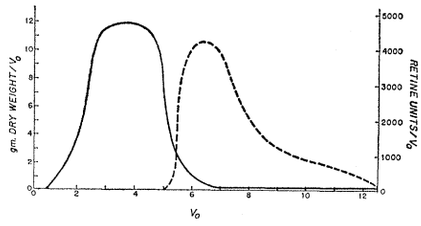

Fig. 2. Filtration of an extract of 1000 liters of urine through Sephadex G–100 gel column. Solid line, distribution of the total dry weight in the eluate. Dotted line, the distribution of the biological activity in the void volumes, expressed in retine units.

unit. The purified preparation of retine dialyzes through cellophane, indicating a molecular weight of less than 1000. The behavior on various Sephadex columns suggests a molecular weight of about 400 (*2*).

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Clearance of Bacteria by the Lower Respiratory Tract

Abstract. *The disappearance of inhaled bacteria from the lungs of mice is rapid and predictable over an 8-hour period following pulmonary implantation by an aerosol. This indicates that important clearance mechanisms are active in the bronchopulmonary tree. The constant pattern of clearance offers an experimental system in which the effects of various conditions relevant to respiratory infection can be estimated quantitatively. Herein, the effects of hypoxia, alcohol, cigarette smoke, and cortisone are described.*

The fact that the normal lower respiratory tract is free of viable bacteria (*1*) is indeed remarkable, since bacterial contamination by inspired air and the aspiration of upper respiratory tract secretions is a common and almost inevitable occurrence. In contrast, bacterial pathogens can be isolated from the bronchial secretions of patients with chronic bronchitis during periods of relative well-being (*1*). These findings indicate that antibacterial mechanisms which are effective in the normal bronchial tree are markedly impaired in subjects with chronic bronchitis. This report describes a quantitative method for studying the capacity of the lower respiratory tract to dispose of inhaled bacteria.

Aerosols were generated from a buffered suspension (*p*H 7.3) of bacteria contained in eight glass nebulizers. The spray from the nebulizers was directed into mixing cylinders through which a secondary airflow of 2830 lit./minute was drawn. The larger air volume mixed the aerosol and carried it into a plexiglass chamber holding 200 white mice. Swiss Webster male mice were exposed for a period of 30 minutes to aerosols of a coagulase-positive *Staphylococcus aureus* (FDA 209P) which has relatively low virulence for the mouse. It was ascertained that the lungs of this strain of mice were normally sterile. After exposure, one group of mice was killed immediately, and the others were grouped and killed at hourly intervals thereafter; the lungs from the mice in each group were removed aseptically, homogenized, and diluted. Nutrient agar pour plates were made of the lung homogenates. After incubating the plates for 48 hours at 37°C, the numbers of viable bacteria in the lungs of each mouse was calculated from the product of the number of plate colonies and the dilution factor. Deposition refers to the numbers of viable organisms retained in the lungs at the end of the exposure time; and mean deposition values for groups of mice correlated directly with nebulizer and air concentrations of bacteria, and lung and body weights.

As illustrated in Fig. 1, the lungs of mice killed during the postdeposition period showed a rapid decrease in the numbers of viable staphylococci. The mean numbers of viable bacteria retained was determined at the designated time intervals; and by subtracting these values from the mean numbers originally deposited, the mean numbers of staphylococci cleared were derived. In addition, the numbers retained were expressed as a percentage of the deposition, and the percentage cleared was obtained by subtracting the percentage retained from 100 percent. At the end of 1 hour, 45 percent of the bacteria deposited had cleared; after 2 hours, 70 percent; 3 hours, 80 percent; 4 hours, 88 percent, and after 6 hours, 95 percent of the staphylococci had cleared. The clearance curve was remarkably constant for bacterial depositions (at 0 hours postdeposition) from 1.0 to 2.5 $\times$ 10^5, and it is identical with the pulmonary clearance pattern for smaller numbers of bacteria (*2*). It is emphasized that the term "clearance" refers to that number of deposited bacteria whose presence in a viable state can no longer be demonstrated. During the experimental period they might have been exhaled, killed, or actually removed from the lungs. Lungs which were removed after deposition and incubated at 37°C showed bacterial colonization. These results indicate that clearance is an active process and does not represent the natural death rate of the organism in pulmonary tissue.

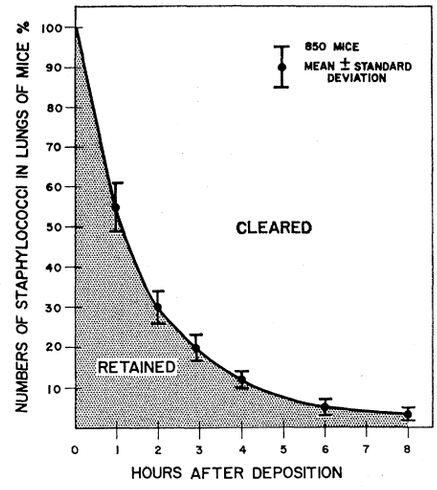

Fig. 1. The clearance of staphylococci from the lungs of mice. Each point on the curve represents the mean percentage of three or more studies with an average of 50 mice in each study. Example: deposition 100,000 ± 13,000; retained at end of 1 hour, 55,000 ± 7000; 2 hours, 30,000 ± 5000; 3 hours, 20,000 ± 3500; 4 hours, 12,000 ± 2500; 6 hours, 5000 ± 1000; and 8 hours, 4000 ± 1000.

The pulmonary clearance of staphylococci was then determined by this method in mice subjected to hypoxia, cigarette smoke, alcohol, and cortisone. The percentage of deposited organisms which were cleared in 4 hours by the normal (control) Swiss Webster male mice was compared with corresponding results obtained with mice subjected to each experimental condition. However, the individual and comparative effects of the different variables are made more apparent, quantitatively, by relating the experimental and control results in each circumstance in terms

of the ratio of the numbers of bacteria retained: (experimental/control). This calculation is designated the relative retention ratio. The differences between the results obtained with the mice subjected to the experimental conditions and those obtained with the corresponding control mice were all highly significant ($p < .001$). Hypoxia, cigarette smoke, and alcohol were introduced and maintained for the first 4 hours following bacterial deposition. Mice, in an atmosphere of 10 percent oxygen (90 percent nitrogen), showed 70 percent clearance and a relative retention ratio of 2.5 (that is, they retained 2.5 times as many bacteria as the controls). Inhalation of cigarette smoke at subtoxic (nicotine) concentrations reduced clearance to 50 percent, and the retention ratio was 4.5. Intraperitoneal injections of 1 ml of 12 percent ethyl alcohol rendered mice stuporous, and clearance dropped to 62 percent, with a retention ratio of 3.6. Cortisone was administered in three divided doses to mice prior to exposure; and at a total dose of 1.22 mg of hydrocortisone (injected subcutaneously) per gram of body weight the animals appeared sick and lost weight. However, they showed 80 percent clearance with a retention ratio of 2.4.

This study offers an effective method for estimating quantitatively pulmonary resistance to airborne bacteria. The method includes: (i) the production of bacterial aerosols of controlled concentrations and particle size from which predictable numbers of bacteria can be implanted in the lungs of experimental animals; (ii) the demonstration of the rapid clearance of the bacteria; and (iii) the measurement (assay) of significant reduction in clearance produced by certain conditions which are often implicated as contributory factors in pulmonary damage and infection. The use of an organism of low virulence which clears rapidly provides a convenient system in relation to time, avoids complicating infection, and lends greater significance to conditions which reduce clearance. In this study, smoke inhalation and alcohol caused most interference; cortisone and hypoxia caused less, but significant, impairment. The disposal of foreign particles from the bronchopulmonary tree is most often attributed to the cleansing actions of the muco-ciliary stream (*3*) and alveolar phagocytes (*4*). With bacteria, immunologic mechanisms must also be considered. Studies of bacterial clearance during the selective blockade of these mechanisms may help to clarify their relative roles in resistance to pulmonary infection (*5*).

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Temperature Regulation and Metabolism in Mexican Freetail Bats

Abstract. *Body temperatures of* Tadarida mexicana *in their natural cave environment were usually maintained at high levels, even when ambient temperatures were low. Oxygen consumption rates were correspondingly higher in low environmental temperatures. However, in laboratory tests, body temperatures and metabolic rates are fairly dependent on ambient temperature.*

Bats of the suborder Megachiroptera have been reported to regulate their body temperatures at fairly constant levels (*1*, *2*). In contrast, body temperatures and metabolic rates of resting Microchiroptera can drop to lower levels (*3*, *4*). Microchiroptera have even been referred to as poikilothermic (*5*). Although there has been some suggestion that they are not always so temperature labile (*2*, *4*, *6*), a detailed study of the temperature response of Microchiroptera in nature, particularly of species which appear not to hibernate, seems lacking. The following data were collected on a migratory species, the Mexican freetail bat, *Tadarida mexicana,* under laboratory and field conditions.

Bats from a variety of Texas and Oklahoma caves were studied throughout most of the year 1959. Rectal temperatures (*7*) were usually obtained within 30 seconds after the bat was removed from the cave ceiling. The bat was killed by dislocation of the cervical vertebrae just prior to the measurement. In spite of variations in the ambient temperature between 12° and 36°C, the *Tadarida* usually had resting temperatures (during the day) between 32° and 42°C (Fig. 1). Field observations during the freetail's stay in the southwestern United States and in the winter in Mexico also indicated that body temperatures are usually sufficiently elevated to permit flight. Young bats, incapable of flight, also had rectal temperatures well above the environmental temperature (Fig. 1). Measurements over a 24-hour period in the cave did not reveal any pronounced depression of body temperatures. Although the temperature of freetails is not as stable as in many mammals, it appears to be less labile than previously considered.

Oxygen consumption was measured throughout a 12-hour period in the cave and was found to be consistent with the pattern obtained for the body temperature. Determinations were made by a direct volumetric technique (*8*). Bats were captured as they returned to the cave in the morning and placed in metabolic chambers situated within the cave. The chambers were subject to normal changes in air temperature, noise, and light within the cave, but they were shielded from disturbances created by the operator. Average metabolic rates were higher at colder temperatures; the lowest rates were recorded in the summer months when environmental temperatures were be-

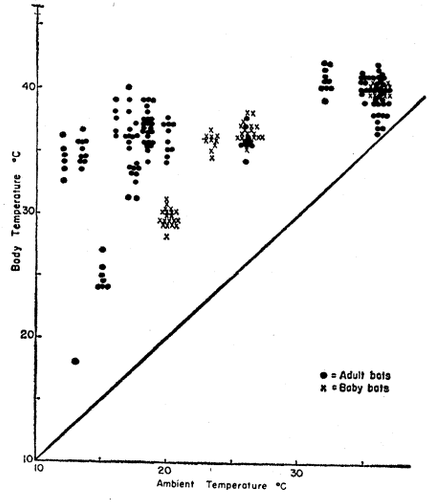

Fig. 1. Body temperatures of free-tailed bats in the cave environment.