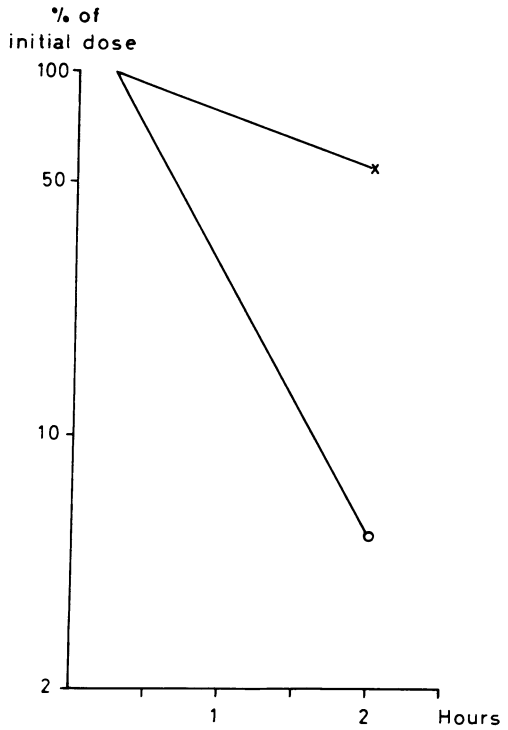

FIG. 1. *Decrease in viable bacteria (Escherichia coli) in whole lung (×) and in airway wash-out fluid (○).*

Although the wash-out fluid cannot be expected to remove all bacteria from the minor airways and alveoli, the presence of phagocytes in the airway wash-out fluid and the almost complete absence of phagocytes in the trachea wash-out fluid indicate that at least a certain number of the peripheral airways are subject to flushing with removal of free or loosely attached phagocytes. The difference in disappearance rates of viable bacteria and autoradiographically detect-

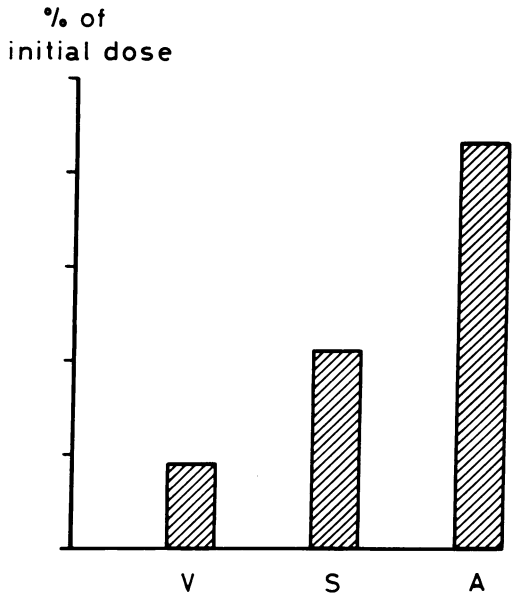

FIG. 2. *Number of Escherichia coli present in airway wash-out fluid 2 hr after exposure. V = viable; S = viable after saponin treatment; A = autoradiographically counted.*

TABLE 1. *Number of viable Escherichia coli cells in trachea wash-out fluid before and after saponin treatment*

Time after exposure									
0 hr		1 hr		2 hr		3 hr		4 hr	
V[a]	S[a]	V	S	V	S	V	S	V	S
117	130	55	56	44	30	4	0	6	4
81	42	271	382	6	8	0	2	20	22
153	160	15	12	0	0	329	440	9	12
139	144	106	108	14	22	60	46	166	160
25	18	6	4	21	8	0	0	1	0
10	40	17	34	10	0	1	10	0	2
118	150	19	24	11	16	170	310	0	0
123	108	14	82	1	0	7	24	0	0
330	1,228	11	6	9	14	0	4	0	2
108	206	62	208	42	42	0	0	1	0

[a]V = viable E. coli cells before treatment; S = viable *E. coli* cells after saponin treatment.

able bacteria from the airway wash-out fluids could then be due to an uptake of bacteria by phagocytes, whereby the bacteria cannot be cultured but are autoradiographically detectable. This theory is supported by the observed difference in removal rates of viable bacteria with or without treatment of the wash-out fluid with saponin. The increased yield of bacteria when saponin is added to the fluid indicates that some bacteria initially retain their viability when within or adherent to the phagocytes.

The reduction in the number of bacteria in airway wash-out fluids is thus, apart from being a determinant of mucus transport, also due to the removal of bacteria from the airways by *uptake in the phagocytes.*

The decrease in number of viable bacteria in the whole lung preparations is obviously the combined effect of mucus removal and of the bacteriocidal effect of the phagocytes.

Another elimination mechanism may be considered, namely, the removal of phagocytized bacteria out of the lung via an interstitial route. Cultures from blood and autoradiographic preparations from liver, kidney, and spleen did not reveal the presence of any bacteria, thus excluding the possibility that direct elimination via the blood plays a major role in the present experiments and with the types of bacteria studied here.

To be able to test the elimination mechanism discussed here, one would have to use two different organisms, one of which is subject to only one of the elimination mechanisms. Spores seem to be suitable for this purpose.

The efficiency of the mucus removal, of the phagocytic removal from the airways, and of the bactericidal effect of the phagocytes in the lung can thus be tested with the following model.

The animals are exposed to an aerosol containing radioactive spores and nonradioactive bacteria, e.g., *E. coli.* The reduction in the number of spores in whole lung grinding will then give an estimate of the capacity of the mucus removal. The reduction in the number of viable bacteria in wash-out fluids from the airways will give an estimate of the capacity of the phagocytic and mucus removal, and the reduction of viable bacteria in whole-lung grindings will provide an index of the bactericidal activity of the phagocytes in the lung and of mucus removal. Effects on any of the above functions can then be evaluated, provided that the three functions are tested simultaneously and that the effects are expressed as deviations from the normal.

Preliminary results from experiments performed with the above model indicate that the mucus removal during the first 2 hr after exposure accounts for about 15% of the total removal under the experimental conditions used. This rate agrees with results from experiments where the elimination of monodisperse plastic aerosols of various size ranges has been followed (Holma, *personal communication*). Experiments are in progress wherein the possible effects on any of the three elimination mechanisms discussed above of continuous exposure to low doses of SO_2 and to dust are being evaluated.

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