Deposition and Clearance of Monodisperse Aerosols in the Calf Lung: Effects of Particle Size and a Mucolytic Agent (Bromhexine)

Christine P. Davies and A.J.F. Webster*

ABSTRACT

Mucociliary clearance and retention of monodisperse aerosols of radiolabelled polystyrene particles of both 3.3 μm and 5 μm diameter were investigated in four healthy calves and two sick calves. The effect of the mucolytic agent bromhexine was also assessed at two dosage levels.

There were significant differences ($P<0.05$) in clearance rate constant between calves, but similar patterns of clearance for each calf. These characteristics of mechanical lung clearance did not vary over a two month period. Values of clearance rate constant and percentage retention varied significantly ($P<0.001$) between the two different particle sizes, 5 μm particles giving faster clearance and lower retention of particles than 3.3 μm particles. Bromhexine at the recommended dose of 1.6 mg/kg$^{0.75}$ caused a significant ($P<0.05$) increase in clearance rate in both healthy and sick calves, but affected percentage retention only in sick calves.

This study illustrates the variation in mucociliary clearance rates shown by individuals and also underlines the importance of particle size in aerosols used for studies of pulmonary deposition and clearance. The work also indicates that bromhexine may be of use in the therapy of respiratory disease in calves.

Key words: Respiratory system, lung, bromhexine, cilia, aerosols, cattle.

RÉSUMÉ

Cette expérience portait sur quatre veaux sains et sur deux, malades. Elle consistait à étudier la clairance mucociliaire et la rétention d'aérosols de particules radioactives de polystyrène, de 3,3 μm et 5 μm de diamètre. Elle visait aussi à évaluer l'effet de deux doses différentes de l'agent mucolytique bromhéxine.

La constante du taux de clairance individuel afficha des différences significatives ($P<0,05$), tandis que les profils de clairance individuels s'avérèrent semblables. Ces caractéristiques de la clairance pulmonaire mécanique ne varièrent pas, au cours d'une période de deux mois. Les valeurs de la constante du taux de clairance et le pourcentage de rétention varièrent de façon appréciable ($P<0,001$), selon les dimensions des particules précitées: celles de 5 μm entraînèrent une clairance plus rapide et une rétention plus faible que celles de 3,3 μm. La bromhéxine, à la dose recommandée de 1,6 mg/kg^{0,75}, causa une augmentation significative ($P<0,05$) du taux de clairance, tant chez les veaux sains que chez les malades, mais elle affecta le pourcentage de rétention, seulement chez les malades.
viduelle du taux de clairance mucociliaire; elle souligne aussi l'importance des dimensions des particules, dans les aérosols utilisés pour l'étude de leur dépôt dans les poumons et de leur clairance ultérieure. Elle indique également la possibilité d'utiliser la bromhéxine dans la thérapie des maladies respiratoires des veaux.

Mots clés: système respiratoire, poumons, bromhéxine, cils, aérosols, bovins.

INTRODUCTION

Mucociliary clearance is a nonspecific lung defence mechanism which is an important first line of defence against potential disease-causing agents. Natural variations in mucociliary clearance and external factors influencing this mechanism may have important consequences for the onset and therapy of respiratory disease.

Clearance of live bacteria from the calf lung has usually been studied using the technique of sequential slaughter (1,2). This method does not distinguish between mechanical clearance by the mucociliary apparatus and the biological balance between pathogenic organisms and host defences at epithelial surfaces. It also necessitates the slaughter of large numbers of calves and precludes repeated measurements in the same individual, which are essential for the study of effects of external agents on lung deposition and clearance. Mechanical clearance from the lung can be assessed by the external monitoring of the clearance of inhaled, radioactive, inert aerosols. This technique is well established in human medicine (3,4) and has also been used in calves (5). By creating monodisperse aerosols of known particle size it is possible to influence sites of deposition of particles within the lung (6,7).

The mucolytic agent bromhexine (N-cyclo-hexyl-N-methyl-[2-amino-3, 5 dibromobenzyl]-amine hydrochloride: Bisolvon[R]; Boehringer Ingelheim) has been the subject of several studies in human medicine which have produced conflicting results. The drug did not significantly increase the rate of mucociliary transport or the amount of sputum expectorated in a study of human bronchitic patients (8), but a

*Department of Animal Husbandry, University of Bristol, Langford House, Langford, Bristol BS18 7DU, United Kingdom.
Submitted October 9, 1986.

different study found a significant increase in the clearance rate of similar patients after 14 days' dosage with bromhexine at 48 mg/day (9). In veterinary medicine, bromhexine was found to halve the protein content of tracheal mucus in lambs and to increase the quantity of mucus by 25% (10), but measurements have not been made on the effect of bromhexine on mucociliary clearance from the lungs of animals.

The present study used a noninvasive radio-aerosol technique developed previously (5) to investigate how mucociliary clearance and the retention of inhaled particles in the lung are affected by particle size — comparing particles of 3.3 μm and 5.0 μm diameter — and by the mucolytic drug bromhexine.

MATERIALS AND METHODS

ANIMALS

Six dairy-bred bull calves were used. Four of these (A, H, B, M) were healthy animals showing no symptoms of respiratory disease. They were purchased at about two weeks of age in December 1985 and used for clearance studies in March and May, 1986. A further two calves (J, N) which showed clear clinical symptons of chronic pneumonia (Malaise, persistent cough, tachypnoea, reduced appetite) were obtained at the end of May at about three months of age and used during June/July, 1986.

All calves were housed on straw in separate pens in a well-ventilated calf house. The calves were familiarized with all experimental procedures before any clearance studies commenced. The treatment of the calves followed the guidelines given by "Guide to the Care and Use of Experimental Animals" Volumes I and II.

EXPERIMENTAL DESIGN

Initially it was intended only to observe the effects on mechanical clearance and retention of 3.3 μm particles in four healthy calves given bromhexine at 1.6 mg/kg$^{0.75}$ body mass (S), which corresponds to the standard dose recommended for clinical use, a double dose of bromhexine at 3.2 mg/kg$^{0.75}$ (D), or a control dose of water (C), each involving the intra-oesophageal administration of 100 mL water 30 min prior to aerosol delivery. This initial trial followed a modified Latin Square design (Table Ia) and was carried out during March 1986. It was subsequently decided to extend the experiment by investigating the clearance and retention of 5 μm particles and also by examining the effects of bromhexine on calves with clinical signs of chronic pneumonia. A trial using the four healthy calves with 5 μm particles was carried out in May. Control trials were then repeated using 3.3 μm particles to determine whether any change in the pattern of clearance and retention had occurred between March and May.

A modified Latin Square design was also used with the two sick calves using the same dosage levels of bromhexine (Table Ib). To minimize technical problems with the delivery system, measurements using 5 μm particles were done first (June) and were followed by measurements using 3.3 μm particles (June/July).

TABLE I. Experimental Designs

Calf	Week	1	2	3
(a) **Healthy calves**				
A		C	S	D
H		S	C	D
B		D	C	S
M		C	D	S
(b) **Sick calves**				
J		S	D	C
N		C	S	D

C = Control
S = Single dose of bromhexine (1.6 mg/kg$^{0.75}$)
D = Double dose of bromhexine (3.2 mg/kg$^{0.75}$)

AEROSOL EXPOSURE

The aerosol consisted of ^{99m}Tc-labelled monodisperse polystryene spheres of either 3.3 μm or 5 μm aerodynamic diameter, prepared from a radiolabelled solution of polystyrene in xylene (11) and aerosolized using a May spinning disc (5,12). The diameter of the particles was controlled by altering the concentration of the solution and the rotational speed of the disc.

The diameter and number of particles in the aerosol were checked in each experiment using an Aerodynamic Particle Sizer (T.S.I. Incorporated, St. Paul, Minnesota). The degree of leaching of the Tc label from the particle was also periodically measured and found to be less than 3%.

The aerosol contained, on average, 36 particles/cm^3 using 3.3 μm particles and 9 particles/cm^3 using 5 μm particles. These concentrations corresponded to an estimated activity at the calf's nose of 0.0019 μCi/L for 3.3 μm particles and 0.0017 μCi/L for 5 μm particles. The aerosol was delivered directly to the calf (6) and the period of aerosol delivery was usually 10 min.

The mean initial activity counted at the lung was 0.02 μCi for 3.3 μm particles and 0.008 μCi for 5 μm particles. The difference in these two values is due partly to the greater loss by impaction of 5 μm particles along the aerosol delivery line, but probably mainly due to losses in the upper respiratory tract.

Respiratory rate was monitored throughout aerosol administration and, for healthy calves, averaged at 27 breaths/min (SEM, Standard Error of the Mean, = 5).

MEASUREMENT OF CLEARANCE AND PERCENTAGE RETENTION

Measurement of radioactivity retained in the lung commenced as soon as possible after aerosol exposure, the time interval being, on average, 7 min. The counting equipment used comprised two sodium iodide gamma detectors (E.G. & G. Ortec & Co. Ltd., Bracknell, U.K.) mounted in a specially constructed saddle (W.E.S. Garrett, Draycott, Cheddar, U.K.) over a region corresponding to the junction of apical and middle lobes. Counts were taken at 2 or 5 min intervals for a period of 30 s for at least 90 min which was generally sufficient time to observe the first phase of clearance (5). In most cases, further measurements were taken, including 24 h counts.

Counts were corrected for background and decay and their natural logs were regressed on time to deduce clearance rate constant according to the relationship

$$Qt = C_e^{-kt} + R \qquad (5)$$

where Qt = total activity retained at the lung at time t (h)
C = activity at time 0, approximately distributed in ciliated lung regions
k = clearance rate constant (h^{-1})
R = activity approximately distributed in nonciliated lung regions (i.e. activity

retained after clearance or alveolar deposition).

Values of R at 90 min after the end of aerosol delivery were used to calculate the percentage retention, r(%), of aerosol using

$$r(\%) = \frac{R}{Qo} \times 100$$

In most, but not all cases, (noticeably the sick calves N and J which are discussed later), values of r obtained at 90 min were similar to those obtained at 24 h, which are generally considered to reflect alveolar deposition.

STATISTICAL ANALYSIS

Three-way analysis of variance (13) for both k and r was carried out for the healthy calves, sick calves and all calves together to compare variation due to particle size, bromhexine and inter-calf variation. This was done using the computer package "Genstat" (Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Herts., U.K.).

A further analysis of variance was carried out to compare the results obtained in March with those obtained in May for healthy, control calves which inhaled 3.3 μm particles.

RESULTS

Figures 1 and 2 present patterns of clearance for individual calves at each particle size and dosage level and illustrates that the shape of the clearance curve tended to be consistent within each calf at each particle size. Individual values of clearance rate constant (k, h^{-1}) and percentage retention at 90 min (r,%) are given in Table II along with means for healthy calves, sick calves and all calves combined. Ranges of values for all calves with no drug treatment (controls) are as follows:

3.3 μm particles: $k(h^{-1})$: 0.15 - 0.45 (mean 0.33)
r(%) : 62 - 85 (mean 75)
5 μm particles: $k(h^{-1})$: 0.38 - 1.4 (mean 0.90)
r(%) : 39 - 61 (mean 53)

Examination of values of clearance rate constant and percentage retention obtained with 3.3 μm particles in healthy, control calves in March and in May show considerable variation between calves but no significant difference between these two months (Table III). Therefore, differences obtained between 3.3 μm and 5 μm particles in healthy calves cannot be attributed to changes occurring over time.

Means and SEMs for particle size and dosage level obtained from the analyses of variance of values of k and r for healthy calves, sick calves and all calves together are shown in Tables IV and V respectively. Table IV shows that there were clear, highly significant ($P<0.001$) effects of particle size on both clearance rate constant (k) and percentage retention (r) in healthy calves, and in all calves grouped together. Particle size was significant only at the 5% level for values of k in sick calves.

Bromhexine significantly ($P<0.05$) affected clearance rate constants in all calf groups (Table V) and similarly

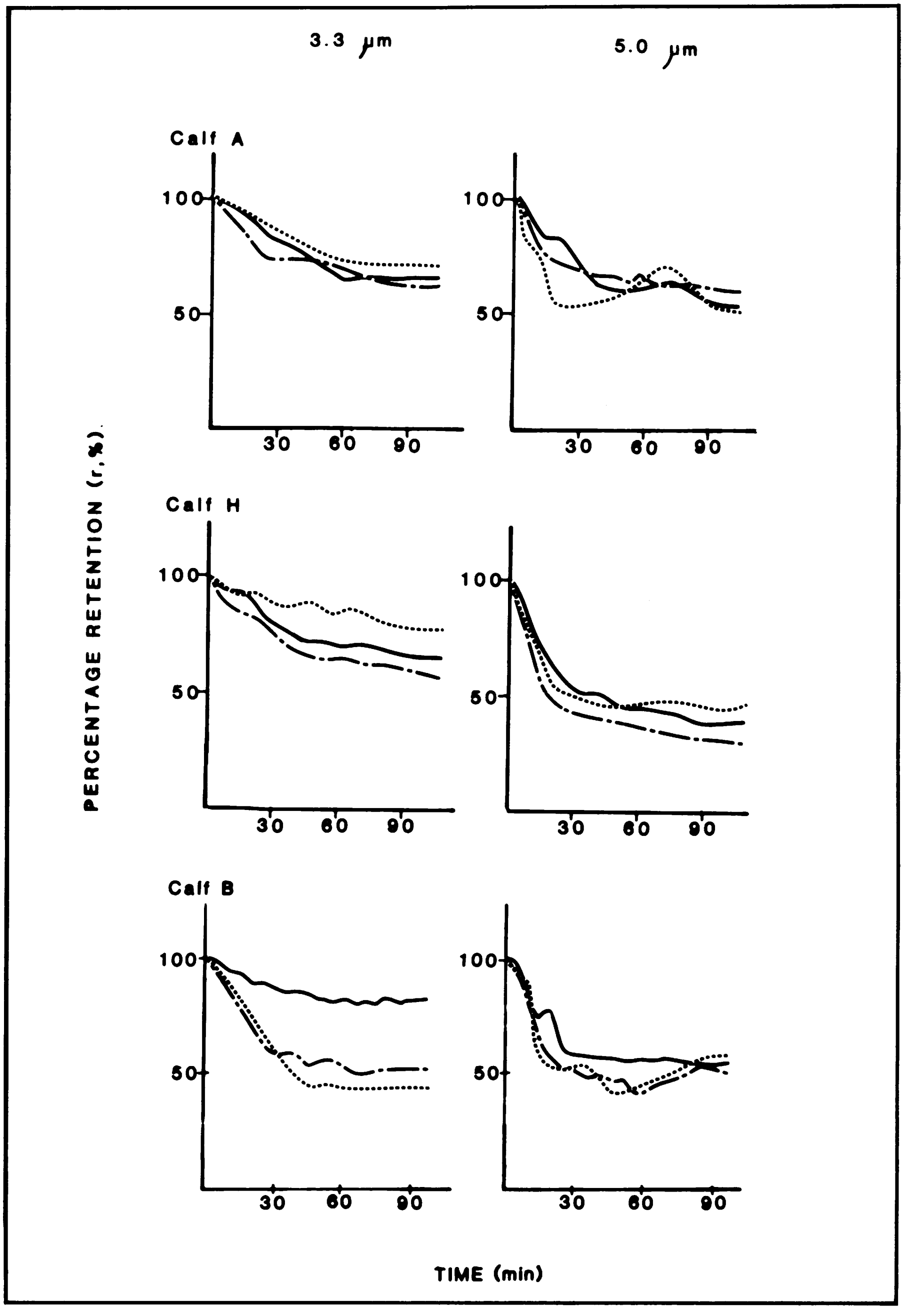

Fig. 1. Clearance curves for calves A, H and B (all healthy) to illustrate the effects of particle size with no bromhexine (————), bromhexine at 1.6 mg/kg$^{0.75}$ (▪ ▬ ▪ ▬) and bromhexine at 3.2 mg/kg$^{0.75}$ (.........).

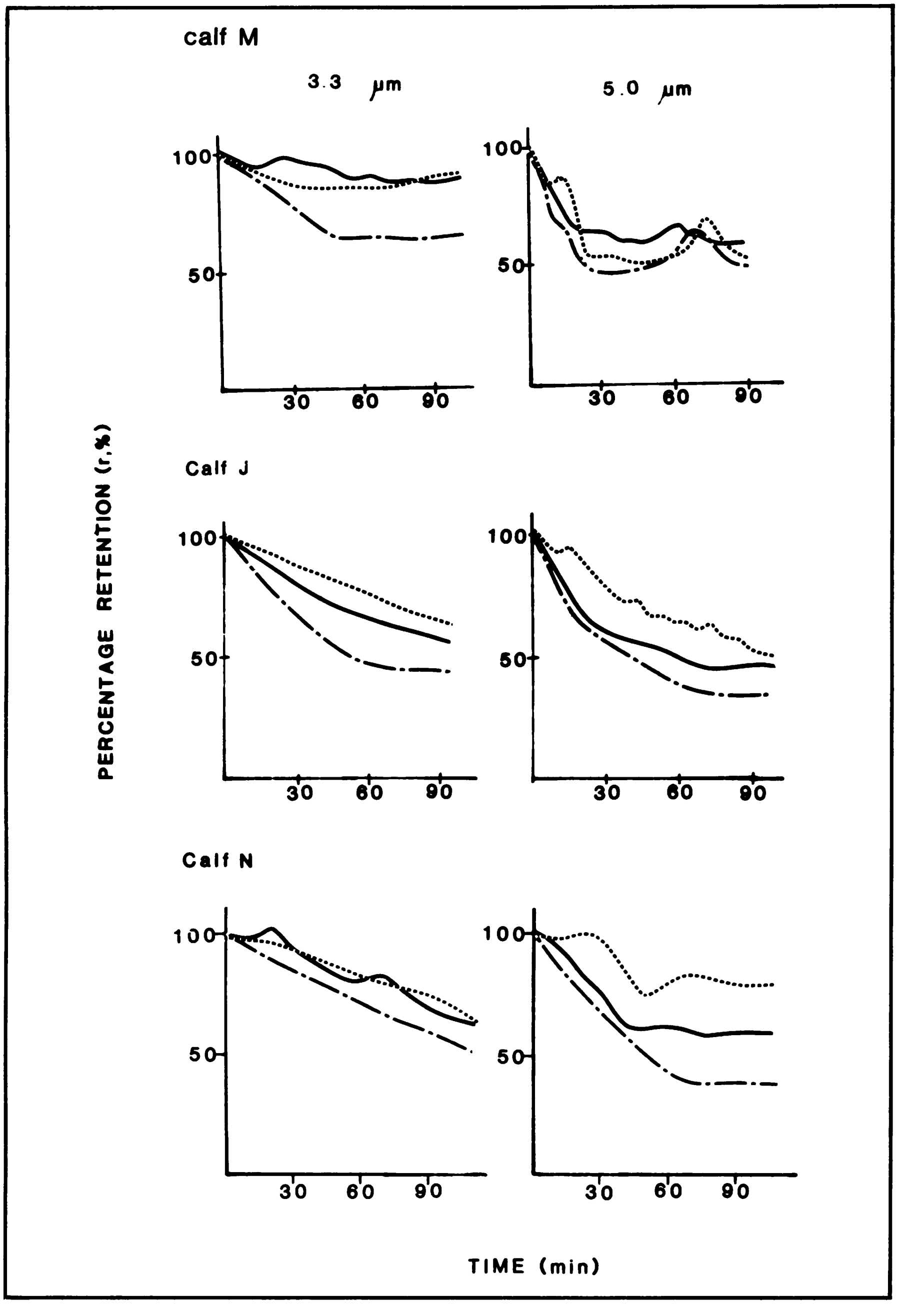

Fig. 2. Clearance curves for calf M (healthy) and calves J and N (sick) to illustrate the effects of particle size with no bromhexine (———), bromhexine at 1.6 mg/kg$^{0.75}$ (• — • —) and bromhexine at 3.2 mg/kg$^{0.75}$ (··········).

affected percentage retention in all groups except the healthy calves. The effect of dose level of bromhexine can be determined by examination of the means and SEMs in Table V. In all calf groups the standard dose of bromhexine (1.6 mg/kg$^{0.75}$) gave significantly ($P<0.05$) different values of k from controls, whereas the double dose did not differ consistently from control values. Similarly, the standard dose of bromhexine significantly ($P<0.05$) altered values of r in sick calves and in all calves combined, but not in healthy calves.

The double dose of bromhexine had no significant effect on r in healthy calves. Additionally, when the double dose of bromhexine was given in association with 5 μm particles, we frequently observed peaks on the clearance curve or upturns in percentage retention, suggestive of mucus refluxing (Fig. 1).

An interaction between particle size and dosage level of bromhexine was not evident from the analyses of variance indicating that the effect of bromhexine is independent of particle size.

DISCUSSION

The results showed that each calf tended to have its own consistent pattern of clearance at each particle size (Fig. 1). Characteristic patterns of this kind have been observed in studies of mucociliary transport in humans (4). However, the shape of the clearance curve (Fig. 1) and the clearance rate constant (Table II) differed between animals and this inter-individual variation has been observed in humans (4,14) and donkeys (15). Such variation in healthy individuals may be a consequence of anatomical and/or physiological variation which has implications for the incidence of respiratory disease in calves.

In the present study it was found that clearance rate constant and percentage retention values did not vary significantly over a two-month time period, corresponding to an increase in age from three months to five months. However, it is possible that calves younger than this may have quite different clearance rates and this may be of importance with regard to the development of respiratory disease in very young calves.

The highly significant differences in clearance rate constant evident between 3.3 μm and 5 μm particles are consistent with values quoted in human studies (6,7). These differences are probably due to the fact that 5 μm particles reaching the lungs are preferentially deposited in larger airways and are therefore transported by faster sections of the mucociliary escalator. The 3.3 μm particles penetrate further into the lung and deposit in smaller airways where mucociliary clearance is slower (7). This more peripheral deposition also accounts for the significant difference in values of percentage retention obtained for the two particle sizes. The range of values of clearance rate constant obtained using 3.3 μm particles falls within the range of 0.15 - 2.27 h^{-1} previously obtained in calves in this laboratory (5), but this range is much narrower than that previously observed. The values of percentage retention obtained using 3.3 μm particles in control trials also fell within a narrow

TABLE II. Individual Values of Clearance Rate Constant (k, h^{-1}) and Retention (r,%) for all Calves

		Dose of Bromhexine					
		Control		Single		Double	
Particle Size		3.3 μm	5 μm	3.3 μm	5 μm	3.3 μm	5 μm
Calf	Item						
Healthy							
A	k	0.42	0.82	0.51	0.97	0.35	1.54
	r	66	57	62	62	73	57
H	k	0.45	1.4	0.57	2.08	0.24	1.98
	r	66	39	58	35	77	48
M	k	0.15	0.89	0.56	1.5	0.25	1.35
	r	85	61	62	51	87	52
B	k	0.19	0.86	0.65	1.67	0.88	1.7
	r	82	55	55	52	43	59
Mean	k	0.30	0.99	0.57	1.56	0.43	1.64
	r	75	53	59	50	70	54
Sick							
J	k	0.4	1.04	0.81	1.29	0.37	0.58
	r	62	48	47	41	64	52
N	k	0.36	0.38	0.43	0.81	0.23	0.21
	r	66	60	58	37	68	79
Mean	k	0.38	0.71	0.62	1.05	0.3	0.40
	r	64	54	52	39	66	66
Grand	k	0.33	0.90	0.59	1.39	0.39	1.23
Mean	r	71	53	57	46	69	58

TABLE III. Values of Clearance Rate Constant, $k(h^{-1})$ and Percentage Retention, r(%) for Healthy, Control Calves, using 3.3 μm Particles in March and May

		Month		
Calf	Item	March	May	
A	k	0.42	0.78	
	r	66	63	
H	k	0.45	0.7	
	r	66	72	
B	k	0.19	0.13	
	r	82	88	
M	k	0.15	0.10	
	r	85	92	
Mean	k	0.30	0.43	SEM = 0.11
	r	75	79	SEM = 2.3

[SEM represents Standard Error of the Mean]

TABLE IV. Means and SEMs for Clearance Rate Constant, $k(h^{-1})$ and Percentage Retention, r(%) at Different Particle Sizes

		Particle Size			
Item	Calves	0.3	5.0	SEM	Significance Level
	Healthy	0.41[a]	1.40[b]	0.10	0.1%
k	Sick	0.43[a]	0.72[b]	0.09	5%
(h^{-1})	Combined	0.43[a]	1.17[b]	0.11	0.1%
	Healthy	68.0[a]	52.3[b]	4.2	1%
r	Sick	60.1[a]	52.8[a]	4.3	n.s.
(%)	Combined	65.6[a]	52.5[b]	3.2	0.1%

[Values in the same line having different superscripts differ significantly]

range, unlike the range of 14-76% quoted (5).

The sick calves showed significant differences in clearance rate constant due to particle size only at the 5% level. At their time of purchase, these calves exhibited symptoms of pneumonia, but during the course of the experiments their clinical condition improved considerably. Therefore, in the case of these calves, time may have confounded the effect of particle size. The change in the health of the "sick" calves may also explain the similarity in mean values of k and r obtained for "sick" and healthy calves (Table II).

Bromhexine, at the standard dose level (1.6 mg/$kg^{0.75}$), appeared to cause a consistent improvement in mucociliary clearance rate. This is presumably a consequence of its mucolytic action, reducing mucus viscosity such that movement of mucus by cilia is made easier (16,17). The lack of dose-size interaction in the analysis of variance carried out indicates that the effect of bromhexine is independent of particle size (at least within the range 3.3 μm to 5 μm) and therefore it is likely that bromhexine is effective in both large and small airways.

The standard dose of bromhexine significantly reduced percentage retention values in sick calves but not in healthy calves. The fact that percentage values were not significantly different with or without bromhexine in healthy calves, despite a significant increase in clearance rate constant, confirms that percentage retention values at 90 min were reasonable approximations of alveolar deposition in nearly all these trials. It is likely that percentage retention values differed significantly in the sick calves because clearance proceeded slowly (this is particularly evident in calf N with no clear "first phase" of clearance [Fig. 2]) and hence percentage retention values obtained in control trials were not good approximations of alveolar deposition. The improved clearance caused by bromhexine would remove more particles from ciliated lung regions within a given time, thus giving a lower value of percentage retention and therefore a lower apparent value for alveolar deposition.

In some calves (Figs. 1 and 2) bromhexine at 3.2 mg/$kg^{0.75}$ appeared to cause the phenomenon of "refluxing". It seems that this dose reduced mucus

TABLE V. Means and SEMs for Clearance Rate Constant, $k(h^{-1})$ and Percentage Retention, r(%) at Different Dosage Levels of Bromhexine

Item	Calves	Dose			SEM	Significance Level
		Control	Single	Double		
k	Healthy	0.65^{a}	1.06^{b}	1.04^{b}	0.12	1%
	Sick	0.55^{a}	0.84^{b}	0.35^{a}	0.11	5%
	Combined	0.61^{a}	0.99^{b}	0.81^{ab}	0.13	5%
r	Healthy	63.0^{a}	54.6^{a}	62.0^{a}	5.2	n.s.
	Sick	59.0^{a}	45.8^{b}	65.8^{a}	5.3	5%
	Combined	62.3^{a}	51.7^{b}	63.3^{a}	4.0	5%

[Values in the same line having different superscripts differ significantly]

viscosity to a point beyond the optimum level (18) whereby the cilia become less effective at transporting it and it consequently falls backwards. This effect has been observed by other authors (15). It was seen primarily using 5 μm particles, presumably due to a greater abundance of mucus glands or perhaps a thicker mucus layer in the large airways where 5 μm particles would deposit (19).

However, the positive effects of the standard recommended dose of bromhexine on clearance and retention of inhaled particles indicates that this drug may be of use in the therapy of respiratory conditions in veterinary medicine. It was previously found that cattle naturally infected with bovine parainfluenza-3 recovered more rapidly if bromhexine had been administered (intramuscularly) than if they had received antibiotics alone (20), although part of this effect may have been due to bromhexine causing an increase in the antibiotic content of respiratory mucus (21). Similar results were obtained in a study of bronchopneumonia in cattle (22) and in a study using horses (23) which showed a marked improvement in the clinical and radiological symptoms of respiratory disease after receiving 15-20 g bromhexine in daily feeds for 10-45 days. Additionally, prophylactic use of bromhexine was observed to reduce the incidence of respiratory disease in calves and the amount of antibiotic agents used to treat calves developing respiratory disease (24).

The present study indicates that the effects of bromhexine are rapid in onset. This agrees with electron microscopic observations (25) and contrasts with one claim (26) that the beneficial effects of bromhexine in humans were evident only seven days after administration. These authors also reported that bromhexine may cause a decrease in airways resistance of about 30% in roughly 20% of cases. Such a decrease is presumably due either to the liquification of viscid mucus or to bronchodilation. The lack of effect of bromhexine on percentage retention in the present study indicates that such effects are unlikely to occur during the 30 minute period immediately following the administration of bromhexine.

ACKNOWLEDGMENTS

The authors thank Dr. A. Clarke for his assistance during experiments and Mr. M. Badman for his care of the calves. The senior author thanks Boehringer Ingelheim for financial support.

REFERENCES

1. **LILLIE LE, THOMSON RG**. The pulmonary clearance of bacteria by calves and mice. Can J Comp Med 1972; 36:129-137.
2. **LOPEZ A, MAXIE MG, SAVAN M, RUHNKE HL, THOMSON RG, BARNUM DA, GEISSINGER HD**. The pulmonary clearance of *Pasteurella haemolytica* in calves infected with bovine virus diarrhea or *Mycoplasma bovis*. Can J Comp Med 1982; 46:302-306.
3. **AGNEW JE, SUTTON PP, PAVIA D, CLARKE SW**. Radioaerosol assessment of mucociliary clearance: towards definition of a normal range. Br J Radiol 1986; 59:147-151.
4. **CAMNER P**. The production and use of test aerosols for studies of human tracheobronchial clearance. Environ Physiol 1971; 1:137-154.
5. **JONES CDR, BULL J**. Deposition and clearance of radio-labelled monodispersed polystyrene spheres in the calf lung. Res Vet Sci 1987; 42: 82-91.
6. **TASK GROUP ON LUNG DYNAMICS**. Deposition and retention models for internal dosimetry of the human respiratory tract. Health Physics 1966; 12:172-207.
7. **LIPPMANN M, YEATS DB, ALBERT RE**. Deposition, retention and clearance of inhaled particles. Br J Indust Med 1980; 37:337-362.
8. **MOSSBERG B, PHILIPSON K, STRANDBERG K, CAMNER P**. Clearance by voluntary coughing and its relationship to subjective assessment and effect of intravenous bromhexine. Environ J Respir Dis 1981; 62:173-179.
9. **THOMSON ML, PAVIA D, GREGG I, STARK JE**. Bromhexine and mucociliary clearance in bronchitis. Br J Dis Chest 1974; 68:21-27.
10. **HOFFMAN W, GRUNDER HD**. Influence of secretolytic drugs (particularly bromhexine) on the volume and consistency of tracheal secretions of healthy calves and lambs. Tierarztl Umsch 1982; 37:414-424.
11. **FEW JD, SHORT MD, THOMSON ML**. Preparation of ^{99m}Tc-labelled particles for aerosol studies. Radiochem Radioanal Lett 1970; 5:275-277.
12. **MAY KR**. Spinning-top homogeneous aerosol generator with shockproof mounting. J Sci Instrum 1966; 43:841-842.
13. **SOKAL RR, ROHLF FJ**. Biometry. 2nd ed. San Francisco: W.H. Freeman and Company, 1981: 372-396.
14. **PAVIA D, AGNEW JE, LOPEZ-VIDRIERO MT, CLARKE SW**. Mucociliary clearance as a host defence mechanism. Bronchial Secretions Update 1985: CA 85001.
15. **BERGER J, ALBERT RE, SANBORN K, LIPPMANN M**. Effects of atropine and methacholine on deposition and clearance of inhaled particles in the donkey. J Toxicol Environ Health 1978; 4:587-604.
16. **BRUCE RA, KUMAR V**. The effect of a derivative of vasicine on bronchial mucus. Br J Clin Pract 1968; 22:289-292.
17. **ENGELHORN R, PUSCHMANN S**. The pharmacological investigation of a substance with mucolytic properties. Arzneimittelforsch 1963; 13:474-480.
18. **LITT M**. Mucus rheology. Arch Intern Med 1970; 126:417-423.
19. **LOPEZ-VIDRIERO MT**. Lung secretions. In: Clarke SW, Pavia D, eds. Aerosols and the lung. London: Butterworths 1984: 19-48.
20. **KOCH H**. Experience in practise with the use bisolvon in bovine parainfluenza. Tierarztl Umsch 1976; 31:68-70.
21. **KOTZIAN J, ABDOU MA-F, SALAMON E**. Increase in the oxytetracycline content of bronchial secretion of calves after simultaneous administration of bisolvon (bromhexine). Tierarztl Umsch 1976; 31:401-402.
22. **GRANDEL ERHARD, GRANDEL ELMAR**. Metaphylactic use of bisolvon for bronchopneumonia in cattle. Tierarztl Umsch 1976; 31:20-22.
23. **PEARCE HG, WYBURN RS, GOULDEN BE**. A clinical evaluation of bisolvon for treatment of some equine respiratory diseases. NZ Vet J 1978; 33:20-30.
24. **DESOIZE G, VAUGON M**. Effects of bromhexine hydrochloride in the prevention and treatment of respiratory disease in calves for slaughtering. Bull Soc Vet Prat France 1982; 66:79-90.
25. **HARADA Y, SASAKI H, GAAFAR H**. The effect of an expectorant on the tracheal mucosa — a scanning-electron microscopic study. Otorhinolaryngology 1977; 39:113-121.
26. **GUPTA SK, KISRA K**. Bromhexine and COPD. J Assoc Physicians India (letter) 1985; 33:250-251.