CHAPTER FOUR

IN VIVO STUDIES

CHAPTER 4 - IN VIVO STUDIES

4.1 INTRODUCTION

The deposition and distribution of aerosol particles *in vivo* may be studied by labelling the aerosol with a suitable γ -emitting radionuclide. The simplest device for the external monitoring of gamma-emitting radionuclides within the body consists of a small collimated scintillation detector. When placed over an organ, the detector provides a means of measuring changes in tracer concentration with time. Radionuclide imaging however requires the use of more sophisticated devices, such as the gamma camera, having a field of view of 40 cm diameter (Gottschalk & Beck, 1968). Because the technique is non-invasive, there is a minimum influence on normal biological functions and experiments may be repeated in the same subject (Horton, 1974; Taplin, 1979).

4.1.1 Selection of animal models

Experimental aerosol formulations for *in vivo* studies sometimes contain materials on which there is inadequate toxicological data. The dosage requirements and measurements of radiation dose are not as critical for animal studies, and repeated experiments using the same animal may be carried out. This is particularly useful when direct comparisons are required between different aerosol formulations.

Inhalation experiments in animals have largely been confined to toxicological testing of substances such as cigarette smoke in rabbits (Diamond *et al.*, 1975) and tungstic oxide in beagle dogs (Aamodt, 1975). Some research has used 'model' particles such as polystyrene and teflon administered as aerosols to rabbits to establish retention and clearance rates (Holma, 1968; Svartengren *et al.*, 1981).

However, animals have not been used extensively for in vivo studies of therapeutic aerosols, particularly metered-dose inhalers. This is because of the inherent disadvantages which include the gross anatomical and physiological differences, lack of control over breathing pattern and difficulty in aerosol administration. Schlesinger & McFadden (1981) compared the morphometry in the upper bronchial tree of human, dog, donkey, hamster, rabbit and rat species in terms of branching angles, diameter and length ratios and general branching patterns of the airways.

The results show the greatest differences from man in the rabbit and rat, and the least differences in the dog. However, Schreider and Raabe (1981) have shown that the turbinate region in the nasopharyngeal airways of the dog is considerably more complicated than that of man.

The breathing characteristics in various species have been described by Crosfill and Widdicombe (1961) and Table 1.1 summarises some of the data from this study. Table 4.1 demonstrates the gross differences in respiratory parameters from human, dog and rabbit species. The New Zealand white rabbit and beagle dog were the species used in this study.

Using conscious animals prevented interference with the autonomic control of breathing, so that studies of aerosol deposition under abnormal breathing patterns were not possible. Thus the effect of manoeuvres such as breath-holding, for example, may not be evaluated in species other than co-operative human subjects.

Studies of therapeutic aerosols in animals also require oral inhalation to mimic clinical use of nebulised aerosols and metered-dose inhalers. Previous conscious animal studies used head or whole-body exposure chambers which favoured nasal inhalation (Swann, 1972; Smith & Spurling, 1974;

Table 4.1 Values for respiratory measurements in man, dog and rabbit. (Crossfill & Widdicombe, 1961) Means and ranges are given.

	Body Weight (kg)	Lung Weight (g)	Tidal Volume (ml)	Minute Volume (l/min.)	Frequency (breaths/min.)	Mean alveolar diameter (μm)
Man	70	1065	400	6.4	16	166
Rabbit	2.4 (2.05-3.0)	9.1 (7.0-10.5)	15.8 (11.5-24.4)	0.62 (0.37-0.89)	39 (32-53)	94
Dog	12.6 (10.0-15.5) 13.1 ± 0.2*	82 (44-105) 75 ± 8*	144 (122-176) 204 ± 20*	3.1 (0.8-5.5) 4.5 ± 0.2*	21 (6-31) 23 ± 2*	74

* data from Park et al. (1970)

Campbell, 1976). Few published studies used oral administration of aerosols to conscious animals (Malton et al., 1982a) because of the attendant difficulties. In addition, administration of MDI's requires actuation of the inhaler at the correct point in the breathing cycle so respiratory monitoring is necessary. This was achieved in the present study by modification of the device developed by Poynter & Spurling (1971).

The first experimental animals used in this study were New Zealand white rabbits. Four females were used, varying in weight from 1½-4kg throughout the study. However the majority of the results presented were obtained from two rabbits, each 3kg. Rabbits were chosen on the basis of availability and economics. They are relatively easy to handle, docile and a suitable size for gamma camera imaging (Wilson & Hardy, 1982). Use of smaller rodents such as rats or guinea pigs, was considered unsuitable for this study, since the image detail in the small lung area would have been minimal. The rabbit is, however, a very efficient nose-breather and it was necessary to develop an oral dosing method for aerosols to achieve suitable lung images (Halpern & Schlesinger, 1980).

The results for the rabbits were extremely variable, a lengthy training period was necessary and the animals soon became too large to handle easily. More detailed lung images required larger animals to quantify differences in deposition pattern as a function of aerosol parameters. Beagle dogs were chosen because they were available from a closed colony, and each animal was selected with a temperment suitable for intensive training and a lengthy experiment (ie. not too timid or over-eager, friendly and alert). Two male beagle dogs were used during the study each varying in weight:

Dog 1 - 10.5kg (Dec. 81) to 11.8kg (Feb. 83).
Dog 2 - 11.6kg (Dec. 81) to 14.8kg (Feb. 83).

They were trained over a period of three months to accept the administration device and to stand in the imaging sling. Both dogs were amenable to training and were easy to handle during dosing and imaging procedures.

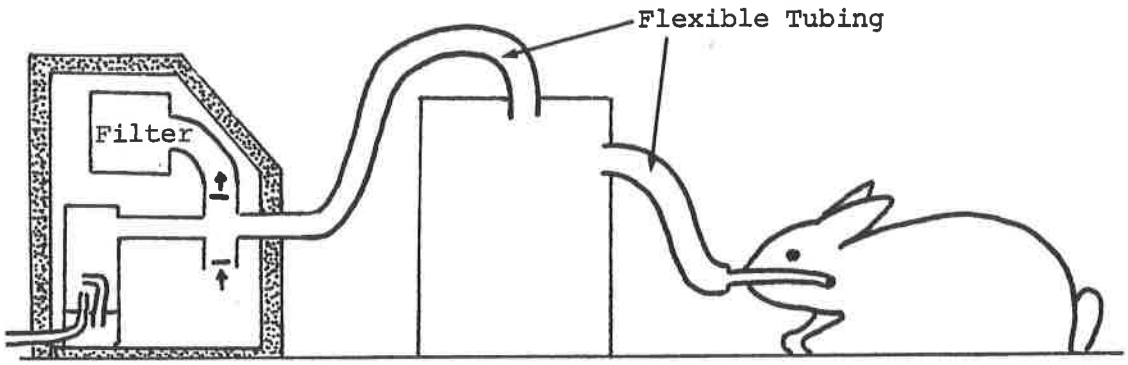
4.2 METHODS

4.2.1 Production and delivery of aerosols

HSA millimicrosphere suspensions and aqueous salbutamol solutions were nebulised in the shielded CIS nebuliser (see Chapter 2). Administration to both rabbits and dogs was achieved via a flexible plastic tube (30cm long, 2cm i.d.) to the administration device (see section 4.2.2.). The nebuliser was operated in all experiments at a flow rate of 8 l/min input air. Administration time to the rabbits ranged from 5 to 10 minutes and averaged 8 minutes. Delivery of nebulised aerosol to the dogs continued for 4 minutes with a one minute pause in administration if required. A 10 litre aspirator was used as a chamber for drying the nebulised aerosol before inhalation via an additional length of flexible tubing (Fig. 4.1).

It was not found possible to administer aerosols from metered-dose inhalers to rabbits to give satisfactory lung images presumably due to the high velocity and concentration of the aerosol spray causing unacceptably high losses in the administration tubing. The low tidal volume of the rabbits allows only a small volume of aerosol to be inhaled. However, the labelled preparations in metered-dose inhalers were administered successfully to the beagle dogs using a single dose of two shots in each experiment. The adminis-

Fig 4.1 Administration of a Nebulised Aerosol via a Drying Chamber to Rabbits.



tration chamber was adapted to fit an actuator, and the inhaler was fired directly into the chamber. The can was shaken for approximately thirty seconds immediately before dosing, then two shots were fired at the beginning of an inspiration, allowing one complete breath between the two shots.

The activity present in each dose from the MDI was assessed at the beginning of each experiment by the following method. The freshly prepared inhaler was primed by firing twice and then two shots were fired into a 5 litre plastic drum, shaking the can between each actuation. The drum was sealed, placed on the gamma camera face, and counted for one minute.

After each in vivo experiment the quality control of the formulation in the MDI was established. The inhaler was fired into the eight-stage Andersen Sampler (see Chapter 2) and the activity and mass size distributions of the technetium-99m and salbutamol respectively were assessed (see Chapter 3).

4.2.2 Administration Systems

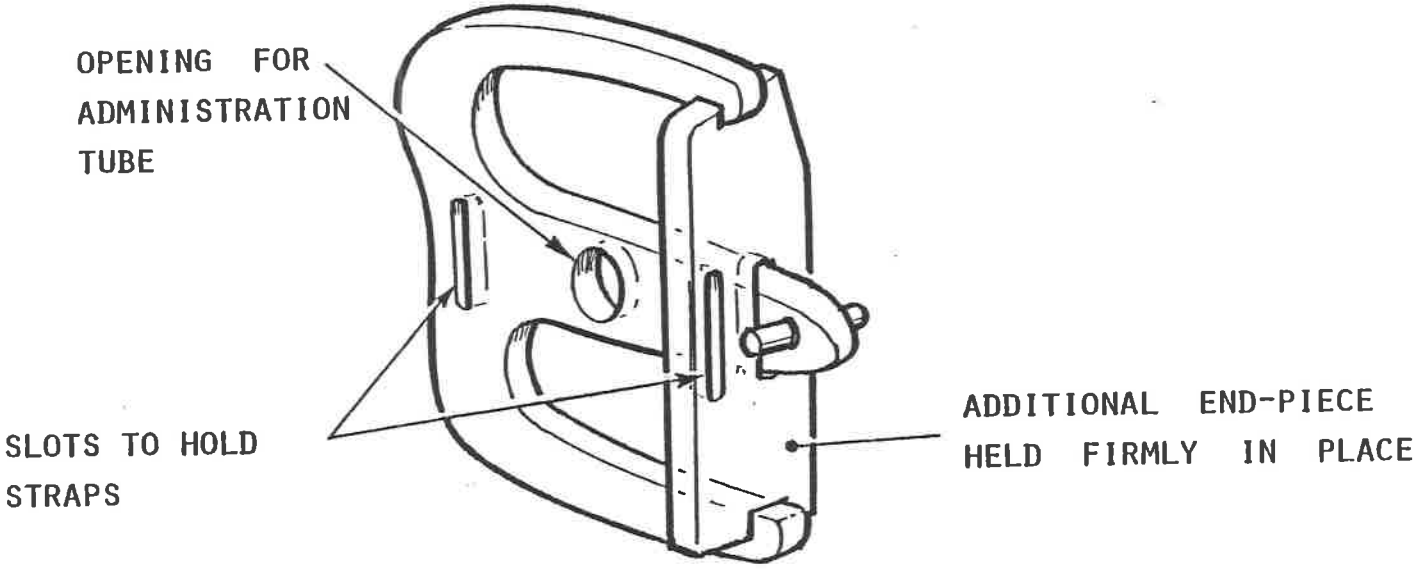
The administration devices for both rabbits and dogs were designed to bypass the nose by introducing a delivery tube into the pharynx of the animal. A considerable amount of training was required for acceptance of either device.

The training involved introducing the delivery devices into the mouth and oropharynx of the animals for increasing periods of time. The beagle dogs were very amenable to training; the rabbits required up to three months of daily attention and two of the rabbits were rejected from the study due to difficulties in training.

The oral delivery device (O.D.D.) used for rabbits is shown in Fig. 4.2, modified from the original device of Halpern & Schlesinger (1980). Constructed in 'Perspex' and inserted sideways into the mouth, the 'E' shaped device firmly clamps the jaws around the central portion through which the administration tube is inserted. The end-piece on the 'E' and straps around the head maintain the O.D.D. in the correct position after insertion. All surfaces are smooth, cushioned with silicone tubing and manufactured to fit each rabbit to avoid any discomfort. Training of the rabbits involved gradual introduction of simpler devices for increasing time periods; rabbits which would not accept the device were discarded from the study. The delivery tube was initially silicone tubing and later a modified rubber endotracheal tube. The tube was greased for easy insertion and marked so that 6-7cm was inserted on each occasion. The O.D.D. and tube were positioned with the rabbit on its back; careful training ensured the minimum of stress in the animal. Finally, the nose was sealed by placing a tightly fitting latex glove over the nose and mouth region. A diagram of the complete device in place is shown in Fig. 4.3.

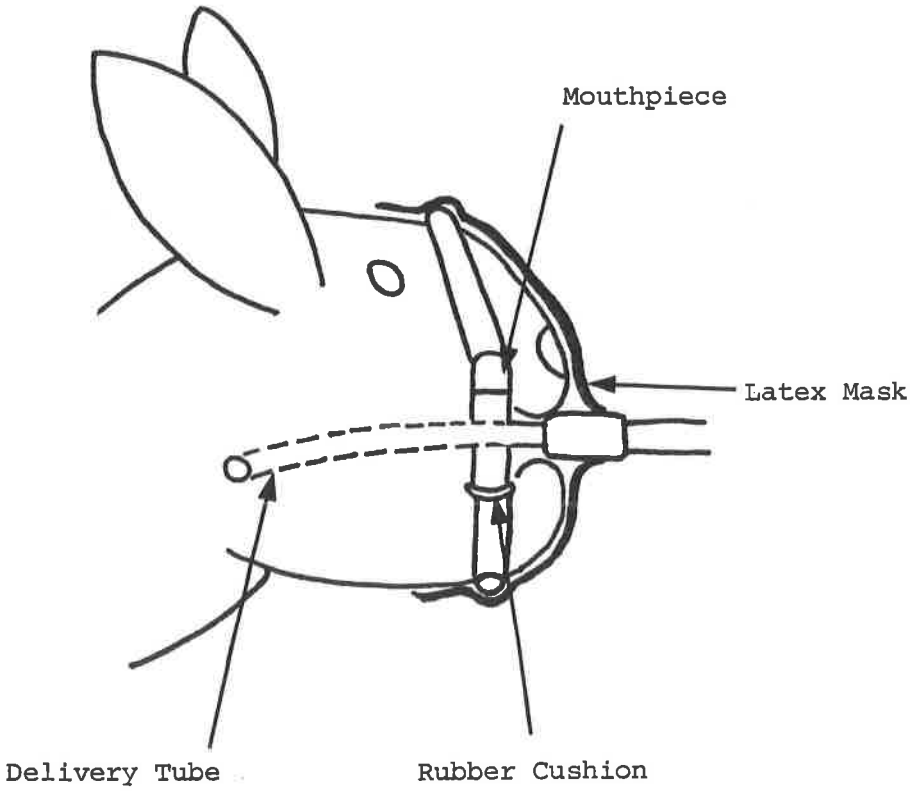
Fig. 4.4 shows the administration chamber used for all in vivo experiments in beagle dogs (Poynter & Spurling, 1971). It consists of a hollow cylinder containing a stretched latex sleeve. At one end a curved rubber tube (approx. 15mm i.d.) is inserted into the animal. The other cylinder end is adapted to fit a spirometer tube and delivery tube from a nebuliser or MDI (Fig. 4.5). The latex sleeve expands and contracts with the respiratory manoeuvres so that a metered-dose inhaler may be fired at the required point in the respiratory cycle. Movement of the sleeve also indicated oral breathing through the device and thus correct positioning of the rubber tube in the pharynx above the epiglottis (Fig. 4.6). Some nasal breathing was observed

Fig 4.2 ORAL DELIVERY DEVICE FOR ADMINISTRATION OF AEROSOLS TO CONSCIOUS RABBITS.



(designed and manufactured by B. Warehand, Process Development Department Glaxo Operations Ltd.)

Fig 4.3 Oral Delivery Device in place in the Rabbit.



(Halpern & Schlesinger (1980) J.Toxicol. Environ. Hlth.

6 751-755)

Fig 4.4

EXPANSION CHAMBER FOR ADMINISTRATION OF AEROSOLS TO CONSCIOUS DOGS.

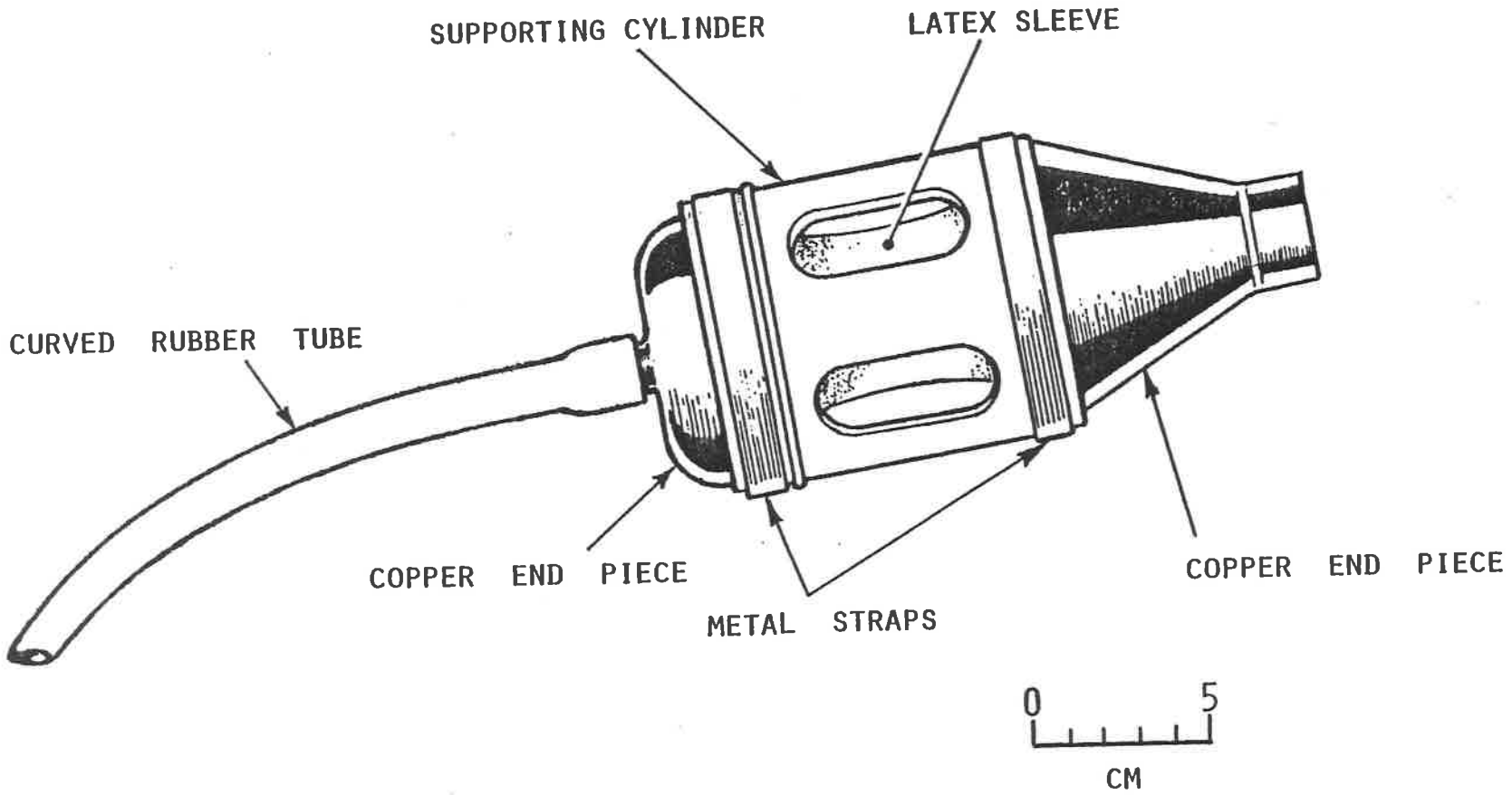
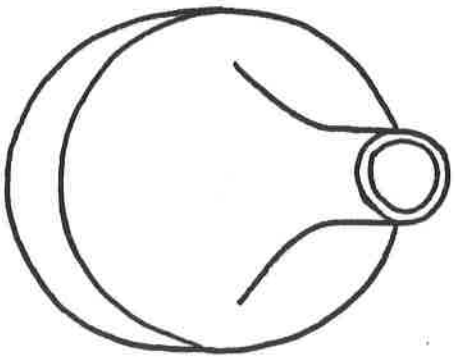
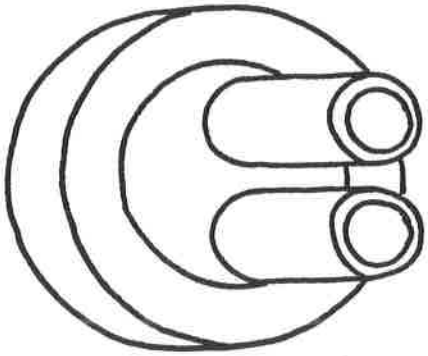


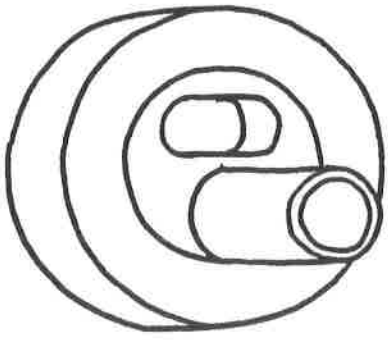
Fig 4.5 Administration Chamber Adaptors.



A Aerosol Administration Only.

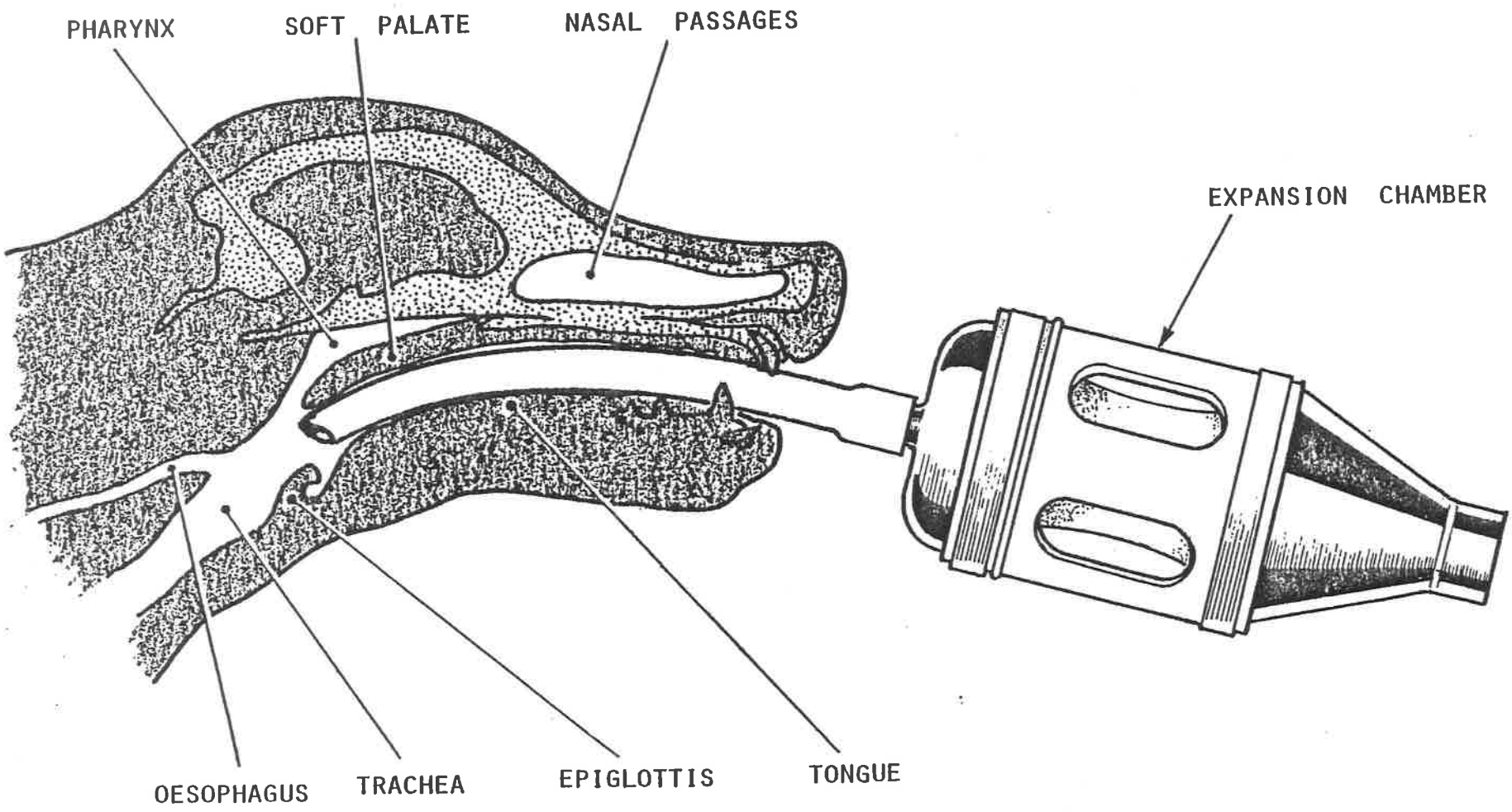


B Nebulised Aerosol Administration and Spirometer Monitoring.



C MDI Aerosol Administration and Spirometer Monitoring.

Fig 4.6 ADMINISTRATION DEVICE IN POSITION



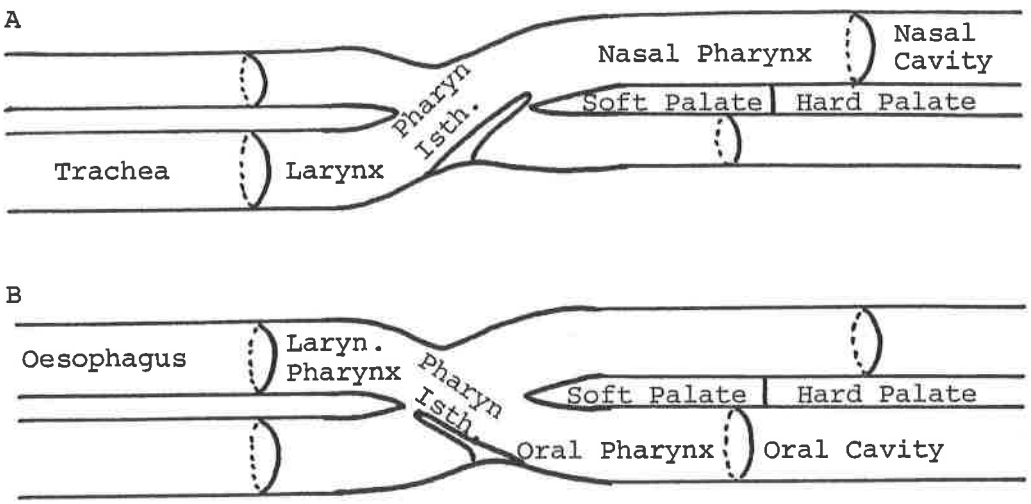
in the larger dog presumably due to the relative sizes of the tube and pharynx; this was prevented during dosing by sealing the nose. The positioning of the tube was critical. Insertion too far caused discomfort and when not far enough allowed complete nasal breathing. The position of the end of the tube and posture of the head should ensure a clear airway passage into the lung by allowing the epiglottis to drop back slightly. The epiglottis regulates the amount of air entering the lungs, Fig. 4.7. illustrates the action of the epiglottis in the beagle dog during respiration and deglutition (Miller et al., 1964). The positioning of the tube was assessed by using Krypton-81m gas. Each dog was placed in front of the camera in its normal dosing position (ie. sitting upright, with the head slightly raised), and a Tc-99m marker placed on its collar to facilitate maintenance of this position. The rubber tube of the administration chamber, previously painted inside with a sodium pertechnetate (Tc-99m) solution, was inserted into the animal and the position recorded. With the tube and dog in the same position, Krypton-81m gas was administered until a satisfactory image of the respiratory airways was obtained, this taking approx. 2 mins.

4.2.3 Respiratory monitoring

No respiratory monitoring was carried out on the rabbits, published data for breathing rate and tidal volume were assumed to apply for the dosing of fully trained rabbits.

A simple bell spirometer was used to monitor the breathing pattern of the beagle dogs. The appropriate adaptor (Fig. 4.5) was fitted on the administration chamber so that the spirometer tubing was attached throughout the experiment. Two different adaptors were used for either nebulised aerosol or aerosols from metered-dose inhalers. Each connection fitted tightly, so that the spirometer trace

Fig 4.7 Diagram Showing Relation of Portions of Pharynx to Oesphagus and Trachea. (From Miller et al, 1964).



A. During normal respiration.

B. During deglutition.

obtained on a flat-bed recorder was an accurate representation of the respiratory movements. The spirometer trace was calibrated before each experiment with a 2 litre syringe marked at 100ml intervals. Fig. 4.8 shows the type of trace obtained.

For each experiment, the dog was held in position in front of the spirometer in the same position as used for dosing. The administration chamber was inserted, and the spirometer tubing connected. The actuator of the MDI was also sealed into place on the chamber adaptor to complete the closed system. The dog then breathed into the spirometer and an initial trace was recorded for a few minutes (Fig. 4.9). The delivery tube was removed and the dog allowed to rest for a few minutes. A second trace was recorded with the chamber in position, and after about a minute of steady breathing, two shots were fired from the aerosol, allowing one complete breath between the actuations. Immediately after the second trace the gamma camera pictures were taken. After the imaging was complete, a third spirometer trace was recorded.

When a nebulised aerosol was administered, a simultaneous spirometer trace was not possible, so traces were only recorded before and after dosing.

Occasionally, the spirometer traces were abnormal due to incorrect tube positioning and/or a nervous dog. In these cases the experiment was delayed until a regular trace was obtained.

The breathing rate and tidal volume were calculated from each trace by computing the results using a digitiser. The end-points of inspiration and expiration for ten complete breaths from each trace were recorded as x, y co-ordinates using the digitiser. A computer program converted these

Fig 4.8 Spirometer traces of breathing patterns of dog.

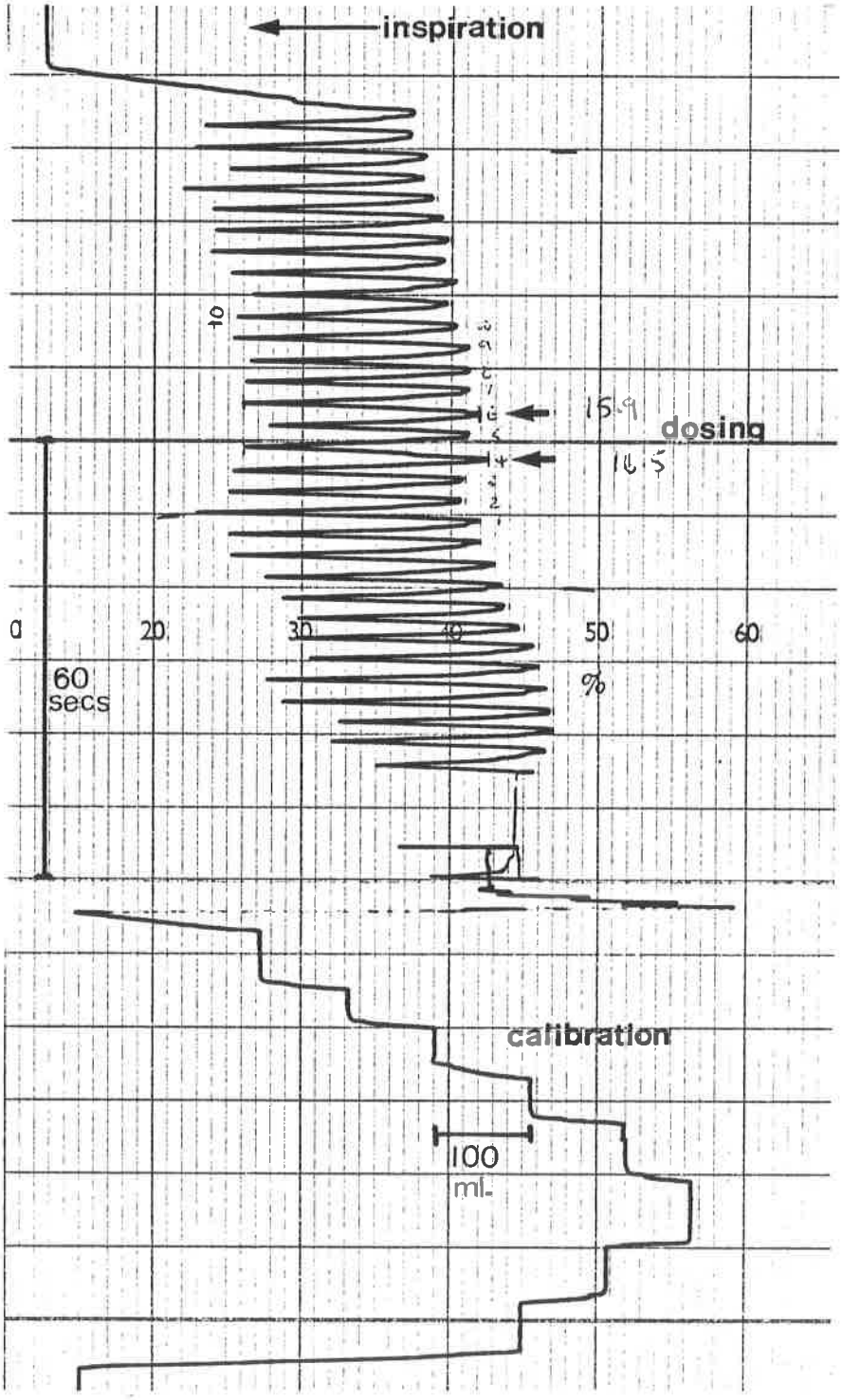
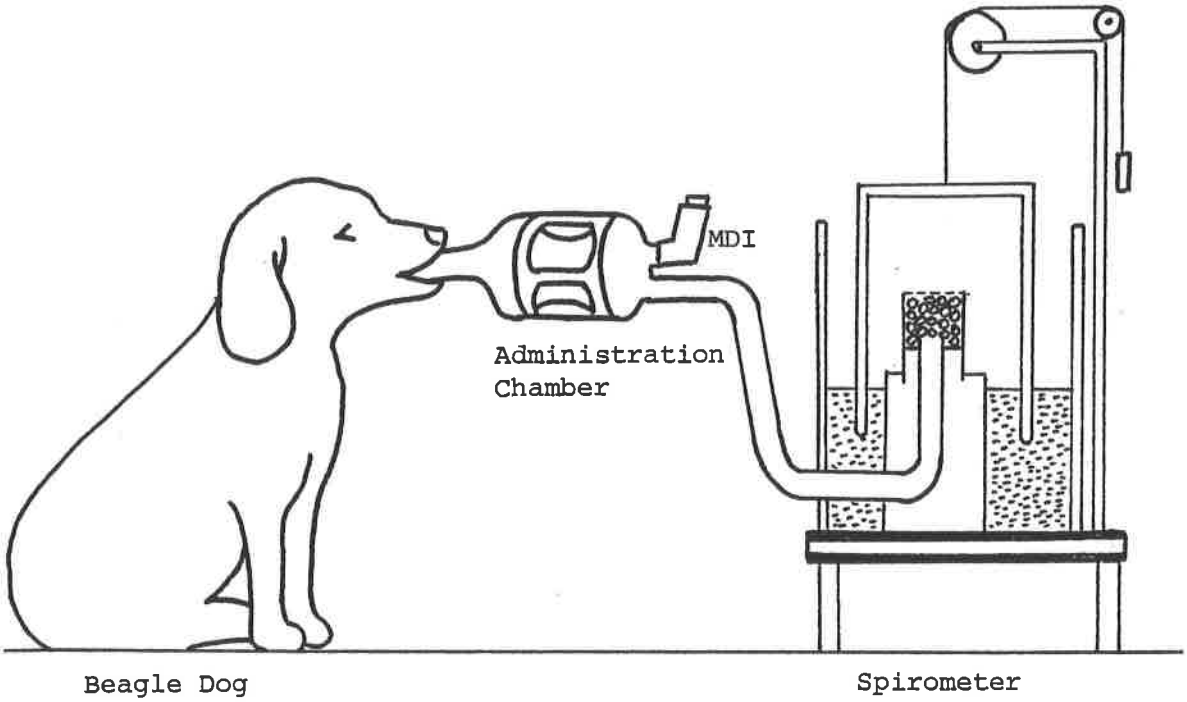


Fig 4.9 Administration of MDI to a Dog with Spirometer Monitoring.



data to tidal volume, breathing rate and minute volume, and calculated the mean and standard deviation for each parameter.

In addition, the inherent variability of breathing pattern during aerosol inhalation was established using placebo MDI's. The experiments were carried out in a constant temperature room, using the same handler and at the same time of day to eliminate as many environmental variables as possible. The spirometer traces were recorded and assessed in the same way as previously.

4.2.4 Gamma camera imaging

A diagram of an Anger type scintillation camera is shown in Fig. 4.10. The detector consists of a large diameter, 1cm thick, thalium-activated crystal of sodium iodide which is optically coupled to an array of photomultiplier tubes. Between the subject and the crystal is a perforated lead plate collimator which accepts only parallel gamma rays perpendicular to the camera face. Other designs of collimator permit magnification of the object. Gamma radiation interacting with the crystal produces light, the position and intensity of which is calculated from the relative strengths of the photomultiplier signals. The gamma camera used (General Electric Maxicamera II) was fitted with a low energy, high resolution, parallel hole collimator.

The images are recorded on magnetic media and later analysed (computer, disc drive and monitor from Link Systems Ltd., analysing computer from Nodecrest Medical Systems). A cursor is used to draw round a region of interest of the image on a TV screen. The area of each region is defined by the number of small squares or pixels (picture elements) of the screen. A computer is used to calculate the number of counts within a region of interest over a

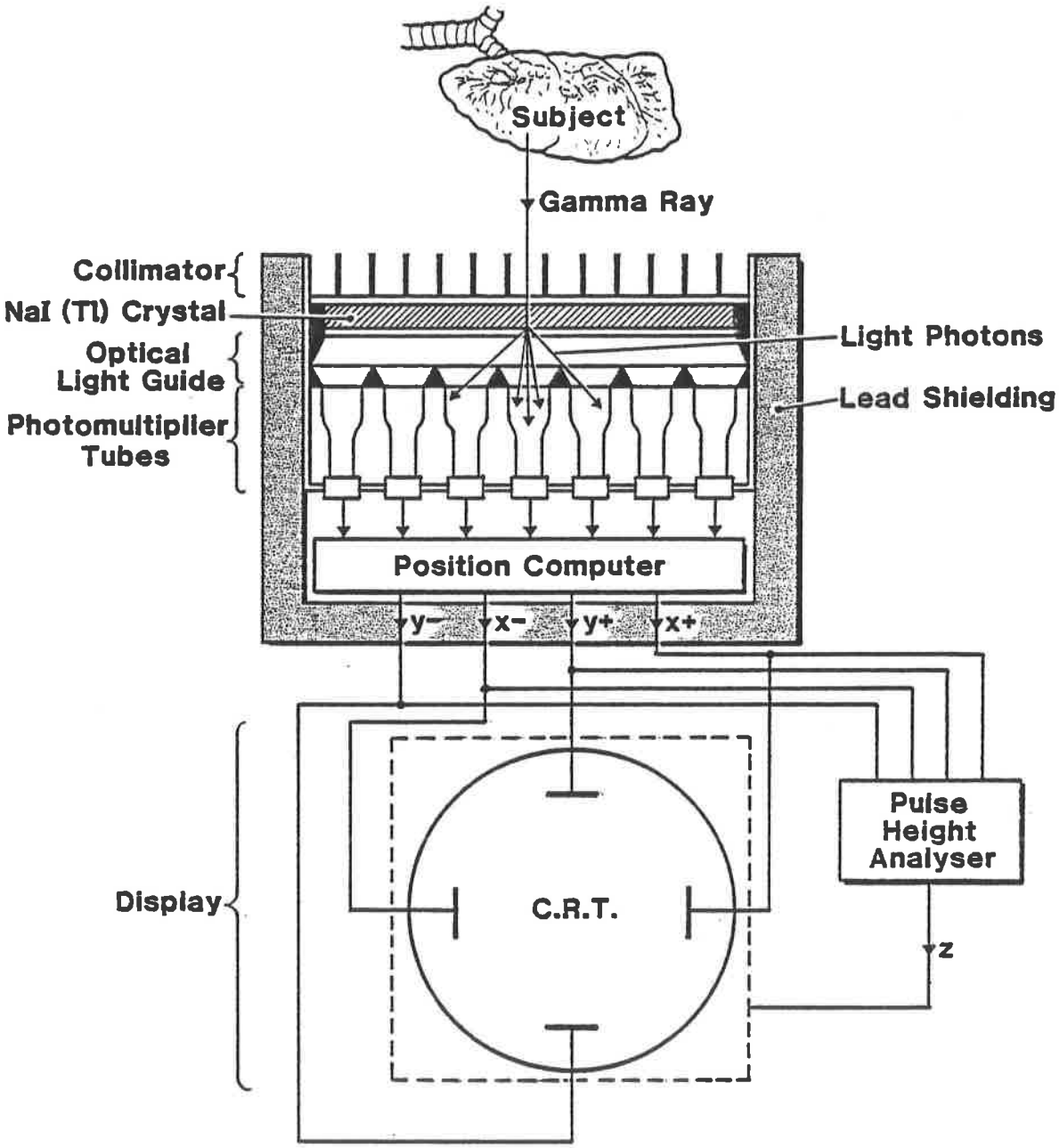


Fig. 4.10 Anger-Type Gamma Camera

specified time period. The counts from the regions of interest are corrected for background activity and radioactive decay.

The corrected counts for each region of interest (ROI) are then calculated from the equation:

$$\text{Corrected counts} = \text{no. of counts in ROI} - \text{no. of background counts} \times \frac{\text{no. of pixels in ROI}}{\text{no. of pixels in background ROI}}$$

The correction for radioactive decay uses the equation:

$$A_T = A_O \exp^{-\lambda T_{\frac{1}{2}}}$$

[$\lambda = 0.1155 hr^{-1}$, $T_{\frac{1}{2}} = 6$ hrs, for Tc-99m]

Modifications to the Anger gamma camera take images of the subject from several directions to obtain tomographic (or slice) images of the subject. Several reviews of instrumentation have recently been published. (British Medical Bulletin, 1980; Ter-Pogossian et.al., 1980).

Radioactive gases are used routinely in nuclear medicine as ventilation measurements indicate the presence of many respiratory diseases (Taplin, 1979). In the present work initial gamma camera studies using Krypton-81m gas provided ventilation images of the subjects. The gas from a Krypton-81m generator was diluted with humidified air (approx. 1 litre min-1) and delivered to a face mask via 3mm plastic tubing. This allowed good estimates of lung size and outline for comparison with subsequent aerosol studies.

The methods used for gamma camera imaging of the rabbits and beagle dogs after administration of radiolabelled aerosols is described below.

The rabbit was positioned on the camera face, with the O.D.D. removed, 2-3 minutes after completion of dosing. Static images of the anterior thorax were recorded for a preset duration of 180 seconds and the time of imaging noted. Three regions of interest (ROI) were assessed for each image: total lungs, stomach and head/pharyngeal region. In addition, well-defined lung images were assessed for central and peripheral deposition, and lung clearance was monitored by measuring the reduction in activity in the lung region of interest over a period of time.

Imaging of the beagle dogs with the gamma camera employed a sling, specifically designed for this purpose (Fig. 4.11). Immediately after dosing, the dog was placed in the sling, held in place by an elasticated bandage around the thorax, and dorsiventral images recorded (Fig. 4.12). Initial static images were two minutes after the end of the dosing, duration 60 seconds. Simultaneous viewing of the head, thorax and stomach of the dog was not possible due to the limited field of view of the parallel hole collimator. Two 1 minute images of the head and thorax/stomach regions were taken sequentially, 1-2 mins. apart, and the initial deposition patterns calculated from the total counts of both views. The interval after dosing was taken as the intermediate time between the views to the nearest minute. In some studies lateral views of the dog were also taken. Counts were obtained from the following regions of interest: total lungs, central and peripheral lungs, stomach, head and pharyngeal (or upper respiratory tract, URT). Central and peripheral lung areas were assessed at different times using a different computer, giving slight variations in total lung counts. Templates were used for the ROI's so that the areas were approximately constant for every study. The initial dorsiventral images were compared in different experiments, although additional dynamic and clearance studies were also completed.

Fig 4.11 The Dog Sling.

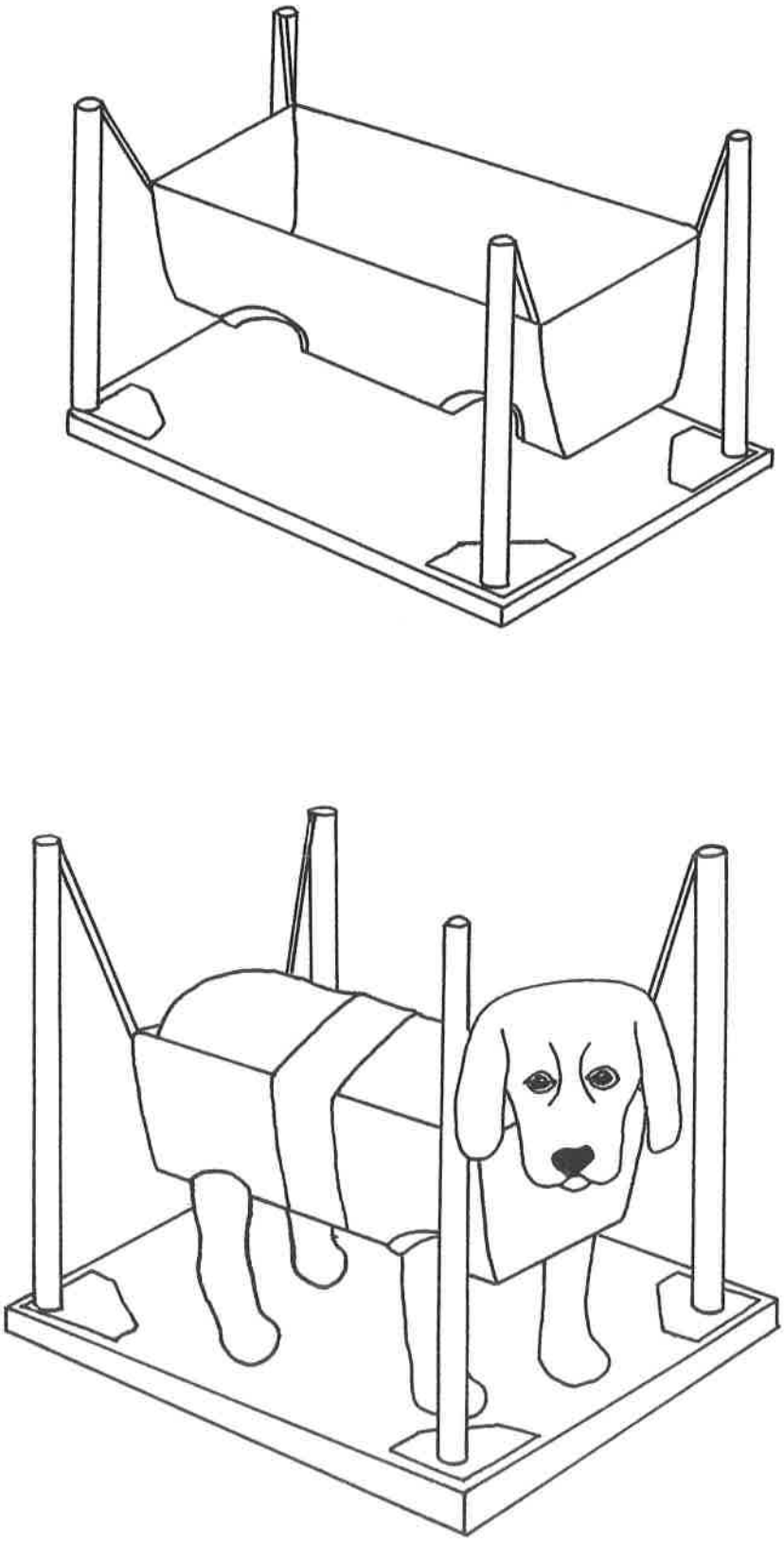
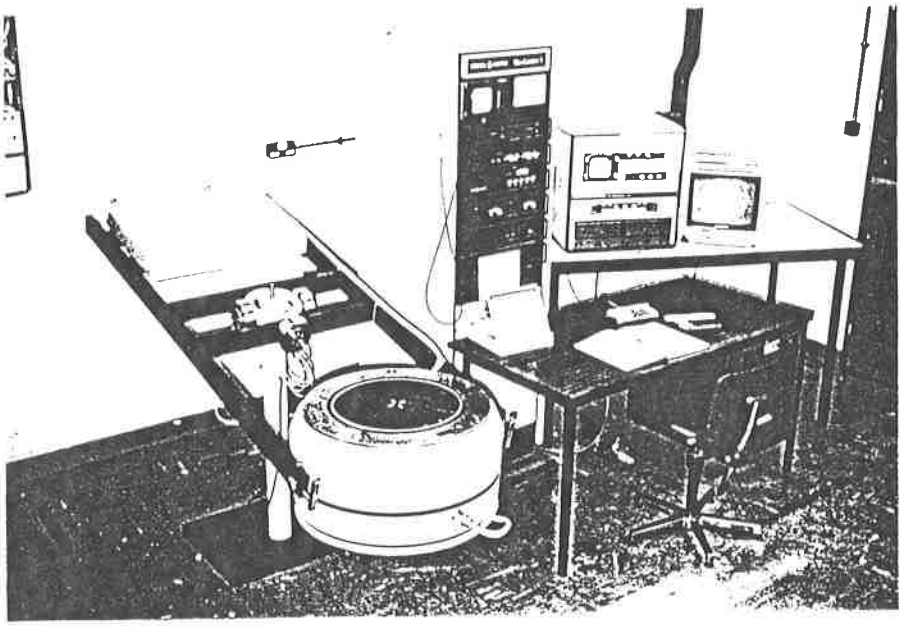
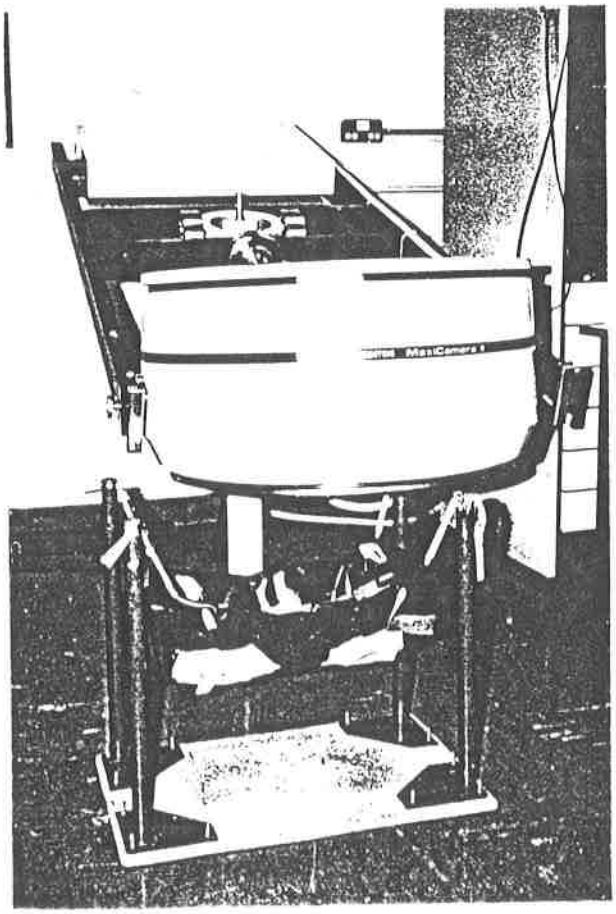


Fig 4.14. The Gamma Camera.



General View
including
computer



Camera head
and dog sling

At the end of each experiment, the administration chambers used for each dog were also counted for γ -activity, together with nebuliser tubing if used, by placing them directly on the camera face.

4.3 RESULTS

4.3.1 Aerosols

The results from the particle size measurements of both the human serum albumin (HSA) and nebulised salbutamol formulations are described in Chapter 3. The mean activity diameter of the HSA was measured as 2.18 $\pm$ 0.22µm (n = 6). Measurement of the mass and activity distributions of aerosolised Tc-labelled salbutamol solution gave 1.50µm and 1.42µm as MMAD and AMAD, respectively.

The Tc-Ph_4AsCl/salbutamol formulation was used for most *in vivo* studies in the beagle dogs. Each prepared MDI was assayed for particle size distribution (see Chapter 3) after the *in vivo* experiment. The results are presented in Appendix 4.1 and summarised in Table 4.2. The MDI's were divided into four groups according to the measured size and activity distributions. The criteria for selecting each group are summarised below:

Group 1 - Difference between mean diameters < 0.6µm
Difference between σg < 0.3

Group 2 - Difference between mean diameters 0.6 > 1.5µm

Group 3 - Difference between mean diameters > 1.5µm

The inhalers used in Group 4 contained specially prepared salbutamol with a larger mean particle size than standard micronised drug which was used in Groups 1-3.

Table 4.2 Summary of the size distribution results of salbutamol MDI's containing Tc-Ph_4AsCl.

EXPERIMENT GROUP	SALBUTAMOL			TECHNETIUM-99m	
	Mean Diameter D_{gw} (μm)	σg	Dose/shot (μg)	Mean Diameter D_{act} (μm)	σg
1 n= 7	3.0 (1.1)	1.7 (1.2)	24.6 (1.1)	3.2 (1.1)	1.7 (1.2)
2 n= 3	3.6 (1.1)	2.5 (1.1)	17.0 (1.3)	4.5 (1.1)	2.0 (1.2)
3 n= 5	3.7 (1.1)	2.6 (1.2)	15.4 (1.3)	6.9 (1.2)	2.6 (1.3)
4 n= 7	5.0 (1.3)	3.1 (1.6)	15.8 (1.3)	4.8 (1.5)	2.2 (1.2)

n = number of experiments

Note Values are geometric mean of results (geometric standard deviation).

Table 4.3 Summary of breathing patterns in beagle dogs during dosing with placebo metered dose inhalers (median values (range) of 10 experiments)

	Dog 1	Dog 2
Tidal volume (ml)	238 (170-336)	338 (204-453)
Breathing Rate (min^{-1})	29 (16-50)	21 (16-32)
Inspiratory Flow Rate (l min^{-1})	11.7 (7.8-28.6)	11.5 (9.3-17.9)
Minute Volume (ml)	5805 (4289-12770)	6526 (4479-9461)

The activity and particle size distributions of MDI's in Group 1 were found to match well. It was assumed, therefore, that camera images produced from deposition of these aerosols indicated the deposition sites of the salbutamol.

The double distributions in Groups 2, 3 and 4 did not correspond, so that deposition images were not representative of the salbutamol lung distribution. Nevertheless, the γ-camera images may be correlated with activity mean diameters since this parameter was measured in each MDI used.

Appendix 4.2 shows the dose per shot data (summarised in Table 4.2) obtained for each group of MDI's from the Andersen sampler assays. The dose/shot results for Groups 2-4 are considerably lower than the theoretical value of 26.8µg salbutamol per shot.

4.3.2 Breathing patterns

The variability in breathing pattern before, during and after aerosol dosing was assessed in both dogs using a bell spirometer and placebo MDI's (ie. containing oleic acid and propellants only). The results, measured during dosing in ten experiments are shown in Appendix 4.3 and summarised in Table 4.3. The mean values show significant differences between the two dogs, with Dog 2 exhibiting a slower ($p < 0.01$) and deeper ($p < 0.001$) breathing pattern than Dog. 1. Similar differences were shown during dosing with active MDI's from Groups 1-4. These results are detailed in Appendix 4.4 and summarised in Table 4.4. The tidal volume is significantly greater in Dog 2 than Dog 1 ($p < 0.05$) but there are no differences between the groups of MDI's. The variation in tidal volume was significantly greater in Dog 1 than Dog 2 ($p < 0.001$). Dog 1 also had a significantly higher breathing rate than Dog 2 ($p < 0.05$) irrespective of aerosol type.

Table 4.4 Summary of breathing patterns in beagle dogs during dosing with active metered-dose inhalers (Groups 1-4) Median results (and range)

			Dog 1		Dog 2	
Group 1	Tidal volume (ml)		248	(180-293)	269	(225-363)
	Breathing rate (min^{-1})	(n=6)	20.5	(15-22) (n= 6)	16	(15-22)
	Insp. flow rate (l min^{-1})		9.8	(6.6-13.5)	11.0	(8.0-12.8)
	Minute volume (ml)		4564	(3742-6154)	5091	(4036-5601)
Group 2	Tidal volume (ml)		252	(223-257)	276	(223-422)
	Breathing rate (min^{-1})	(n=3)	23	(15-31) (n=3)	17	(17-24)
	Insp. flow rate (l min^{-1})		11.8	(9.1-15.4)	12.9	(12.8-17.4)
	Minute volume (ml)		5328	(3829-8212)	6621	(3812-7520)
Group 3	Tidal volume (ml)		260	(200-381)	303	(192-358)
	Breathing rate (min^{-1})	(n=5)	18	(13-38) (n=5)	16	(14-20)
	Insp. flow rate (l min^{-1})		9.2	(7.9-16.8)	11.7	(8.2-12.8)
	Minute volume (ml)		5256	(3835-7803)	5237	(3961-5989)
Group 4	Tidal volume (ml)		231	(75-272)	237	(176-324)
	Breathing rate (min^{-1})	(n=7)	24	(11-37) (n=6)	20	(16-30)
	Insp. flow rate (l min^{-1})		9.4	(5.7-16.7)	12.4	(7.9-37.1)
	Minute volume (ml)		4016	(2799-8536)	5305	(2825-6338)

n = number of experiments

AMAD of Groups:- Grp. 1 - 3.2µm, Grp. 2 - 4.5µm, Grp. 3 - 6.9µm, Grp. 4 - 4.8µm.

4.3.3 Administration devices

Detailed results showing the percentage of aerosolized activity deposited in the administration devices and tubing are shown in Appendices 4.5 and 4.6 for HSA and metered-dose aerosols, respectively. These results are summarised in Table 4.5. The HSA deposition results show that 50-60% and 9-10% of the nebulised aerosol is lost in the administration tubing and the chamber, respectively. The small percentage lost in the administration chamber contrasts sharply with the large deposits (70-90%) from metered-dose inhalers. The differences in deposition pattern in the chamber from the two types of aerosol are clearly shown in Fig. 4.13. Deposition of nebulised aerosol on one side of the chamber and tube (Fig. 4.13-A) is due to sedimentation of the droplets during the 4 minutes administration time. The chamber deposition pattern for MDI's (Fig. 4.13-B) is due to impaction of the high velocity, larger spray droplets on the walls of the chamber.

The relationship for each dog between % deposition in the administration device and % deposition in the glass throat of the Andersen Sampler is shown graphicaly in Fig. 4.14. The r values of 0.76 and 0.64 calculated for dogs 1 and 2 respectively show there is no significant correlation between these two variables. However both dogs show a positive trend of increasing deposition in the throat with increasing deposition in the administration device.

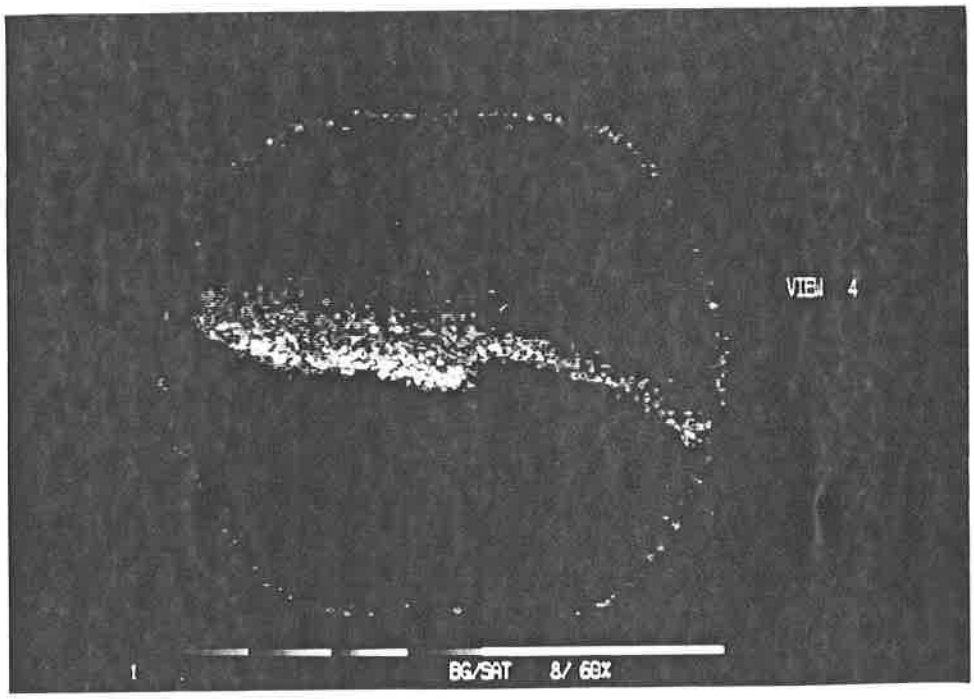
The wide scatter in results probably reflects differences in breathing pattern and MDI formulation.

Table 4.5 Summary of deposition data including administration devices (median values and range)

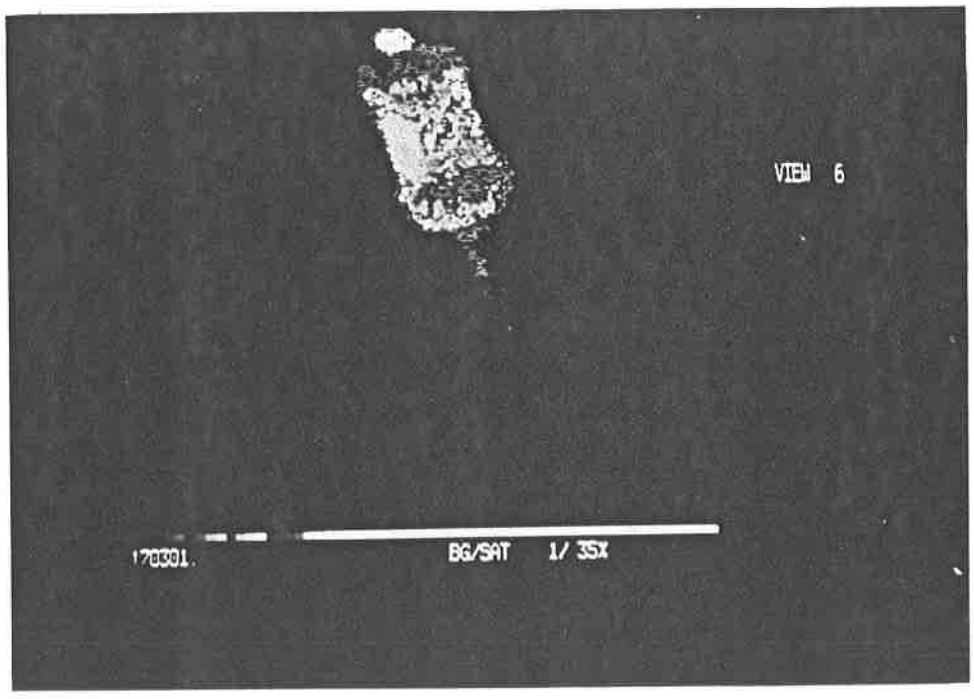
Dog 1

Experiment	n	% Admin. Tubes	% Chamber	% Total Body	% Lungs	% Stomach	% Head & URT
HSA	6	60.5 (51.2-68.4)	10.1 (4.8-15.2)	31.1 (21.1-40.7)	18.1 (13.1-26.7)	4.2 (1.9-7.4)	6.6 (2.0-12.9)
Tc-PhyAsCl Group 1 AMAD 3.2µm	7	-	69.1 (60.7-87.0)	30.9 (12.9-39.3)	23.0 (9.5-31.3)	2.2 (1.5-4.8)	4.6 (1.5-7.2)
Tc-PhyAsCl Group 2 AMAD 4.5µm	3	-	83.0 (79.4-83.7)	17.0 (16.3-20.6)	10.2 (9.5-15.4)	2.9 (2.7-3.8)	3.0 (2.5-3.9)
Tc-PhyAsCl Group 3 AMAD 6.9µm	5	-	88.1 (82.1-92.9)	11.9 (7.1-17.9)	7.0 (3.0-11.9)	1.9 (0.7-3.0)	2.6 (1.8-3.4)
Tc-PhyAsCl Group 4 AMAD 4.8µm	7	-	81.1 (73.6-95.8)	18.9 (4.2-26.4)	7.5 (2.1-15.4)	1.6 (0.9-15.1)	3.5 (1.0-5.6)
Dog 2							
HSA	5	51.0 (44.0-66.6)	9.9 (5.9-11.9)	39.1 (27.5-44.1)	19.2 (12.9-27.5)	6.6 (5.4-15.4)	10.1 (7.2-19.4)
Tc-PhyAsCl Group 1 AMAD 3.2µm	7	-	75.1 (61.8-92.2)	24.9 (7.8-38.2)	4.3 (1.3-16.5)	3.7 (1.4-8.8)	7.8 (3.8-28.7)
Tc-PhyAsCl Group 2 AMAD 4.5µm	3	-	88.5 (74.1-90.2)	11.5 (9.8-25.9)	2.5 (1.6-4.1)	4.8 (2.1-16.4)	5.5 (4.1-6.0)
Tc-PhyAsCl Group 3 AMAD 6.9µm	5	-	92.8 (86.6-94.9)	7.2 (5.1-13.4)	1.7 (0.5-4.0)	2.8 (0.2-6.5)	2.9 (2.3-8.8)
Tc-PhyAsCl Group 4 AMAD 4.8µm	7	-	89.1 (78.1-94.2)	10.9 (5.8-21.9)	1.5 (1.1-16.6)	2.1 (1.6-10.3)	6.3 (3.6-13.2)

Fig 4.13. Gamma camera images of the dog administration device after administration of a nebulised aerosol (A) and a metered-dose inhaler (B).

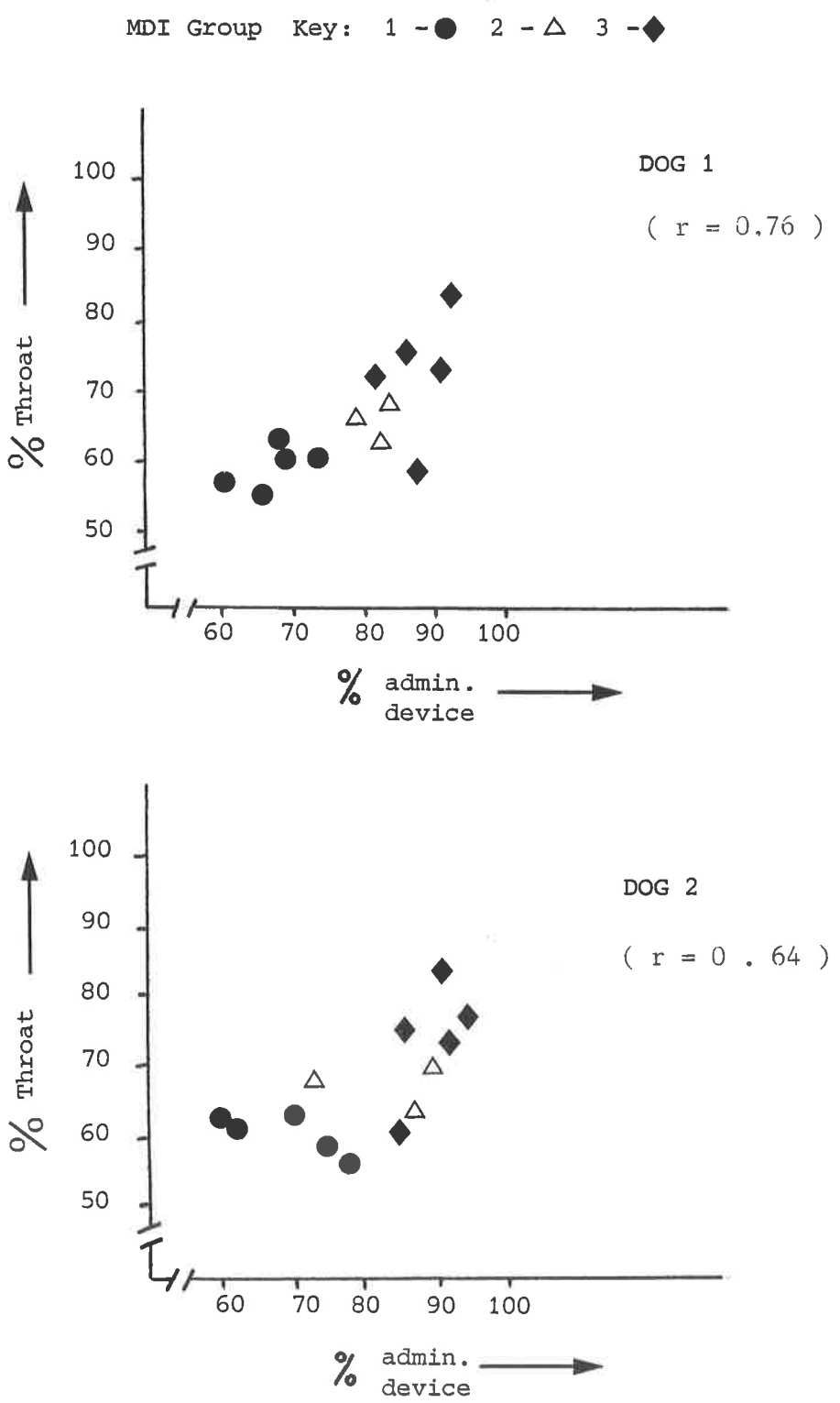


A



B

Fig. 4.14 % Deposition in Administration Device vs % Deposition in Andersen Sampler Throat.



Losses of nebulised HSA aerosol in the rabbit administration tubing were 84% and 85.9% (see Appendix 4.5), with corresponding lung deposition figures of 4.0% and 2.8%. These losses are much higher than the corresponding results in dogs.

Gamma camera imaging

Appendix 4.7 details the rabbit experimental results for HSA millimicrospheres, which are summarised in Table 4.6. The deposition results are expressed as percentage of the total activity in the defined regions of interest. The two rabbits show very different relative body distributions. Rabbit B has more than twice as much aerosol deposited in the lungs as Rabbit A. The latter shows more stomach deposition of aerosol and the head deposition is about the same. These results may be explained by differences in relative positioning of the administration tube between the two rabbits. Rabbit A is slightly larger than B, and also did not tolerate the oral delivery device as effectively as Rabbit B. The end of the tube may therefore direct aerosol into the oesophagus rather than the trachea in Rabbit A. The lower air flow through the tube (due to increased nasal breathing) would also minimise lung deposition. The length of the tube inside this animal should have been increased slightly to allow for the larger body size, to encourage intratracheal aerosol delivery.

Results from deposition of HSA millimicrospheres in beagle dogs are shown in Appendix 4.8 and summarised in Table 4.6. The consistent results show that the combination of the

Table 4.6 Summary of HSA deposition results (rabbits and dogs)

Animal	% Total Lungs	% Stomach	% Head & Urt	% Peripheral Lungs (of Total Lungs)
Rabbit A	12.1 (10.5-14.5) (n = 5)	61.1 (47.3-76.8) (n = 5)	24.8 (8.8-42.2) (n = 5)	-
Rabbit B	27.8 (20.0-35.0) (n = 6)	45.5 (36.8-65.4) (n = 6)	29.3 (11.7-36.3) (n = 6)	28.9 (18.7-36.8) (n = 4)
Dog 1	65.3 (53.1-80.6) (n = 7)	12.2 (8.6-18.1) (n = 7)	18.9 (9.9-34.6) (n = 7)	19.9 (11.5-31.0) (n = 7)
Dog 2	52.5 (33.0-62.2) (n = 6)	16.9 (14.2-35.5) (n = 6)	28.8 (20.2-49.7) (n = 6)	18.9 (10.9-30.6) (n = 7)

Notes: Figures represent median (range) n = number of experiments

Mean activity diameter of HSA millimicrospheres: 2.2µm

Table 4.7 Comparison of deposition data in dogs for nebulised HSA and nebulised salbutamol aerosols.

	AMAD (µm)	% TOTAL LUNGS	% STOMACH	% HEAD & URT	% * PERIPHERAL
HSA (both dogs) n= 13	2.2	56.0 (33.0-80.6)	15.0 (8.6-35.5)	25.9 (9.9-49.7)	19.4 (10.9-31.0)
Salbutamol -Tc-99m (both dogs) n= 4	1.4	58.4 (42.9-79.1)	8.9 (6.4-14.1)	34.8 (10.8-43.0)	20.8 (19.6-21.0)

* % of total lung counts, see Appendix 4.9.
Values are median (and range)

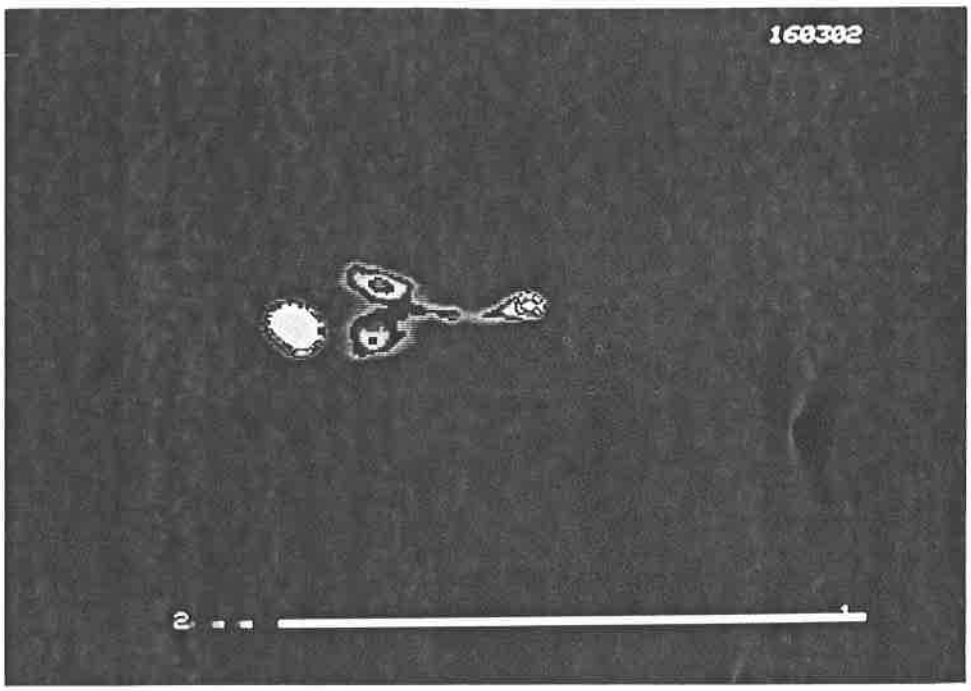
method of administration and the aerosol particle size provides efficient lung deposition. The nebulised suspension is rebreathed from the administration apparatus over a period of four minutes. At least 50% of the inhaled aerosol is deposited in the lungs with a higher percentage obtained from Dog 1.

Fig.4.15 shows the type of gamma camera images obtained in the rabbit and dog after inhalation of HSA millimicrospheres.

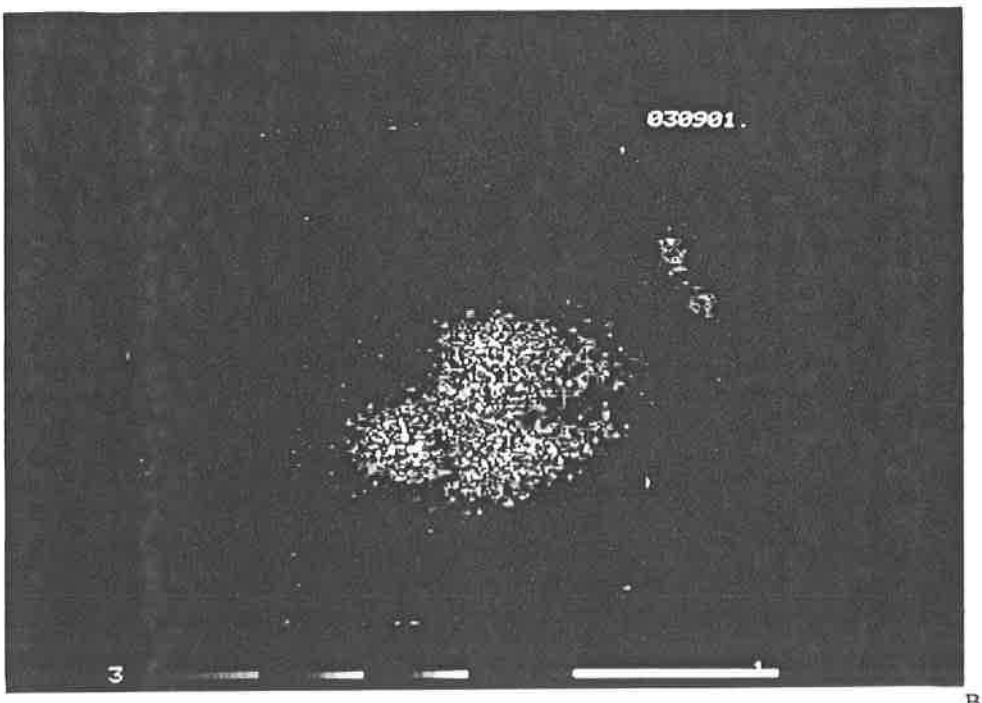
Appendix 4.10 and Table 4.7 show results obtained from administering nebulised salbutamol aerosols to beagle dogs. The median % regional deposition results show remarkable similarities with the nebulised HSA results (Table 4.7) are presumably due to slight differences in breathing pattern.

Data from clearance experiments in the rabbit and dog are shown in Appendices 4.11 and 4.12. Figs. 4.16 and 4.17 show these results in graphical form. Both graphs show very little lung clearance in the relatively short times of measurement.

Fig 4.15 Gamma camera images obtained in the rabbit (A) and beagle dog (B) after inhalation of HSA millimicrospheres. (AMAD 2.2um - measured by the Andersen Sampler)



A



B

Fig 4.16 Clearance of HSA Millimicrospheres Aerosol in Rabbit B.

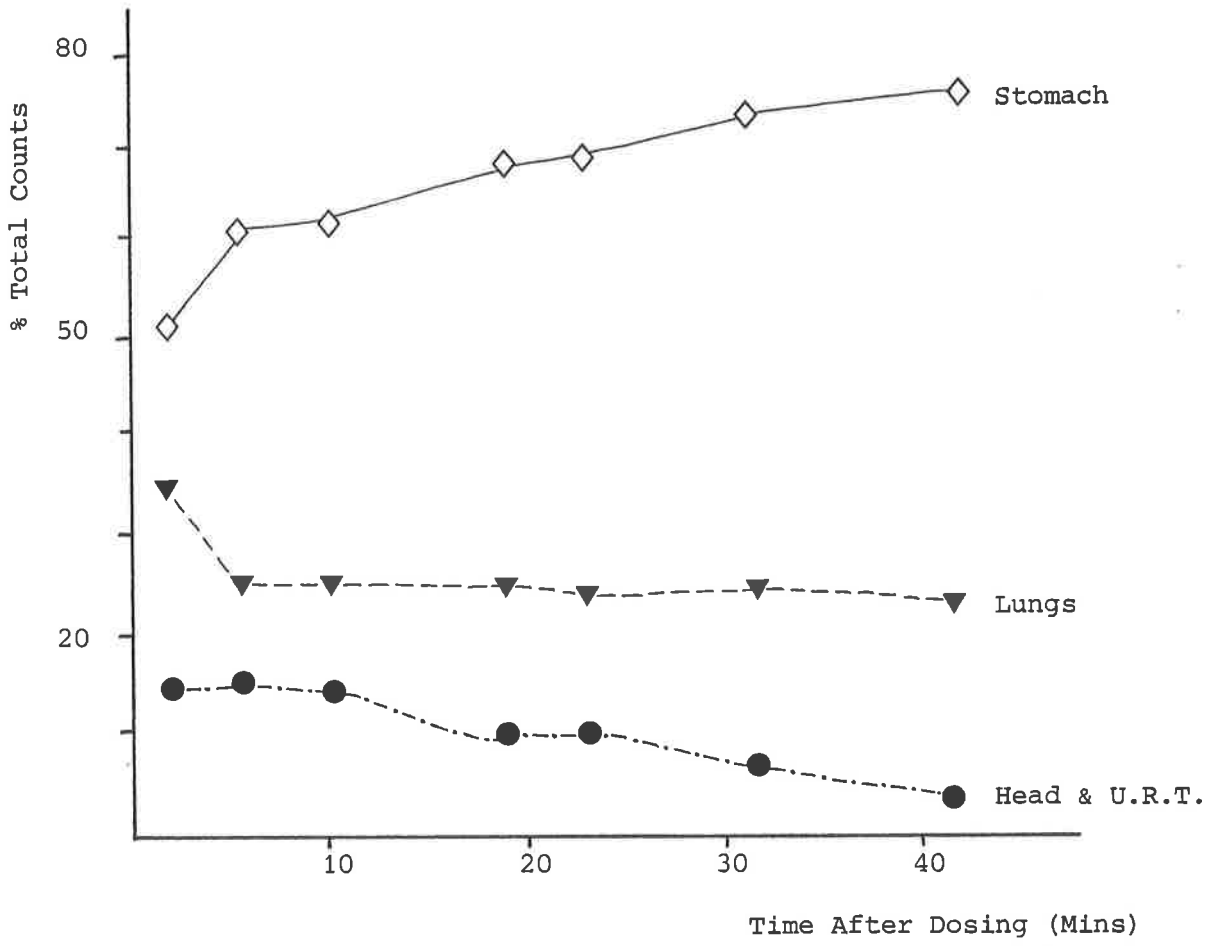
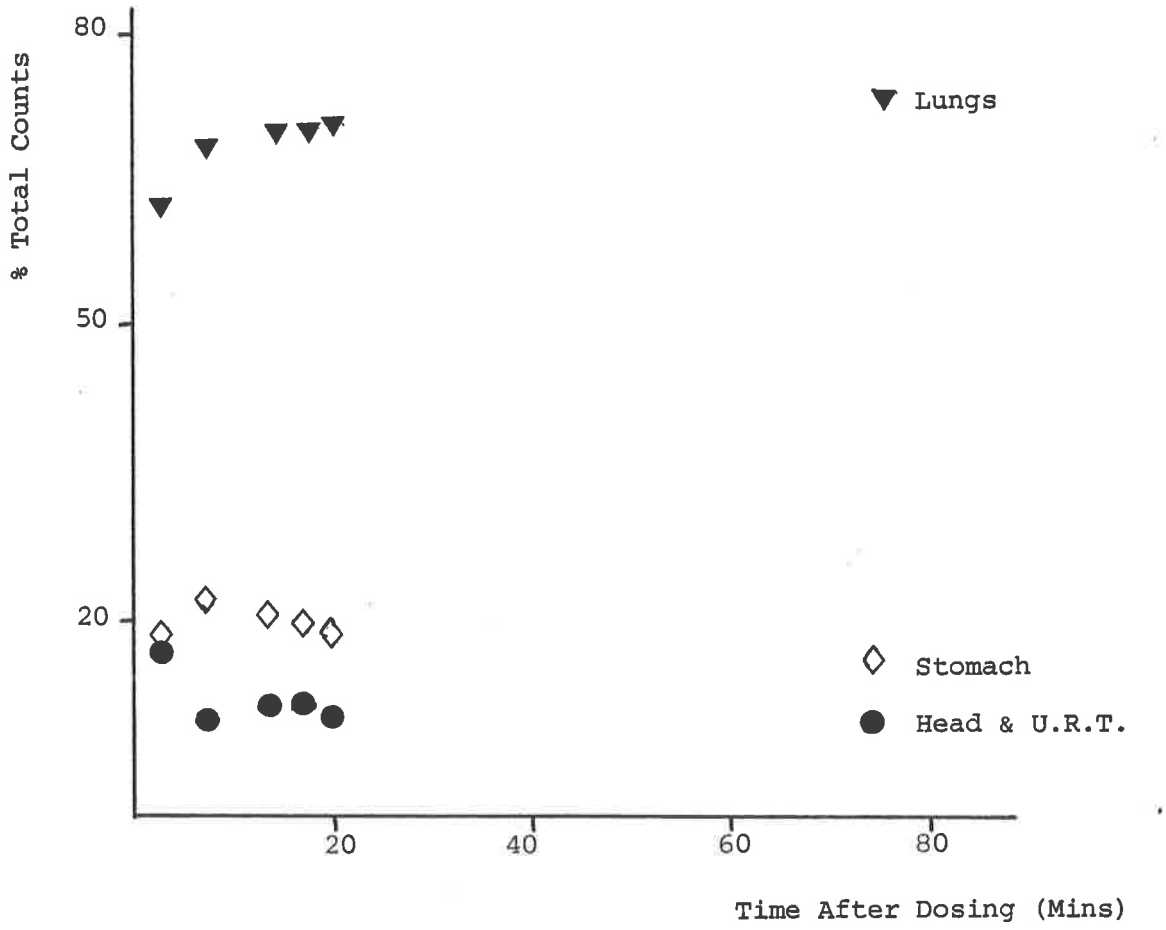


Fig 4.17 Clearance of HSA Millimicrospheres Aerosol in Dog 1.



Appendix 4.13 presents the detailed results of the in vivo experiments in Dogs 1 and 2 using Tc-Ph_4AsCl labelled inhalers from Groups 1-4. Table 4.8 summarises these results, showing the median and range of values for each group. Figs. 4.18 and 4.19 represent the summarised results in graphical form and include the nebulised HSA deposition results for comparative purposes. Figs. 4.20 and 4.21 illustrate the type of gamma camera images which are obtained from each dog for MDI's Groups 1-3.

The results for Group 1 MDI's clearly show the differences in deposition pattern in the two dogs. Although the % actuated dose deposited in vivo was about the same, (Table 4.9), with no significant difference at the 5% level; the regional distribution of this dose is very different for each dog. Dog 1 received more than five times the lung dose of Dog 2 ($p < 0.01$). In addition, Dog 2 had more than 3 times the dose of Dog 1 deposited in the head and upper respiratory tract (URT).

Table 4.9 also shows the similarities in deposition in each dog between nebulised and metered-dose aerosol deposition. When the activity losses in the administration tubing and device are included, the percentage deposition figures are very similar for the two dogs and two types of aerosol. The lower deposition percentage in the administration chamber with nebulised HSA does not include the large losses in the tubing. Thus the % total body dose is about the same for all aerosols. However, Dog 2 shows a large difference in % lung deposition between nebulised and metered-dose aerosols which suggests that the method of administration of an aerosol has a large influence on the inhalation pattern in this dog.

Table 4.8 Summary of deposition results from metered-dose inhalers containing salbutamol and Tc-Ph_4AsCl

ANIMAL	EXPERIMENT GROUP	% TOTAL LUNGS	% STOMACH	% HEAD & URT	TECHNETIUM-99m MEAN DIAMETER (μm)
Dog 1	1 n=7	76.4 (54.1-81.0)	8.7 (6.0-18.2)	15.2 (10.1-27.6)	3.2 (1.1)
	2 n=3	59.9 (58.5-74.9)	17.8 (13.0-22.2)	17.9 (12.1-23.8)	4.5 (1.1)
	3 n=5	51.0 (41.8-71.2)	16.8 (10.0-21.0)	28.0 (16.1-48.2)	6.9 (1.2)
	4 n=7	55.6 (27.5-76.3)	21.6 (6.4-59.7)	20.6 (12.8-29.5)	4.8 (1.5)
Dog 2	1 n=7	16.3 (7.8-69.0)	17.4 (11.5-31.2)	53.5 (16.0-77.6)	3.2 (1.1)
	2 n=3	16.4 (15.7-22.0)	42.2 (22.2-63.2)	35.8 (21.1-61.4)	4.5 (1.1)
	3 n=5	30.1 (7.2-33.2)	34.2 (4.9-54.8)	38.0 (21.4-66.4)	6.9 (1.2)
	4 n=7	18.3 (6.9-76.0)	19.5 (7.5-48.8)	45.0 (16.5-80.0)	4.8 (1.5)

Notes n = number of experiments
median values (range range) are shown.
AMAD values for Experiment Groups:-
Grp. 1 - 3.2μm, Grp. 2 - 4.5μm, Grp. 3 - 6.9μm, Grp. 4 - 4.8μm

Fig 4.18 Fractional Deposition of Aerosols In Dog 1.
(median values and ranges)

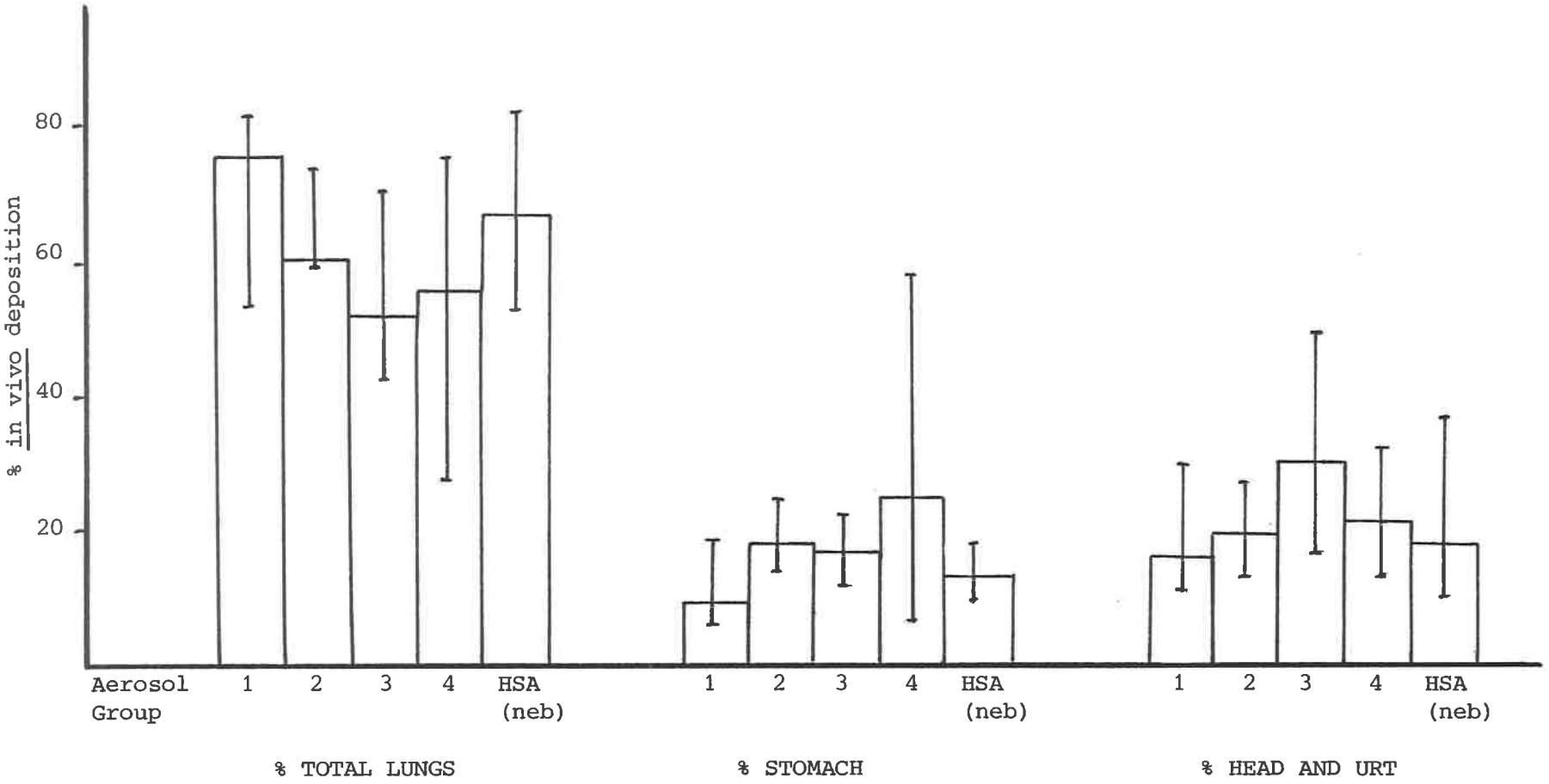


Fig 4.19 Fractional Deposition of Aerosols in Dog 2.
(median values and ranges)

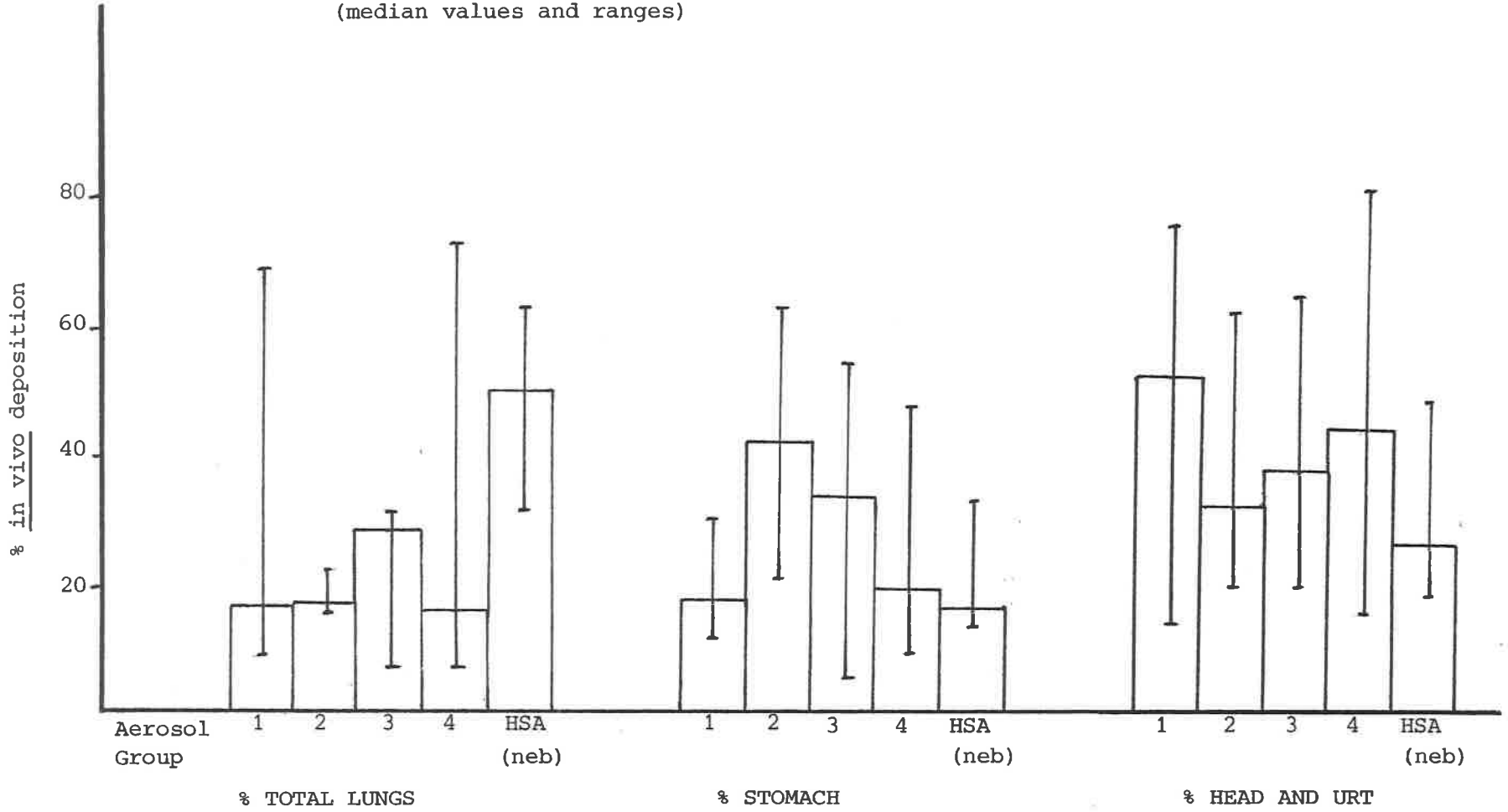
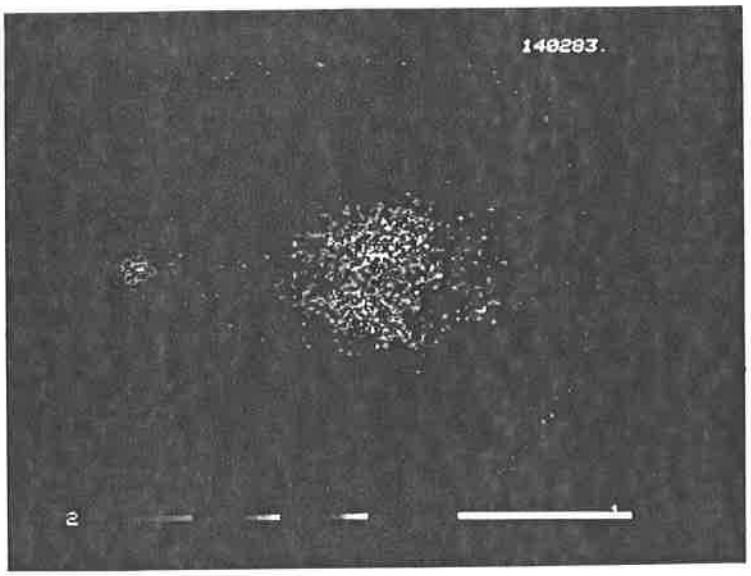
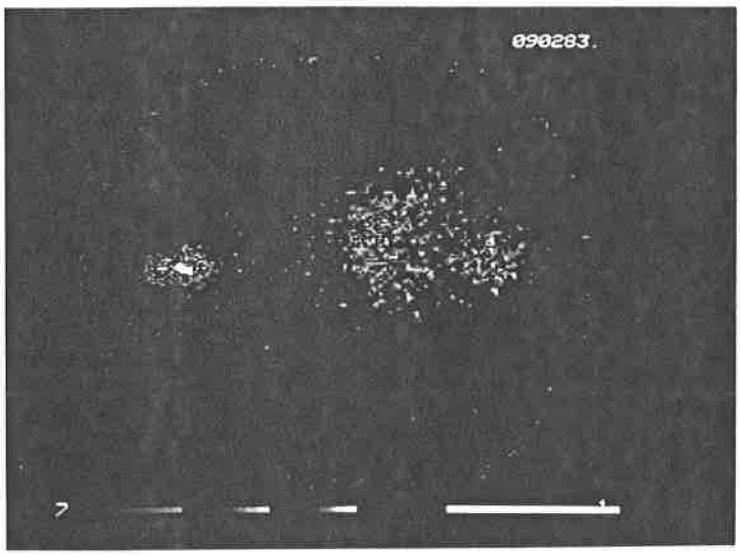


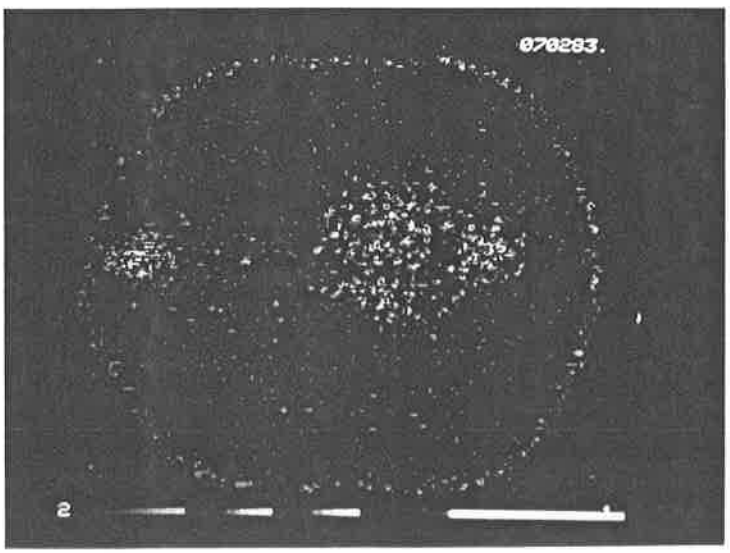
Fig 4.20 Gamma camera images showing lung deposition from MDI's (Groups 1-3) in Dog 1.



MDI
Group 1

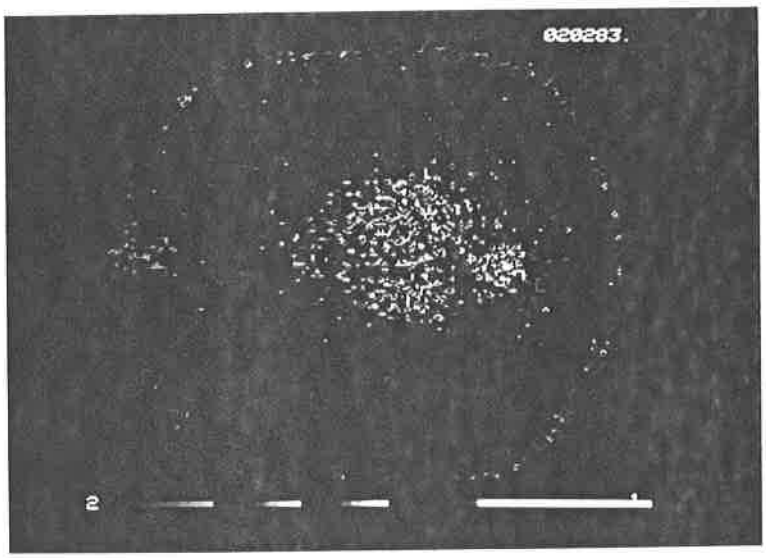


MDI
Group 2

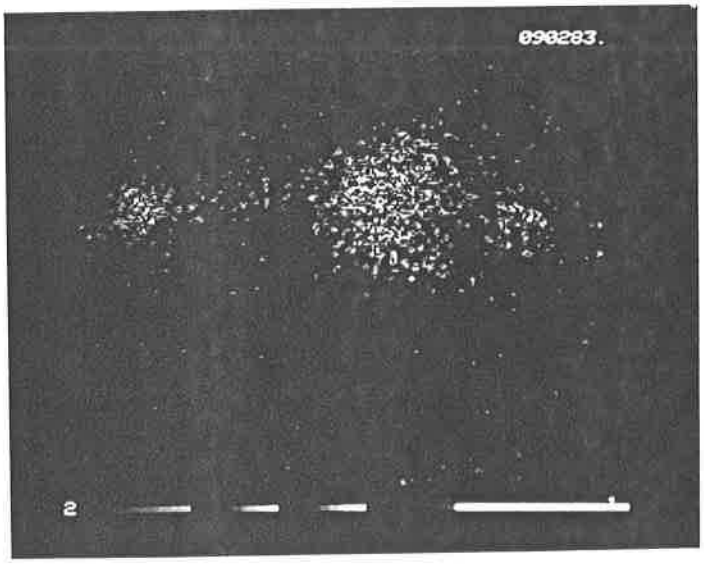


MDI
Group 3

Fig 4.21 Gamma camera images showing lung deposition from MDI's (Groups 1 and 2) in Dog 2.



MDI
Group 1.



MDI
Group 2.

Table 4.9 Comparison of deposition results for nebulised HSA and MDI's Group 1 (median values and range)

	DOG 1		DOG 2	
	HSA n=6	Group 1 MDI n=7	HSA n=5	Group 1 MDI n=7
% Admin. Tubes	60.5 (51.2-68.4)	-	51.0 (44.0-66.6)	-
% Chamber	10.1 (4.8-15.2)	69.1 (60.7-87.0)	9.9 (5.9-11.9)	75.1 (61.8-92.2)
% Total Body	31.1 (21.1-40.7)	30.9 (12.9-39.3)	39.1 (27.5-44.1)	24.9 (7.8-38.2)
% Lungs	18.1 (13.1-26.7)	23.0 (9.5-31.3)	19.2 (12.9-27.5)	4.3 (1.3-16.5)
% Stomach	4.2 (1.9-7.4)	2.2 (1.5-4.8)	6.6 (5.4-15.4)	3.7 (1.4-8.8)
% Head & URT	6.6 (2.0-12.9)	4.6 (1.5-7.2)	10.1 (7.2-19.4)	7.8 (3.8-28.7)

AMAD of HSA = 2.2μm

AMAD of Grp. 1 = 3.2μm

Fig. 4.18 shows particle size differentiation in Dog 1 for MDI's Group 1-3. As the AMAD increases, the % total lung deposition decreases. This effect is also shown in % chamber deposition for Groups 1-3 (see Table 4.5, $p < 0.01$). Fig. 4.22 shows the relationship for both dogs between % total lung dose and AMAD for MDI's Groups 1-4. Dog 1 gave significantly higher % lung dose results than Dog 2 ($p < 0.001$). Dog 1 also showed a significant correlation between % lung dose and AMAD ($p < 0.01$), although Dog 2 did not. The wide scatter of these results in both dogs reflects the number of variables involved such as the differences in breathing pattern.

Fig. 4.23 illustrates an experiment using Krypton -81m gas to establish the profile of the upper airways in both dogs. The images shown are lateral views, taken during administration of the radioactive gas via the administration device. The arrows marked indicate the points where the gas leaves the administration tube in the pharynx. Dog 2 shows a sharp bend in the airway at this point, whereas Dog 1 has a clear, straight airway passage into the lungs. This difference in airway shape in Dog 2 may be due to the positioning of the administration tube or the posture of the head which governs the position of the epiglottis. The inhaled gas or aerosol may therefore travel a more tortuous respiratory passage in Dog 2 which may explain the lower lung deposition figures.

Fig. 4.22 Relationship Between % Deposition in Total Lungs and AMAD.

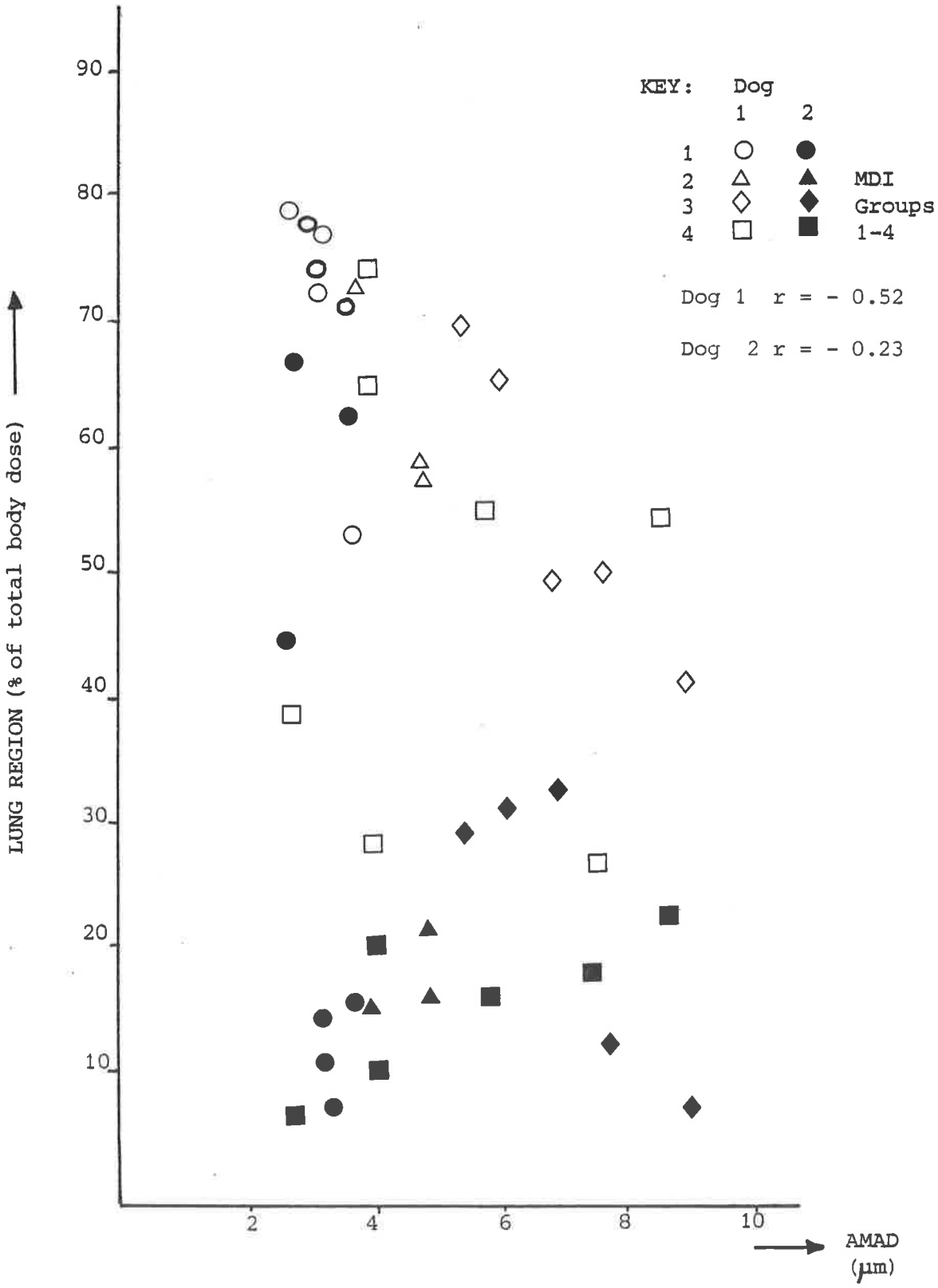
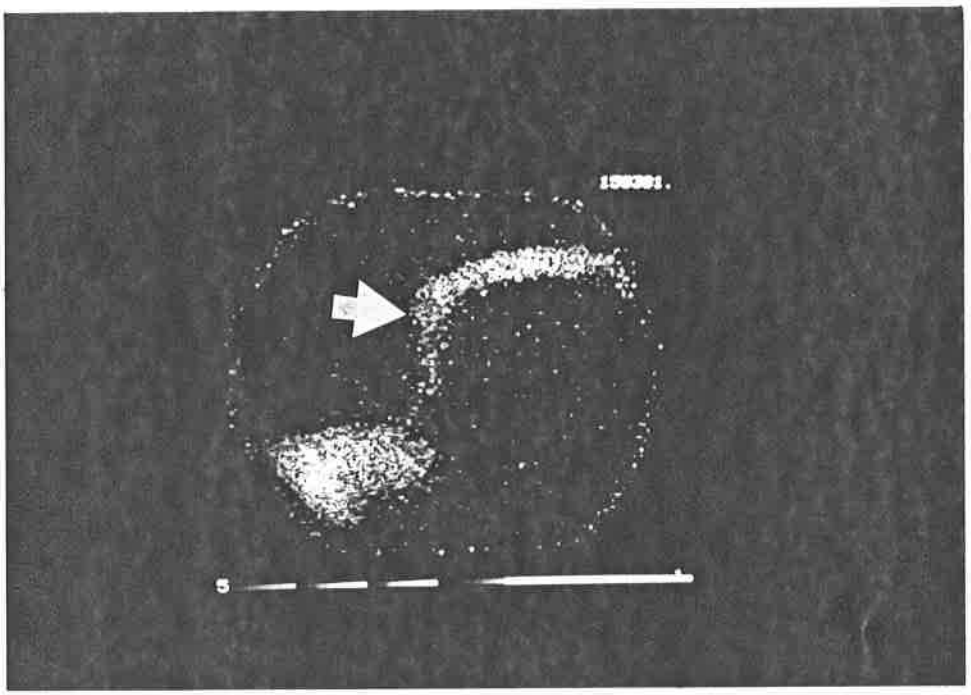
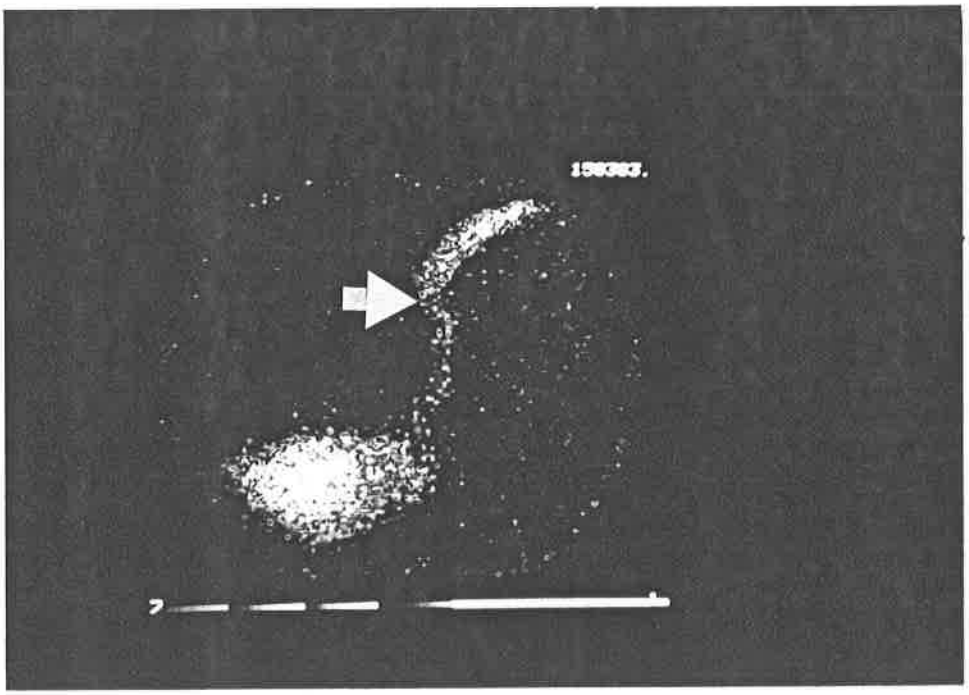


Fig. 4.23. Gamma camera images of administration tube position and respiratory airways using Krypton-81 m gas. (lateral views).



A - Dog 1.



B - Dog 2.

Assessment of lung images

The total lung deposition of activity has been shown to decrease for metered-dose aerosols of increasing mean diameters in Dog 1 (Section 4.3.4, Table 4.8). In addition, comparisons of lung deposition patterns were made to assess the effect of particle size. Two methods were used to compare the γ-camera images. These were the measurement of the activity ratio in peripheral and total lung areas, and the comparison of activity distribution in cross-sectional slices of the lung image (Garrard et al., 1981, Dolovich et al., 1981b). It is assumed that peripheral lung deposition indicates the relative lung dose in the small airways.

The percentage activity of HSA aerosol deposited in the peripheral area of the lungs was calculated as approximately 20% for the dog and 28% for the rabbit, as shown in Appendix 4.9 and Table 4.6. The same method used in assessment of deposition images from metered-dose aerosols in dog 1 gave mean values of 15-18%, with no significant differences between aerosol groups of differing activity mean diameters (Appendix 4.9).

This technique may not be accurate however, because the peripheral region is arbitarily defined due to poor definition of lung anatomy related to gamma images in the rabbit and dog. It is also difficult to reproduce the same area in different studies because the lung region image is relatively small, particularly in the rabbit experiments.

The data obtained from taking cross-sectional slices across gamma camera images in the manner illustrated in Fig. 4.24 is shown in Appendix 4.14. By smoothing and replotting the activity per pixel across the image, detailed comparisons were made of deposition patterns from different aerosols (Fig. 4.25). The peak heights of their widths and distribu-

Fig. 4.24. Gamma camera image to show positions of cross-sectional slices taken for regional deposition data. (Appendix 4.14 and Fig. 4.27.)

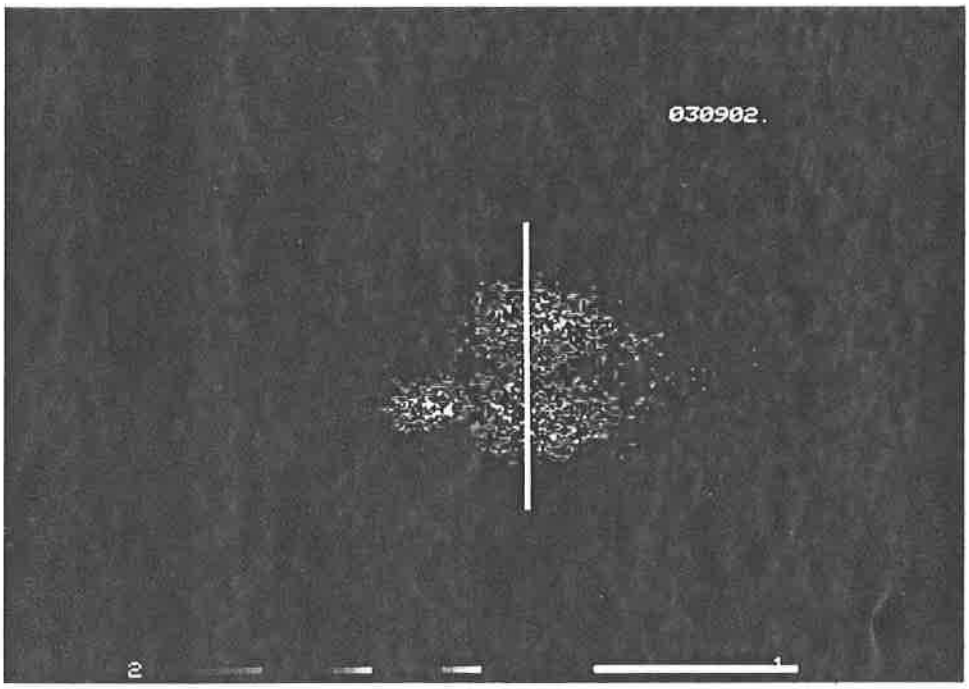
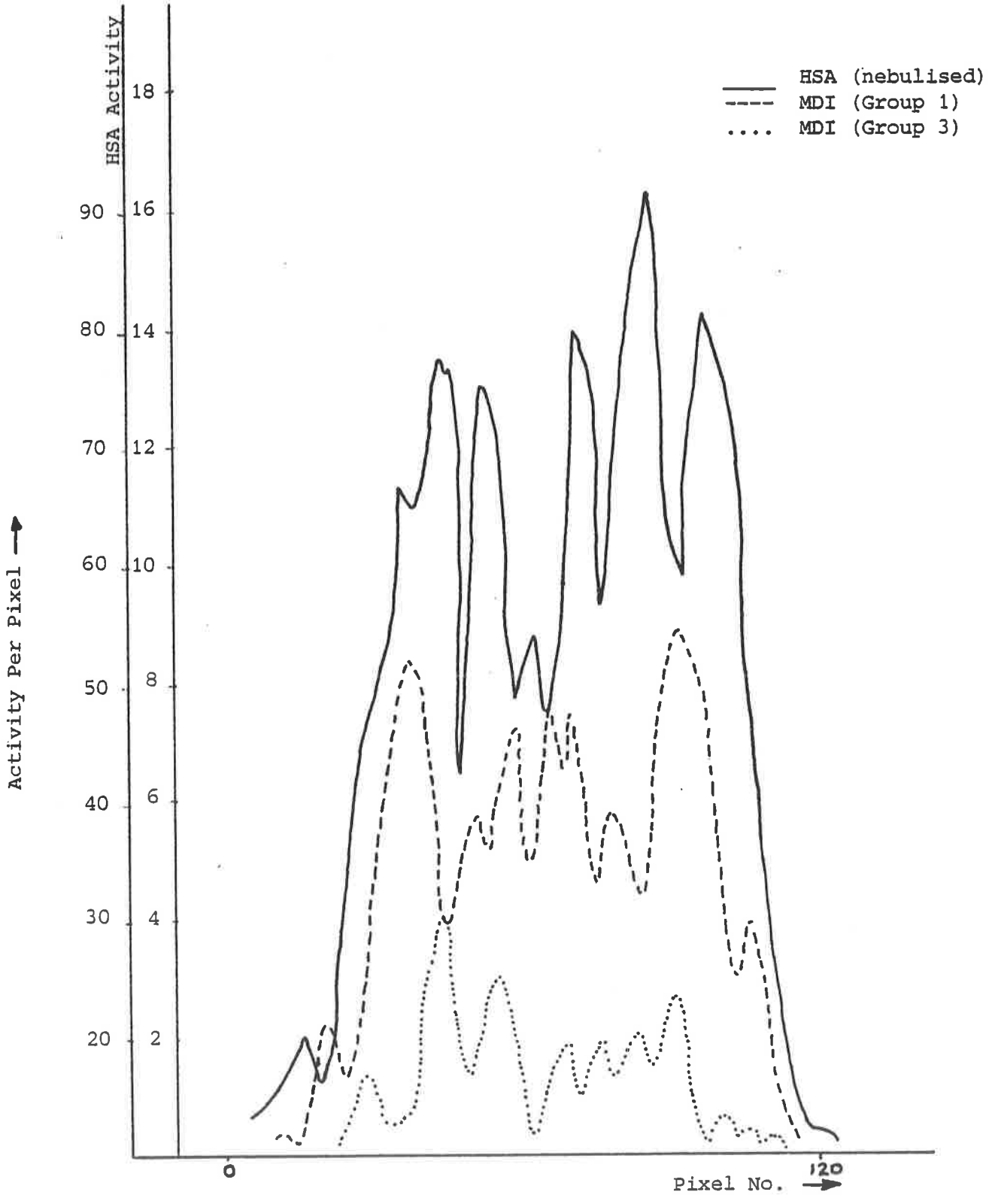


Fig 4.25 Cross Sectional Distribution of Activity in Gamma Camera Lung Images (Dog 1).



tion indicates the pattern of distribution. Assessment of lateral cross-sections of the lung image may be used to provide a measurement of the central/peripheral lung distribution without the errors involved in arbitarily measuring peripheral areas. Fig. 4.25 shows a smaller, narrower pattern of peaks for MDI Group 3 (AMAD 6.9μm) than for HSA aerosol (AMAD 2.2μm). This indicates that the aerosol with a larger mean size has a more centrally deposited pattern of distribution within the lung.

Figs. 4.15 and 4.20 show the type of gamma camera images used for this assessment.

4.4 DISCUSSION

In much of the *in vivo* work in the present study, the initial aerosol deposition pattern in the lungs of two beagle dogs was compared using aerosols administered from MDI's with three different activity median aerodynamic diameters (AMAD). The methods for inhaler preparation were validated and the aerosols in the first group with an AMAD of 3.2 (G.S.D. 1.1) were shown to contain correctly radiolabelled salbutamol. Groups 2 and 3 showed discrepancies between the activity and drug particle size distributions when measured by cascade impaction, with the activity distribution having a large mean size than the drug distribution. The increased activity mean size may have been caused by precipitation of the radiolabelled complex from a concentrated solution. This process would have been accelerated by the rapid cooling encountered on addition of the volatile propellants to the formulation. However, since the distributions of drug and activity were measured for each inhaler used in the *in vivo* experiments, the deposition patterns were correlated with AMAD, regardless of the mean size of salbutamol in each aerosol. The results therefore related lung deposition to particles of known aerodynamic size, but some of them may not be entirely composed of salbutamol.

The MMAD of salbutamol in the radiolabelled Group 1 formulation used in the present study was measured as 3.0 (G.S.D. = 1.1) using an Andersen Sampler eight-stage cascade impactor. These results are similar to those measured by impaction methods on various pressurised inhalers (Bell et al., 1973; Nilsson et al., 1977). A deliberately inefficient micronising process produced salbutamol powder with a coarser, more polydisperse size distribution. This was intended for comparative in vivo studies with the standard micronised material, as used in the Group 1 radiolabelled formulation. However, no significant differences were found in lung deposition patterns between the two aerosols with AMAD's of 3.2μm (σg = 1.7) and 4.8μm (σg = 2.2). This could be explained by the substantial proportion of particles from each aerosol being in overlapping size ranges due to the polydispersity of each distribution. Gonda (1981) has shown that the fractional deposition of polydisperse aerosols becomes increasingly independent of MMAD with increasing polydispersity, for aerosols less than 10μm.

The problems of preparing micronised drug aerosols with polydisperse distributions of different mean sizes were experienced by Rees et al., (1982). They used a zig-zag classifier to produce three broad size distributions of micronised terbutaline for use in MDI's, which were referred to as <5μm, 5-10μm and 10-15μm. Considerable proportions of each distribution were found to overlap between the ranges. However, these authors showed that the clinical response to the <5μm fraction was significantly greater than the others. Similarly, Godfrey et al., (1974) also showed a significant difference in response to sodium cromoglycate after exercise challenge, when comparing monodisperse particles of drug of mean sizes 2 and 11.7μm.

In the present work two types of conscious animal models have been used for the in vivo studies. Animal models

have the advantages of allowing repeated studies of different radiolabelled formulations in the same subject and of enabling materials to be used which have not been adequately tested toxicologically for human use. The distribution of γ-radiolabelled salbutamol aerosols in the initial phase was examined with a gamma camera after inhalation in rabbits and beagle dogs.

The further use of these animal models would allow lung deposition data to be obtained at an early stage in the research and development of an aerosol of a new drug. Most animal work on therapeutic aerosols has involved only toxicological testing (Clarke, 1973; Smith & Spurling, 1974). Recently, however, dogs (Tilov et al., 1978) and horses (Theodorakis et al., 1982) have been used to study respectively the deposition patterns of fenoterol from MDI's and sodium cromoglycate from nebulised aerosols. Tilov et al., (1978) employed a fluorescent tracer, ethidium bromide, with similar particle size and MDI formulation to a fenoterol aerosol. They inferred that the fluorescent lung deposition patterns represented the deposition sites of fenoterol, the two MDI's being administered simultaneously. This technique has the disadvantages of requiring sacrifice of the animal to obtain results, and the use of anaesthetics in administration which may affect respiration. In the present study, MDI's were administered to conscious dogs with a delivery device developed by Poynter & Spurling (1971). This allowed studies of different formulations in the same subject, and avoided the influence of anaesthetics on respiration (Dain et al., 1975).

The many experimental variables in studies with conscious animals which could affect lung deposition are listed in Table 4.11. For a given experiment, all the variables except the breathing pattern and posture of the animal were controlled.

Table 4.11 Variables in in vivo animal experiments.

1. Type of aerosol (nebulised, MDI etc.)
2. Activity mean aerodynamic diameter of aerosol
3. Breathing pattern - tidal volume
   - respiratory frequency
   - breath holding
4. Posture of animal during dosing
5. Drug and radioactive dose
6. Animal model used.
7. Administration system - intrapharyngeal tube, face mask etc.
8. γ-camera details - collimator, sensitivity, resolution
   γ- radionuclide used, γ-energy.
9. γ-camera views - lateral/dorsiventral for best views of ROI's.
10. Movement of animal during dosing and imaging
11. Environment - temperature, humidity, noise
12. Operators - handling of animal
13. Time involved in dosing, preparing for imaging and imaging itself.

Previous in vivo studies on rabbits have used monodisperse, inert particles, anaesthetised animals and intratracheal tubes for administration (Holma, 1968). In the present work, a conscious rabbit model was used which employed an oral delivery device (Halper & Schlesinger, 1980) to avoid filtration of the aerosol by the nasal passages. Nebulised aerosols of human serum albumin were administered successfully, with up to 30% of the initial dose distribution (excluding the administration devices) deposited in the lungs. However, the substantial losses of activity in the administration tubing, due to settling or impaction of nebulised droplets, increases the initial quantities of activity necessary to obtain satisfactory lung images. Regional lung deposition studies in the rabbit model are unsatisfactory because of the small gamma camera images obtained. These disadvantages make the use of conscious rabbits for inhalation studies impractical unless considerations of economy and availability are more important.

The beagle dog was a suitable model for both nebulised and metered-dose aerosols. It was particularly valuable because it was easily trained and large enough to provide a detailed γ -camera lung image. Both these factors are advantages over the rabbit model used earlier in this study. Published work by Schlesinger & McFadden (1981) suggests that the upper bronchial tree anatomy of the dog is most similar to that of the human when compared with several experimental species. There is support for the beagle dog being a satisfactory biological model for studying respiratory tract deposition of inhaled polydisperse aerosols, particularly with regard to the initial dose distribution (Cuddihy et al., 1973).

The delivery device used with the beagle dogs consisted of a moveable rubber sleeve inside a chamber which was attached to a rubber tube. Breathing took place through the tube,

the end of which was positioned in the pharynx, and movements of the sleeve enabled co-ordination of MDI firing and inspiration to be achieved. This device proved to be a relatively efficient means of dosing the dogs. Up to 25% of the actuated dose was deposited in the lungs, presumably because the aerosol was delivered directly into the pharynx of the dog.

Inhalation of Krypton-91m gas, used in ventilation studies to outline the respiratory airways (Kawakami et al. 1981), was employed in the present study to visualise the upper respiratory tract profile. The natural posture of each dog during dosing in the seated position, corresponded to the airway profile as demonstrated by Krypton-81m scans. Dog 2 sat upright with the head held back, producing a sharp bend in the airway at the end of the dosing tube, and thereby encouraging high aerosol deposition at this point. Dog 1 stretched forward, producing a straight airway passage, and achieved higher lung deposition. Large differences in dose distribution in beagle dogs using salbutamol aerosols from MDI's have been shown by Martin et al., (1971) who employed tritiated drug in a series of metabolic studies. They showed that the plasma activity in two dogs showed peaks at five minutes and two hours which suggests absorption from the lungs and stomach respectively, reflecting gross differences in initial dose distribution. Martin et al., (1971) also showed 10-22% of the dose deposited in the lungs which corresponds to the results of the present study. A similar administration device was used, and a similar suspension formulation of salbutamol in a metered-dose inhaler.

Excellent γ-camera lung images were obtained from the beagle dog experiments in the present study and differentiation was achieved in the lungs of aerosols with different particle size distributions. The two dogs used gave markedly different results and served to illustrate the wide range

of response normally exhibited in in vivo studies. Dog 1 showed a clearer relationship between % lung dose and AMAD than dog 2, the total lung dose increasing with decreasing AMAD ($p < 0.05$). The lung deposition values achieved in dog 1 (21.3 ± 7.0% for AMAD 3.2 ± 0.03µm) are approximately double those found in human lungs in a comparative study (Newman, 1981a). However, the high lung deposition in this dog provided detailed lung images which were qualitatively shown to differentiate between aerosols of different mean diameters. Using the technique of Garrard et al., (1981), cross-sectional slices of lung activity were compared and aerosols with larger mean diameters were shown to deposit more centrally in the respiratory airways.

The high velocity delivery of metered-dose inhalers is likely to cause high impactional deposition at the end of the dosing tube, which is positioned in the oropharynx. This would give high % head and upper respiratory tract (URT) deposition values and lower lung deposition, as found in dog 2. A sharp bend in the airway of dog 2 at the distal end of the dosing tube probably contributed to the high % deposition at this point. It may be argued that this dog provides a suitable model to assess the oropharyngeal deposition caused by the high velocity delivery and sharp bend in the airway in human subjects.

The low lung deposition and high URT and mouth deposition in Dog 2 for MDI's from Group 1 (AMAD 3.2 ± 0.3µm) resembles the initial dose distribution of Teflon aerosols in humans. Dog 2 achieved 7.8 ± 5.8% lung deposition with Group 1 aerosols which is similar to the 8.8% found in humans (Newman et al., 1981a) with an aerosol of similar particle size.

The proportion impacted in the delivery device (70-90%) was comparable with the oral deposition fraction of 80% in

humans measured by Newman et al. (1981a). In this respect, the delivery device used for administering aerosols to dogs has the same function as an oral delivery chamber (spacer), which reduces oropharyngeal deposition in clinical use by minimising impaction of the high velocity spray in this region (Moren, 1978). Spacers have also been claimed to improve the lung deposition fraction (Newman et al., 1981b). This could be caused by allowing more time for the propellant droplets to shrink by evaporation before reaching the impaction sites in the airways, or probably more significantly, by avoiding the co-ordination problems often associated with administering an MDI. The co-ordination of firing was not applicable in this study but the expansion chamber permitted inhalation of a single actuated dose during more than one breath. This allows rebreathing of any aerosol which is exhaled and could increase the dose delivery to the small airways.

The proportion of dose deposited in the delivery device was related to that deposited in vitro in the glass throat of the Andersen Sampler, which was used to mimic the oropharynx. The dose fraction deposited in the glass throat (about 60%) was generally less than that deposited in either the dog delivery device or clinically in the human oropharynx. This difference could be a result of increased impactional deposition in vivo below the larynx due to the laryngeal jet effect (Dekker, 1961; Chan et al., 1980). No attempt was made to incorporate an artificial larynx in the Andersen glass throat so lower deposition would be expected in this device than in vivo. The increased deposition in the delivery device may be due to several factors. These include the complex airflow patterns within the chamber which could increase wall losses; and sedimentation of non-impacted particles. The latter factor is related to time in the chamber and could be increased by hygroscopic growth of the particles. It could also be increased by the lower airflow through the device compared with the Andersen glass throat.

The inherent biological variability in aerosol deposition results has been demonstrated in humans (Stahlhofen et al., 1981) and was also evident in this study. The two dogs gave very different results, due to differences in their size (and therefore airway dimensions), posture and breathing pattern. The lack of control over breathing pattern is a major disadvantage of using conscious animals. Large numbers of subjects and experiments are required to ensure valid statistical comparisons at suitable breathing rates. The intra-subject variability in the dogs was also significant. For example, the tidal volume in dog 1 varied by 20-25%, and the total lung dose in dog 2 varied by 24-45%.