Mucociliary Clearance from the Calf Lung

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ABSTRACT

Mucociliary clearance rate constants for ten calves were obtained using Technetium 99m-sulphur colloid complex. The mean rate constant measured at the lung for all calves was 0.012 (± 0.009) min^{-1} (half-life 58 minutes). The clearance rate constants obtained from measurements made at the larynx were higher (0.02 ± 0.007 min^{-1}), although not significantly higher, than those determined from measurements taken at the lung. The constants can be used to predict the particle burden on the lungs of calves kept under husbandry systems of varying air hygiene.

Key words: Lung clearance, mucociliary clearance, calf, rate constant, larynx, technetium.

RÉSUMÉ

Cette étude visait à obtenir des constantes du taux de clairance muco-ciliaire, chez dix veaux, en utilisant le complexe: technétium 99m-colloïde sulfureux. La constante moyenne de ce taux, comme le révéla sa détermination dans les poumons de tous les veaux, atteignit 0,012 ± 0,009 min^{-1}, par rapport à une demi-vie de 58 minutes. La détermination de cette valeur, dans le larynx, donna une valeur sensiblement plus élevée de 0,02 ± 0,007 min^{-1}. Ces constantes peuvent servir à prédire le fardeau pulmonaire de particules, chez des veaux qu'on garde dans des conditions de régie qui comportent des variations de la qualité de l'air ambiant.

Mots clefs: clairance pulmonaire, clairance muco-ciliaire, veau, constante du taux de clairance, larynx, technétium.

INTRODUCTION

The clearance of viable bacteria from the calf lung is essential if infection is to be avoided. In some calf houses the airborne challenge to the calf can be as high as 5000 bacteria colony-forming particles (BCFP) deposited in the lung per hour. Such high airborne BCFP concentrations are known to exacerbate pneumonic conditions (1). It appears, moreover, that the magnitude of this challenge will vary with climatic environment, particularly relative humidity (2). Previous clearance studies with calves have used a technique of sequential slaughter (3,4,5) which does not distinguish between mechanical clearance of bacteria via the mucociliary apparatus and biocidal clearance via macrophages and other antibacterials (6). The technique is also extravagant in its use of calves. There is a need to determine the relative contribution that mucociliary clearance makes to overall lung clearance in calves. Also, there is an equal need for alternative sampling techniques that do not require slaughter, particularly for the study of environmental effects on deposition and clearance of BCFP.

The experiment reported here measured mucociliary clearance rate constants for calves at 50% and 80% relative humidity (r.h.) using an aerosol of Technetium 99m (^{99m}Tc). Technetium 99m is a low energy γ emitter and is commonly used for diagnostic purposes in humans (7,8). An attempt was also made to determine mucociliary clearance rate constants from measurements taken at the larynx.

MATERIALS AND METHODS

CALVES

Ten, four week old Friesian male calves (Numbers 1-10) were used. The calves were purchased from a local market and housed on site for three weeks before the experiment. They were kept individually in wooden crates at a constant temperature of 16°C and an r.h. of 80%, or 50%.

AEROSOL EXPOSURE

A polydisperse aerosol of Technetium 99m-sulphur colloid complex (^{99m}Tc) in distilled water with a mass median aerodynamic diameter of 3.4 μm was produced using a DeVilbiss '65 ultrasonic nebulizer (DeVilbiss Health Care UK Ltd.). The Malvern 2200 Particle Sizer (Malvern Instruments Ltd., Spring Lane, Malvern) was used to measure the characteristics of the aerosol produced. Eighty-six percent of the total aerosol mass was in particles below 10 μm in diameter. The calves were exposed to the aerosol

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using the apparatus illustrated in Fig. 1. Air supplied to the calf contained 7.4×10^4 Bq l^{-1} (2 μCi l^{-1}) and each calf was exposed to the aerosol for a period of 10 min. The estimated total dose of radioactivity received by each calf was between 1.5×10^7 and 3.0×10^7 Bq (400-800 μCi).

MEASUREMENT OF CLEARANCE

The activity within the calf lung was monitored every ten minutes for 180 minutes using a sodium iodide crystal counter with the detecting probe positioned over the fifth and sixth ribs immediately caudal to the triceps to achieve maximum counts. Three ten second counts were taken on each side of the animal for each observation. The activity at the larynx was measured every 20 minutes over the same time period for calves 7-10 only at 80% r.h., by swabbing the larynx in a standard manner with a laryngeal swab (Exogen Ltd., Clydebank, Glasgow, Scotland) and performing counts on the cotton buds of the swabs. Final counts were taken 24 h after the initial exposure. There was no leaching of activity from the ^{99m}Tc colloid.

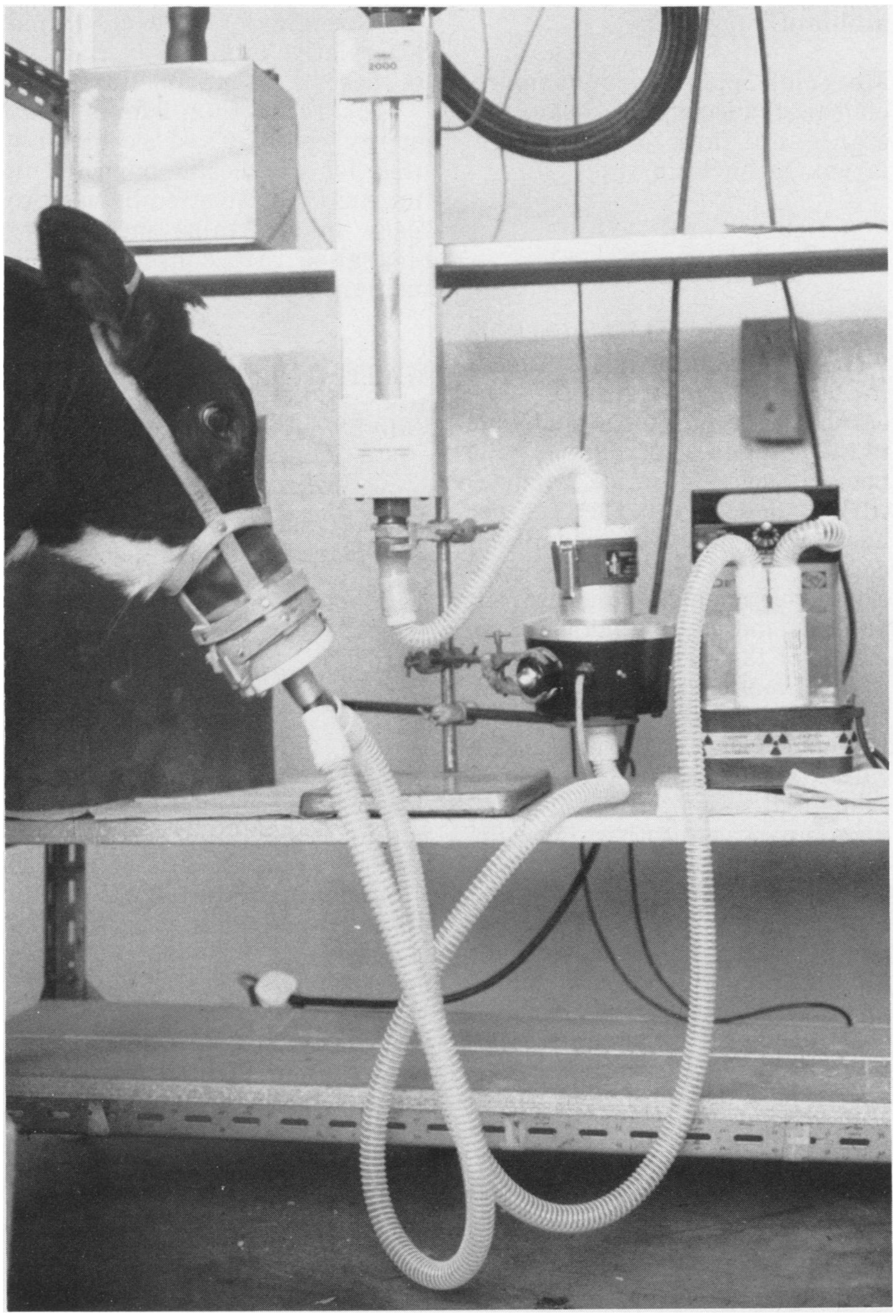

Fig. 1. Apparatus for exposing calves to an aerosol of ^{99m}Tc-sulphur colloid complex.

DETERMINATION OF CLEARANCE RATE CONSTANTS

Clearance of the ^{99m}Tc from the lung was described by the following equation:

$$Q_{(t)} = A + M e^{-kt}$$

where Q is the radioactivity measured at time t, A is the total activity deposited in the alveolar portion of the lung and not cleared by the mucociliary apparatus and M is the activity removed via the ciliated airways at a rate proportional to the amount present (k). The rate of removal of ^{99m}Tc from the alveolar portion was considered negligible over the time period monitored and the activity at 24 hours was taken to be equivalent to this alveolar portion A (9). Regressing $\log_e Q_{(t)}$ on time yielded curves with slopes of k.

The flow of ^{99m}Tc at the larynx was dependent on the rate of change of Q and described by the equation:

$$F_{(t)} = - \frac{dQ}{dt} = k M e^{-kt}$$

where F is the flow of ^{99m}Tc in particles min^{-1}. The activity obtained on the laryngeal swab (S) was taken to be a function of F such that:

$$S_{(t)} = \mu k M e^{-kt}$$

where μ is some function of the swabbing procedure. Regressing $\log_e S_{(t)}$ on time would again yield a curve of slope k. This relationship between F and Q depends on there being no time lag between the deposition of ^{99m}Tc and its appearance at the larynx — it assumes that the system is "primed". There is also the assumption that the flow of ^{99m}Tc (in particles min^{-1}) is independent of the position in the respiratory tract. It is known that the mucus velocity in any ciliated airway increases from the terminal bronchioles to the larynx (10).

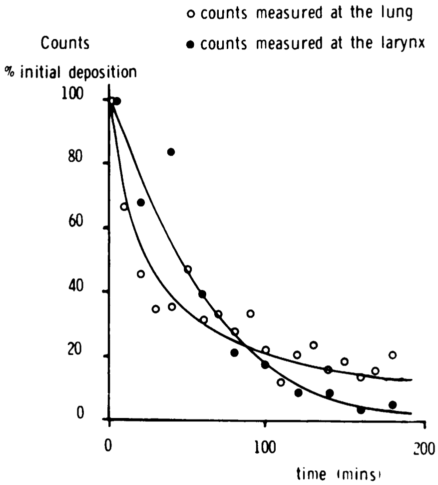

Fig. 2. Mean clearance curves of ^{99m}Tc-sulphur colloid complex from the lungs of calves measured at the lung and larynx (Lung curve corrected for 24 hour count).

Whether this increase is reflected in the total particle flow min^{-1} at a given level would depend on the airway dimensions. Any discrepancy between the two k values derived by the two methods would question these assumptions.

RESULTS

Mean clearance curves measured at the lung and larynx are shown in Fig. 2. The overall clearance rate constants were 0.012 (s.d. 0.009) and 0.020 (s.d. 0.007) min^{-1} for the lung and larynx respectively. Expressed as half-lives these constants are 53 and 34 min. The individual rate constants for each calf are given in Table I. The mean mucociliary clearance rate constant for calves at 80% r.h. was 0.013 (s.d. 0.01) and was 0.011 (s.d. 0.005) at 50% r.h. There was no significant difference between the rate constants obtained at the two r.h. (Table II). The differences between the rate constants obtained at the two sites (the lung and larynx) were not statistically significant, partly because of the large differences between animals for rate constants measured at the same site (Table III). The shape of the lung clearance curve in Fig. 2 suggests that clearance had not been adequately corrected for alveolar deposition and so would tend to yield lower rate constants.

TABLE I. Mucociliary-clearance Rate Constants for Technetium 99m-Sulphur Colloid Complex from the Lungs of Calves Measured at the Lung and Larynx

		Clearance Rate Constant (min^{-1})[a]	
	Animal	Lung	Larynx
50% r.h.	1	0.012	
	2	0.007	
	3	0.006	
	4	0.019	
	5	0.010	
80% r.h.	6	0.021	—
	7	0.004	0.020
	8	0.032	0.013
	9	0.006	0.020
	10	0.002	0.030

[a]Clearance rate constants (k) may be converted to half-lives ($t_{1/2}$) using the following relationship:

$$t_{1/2} = \frac{\log_e 2}{k}$$

DISCUSSION

The mean clearance rate constant obtained at the lung for all calves was 0.012 min^{-1}. Studies using ^{99m}Tc tagged particles in donkeys have derived values of 0.003 to 0.05 min^{-1} (11) and a range of values from 0.004 to 0.02 min^{-1} were obtained from measurements taken on human patients with and without asthma (12). Thus, the results of the present experiment give mucociliary clearance rate constants within the range of those reported by other authors, using a similar technique.

The rate constants are considerably higher than clearance rate constants for viable bacteria calculated from studies of total lung clearance in calves by Veit and Farrell (5), Lillie and Thomson (3) and Lopez *et al* (13). Values derived from the results of these authors are respectively 0.007, 0.005 and 0.004 min^{-1}. This indicates that net biocidal clearance is slower than mechanical clearance. Rylander (14) demonstrated in guinea pigs that the clearance of viable *Escherichia coli* from the "airways" (presumably, mucociliary and biocidal clearance) was faster than clearance from the "lung" where biocidal clearance predominates. The clearance rate constants were 0.012 and 0.006 min^{-1} respectively, suggesting that mechanical and biocidal clearance rate constants, if additive, are of the same order. These observations of low biocidal clearance rates are curious when compared with the rates of phagocytosis by alveolar macrophages obtained *in vitro* by Maheswaran *et al* (15). These workers reported a 90% uptake of *Pasteurella haemolytica* by macrophages after 30 minutes' incubation, which would give a clearance rate constant of 0.077 min^{-1}. Phagocytosis of *Staphylococcus aureus* by human leucocytes was found to be faster than the subsequent intracellular killing but even so, rate constants of 0.08 were measured by the latter (16). This intra-

TABLE II. Analysis of Variance of Mucociliary-clearance Curves of Technetium 99m from the Lungs of Calves Kept at 80% or 50% Relative Humidity

Source of Variation	df	Sums of Squares	Mean Square	F
Regression	1	11.637	11.637	
Intercepts	8	37.779	4.722	
Slopes: between humidities	1	0.438	0.438	0.6 NS
within humidities	7	5.083	0.726	
Residual	138	19.345	0.140	
Total	155	74.280		

TABLE III. Analysis of Variance of Mucociliary-clearance Curves of Technetium 99m from Calves Lungs, Measured at the Lung and the Larynx

Source of Variation	df	Sums of Squares	Mean Square	F
Regression	1	10.644	10.644	
Intercepts	8	54.944	6.868	
Slopes: between sites	1	1.028	1.028	1.16 NS
within sites	7	6.222	0.889	
Residual	106	17.717	0.167	
Total	123	90.556		

cellular killing is reduced in alveolar macrophages at high challenge levels (15). Al-Izzi *et al* (17) achieved total lung clearance rates for viable bacteria closer to the values that would be expected from the *in vitro* work (0.016 min^{-1}). However, it appears that the rate of killing of bacteria in the calf lung is slower than would be expected from *in vitro* studies. Also, for viable bacteria deposited on the ciliated airways, mechanical clearance is, quantitatively, a more important means of removal than biocidal clearance.

The variation in mucociliary clearance between individuals of the same species has been widely reported (18), and may partly account for the variation in total lung clearance observed by many authors (13). Some of the variation in the present experiment may be explained by the differences in the site of initial deposition of the aerosol between the calves (8). There was, however, no consistent relationship between the 24-hour retention levels and the clearance rate constants (the mean retention level was 50% of the initial dose). Thus, deposition site alone could not explain all the variation observed. Other authors have investigated these differences between individuals in greater detail and a recent review has been published by Pavia *et al* (8). Mucociliary clearance rates have been reported to vary with age of animal, sleep, environmental pollutants and various disease states. It is undisputed, though, that individuals with poor clearance ability have an increased susceptibility to lung disease. The rate of clearance via the mucociliary apparatus could be a crucial characteristic for the housed calf (19).

The relationship between the clearance rate constants measured at the two sites, described by an equation earlier in the paper, has not been disproved. However, although there was no significant difference between the rate constants obtained at the two sites, those at the larynx were usually higher than those at the lung. This may have been a consequence of the experimental technique. The use of a polydisperse aerosol may have invalidated the second assumption, while ensuring the condition required by the first, allowing too large a portion of the aerosol to be deposited in the upper airways.

The relationship between the clearance rate constants measured at the two sites can be further investigated in future experiments using monodisperse aerosols. The mean mucociliary clearance rate constant (0.012 min^{-1}), derived from the present experiment, may be used to predict the levels of inhaled bacteria retained at any one time in the lungs of calves housed under varying conditions of air hygiene. Assuming that net biocidal clearance in the ciliated airways is negligible in comparison to mechanical removal, the sustained load of viable bacteria in these airways can be estimated from the following relationship:

$$M_{(t)} = \frac{d}{k} (1 - e^{-kt})$$

where d is the bacterial deposition rate (bacteria min^{-1}) and is constant. $M_{(t)}$ achieves an equilibrium value at $\frac{d}{k}$. Thus, a calf reared extensively where d = 2 would sustain, on average, 150 bacteria in its lung. A housed calf, however, under conditions where $d = 5 \times 10^3$ would sustain 3.8×10^5 bacteria in its lung. With individual calf variation in k this figure will also vary, possibly by a factor of 20 between calves.

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