ADAPTION AND USE OF AN ULTRASONIC NEBULISER FOR INHALATIONAL STUDIES IN LABORATORY ANIMALS

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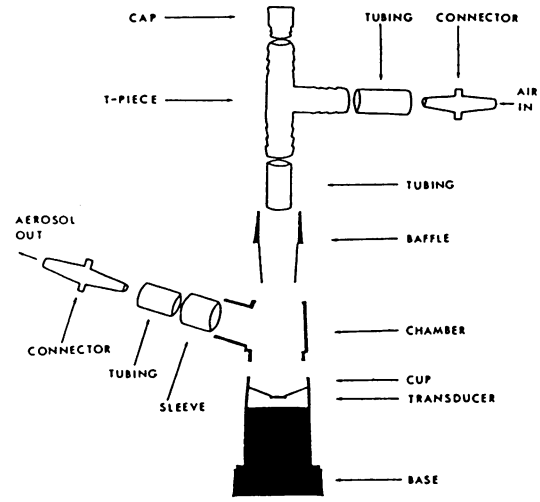
Aerosol studies in laboratory animals are often compromised by the experimental method in which the aerosol is both generated and administered. This is especially true in cases where simultaneous measurements of accompanying changes in airway metameters of mechanically ventilated animals are required. In the present study we describe the simple adaptation of a commercially available ultrasonic nebuliser which can be permanently installed in the afferent limb of the ventilator circuit. This technique allows artefact-free measurement of changes in airway metameters (in this instance pulmonary inflation pressure) before, during, and after aerosol administration of drugs under test.

A Pulmosonic nebuliser (model 2512) was obtained from Devilbiss Health Care (U.K.) Limited of Feltham Middlesex U.K. and adapted as shown in the adjacent diagram. The medication chamber, baffle, and mouthpiece were removed from the nebuliser assembly. A hollow segment approximately 2cm in length was cut from the plastic mouthpiece. This formed a sleeve for the subsequent insertion of a 2.5cm length of polythene tubing (1.65cm o.d., 0.75cm i.d.) into the mouthpiece aperture of the medication chamber and fitting of a plastic connector (Portex 700/150/634 or equivalent). The check valve was removed from the baffle assembly and discarded. A plastic T-piece (Portex 700/160/020 or equivalent) fitted with a 3cm length of polythene tubing (2.1cm o.d., 1.5cm i.d.) was introduced into the top end of the baffle assembly which was then re-inserted into the medication chamber and the join sealed with araldite or PTFE tape. A plastic cap (Sarstedt 65.973) was fitted to the opposite arm of the T-piece and a further length of polythene tubing (3.5cm long, 1.65cm o.d., 0.75cm i.d.) fitted to the side arm allowing subsequent insertion of a plastic connector (Portex 700/150/634 or equivalent). The adapted nebuliser assembly was then directly connected into the afferent limb of the ventilator circuit and arranged so that inspired air passed through the medication chamber before entering the lungs of anaesthetised animals (rats, guinea-pigs, cats) via a tracheal cannula. In these animals pulmonary inflation pressure, an index of intrathoracic airway calibre, (Dixon and Brodie 1903) was measured from a lateral port in the afferent limb of the ventilator circuit. Actuation of the nebuliser did not cause any discernable artefacts in this measurement.



The above method is advantageous in that firstly, the aerosol is administered directly to the lungs instead of first passing through either the ventilator or a separate shunt circuit. Secondly continuous quantitative measurement of changes in lung function is possible throughout the aerosol administration of test substances.

Dixon, W.E. and Brodie, T.G. (1903). J. Physiol. 29, 97-173.

QUANTITATIVE ASSESSMENT OF THE EFFECTS OF ABSOLUTE ETHANOL ON THE RAT GASTRIC MUCOSA

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The evaluation of ethanol-induced gastric mucosal damage in the rat is frequently carried out using subjective scoring systems. However, Hollander et al (1982) and more recently Witt et al (1985) and Szabo et al (1985) have described objective methods for the quantification of gastric damage in the rat. The latter two methods involved the use of transmission densitometry and a micro-processor assisted planimetry respectively.

The method we will demonstrate employs an 'on-line' computer-assisted digitisation of a video picture. The glandular portion of excised rat stomachs are laid on a plain black ground, under water to eliminate reflections, and the mucosal surface is viewed by a closed circuit TV camera. This is connected to an Eltime image digitiser controlled by a BBC microcomputer. Images are digitised to give 256 x 256 pixels at 64 grey levels. Prior treatment of the glandular mucosa of the stomach with 0.1N HCl increases the contrast between the intact and damaged areas, such that the digitised image is differentiated into three bands: the black background, the damaged mucosal surface, and the intact mucosal surface. The areas of these bands are computed and the area of damage is expressed as a proportion of the total area of the gastric glandular mucosa. A white disc placed into the field of view of the camera, serves to calibrate the grey levels, and this allows the severity of damage to be calculated.

Validation of the computation of areas has been carried out using uniformly coloured discs of paper superimposed on a white background. A highly significant correlation (by the method of Pearson) was obtained ($r=0.99$, $n=15$) between the measured and calculated areas. The severity of ethanol-induced gastric mucosal damage was validated by computing the areas of damage from rats treated with 0, 0.5, 1.0 or 2.0 ml p.o. absolute ethanol. These areas were compared with a mean of macroscopic 'blind' subjective scores obtained by two observers. The Spearman rank correlation coefficient between the subjectively scored and computed areas was $r=0.93$ ($p<0.001$, $n=21$).

The gastric mucosal protective effects of PGE_2 (0.1mg/kg p.o., pre-dosed 0.5h prior to 1ml absolute ethanol p.o.) were studied in the rat. By macroscopic 'blind' subjective scoring, PGE_2 reduced the ethanol-induced mucosal damage by 71% ($p<0.002$ Mann-Whitney 'U' test). Quantification of the same mucosal damage revealed a reduction of damage by 76% ($p<0.001$ Mann-Whitney 'U' test) compared with controls. The Spearman rank correlation coefficient of this data was $r=0.87$ ($p<0.001$, $n=18$).

These results demonstrate that this 'on-line' quantitative method is capable of producing results that are similar to subjective scores, but with the advantage of objectivity. The method can also detect the activity of drugs which reduce ethanol-induced gastric mucosal damage in the rat.

Hollander, D. et al (1982) J.Lab.Clin.Med, 100 (2), 296
Witt, C. et al (1985) J.Pharm.Methods. 13, 109
Szabo, S. et al (1985) J.Pharm.Methods. 13, 59

A MICROCOMPUTER SYSTEM TO MEASURE FOREARM BLOOD FLOW AND FINGER TREMOR IN MAN

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Plethysmography using a mercury in silastic strain gauge has been widely used to measure the changes in peripheral blood flow caused by drugs. The measurement of finger tremor has also been used to assess drug effects in man. In each case the output from a transducer which converts a physical change into an electrical signal is used to quantify effect. In the past a major difficulty of these techniques has been the analysis of the signal produced. We have developed a system to overcome these difficulties using an Apple IIe microcomputer fitted with a data acquisition board (McAllister et al., 1983), dual disk drives and Epson FX-80 printer.

For plethysmography the software controls a compression system for inflating and deflating the cuffs, simultaneously storing and displaying the amplified signal from the strain gauge in eight second epochs, calculating the least squares regression line of the initial increase in forearm circumference following venous occlusion, averaging the slopes of ten measurements and providing a hard copy of the traces (Figure 1). Software is also available to measure venous capacitance.

For tremor the amplified output from a piezoelectric accelerometer attached to the dorsum of the middle phalanx of the middle finger is sampled in eight, eight second epochs. This output is subjected to fast Fourier analysis. A copy of the average power frequency curve (Figure 2) and the tremor power with standard deviation in each frequency band is printed.

Figure 1

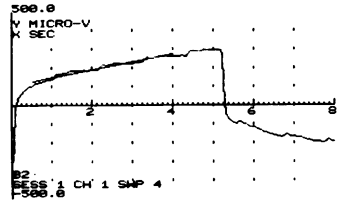
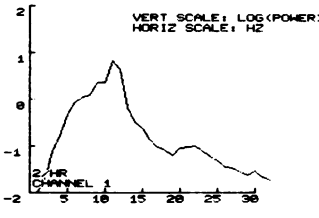


Figure 2



Programs are also available to use the microcomputer to record auditory, visual or somatosensory evoked responses and electronystagmography. The software could also be developed to acquire and analyse similar signals from other physiological transducers.

McAllister, H.G., Armstrong, G.A., McClelland, R.J. & Linggard, R. (1983) Br. J. Audiol., 17, 275-277.