HUMAN REFLEX BRONCHOCONSTRICTION AS AN ADJUNCT TO CONJUNCTIVAL SENSITIVITY IN DEFINING THE THRESHOLD LIMIT VALUES OF IRRITANT GASES AND VAPOURS.

BY

ROBERT BARRIE DOUGLAS, B.Sc., M.Tech.

TUC Centenary Institute of Occupational Health
London School of Hygiene and Tropical Medicine.

A thesis submitted for the
Degree of Doctor of Philosophy in the
University of London.

1974

When you can measure what you are speaking of and express it in numbers you know that on which you are discoursing. But when you cannot measure it and express it in numbers, your knowledge is of a very meagre and unsatisfactory kind.

LORD KELVIN

ABSTRACT

The thesis describes studies on acute exposures of human lungs and eyes to irritant gases and vapours and the physiological response of these organs. The bronchoactivity of the gases and vapours after inhalation was measured objectively by constant volume whole body plethysmograph. The threshold sensitivity of the eyes was assessed subjectively by exposure inside tight fitting goggles.

Considerable effort was devoted to the development of methods for the production of known, low concentrations of gases and vapours in air. Sulphur dioxide and ammonia were prepared by static dilution in a Douglas bag. Vapours were produced by continuous generation of vapour at constant temperature and subsequent dilution.

Mean dose response curves were obtained for sulphur dioxide and ammonia inhalation by randomised double blind Latin square exposures using twelve subjects and four concentrations for each gas.

Examples of other irritants were chosen so as to illustrate the effect of straight and branched chains (ketones), double bonds (aldehydes) and of increasing molecular weight in homologous series. A chlorinated hydrocarbon (trichloroethylene) was also included.

The effect of increasing molecular weight from acetone to the pentanones was an increase in irritancy (acetone and methyl ethyl ketone are not irritant). The branched chain pentanone (3-methyl-butan-2-one) was less irritant than the two straight chain isomers. Moving the oxygen atom from the 3- to the 2- position also increased irritancy.

Formaldehyde was found to be more irritant than acetaldehyde and acrolein more irritant than either of these; this may be attributed to the presence of the unsaturated double bond in acrolein.

A correlation was found between the threshold of irritancy as measured at the eye and the threshold of bronchoactivity. Further, the evidence suggests that reflex bronchoconstriction may be present at concentrations below those necessary for irritancy at the eye.

The implications of these findings are discussed with reference to the establishment of Threshold Limit Values for industrial exposures. However, although these experimental findings on volunteers were, it is believed, adequate to support the above conclusion further observations, especially near the threshold level would be necessary before they are used as an adjunct to other physiological responses in establishing the TLVs.

A chapter on miscellaneous studies examines the bronchoactivity of methoxyflurane (Penthrane) an anaesthetic and obstetric analgesic. The results indicate that it is a bronchodilator in normal adults. This chapter also includes a comparison of residual volume obtained by body plethysmography and by the method of helium dilution.

CONTENTS

CHAPTER 1.

INTRODUCTION.

Much evidence has been adduced for and against the existence of physiological thresholds. This however is a different question than deciding on a Threshold Limit Value (TLV) which is a hygiene standard. Such a standard is continuously under review and may be adjusted in the light of reports of adverse effects and the numbers of such cases.

The soundest way to establish a value is by taking a large body of existing data on levels of exposure and number of years together with health records. It is then possible to state with precision the probability of disease associated with a given exposure (Roach, 1970). Even then the decision has to be made as to what is an acceptable risk of disease.

In the absence of existing data, arrival at a TLV is necessarily a much more iterative process.

Threshold limit values refer to airborne concentrations of substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed day after day without adverse effect. Because of wide variation in individual susceptibility, however, a small percentage of workers may experience discomfort from some substances at concentrations at or below the threshold limit, a smaller percentage may be affected more seriously by aggravation of a pre-existing condition or by development of an occupational illness.

Threshold limit values refer to time-weighted concentrations for a 7 or 8-hour workday and 40-hour workweek. They should be used as guides in the control of health hazards and should not be used as fine lines between safe and dangerous concentrations. (Exceptions are the substances listed in Appendices).

Threshold limits are based on the best available information from industrial experience, from experimental human and animal studies, and, when possible, from a combination of the three. The basis on which the values are established may differ from substance to substance; protection against impairment of health may be a guiding factor for some, whereas reasonable freedom from irritation, narcosis, nuisance or other forms of stress may form the basis for others.

Threshold Limit Values for 1973
Department of Employment. Technical Data Note 2/73

It is advantageous if an initial value can be chosen based only on acute exposures thereby providing minimum risk and inconvenience to the subjects together with minimum difficulty in obtaining the necessary volunteers. The whole body plethysmograph is a very sensitive instrument for measuring broncho-constriction caused by inhalation of irritant gases and vapours. Approximately half of the substances listed by the American Conference of Governmental Industrial Hygienists have a TLV which is set on grounds of irritancy: often irritancy at the eyes. Thus the work described in this thesis is pursuant to the question "can the whole body plethysmograph provide objective quantitative measures as an adjunct to subjective assessments of irritancy at the eye for setting TLVs ?"

The rest of this chapter is devoted to a discussion of the chemical sense as described by Moncrief (1967) and Keele (1964) followed by a brief discussion of lung vagal reflexes and finally by an examination of some methods of preparing known, low concentrations of gases and vapours in air.

Chapter 2 describes the methods and Chapter 3 describes the development of methods. Chapter 4 describes the sulphur dioxide experiments and Chapter 5 the ammonia experiments.

Chapters 6 and 7 deal with the ketone results and aldehyde results respectively. Chapter 8 contains a discussion of the results and Chapter 9 contains the results of miscellaneous studies. Chapter 10 is an examination of some sources of error. The appendix contains a printout of the individual results of the sulphur dioxide, ammonia and trilene results. The ammonia and trilene results were used as secondary data in Chapter 10 .

1.1. THE COMMON CHEMICAL SENSE.

Man is equipped with five classically recognised senses: sight, hearing, touch, taste and smell. The first three are physically excited whereas the last two, taste and smell, are stimulated by chemical agencies and are known as the chemical senses (Moncrief 1967). However, in addition to these two chemical senses there is a third chemical sense. This may be aroused by the action of irritants on exposed mucous membranes such as those of the mouth and nasal cavities, the eye, the respiratory tract, and anus and reproductive openings. Parker (1922) has called this the "Common Chemical Sense" . It is the most primitive of the chemical senses from which the more specialized senses of taste and smell have evolved. Whereas these differentiations cater for the provision of nutrition, the common chemical sense serves to promote rejection of, or withdrawal from,

noxious chemicals in the immediate environment.

Chemical sensibility occurs even in the simplest living organisms. The unicellular protozoa, such as the amoeba or paramecium react appropriately to nutrient materials and to chemical irritants. However, they apparently possess no localized receptors (Keele, 1964) nor have they nerve elements to subserve these responses. Indeed the whole body is susceptible to stimulation. If a paramecium is cut into pieces, each separate piece responds to chemicals such as 1% sodium chloride solution or 0.05% sulphuric acid with the same withdrawal movements seen in whole animals (Autrum, 1959). The sea-anemones contract in response to chemical irritation, the jelly-fishes, the flat worms and the earthworms all show a negative tropism to chemicals and get away from them as fast as possible. The legs of the lobster are covered with tens of thousands of sensory bristles, many of them chemically sensitive.

In Diptera (flies) each labellar hair is supplied by three neurones, one of which ends near the base of the hair and responds to mechanical stimuli; the other two run through to the tip of the hair and their receptors respond to chemical stimuli. Of the two chemoreceptors one is specifically stimulated by sugar which evokes small electrical potentials in the corresponding neurone and promotes a positive feeding

response i.e. extension of the proboscis and imbibition of liquid. The "non-sugar" receptor is stimulated by salts, acids, alcohols and many other irritants which evoke large electrical potentials in its neurone and a negative or rejection reaction by the fly (Hodgson, 1955; Dethier, 1955; Beidler, 1961).

In fishes the chemical sense is important to the survival of the creature and is distributed over the whole body surface. It is mediated via the fifth cranial (trigeminal) nerve. This is in marked contrast to the organs of olfaction and gustation. Olfaction is confined to the olfactory pits and is mediated by the olfactory nerve, whereas taste is widely distributed over the flanks, barbels and pectoral fins as well as the mouth, and is mediated by the seventh nerve.

In amphibia the taste buds are confined to the mouth and pharynx but the common chemical sense is distributed over the whole surface of the body. As in fishes it is mediated by receptors belonging to free endings of spinal and cranial (fifth) nerves.

In man and other mammals, whose skin is horny or covered with hair, chemical sensibility of all kinds including the common chemical sense appears to be restricted to defined regions. The taste buds are confined to the mouth, the olfactory epithelium to the nose and the common chemical sense to the mucous membranes.

Evidence for the separate existence of the Common Chemical Sense.

1. Experiments with taste and smell.

Parker (1910) showed that when a piece of meat was held close to the flank of a catfish, (Amiurus), it would turn around and snap at it, since the flank is covered with taste-buds, but that if one of these fishes has the nerves serving the taste organs and the lateral line organs cut, it no longer snaps at the bait. Such a fish is, however, sensitive to sour, saline and alkaline solutions, but not to sweet. As the only receptors left in working order are the free nerve terminals, it must be these which are operative.

In the same way this type of nerve ending is the only one that occurs in parts of the skin of the dogfish, (Mastelus), which is very sensitive to acids and alkalis, less so to salts and bitter substances, and not at all to sugar solution.

Lashley and Sperry (1943) have shown that normal rats readily distinguish between the smells of oil of wintergreen (Methyl salicylate) and bread and milk. If the anterior thalamic nuclei are destroyed they still make the distinction without difficulty, but if the olfactory bulb is removed, their ability to distinguish between the smells is permanently abolished. Therefore this ability depends on olfactory and not on trigeminal stimulation.

In man it is common to refer to the "smell" of raw onion or of ammonia. However, those persons who have suffered destructive lesions of the olfactory apparatus or its nervous connections are quite anosmic to trace smells such as essential oils, perfumes and food flavours yet are still normally cognizant of ammonia, chlorine and other such irritants, resulting from stimulation of fifth nerve endings subserving the common chemical sense. Beidler (1957) has made simultaneous recordings in rabbits of the nerve impulses in multifibre preparations of the non-medullated fibres of the olfactory nerve and the fifth nerve. He found that amyl acetate and other odours, in connentrations which were not objectionable to man, evoked impulses in both olfactory and fifth nerve fibres, thus suggesting that stimulation of the common chemical sense plays a role in the appreciation of odours, even in non-irritant concentrations.

With regard to the sense of taste, a study by Harris (1952) of several hundred patients with fifth nerve lesions led to the conclusion that in most people, taste perception is effected by a blending of fifth nerve sensibility with the primary gustatory function of the chorda tympani and vidian nerves.

Thus as far as smell and taste are concerned the common chemical sense can play a physiological role,

though Keele (1964) points out that excessive stimulation of this sense is painful.

2. Experiments with pain and touch.

Parker (1922) regarded pain and the common chemical sense as distinct sensations. He wrote "The curious feeling that comes from vapours that irritate the eyes, nose or even the mouth, has not the remotest relation to touch, smell or taste and is only distantly related to pain. Pain, however, is easily separated from the common chemical sense by the use of cocaine (Cole, 1910; Crozier, 1916), and we are therefore entirely justified in concluding that the common chemical sense is a true sense with an independent set of receptors and a sensation quality entirely its own". Thus Parker differentiated between the common chemical sense and pain on the basis of (i) quality of sensation (ii) sensitivity to cocaine and (iii) the existence of different receptors. To these we may add (ii.a.) the evidence adduced by Jancso (1960) for chemical desensitization.

2 (i) Quality of sensation.

Keele (1964) points out that if Parker is to be interpreted as meaning that the sensations aroused by the actions of irritating vapours on the eyes, nose and mouth are different from those evoked in the skin or mucous membranes by pinprick, no one will disagree,

but this does not necessarily mean that the sensations are fundamentally distinct from one another. Some investigators (Lewis and Hess 1933; Lewis (1942)), decided that the quality of the sensation is determined not so much by the nature of the stimulus as by the duration. For instance a brief electric shock and a quick tug on a single hair were indistinguishable from a needle-prick. However these same stimuli maintained for a period or the application of mustard oil or chloroform to the skin produced pain that was indistinguishable from that produced by prolonged heating of the skin. Keele (1964) found that the application of chemicals to an exposed blister base may produce burning or pricking depending on the chemical.

2 (ii) Sensitivity to cocaine.

Parker (1922) referred to the actions of cocaine in differentiating the common chemical sense from pain. In one case the tail of a lancetfish, (Amphioxus), was subjected to twenty sequential applications of a 0.025 molar solution of nitric acid and then did not respond to further applications of this accid. The chemical receptors were fatigued but not the touch receptors, since the fish reacted when its fatigued tail was touched with a light brush. On another occasion a fish had its tail stroked with a brush thirty times, and then, when it was fatigued to touch and did not respond to further strokes, it still

did respond to stimulation of the skin with weak acid.

In another case Sheldon (1909) treated the surface of a dogfish with 2% cocaine solution and found that responses to touch were abolished within 10-20 minutes, whereas chemical sensitivity remained for much longer, although it too finally disappeared. Cole (1910) immersed the legs of spinal frogs into 1% cocaine solution until there was no response to superficial pricking and scratching with a needle, and to pinching of the skin with forceps. After this there was still a reflex response when the legs were dipped into 3M NH_4CL solution. Crozier (1916) recorded similar findings using ½% cocaine and a dilute solution of formic acid as the chemical irritant.

These findings certainly show that the chemical irritants act on different receptors from those which react to noxious mechanical stimuli, but it is possible that the irritants penetrate further than cocaine and excite deeper sensory nerves than those stimulated mechanically. However, there is another line of evidence which stongly supports the view that chemonociceptors differ from mechanonociceptors in the skin and mucous membranes of rat, guinea-pig and man.

2 (ii.a) <u>Chemical desensitization</u>.

Jancso (1960) has furnished strong evidence that chemonociceptors are susceptible to chemical

desensitization. The chemical desensitizing agent employed was 0.5% alcoholic capsaicin solution which is a pungent principle present in the seed and fruit of various species of capsicum. The fruits are known as chillies or red peppers and when dried and ground they form cayenne pepper which is used as a condiment.

Local desensitization.

Local desensitization was demonstrated in animal and in human skin. When one ear of a rat was treated with 0.5% alcholic capsaicin solution five times at two hourly intervals the initial application caused apparent pain, increased sensitivity to touch, intense hyperaemia and oedema, vigorous scratching of the ear and reflex muscular twitching. The treated ear became 2.6°C warmer than the untreated ear. With subsequent treatments, pain, hyperaemia and temperature rise decreased and were completely absent after the fifth treatment. If twentyfour hours later both ears were treated with the capsaicin analogue vanillillye - n - decoylamide the desensitized ear failed to respond though the normal ear became hyperaemic and painful.

In human facial skin a similar reaction occurred. When 0.5% alcoholic capsaicin solution was applied to the freshly shaved cheek on one side, the initial application caused intense burning pain and bright red hyperaemia with obvious oedema of the

skin. The treated side became 3.8°C warmer than the untreated side. With repeated applications to the same side the response progressively diminished until after the eleventh application there was no reaction to capsaicin. Jancso (1960) also noted that the side desensitized to capsaicin showed no reaction to a ten percent solution of ammonia which caused burning pain and hyperaemia on the normal cheek. The desensitization lasted for at least twentyfour hours. The desensitized facial skin retained its normal sensitivity to touch, slight tickling and needle pricking thus indicating "that capsaicin eliminated selectively the chemical pain stimuli".

General desensitization.

Jancso (1958) and Jancso and Jancso - Gabor (1959) have shown that repeated parenteral administration of capsaicin to rats and guinea pigs can induce a generalized desensitization, the pain receptors throughout the body becoming insensitive to chemical but not to physical stimuli. This induced refractory state may last for months or even for the lifetime of the animal.

Desensitized guinea-pigs showed no signs of irritation even in a strong mist of ammonia or chloracetophenone solution which caused blepharospasm, intense lacrimation and violent scratching of the

nose in normal animals. Instillation of formalin, nicotine, veratrine, allyl alcohol solution or hypertonic saline solution into the eye caused no lacrimation or chemosis such as occur with these substances in normal animals. Smearing the nose with mustard oil or formic acid produced no scratching, lacrimation or sneezing in desensitized animals. Even dousing the nose with concentrated capsaicin solution failed to elicit sneezing whereas normal guineapigs after such treatment sneezed 10-20 times consecutively. There were no defensive reflex responses after rubbing the skin with xylene, mustard oil or chloracetophone solution. In contrast, corneal and sneezing reflexes could be easily elicited by tactile stimuli and the thresholdfor pain aroused by pinching, pricking, heat or an electric current was unaltered.

It has been observed, however, (Jancso 1960) that other chemical agents can still produce inflamatory reactions in desensitized or denervated organs to the same degree as in normal organs. The histamine liberator, compound 48/80 , 5 - HT , (hydroxy tryptamine), dextran and egg albumin come in this category and Jancso has attributed their independence of innervation to the fact that they do not stimulate nerve-endings concerned with pain. However, Keele (1964)

has pointed ou. that this is not true for compound 48/80 which evokes both pain and itch and that 5 - HT also produces pain in human skin.

Desensitization of eyes.

Jancso, Jancso - Gabor and Takets (1961) have shown that aplication of nicotine tartrate or ACh,(actylcholene), (2.5%) to the eye of a rat or guineapig produces immediate signs or irritation in the form of blepharospasm, lacrimation and scratching, followed by an inflammatory reaction comprising hyperaemia and oedema of the conjunctiva. All these effects could be prevented by desensitizing the nerve-endings in the following ways:

1. By repeated applications of nicotine or Ach.
2. By local application of ganglion - blocking drugs e.g. hexamethonium.
3. By systemic administration of ganglion - blocking drugs.

However, when the sensory nerve-endings in the eye (or in the nasal mucosa) have been desensitized in one of the above - mentioned ways irritation by substances such as capsaicin, piperine, formalin or chloracetophenone was in no way reduced, and a normal corneal reflex could be elicited by touch.

On the other hand, in an eye which had been desensitized to capsaicin, by local or systemic

administration of this substance (Jancso 1960), the actions of nicotine and Ach were completely prevented, though responses to noxious physical stimuli were unaltered.

2 (iii) Existence of different receptors.

Parker's (1922) claim that the common chemical sense has an independent set of receptors, said to be the free nerve endings of spinal and cranial nerves, is true only in comparison with the specialized receptors for the chemical senses of taste and smell. Free nerve endings in skin and mucous membranes are now known to be concerned not only with pain and common chemical sense but also with sensations of touch, pressure and temperature.

Application of the chemical sense.

Thus we have a sense which is chemically mediated. It is fortuitous that it does not enjoy the exquisite sensitivity of the sense of smell and the accompanient gross variation between individuals. It is better thought of in comparison with sensibility to heat. Humans find water at 40°C to give little sensation of heat whereas water at 80°C produces quite unacceptable pain. Somewhere in this narrow range lies a threshold which varies only little between individuals. Thus we have tried to use this property to try to

determine a threshold of irritancy for irritant gases and vapours. Keele (1964) suggests that the eye may be the most chemically sensitive organ being three to four times more sensitive than the nose and thus the eyes were selected for exposure. Keele (personal communication) has pointed out that it is not possible to expose each eye to a different gas and make a comparison of irritancies because of the possibility of irritation in one eye producing irritation in the other. The correct strategy is to expose both eyes together administering different concentrations on different occasions. Histologically the conjunctiva contains fewer free nerve endings than the cornea from which sensations of warmth, cold, touch and itch may be elicited as well as irritation (Lele and Weddell (1956)). The cornea contains many free nerve endings served by non-myelinated axons of different diameters (Weddell and Miller (1962) and the terminal arborizations intertwine and overlap.

1.11. LUNG REFLEXES.

Lung reflexes have been reviewed by Widdicombe (1964) and Widdicombe and Sterling (1970). Three vagal afferent systems have been observed which mediate respiratory reflexes in mammals Glogowska and Widdicombe (1973). These are:

1. Pulmonary stretch receptors.

These are slowly adapting myelinated fibres

found in the smooth muscle of the airways extending from the trachea down to the bronchioles which Adrian (1933) concluded were responsible for the Hering-Breuer inflation reflex. In man this reflex is very weak and bilateral vagotomy does not cause slow, deep breathing as observed in other mammals. Experimental evidence by Guz et al (1966); and Guz and Widdicombe (1968) showed that bilateral anesthetization of the vagus nerves caused no change in the pattern of quiet breathing or in end-tidal P_{CO_2}.

Work on animals has shown that increased activity is produced by pulmonary congestion and atelectasis. Also, stimulation of these receptors causes a reflex relaxation of tracheobronchial smooth muscle (Widdicombe and Sterling (1970)). However they are insensitive to pathological changes such as micro-embolism, mild bronchoconstriction and inhalation of irritants and dust.

2. Type - J receptors.

First reported by Paintal (1970) type - J receptors lie in the alveolar wall and have non-myelinated vagal afferent fibres. They are stimulated by microembolism, congestion and oedema and also by inhalation of irritant gases and of halothane. The reflex action is to cause rapid shallow breathing, hypotension and bradycardia.

3.(a). Cough receptors.

The cough receptors are concentrated at the carina and at bronchial bifurcations and decrease in number in smaller bronchi. They are found superficially between the epithelial cells and are relatively insensitive to chemical irritants. However they are very sensitive to mechanical stimulation for example by inhalation of carbon dust (Widdicombe et al (1962)). At low concentration this produces bronchoconstriction but at high concentrations it also elicits coughing.

3.(b). Lung irritant receptors.

These are found in the epithelial layer of the intrapulmonary airways from the trachea to the large bronchioles. They have myelinated fibres in the vagus nerves which produce reflex bronchoconstriction and hyperpnoea. They are more sensitive to chemical than to mechanical irritation. The receptors may be stimulated by inhalation of irritant gases, pulmonary microembolism, cigarette smoke, carbon dust, intravenous histamine and also histamine aerosol. In man they are thought to contribute to the sensation of breathlessness (Sellick and Widdicombe (1971)). They are also stimulated by bronchoconstriction and hyperpnoea thus providing a reinforcing positive feedback which may prolong any response. The bronchoconstriction can be abolished or prevented by isoproterenol, indicating that the effect is

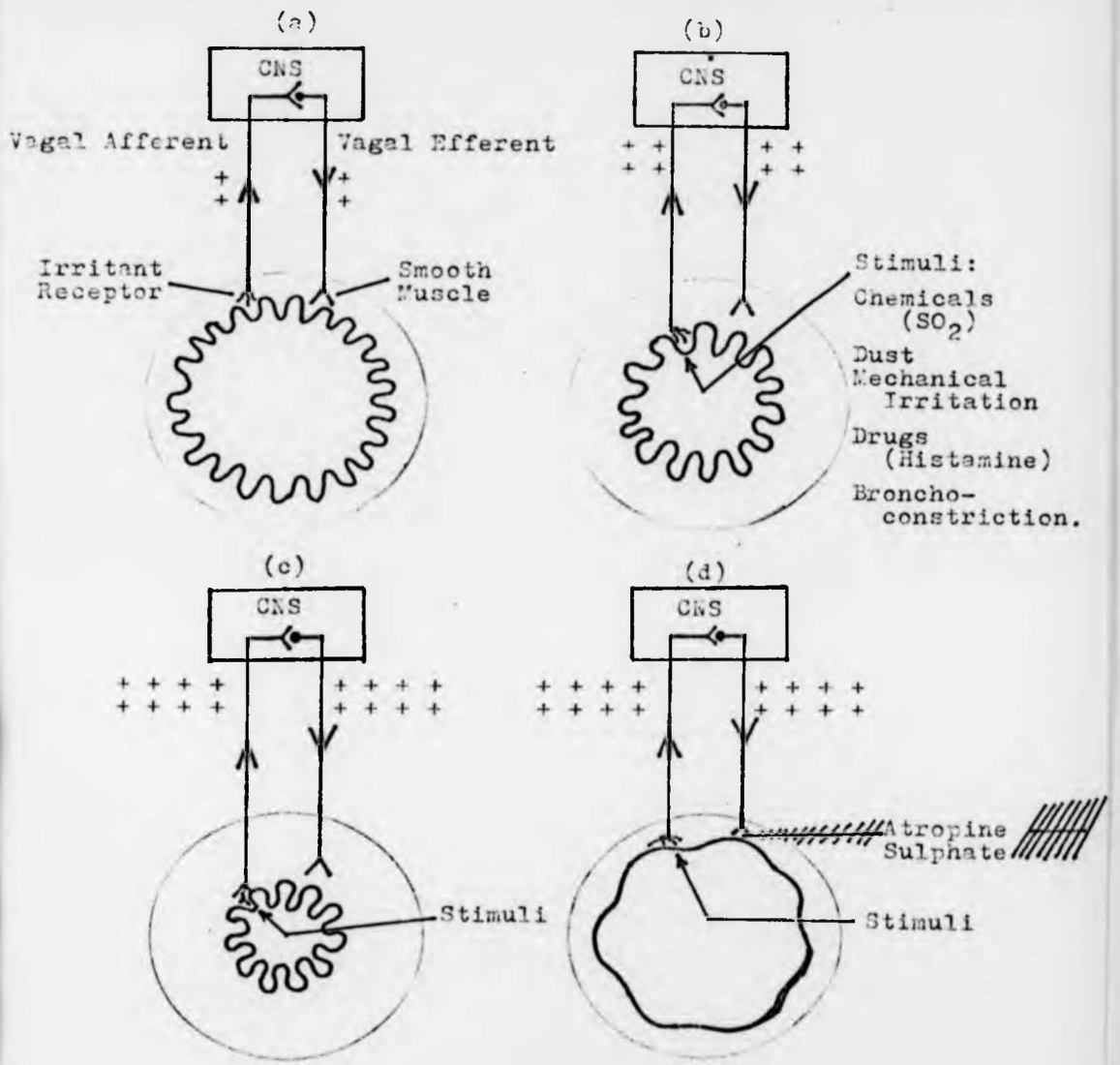


Fig.1.1. Showing the relationship between activity of irritant receptors and tone in airway smooth muscle.
(a) In normal subjects there exists a small degree of bronchomotor tone.
(b) Stimulation of irritant receptors increases vagal activity (++) associated with broncho-constriction (note the inclusion of broncho-constriction in list of stimuli).
(c) More sensitive airways suffer greater bronchoconstriction.
(d) Atropine sulphate blocks postganglionic parasympathetic pathways to airways preventing bronchoconstriction.

due to contraction of airway smooth muscle. Atropine also blocks the bronchoconstriction suggesting that the effect is mediated via postganglionic cholinergic pathways.

Different subjects show greater and lesser sensitivities to stimulation of irritant receptors. Fig. 1.1. after Nadel (1973) depicts the mechanisms by which reflex bronchoconstriction is thought to occur.

1.III. SOME METHODS OF PREPARING KNOWN, LOW CONCENTRATIONS OF GASES AND VAPOURS IN AIR.

1. Static Methods.

One technique is to allow a solution in a thermostatically controlled closed container to come to equilibrium with its vapour. Henry's law states that the partial pressure in the vapour phase is proportional to the concentration in the solution. It is usual to make the volume of the liquid large compared with the vapour space so as to leave the concentration in the solution unchanged. Burnett and Swaboda (1962) employed this method to obtain samples of argon containing known concentrations of ethanol or acetone vapour from aqueous solutions which they used to calibrate an argon ionization detector.

Hill (1961) made up low concentrations by evacuating a steel cylinder, admitting a small quantity

of the required vapour and then filling up with the diluent gas to about a thousand pounds per square inch pressure. However, under these conditions physisorption at the walls reduces the concentration of vapour. For instance an attempt to produce 200ppm of ethanol actually produced 142ppm.

Pate et al (1963) prepared 1ppm of SO_2 in air by adding 0.1ml of SO_2 from a gas tight syringe to 100 litre of air in a Mylar bag. Altshuller et al (1962) record similar techniques and adopt the precaution of filling and emptying the bag several times in order to precondition them. They also conclude that many dry, stable gases and vapours may be stored in this way. The importance of the effect that the presence of water may have on the concentration is stressed by Baker and Doerr (1959).

2. Dynamic Methods.

The "log bottle" technique of Lovelock (1961) refined by Fowliss and Scott (1963) has been employed successfully for producing steadily reducing concentrations of organic vapours but it does rely on the assumption of thorough rapid mixing. Hill and Newell (1965) describe the use of a slow injector built and developed to overcome the limitations of commercially available instruments. Hersch (1969) reviews fourteen different methods of continously generating experimental pollutants in air.

CHAPTER 2

METHODS AND SUBJECTS

Introduction.

This chapter describes the body plethysmograph used for measuring bronchoactivity of irritant gases and vapours, the eye exposure experiments, the methods for producing known low concentrations of gases and vapours in air, the methods of measurement and details of subjects.

Body plethysmograph.

The central instrument for this study is the constant volume whole body plethysmograph due to Dubois et al (1954). (see also Dubois et al 1956(a); 1956(b)). For a subject panting inside an airtight box it is possible to obtain the lung volume and the airways resistance and this technique has been described by previous workers in this department (Pelzer, A.M. 1965 ; Kamburoff, P.L. 1969).

Theory.

As part of the manoeuvre the subject pants against a closed shutter producing complementary

compressions and rarefactions of the fixed masses of gas in the lungs and in the box. The compressions and rarefactions of the gas in the lung are isothermal and Boyle's Law is applicable.

$$PV = \text{const.} \qquad \ldots\ldots\ldots(1)$$

Differentiating with respect to pressure:

$$P \frac{dV}{dP} + V = 0 \qquad \ldots\ldots\ldots(2)$$

$$\text{Thus } V_{TG} = \frac{(P_{atm} - 47)}{10} \; 13.6 \; \frac{\Delta V}{\Delta P} \qquad \ldots\ldots\ldots(3)$$

where V_{TG} = Thoracic gas volume in litres.

P_{atm} = Barometric pressure (mm Hg).

47 = SVp of water at 37°C.

ΔV = Change in box volume or lung volume measured plethysmographically.

ΔP = Change in alveolar pressure measured at mouth during panting against closed shutter (at zero flow alveolar pressure = mouth pressure).

Airways resistance is defined as

$$RAW = \frac{\text{driving pressure}}{\text{flow}}$$

from (3) above

$$P_{alv} = \frac{(P_{atm} - 47) \times 13.6 \times \Delta V}{10 \times V_{TG}}$$

The flow is measured with a Fleisch pneumotachograph.

Equipment.

In practice R_{AW} and V_{TG} are calculated by combining two measurements from the oscilloscope screen. Initially flow is displayed on the vertical axis against box pressure on the horizontal axis. A characteristic shaped loop is obtained similar to a hysterisis loop (Fig. 2.1.). A rotating transparent disc scribed with a number of parallel chords is located in front of the screen. The disc is rotated to align the parallel chords with the inspiratory portion of the loop at a point 10mm below the centre of the screen. At this point the flow is about 0.5 l sec^{-1} and it is fair to assume a linear relationship between pressure and flow. The angle of chords to the vertical (α) is noted from a scale on the perimeter.

A shutter is then closed at end expiration which occludes the mouthpiece while the subject continues to pant. The variations in lung pressure are now displayed on the vertical axis against box pressure on the horizontal axis, producing a straight line (Fig 2. 2.). The angle of inclination to the vertical (β) is noted.

SHUTTER OPEN

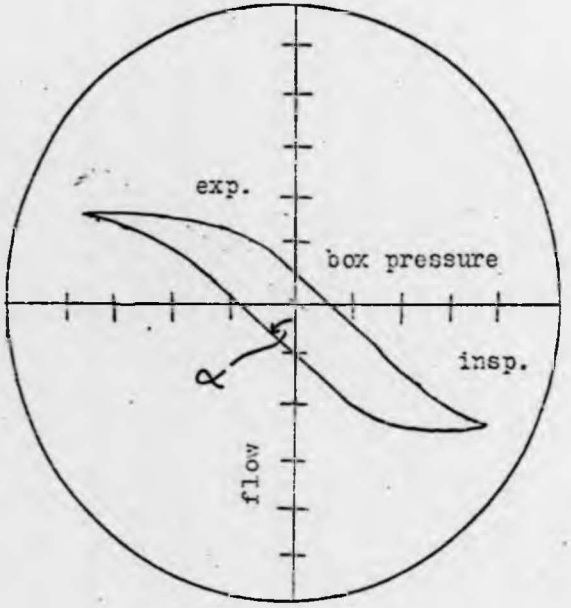


Fig.2.1.

SHUTTER CLOSED

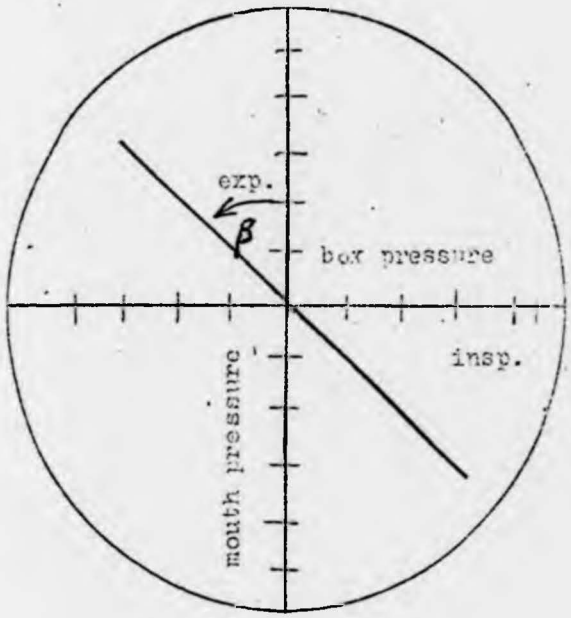


Fig.2.2.

Figs.2.1 and 2.2. SHOWING APPEARANCE OF OSCILLOSCOPE SCREEN.

The equations used are:-

$$R_{AW} = \frac{\cot\beta}{\cot\alpha} \times \frac{M}{F} - R_{eq}$$

M = mouth calibration factor.

F = flow calibration factor.

R_{eq} = resistance of pneumotachograph.

$$V_{TG} = \frac{(P_{atm} - 47) \cdot BVF \cdot BCF}{10 \cdot 2 \cdot \cot\beta}$$

BVF = Body volume factor (to allow for box volume reduction due to subject).

BCF = Box calibration factor.

The box has a volume of 600 litres and the Fleisch pneumotachograph carries a heated screen to prevent condensation and to minimise the difference in temperature between inspired and expired air (Dubois et al, 1956.). It also has sufficient dead space to permit a certain amount of rebreathing; this is because the expired air is richer in CO_2 and the inspired air is richer in oxygen. The oscilloscope screen also carries two horizontal lines at 3cm. above and below the median diameter. The box and mouth pressures are each measured by strain gauge transducer. The presence of the subject in the box warms the air and causes a drift in box pressure until thermal

equilibrium is achieved. The pressure is restored to atmosphere by momentarily opening a shutter in the side of the box. Cumming (1961) and Lloyd and Wright (1963) did not use a shutter but simply drilled a hole in the plethysmograph.

Procedure.

The subject*is seated in the plethysmograph with head held upright and wearing a nose clip (Fig.2.3). The procedure is explained to the subject who practices with the door off. The subject executes a shallow panting manouevre in time to a metronome confining the oscilloscope loop between the two lines mentioned above. The cheeks are held against the side of the teeth to prevent movement during the period of panting against the closed shutter. It is most important that the subject does not leak around the mouthpiece. An advantage of the panting manouevre is that it abducts the vocal chords reducing the component due to the resistance of the larynx (Pride, 1970). The observer notes the values of α and β and using the formulae given above is able to compute R_{AW} and V_{TG}. The reciprocal of resistance is known as conductance (G_{AW}). It is found that there is a sigmoid relationship between G_{AW} and V_{TG} which is approximately

---

*Smokers refrain for one hour prior to test.

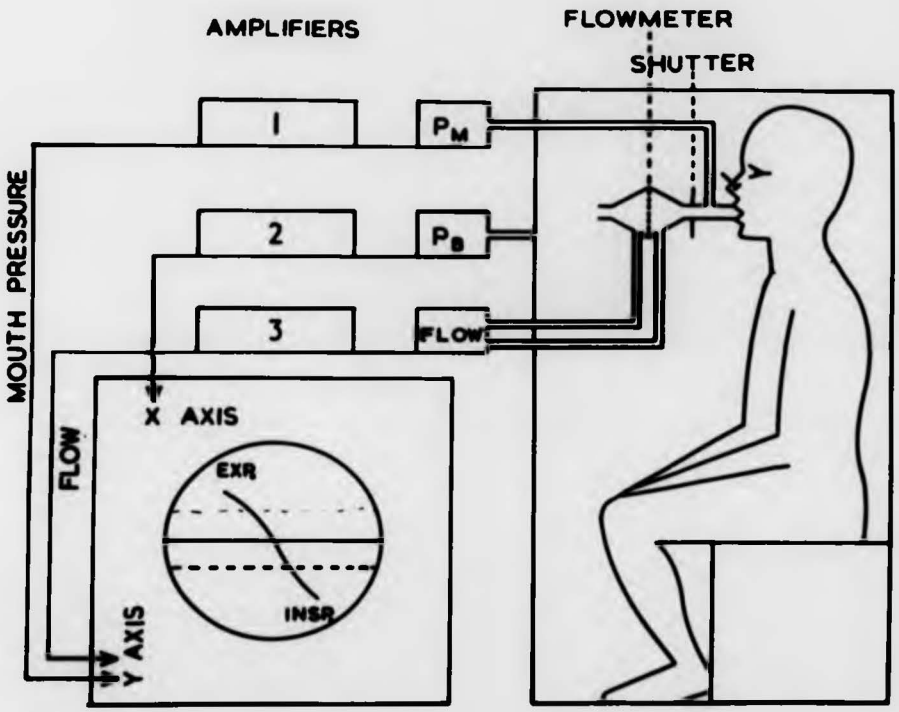


Fig. 2.3. Constant volume whole body plethysmograph.

linear over the middle range. The slope of this line (G_{AW} / V_{Tu}) is called specific conductance (SG_{AW}) and is taken as the most stable measure of conductance (Pelzer and Thomson, 1969).

Calibrations.

1. Box Calibration Factor.

The box pressure was calibrated by a dynamic method. A metal bellows pumped air sinusoidally into and out of the box and the excursion of the oscilloscope spot on each range noted for each volume setting. Typical values obtained are shown in Fig. 2.4.

The volume pumped was set by adjusting the position of the bellows linkage arm in a slot on the electric motor driven wheel. The volume was estimated by disconnecting the wheel from the electric motor and the bellows from the box. The bellows was then connected by rubber tubing to a 100 ml. glass syringe with a water manometer measuring the pressure in the tubing. The wheel was then turned slowly by hand to expel the air from the bellows. At the same time a second person withdrew the plunger from the syringe so as to maintain the pressure in the connecting tube

linear over the middle range. The slope of this line (G_{AW} / V_{TG}) is called specific conductance (SG_{AW}) and is taken as the most stable measure of conductance (Pelzer and Thomson,. 1969).

Calibrations.

1. Box Calibration Factor.

The box pressure was calibrated by a dynamic method. A metal bellows pumped air sinusoidally into and out of the box and the excursion of the oscilloscope spot on each range noted for each volume setting. Typical values obtained are shown in Fig. 2.4.

The volume pumped was set by adjusting the position of the bellows linkage arm in a slot on the electric motor driven wheel. The volume was estimated by disconnecting the wheel from the electric motor and the bellows from the box. The bellows was then connected by rubber tubing to a 100 ml. glass syringe with a water manometer measuring the pressure in the tubing. The wheel was then turned slowly by hand to expel the air from the bellows. At the same time a second person withdrew the plunger from the syringe so as to maintain the pressure in the connecting tube

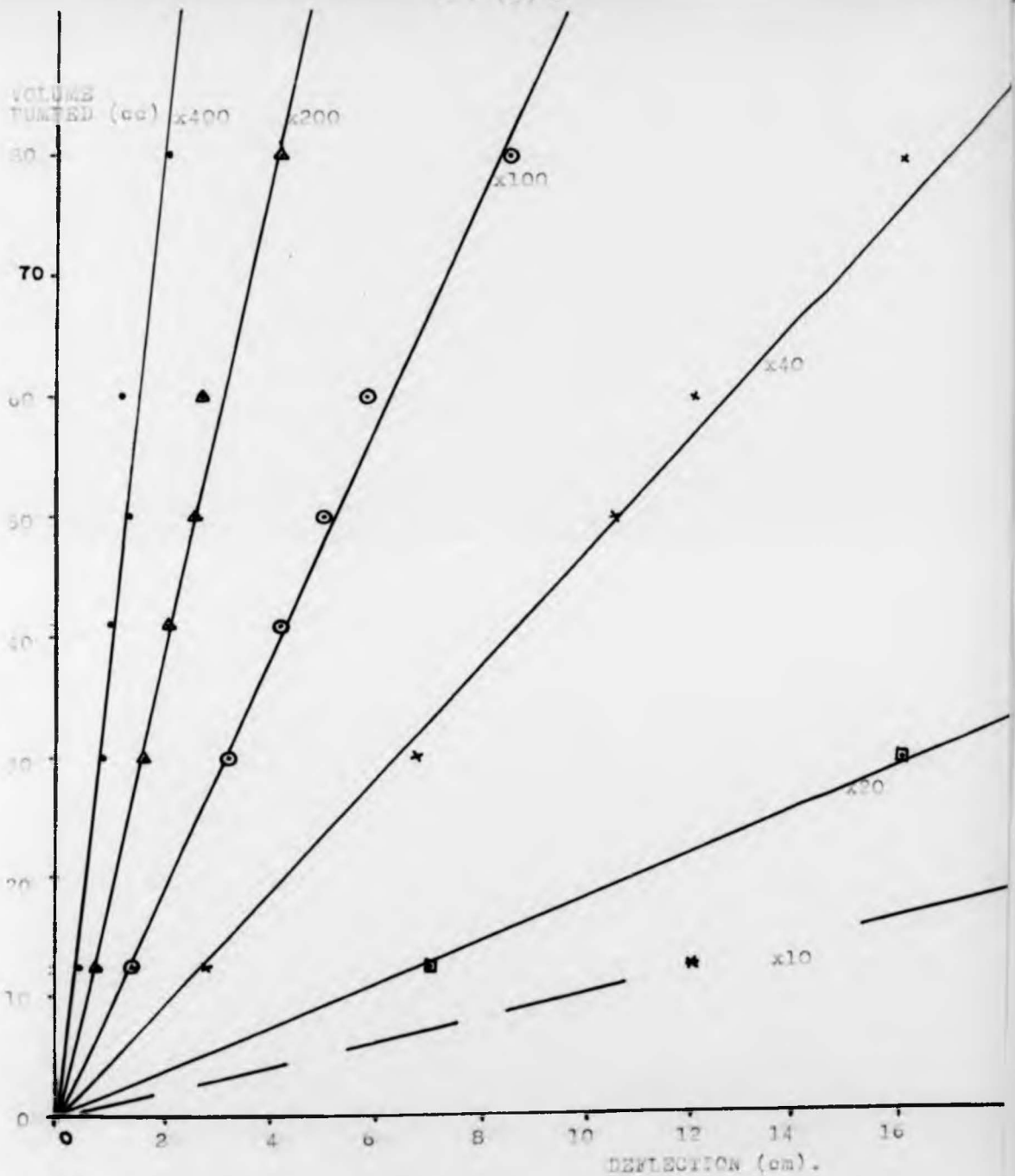


Fig.2.4. Box Calibration Curves.

at atmospheric pressure as indicated by the water manometer.

The box calibration has been investigated further as described in chapter 10.

2. Flow Calibration Factor.

The flow calibration factor (that flow required to produce unit excursion (cms.) on the oscilloscope screen) was determined by passing air serially through the pneumotachograph and a rotometer (0.200 l min^{-1}) and noting the corresponding deflection on the oscilloscope for each range. Typical values are shown plotted in Fig. 2.5.

3. Mouth Calibration Factor.

The mouth calibration factor (that pressure required to produce unit excursion on the oscilloscope screen) was determined by connecting the transducer to a water manometer and noting the deflection on each range for different pressures. Typical values are shown in Fig. 2.6.

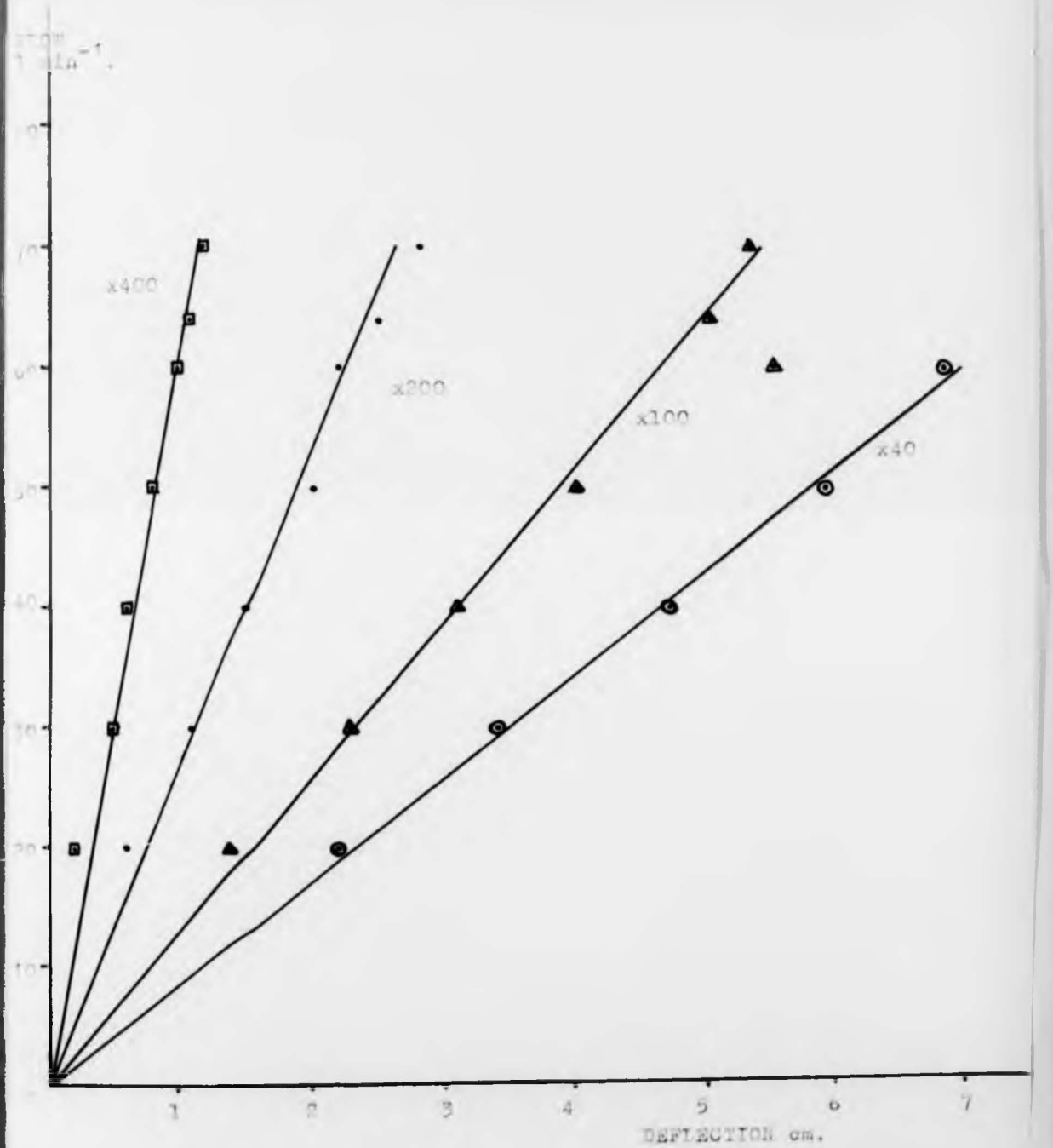


Fig.2.5. Flow Calibration Curves.

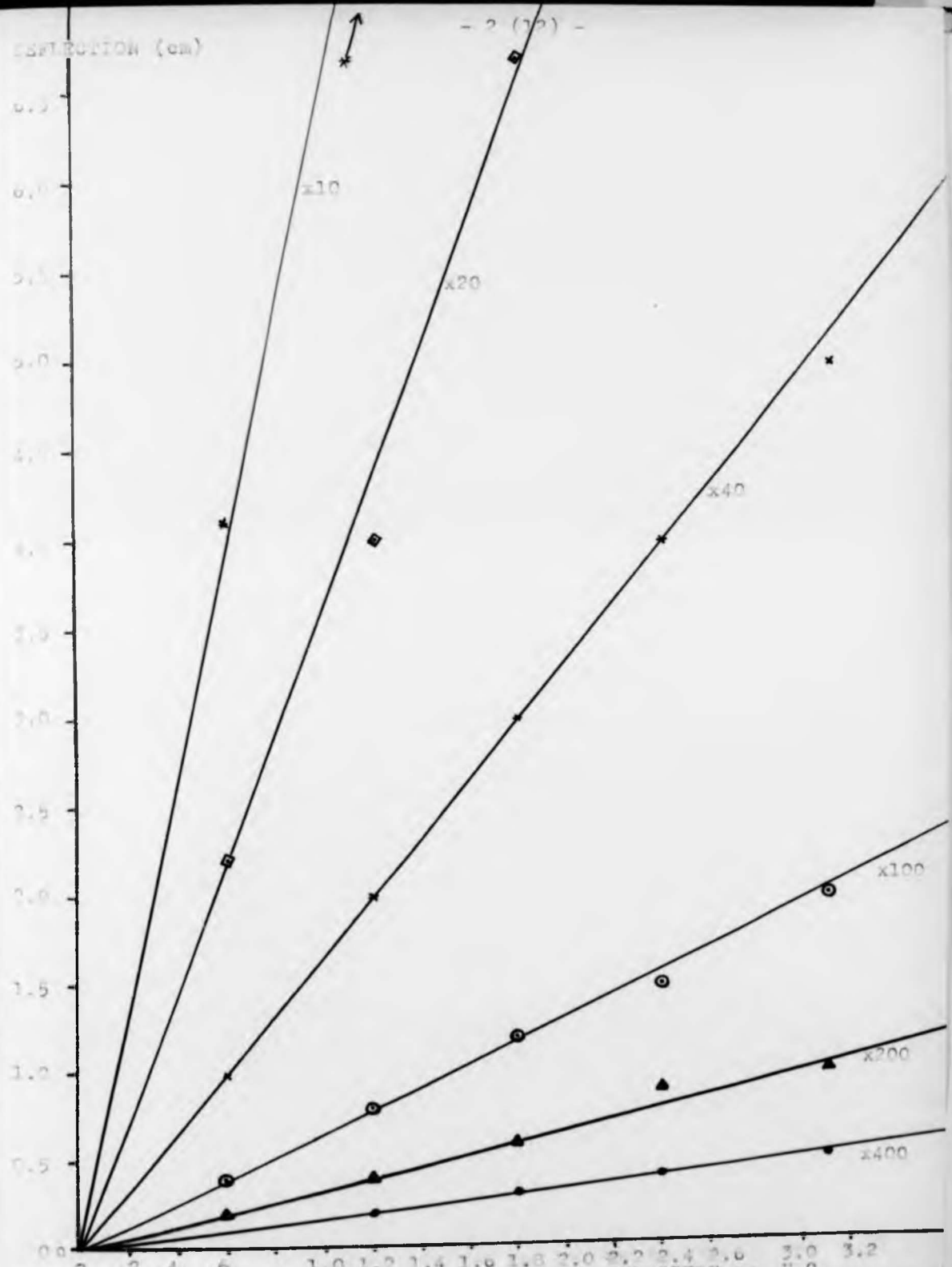


Fig.2.6. Mouth Pressure Calibration Curves.

EYE EXPERIMENTS.

An apparatus was designed and constructed to expose the eyes exclusively to known concentrations of gasses and vapours (Fig. 2.9.). This included a proprietary pair of goggles modified by the addition of polypropylene connecting tubes. The goggles received the vapour or gas via stainless steel tubing and are voided to the outside of the building.

The vapours and gases supplied to the goggles were produced in the same way as those for the innalation studies using the same equipment. The delivery system of that equipment (Fig. 2.10) contained provision for on-line monitoring of dose concentration by a dual flame ionisation gas chromatograph (Perkin Elmer FIII). Continuous supply to the goggles (and/or the chromatograph) was maintained by an oil free oscillatory pump drawing vapour from the production unit via 1/8" o.d. stainless steel tubing and passing it to a resettable needle valve (Edwards type OSID). From here the stainless steel tubing was constrained to adopt the form of two shallow helixes each in a separate plane providing two degrees of freedom of movement to the end of the stainless steel tubing. Thus the subject was able to move his head easily whilst wearing the goggles. The mild steel inlet and outlet ports of the pump have been adapted by the

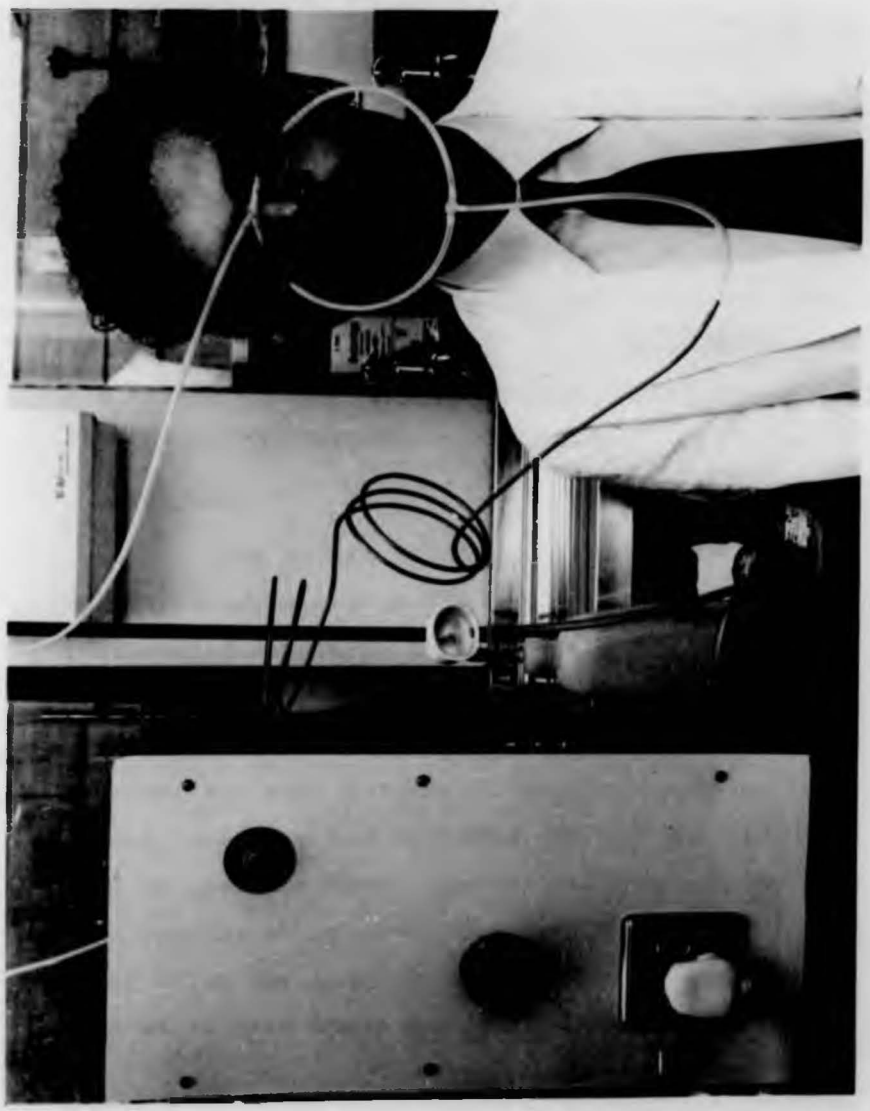


Fig. 2.7. Apparatus for exposure of eyes.

addition of ¼" o.d.,copper tubing silver soldered to the ports. Connection of the copper tubing to the stainless steel is by Swagelock compression couplings (brass with copper olives). Two Swagelock "T"-pieces were inserted in the line between the pump and the needle valve. The first one to incorporate a manually operated bypass valve in the system which voided to the outside of the building. The second was of the nature of a flow splitter delivering a continuous supply to the gas sampling loop of the gas chromatograph.

Procedure.

The nature of the experiment and procedure was first explained to the subject. The subject was then seated wearing a nose clip and the goggles which were retained by an adjustable rubber band. The subject closed his eyes and the pump was switched on by the observer who also started a stopclock. After twenty seconds the pump was switched off and the subject told to open his eyes. He was informed of the time from opening every five seconds for fifteen seconds, after which the goggles were removed. The observer then asked "was there any irritation?".

The fifteen second period was chosen to standardise the exposure for each subject. This was

a uniform brief exposure rather than a time exposure. The object here was to ascertain the acute effect and not the effect of long exposures. We feel this to have been justified on several counts. Initially our experience of manufacturing low concentrations reproducibly for inhalation had led us to developing methods of continuous generation instead of static methods. The same advantages of minimal losses due to sorption appertain equally to the goggles as for the breathing system. However, it is found that a flow of pure air across the open eye can of itself be irritating. Thus the strategy adopted was to fill the goggles by flushing through for twenty seconds with the continuously generated vapour. During this period the eyes were closed, the eyelids serving a dual role. Firstly to protect the chemical sensors in the eye during filling of the goggles. Secondly to avoid presenting the interior of the goggles with a wet surface during this period. It would seem likely that when the flow is switched off and the eyes opened, the concentration in the goggles would fall as a function of time, due mainly to sorption in the eye. Finally concentrating on the acute effect and limiting the time of exposure to fifteen seconds has merit in limiting the toxicity to the subject.

Calibration of needle valve controlling flow through goggles.

It was necessary to ensure that the flow supplied to the goggles was sufficient to wash out the dead space of the system and to make sure that losses of even the most soluble gases and vapours were immediately made good. The needle valve controlling the flow was calibrated over a range of settings with the line to the chromatograph sampling loop alternately open and occluded. A GAP flowmeter was used with interchangeable components to cover the complete range. The flowmeters were marked in units which were interpreted by means of calibration charts supplied with the instrument. Results are shown corrected to litres per minute in Table 2.1. , and presented graphically in Fig. 2.8. The eye experiments were performed with a needle setting of four turns and with the chromatograph line occluded, corresponding to a flow of 2.7 litre min^{-1} .

The opportunity was also taken to examine the reproducability of setting of the needle valve. No error could be detected in re-setting over the range investigated.

TABLE 2.1.

CALIBRATION OF NEEDLE VALVE CONTROLLING FLOW THROUGH GOGGLES.

Needle Setting	GLC line open l min^{-1}	GLC line occluded l min^{-1}	Flow Meter
1 turn	0.32	0.40	A1
1½ "	0.62	0.72	
2 "	0.84	1.08	
3 "	-	2.1	A6
3½ "	1.8	2.5	
4 "	2.1	2.7	
4½ "	2.2	3.0	
5 "	2.3	3.2	
5½ "	2.5	3.3	
6 "	2.6	3.6	
6½ "	2.7	3.6	
7 "	2.7	3.7	

GLC = Gas liquid chromatograph.

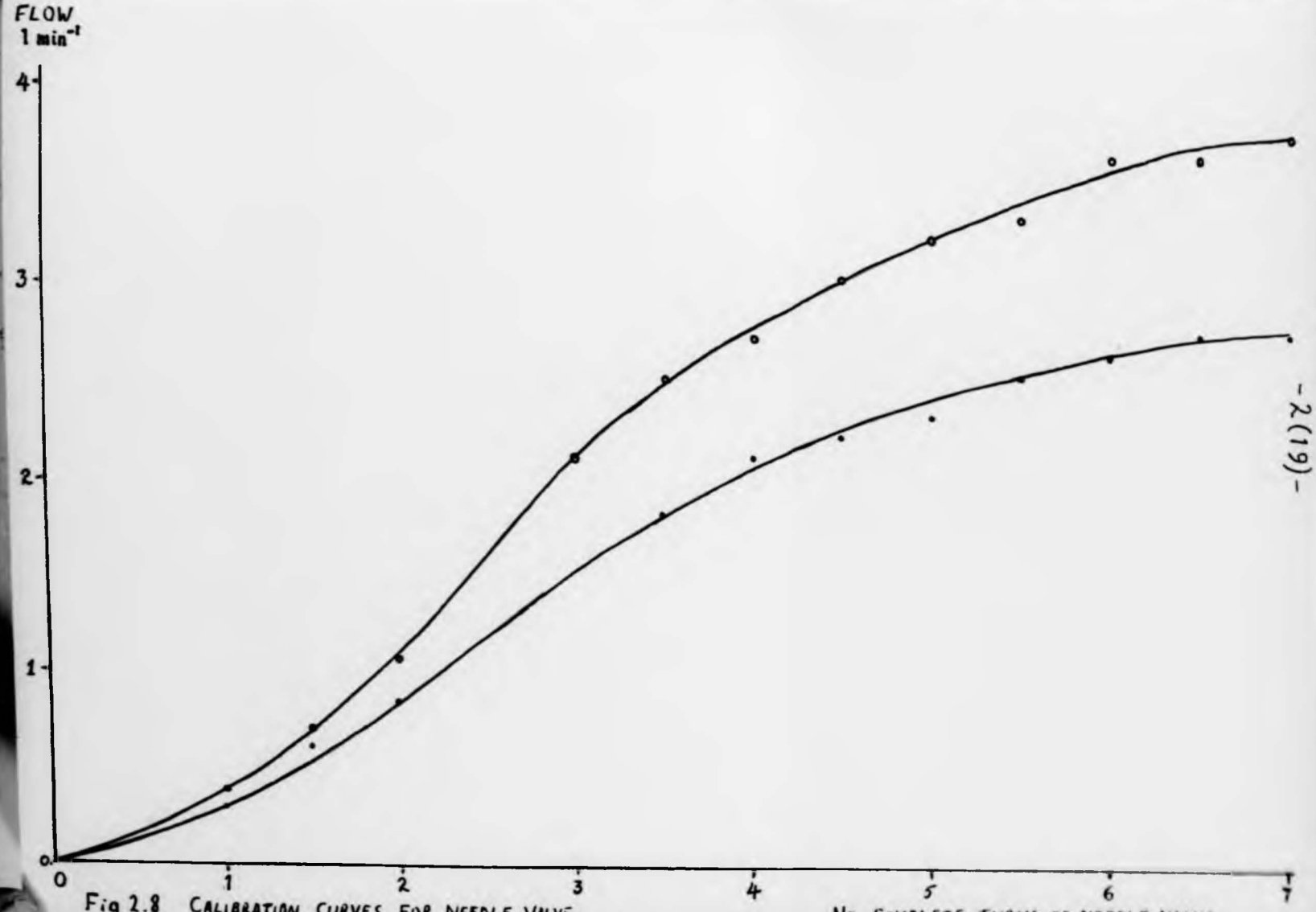


Fig 2.8 CALIBRATION CURVES FOR NEEDLE VALVE.

A SYSTEM FOR CONTINUOUS PRODUCTION OF KNOWN CONCENTRATIONS OF VOLATILE LIQUIDS AS VAPOURS IN AIR FOR INHALATION AND EXPOSURE OF THE CORNEA.

Equipment.

A stabilised airflow from a laboratory compressed air line was divided at a T-piece, the major fraction being diverted via a rotameter to perform subsequent dilution. The minor fraction was bubbled through a dreschel bottle containing the volatile liquid and immersed in a hot bath at fortysix degrees centigrade any aerosol present being removed by a filter. The flow then passed serially through three dreschel bottles (modified as shown in Fig.2.9.) supported in a refrigerated bath at twenty degrees centigrade. These bottles act as condensers from which the emergent stream was considered to be fully saturated at twenty degrees centigrade with the volatile vapour. The vapour humidity was the same as that supplied by the compressor which was ambient. However when the system was rebuilt for use with acrolein a drying tube of silica gel was included for the air going through the bubbler. The saturated stream was diluted by the major fraction (either single or double dilution)to provide the required concentration. It was then admitted to the main breathing tube via an aluminium clad rubber bung. Thorough mixing was achieved by the interposition of two wire mesh baffles. It is most important to ensure that the

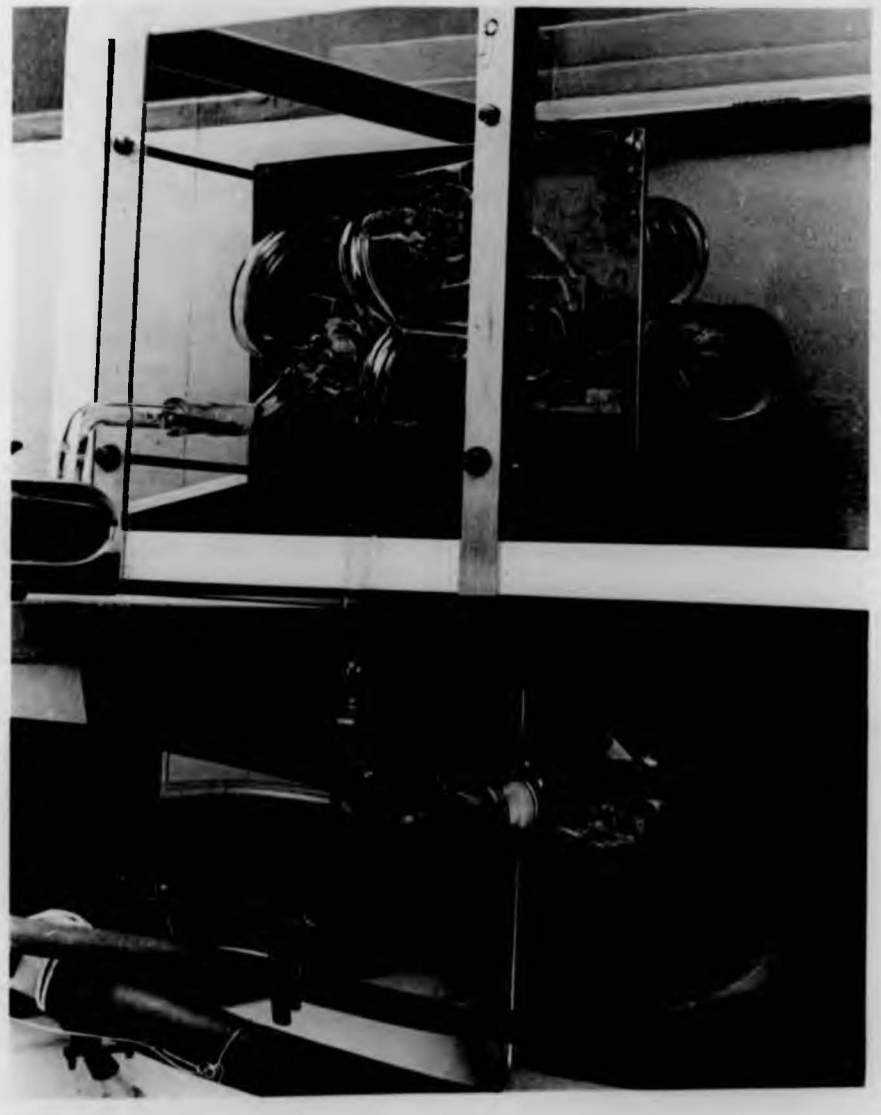


Fig.2.7. Bubbler and condensing chain.

contents of the main breathing tube are as homogeneous as is possible. In the first place human volunteers are invited to inhale from the system and secondly the sampling for the chromatographic estimation of concentration and for the eye exposures is via stainless steel tubing localised at a point in the air stream. The breathing tube was constructed from glass to minimise chemisorption and physisorption at the walls. Fig. 2.10. gives a general view of the system.

Procedure.

Wearing a nose clip the subject inhaled ten breaths of one litre each through the mouth. The volumes were measured by a Wright respirometer (Fig. 2.11.)

MEASUREMENT OF CONCENTRATIONS.

The system for continuous generation described above is an absolute method producing concentrations predictable from a knowledge of the saturated vapour concentration and the dilution ratio. However the concentrations were always checked by Kitagawa gas detector tubes and by gas chromatography (formaldehyde was measured by a colorimetric method).

The chromatograph was employed in conjunction with a standard cylinder of 870ppm acetone. A series of alternate samples of acetone and vapour for analysis

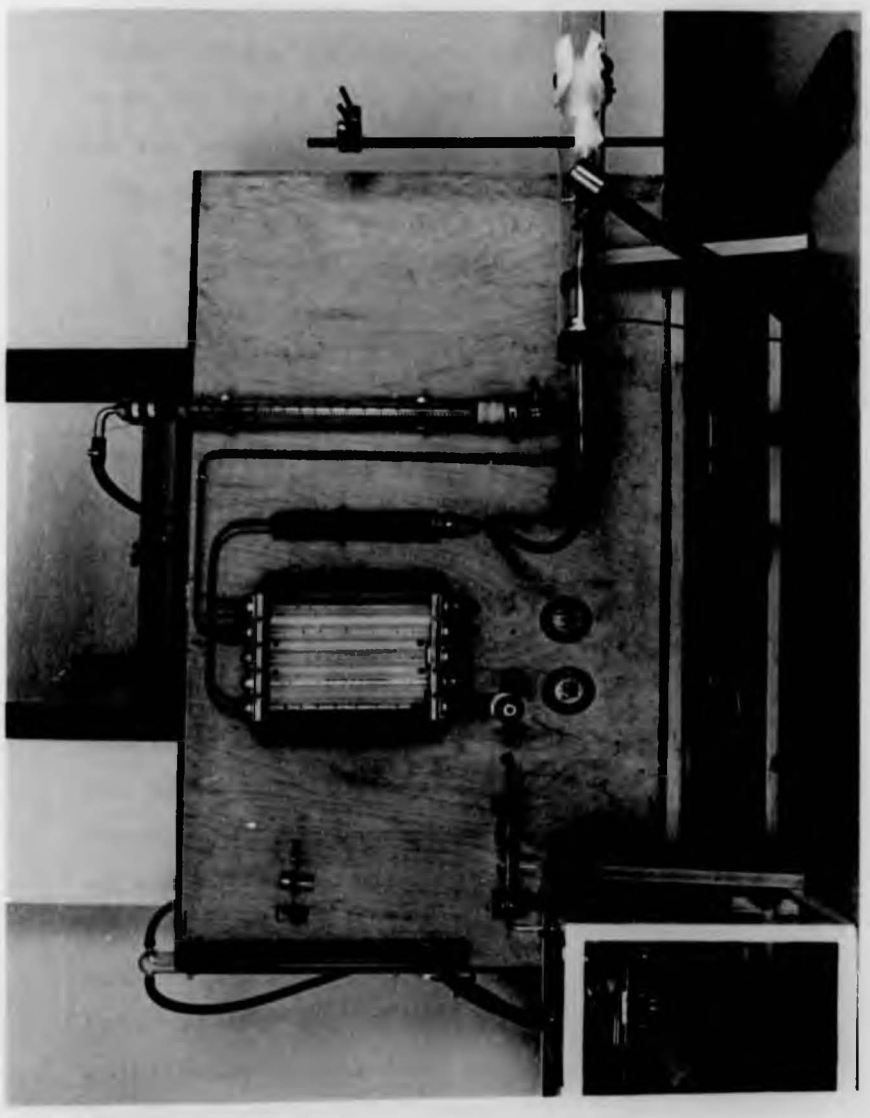


Fig.2.10. General View of Dilution System.

were injected via the gas sampling loop. (The inlet was modified by the addition of brass switching valves silver soldered to the stainless steel inlet.) From the known sensitivity (manufacturers catalogue) it was possible to reconcile the actual concentration with the predicted. Fig.2.12. shows typical chromatogram.

Methods of measurement of formaldehyde.

It was not possible to obtain formaldehyde gas in cylinders. Therefore it was decided to obtain formaldehyde in air by bubbling air through formalin solution in the system described above. However although the dreschel bottle was thermostated and the flow held constant it was not possible to predict the concentration produced in the air. The concentration was measured directly by a method due to Sawicki et al (1961) as modified by Hauser and Commins (1964). A sample of the generated atmosphere was drawn continuously through an impinger containing twenty mls. of 0.05% 3-methyl-2-benzothiazolone hydrazone for a measured time. When the flow was switched off the reagent was allowed to stand for one hour. Then ten mls. were pippeted into a test tube and two mls. of oxidising agent, an aqueous solution containing 1.6% sulphamic acid and 1.0% ferric chloride were added and allowed to stand for a further twelve minutes. During this time the solution was observed to turn blue. At the same

Fig.2.11. Subject inhaling ten breaths of 1 litre each.

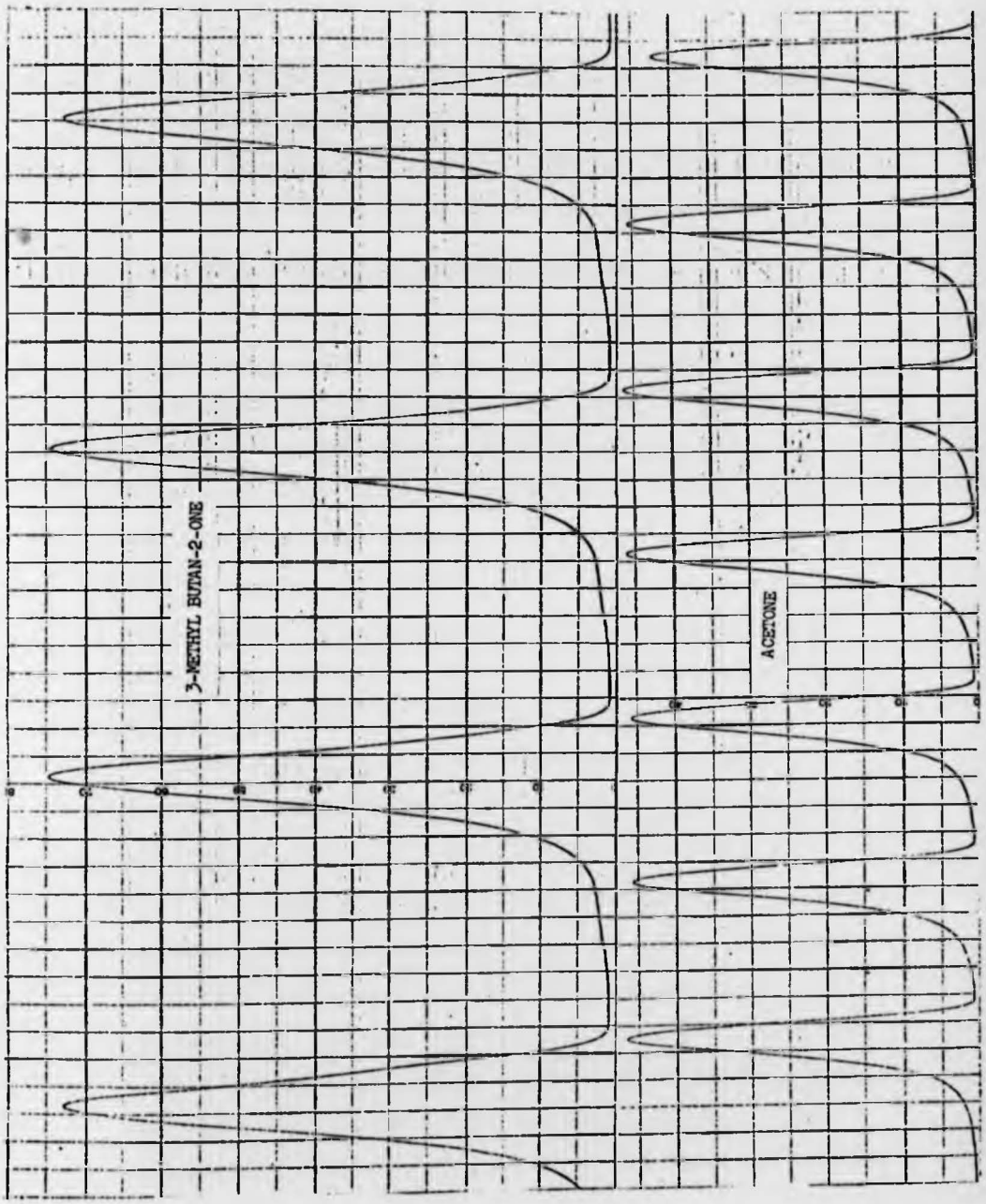


Fig.2.12. TYPICAL CHROMATOGRAMS.

time, a blank was prepared by adding 2ml of reagent (sulphamic acid and ferric chloride) to 10ml of MBTH solution. The sample and the blank were then examined at 628nm in a spectrophotometer.

Calibration of spectrophotometer.

Twenty mls. of 0.05% 3-Methyl-2-benzothiazolone hydrazone are pipetted into each of seven test tubes and stoppered. To each of six of these is added a different quantity of 0.01% formalin solution; the seventh serving as a blank. The amounts added by Eppendorf pipettewere twenty, fifty, one hundred, one hundred and fifty, two hundred and three hundred microlitres. All seven were then allowed to stand for one hour. At the end of this period four mls. of oxidising agent, an aqueous solution containing 1.6% sulphamic acid and 1.0% ferric chloride, were added to each test tube and allowed to stand for a further twelve minutes.

The Beer's Law curve was obtained by two methods. Firstly by balancing the instrument with distilled water in each cell and sequentially introducing the blank and the six standards to the sample cell: care being taken to thoroughly rinse out the sample cell with each new solution. The recorded absorbance of the blank was then subtracted

from each of the values recorded for the standards. The net absorbance for each standard was then plotted against concentration. The second method was to place blank solution into both sample and reference cells. The absorbance due to each standard is then obtained directly.

Beer's Law was obeyed from five to at least one hundred and twentyfive micrograms per hundred mls. of solution. (Fig.2.1a.)

Fig. 3.13. Spectrophotometer calibration chart.

Subjects.

The subjects were healthy volunteers with normal ventilatory indices and were either students or staff of the London School of Hygiene and Tropical Medicine. There were 21 men and 18 women and the median age was 23 years (range 16 - 64). For the men mean forced expiratory volume in one second was 4.19 ± 0.29litre. the mean vital capacity was 4.95 ± 0.30litre, capacity ratio was 84.87 per cent. ± 3.35 per cent. For the women the values were 3.31 ± 0.14 litre, 3.85 ± 0.15litre, and 86.18 ± 1.89 per cent respectively. A further six persons took part in the eye experiments only and of the fortyfive subjects, thirteen were smokers (range 448 - 11,607 pack years).

Many of the subjects had direct research experience and all of them were intelligent. The experiment was explained to them and it was suggested to the subjects that of the doses they were to receive (lungs and eyes) one or more of these might be a blank. It is arguable that suggestible persons might bronchoconstrict on fresh air as is known to

occur in asthmatics who believe they are inhaling an allergen. Against this possibility was the finding that bronchodilatation was occasionally observed in individuals with smaller and even medium doses of SO_2 although the average for all individuals was always bronchoconstriction for all doses administered.

Atopy.

A definition of atopy (Dr. Kevin Carrol pers. comm.) is:

"The propensity of a person to produce reaginic antibody (IgE) to everyday exposures of common allergens in their environment".

It was not practicable to measure the IgE level in the subjects here described and for the purposes of this study, those subjects who responded to one or more of three common allergens (grass pollen, house dust mite and aspergillus) were described as atopic. (see Appendix B). Pepys (1969) states: "The prick test is preferable..... to the intracutaneous test since fewer reactions are

obtained and these show a much better correlation with the clinical findings and a good correlation with inhalation tests".

It is against official policy to do antigen inhalation tests in this school.

Control of Experiments with Air.

In planning the more intensive early trials (e.g. SO_2 and NH_3) the question arose whether to incorporate a blank which is usually advisable in testing subjective responses. Random trials of this nature indicated that suggestible individuals were rare in population examined. It was decided that in all cases a subirritant response would be mandatory. From prior experience the doses chosen usually straddled the threshold but if a positive response was obtained from the calculated sub threshold further lower doses were given, with attention to randomisation, until a sub threshold response was achieved. It was felt that the gain on the positive response side more than compensated for omission of a routine blank.

Timing of inhalation of irritants, entry to box, box temperature.

The subjects inhaled ten one litre breaths at their own speed. A clock was started at the first breath. The time for inhalation showed little variability being about thirty or thirtyfive seconds. The subject was then seated in the plethysmograph and allowed to come to thermal equilibrium. This was determined by observing when the constant drift of the oscilloscope spot had ceased. The pressure in the box was brought back to atmospheric by the operation of a solenoid activated shutter in the wall of the box.

Fig. 2.14. shows the temperature in the plethysmograph plotted as a function of time from a subject (height 193cm, weight 85.7kg,) entering the box, using the data in Table 2.2.

Table 2.2.

time (Mins.)	temperature.(^{o}C)
0	22.2
1	23.6
2	24.1
3	24.0
4	24.2
5	24.0

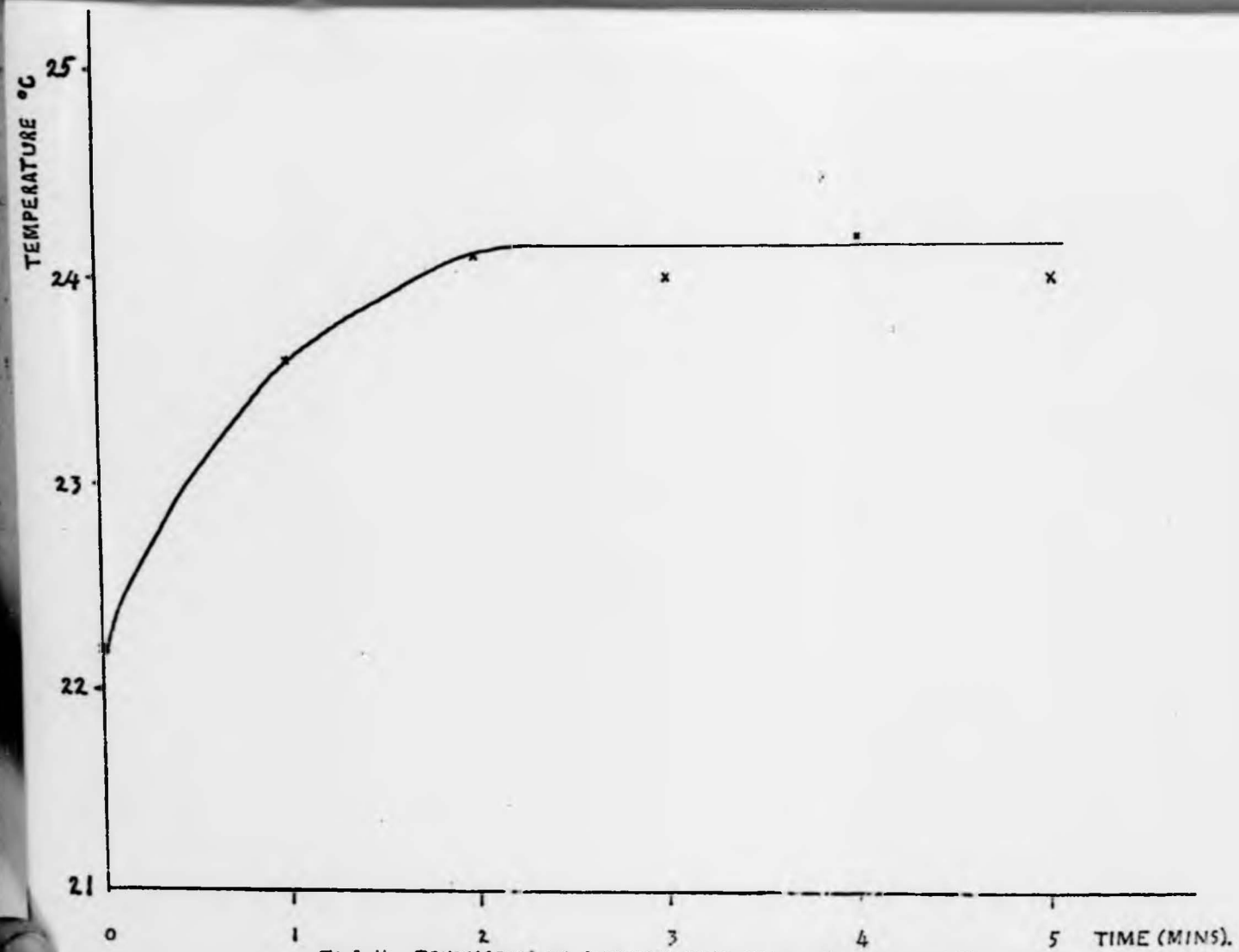


Fig 2.14. TEMPERATURE OF PLETHYSMOGRAPH POST ENTRY OF SUBJECT.

CHAPTER 3

DEVELOPMENT OF METHODS.

The first gas investigated, sulphur dioxide, was undertaken with the production of standard atmospheres in a Douglas bag. The concentration required was produced by known dilution of sulphur dioxide with air and the concentration confirmed by Kitagawa gas detector tubes. Further repeated samples showed that the concentration in the Douglas bag was stable with time. Attempts to repeat this method for ammonia were not successful. The concentration was found to decay exponentially with time and drying of the diluting air only delayed the rate of decay. (Later experiments with sulphur dioxide and ammonia were performed using continuous dilution from cylinders using the developed methods described below).

Following the difficulties with static dilution it was resolved to develop a method of continuous generation. The vapour selected for investigation was trichloroethylene of anaesthetic quality (Trilene) and although it does not appear to have been bronchoactive for an acute exposure it provided valuable experience.

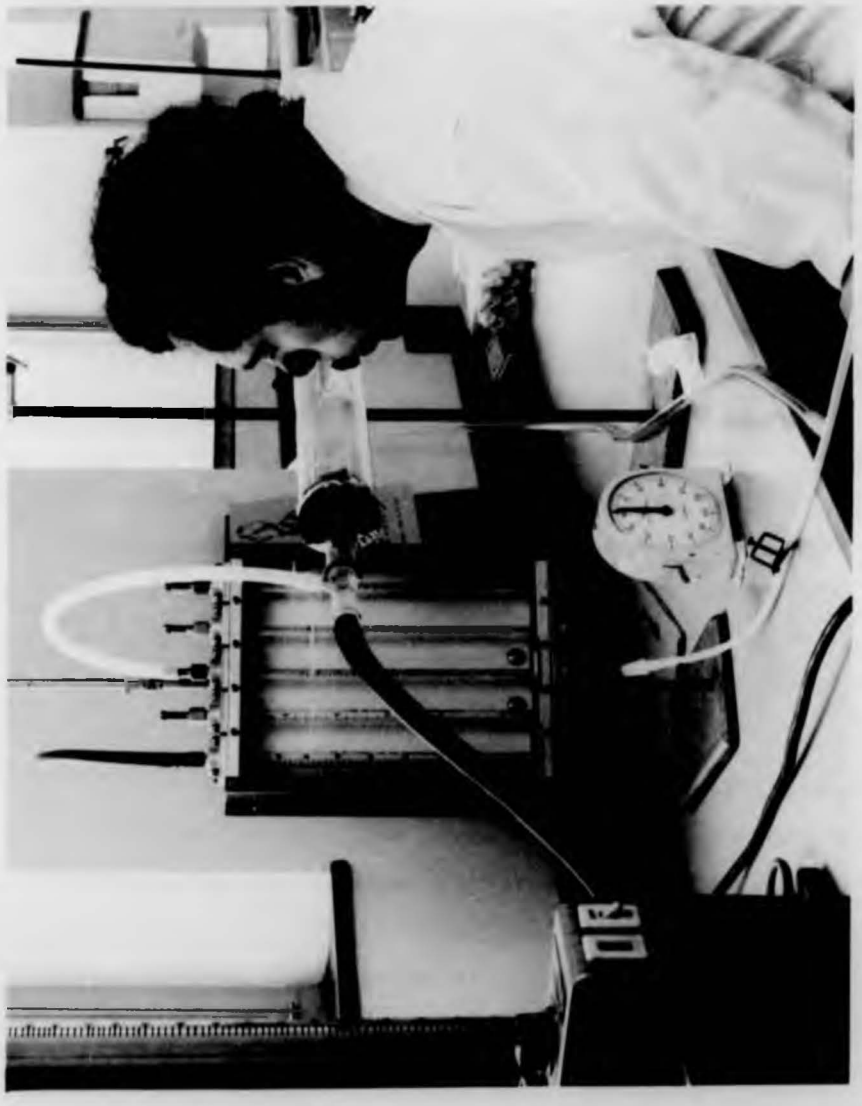


Fig. 3.1. Glass Breathing System.

An apparatus was assembled in which air was bubbled through a dreschel bottle thermostated at 20°C and containing trilene. This stream was diluted to produce a concentration predicted from knowledge of the saturated pressure at 20°C and the subject innaled from a glass breatning system shown in Fig. 3.1. The concentrations were checked by Kitagawa tubes and it became apparent that the concentrations were too low. It was concluded that the air passing through the bubbler was not one hundred per cent saturated (this is supported by the analysis in appendix A). To achieve full saturation the bubbler was raised to 46°C and followed by a chain of condensing bottles in a refrigerated bath at 20°C . Room temperature was 24°C and this obviated the danger of further condensation in the tubing leaving the refrigerated bath.

Fresh estimations of concentration produced values which were too high. This was investigated by passing a thermo-couple into the condensing chain see Fig. 3.2. and checking if the temperature was in fact 20°C . The temperature was indeed 20°C and a solution to the puzzle was proposed by Dr. C.N. Davies who suggested that aerosol formed in the bubbler may be transported along with the saturated vapour and

Fig.3.2. The case-sample investigation.

unable to evaporate until the first dilution after the condensing chain. A duraluminium filter holder containing a disc cut from a Martindale mask filter was interposed between the bubbler and the condensing chain. Magnesium oxide coated slides were held in the vapour stream with and without the filter. Without the filter craters, the result of impact, were observable under the light microscope (size approximately 25 μ). With the filter condensation phenomena only were observable (see Figs.3.3 , 3.4.).

Following the successful completion of the ketones study it was decided to investigate the irritant properties of aldehydes beginning with acrolein. The system described above although rigorously checked for leaks using a water manometer allowed acrolein to escape into the laboratory. It was suggested by chemists that it was possible for the acrolein (TLV 0.1ppm) to permeate the walls of the plastic connecting tubing. Whatever the reason it was decided to reconstruct the system from glass and stainless steel. It was decided to replace the duraluminium filter holder with an all glass component. The method devised was to turn the rim of a B-34 glass cone in a coal-gas and oxygen flame to orange heat. It was then placed onto an all glass fibre filter

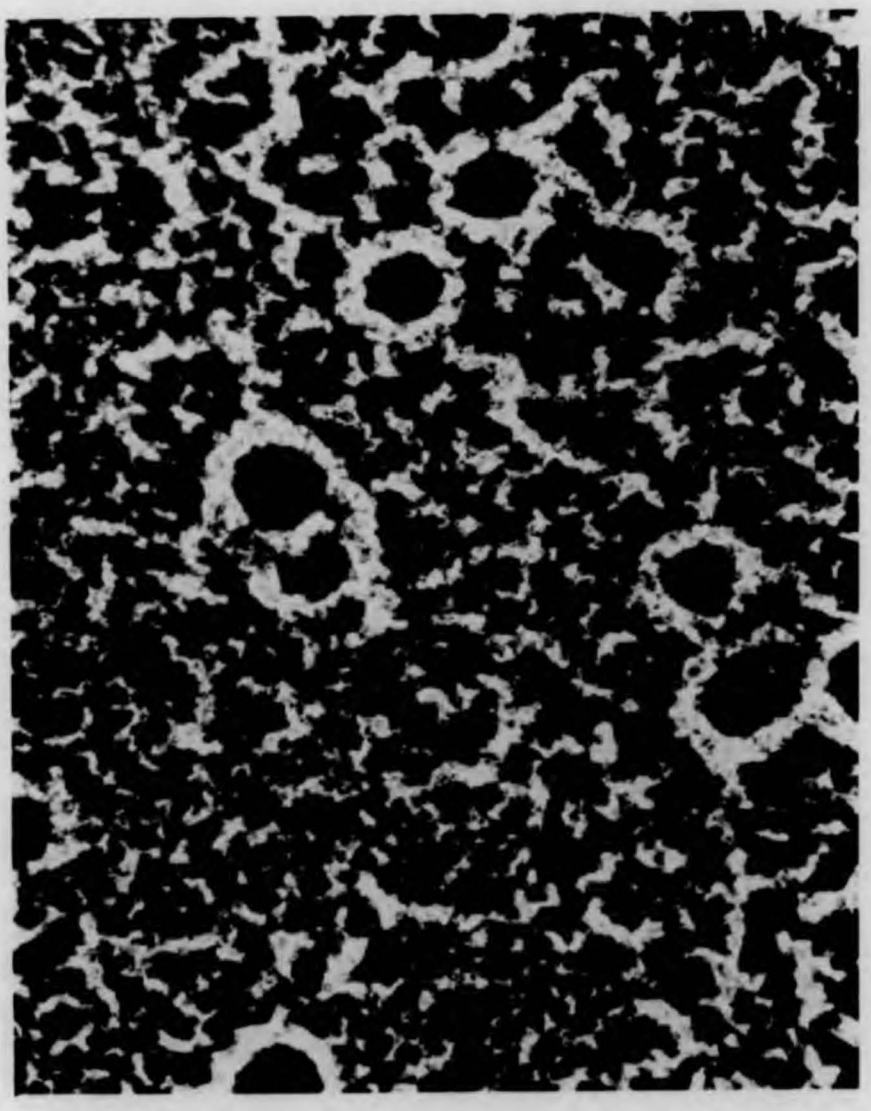


Fig. 3.3. MgO slide without bubbler filter.

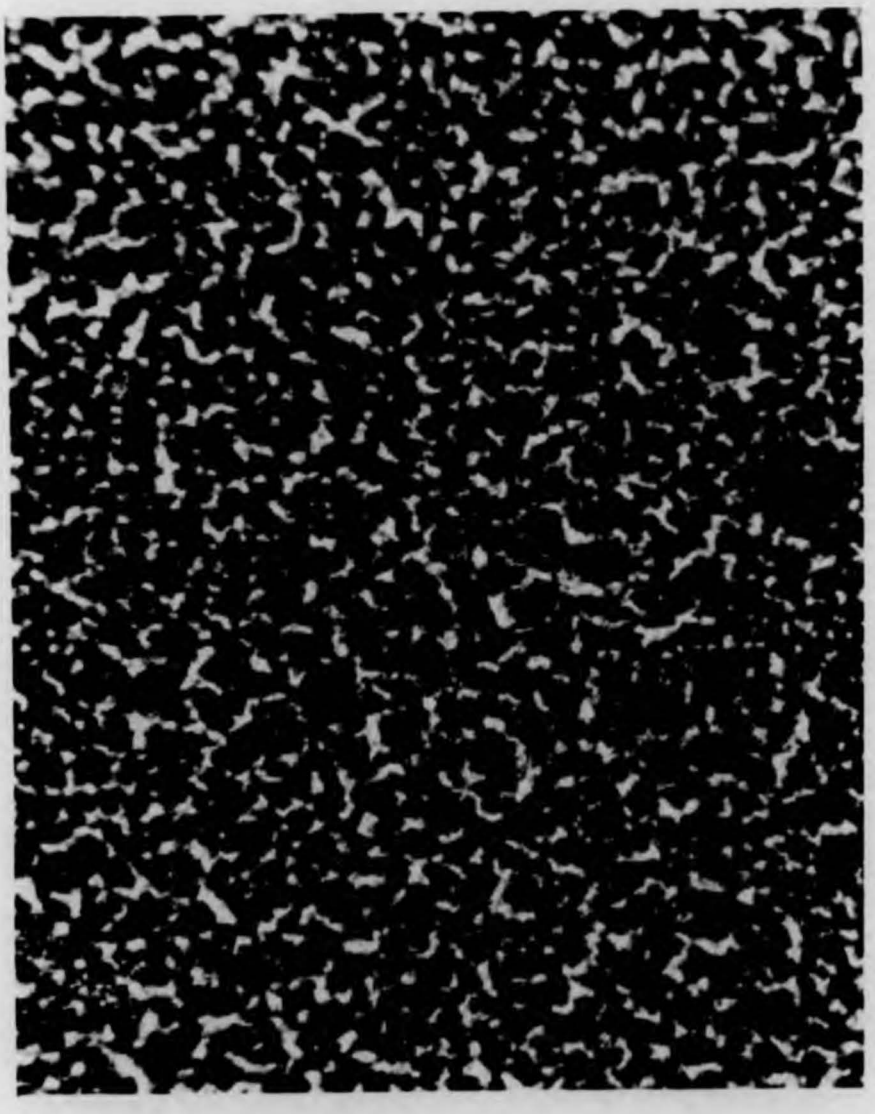


Fig.3.4. MgO slide with bubbler filter.

paper and with a rolling action a disc was cut out and sealed across the cone (Fig. 3.5.). Pressure testing revealed the seal to be stronger than the paper itself. The assembled apparatus is shown in Fig. 3.6.

In the trilene experiment the Mark I goggles (Fig. 3.7.) were used successfully and suggestions were made for improvement. These were incorporated in the Mark II goggles shown in Fig. 3.8 . Subjects had commented that a "slight coolness" could be felt on the closed eyelids during filling of the goggles. Others had commented that one eye felt cool and not the other and on occasion that one eye suffered more irritation than the other. Thus the supply of vapour to the goggles was restructured into a parallel configuration supplying each goggle at the same time. The effluent from the goggles was voided via a tube from the bridge of the nose. The coolness experienced by the inflowing vapour impinging on the eyelid was obviated by cementing deflector tubes onto the inlet points inside the goggles. Cut obliquely from plastic tubing, these deflected the stream onto the front window of the goggles.

Fig. 3.5. Showing fibre-glass filter sealed to cone.

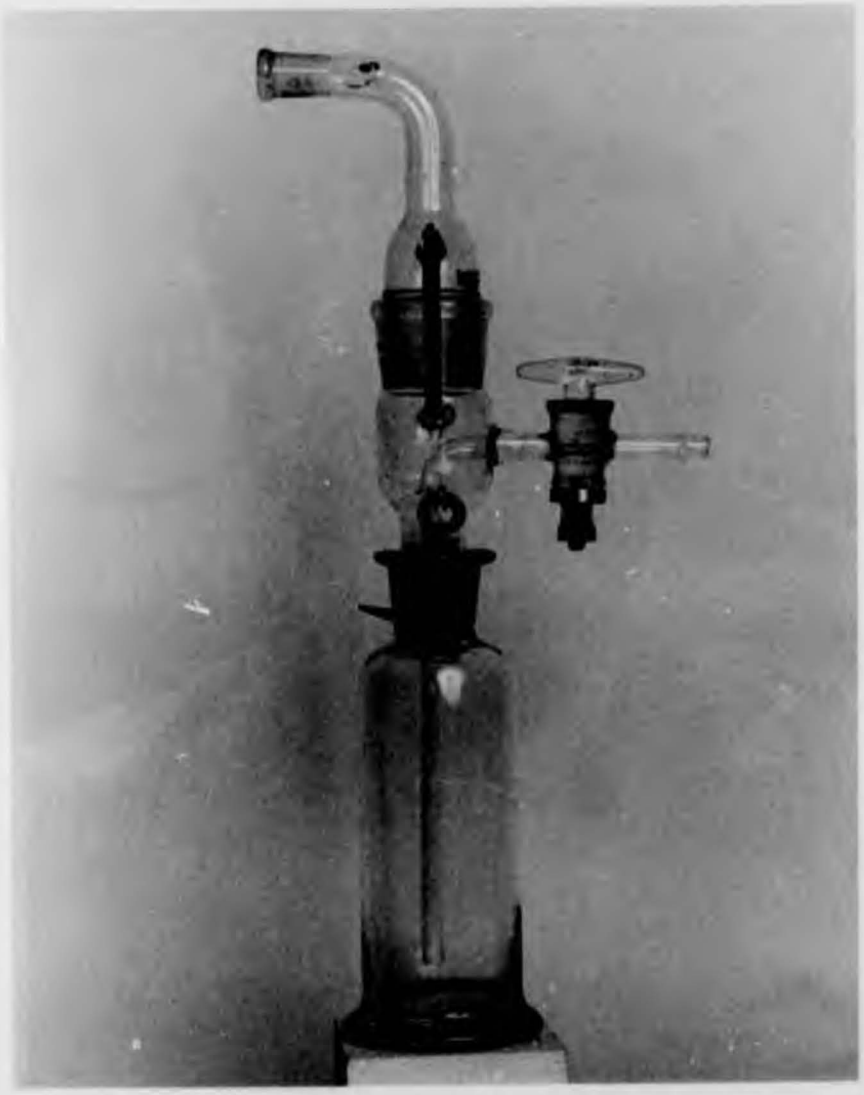


Fig.3.6. Bubbler and filter assembled.

Fig.3.7. Mark I goggles.

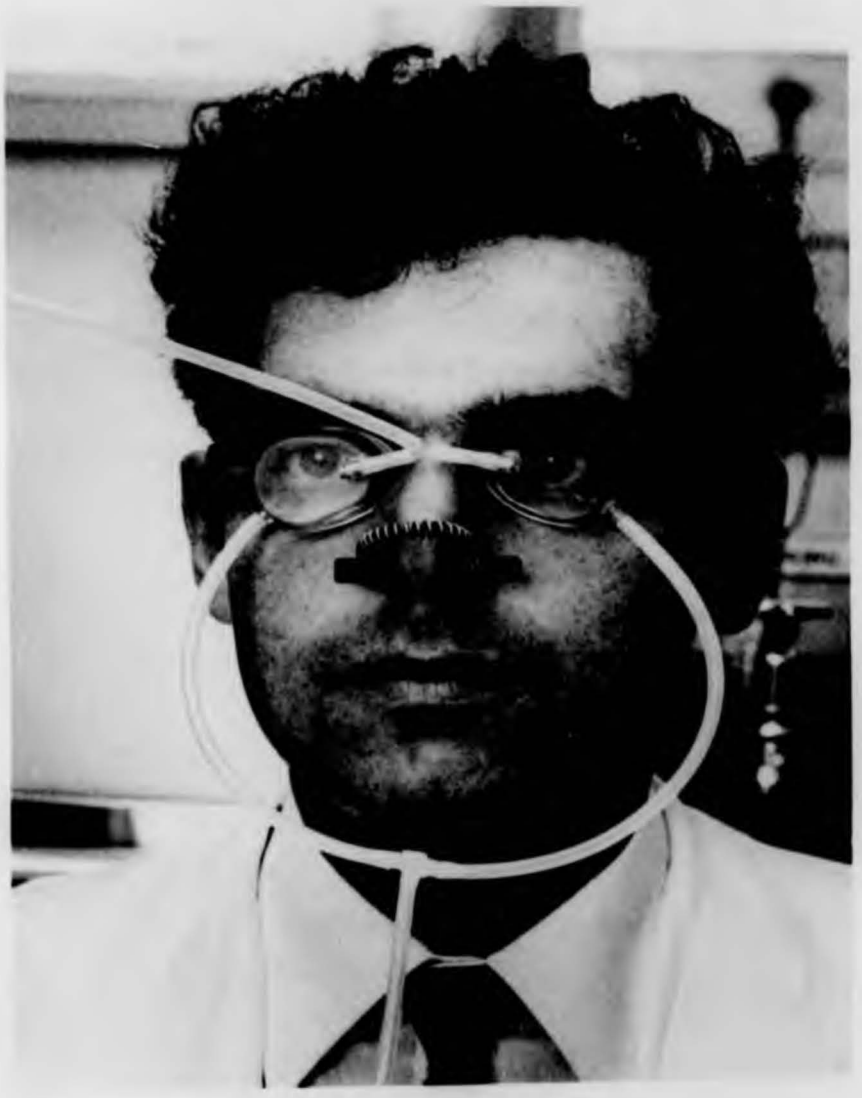


Fig.3.8. Mark II goggles.

Effect of Varying Number of Breaths.

Pursuant to the general policy of obtaining information with minimum exposure of the subject, the effect of taking three breaths of pentan-3-one was compared with the effect of the standard ten breaths.

Results.

At 600ppm the subject chosen produced 38.35% drop in specific conductance with ten breaths of one litre each (total 10.04 litres). The subject commented "Distinctive taste. Slight warming sensation in trachea after 2 mins. Feeling of chest tightness not felt until after approximately 3 mins. Difficult to keep up pace of panting".

Seven days later the same subject took three breaths (total 4.95 litres) of the same dose. This produced 9.03% drop in specific conductance and the subject commented "Same taste, could not feel any bronchoconstriction".

Conclusion.

It was decided to continue with the policy of taking ten breaths.

CHAPTER 4.

SULPHUR DIOXIDE.

Introduction.

Sulphur dioxide is a common pollutant of the atmosphere arising chiefly from industrial, commercial and domestic combustion of fossil fuels. It is produced during the smelting of sulphur ores and is used in the bleaching of paper and the preservation of some foods. Urban concentrations are usually in the region of 0.2ppm but local maxima may reach 2ppm.

Sulphur dioxide was selected as the first gas for investigation because it is widely encountered and known to be bronchoconstricting. The first person to record this was Bernardino Ramazzini (1633 - 1714) Fig 4.1. In his book Diseases of Workers (1713) he describes how a baker found that balls of sulphur used for lighting the oven had caught fire. He managed to stamp them out but was tormented with a cough and had "great difficulty of breathing; for the vesicular structure of the lungs was constricted by the strong acid fumes".

Kehoe et al (1932) conducted a survey of workers in a refrigerator factory: SO_2 exposure 20 - 30 ppm with peaks of 55 - 70 ppm. The control group was exposed to CO. There was nothing peculiar

Fig. 4.1.

in the appearance of the roentgenograms of the exposed subject and nothing from an X-ray point of view to indicate the existence of injury to the lungs or bronchi as a result of the exposure. However, the exposed group had greater fatiguability and more shortness of breath on exertion. No correction was made for possible differences in smoking. Two other points emerge from Kehoe's study which may in 1932 have been less significant. Firstly abnormal reflexes were higher in the exposed group. Kehoe deduces that this represents a degree of variation in general irritability, (Kasl 1964 found a strong association between aggressive mood and illness behaviour). Secondly duration of colds was extended on average by a factor of 2.3 times. This is supported by the total man - weeks of colds (1,141 exposed, 462 controls) and the number of men having colds lasting all winter (40 exposed, 13 controls). It is possible that today sickness absence could be affected.

Anderson (1950) examined the workers at Abadan on behalf of the Anglo Iranian Oil Company. Introducing pulmonary function measurement to this problem he measured vital capacity as the best index available. In one plant he found the exposed

workers to have a significantly higher mean vital capacity: in the other plant it was higher but not significant. Daily concentrations of SO_2 varied between 0 and 25 ppm. with occasional peaks of 100 ppm. Radiographic findings yielded no difference between exposed and controls. Anderson concluded "No evidence of adverse effects on health could be found".

Ferris et al (1967) made a study of workers exposed to SO_2 (0 - 7 ppm) in a pulp mill. They were able to construct maximum flow volume curves by measuring the slope of the last three F.V.C. curves at the specified lung volumes from the tracings obtained on a Stead-Wells spirometer with a fast paper speed and averaging them. This may leave something to be desired in terms of sensitivity. No significant effects were found attributable to SO_2 although men exposed to chlorine "had a somewhat poorer respiratory function and more shortness of breath".

Both Kehoe and Anderson discussed the different acclimatization times of different workers and Ferris went further to suggest that a considerable degree of self selection had taken place.

Subjects not exposed occupationally to SO_2 have been studied by several investigators who examined their response to brief exposures of SO_2 . Amdur et al (1953) reported shallow, tachypnea following ten minute exposures of 1 to 8ppm SO_2 . However, Lawther (1952) and Sim and Pattle (1957) found little change in breathing pattern. Strandberg (1964) suggested that in rabbits the scrubbing efficiency of the upper respiratory tract is much greater at higher than at low concentrations. Thus a disproportionately greater percentage of sulphur dioxide may reach the lung at low concentrations. Amdur (1966) working with guinea pigs claimed that this accounted for the changes observed by her in slope of the dose response curve.

Speizer and Frank (1966) considered the attention imposed upon an initial concentration of SO_2 by inhalation through the human nose. They concluded that during quiet breathing of an initial average concentration of 16.1ppm virtually all of the SO_2 was absorbed by the nose and further that it re leased significant amounts of SO_2 with the subsequent expiration. Anderson (1974) claims that ciliastasis is produced in the anterior naso pharynx by SO_2 .

Alarie et al (1970) suggested that too short exposures had been used in earlier experiments. Thus

they exposed guinea pigs to low concentrations (0.13, 1.01 and 5.72ppm) for fiftytwo weeks. Apart from some alterations in the livers of those exposed to the highest concentration there were no detrimental effects.

Frank et al (1962) exposed humans to average levels of sulphur dioxide of 1, 5, and 13ppm while seated in a constant pressure body-plethysmograph. Pulmonary flow resistance was measured by oesophageal balloon during spontaneous breathing. Exposures varied between ten and thirty minutes. With one exception the group (of eleven subjects) showed no significant increase in pulmonary flow resistance at 1ppm . At both 5 and 13ppm flow resistance was elevated, the change being greater at 13ppm . The change occurred within one minute of exposure, increased after five minutes but, on the average, showed no further change after ten minutes. Four subjects were exposed to 5 or 13ppm of SO_2 for thirty minutes without exhibiting increases in flow resistance beyond the first ten minutes. In addition, at 1ppm a prolongation of exposure to thirty minutes did not increase the likelihood of a significant rise in flow resistance.

From the community environment viewpoint, studies in the United States (Public Health Service, (1969)) have shown that a proportion of the sulphur

dioxide emitted into the atmosphere undergoes oxidation, leading eventually to the formation of sulphuric acid and particulate sulphates. Atkins et al (1972) have shown similar processes taking place in England. Amdur (1971) has reviewed the toxicology of aerosols formed by oxidation of sulphur dioxide. She concluded that the sulphur acid and particulate sulphates engendered have a greater potency for irritation than sulphur dioxide. She also stresses the importance of particle size and found that submicronic particles were the most potent.

Methods.

The methods were as described in the chapter on methods. Four doses (5, 15, 30 and 80ppm SO_2) were administered to four subjects in a double blind experiment using a randomised block Latin square design. The experiment was repeated with three groups making a total of twelve subjects.

Results.

Table 4.1 shows the mean change in specific conductance elicited by each of the four doses administered to twelve subjects. The following percentage changes were obtained, ten pairs of oscilloscope readings were obtained as control values for the subject (see methods) in two blocks of five readings. The first

TABLE 4.2 GIVES VITALOGRAPH READINGS.

TABLE 4.1.

SULPHUR DIOXIDE: MEAN CHANGES IN SPECIFIC CONDUCTANCE FOR FIVE SUBJECTS.

Dose ppm.	$\Delta SG_{A.W.}$	Standard Error
5	11.0	1.5
15	16.7	2.7
30	21.8	3.3
80	33.8	3.3

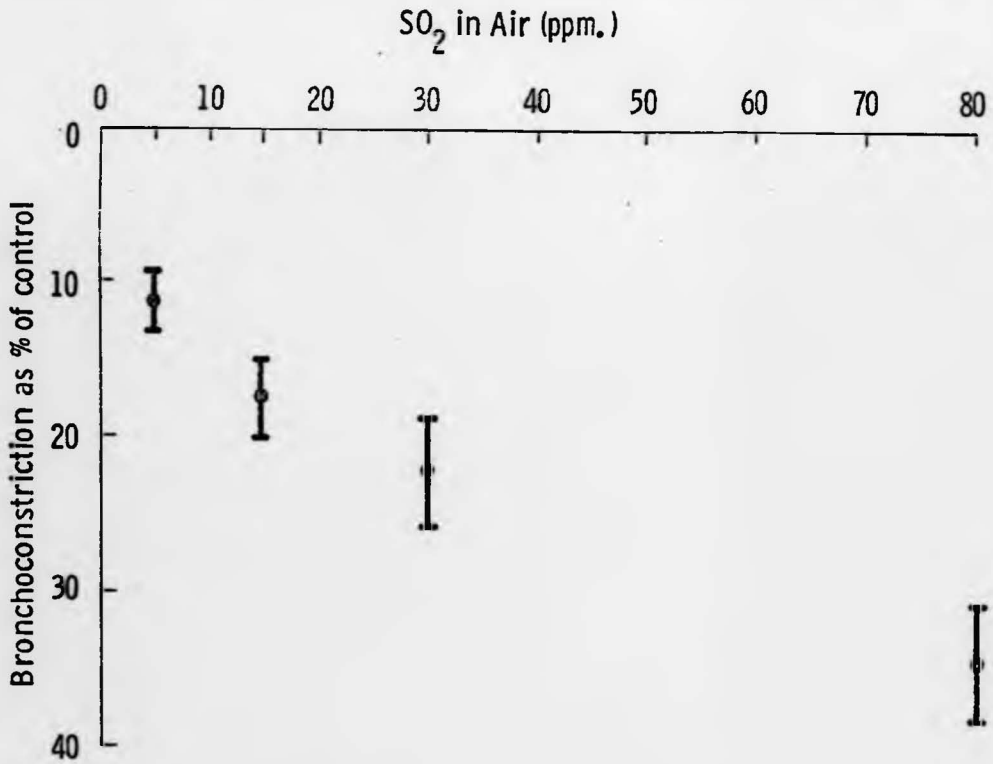


Fig.4.2. THE EFFECT OF SO_2 AS BRONCHOCONSTRICTOR IN TWELVE SUBJECTS

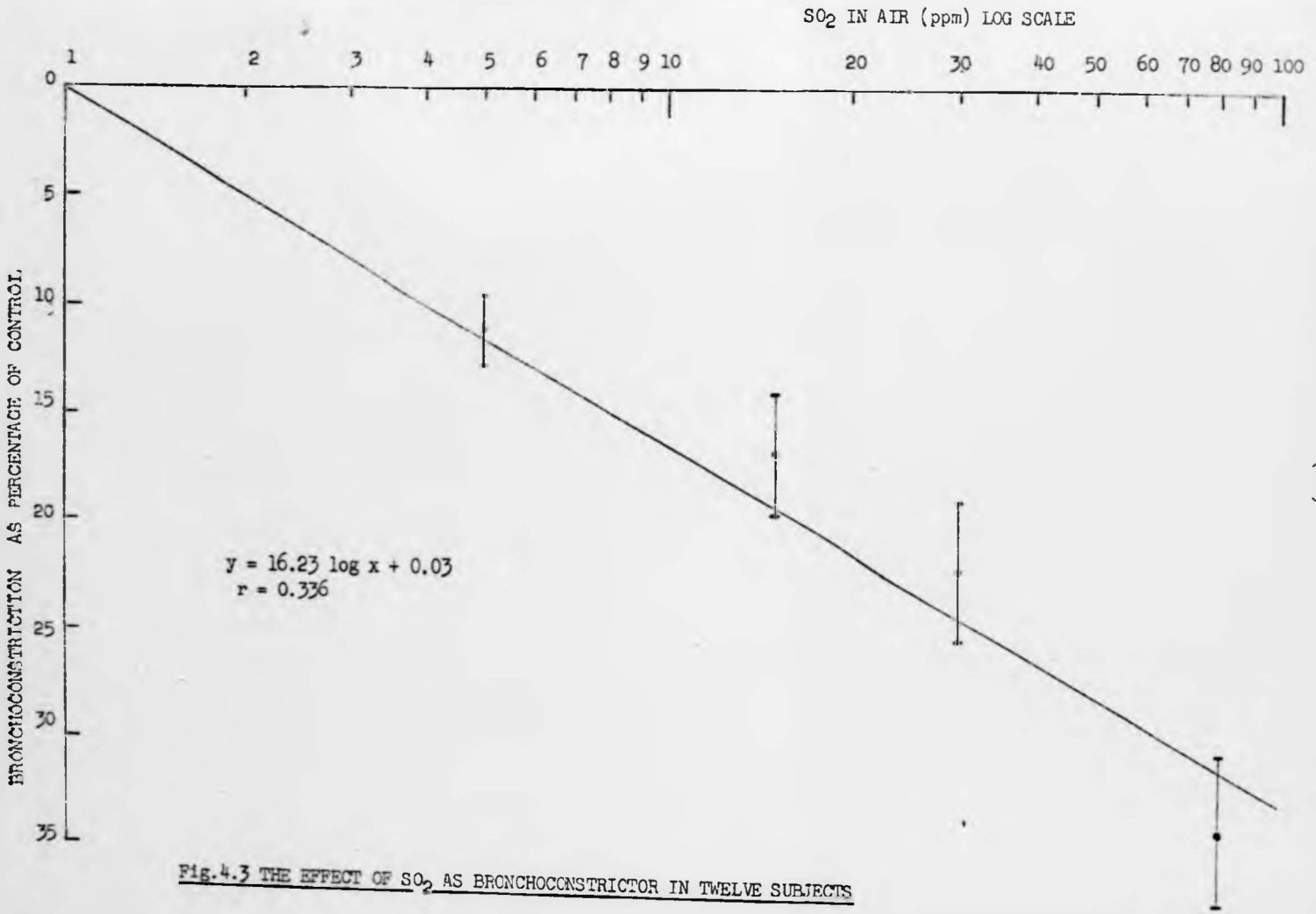


Fig.4.3 THE EFFECT OF SO_2 AS BRONCHOCONSTRICTOR IN TWELVE SUBJECTS

pair of each five was discarded and the remaining eight pairs used to compute values of airways resistance (RAW) thoracic gas volume (VTG) and specific conductance (SGAW). These were averaged to provide the control values. Following the inhalation of the gas, two groups of five more pairs were obtained and again the first reading was discarded from each group. The mean of each group was then compared with the control value and the change expressed as a percentage of control. The average percentage changes in specific conductance for the first post gas group are shown plotted in Fig. 4.2 and are replotted on a logarithmic scale of dose in Fig. 4.3 . The least squares regression line was computed as

$$y = 16.23x + 0.03$$

$$(r = 0.336 \quad p < .01)$$

where y = percentage change SGAW

and x = log dose

The intercept on the dose axis was 0.99ppm.

Subjectively the effect of SO_2 inhalation was recorded as irritant but not particularly unpleasant. Additionally the values of resistance, specific conductance and lung volume obtained without discarding the first pair of each five pairs of readings are given in appendix C.

The intercept of the least squares dose response curve may conveniently be regarded as a notional "threshold" for bronchoconstriction which permits comparison of one vapour with another and also provides a means of comparing the results of the inhalation experiments with those of the eye experiments. It must be strongly emphasised however that this extrapolation outside the measured range does not purport to establish a value for a physiological threshold. This could only be achieved by taking a large number of readings especially in the vicinity of the "threshold". The techniques employed here can at best provide only an indication of where the "threshold" might lie. Any attempt to exploit body plethysmographic measurements as a contributing factor in determining TLVs would have to be strictly limited to the range of concentrations actually administered. In addition it would require a specifically planned experiment for each gas or vapour under investigation in which a large number of subjects would be exposed - preferably for long periods. This of course is

expensive and long exposures require some estimate of the risk, if any, to the subject. There are indications that the dose response curve obtained by an acute exposure of ten breaths may not differ too greatly from that obtained by prolonged exposure of up to 30mins. (Frank et al 1962).

Eye Exposures.

Two subjects received eye exposures using the goggles shown in Fig.2.7 (see Methods). The doses administered on separate occasions were 10, 25, 42, 88 and 166ppm . At doses up to 88ppm no irritation was detected but at 166ppm one subject (RD) reported "irritating immediately and more so after ten seconds plus lacrimation". The other subject (SMF) reported "definite irritation (quite severe on opening eyes) worn off after ten seconds. Some lacrimation".

Discussion.

The intercept of the least squares dose response curve at 0.99ppm may be interpreted as being a notional "threshold" for bronchoconstriction. While bearing in mind that this is an extrapolation outside the measured range of doses it is none the less interesting that Frank et al (1962) found no significant bronchoconstriction at 1ppm with exposures ranging from five to thirty minutes. At 5ppm they obtained an average 40% increase in broncnoconstriction (the value in this present study being 11%) and at 13ppm 73% (this study obtained 17% at 15ppm). Thus Frank et al obtained a steeper dose response curve but the same intercept. It is suggested that the need for the subject to take only ten breaths may in some respects represent an advantage when investigating broncho-active gases and vapours.

TABLE 4.2.

VITALOGRAPH RESULTS OF SUBJECTS OF SO_2 EXPERIMENT.

No	Name	Sex	FEV	%pred	VC	%pred	FEV/VC	%pred
1.	D.H.	M	3.51	81	5.72	113	61.4	75
2.	A.W.	M	4.54	109	4.70	94	96.6	117
3.	J.C.(a)	F	3.67	123	4.03	115	91.1	106
4.	J.C.(b)	F	3.51	113	4.00	109	87.7	101
5.	E.O.	F	3.19	109	4.19	122	76.1	89
6.	P.K.	F	2.43	86	2.86	89	85.0	99
7.	P.P.	M	2.73	98*	3.24	101*	84.3	107
8.	D.C.	M	5.54	125	6.31	117	87.8	107
9.	G.D.	M	3.54	111	4.55	109	77.8	108
10.	M.D.	F	3.11	105	3.88	112	80.2	93
11.	S.F.	F	3.66	117	4.23	113	86.5	101
12.	C.B.	F	4.36	158	4.74	148	92.0	108

The best reading is taken (out of 5) and corrected to BTPS. Predicted values obtained from Cotes "Lung Function" 1968.

*Predicted values for Indians and Pakistanis based on data of Hearn also Hunt (unpublished) and Cotes and Malhotra, Cotes "Lung Function" 1968.

CHAPTER 5.

AMMONIA.

TLV = 25ppm

formula = NH_3

Introduction.

Ammonia is produced as a by-product in the distillation of coal, by the action of steam on cyanamide, and by the cataytic combination of nitrogen and hydrogen gases at high temperature and pressure. It is used in the manufacture of fertilizers, nitric acid, explosives, dyes, plastics, in refining petroleum and as a refrigerant. The physical and chemical properties of ammonia are listed below in Table 5.1.

Table 5.1.

Physical and chemical properties of ammonia.

Physical state : colorless gas

Molecular weight : 17.03

Melting point : $-77.7^{\circ}C$.

Boiling point : $-33.35^{\circ}C$.

Solubility : 90g.in 100ml. water at $0^{\circ}C$.

The bronchoactivity experiments were performed initially using twelve subjects with methods similar to those used for the sulphur dioxide experiment. Due to difficulties of dose preparation using static methods

(see methods chapter) a second bronchoactivity experiment was performed later using techniques developed from the ketone experiments.

First Bronchoactivity experiment.

Methods.

The equipment and procedure were as described in the chapter of methods using static dilution in a Douglas bag.

Results.

The mean changes in specific conductance for twelve subjects are shown graphically in Fig. 5.1. using the data from Table. 5.2. Table 5.3. gives the forced expiratory volume in one second (FEV_1) in litres, the forced vital capacity (FVC) in litres and the ratio (FEV_1/FVC) expressed as a percentage and measured using a Vitalograph. Subjects reported increased salivation while inhaling the gas. One subject described the response as "raining from the roof of my mouth". The results were later re-computed for each individual using all five replicates and these are included as appendix C

Second bronchoactivity experiment.

Methods.

Equipment.

The continous generation system used for

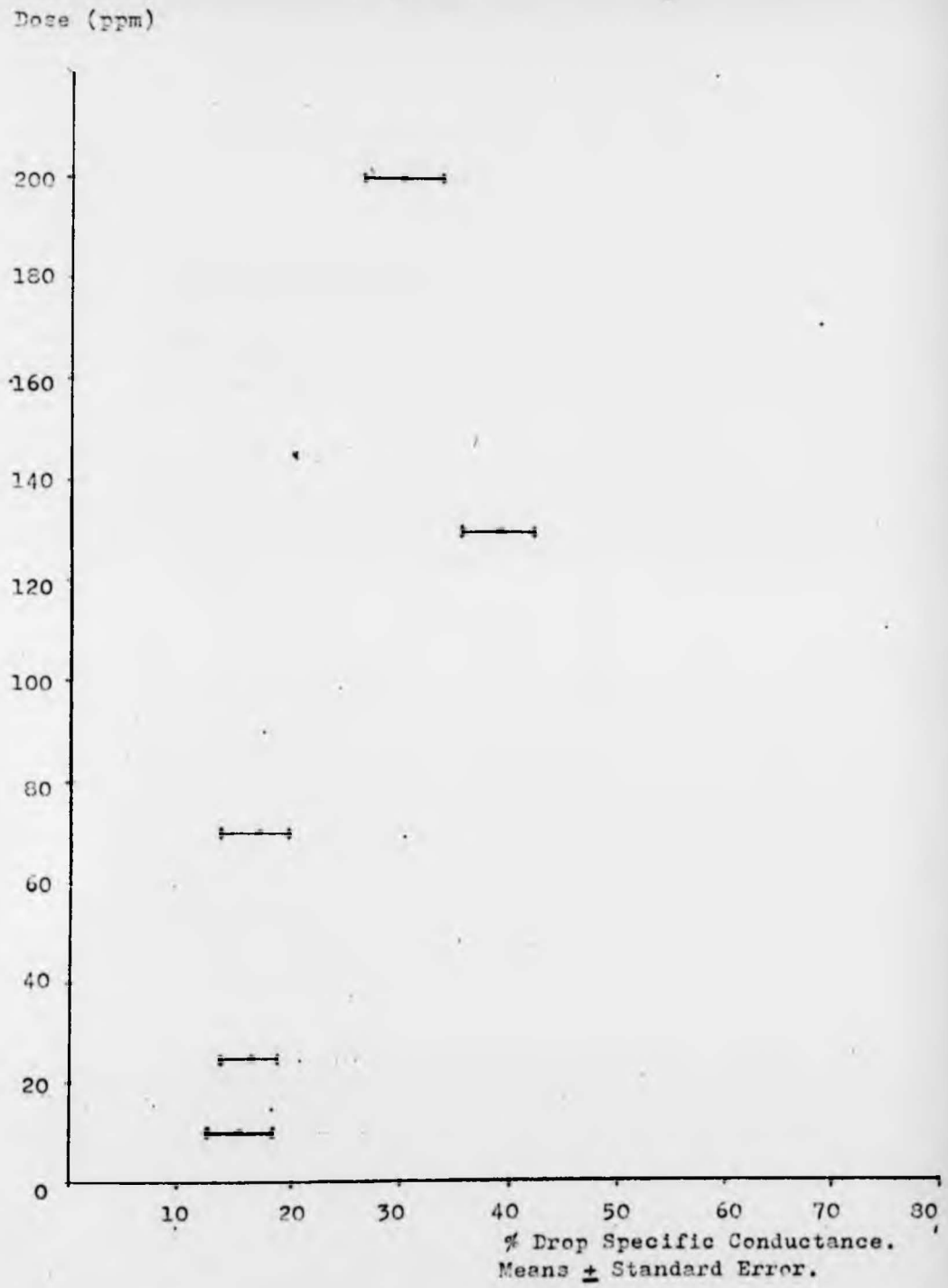


Fig. 5.1. Dose response curve for ammonia using static dilution.

TABLE 5.2.

AUXOLITA: MEAN CHANGES IN SPECIFIC CONDUCTANCE WITH TWENTY SUBJECTS.

Dose (ppm)	ΔSC_{AV}	Standard Error
10	15.2	2.2
25	16.2	2.2
70	17.0	2.5
130	28.8	3.2
200	30.0	3.4

TABLE 5.3

VITALOGRAPH RESULTS OF SUBJECTS OF NH_3 EXPERIMENT.

No	Name	Sex	FEV	%pred	VC	%pred	FEV/VC%	%pred
17.	E.O.	F	3.19	109	4.19	122	76.1	89
18.	P.P.	M	2.73	98*	3.24	101*	84.3	107
19.	J.C.	F	3.51	113	4.00	109	87.7	101
20.	P.K.	F	2.43	86	2.86	89	85.0	99
21.	D.P.	M	4.20	108	4.52	98	92.9	115
22.	M.D.	F	3.11	105	3.88	112	80.2	93
23.	D.C.	M	5.54	125	6.31	117	87.8	107
24.	G.D.	M	3.54	111	4.55	109	77.8	108
25.	M.H.	M	4.21	108	4.91	106	85.7	105
26.	S.G.	F	3.48	119	3.54	98	98.3	112
27.	A.W.	M	4.54	109	4.70	94	96.6	117
28.	R.W.	F	3.40	116	3.60	105	94.4	110

The best reading is taken (out of 5) and corrected to BTPS.
Predicted values obtained from Cotes "Lung Function" 1968.

*Predicted values for Indians and Pakistanis based on data of Hearn also Hunt (unpublished) and Cotes and Malhotra, Cotes "Lung Function" 1968.

the ketone experiments was modified for use with a cylinder of gas described in the sulphur dioxide eye experiments.

A cylinder nominally 800ppm of ammonia in air was employed and doses for inhalation were prepared using a single diultion. Fig. 5.2. shows kitagawa readings of different doses plotted against nominal values using the data of Table 5.4.

The data may also be used to provide estimates of the cylinder concentrations (nominally 800ppm) by dividing by the proportional dilution (see Table 5.4.). However, although the detector tubes are convenient for providing a simple check on the dilutions, they are not good enough to provide a reliable estimate of the cylinder concentrations. An atmosphere of ammonia in air was generated in the breathing system as used for the subjects and the concentration measured by absorbing ammonia in standard acid and back titrating with standard base using methyl red - bromocresol green as indicator. The acid used was 1/10 molar HCL diluted to 2×10^{-3} molar and the base was a concentrated volumetric solution of Na OH diluted to 10^{-3} molar.

The ammonia in air was drawn through

100ml of standard acid in a fritted bubbler for ten minutes at one litre a minute. Ten mls was then placed in a conical flask with four drops of indicator solution. This was then titrated with standard base.

Immediately following the bubbling six kitagawa tubes from two boxes A and B were used to sample. The time to draw six tubes was approximately 30 mins. and the results are given in Table. 5.4(a). It may be seen that on average the kitagawa tubes are reading 9.17% (Range 4.31% - 14.14%) below the estimate from titration. The estimates of cylinder concentration obtained by dividing by the proportional dilution had a mean value of 764.4ppm. If one accepts that the kitagawa tubes read low, then increasing this by 9.17% gives an estimate of 834.49ppm (797.34ppm and 872.49ppm if the values of 4.31% and 14.14% are uses respectively). This is approximately 4% more than the manufacturers estimate of 800ppm but it is not possible to reject this value due to the range of estimates.

Table 5.4. AMMONIA CYLINDER DILUTIONS vs. KITAGAWA TUBE ESTIMATIONS. plus CALCULATED CYLINDER CONCENTRATIONS.

PROPORTION OF NH_3 CYLINDER (800ppm).	KITAGAWA TUBE ESTIMATION(ppm)	CALCULATED CYLINDER CONCENTRATION (ppm).
0.090	74	822.2
0.168	120	714.3
0.200	150	750.0
0.250	220	880.0
0.335	200	600.0
0.500	410	820.0
		mean 764.4

Table 5.4(a).

TITRATION ESTIMATES OF NH_3	KITAGAWA ESTIMATES OF NH_3
1. 292 ppm.	1. 260 ppm (A)
2. 289 ppm.	2. 280 ppm (B)
3. 288 ppm.	3. 270 ppm (A)
4. 290 ppm.	4. 250 ppm (B)
	5. 275 ppm (A)
mean 290 $\pm$ 1.5 ($p < .05$)	6. 245 ppm (B)
	mean 263 $\pm$ 12.7 ($p < .05$)

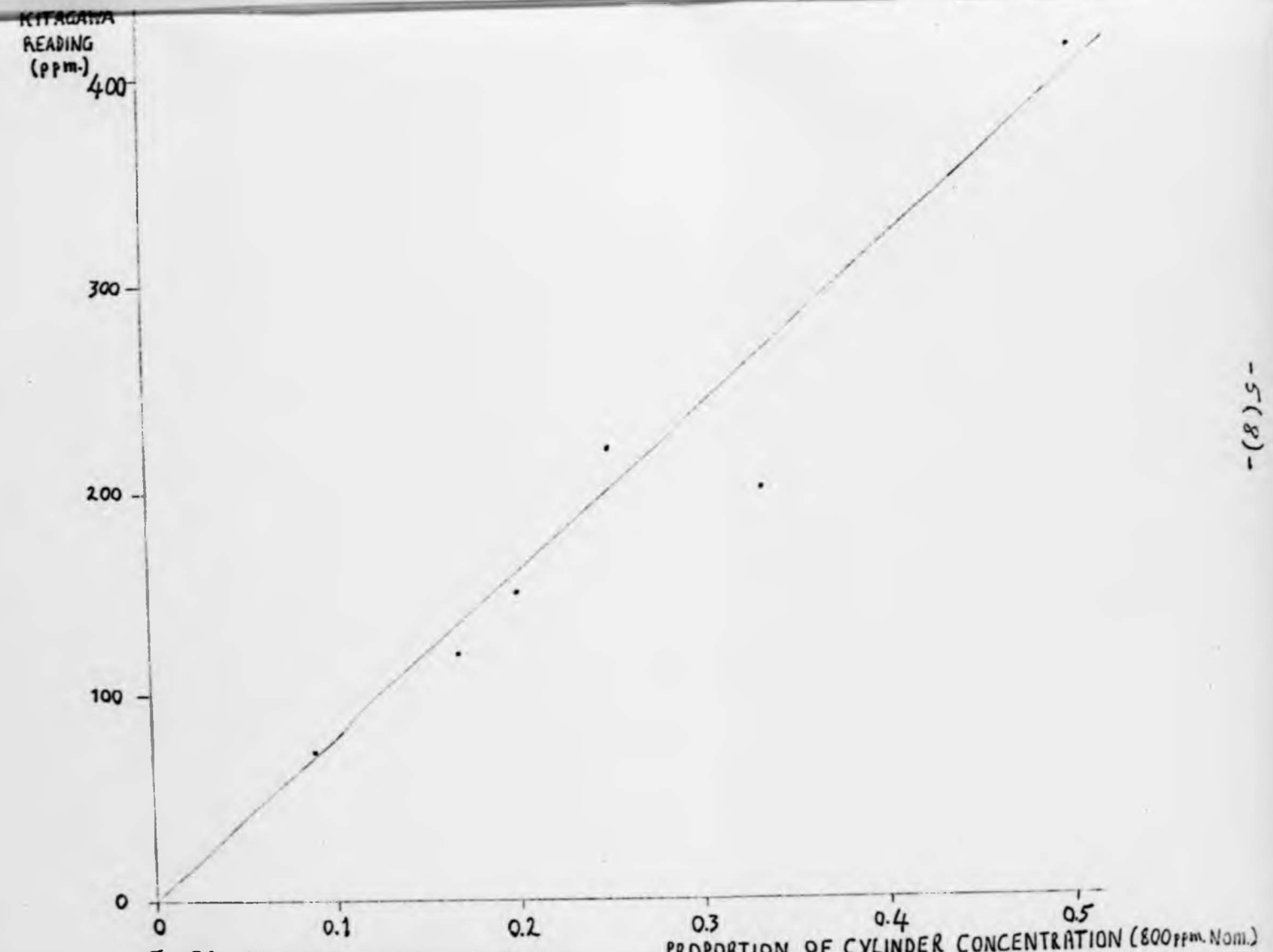


Fig 5.2. DILUTION CALIBRATION CURVE.

The second subject (ML) found 200ppm to produce a "slight burning in the throat" while at 300ppm described a "desire to cough after the first breath and grating feeling in the throat". The largest dose (400ppm) elicited "throat irritation after the second breath which became worse after the fifth breath". The thirst subject (DD) found 200ppm to produce a "burning sensation, lacrimation and salivation" but "no desire to cough". Subject HR found 200ppm to produce "burning in the throat after the first breath and very difficult to take the tenth breath". However, these results are qualified by the fact that the subject had previously been briefly inside an exposure chamber contaminated with flax dust. The final subject (MH) found 200ppm to produce a "barely controlable urge to cough which was far more worrying than the burning sensation in the throat". At 300ppm "the effect was initially worse than last time but with conscious relaxation it was possible to cope with this concentration for ten breaths".

Table 5.6 contains the constants of the straight lines shown in Fig. 5.3 The threshold of the dose response curves range from thirtyfive ppm to one hundred and fortyeight ppm (arithmetic mean 84.8ppm, geometric mean 74.6 ppm).

TABLE 5.5

AMMONIA

SUBJECT	CONTROL RAW	CONTROL VTG	CONTROL SGAW	% CHANGE FROM CONTROL RAW	% CHANGE FROM CONTROL VTG	% CHANGE FROM CONTROL SGAW	DOSE	ATOPY	COMMENTS
S.W.	0.55	4.40	0.41	-35.0	-4.8	28.6	200	NA	S: BURNING AT BACK OF THROAT, CHOKING; O: CAME OFF AFTER 5 BREATHS.
S.W.	0.55	4.32	0.43	-83.7	12.8	38.8	300	NA	S: -
M.L.	0.44	4.25	0.54	-42.9	-1.0	30.7	200	NA	S: SLIGHT BURNING IN THROAT.
M.L.	0.47	4.11	0.52	-54.5	5.4	32.2	300	NA	S: DESIRE TO COUGH AFTER 1ST BREATH. GRATING FEELING IN THROAT.
M.L.	0.47	4.06	0.53	-74.2	-3.2	45.3	400	NA	S: THROAT IRRITATION AFTER 2ND BREATH, BECAME WORSE AT 5TH BREATH.
D.D.	0.59	5.99	0.28	-41.6	-2.7	31.3	135	NA	S: -
D.D.	0.57	3.80	0.47	-41.4	-18.9	40.6	200	NA	S: BURNING SENSATION, LACRIMATION, SALIVATION, NO DESIRE TO COUGH.
H.R.	0.63	4.64	0.34	-36.1	7.2	31.4	200	A	S: BURNING IN THROAT AFTER 1ST BREATH. VERY DIFFICULT TO TAKE 10TH BREATH. O: HAD BEEN IN CONTAMINATED FLAX CHAMBER.
M.H.	0.48	3.40	0.62	-28.8	-9.2	24.5	200	NA	S: BARELY CONTROLABLE URGE TO COUGH WHICH WAS FAR MORE WORRYING THAN THE BURNING SENSATION IN THE THROAT.
M.H.	0.50	3.36	0.60	-26.9	-29.8	37.4	300	NA	S: THE EFFECT WAS INITIALLY WORSE THAN LAST TIME BUT WITH CONSCIOUS RELAXATION IT WAS POSSIBLE TO COPE WITH THIS CONCENCRATION FOR 10 BREATHS.

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

S = SUBJECTIVE COMMENT.
O = OBSERVER COMMENT.

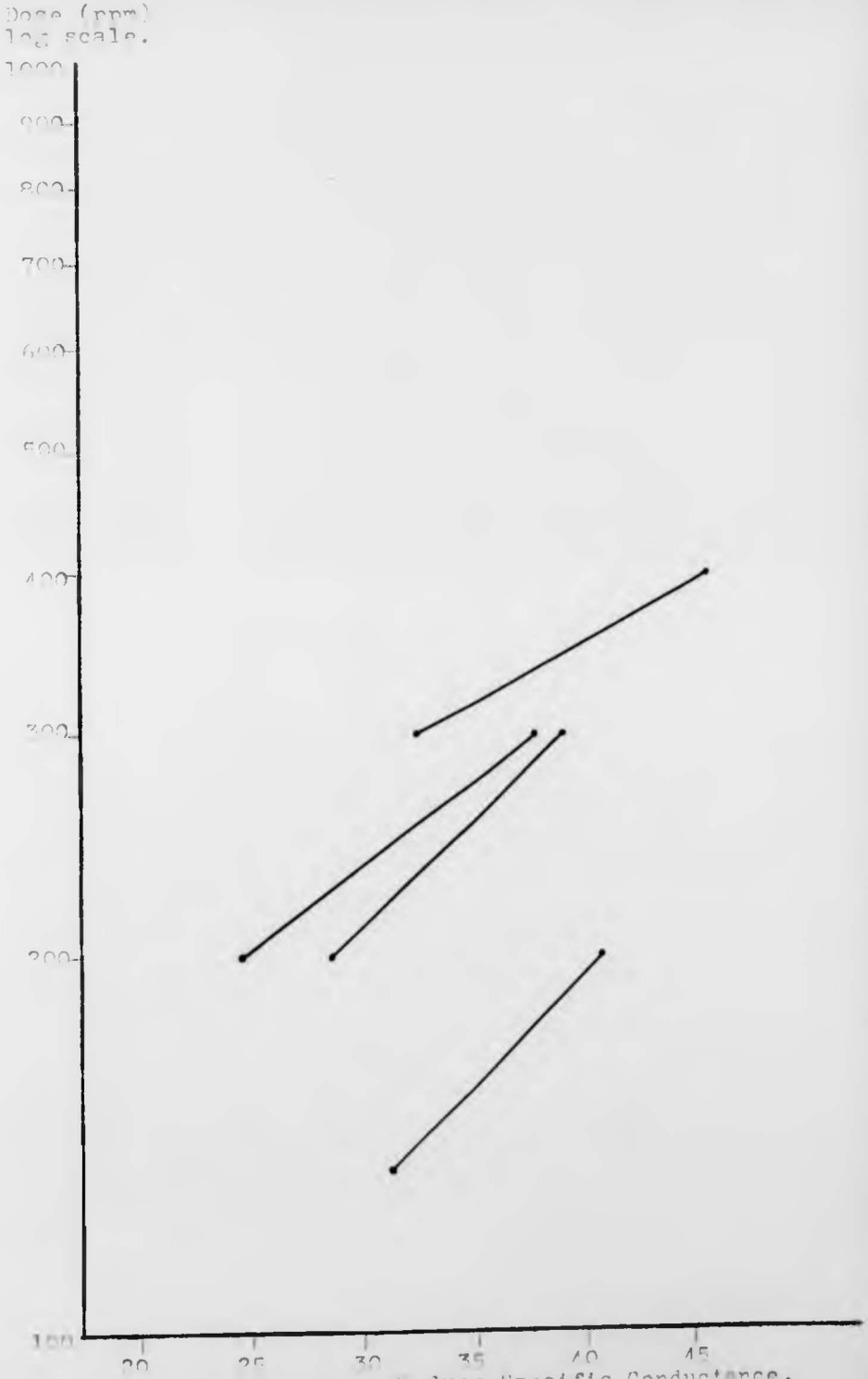


Fig. 5.3. Dose response curves ammonia.

TABLE 5.6

AMMONIA

SUBJECT	ATOPY	EYE THRESHOLD DOSE PPM	LUNG THRESHOLD DOSE PPM	SLOPE OF DOSE RESPONSE CURVE
S.W.	NA	110	63.83	57.62
M.L.	NA	110	147.6	104.6
D.D.	NA	59	35.89	54.37
M.H.	NA	110	91.83	72.85
A.H.	NA	110		
H.S.	A	110		
S.M.F.	A	100		
R.D.	NA	100		

Eye experiments.

Table 5.6 gives the results of the ammonia eye experiments. The first subject listed (SW) found 59ppm to have no effect and 110ppm and 160ppm to be irritant. The subject reported reported 110ppm as "less irritant than the other (160ppm) dose". The second subject (ML) described 160ppm as irritant commenting "I felt it right away. Twice as nasty as the first dose (110ppm). It continued for fifteen seconds". At 59ppm the subject reported "no sensation". The third subject (DD) reported of 59ppm "slight irritant effect immediately; disappeared before five seconds. Slight lacrimation." At 110ppm the subject reported "intense irritation". Subject (MH) found "no effect" at 59ppm and at 110ppm reported "certainly different from first dose, very slight stinging which continued after removing goggles". At 160ppm subject remarked "irritant I felt that one right away". Subject (AH) remarked for 59ppm "no, not irritant" , for 110ppm recorded "could just feel something irritant immediately on opening eyes". At 160ppm the subject reported "certainly stronger than the first one (110ppm)". Subject (HS) found 59ppm to produce "no effect whatsoever"and 110ppm to produce "smarting at four or five seconds which then wore off". In previous experiments subjects SMF and RD had recorded thresholds for irritation of 100ppm .

CHAPTER 6.

KETONES.

Introduction.

Historically, some of the first commercially available ketones were produced by fermentation of grain or by the destructive distillation of wood. In recent years, however, more and more of the commercial ketones are made by organic synthesis. In general, the usual method involves the dehydration or the oxidation of an appropriate secondary alcohol using suitable catalysts and conditions. The petrochemical industry is becoming an increasingly important source of the raw materials for making ketones.

In industrial operations, ketones find three major uses: as solvents for a wide variety of materials, as raw materials or intermediates in organic syntheses, and for speical uses such as in perfumes. They may be encountered in the manufacture of smokeless powder and other explosives, varnishes, lacquer and varnish removers, plastics, rubber, artificial silk and leather, lubricating oils, cosmetics, pharmaceuticals, perfumes, and many organic chemicals. They are used widely as solvents for dyes, oils, fats, tars, waxes, and many natural and synthetic resins and gums. They are to be found in many consumer items such as synthetic coatings, dopes, and adhesives.

The ketones are generally quite stable chemically but all of them are flammable to some degree. It is important, therefore, that due consideration be given to this property in their industrial handling.

The physical and chemical properties of the five ketones investigated are listed in Table 6.1. Acetone is the first member of the homologous ketone series. The second is methyl ethyl ketone (MEK) or 2-butanone while the third member has three isomers. These are pentan-2-one, pentan-3-one and 3-methylbutan-2-one. This last isomer has a branched chain structure.

Industrial exposure to the commonly used ketones has been occuring to an appreciable degree for many years. The vapours of the saturated aliphatic ketones generally are classed as narcotic. Patty (1967) comments that concentrations required to cause frank narcosis are not breathed voluntarily giving irritation to eyes and respiratory passages as the reason; (this finding is not supported fully by the experiments here reported (see acetone results)). Patty also points out that lower concentrations which can be breathed without discomfort, may cause impairment of judgment and thereby create a secondary hazard. Generally, toxicity, irritation, and narcotic potency of the aliphatic ketones increases with increasing molecular weight; irritation and toxicity also increase

TABLE 6.1.

KETONES - PHYSICAL AND CHEMICAL PROPERTIES

Name	Molecular formula	Structural formula	M.Wt.	Solbty.g/100ml. H_2O	Ether	B.P.
Acetone	C_3H_6O	$CH_3.CO.CH_3$	58.8	∞	∞	56.5°C
2-Butanone (Methyl Ethyl Ketone)	C_4H_8O	$CH_3.CH_2-CO.CH_3$	72.10	35@10°C 19@90°C	S	79.6°C
3 methyl butan-2-one	$C_5H_{10}O$	$(CH_3)_2.CH.CO.CH_3$	86.13	.61@30°C	∞	88.9°C
Pentan-2-one	$C_5H_{10}O$	$CH_3.CH_2.CH_2.CO.CH_3$	86.13	.63@30°C	∞	103.3°C
Pentan-3-one	$C_5H_{10}O$	$CH_3.CH_2.CO.CH_3.CH_3$	86.13	4.7@20°C 3.8@100°C	∞	102.7°C

S = solubility between 10 - 50 g 100 ml.$^{-1}$.

Sources: see Table 7.1.

with unsaturation. In liquid form, the common ketones are painful and irritating to the eyes and prolonged and/or repeated contact with the skin may cause detrimental effects.

BLANK PAGE

P.T.O.

ACETONE.

TLV 1000ppm.

formula = C_3H_6O

Methods.

The equipment and procedure were as described in the chapter on methods. The saturated vapour pressure of acetone at 20°C was obtained by interpolation from the graph shown in Fig. 6.1. to be 180 mm Hg.

Bronchoactivity Experiment.

Results.

Plethysmographic results were obtained for only one subject and these are shown below in Table 6.2.

Table 6.2.

ACETONE RESULTS

SUBJECT	DOSE (ppm)	CONTROL VTG	CONTROL RAW	CONTROL SGAW	% CHANGE VTG	% CHANGE RAW	% CHANGE SGAW
SMF	8,000	3.28	0.61	0.50	-1.7	-4.5	5.8

Subjective sensations.

A dose of 8,000ppm produced virtually no bronchoconstriction. The ten breaths were inhaled only with great difficulty although there was no

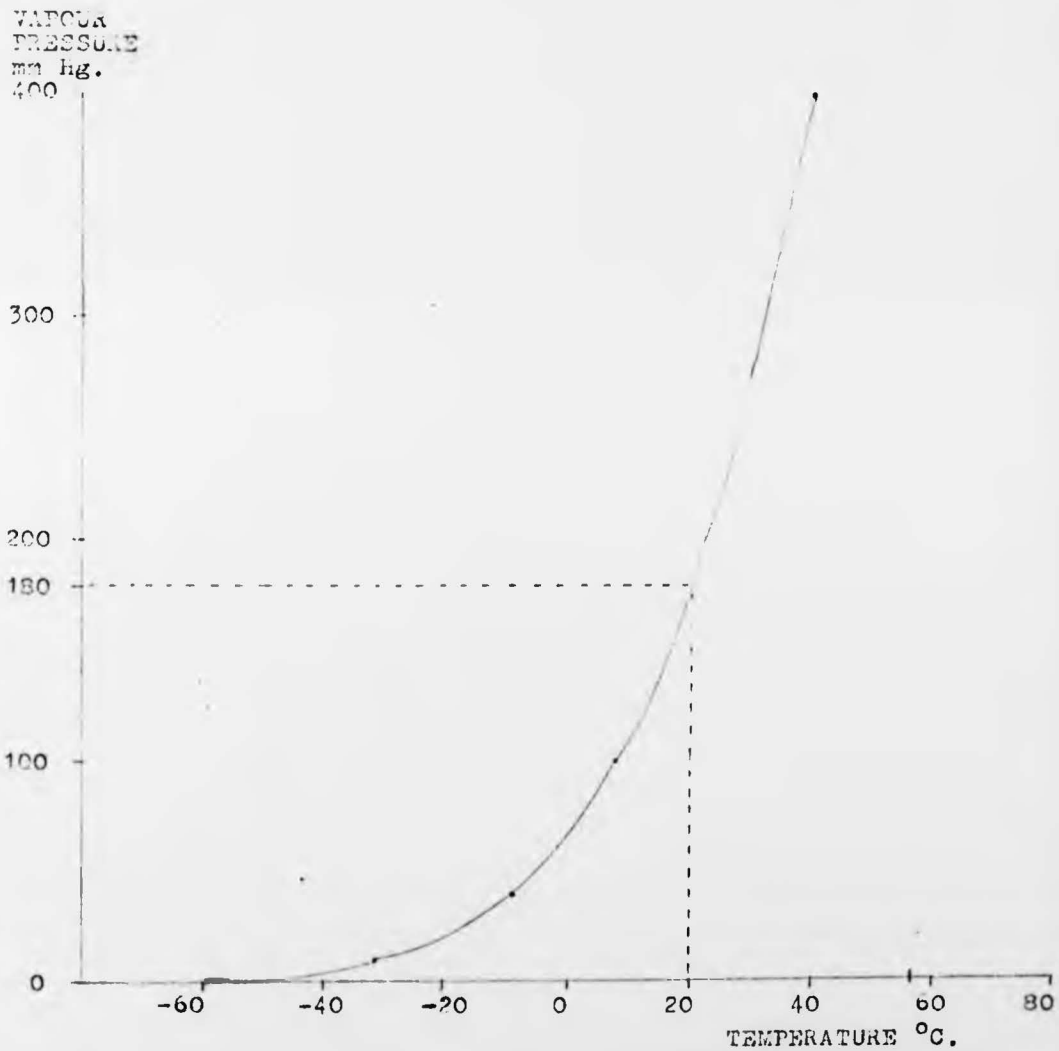


Fig. 6.1. Saturated Vapour Pressure Curve for Acetone.

feeling of irritation in the trachea. However the effects elicited were most unpleasant including nausea and a "mild anaesthetic feeling". In addition the subject recorded "vasodilation initially : facial and peripheral which was rather unpleasant". Pallor was observed in the subject after 4.5 minutes. A second subject (RD) inhaled 6,000ppm and succeeded in taking ten breaths only with difficulty. The effects were nausea, suffocation, slight dizziness and a strong desire to withdraw from the mouth piece. There was however no feeling of irritancy.

It was concluded that further exposures should not be attempted.

Acetone Eye Experiments.

Results.

Two subjects received eye exposures of acetone. At 1,000ppm subject one (RD) felt "nothing" and the other, Subject two (SMF), experienced "cold on opening eyes". At 2,000ppm subject one experienced a "feeling of lacrimation to a small, but definite, extent". Subject two recorded a "stinging feeling on opening the eyes which took a while to wear off". At 4,000ppm subject one felt "stinging rather than lacrimation; gone after ten seconds". Subject two felt "stinging but gone after five seconds". At 10,000 ppm subject one was "conscious of lacrimation and

desire to shut eyes - gone after ten seconds. Not irritant as such". To an observer the subjects eyes appeared "watery". Subject two recorded "lacrimation and desire to shut eyes which continued for the full fifteen seconds. Not really irritant". Again, to an observer the subject's eyes appeared "watery".

Conclusion.

Acute exposures of the eyes to acetone do not produce very great irritation and it seems that at elevated concentrations (10,000ppm) all irritation is obviated by lacrimation.

METHYL ETHYL KETONE (2 - BUTANONE).

TLV 200ppm.

formula = C_4H_8O

Methods.

The equipment and procedure were as described in the chapter on methods. The saturated vapour pressure of acetone at 20°C was obtained by interpolation from the graph shown in Fig. 6.2. to be 80 mm Hg.

Bronchoactivity Experiments.

Results.

The results are shown below in Table 6.3.

Table 6.3.

METHYL ETHYL KETONE RESULTS

SUBJECT	DOSE (ppm)	CONTROL VTG	CONTROL RAW	CONTROL SGAW	% CHANGE VTG	% CHANGE RAW	% CHANGE SGAW
MT	1,600	3.03	0.50	0.69	-14.8	-9.9	23.1
SMF	1,600	4.42	0.64	0.36	- 2.1	0.7	1.7

Methyl ethyl ketone (MEK) was administered to two subjects at 1,600ppm producing bronchoconstriction (23.1% drop in specific conductance) in one subject and virtually no change (1.7% drop in specific conductance) in the other subject.

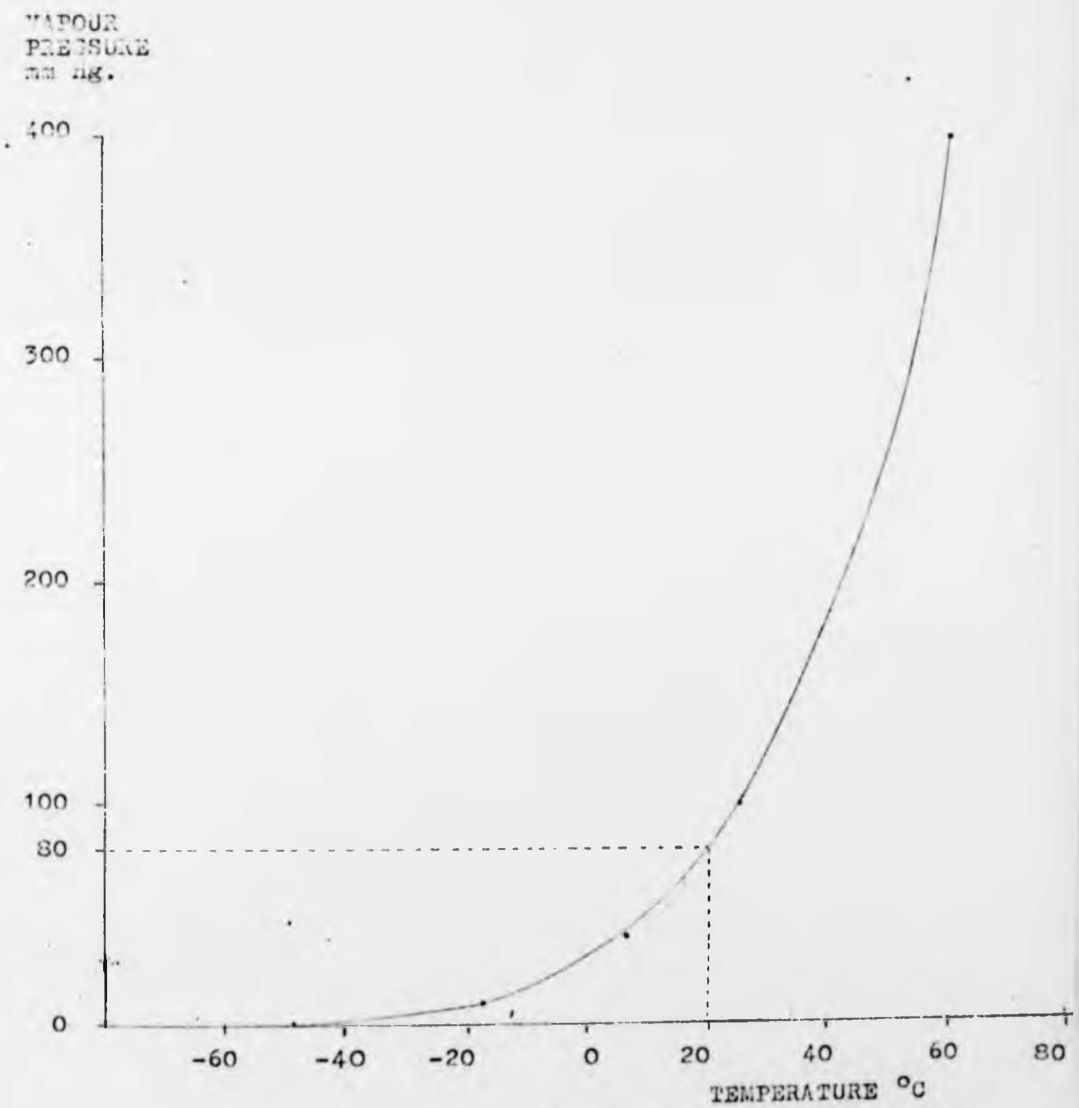


Fig. 6.2. Saturated Vapour Pressure Curve 2-Butanone.

Subjective sensations.

After inhalation the first subject above did not comment. The second subject described the effect "like drinking water used for washing hospital corridor floors". A third subject who inhaled the same concentration spat into the sink and described the effect as "horrible". A fourth subject (RD) confirmed this ketone was subjectively very distasteful and although not unbreathable was likely to be unacceptable to the average volunteer.

Eye Experiments.

Results.

Four subjects received acute eye exposures of MEK. The first (DP) experienced a "slight tingling" at 200ppm and "nothing" at 100ppm and 400ppm. The second subject (RD) reported "very slight lacrimation" at 100ppm and 200ppm, "nothing" at 400ppm and at 800ppm "some lacrimatory sensation on opening the eyes together with a suggestion of irritancy lasting for three seconds". The third subject (SMF) experienced "nothing" at 100ppm, but "irritancy, lasting for five seconds" at both 200ppm and 400ppm. At 800ppm this subject recorded "a bit of irritancy lasting for three seconds". The fourth subject (MT) found that 400ppm produced a sensation described as "faintly cool but no irritancy". At 800ppm there was "no effect".

Conclusion.

In concentrations up to 800ppm, MEK appears to be non irritant in three subjects and although a fourth subject found slight irritancy this was very transient.

PENTAN - 3-ONE

TLV not known

formula $C_5H_{10}O$

Methods.

The equipment and procedure were as described in the chapter on methods. The saturated vapour pressure of acetone at 20°C was obtained by interpolation from the graph shown in Fig. 6.3. to be 11 mm Hg.

Bronchoactivity Experiment.

Results.

Table 6.4. summarizes the results of inhalation of Pentan - 3-one which are shown graphically in Fig.6.4.

All six subjects showed a degree of bronchodilation at doses below that required for bronchoconstriction. Moreover the dose level at which reversal occurred varied between individuals. Two subjects (MT and SH) were still bronchodilating at 600ppm but increasing the dose to 800ppm produced marked bronchoconstriction with 43.5% and 52.7% drop in specific conductance respectively. A third subject (LI) showed bronchodilation at 400ppm and bronchoconstriction at 600ppm. The remaining three, all atopics, bronchodilated at 200ppm and bronchoconstricted at 400ppm (HR) and 600ppm (HS and SMF).

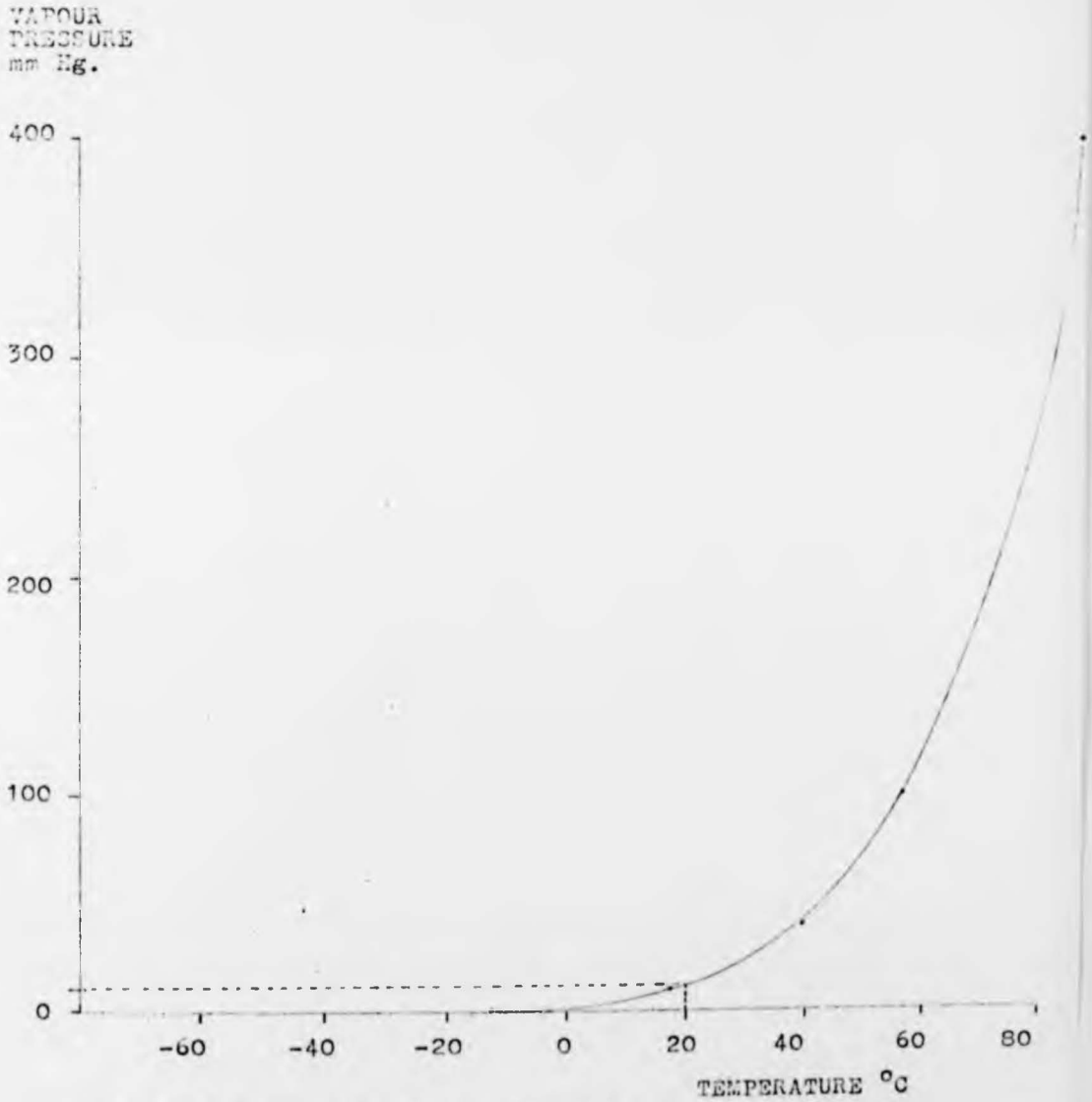


Fig. 6.3. Saturated Vapour Pressure Curve Pentan-3-one.

TABLE 6.4

PENTAN 3-ONE

SUBJECT	CONTROL RAW	CONTROL VTG	CONTROL SGAW	% CHANGE FROM CONTROL RAW	% CHANGE FROM CONTROL VTG	% CHANGE FROM CONTROL SGAW	DOSE	ATOPY	COMMENTS
M.T.	0.74	3.17	0.46	15.0	-0.1	-10.3	400	A	S: SLIGHT IRRITANCY IN TRACHEA PLUS FEELING OF ANAESTHESIA.
M.T.	0.54	3.46	0.54	26.1	-9.2	-25.0	600	A	S: INITIAL SENSATION OF ANAESTHESIA WHICH WORE OFF QUICKLY.
M.T.	0.53	3.33	0.57	-93.1	9.2	43.5	800	A	S: SENSATION FELT IN THE THROAT, DESCRIBED AS NOT IRRITANT.
S.H.	0.60	3.65	0.45	-20.0	1.7	16.0	200	NA	S: -
S.H.	0.56	3.66	0.47	6.9	0.5	-8.6	600	NA	S: COULD FEEL IT IN THE TRACHEA BUT IT WASN'T IRRITANT.
S.H.	0.50	3.35	0.60	-157.6	-10.6	52.7	800	NA	S: -
L.I.	0.60	3.02	0.55	14.9	-17.4	-1.2	400	NA	S: -
L.I.	0.60	3.16	0.53	-14.4	8.9	4.4	600	NA	S: -
H.R.	0.50	4.12	0.49	25.3	-13.1	-17.3	200	A	S: -
H.R.	0.64	3.68	0.43	-8.0	-3.1	10.6	400	A	S: TIGHTNESS IN LUNGS (INDICATED BOTTOM OF TRACHEA).
H.S.	0.75	6.34	0.21	-18.0	14.5	-1.7	200	A	S: -
H.S.	0.59	6.60	0.26	-31.6	13.2	12.4	600	A	S: -
S.M.F.	0.58	3.39	0.52	0.9	4.8	-6.2	200	A	S: -
S.M.F.	0.56	3.28	0.55	-65.0	1.4	38.4	600	A	S: WARMING SENSATION IN TRACHEA AFTER 2 MINS. CHEST TIGHTNESS IN 3RD MINUTE.
S.M.F.	0.56	3.88	0.46	-17.0	3.6	9.6	600*	A	S: -
S.M.F.	0.62	3.33	0.49	-7.3	2.0	4.5	1600**	A	S: UNPLEASANT BURNING IN TRACHEA CONTINUING FOR ½ HOUR. O: ON BASIS OF SINGLE BREATH DECIDED 3 BREATHS THE MAXIMUM FEASIBLE DOSE.

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

* = 5 BREATHS ONLY
** = 3 BREATHS ONLY

S = SUBJECTIVE COMMENT
O = OBSERVER COMMENT

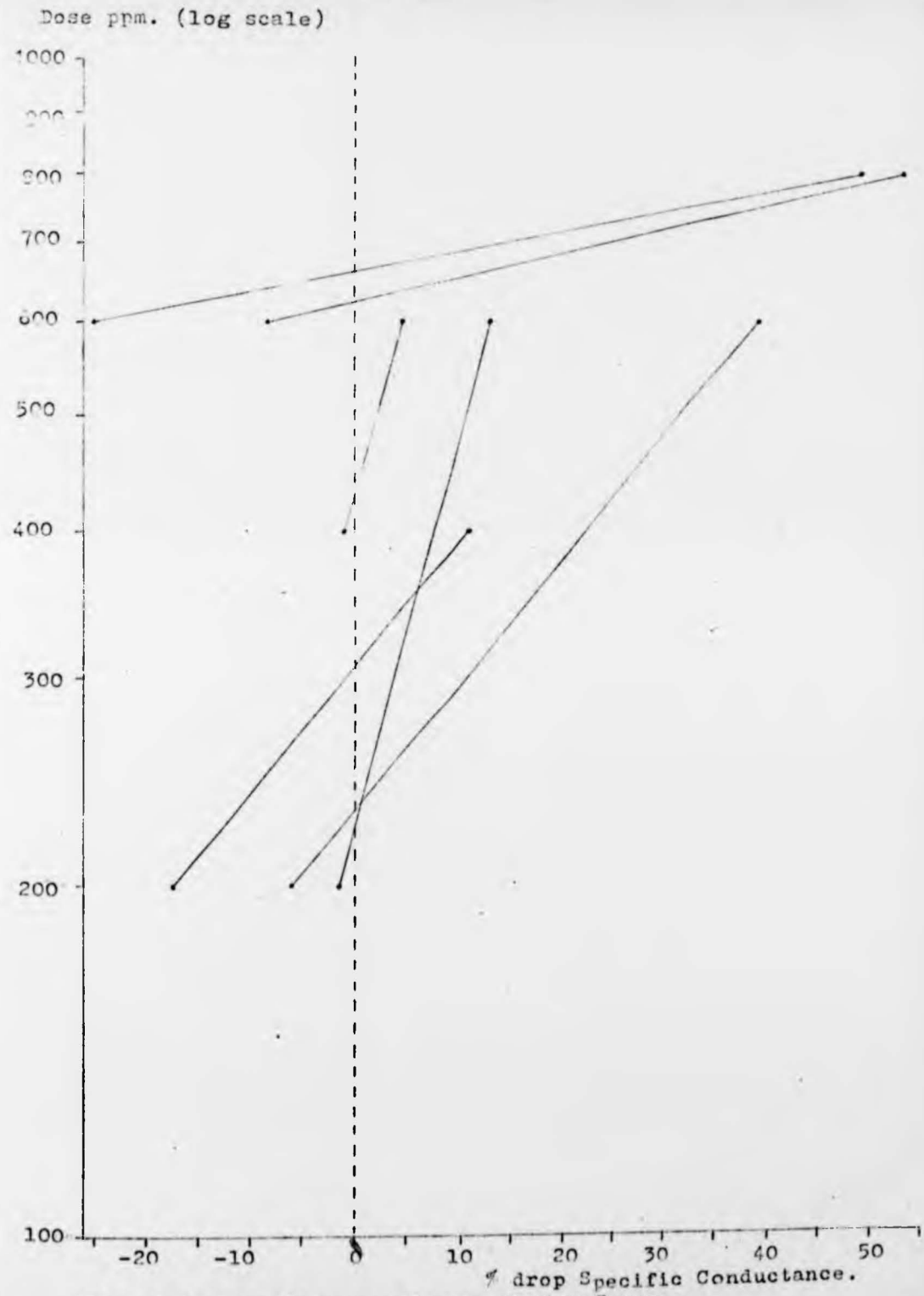


Fig. 6.4. Dose Response Curves Pentan-3-one.

This vapour was also used to investigate the effect of varying the number of breaths (see Methods)

Subjective sensations.

Some comments made by the subjects about the effect of pentan - 3-one are given in Table 6.4 It was generally regarded as producing unpleasant sensations localised in the trachea in contrast to acetone which was more general in its effect. In two subjects it produced burning and chest tightness which persisted for up to fifteen minutes. Another subject particularly emphasised the necessity for him to spit into the sink after inhalation.

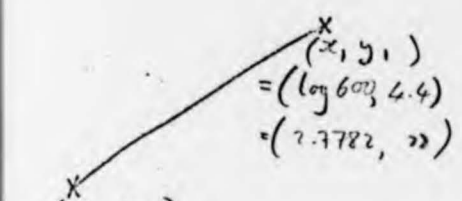
The initial bronchodilation could possibly be accounted for by relaxation of the airways smooth muscle. The effect of muscle tone on airways resistance has been reviewed by Widdicombe and Sterling (1970) and relaxation of muscle tone is usually only produced by stimulation of pulmonary stretch receptors.

Table 6.5 is a specimen of the calculation used to obtain the slope and intercept of the dose response curves. Fig. 6.4 shows the dose response curve for pentan - 3-one using the data of Table 6.4 The intercept on the abscissa may be considered as

name C. I

TABLE 6.5.

gas part-3-one TYPICAL CALCULATION OF INTERCEPT a AND SLOPE b. (STANDARDISED LAYOUT).



	N	L	
Δy	5.6	0.7482	
Δx	0.1761	$\bar{1}$.2458	sub
[b]	31.80	1.5024	
(x_1)	2.7782	0.4438	add
$(x_1)[b]$	88.35	1.9462	
$y_1 - x_1[b] = a$	(-)83.95	1.9243	
[b]	31.80	1.5024	sub
a/b	(-)2.642	0.4219	

88.35
4.4
83.95

Threshold dose = antilog $(-a/b)$

= antilog (+2.642)

= 438.5 ppm

the lung threshold dose for bronchoconstriction. The calculated values are shown in Table 6.6. The arithmetic mean of the lung thresholds is 414ppm and the geometric mean 377ppm. One subject (LI) is of interest. His intercept of 439ppm is quite close to the mean. He suffers from allergic rhinitis but his prick test results were negative for grass pollen, house mite and aspergilus. The remaining five subjects fall into two groups: the sensitive and the less-sensitive. Students "t"-test reveals that the mean thresholds for the two groups were significantly different ($t = 9.9$, $p < .01$).

The mean for the more sensitive group is 253.4ppm and for the less sensitive 645ppm. Thus a TLV of 200ppm may be too high to avoid bronchoconstriction in all individuals.

Eye Experiments.

Results.

Table 6.6. summarises the results of the eye experiments. Eight subjects were exposed to doses of two, four, eight and sixteen hundred ppm of pentan - 3-one. Six of the subjects showed a response at eight hundred ppm. One of the subjects (MLT) felt irritation which wore off in five seconds and described it as "a minimum irritant sting", while another subject (SMF) felt "stinging for fifteen seconds plus slight lacrimation".

TABLE 6.6.

PENTAN-3-ONE

SUBJECT	ATOPY	EYE THRESHOLD DOSE PPM	LUNG THRESHOLD DOSE PPM	SLOPE OF DOSE RESPONSE CURVE
S.M.F.	A	800	232.8	93.46
M.T.	A	800	668.3	548.4
L.I.	NA	800	438.5	31.80
S.H.	NA	800	622.3	490.8
H.S.	A	800	219.8	40.86
H.R.	A	4-800	307.6	92.68
R.D.	NA	1,600		
M.L.T.	NA	800		

At 400ppm this same subject (SMF) could detect a "very slight non irritant sensation on opening the eyes which was gone within two seconds". Subject MLT also reported a "slight sensation, gone in three seconds" at four hundred ppm, as did SH, LI and HS. MT experienced no such sub-irritant sensation at 400ppm but at 800ppm reported "definitely irritant for fifteen seconds although wearing off a little towards the end".

Of the two remaining subjects (HR) observed at 400ppm "very little irritancy - somewhere round the threshold". At 800ppm he reported simply "definitely irritant". Also at 800ppm subject RD experienced "cold for the first five seconds on opening eyes but not irritancy". At 1600ppm this subject observed "stinging for fifteen seconds plus slight lacrimation".

Conclusion.

An acute exposure of the eyes to pentan - 3-one produces a sub-irritant sensation in most people at 400ppm which becomes unmistakably irritant at 800ppm.

PENTAN - 2-ONE

TLV = 200ppm.

formula = $C_5H_{10}O$

Methods.

The equipment and procedure were as described in the chapter on methods. The saturated vapour pressure of acetone at 20°C was obtained by interpolation from the graph shown in Fig. 6.5. to be 11mm Hg.

Bronchoactivity Experiment.

Results.

Table. 6.7. summarizes the results of inhalation of Pentan - 2-one which are shown graphically in Fig.6.6.

All four subjects showed bronchoconstriction in response to the larger of the two doses. Three of the subjects showed virtually no response at 400ppm, while the fourth, an atopic, showed a 57.4% increase in resistance and a 40.2% drop in specific conductance. At 200ppm this subject had slight bronchoconstriction (29.4% increase in resistance and 24.7% drop in specific conductance). Of the other three, one showed 26.7% fall in specific conductance at 600ppm and the other two showed drops in this index of 37.0% and 30.6% at 800ppm. In all cases of bronchoconstriction, the percentage increase in airways resistance was greater than the drop in specific conductance shown graphically in Fig.6.6.

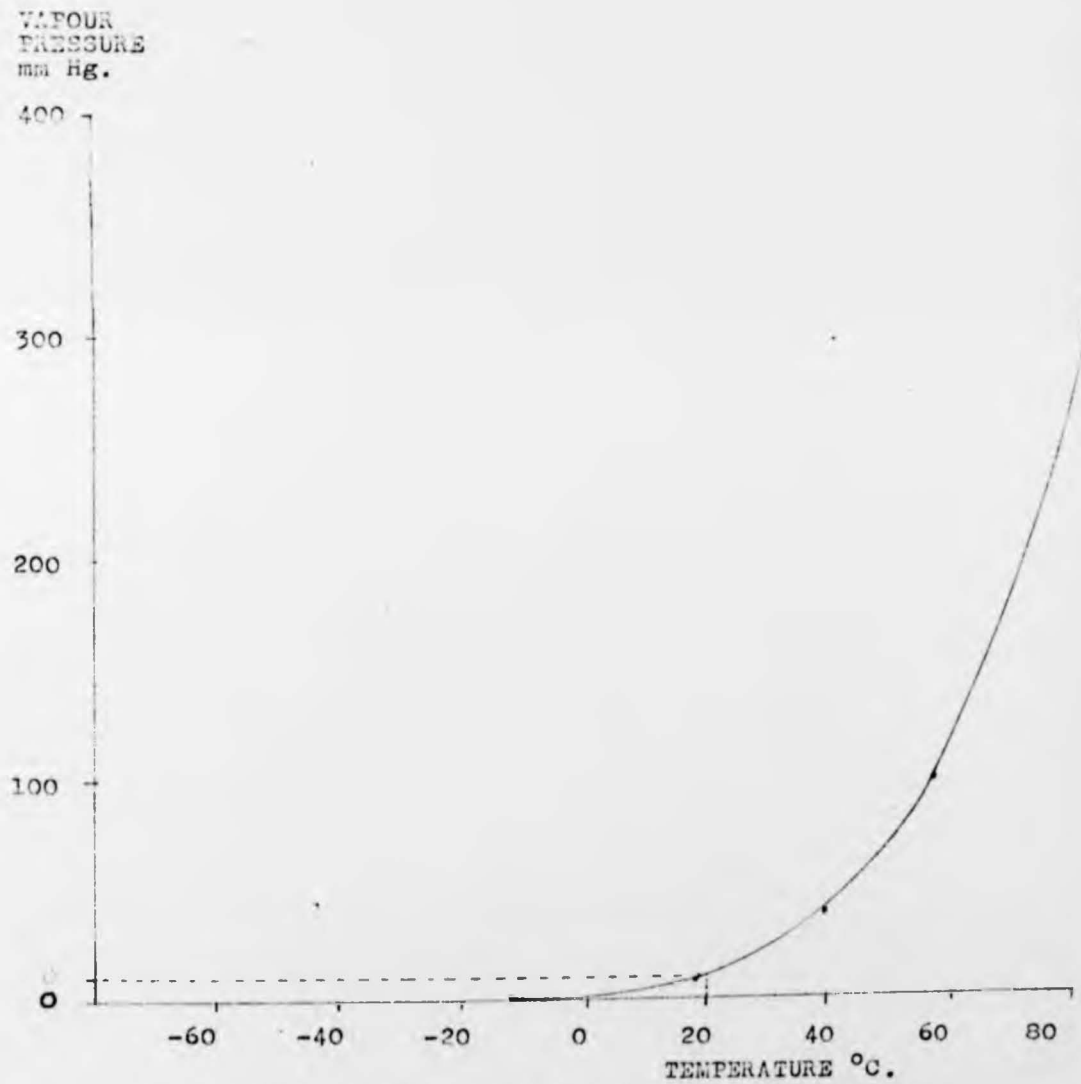


Fig. 6.5. Saturated Vapour Pressure Curve Pentan-2-one.

TABLE 6.7.

PENTAN 2-ONE

SUBJECT	CONTROL RAW	CONTROL VTG	CONTROL SGAW	% CHANGE FROM CONTROL RAW	% CHANGE FROM CONTROL VTG	% CHANGE FROM CONTROL SGAW	DOSE	ATOPY
S.M.F.	0.63	3.46	0.46	-29.4	-2.6	24.7	200	A
S.M.F.	0.62	3.49	0.46	-57.4	-5.8	40.2	400	A
P.G.	0.60	3.47	0.49	0.9	3.5	-4.3	400	NA
P.G.	0.64	3.39	0.46	-37.2	0.1	26.7	600	NA
P.M.	0.68	3.83	0.39	-1.6	-1.8	3.4	400	NA
P.M.	0.60	3.86	0.44	-54.8	-1.9	37.0	800	NA
M.T.	0.61	3.25	0.51	1.6	-0.3	-1.3	400	A
M.T.	0.64	3.36	0.47	-41.1	-1.6	30.6	800	A

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

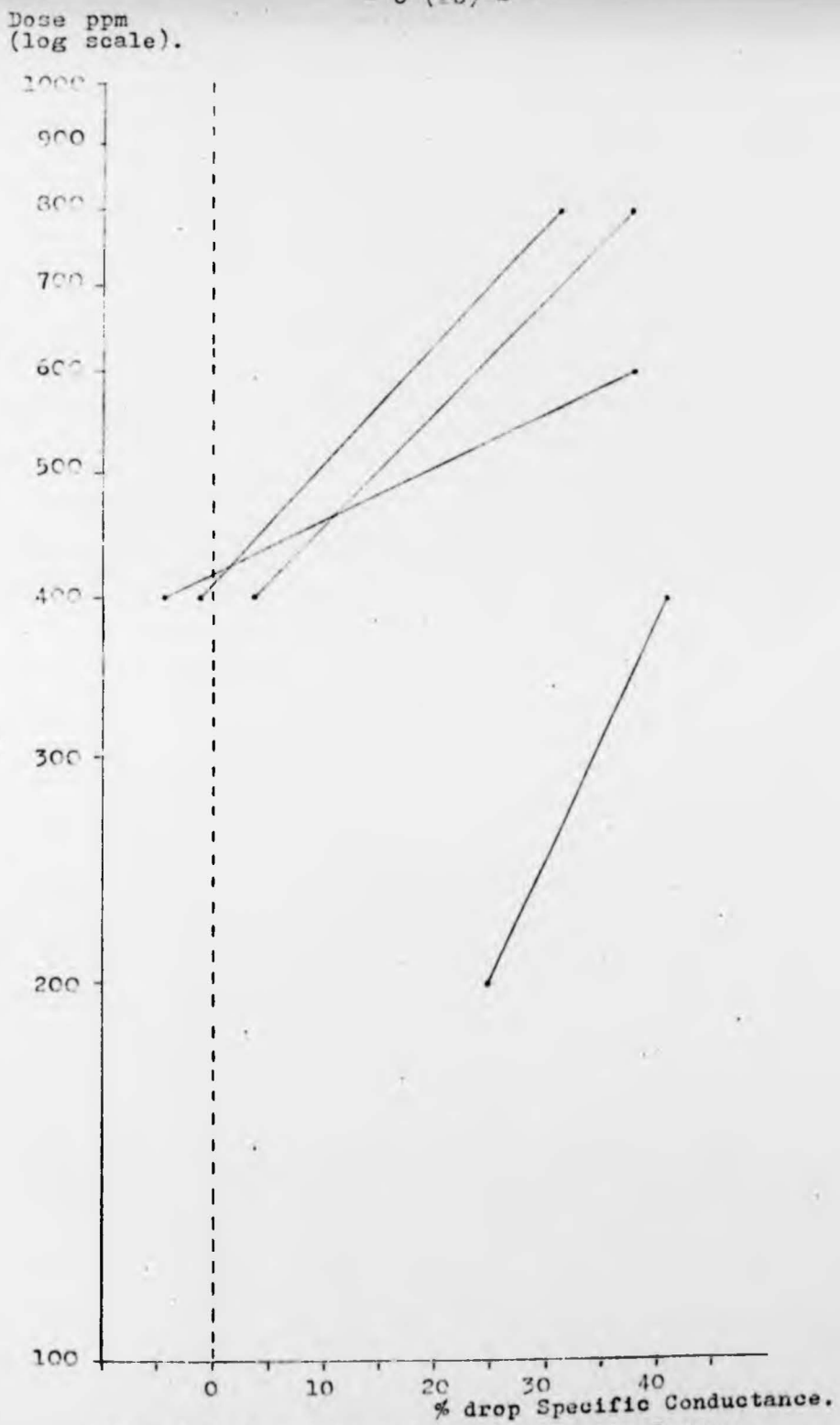


Fig. 6.6. Dose Response Curves Pentan-2-one.

TABLE 6.8.

PENTAN-2-ONE

SUBJECT	ATOPY	EYE THRESHOLD DOSE PPM	LUNG THRESHOLD DOSE PPM	SLOPE OF DOSE RESPONSE CURVE
S.M.F.	A	400	62.95	49.82
M.T.	A	800	408.3	106.3
P.G.	NA	800	421.7	176.0
P.M.	NA	800	349.9	112.9
R.D.	NA	800		
L.I.	NA	400		
S.H.	NA	400		
H.S.	A	400		
H.R.	A	400		
M.L.T.	NA	400		

Subjective sensations.

Pentan - 2-one was the most bronchoactive of the ketones investigated. The subjects commented on the "taste of pear drops" after inhalation. The intercept values, together with the slopes of the dose response curves are summarized in Table 6.8. Students "t"-test revealed the lung threshold dose for the sensitive subject (63ppm) to be significantly different from the mean threshold dose (393ppm) for the other subjects ($t = 15.70$, $p < .01$). The existing Threshold Limit Values for Pentan - 2-one is quoted at 200ppm (Amer. Conf. Govt. Ind. Hyg. 1973). This would seem to be appropriate for three of the subjects but not necessarily for the sensitive subject.

Eye Experiments.

Results.

In this experiment, the Mark II goggles Fig.3.3 with the parallel supply and gas stream deflectors were introduced. Two subjects were exposed to two, four, eight and sixteen hundred ppm. From the first subject, (RD), 200 and 400ppm elicited no response. 800ppm produced "stinging for eight seconds and then lacrimation", while 1600ppm produced "severe stinging for ten seconds when lacrimation begins, stinging continued at lower degree for full fifteen seconds. Noticeably stronger than 800ppm. The second subject found nothing at 200ppm but with

400ppm experienced "definite stinging throughout" and lacrimation. 800ppm produced "stinging throughout but indistinguishable from 400ppm" and again lacrimation. At 1600ppm there was "very severe stinging throughout the fifteen seconds which made it difficult to keep the eyes open. Definitely more irritant than the other two".

Eight other subjects were exposed to the above doses except sixteen hundred ppm and in addition one at 100ppm. Three of the subjects were also exposed to zero dose which produced no effect. The thresholds for the ten individuals are recorded in Table 6.8

Conclusion.

Six persons had a threshold eye irritation at 400ppm and four persons at 800ppm following an acute exposure to pentan - 2-one. This supports the existing TLV of 200ppm.

3 - METHYL - BUTAN - 2-ONE

TLV not known

formula = $C_5H_{10}O$

Methods.

The equipment and procedure were as described in the chapter on methods. The saturated vapour pressure of acetone at 20°C was obtained by interpolation from the graph shown in Fig. 6.7. to be 21 mm Hg.

Bronchoactivity Experiment.

Results.

The results of two trials are tabulated in Table 6.9. Fig. 6.8. shows the dose response curves obtained for the first trial in which four subjects inhaled two doses, 800ppm and 1600ppm. A fifth subject (PM) inhaled 800ppm only. There were only small and inconsistent changes at these low doses and although it can be seen that with one exception the small changes observed were bronchoconstricting it was decided that insufficiently high doses had been administered.

The experiment was repeated later using (Fig.6.9.) doses up to 2400ppm and four new subjects. Two subjects (PC and AB) showed considerable bronchoconstriction at 1600ppm. PC and AB showed respectively 43.2% and 33.8% drops in specific conductance. At 1200ppm the falls were 32.4% and 19.7% respectively. The other

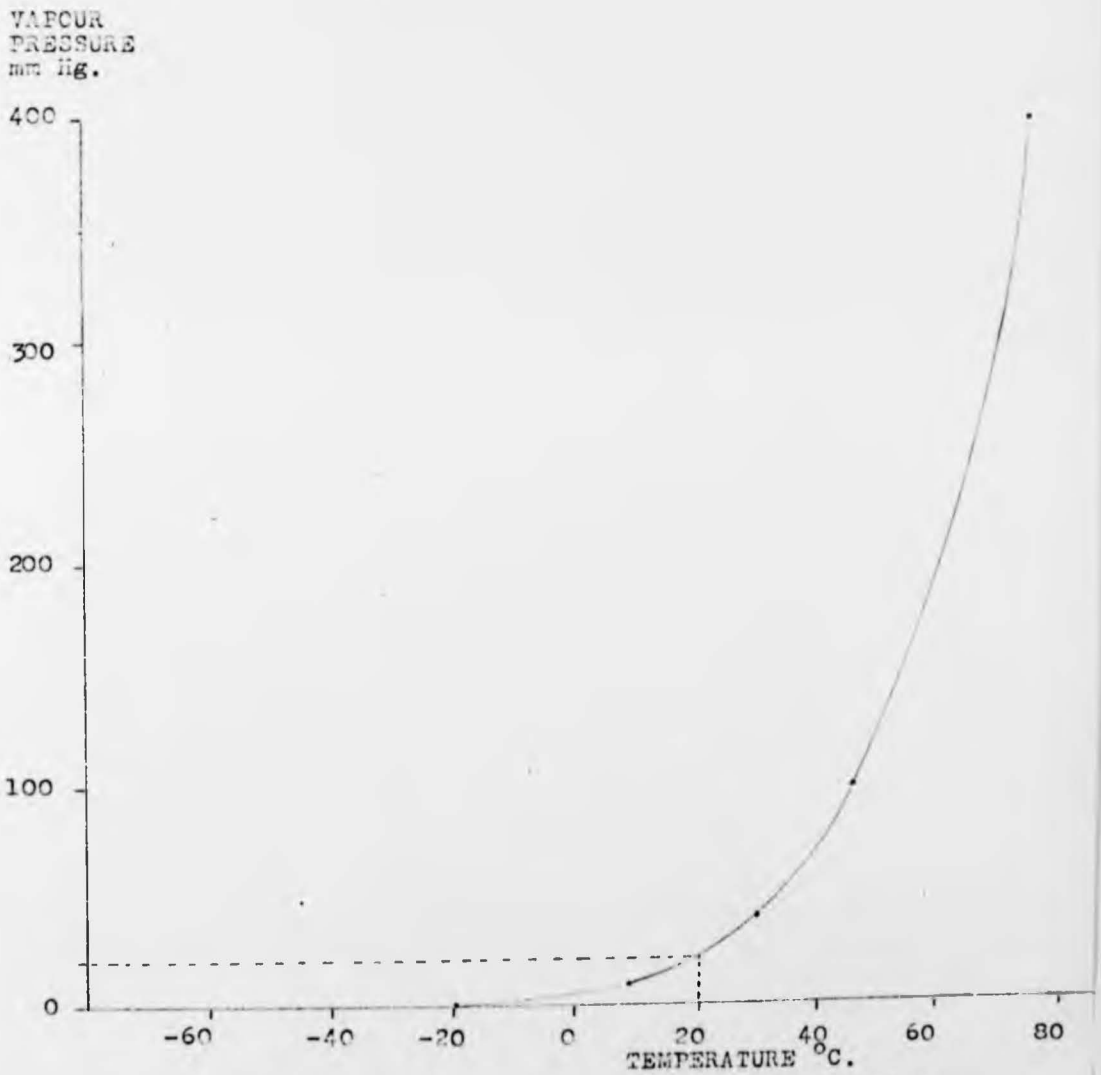


Fig. 6.7. Saturated Vapour Pressure Curve 3-Methylbutan-2-one.

TABLE 6.9

2-METHYLBUTAN-2-ONE

SUBJECT	CONTROL RAW	CONTROL VTG	CONTROL SGAW	% CHANGE FROM CONTROL RAW	% CHANGE FROM CONTROL VTG	% CHANGE FROM CONTROL SGAW	DOSE	ATOPY	COMMENTS
M.T.	0.50	4.68	0.43	-1.4	-11.0	11.9	800	A	S: -
M.T.	0.56	4.05	0.44	-24.3	12.1	8.9	1600	A	S: -
P.G.	0.61	3.62	0.45	-6.9	-0.5	6.7	800	NA	S: -
P.G.	0.54	3.73	0.51	5.2	-8.6	2.7	1600	NA	S: UNPLEASANT AND SENSATION OF WARMTH IN TRACHEA.
H.S.	0.59	7.32	0.23	-11.6	4.3	7.3	800	A	S: -
H.S.	0.53	5.04	0.39	2.9	6.9	-9.5	1600	A	S: -
L.I.	0.57	4.88	0.36	-1.9	-9.8	10.3	800	NA	S: -
L.I.	0.56	3.78	0.47	-16.3	2.0	14.7	1600	NA	S: -
P.M.	0.56	5.04	0.37	-2.0	2.1	0.8	800	NA	S: -
C.C.	0.43	4.30	0.54	-22.4	-6.2	23.0	1600	A	S: -
C.C.	0.49	4.56	0.45	-61.6	-13.5	45.6	2400	A	S: -
M.B.	0.53	3.94	0.48	-21.9	13.6	5.1	1600		S: -
M.B.	0.35	4.31	0.68	-86.9	4.9	44.5	2400		S: -
P.C.	0.54	3.27	0.57	-38.7	-7.9	32.4	1200	NA	S: -
P.C.	0.37	4.23	0.64	-70.1	-3.2	43.2	1600	NA	S: -
A.B.	0.62	2.74	0.59	-24.6	-0.6	19.7	1200		S: -
A.B.	0.53	3.09	0.63	-46.2	-3.5	33.8	1600		S: -

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

S = SUBJECTIVE COMMENT.

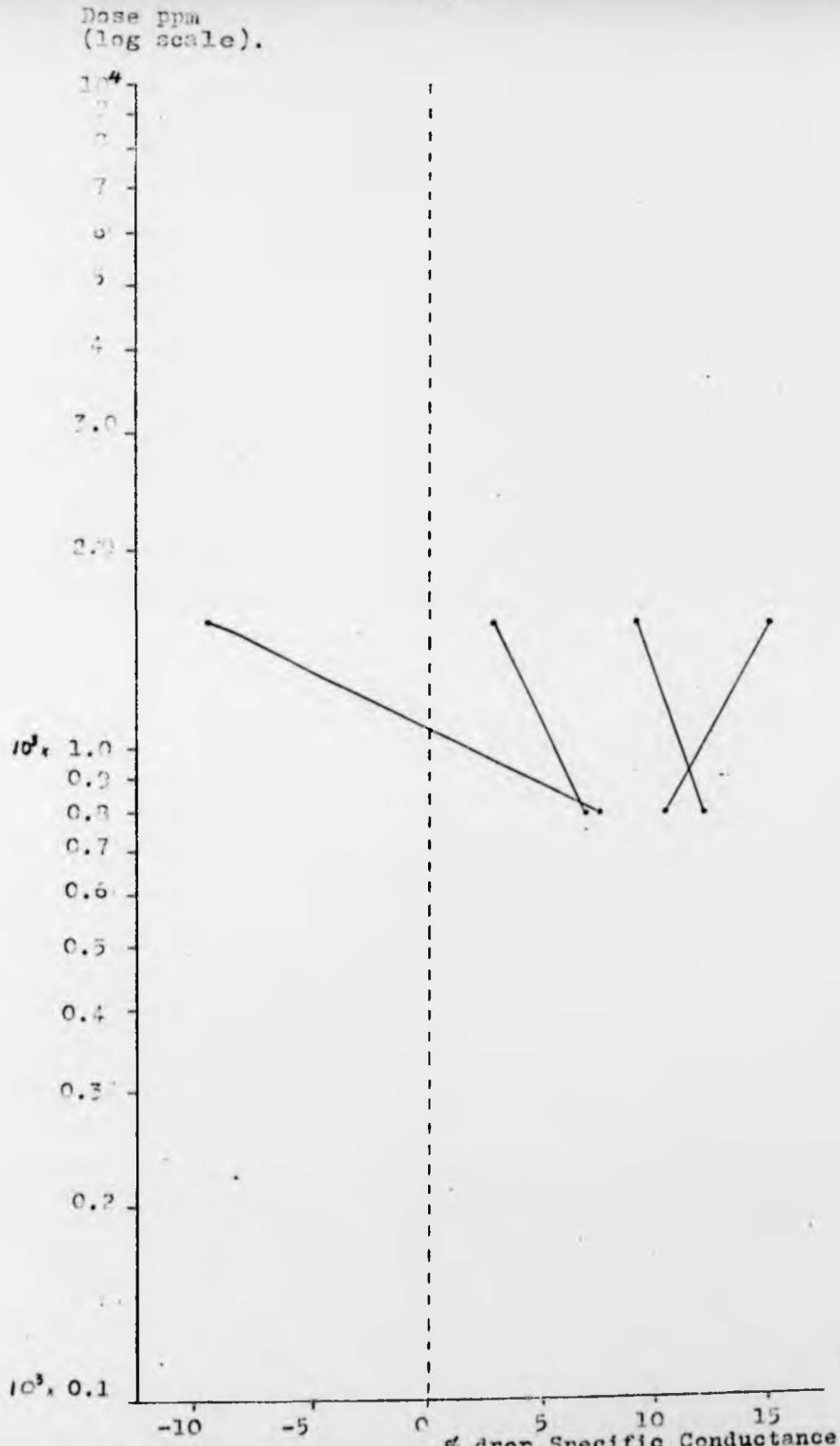


Fig.6.8. Dose Response Curves 3Methylbutan-2-one (1st trial).

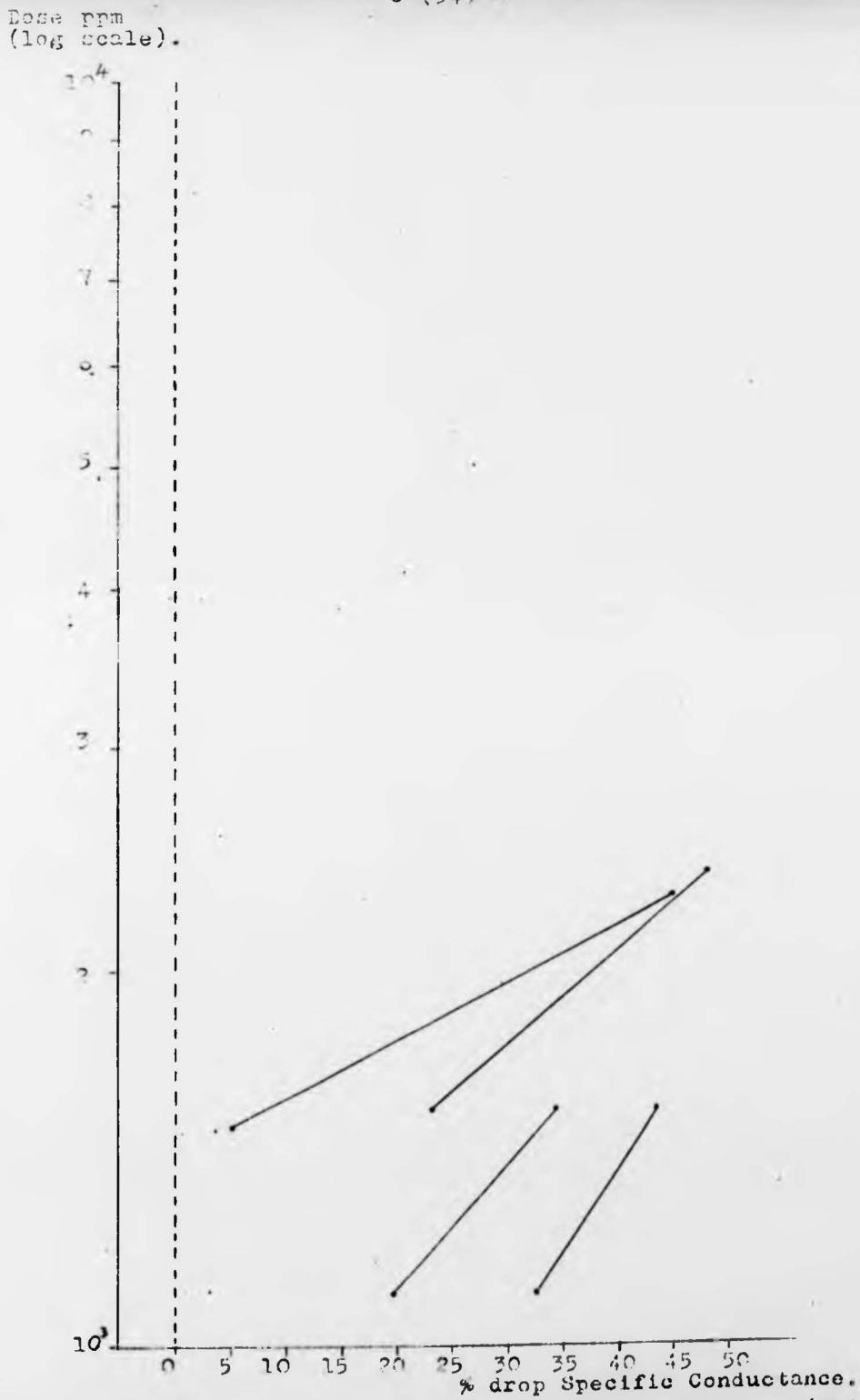


Fig.6.9. Dose Response Curves 3-Methylbutan-2-one (2nd trial

TABLE 6.10.

3-METHYLBUTAN-2-ONE

SUBJECT	ATOPY	EYE THRESHOLD DOSE PPM	LUNG THRESHOLD DOSE PPM	SLOPE OF DOSE RESPONSE CURVE
M.T.	A	>1600		
P.G.	NA	1600		
H.S.	A	1600		
L.I.	NA	1600		
C.C.	A		1057	128.3
M.B.	-		1393	369.1
P.C.	NA		505.8	86.48
A.B.	-		799.8	112.9
S.M.F.	A	1600		
R.D.	NA	2400		
P.M.	NA	1600		
H.R.	A	1600		

two subjects (CC and MB) were less susceptible at 1600ppm showing 23.0% and 5.1% drops in specific conductance respectively. However at 2400ppm the changes were 45.6% and 44.5% respectively.

Subjective sensations.

This isomer was generally regarded as the most unpleasant of the pentanones but the least irritant to inhale.

The parameters of the dose response curves for the second trial are given in Table 6.10. The thresholds range from 506ppm to 1393ppm with an arithmetic mean of 939ppm (geometric mean 879ppm).

Eye Experiments.

The results are summarized in Table 6.11. The eyes of eight subjects were exposed to concentrations of 400ppm and 800ppm. In addition two of these subjects were given 200ppm. None of the subjects experienced any irritation. At 1600ppm six subjects experienced slight but definite irritation. One of the subjects (HR) blinked constantly throughout the fifteen seconds. Of the other two subjects one (MT) experienced a sensation described as "cold but not irritating". The other subject (RD) experienced "a sensation bordering on irritancy for a full fifteen seconds and lacrimation from five until fifteen seconds".

At 2400ppm one subject (RD) experienced "stinging for fifteen seconds. Definite but not so bad as SO_2 ". A second subject (SMF) found "definite but not severe stinging throughout the fifteen seconds. It also stops on removing the goggles: in marked contrast to acrolein." The subject was observed to blink excessively for fifteen seconds.

Conclusion.

These experiments indicate that 1600ppm is the threshold of eye irritancy for an acute exposure to 3 - methyl - butan - 2-one.

CHAPTER 7.

ALDEHYDES.

Introduction.

The name aldehyde is said to be derived from the words alcohol dehydrogenated which refer to the oldest methods of synthesis of aldehydes by the oxidation of alcohols. Thus methyl and ethyl alcohols can be converted to formaldehyde and acetaldehyde respectively. The oxidation of natural gas can also be used as a means of producing formaldehyde. Acetaldehyde may be prepared by reaction of water with acetylene and acrolein may be obtained from the denydration of glycerol.

Industrially aldehydes are of great importance. They are used in the manufacture of resins, as intermediates in the synthesis of alcohols, acid and other chemicals: they are also used in rubber, tanning and paper industries and also in agriculture. Aldehydes are used in the manufacturing of medicinals and dyes while formaldehyde in particular is used as a deodorizing, bactericidal agent and as a hardening agent for proteins.

The physical and chemical properties of the aldehydes studied are listed in Table 7.1. An important characteristic of aldehydes is the tendency to polymerize.

TABLE 7.1

PHYSICAL AND CHEMICAL PROPERTIES OF ALDEHYDES INVESTIGATED.

NAME	MOLECULAR FORMULA	STRUCTURAL FORMULA	M.Wt.	SOLUBILITY g 100 ml.$^{-1}$ H_2O	ETHER	B.P. °C
Formaldehyde	CH_2O	H.CHO	30.03	S	S	-21
Acetaldehyde	C_2H_4O	CH_3.CHO	44.05	∞	∞	21
Acrolein	C_3H_4O	CH_2.CH.CHO	56.06	40	S	52.5

S = solubility between 10 - 50 g 100 ml.$^{-1}$.

Sources: Seidell, "Solubility of Organic Compounds" 3rd edition vol. 2, Van Nostrand (1941).

Merck Index of Chemicals and Drugs 7th edition, Merck & Co. Inc. N.J. U.S.A. (1960).

"Handbook of Chemistry and Physics" 39th edition, The Chemical Rubber Rub. Co. Ohio.

The aldehydes are considered to be irritants. Irritancy is usually greatest in those with low molecular weights. Patty (1967) states that the general and parenteral toxicities of these molecules appear to be related primarily to the irritant properties.

ACROLEIN.

TLV = 0.1ppm

formula = $CH_2 = CHCHO$

Methods.

Equipment.

Preliminary experiments with acrolein were made using the production system used for the ketones. Although the system was shown to be leak-tight using a water manometer, unacceptable concentrations of acrolein escaped into the laboratory air. The explanation was thought to be that acrolein was permeating the polypropylene connecting tubes. Accordingly the system was rebuilt from glass and stainless steel (see methods). The cold bath was maintained at $0^{\circ}C$ throughout its depth by packing with crushed ice. The "hot" bath had melting ice floating on its surface and the temperature was about $2^{\circ}C$.

Procedure.

The procedure was the same as used for the ketones except for a modification to the eye exposure experiments. During the experiments it became apparent that the time course of events for acrolein was different from the ketones. Accordingly longer exposures were introduced with the concentration in the goggles maintained by a trickle flow of 0.4 l min^{-1} .

Measurement.

The concentration of acrolein was calculated

from a knowledge of the saturated vapour pressure of acrolein at 0°C obtained by interpolation from the graph shown in Fig. 7.1 to be 30mm Hg.

Bronchoactivity Experiment.

Results.

Table 7.2 summarizes the results of inhaling acrolein which are shown graphically in Fig. 7.2. Four subjects were exposed to two concentrations each: 5.5ppm and 8.25ppm. Three subjects bronchoconstricted at the lower concentration (mean drop in specific conductance 22.8%). The remaining subject (HR) showed little change at this dose (3.1% increase in resistance and 6.3% drop in specific conductance). At the higher dose, all four subjects bronchoconstricted showing a mean drop in specific conductance of 38.9%.

Subjective sensations.

All four subjects found acrolein to be irritant at both 5.5 and 8.25ppm. At the lower dose the first two subjects (HR and HS) noted a "prickling in the throat" after three breaths. In addition the first subject reported an "aching stiffness in the chest" one and a half hours later when starting to smoke. The third subject (SMF) experienced a "burning

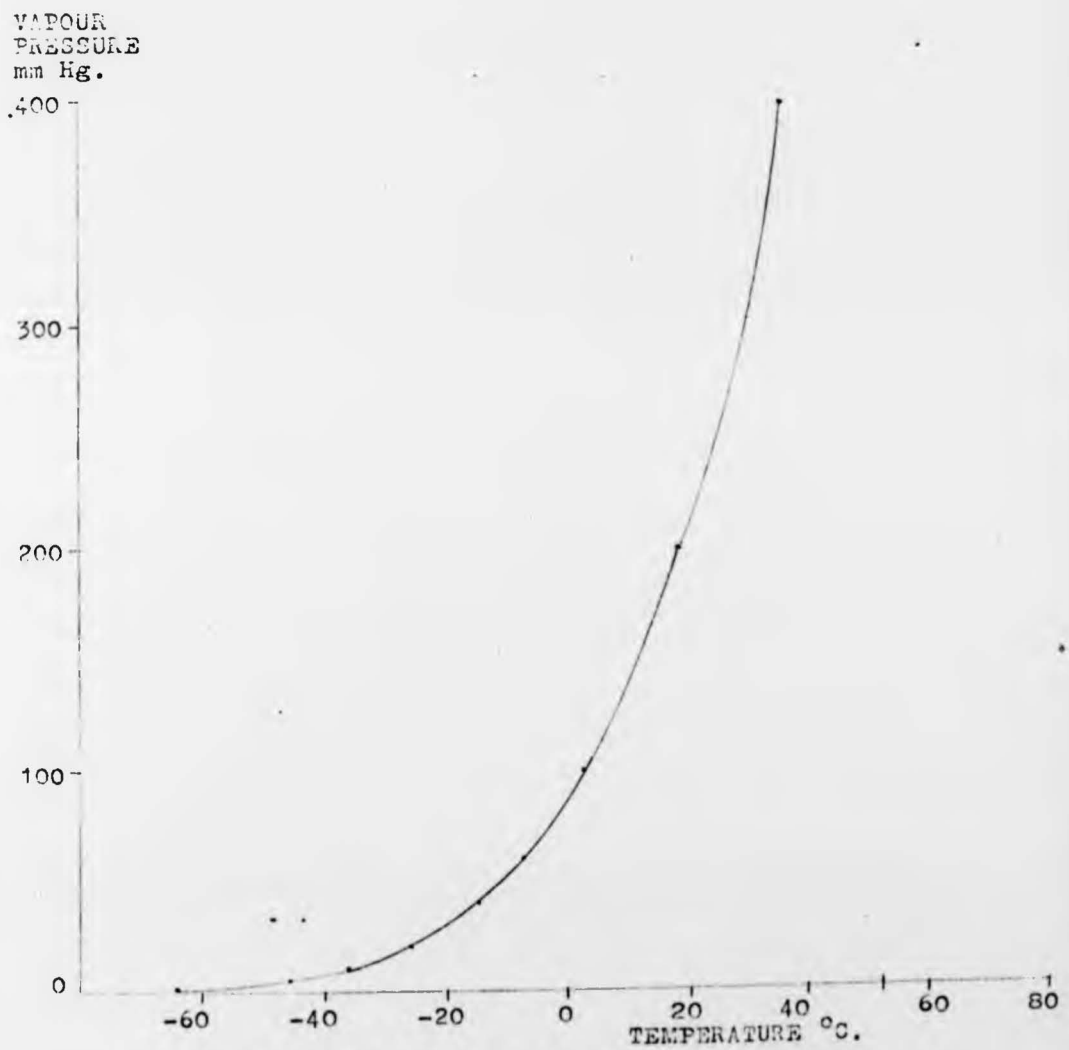


Fig. 7.1. Saturated vapour pressure curve acrolein.

TABLE 7.2.

ACROLEIN

SUBJECT	CONTROL			% CHANGE FROM CONTROL			DOSE	ATOPY	COMMENTS
	RAW	VTG	SGAW	RAW	VTG	SGAW			
H.R.	0.82	6.25	0.20	-3.1	7.8	-6.3	5.5	A	S: PRICKLING IN THROAT AFTER 3 BRSATHS. ACHING STIFFNESS IN CHEST 1½ HOURS LATER WHEN STARTED SMOKING.
H.R.	0.58	6.53	0.26	-60.8	2.7	35.6	8.25	A	S: PRICKLING ON 3RD BREATH INCREASED AFTER 5. 10TH BREATH REQUIRED CONSCIOUS EFFORT.
H.S.	0.68	3.38	0.44	-16.9	-11.5	22.8	5.5	A	S: PRICKLY FEELING IN THROAT AFTER 3-4 BREATHS.
H.S.	0.36	4.41	0.63	-93.7	-0.4	47.0	8.25	A	S: IRRITANT AT 3RD BREATH DESIRE TO COUGH WHICH BECAME WORSE WITH MORE BREATHS. RESIDUAL IRRITATION AFTER 50 MINS.
S.M.F.	0.70	3.75	0.38	-38.2	4.7	23.7	5.5	A	S: BURNING IN TRACHEA AFTER 9 BREATHS. ACHE IN CHEST AFTER 6 MINS. CONTINUING FOR ONE HOUR.
S.M.F.	0.64	3.31	0.48	-57.9	-11.6	43.5	8.25	A	S: BURNING IN TRACHEA AFTER 5 BREATHS GETTING WORSE. STILL SORE 45 MINS. LATER.
G.C.	0.84	4.82	0.24	-20.1	-6.3	21.9	5.5	A	S: BURNING IN THROAT AFTER 6 BREATHS.
G.C.	0.95	5.04	0.21	-48.8	0.9	29.6	8.25	A	S: IRRITATION AFTER 3 BREATHS.

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

S = SUBJECTIVE COMMENT.

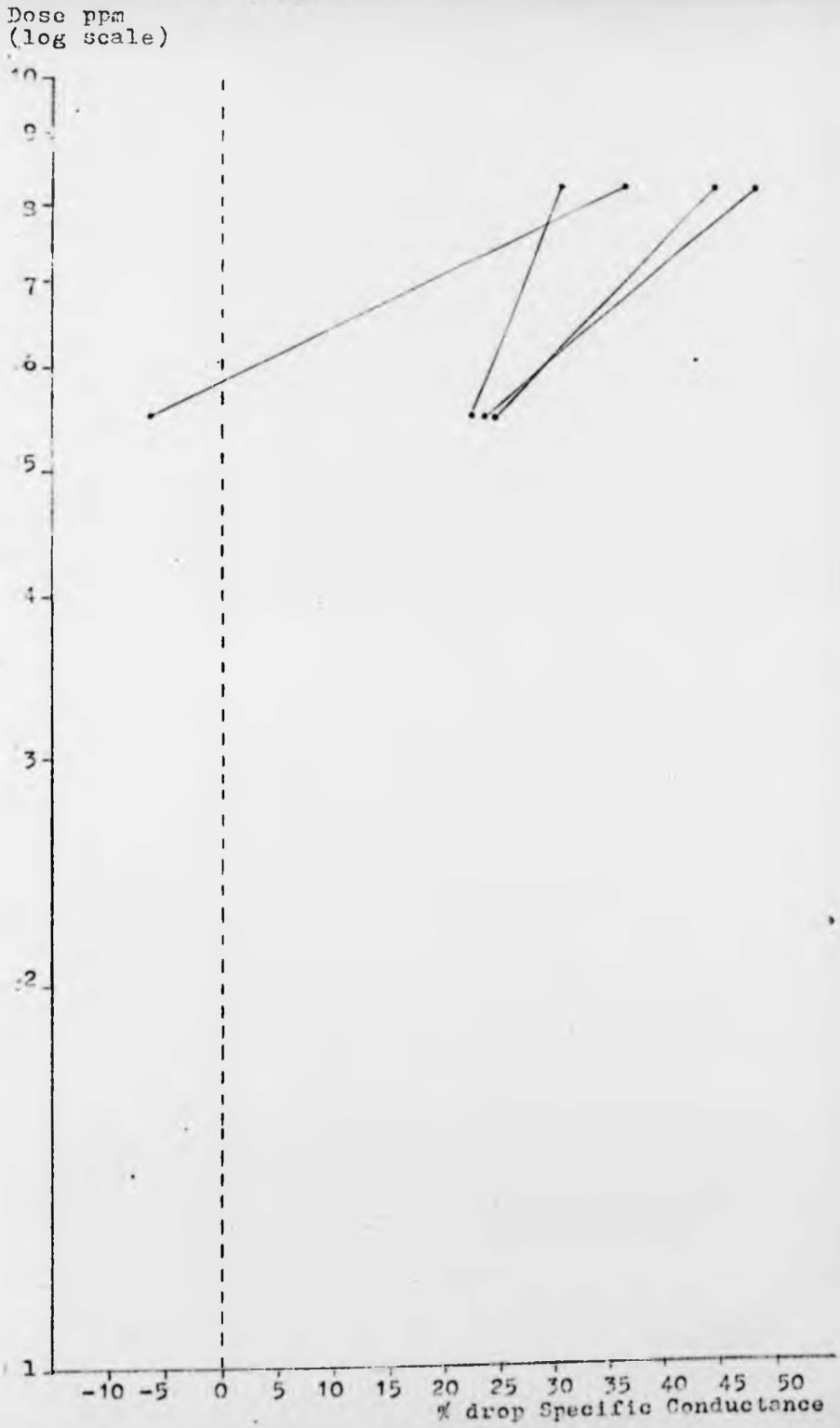


Fig.7.2. Dose response curves Acrolein.

in the trachea after nine breaths", also an "ache in the chest after six minutes continuing for one hour." The fourth subject (GC) noted a "burning in the throat after six breaths".

At the higher dose the first two subjects again reported sensation at the third breath. The first (HR) noted that the "prickling at the third breath increased after the fifth" and that the "tenth breath required conscious effort". The second subject (HS) now reported "irritancy at the third breath" with a "desire to cough which became worse with more breaths"; he had residual irritation after fifty minutes. The third subject (SMF) observed "burning in the trachea after five breaths getting worse thereafter"; the effects were still present fortyfive minutes later. The fourth subject (GC) reported "irritation after three breaths".

Table 7.3. shows the calculated parameters of the curves shown in Fig. 7.2. The intercepts range from 1.9ppm to 5.8ppm with an arithmetic mean of 4.1ppm (geometric mean 3.8ppm).

Eye Experiments.

Results.

Table 7.4. summarizes the results of eye

TABLE 7.3.

ACROLEIN

SUBJECT	ATOPY	EYE EXPERIMENTS		LUNG THRESHOLD DOSE PPM	SLOPE OF DOSE RESPONSE CURVE
		CONC. PPM.	TIME OF ONSET OF IRRITATION		
H.R.	A			5.843	237.9
H.S.	A	12.5	10	5.362	250.9
S.M.F.	A	5.5	60	3.382	112.4
		10.0	11		
		12.5	10		
G.C.	A	12.5	30*	1.970	44.37
R.D.	NA	5.5	35		
		10.0	3		
M.L.T.	NA	5.5	25		
		10.0	10		
		12.5	5		
L.K.	A	12.5	10		
D.P.	A	12.5	10		

* Swims daily in chlorinated pool.

exposures of seven subjects to acrolein. Four subjects experienced irritation beginning at ten seconds when exposed to 12.5ppm. Subject HS remarked "nothing until ten seconds and then getting worse to fifteen seconds". The second subject (SMF) reported "irritant after ten seconds and still building up. A very different irritancy from the ketones. Fading away after about forty seconds." The third subject (LK) observed "stinging after ten seconds; wearing off after sixty seconds". The fourth subject (DP) recorded "stinging after ten seconds and getting worse to fifteen seconds. Wearing off after sixty seconds." A fifth subject also exposed to 12.5ppm (MLT) reported "irritation at five seconds getting rapidly worse till ten seconds". The goggles were removed at ten seconds and the subject reported that after sixty seconds there was "only residual stinging". A sixth subject (GC) also received 12.5ppm and experienced "nothing until fifteen seconds after removal of goggles after which there was irritancy".

Three subjects were also exposed to 10ppm. The first subject (SMF) observed stinging at eleven seconds and the second subject (MLT) made similar comment at ten seconds. The third subject (RD) found it "very irritant from three seconds".

The same three subjects were exposed (one

week later) to 5.5ppm. The first subject (RD) found "slight pricking after thirtyfive seconds which wore off in sixty seconds". The second subject (SMF) reported "nothing until sixty seconds, then soreness in eyes which continued until after ninety seconds". The third subject (MLT) recorded "irritation at twenty-five seconds". A later repeat of this dose for subject MLT with flow maintained as a trickle (see methods) produced "irritation at twentyfive seconds which rapidly became worse inducing involuntary blinking".

Conclusion.

The product of concentration (C) and time to produce irritancy (T) gave a range of CT values of [illegible] ppm secs. to [illegible] ppm secs. The arithmetic mean was [illegible] ppm secs. and the geometric mean [illegible] ppm secs.

FORMALDEHYDE

TLV = 2ppm

formula = HCHO

Methods.

It was not possible to obtain formaldehyde gas in cylinders. Thus the gas was obtained by bubbling air through formalin solution. The concentration was measured by a colourometric method using a spectro-photometer as described in the chapter of methods. Otherwise the equipment and procedure were as used for acrolein.

Bronchoactivity Experiment.

Results.

Table 7.4. summarizes the results of inhaling formaldehyde which are shown graphically in Fig. 7.3. All four subjects received doses of 8.1ppm and 12.2ppm and in addition two subjects (HS and SMF) each received one extra dose, 9.7 and 4.1ppm respectively. Subject NF showed 29.8% drop in specific conductance at the lower dose and 38.8% drop at the higher dose. Subject SW showed 19.3% and 48.1% drop in specific conductance at the lower and higher does respectively. The third subject (HS) showed virtually no change to the lower dose but responded to the higher dose with 53.2% drop in specific conductance. An intermediate dose of 9.7ppm produced 9.2% bronchoconstriction. The fourth

TABLE 7.4.

FORMALDEHYDE

SUBJECT	CONTROL RAW	CONTROL VTG	CONTROL SGAW	% CHANGE FROM CONTROL RAW	% CHANGE FROM CONTROL VTG	% CHANGE FROM CONTROL SGAW	DOSE	ATOPY	COMMENTS
M.F.	0.51	4.72	0.54	-43.3	1.1	29.8	8.13	A	S: VERY MILD SENSATION AFTER 5 BREATHS.
M.F.	0.46	4.37	0.50	-50.4	-8.0	38.8	12.2	A	S: SHARP SENSATION AT BACK OF THROAT DURING LAST 2 OR 3 BREATHS.
S.W.	0.54	4.28	0.45	-19.4	-1.4	19.3	8.13	NA	S: IRRITANT IN THROAT AFTER 5 BREATHS.
S.W.	0.54	3.90	0.48	-91.4	-0.4	48.1	12.2	NA	S: IRRITANT IN THROAT AFTER 6 BREATHS AND BECAME WORSE.
H.S.	0.54	3.70	0.50	4.2	-7.2	0.1	8.13	A	S: SLIGHT PRICKLING IN BACK OF THROAT AFTER 5-6 BREATHS.
H.S.	0.52	3.91	0.50	-9.8	-0.9	9.2	9.72	A	S: SLIGHT IRRITATION IN THROAT AT 3-4 BREATHS.
H.S.	0.35	4.53	0.65	-107.5	-1.3	53.2	12.2	A	S: PRICKLING IN THROAT AFTER 4 BREATHS WHICH GOT WORSE WITH EACH BREATH.
S.M.F.	0.54	3.73	0.49	-20.2	-2.2	18.6	4.06	A	S: -
S.M.F.	0.57	3.57	0.49	-40.4	-2.8	30.8	8.13	A	S: STINGING SENSATION IN THROAT COMMENCING AFTER 2 BREATHS, PERSISTED AFTER INHALATION.
S.M.F.	0.55	3.65	0.51	-74.0	18.7	29.6	12.2	A	S: IRRITANCY IN THROAT WITH 2ND BREATH WHICH BECAME WORSE FOR THE 10 BREATHS.

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

S = SUBJECTIVE COMMENT.

TABLE 7.4.

FORMALDEHYDE

SUBJECT	CONTROL RAW	CONTROL VTG	CONTROL SGAW	% CHANGE FROM CONTROL RAW	% CHANGE FROM CONTROL VTG	% CHANGE FROM CONTROL SGAW	DOSE	ATOPY	COMMENTS
M.F.	0.51	4.72	0.54	-43.3	1.1	29.8	8.13	A	S: VERY MILD SENSATION AFTER 5 BREATHS.
N.F.	0.46	4.37	0.50	-50.4	-8.0	38.8	12.2	A	S: SHARP SENSATION AT BACK OF THROAT DURING LAST 2 OR 3 BREATHS.
S.W.	0.54	4.28	0.45	-19.4	-1.4	19.3	8.13	NA	S: IRRITANT IN THROAT AFTER 5 BREATHS.
S.W.	0.54	3.90	0.48	-91.4	-0.4	48.1	12.2	NA	S: IRRITANT IN THROAT AFTER 6 BREATHS AND BECAME WORSE.
H.S.	0.54	3.70	0.50	4.2	-7.2	0.1	8.13	A	S: SLIGHT PRICKLING IN BACK OF THROAT AFTER 5-6 BREATHS.
H.S.	0.52	3.91	0.50	-9.8	-0.9	9.2	9.72	A	S: SLIGHT IRRITATION IN THROAT AT 3-4 BREATHS.
H.S.	0.35	4.53	0.65	-107.5	-1.3	53.2	12.2	A	S: PRICKLING IN THROAT AFTER 4 BREATHS WHICH GOT WORSE WITH EACH BREATH.
S.M.F.	0.54	3.73	0.49	-20.2	-2.2	18.6	4.06	A	S: -
S.M.F.	0.57	3.57	0.49	-40.4	-2.8	30.8	8.13	A	S: STINGING SENSATION IN THROAT COMMENCING AFTER 2 BREATHS, PERSISTED AFTER INHALATION.
S.M.F.	0.55	3.65	0.51	-74.0	18.7	29.6	12.2	A	S: IRRITANCY IN THROAT WITH 2ND BREATH WHICH BECAME WORSE FOR THE 10 BREATHS.

BRONCHOCONSTRICTION IS INDICATED BY A POSITIVE VALUE OF % CHANGE SGAW AND A NEGATIVE VALUE OF % CHANGE RAW.

S = SUBJECTIVE COMMENT.

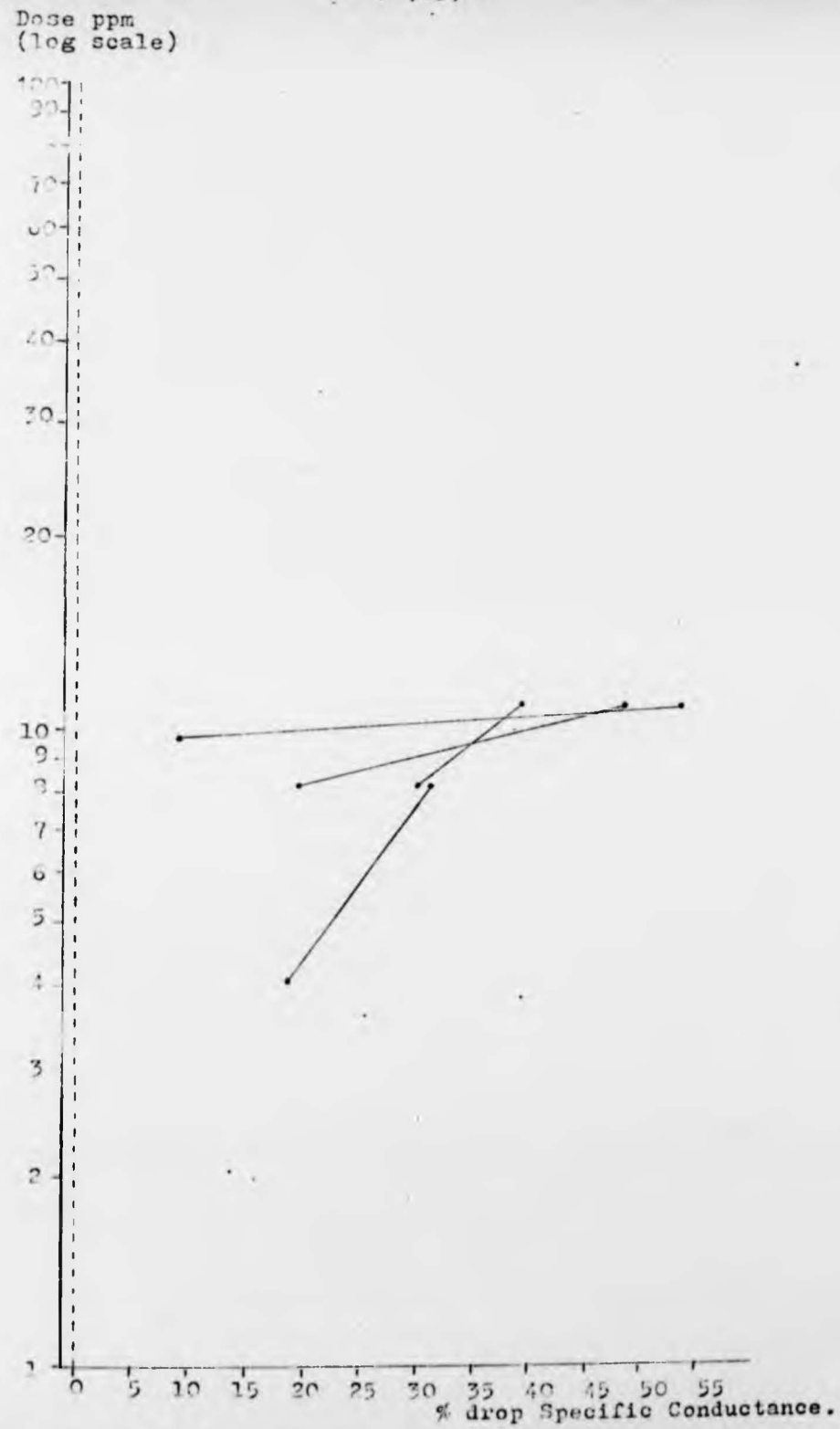


Fig. 7.3. Dose response curves Formaldehyde.

subject (SMF) showed 18.6% drop in specific conductance at 4.1ppm and 30.8% drop at 8.1ppm. At 12.2ppm there was 29.6% drop in specific conductance but this was accompanied by 18.7% drop in thoracic gas volume and 74.0% increase in resistance.

Subjective sensations.

At 8.1ppm three of the subjects experienced a sensation in the throat after five breaths. This was described as "very mild" in the first case, "irritant" in the second case and "slight prickling" in the third. The fourth subject (SMF) described this dose as producing a "stinging sensation in the throat" but commencing after two breaths and persisting after inhalation had ceased. At 12.2ppm the number of breaths to onset of irritation in the throat were substantially the same. Three of the four subjects commented that, as compared with the lower dose the sensation became worse with each breath and the fourth subject (NF) found that the "mild sensation" experienced at the lower dose had now become a "sharp sensation".

The calculated parameters of the curves shown in Fig. 7. 3. are presented in Table. 7. 5. The intercepts range from 1.4 to 9.3ppm with an arithmetic mean of 4.8ppm (geometric mean 3.6ppm). It is noteworthy

TABLE 7.5.

FORMALDEHYDE

SUBJECT	ATOPY	EYE EXPERIMENTS		LUNG THRESHOLD DOSE PPM	SLOPE OF DOSE RESPONSE CURVE
		CONC. PPM.	TIME OF ONSET OF IRRITATION		
N.F.	A	18.3	-	2.136	51.28
		30.3	10 sec		
		30.3	9		
S.W.	NA	8.1	-	6.183	163.3
		12.2	5		
H.S.	A	8.1	-	9.301	453.1
		12.2	10		
S.M.F.	A	8.1	-	1.407	40.41
		12.2	15		
		18.3	8		
R.D.	NA	8.1	-		
		12.2	10		
		18.3	8		
D.P.	A	12.2	-		
		18.3	9		
P.M.	NA	18.3	-		
		24.4	10		
HR	A	6.1	-		
		8.1	5		
		12.2	4		

that there is a delay of several breaths before the onset of sensation and that a disproportionate increase in subjective sensation occurred with only a 50% increase in concentration.

Eye Experiments.

Results.

Table. 7.5. summarizes the reults of the formaldehyde eye experiments. The first subject (NF) found 18.3ppm to be not irritant. At 30.3ppm this subject reported "definitely irritant at ten seconds getting progressively worse to fifteen seconds". Ten days later with the same dose this same subject observed "irritant at nine seconds becoming unbearable by fifteen seconds". The second subject (SW) found 8.1ppm to be not irritant and 12.2ppm to be "irritant at five seconds with lacrimation which lasted until the goggles were removed". The third subject (HS), fourth subject (SMF) and the fifth subject (RD) all found 8.1ppm to be not irritant. Subject HS found 12.2ppm to be "irritant at ten seconds which got worse until fifteen seconds". Subject SMF found 12.2ppm to be irritant at fifteen seconds and subject (RD) experienced "irritation from ten seconds with lacrimation". At 18.3ppm subjects SMF and RD experienced irritation from eight seconds and DP irritation from nine seconds.

The final subject (PM) when exposed to 24.4ppm experienced "irritation at ten seconds increasing to fifteen seconds".

Conclusion.

The product of concentration (C) and time to produce irritancy (T) gave a range of CT values of 61 ppm secs. to 303 ppm secs. The arithmetic mean was 177 ppm secs. and the geometric mean 161 ppm secs.

ACETALDEHYDE.

TLV = 200ppm.

formula = CH_3CHO

Methods.

The equipment and procedure were the same as for the acrolein exposures. Fig.7.4. is the saturated pressure curve for acetaldehyde.

Results.

Table. 7.5. summaries the results of the acetaldehyde eye experiments. All of the eight subjects were exposed on separate occasions to two doses, 1704ppm and 3408ppm. The first subject (RD) exposed to the lower dose found "stinging at fourteen seconds which almost ceased on removing goggles". At the higher dose this subject recorded irritant at seven seconds rapidly becoming worse to fifteen seconds and still getting worse with goggles off until thirty seconds. Conscious blinking made the stinging worse at fortyfive seconds; increasing thereafter up to one minute": residual stinging was present at three minutes. The second subject (PB) exposed to 1704ppm found "irritation in thirtyfive seconds from opening eyes". The conjunctiva were observed to be red (Fig.7.5.). At the higher dose the subject found "irritation three seconds after opening the eyes continuing till ten seconds (when goggles removed) and still present until washing with eyebath then completely gone". The observer noted conjunctival reddening before washing

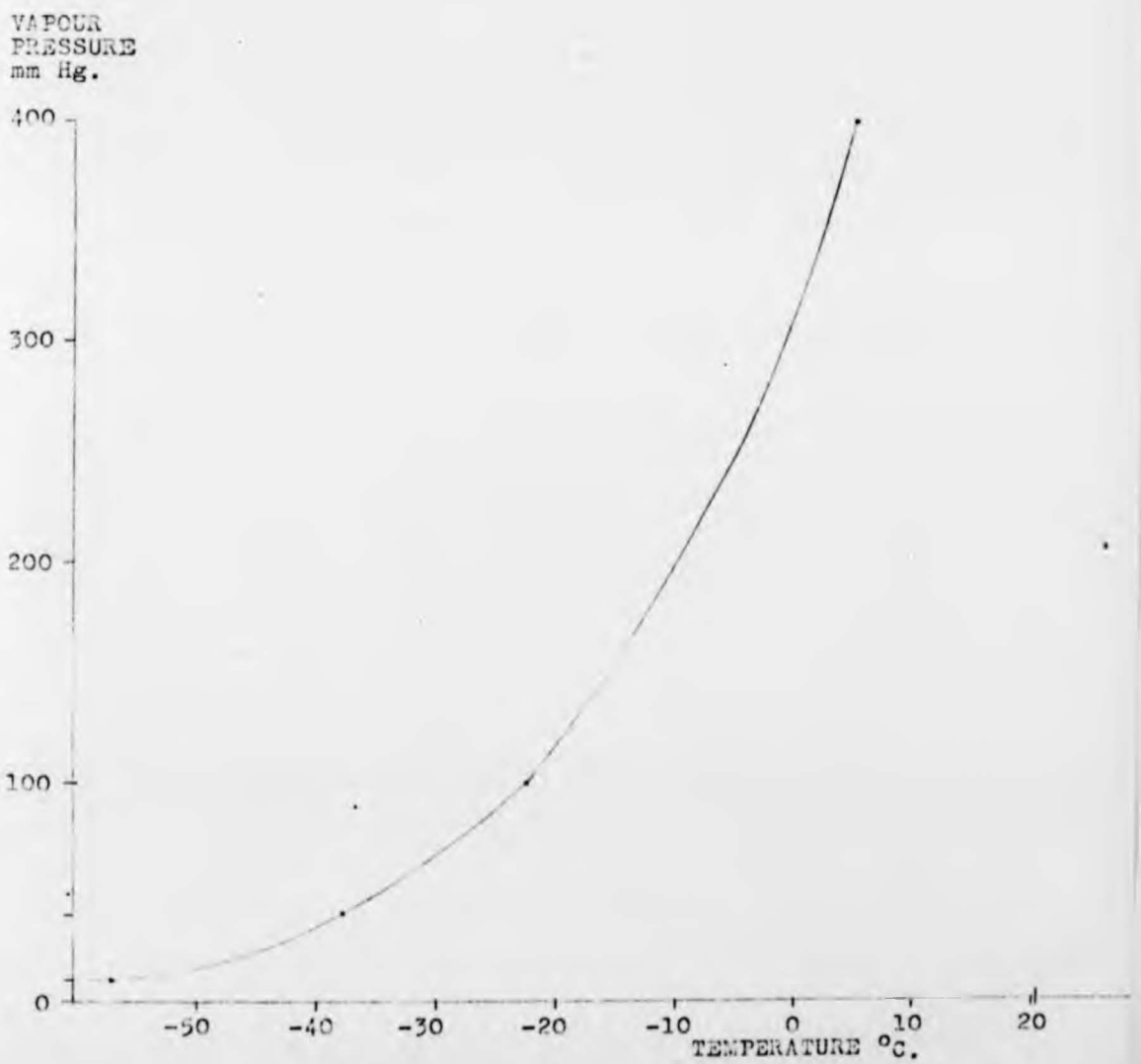


Fig. 7.4. Saturated Vapour Pressure Curve Acetaldehyde.

TABLE 7.5.

ACETALDEHYDE

SUBJECT	ATOPY	EYE THRESHOLD DOSE	
		CONC. PPM	TIME OF ONSET OF IRRITATION IN SECS.
R.D.	NA	1704	14
		3408	7
P.B.	A	1704	35
		3408	3
S.M.F.	A	1704	15
		3408	9
D.D.	NA	1704	80
		3408	50
M.L.T.	NA	1704	185
		3408	85
W.J.		1704	190
		3408	80
S.W.	NA	1704	135
		3408	40
H.S.	A	1704	90
		3408	35

Fig 7.5(a)
Pre Gas

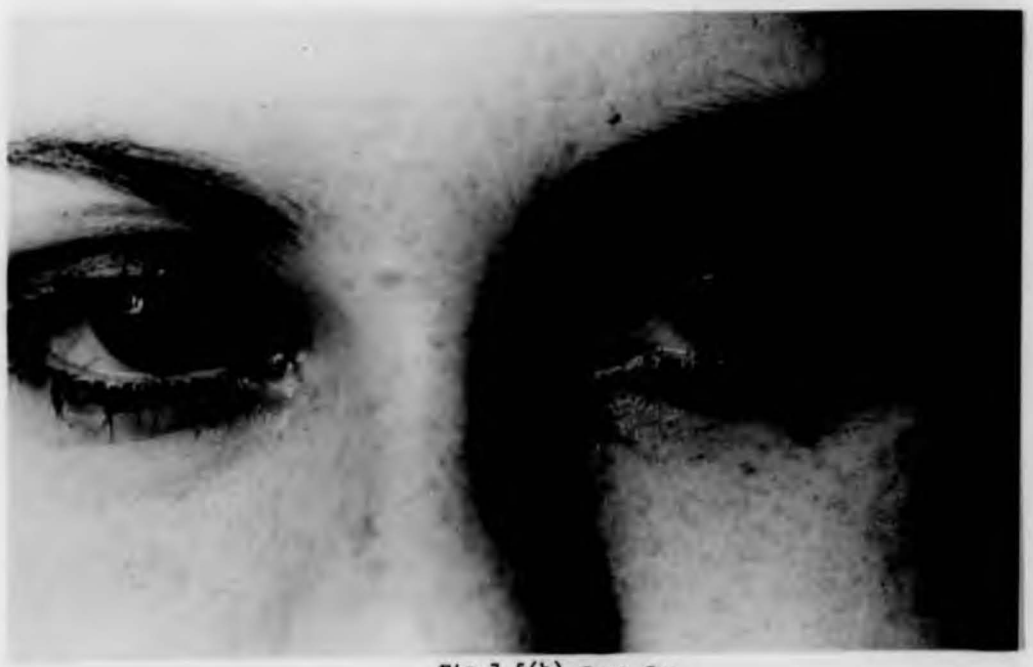


Fig 7.5(b) Post Gas

Fig 7.5(a)
Pre Gas

Fig 7.5(b) Post Gas

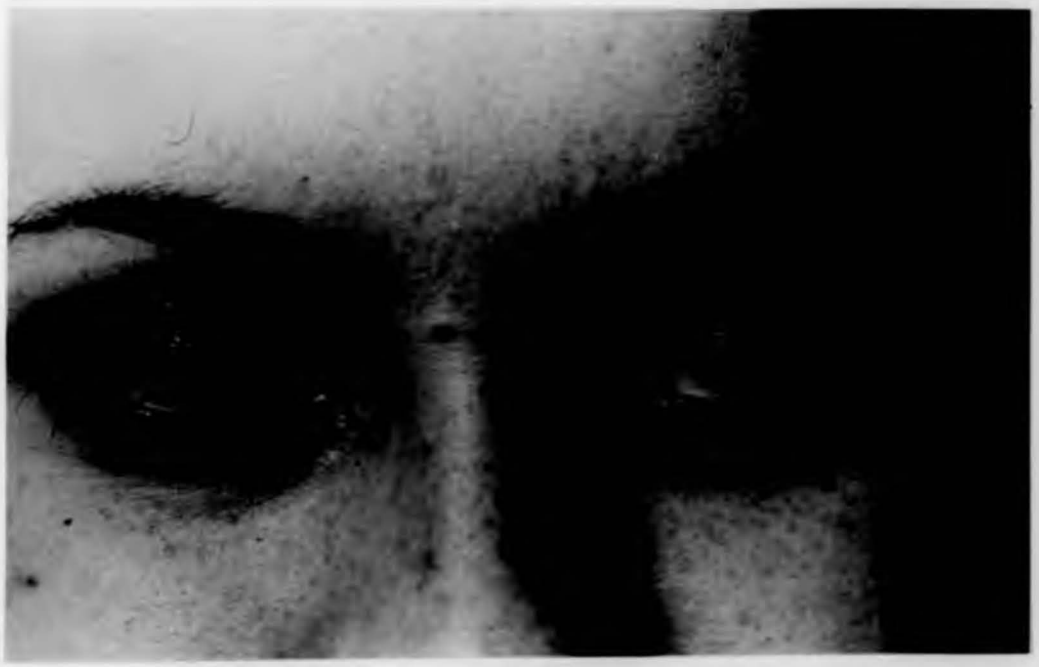


Fig 7.5(c)
Post Washing

Fig 7.5(c)
Post Washing

which was virtually gone afterwards. The third subject (SMF) found the lower dose to produce quite sharp "stinging at fifteen seconds and thought it was going to get worse but it ceased on removing goggles". The observer noted "some reddening of the eyes". At the higher dose the subject observed definite but not severe "stinging at nine seconds. Continued at tne same level though goggles removed at fifteen seconds and still present at one minute. The stinging felt as if it were going to develop and become worse but didn't and remained definite but not severe". The fourth subject (DD) exposed to the lower dose found irritancy at eighty seconds and at ninety seconds commented "getting worse and lacrimation". At one hundred seconds the subject remarked "definite discomfort" and the goggles were removed. The irritation continued up to thirty seconds after removal. Reddening of the eyes was evident a few seconds after beginning the exposure. At the higher dose this subject detected irritancy at fifty seconds described as "continuous". Again the eyes were observed to be red. The fifth subject (MLT) found the lower dose to be irritant from one hundred and eightyfive seconds and at two hundred and forty seconds commented "still getting worse" whereupon the goggles were removed. The eyes were observed to be red and an increased rate of blinking was also recorded. At the higher dose the subject

found irritancy at eightyfive seconds described as "very definite and getting worse until ninetyfive seconds" (when goggles removed) and "still irritant after removal of goggles". Reddening of the eyes was again noted. The sixth subject (WJ) found the lower dose to produce irritancy at one hundred and ninety seconds and the higher dose was "irritant at eighty seconds, getting worse until ninety seconds" (when goggles removed) and "irritation continuing after goggles off". The seventh subject (SW) found the lower dose to be "irritant at one hundred and thirtyfive seconds". The observer noted redness of the eyes after half a minute. At the higher dose the subject recorded irritation in forty seconds. Again the eyes were observed to be pink. The eighth subject (HS) found the lower dose to be irritant at ninety seconds and also remarked on the presence of lacrimation. At one hundred seconds the subject reported "strong irritation" and at one hundred and fifteen seconds "still strong" whereupon the goggles were removed. The eyes were observed to be reddened even after washing. At the higher dose the subject reported irritation at thirtyfive seconds and at fortyfive seconds remarked that it was "stronger with lacrimation". The eyes were observed to be pink.

In addition to the above exposures, seven subjects were exposed to concentrations of eight hundred and fiftytwo ppm for periods up to five minutes. In no instance was irritancy reported but in all cases the eyes were observed to be red. A feature of the reddening produced by acetaldehyde is that the colour is deeper in the corners of the eye although none of the white is unaffected.

Conclusion.

The product of concentration (C) and time to produce irritancy (T) gave a range of CT values of 10,224 ppm secs. to 323,740 ppm secs. The arithmetic mean was 43,562 ppm secs. and the geometric mean 90,440 ppm secs.

CHAPTER 8

ASSESSMENT OF RESULTS.

Introduction.

This chapter assesses the results of the preceding chapters and what conclusions may be drawn while also bearing in mind the question posed in the introductory chapter: "Can the whole body plethysmograph provide objective quantitative measures as an adjunct to subjective assessments of irritancy at the eye for setting TLV's?".

Assessment of results.

Table 8.1 gives the mean eye and lung thresholds for seven of the gases and vapours investigated and these are plotted in Fig. 8.1; the line of identity is also drawn. It must be born in mind that the eye thresholds for the two aldehydes plotted on an axis labelled in ppm (as is appropriate for the other five gases and vapours) are in fact threshold doses with units of ppm secs. Thus the values given may be considered as that concentration in ppm which will produce a response in one second. Ammonia and the ketones show an association between the thresholds at the lung and the eye. The branched chain isomer 3 - methyl - butan - 2-one was less irritant than the other two pentanones. Moving the functional group

TABLE 8.1.

EYE THRESHOLD DOSES VS. LUNG THRESHOLD DOSES

GAS OR VAPOUR	EYE	LUNG
Sulphur Dioxide.	168ppm.	1.0ppm.
Ammonia.	110ppm.	85.0ppm.
Formaldehyde.	177ppm.secs.	4.8ppm.
Acrolein.	153ppm.secs.	4.1ppm.
Pentan-2-one.	560ppm.	311.0ppm.
Pentan-3-one.	800ppm.	414.0ppm.
3-Methyl Butan-2-one.	1600ppm.	939.0ppm.

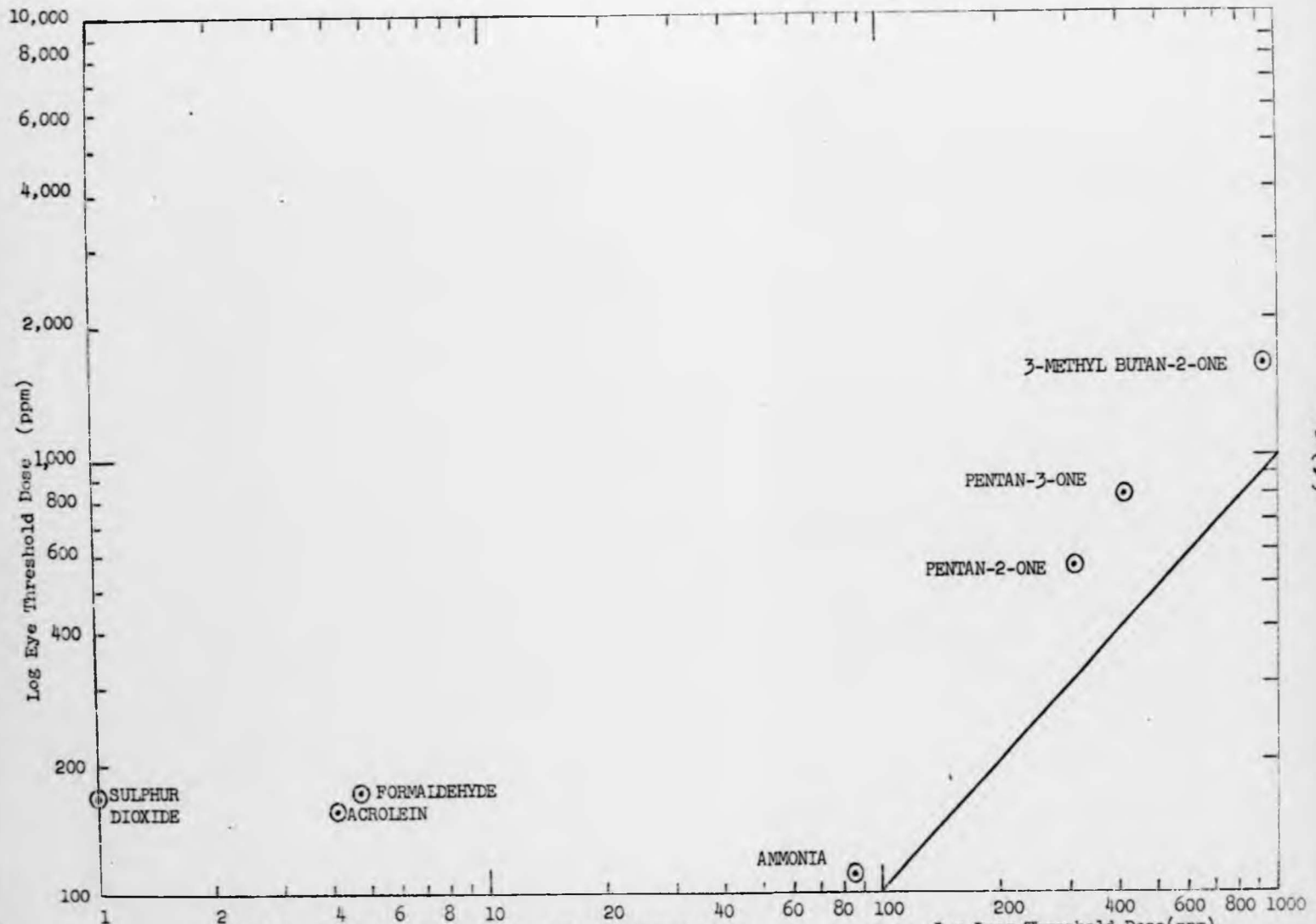


Fig.8.1. LUNG THRESHOLD DOSES vs EYE THRESHOLD DOSES.

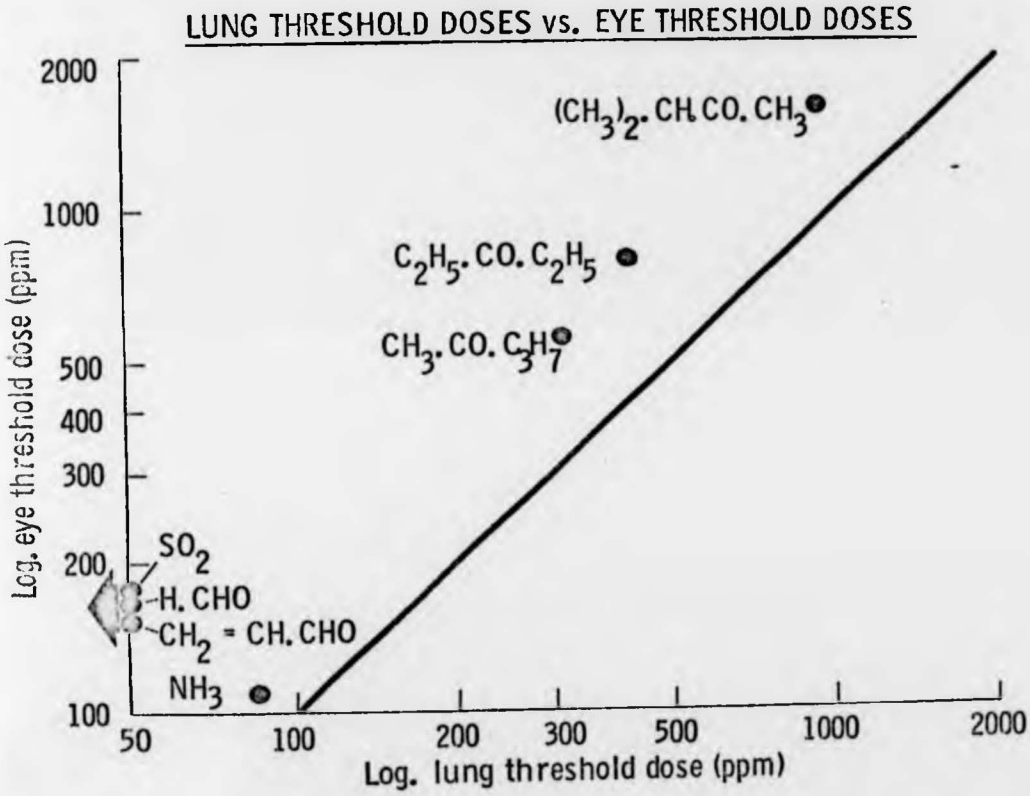

LUNG THRESHOLD DOSES vs. EYE THRESHOLD DOSES
2000
1000
500
400
300
200
100
Log. eye threshold dose (ppm)
$(CH_3)_2$. CH. CO. CH_3
C_2H_5. CO. C_2H_5
CH_3. CO. C_3H_7
SO_2
H. CHO
CH_2 = CH. CHO
NH_3
50
100
200
300
500
1000
2000
Log. lung threshold dose (ppm)

Fig.8.1.

from the three to the two position further increased irritancy. Ammonia was more irritant than the ketones but less than the aldehydes. Patty states that aldehydes may be expected to show reduced irritancy with increasing molecular weight but the presence of the double bond in acrolein produces a molecule which was found to be more irritant than formaldehyde. Thus although the relative positions of the aldehydes are determined it is quite possible to relocate them on the ordinate scale be inserting a different value of time.

The eye threshold for sulphur dioxide was found to be 168ppm for an acute exposure using two subjects. Henderson and Haggard (1943) found eye irritation to be possible at 20ppm using acute exposures. The lung threshold was much lower at 1ppm and nearer to the TLV of 5ppm. The value of 1ppm was in close agreement of that of Frank and Amdur (1964) who used exposures of up to thirty minutes. This indicates that the same information may be obtained with an acute exposure of ten breaths only. However caution would have to be exercised in applying this to aldehydes where time was shown to be important in eye exposures.

The values plotted in Fig. 8.1 all lie

above the line of identity. Does this indicate that the lung is more sensitive to irritants than the eye? Possibly yes since bronchoconstriction is measured at levels below that for irritation at the eye. However this cannot be shown to be so on subjective grounds alone and it may be possible to measure physiological responses of increasing sensitivity in either organ at levels many orders below those required for conscious irritation. This accounts for the major divergence in TLVs listed in the USSR and USA (and Britain) (Sixth report joint ILO/WHO Committee on Occ. Health, 1969). The Russian position appears to be that if any physiological response can be detected at all, the level is too high. For instance levels which abolish a Pavlovian conditioned reflex come within this definition (Izmerov, 1971). The American position, more pragmatic has been given by Hatch (1971, 1972). The Americans attach considerable importance to the degree to which the defence mechanisms, which are a normal part of man's physiological endowment, can safely be drawn upon to offset any result from the offending agent. The Americans employ dose response studies working down from levels of known adverse effects towards levels at which predictions of probable adverse effects can also be made using whatever sensitive measures are appropriate (histological, biochemical, etc.) eventually to a value

which appears to carry no unacceptable risk of adverse effect. This is then subject to continuous revision in the light of subsequent events (Stokinger, H.E., 1969).

A recent British report by Silverman (1974) considers the Russian attitude in relation to his investigations of behavioural responses of rats to trichloroethylene exposures. He notes that the TLV for trichloroethylene in America is 100ppm and in Russia 2ppm . (The Germans, Japanese, Scandinavians, Swiss and Czechs adopt intermediate values between 40 and 75ppm). Silverman found significant changes in behaviour of rats but commented that with exposures which varied from a few hours up to two months and dose levels between 100 and 1000ppm there were no simple dose response relationships and even paradoxical findings. He compared this with the actions of sedative drugs like pentobarbitone where small doses regularly have a paradoxical stimulant effect. Finally he speculated on the implications of his work for humans and finding reports of drowsiness, fatigue and headaches not unexpected, suggested that workers exposed to the TLV concentration of 100ppm trichloroethylene would perform below their peak rate on a self paced task; and under pressure this might lead

instead to increased errors. He also suggests that in an emergency "exposed people would be apt to act first and think later". This is interesting because it draws attention to reliance of British and American TLVs on the deliberately inexact term "adverse effect". Trichloroethylene is known to be of low toxicity to the individual although it is suggested that it may produce subtle changes in mood and behaviour. Many factory workers leave work at 4.30p.m. and get into a motor car to drive themselves home. It is possible that the accident statistics for this group may be altered (or not altered). Thus it may be argued that the strength of the loose term "adverse effect" is that it may cater for any circumstances that may arise.

At the Symposium on Assessment of Exposure and Risk (St. Bartholomew's Hospital, 1972) the first session was entitled "At what stage is the measurable or identifiable effect of a chemical or physical agent to be regarded as pathological?". The discussion reiterated that the TLV is a hygiene stardard and not a physiological threshold.

Many investigators have observed aclimatization to respiratory irritants. Henschler (1962) found that a two hour exposure to 30ppm of nitrogen dioxide significantly increased the rate of breathing for a period of two days, subsequent similar exposures

were tolerated easily. Kleinfeld and Giel (1956) and Challen, Hickish and Bedford (1958) also observed the development of tolerance to ozone. However, Fairchild (1967) has pointed out that although tolerance may develope this does not imply that increasingly serious damage will not occur. Corn and Burton (1967) comment that many workers disagree on the meaning of the word irritant. Patty (1967) suggests that the irritant properties of many molecules indicate probable toxicity. The experiments in this thesis suggest that the reflex bronchoconstriction produced by an acute exposure of irritant gas or vapour may be combined with Patty's suggestion to provide some indication of toxicity in many cases.

To meet the objection that the above correlation is based on extrapolated values the following alternative presentation is now given. Table 8.2. is a summary of the minimum values found to be effective for an acute exposure of the eyes and the lowest doses at which bronchoconstriction has been measured experimentally in human volunteers and which meets the criterion of exceeding 10% that is used as a level of significant bronchoconstriction. These values are of course higher than the notional "thresholds" or intercepts obtained by extrapolation below the measured range, but have the advantage of using actual measured values. Ranking produces a correlation ($R = 0.77$) which is significant at the 5% level. The apparently anomalous value for NH_3 relative to SO_2 may be due to the very high solubility of NH_3 in water (0.329g/g. at 40°C. which is approximately six times that of SO_2. Also, when measured values instead of extrapolated notional "thresholds" are used bronchoconstriction occurs at concentrations below the threshold for irritancy at the eye. It is highly unlikely that the lowest doses at which bronchoconstriction was measured in these experiments are also the lowest doses at which bronchoconstriction can be produced

TABLE 8.2.

LOWEST CONCENTRATIONS AT WHICH EYE IRRITATION AND BRONCHOCONSTRICTION WERE ACTUALLY MEASURED.

GAS	EYE (ppm)	LUNG (ppm)
SO_2	168	5
NH_3	110	200
Pentan - 3 - one	800	600
Pentan - 2 - one	600	500*
2 Methyl butan - 2 - one	1600	1600*
Acrolein	5.5	5.5
Formaldehyde	12.2	8*

*For these gases the lowest dose administered was different for subjects of differing sensitivities and here the median is quoted.

i.e. the true physiological threshold (if it exists and if it can be located experimentally) is going to be found at some lower concentration. There is no way of knowing the precise shape of the dose response curve outside the measured range; however a semi logarithmic curve was found to fit the data of the exhaustive SO_2 experiment within the measured range and so this relationship was adopted. This provides an intercept on the dose concentration axis which permits comparison of one gas or vapour with another. This value may perhaps give a first approximation of the region in which the true physiological threshold is located. It is these intercepts which are plotted as notional lung "thresholds" in Fig. 8.1. producing a picture which is in general agreement with the data in Table 8.2. More specifically, gases which are more irritant to the eyes are also more bronchoactive. Moreover bronchoconstriction is produced at levels below those required for subjective irritancy at the eye when acute exposures are used in both cases.

The general accord between the response of the eyes and the lung to these irritants is supported by the rapid increase in response with

increasing concentration which has been observed with some irritants especially the aldehydes.

Do these findings have any applicability to the problem of setting TLV s? A connection has been adduced in this thesis between human reflex bronchoconstriction and one of the health effects encountered by work people i.e. irritancy as detected at the eye, but this is, at best, only a small part of the knowledge needed when agreeing hygiene standards. Standards of air cleanliness include maximum permissible concentrations, maximum acceptable concentrations, ceiling values and Threshold Limit Values. These standards are a compromise between what is feasible, practicable, economic and socially acceptable and what consequences to health arise from their implementation. However, body plethysmographic measurements may well be considered as a supplement to observations of eye irritation.

The physiological significance of (a) lack of correlation between sensation and bronchoconstriction and of (b) possible sites of action of the irritants and the responses.

(a) Those gases which were irritant generally produced greater subjective sensation (of irritancy) at higher doses. However, at lower doses, subjects could not always detect irritancy although bronchoconstriction took place. This is perhaps not surprising, since subjects can bronchoconstrict after smoking a cigarette without noticing any particular irritancy.

Other sensations such as chest tightness were less frequent and sometimes delayed. That subjective sensations of this nature are less sensitive than external measuring instruments is illustrated by an example given by Dr. M.L. Newhouse (pers.comm.) of a worker exposed to toluene diisocyanate. (TDI). During a three hour afternoon shift the man (aged 42, height 5' 8") had an FVC which fell from 6.8 litre to 3.5 litre and an FEV_1 which fell from 4.8 litre to 1.8 litre. The subjective comment: "I was just beginning to feel a bit tight in the chest"

does indicate the large functional change which can occur with minimal subjective sensation.

Besides sensations of irritancy and chest tightness the subjects of this thesis experienced several sensations simultaneously which did not include irritancy when exposed to acetone. These were nausea, dizziness, revulsion, vasodilation etc., but no irritancy.

(b) <u>possible (i) sites of action of the irritants and (ii) the responses</u>.

(i) <u>Sites</u>. Sellick and Widdicombe (1970) have identified lung irritant receptors in rabbits by the patterns of activity in single vagal myelinated fibres, and by their responses in a variety of conditions in the lungs. The receptors are almost certainly intra - or subepithelial endings in the bronchial or bronchiolar mucosa.

Jeffery and Lynne Reid (1973) have provided definite histological evidence of nerve endings in the mucosa of rats near to the surface which are capable of filling the role of reflex bronchoconstriction.

(ii) The responses. Nine conditions have been shown to increase significantly the discharge in vagal fibres from lung irritant receptors and also to stimulate inspiratory activity. These nine conditions are: inhalation of irritant gases, pulmonary microembolism, anaphylaxis, pulmonary congestion, drug induced bronchoconstriction, asphyxial hyperpnoea, pneumothorax, negative pressure deflations and large inflations of the lungs. In investigating these last two quantitatively, Sellick and Widdicombe (1970) noted that the irritant receptors showed considerable variation in the size of their responses to inflation or deflation and since this variability has been seen with all other stimuli to irritant receptors, they suggested that this may be due to localization of the receptors at different sites in the intrapulmonary airways. This is supported by the histological evidence of Jeffery and Lynne Reid (1973) who found that the concentration of axons was greatest in the upper trachea, and was significantly greater ($p < .05$) than in either the lower trachea or the main bronchus, between which no significant difference was found.

In the upper trachea the concentration of axons in the anterior wall did not differ significantly from that in the posterior wall. The lower trachea and main bronchus resembled each other in having significantly more axons (on average six times as many in the anterior wall ($p < .001$). There were fewer single axons as well as fewer groups in the posterior wall. The decrease in the concentration of axons from the upper to the lower part of the trachea was also most marked in the posterior wall.

The experiments of Stransky et al (1973) showed that stimulation of lung irritant receptors in cats and rabbits by histamine acid phosphate (either intravenously or by inhalation of aerosol) caused tachypnoea and expiratory constrictions of the larynx, and increased discharges in expiratory laryngeal motoneurones.

Further experiments by Szereda - Przestaszewska and Widdicombe (1973) extended this work to chemical irritation of the upper airways by insufflation of ammonia vapour. Again, expiratory increases in laryngeal resistance were measured.

That which follows has been in part abstracted from a recent report by Roach (MRC limited circulation report, 1974).

Procedures for Producing Hygiene Standards in Britain.

In 1965 the British Occupational Hygiene Society (B O H S) appointed a Committee on Hygiene Standards with the objective of formulating hygiene standards for airborne substances and other environmental agents. Prior to that time there were no standards produced in Britain apart from those specifically related to ionising radiations and coal dust. The committees activities are limited to considering isolated agents for which existing American standards (A C G I H) are believed to be most unsatisfactory for use in this country. The procedure for producing a TLV is somewhat different in the two countries.. In America the A C G I H select values which are in large measure secondhand values, being taken from publications in technical journals. Criticism of individual TLVs is deflected to the original references. The procedure of the B.O H S Committee on Hygiene Standards is first to give reasons for the standards it recommends, and then to derive the standards first hand from data in the technical literature or unpublished data made available to the Society. Such a procedure has the advantage that the quality of the recommended standards may have a

degree of uniformity. On the other hand it has the disadvantage of taking a great deal of work to arrive at an acceptable standard.

Developing a hygiene standard entails first finding and critically reviewing relevant new or published evidence. Appraisal entails fitting this evidence into an agreed framework for hygiene standards of air contaminants. The evidence and argument associated with a standard should be capable of presentation in such a way as to show that the hygiene standards used or proposed are practicable, reasonable and consistent with other comparable standards. Roach (MRC limited circulation report) has enlarged on this theme by specifically stating questions which should be answerable in developing a hygiene standard.

Finally the committee recommends standards which it is able to show to its satisfaction will protect the health of a stated high proportion of those exposed to an agent for a working lifetime. The necessary periodic medical examinations to protect the health of the remainder are also specified.

The Future - Scientifically

Roach (MRC limited circulation report) has suggested attempts be made to establish the rules which govern the relationships between:

a. The concentations of chemically similar air contaminants to produce the same adverse effects.

b. The concentrations of physically similar air contaminants to produce the same adverse effects.

c. The human and laboratory animal responses for a concentration of air contaminant at or about the TLV. By response is meant abnormal behaviour, irritation, narcosis, impairment of health or death.

d. The temporary and permanent responses. Such responses as odour, eye irritation, cough, physiological function or the appearance of metabolites in excretions might be temporary and readily observable.

The levels of air concentration which are of interest are low as also is the degree of

expected responses and since the order of magnitude of the response under study should be the same as that which would be accepted by individuals in their daily work, it is therefore important to know what is an acceptable health risk or response. The risk to health accepted in one's choice of place of work rises with the benefits associated with the activity. It would be desirable to obtain uniform knowledge of risk involved with different substances. This could be more readily obtained if the toxicological results of animal experiments could be used as a predictor of the likely human experience.

The Future - Legislatively.

The report of the Committee of Inquiry on Safety and Health at Work under the Chairmanship of Lord Robens was published in July 1972 (HMSO 1972), and was followed by the Health and Safety at Work Act which received the Royal assent on 31st July, 1974. This now means that many persons, about five million, who have not hitherto been covered by health and

safety legislation, are protected for the first time. This is also the first time that the health and safety of the public in connection with the workplace has been covered comprehensively. It is an enabling Act, its flexibility allowing change, expansion and revision to cope with any exigency arising in the future.

The Health and Safety Commission (1st October 1974) and its Executive (1st January 1975) will administer the Act which now defines the obligation of employers, employees, self-employed and those manufacturing and supplying articles and substances for use at work.

Conclusion.

From the above discussion one is drawn ineluctably to the conclusion that getting a TLV is not easy. No two people are the same; some are sensitive individuals, others less so. Some poeple show aclimatization to some substances. In addition, major industrial countries adopt different philosophies as to waht a TLV should be.

In Britain a TLV is taken to be a Hygiene standard set on the best available information at that time. Thus one needs all the information that one can get: Preferably quantitative if one is to follow Kelvin's advice. Roach (1970) has applied the quantitative method to chrysotile asbestos (Fig.8.2.) and states "it is believed that this is the first time a hygiene standard has been associated with such a specified degree of protection". This precise specification follows directly from applying the quantitative method.

The association between eye and lung responses for acute exposures supports the thesis that bronchoactivity is a useful adjunct to eye exposures for setting TLVs.

To the physiologist one can suggest that the lung may be more sensitive than the eye to irritants.

RESPONSE TO CHRYSOTILE ASBESTOS EXPOSURE

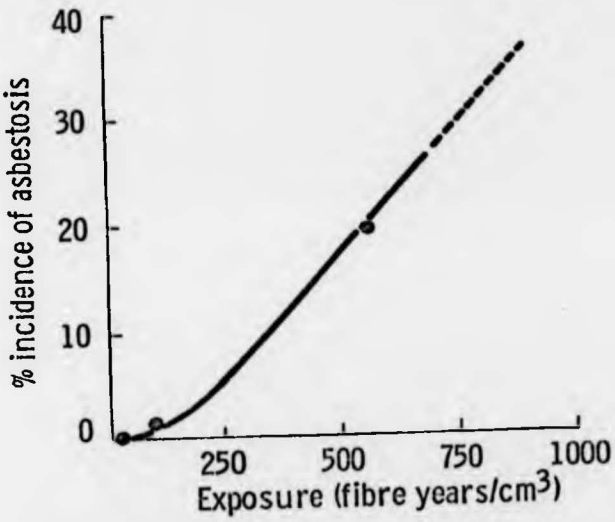


Fig.8.2.

CHAPTER 9

MISCELLANEOUS STUDIES

Introduction.

This chapter describes two experiments additional to the main thesis. The first describes the acute effects of a volatile anaesthetic (methoxyflurane) on airways resistance. The second describes a comparison between estimates of residual volume in 56 patients obtained by body plethysmography and the method of helium dilution.

1. The Acute Effect of Methoxyflurane on Airways Resistance.

$$CHCl_2 . CF_2 . O . CH_3$$

Methoxyflurane is a halogenated ether used as a self administered analgesic in childbirth. The physical and chemical properties are given in Table 9.1.

Methods.

After control values of specific conductance had been obtained the subject remained seated in the plethysmograph and inhaled from a Cyprane Cardiff inhaler through the standard face mask. The inhaler delivers methoxyflurane at a fixed concentration of

TABLE 9.1.

PHYSICAL AND CHEMICAL PROPERTIES OF METHOXYFLURANE.

Chemical formula	$CHCl_2.CF_2.O.CH_3$
M. Wt.	166
B.P. (^oC)	104.7
Vapour Pressure (20^oC)	25 mm Hg.

0.35% v/v. The exhalate was voided externally to avoid contamination of the plethysmograph. Breathing was at tidal volume for 1½ minutes, following which the specific conductance was remeasured immediately.

It was considered worthwhile to repeat the manouevre using an empty inhaler. It is unfortunately not possible to completely dry-out an inhaler once charged and so a dummy inhaler was substituted. The manufacturers leaflet gives the resistance of an inhaler as 0.5" H_2O at 30 l min^{-1} . This was simulated by adding a connector and a length of 10mm i.d. pvc tubing to the standard face mask and corrugated tube. Various lengths were tried and the pressure flow characteristics obtained (Table 9.2; Figs. 9.1. to 9.5.). The resistance at 30 l min^{-1} flow for each length was plotted in Fig. 9.6. It can be seen that 5" of pvc tubing provided the correct value of 0.5" H_2O at 30 l min^{-1} . Several subjects breathed from this dummy inhaler with no effect on airways resistance.

Results.

The results are shown in Table 9.3. Six subjects showed a rise in specific conductance (range 7.2% to 33.6%; mean 20.3%). Bronchodilation also occurred in the seventh subject, but this was overridden by a larger change in lung volume.

TABLE 9.2.

PRESSURE FLOW CHARACTERISTICS FOR FACE MASK AND TUBING.

Flow $l\ min^{-1}$	Pressure in squares* at each tube length "L". L = 0	L = 19.75"	L = 15	L = 0.75"	L = 5
20	-	1.8	1.8	1.2	1.6
25	0.5	2.8	2.7	1.8	2.0
30	0.7	4.0	3.9	2.6	3.0
35	1.2	5.4	5.1	3.4	4.0
40	1.4	7.2	6.8	4.6	5.4
45	1.8	9.2	8.8	6.0	7.0
50	2.0	11.4	10.6	7.2	9.0
55	2.6	13.0	12.5	9.0	10.6
60	3.0	15.0	15.0	11.0	12.4

* one square = 1/6th inch of water.

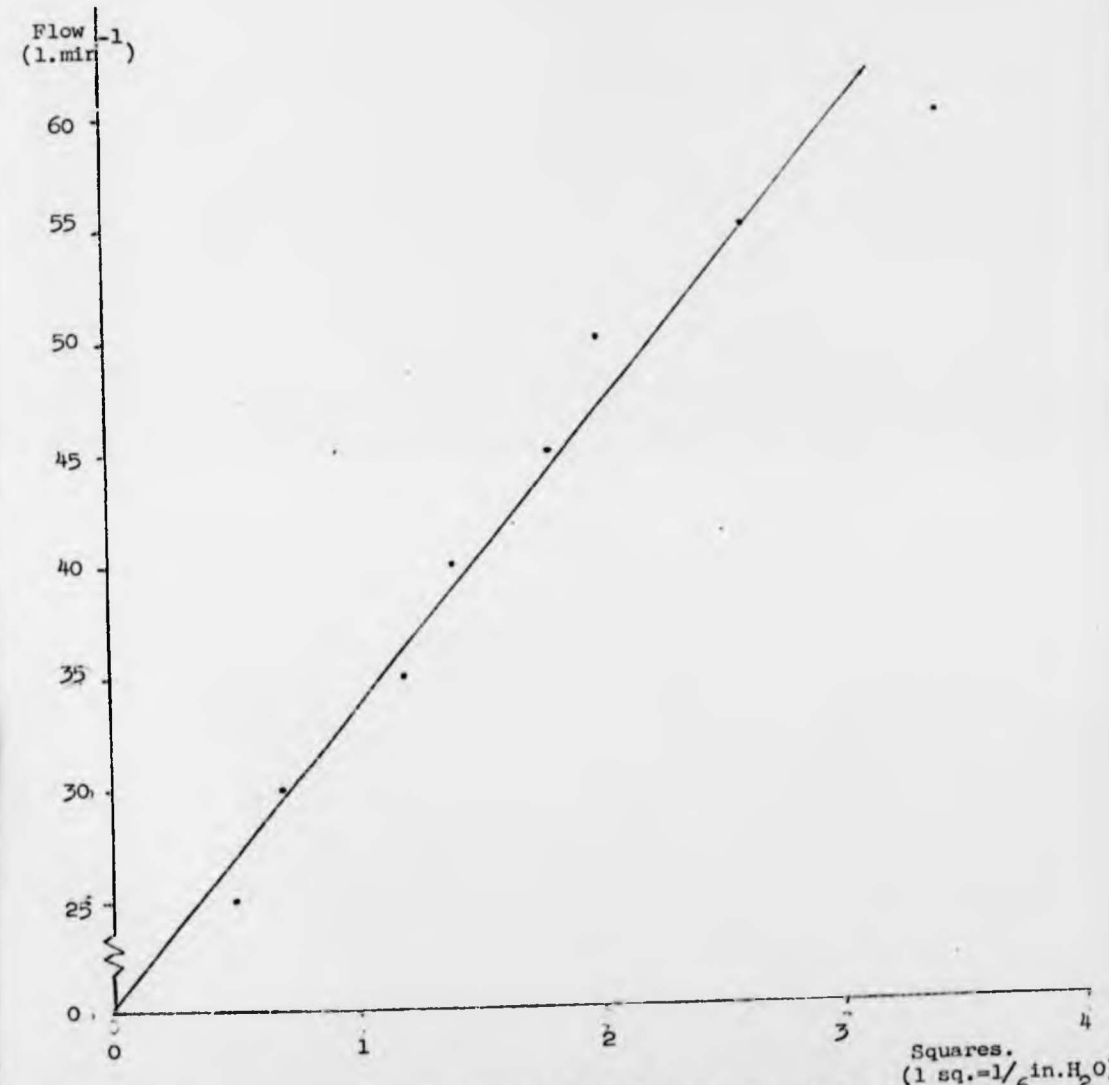


Fig.9. PRESSURE FLOW CURVE FOR FACE MASK, CORRUGATED TUBE AND CONNECTIONS (ZERO ins. EXTRA TUBING).

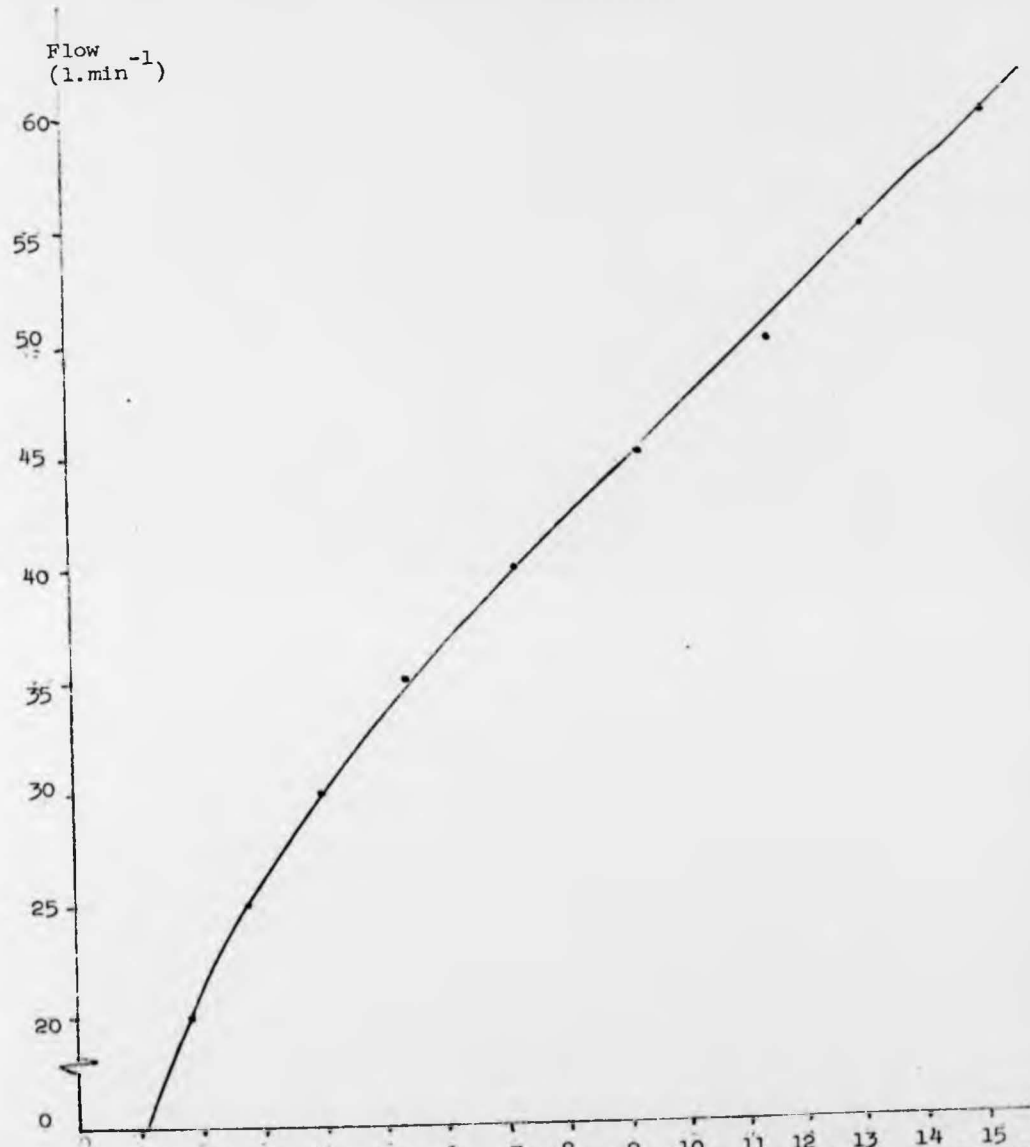


Fig.9. PRESSURE FLOW CURVE 19.75ins EXTRA TUBING.

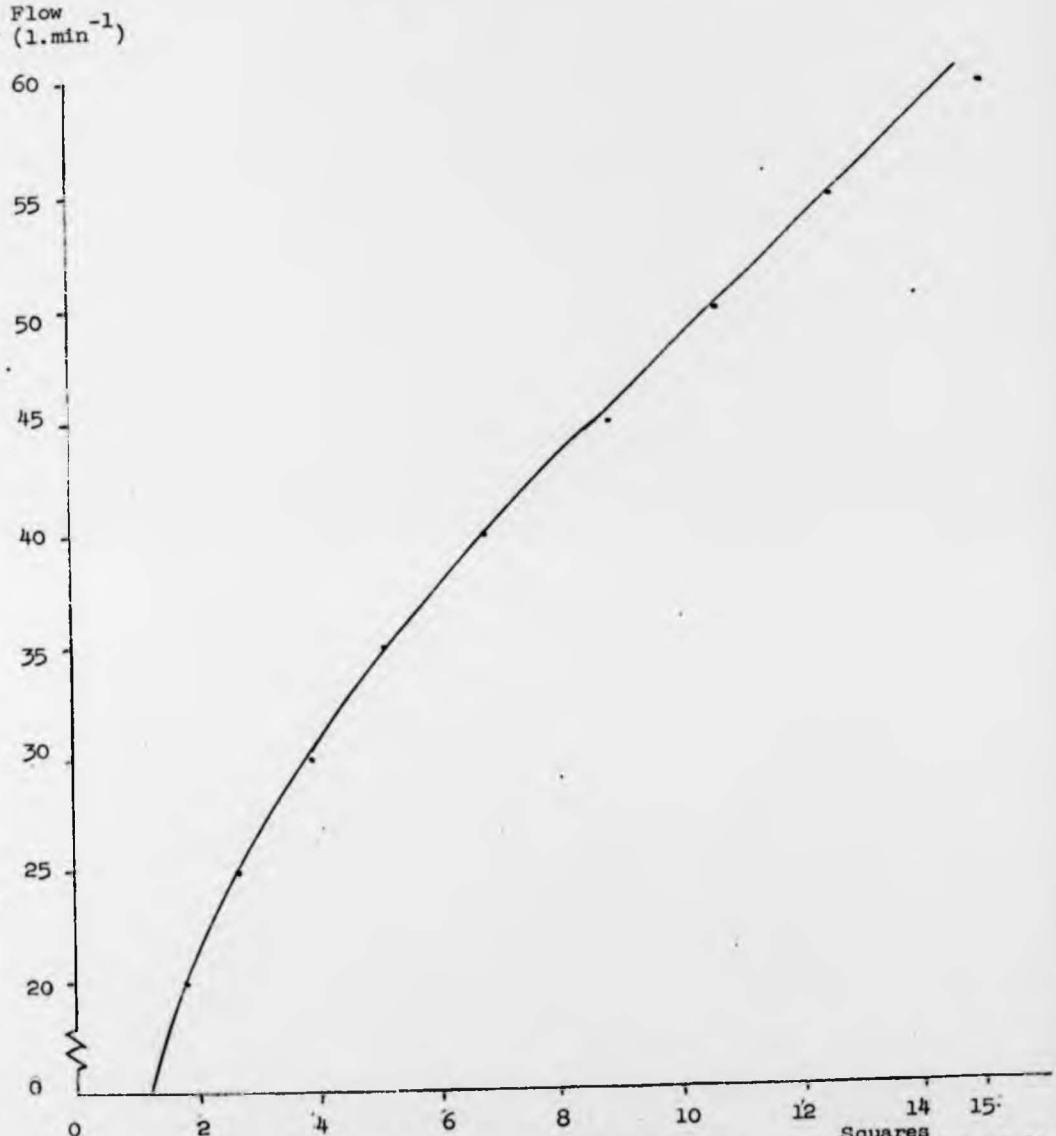


Fig.9. PRESSURE FLOW CURVE 15ins. EXTRA TUBING.

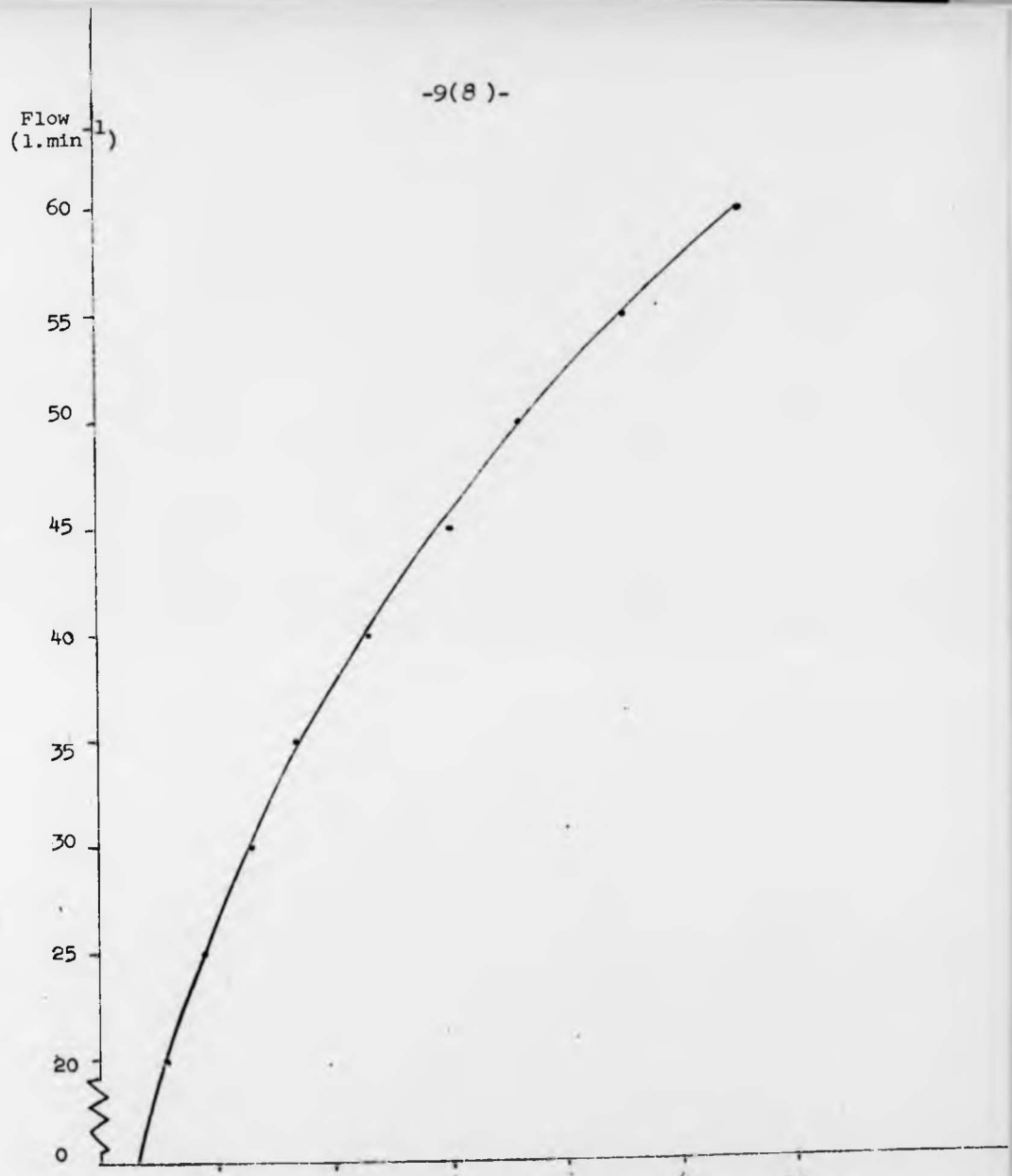


Fig.9. PRESSURE FLOW CURVE 0.75 ins. EXTRA TUBING. (1 sq.=$1/6$in.H_2O)

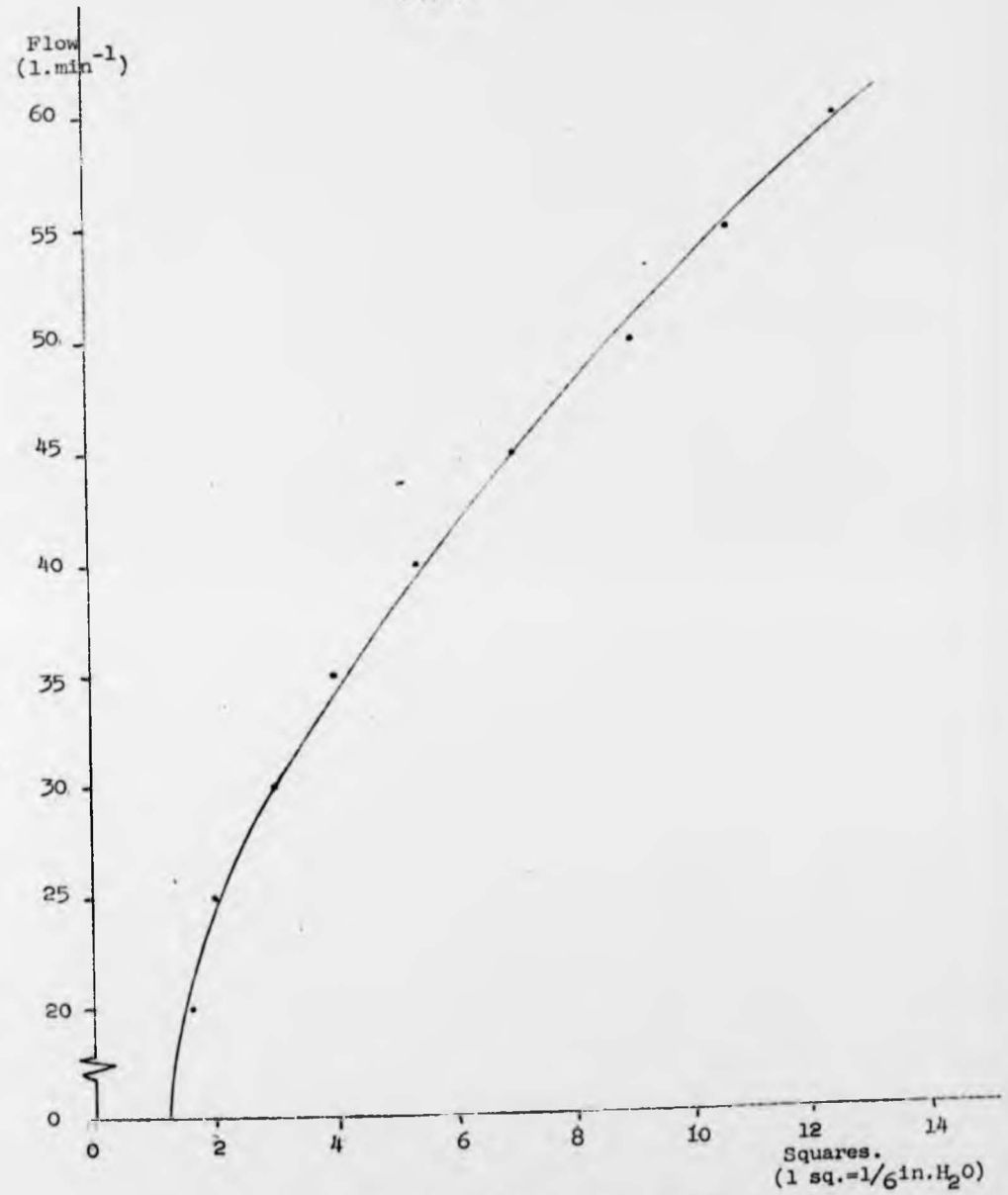


Fig.9. PRESSURE FLOW CURVE 5 ins. EXTRA TUBING.

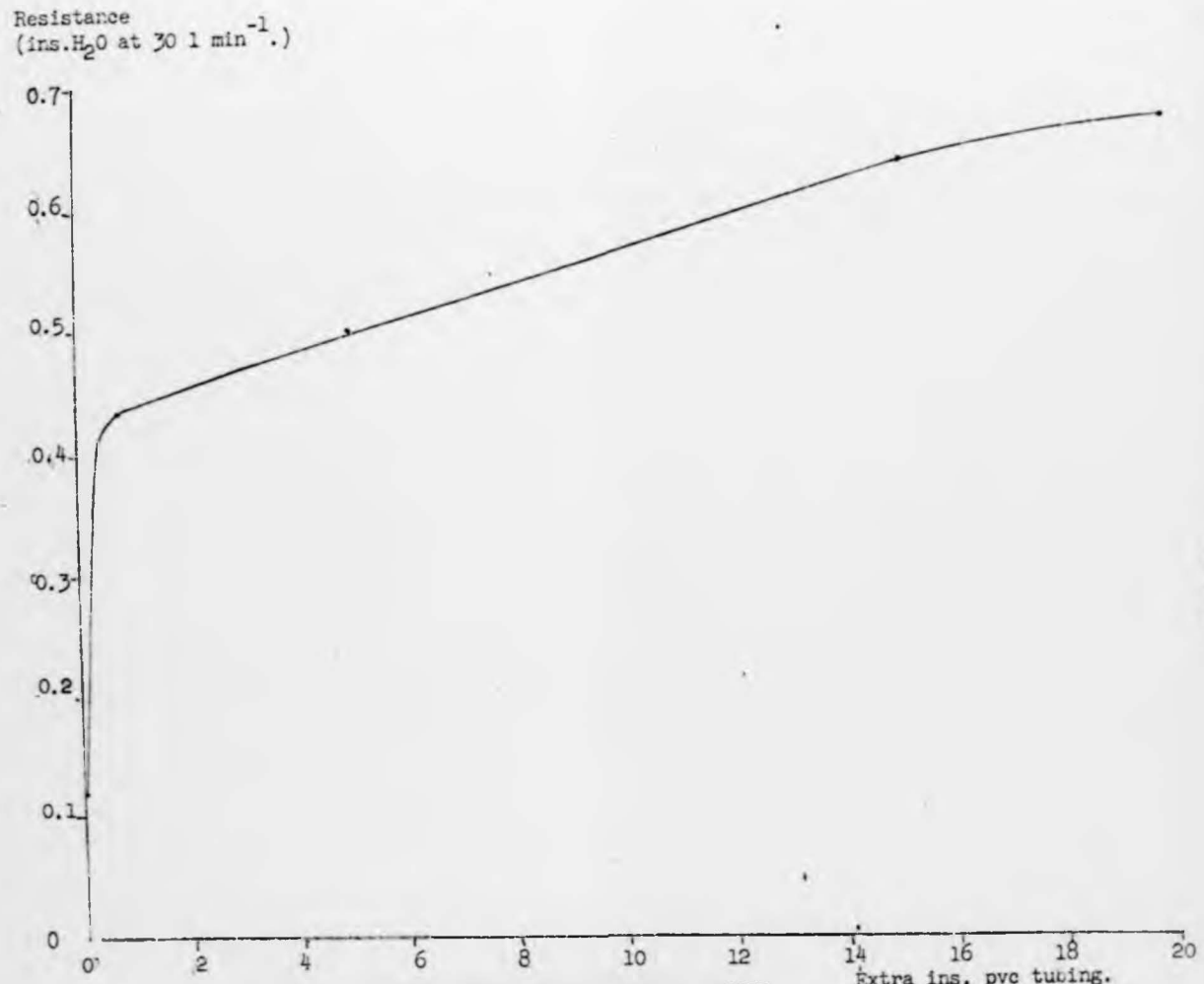


Fig.9. SUMMARY GRAPH SHOWING TUBING LENGTH vs RESISTANCE.

TABLE 9.3.

METHOXYFLURANE.

Subject	CONTROL V_{20}	CONTROL R_{AW}	CONTROL SG_{AW}	% CHANGE V_{20}	% CHANGE R_{AW}	% CHANGE SG_{AW}
VLT	6.46	0.61	0.25	16.3	5.6	-27.4
PJ	3.99	0.71	0.37	5.6	0.7	-7.2
JS	2.15	0.47	1.01	-78.3	25.1	12.5
JC	3.66	0.79	0.35	0.1	20.5	-25.6
AH	3.39	0.64	0.46	13.2	4.5	-19.5
AG	4.16	0.58	0.44	2.2	2.9	-8.4
SP	3.22	0.73	0.43	12.8	13.7	-33.6

Bronchodilation is indicated by a positive value for % change R_{AW} and a negative value % change SG_{AW} .

Conclusion.

These results suggest that consideration might be given to favouring this analgesic for women presenting with airways obstruction or with a history of asthma.

2. Residual Volume by Body Plethysmography and by Helium Dilution.

Methods.

The whole body plethysmograph was modified by the addition of a solenoid operated valve between the subject and the shutter which occludes the mouthpiece during the panting manouevre. This valve communicated the subject to a spirometer outside the plethysmograph to permit spirographic measurement of the vital capacity and its subdivisions. Combining these with the plethysmographic determination of thoracic gas volume at functional residual capacity yielded residual volume, total lung capacity and the ratio RV/TLC % . Another observer obtained these same measurements using the method of helium dilution. The estimates by both methods were made in random order within an hour of each other.

Sixtyfive subjects were examined, most of whom had had industrial exposure to asbestos, but

there were other industrial exposures. Nine subjects were excluded from the study; one because a cleft palate made the helium dilution readings doubtful and the other eight because they could not satisfactorily perform the panting manouevre in the plethysmograph.

Results.

The residual volumes for the 56 subjects are given in Table 9.4 and are plotted in Fig. 9.7 A paired t-test showed a mean difference of 0.62 litre and a t of 3.42. This was significant at the .001 level.

Conclusion.

Estimates of residual volume may be obtained by body plethysmograph or by the method of helium dilution. There is adequate agreement in many cases but there is a significant mean difference of about three or four per cent overall. This may be a response to that group of subjects of say less than three and a half litres by helium dilution and greater than three and a half litres by plethysmograph (see Fig. 9.7).

TABLE 9.4

ESTIMATES OF RESIDUAL VOLUME (IN LITRES) BY BODY PLETHYSMOGRAPH AND BY HELIUM DILUTION.

Helium.	Box.	Helium.	Box.	Helium.	Box.
2.44	4.28	2.80	2.91	2.68	3.59
2.26	0.91	2.85	4.48	2.84	2.80
2.66	4.24	4.24	4.23	2.42	1.92
2.25	2.55	2.37	2.66	3.35	5.67
5.32	5.03	2.57	4.55	2.30	5.01
1.77	1.57	6.06	5.59*	2.25	4.38
3.44	3.64	2.50	3.51	5.03	4.56
1.57	6.89	3.33	4.02	5.01	4.67
3.51	5.15*	1.36	1.91	3.35	3.10
1.64	3.43	3.11	4.26	5.33	5.64
2.95	2.96	4.13	2.97*	5.83	5.81
2.14	5.25	3.64	4.31	2.30	2.34
3.91	3.77	2.45	2.72	3.94	1.07
3.15	3.79	4.77	8.56*	6.54	7.17*
2.23	2.34	2.72	4.56	1.45	2.48
3.05	4.72	2.83	2.30		
2.68	4.00	3.46	2.70		
3.73	3.68*	2.87	2.09		
4.04	4.14	2.41	1.79		
3.75	4.73	4.44	4.58		

* FEV/VC % less than 75% predicted.

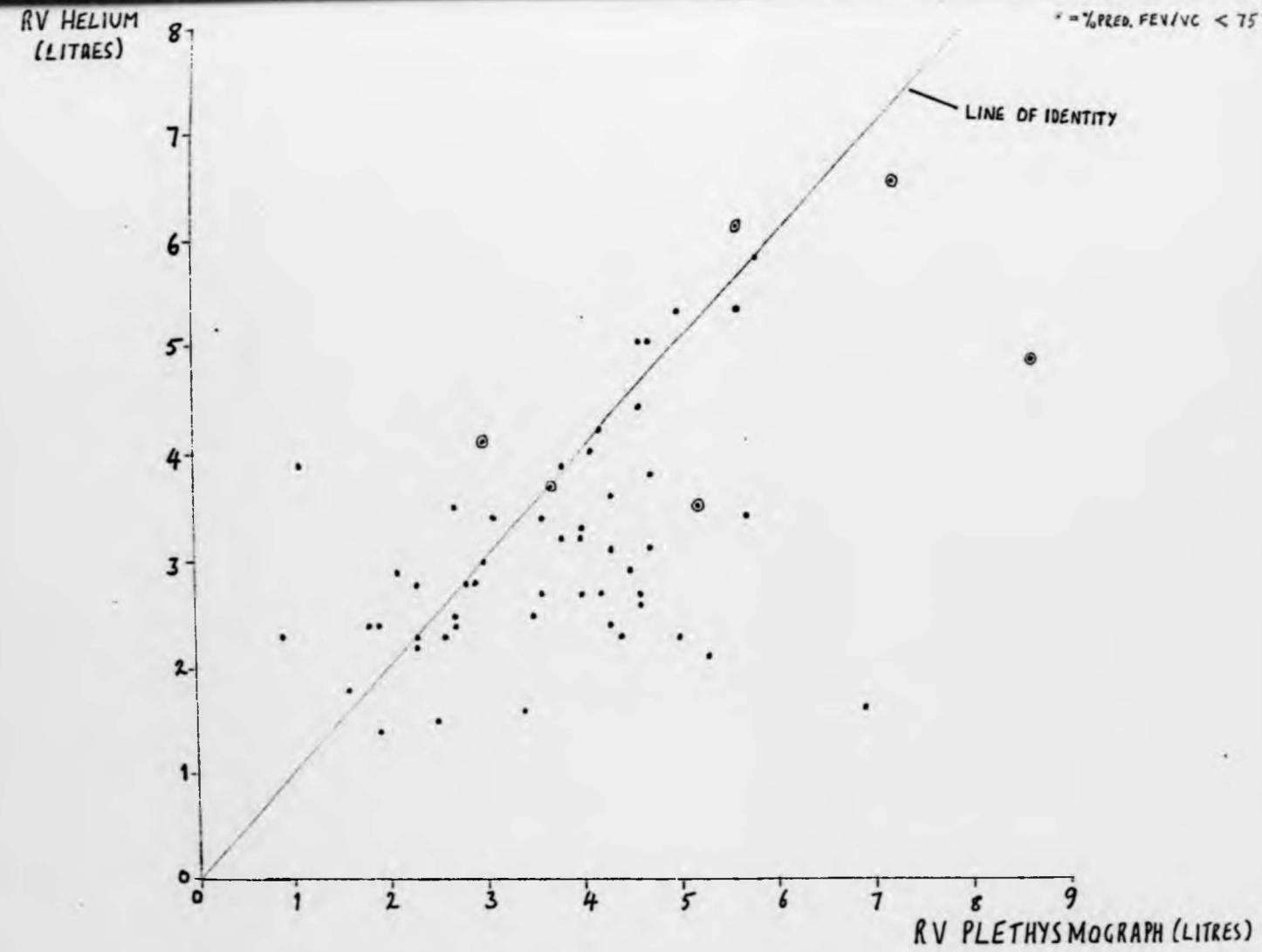


Fig 9.7. RESIDUAL VOLUME BY BODY PLETHYSMOGRAPH AND HELIUM DILUTION.

CHAPTER 10

SOURCES OF ERROR.

Introduction.

This chapter describes three investigations of sources of error. The first was an examination of the errors of measurement of airways resistance and sources of variation.

The second investigation was an examination of the box calibration factor, the frequency with which it should be changed and its dependence on atmospheric variables.

The third investigation tested the efficiency of mixing in the low vapour concentration production system. The absence of thorough mixing can produce regions of high concentration (for example along the wall) which can maintain their integrity for a long distance.

1. Measurement of Airways Resistance.

As described in the methods section the body plethysmograph is operated by an observer to measure the specific conductance, airways resistance and thoracic gas volume of an instructed subject. It was decided to examine the measurements yielded by

the experimental system described above.

Part 1.

The first part of the investigation was to assess physiological variation by measuring the specific conductance of two subjects each weekday at the same time of day for two and one half consecutive weeks (fourteen days for one subject and thirteen days for the other). On each occasion the subject performed the manouevre ten times in two sets of five leaving the plethysmograph between sets.

Treatment of Data.

For each subject the data was treated as a factorial experiment (2 x 14 in one case and 2 x 13 in the other) and a two way analysis of variance was performed using an Olivetti programme developed in the department. The first of each of the five replicates was discarded, leaving two sets of four for each person and day. The data is tabulated in Tables 10.1 and 10.2 and the means are shown for each set and day in graph No 10.1 a. The results of the analysis of variance are shown in Tables 10.3 and 10.4

Conclusions.

1. There is a significant difference in specific conductance between days ($p < 0.001$) and

Days / Repeats	3	4	5	6	9	10	11	12	13	16	17	18	19	20
1	0.2276	0.6135	0.3343	0.3208	0.2659	0.2946	0.2803	0.2278	0.2470	0.1756	0.1719	0.3785	0.2431	0.1982
2	0.3342	0.4256	0.2827	0.3092	0.2274	0.3292	0.2327	0.2042	0.2663	0.2051	0.1886	0.2431	0.2314	0.2042
3	0.3474	0.4155	0.4256	0.3635	0.2616	0.2781	0.2267	0.2748	0.1982	0.1859	0.3127	0.2225	0.2101	0.1823
4	0.2697	0.4822	0.3268	0.3503	0.2906	0.3583	0.2470	0.2191	0.2359	0.1859	0.2616	0.2101	0.2473	0.2023
Mean	0.2947	0.4842	0.3424	0.3359	0.2591	0.3151	0.2467	0.2315	0.2369	0.1881	0.2337	0.2636	0.2329	0.1968
1	0.3426	0.3343	0.3058	0.3208	0.2803	0.3160	0.2241	0.2296	0.2090	0.2357	0.2253	0.2204	0.2431	0.1995
2	0.4021	0.2500	0.4556	0.3670	0.2610	0.2885	0.2116	0.2393	0.2190	0.2037	0.2074	0.2282	0.2101	0.2359
3	0.3630	0.3503	0.3223	0.2981	0.3127	0.2303	0.2247	0.2470	0.2470	0.1859	0.2239	0.2443	0.1911	0.1881
4	0.2945	0.3034	0.2740	0.3982	0.2853	0.3420	0.1881	0.2603	0.2170	0.1859	0.2365	0.2148	0.2282	0.2170
Mean	0.3506	0.3095	0.3394	0.3460	0.2848	0.2942	0.2121	0.2441	0.2230	0.2038	0.2233	0.2269	0.2181	0.2101

Table 10.2. Values of Specific Conductance (SG_{aw}) measured on different days (S.M.F.)

Days / Repeats	4	5	6	9	10	11	12	13	16	17	18	19	20
1	0.3298	0.2763	0.3560	0.4476	0.3014	0.2976	0.4792	0.3205	0.2899	0.3685	0.3994	0.6533	0.4165
2	0.4195	0.3337	0.3694	0.4863	0.5455	0.3462	0.4709	0.3145	0.3553	0.3850	0.4925	0.3994	0.3038
3	0.3937	0.5072	0.3837	0.3920	0.4005	0.3470	0.3909	0.4385	0.3693	0.4568	0.6244	0.5584	0.4274
4	0.3177	0.3837	0.3005	0.4594	0.4479	0.4709	0.3268	0.3397	0.4438	0.4368	0.4925	0.4268	0.2721
Mean	0.3652	0.3752	0.3524	0.4463	0.4238	0.3654	0.4169	0.3533	0.3646	0.4118	0.5022	0.5095	0.3549
1	0.3236	0.4397	0.3740	0.4722	0.3998	0.3779	0.4319	0.2976	0.4253	0.4214	0.4324	0.4341	0.3339
2	0.3177	0.3797	0.3712	0.4331	0.4633	0.3608	0.3826	0.3145	0.3417	0.4654	0.3970	0.4424	0.3999
3	0.4099	0.3634	0.3797	0.4863	0.4216	0.3145	0.3330	0.3291	0.4438	0.4108	0.5259	0.4783	0.4581
4	0.3125	0.4592	0.4222	0.4331	0.3316	0.3416	0.3030	0.3030	0.4290	0.3790	0.5259	0.4533	0.4581
Mean	0.3409	0.4105	0.3868	0.4562	0.4041	0.3487	0.3626	0.3111	0.4099	0.4192	0.4703	0.4520	0.4125

Table 10.2. Values of Specific Conductance (SC_{aw}) measured on different days (J.C.)

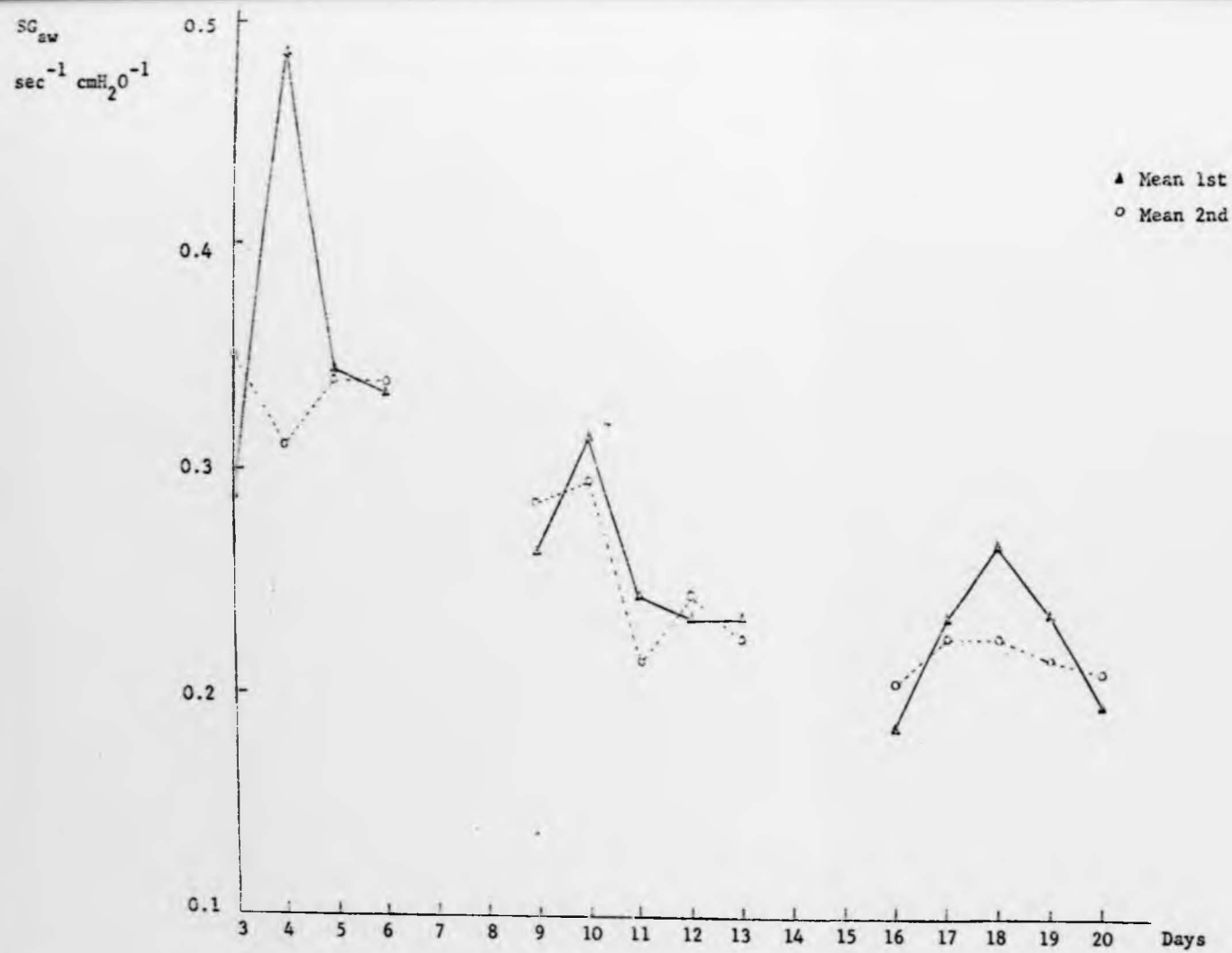


Fig.10.1 Specific conductance (SG_{aw}) on different days. (S.M.F.)

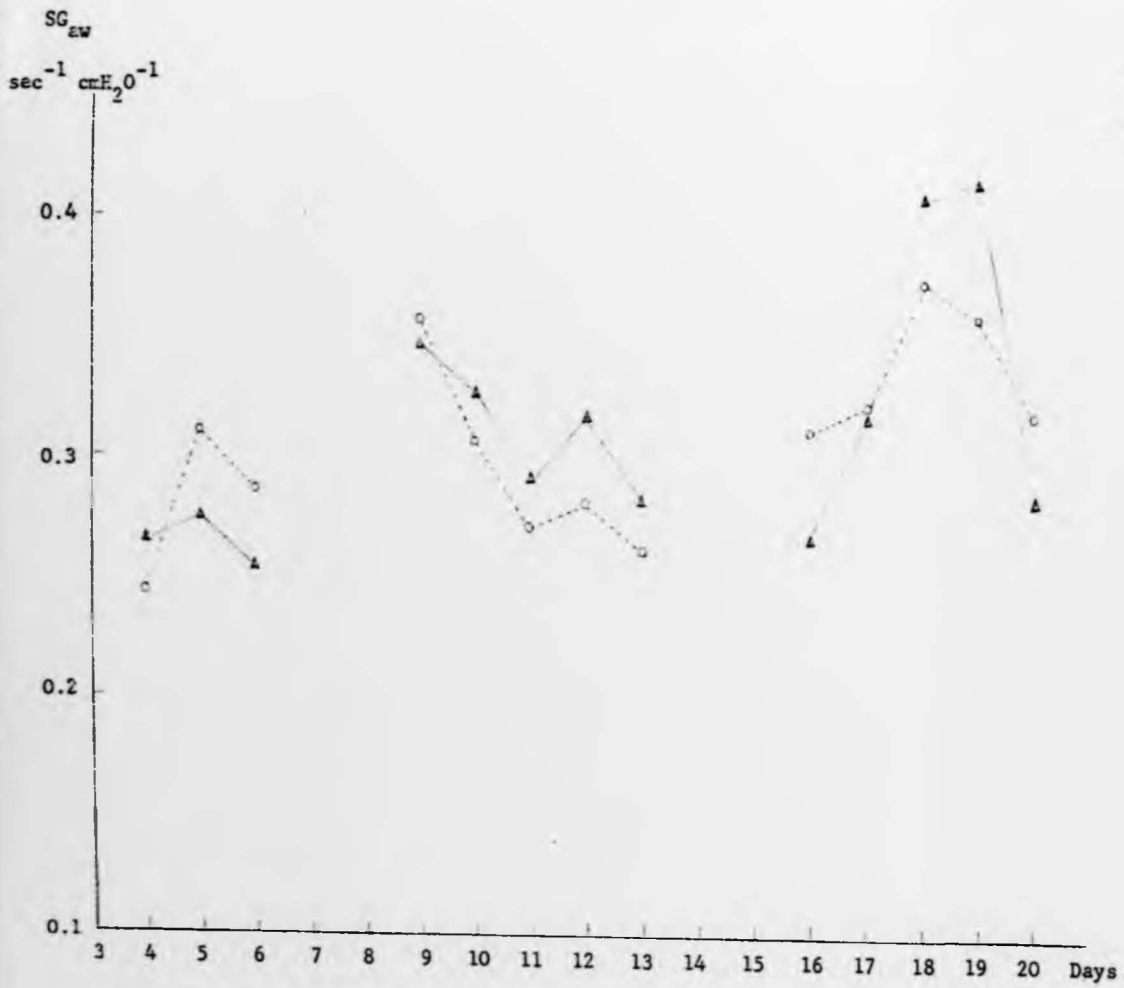


Fig.10.1.a Specific Conductance (SG_{aw}) on different days. (J.C.)

Table 10.3 ANALYSIS OF VARIANCE SUMMARY FOR SPECIFIC CONDUCTANCE (SUBJECT SMF.)

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	p
DAYS	0.3900	13	0.0300	18.75	< 0.001
SETS	0.0061	1	0.0061	3.81	NS
INTERACTION	0.0715	13	0.0055	3.44	< 0.001
RESIDUAIS	0.1344	84	0.0016		
TOTAL	0.6020	111			

Table 10.4. ANALYSIS OF VARIANCE SUMMARY FOR SPECIFIC CONDUCTANCE (SUBJECT J.C.)

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	p
DAYS	0.2148	12	0.01719	4.84	<0.001
SETS	0.0005	1	0.0005	0.14	NS
INTERACTION	0.0380	12	0.0032	0.86	NS
RESIDUALS	0.2886	78	0.0037		
TOTAL	0.5419	103			

therefore experiments to measure the change due to a stimulus require fresh sets of control assessments on each occasion.

2. There is no significant difference between the two sets on any day. On this basis it was decided to dispense with the practice of physically removing the subject from the box between each set and simply to replace this with a rest period seated within the box.

Part 2.

It was decided to enlarge and extend the scope of the investigation. This forms the second part of the study. The control values from the trilene experiment plus ammonia experiment were used as secondary data. In these experiments, the door was not removed from the plethysmograph between sets (see conclusions 2 above). Otherwise the methods were the same.

Treatment of Data.

There were seventeen persons (9 men, 8 women) who came on four separate days. Coding this data onto punched cards enables analysis by computer to include analysis of variation within sets. This had not been possible in the first part of the investigation. Further the first reading was not discarded, the whole five readings in each set being included in the analysis.

The title of the computer program employed is BMD02V - Analysis of Variance for Factorial Design. The program performs the analysis for α , β , cotα , cotβ , cotβ / cotα , VIG, G_{aw}, SG_{aw}.

Cot β / Cot α .

There is a highly significant difference between persons ($p < 0.01$) . It will be seen from the table of means (Table 10.5) that between the lowest value (0.84919) and the highest value (1.67272) there is a difference of almost one hundred percent.

From Table 10.6 it can be seen that between days the difference is highly significant ($p < 0.01$). Reference to the table of means suggests that this is attributable to the first day being high. Why this should be is not known but it reinforces the policy of taking control values on each day.

There is no significant difference between sets thus validating the decision to replace the practice of physically removing the subject between sets with one of simply allowing the subject to rest inside the box.

There is a suggestion of a difference between replicates but the probability ($p < 0.1$) is not generally described as significant. Examination of

PROBLEM NO. A1

Table 10.5. MARGINAL MEANS

MARGINAL MEANS

VARIABLES	CATEGORIES	MEANS
1	1	.93411
	2	.86802
(PERSONS)	3	.84919
	4	1.55425
	5	1.22680
	6	1.19542
	7	1.27815
	8	1.34019
	9	1.19045
	10	1.37676
	11	.89543
	12	1.24892
	13	1.67272
	14	1.38344
	15	1.36724
	16	1.20139
	17	1.22668
2	1	1.23858
	2	1.19127
(DAYS)	3	1.20487
	4	1.21262
3	1	1.23183
(SETS)	2	1.21654
4	1	1.18485
	2	1.26031
(REPLICATES)	3	1.23361
	4	1.22033
	5	1.22131

Table 10.6. ANALYSIS OF VARIANCE COTβ/COTα

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARES	F	P
1(PERSONS)	16	35.48692	2.21793		
2(DAYS)	3	.97758	.32586	48.0	< 0.01
3(SETS)	1	.03972	.03972	7.1	< 0.01
4(REPLICATES)	4	.40262	.10065	0.8	N.S.
12	48	4.89197	.10192	2.2	N.S.
13	16	.98052	.06128	2.2	<0.01
14	64	4.15040	.06485	1.3	N.S.
23	3	.01419	.00473	1.4	N.S.
24	12	.70468	.05872	< 1.0	N.S.
34	4	.12727	.03182	1.2	N.S.
123	48	2.69411	.05613	< 1.0	N.S.
124	192	8.83771	.04603	1.2	N.S.
134	64	2.14190	.03347	< 1.0	N.S.
234	12	.64785	.05399	< 1.0	N.S.
RESIDUAL	192	8.85365	.04611	1.1	N.S.
TOTAL	679	70.95049		< 1.0	N.S.

the means suggests that the first replicate may be low (by about three percent) and the second high (by a similar amount).

The F - value reveals a highly significant interaction for person and day combinations ($p < 0.01$). This is consequential upon there being a difference between persons and also the day to day variation of individuals.

α , β , cot β , cotα .

The results for α , β , cot β , cotα , show significant interactions. This is accounted for by the strong interdependence of alpha and beta. In all cases but one (see above) the interactions disappear in the analysis of cot β / cotα .

2. Box Calibration Factor Experiment.

It was decided to investigate the frequency with which the box should be recalibrated and the influence on the calibration of temperature, humidity, barometric pressure and volume pumped.

Methods.

The methods were as described in the chapter

on methods. The calibration was carried out three times at each range setting using different pump volumes selected at random in order to cover the total range of attenuation. Using the manoeuvre described in Methods the volume was measured before and after the calibration of the box. The syringe was read to the nearest 1 ml. A subsidiary study of the accuracy of measurement was carried out by performing the manoeuvre three times consecutively: the readings of the syringe were identical. Further, repeating three consecutive measurements after the equipment had been left standing over a weekend yielded the same values.

The oscilloscope screen carried a graticule scribed in centimetre divisions. Using this, estimates of the excursion were made by eye to the nearest millimetre for each range setting.

The humidity of the room was measured by whirling bulb thermometer and interpret ed from Tables. The dry bulb thermometer was also used to measure room temperature. The barometric pressure was measured using a Fortin barometer.

The experiment as described, and for three volume settings, was then repeated, morning and afternoon, for ten days.

Results.

An analysis of the data was undertaken using the Olivetti Programma. First the values of the box calibration factor were evaluated and then the correlation coefficient was calculated between box calibration factor and volume pumped, B.C.F. and barometric pressure (P_{ATM}) , BCF and humidity, BCF and temperature. In addition a students t-test was applied to test for a difference between the morning and afternoon estimations. The results of the analysis are summarised in Table **10.7**.

On two ranges (x 100 and x 40) the calibration factor correlated signicantly with volume pumped at the five percent level and one percent level respectively. However for the times ten range the correlation coefficient was positive and on the times forty range negative. During the experimentation the bellows had been observed to bump against the push-rod at certain stroke volumes. This behaviour could be accounted for by some resonance phenomenon which may also have produced the significant but opposite correlation coefficients.

On three ranges (x 40, x 100, x 200) the calibration factor correlated significantly with

TABLE 10.7.

CORRELATION CO-EFFICIENTS BETWEEN BCF AND VARIABLES RECORDED FOR EACH RANGE. (above)

STUDENTS t-test FOR SIGNIFICANCE BETWEEN MORNING AND AFTERNOON RESULTS. (below)

CORRELATION CO-EFFICIENT r

BCF:-

Range	Vol. pumped		PATM		Humidity		Temp	
X10	0.949	*	-0.966	NS	0.224	NS	0.690	NS
X20	-0.288	NS	-0.661	NS	-0.186	NS	0.216	NS
X40	-0.453	**	-0.180	NS	0.021	NS	0.355	*
X100	-0.147	NS	-0.146	NS	0.091	NS	0.339	*
X200	-0.071	NS	-0.072	NS	-0.126	NS	0.309	*
X400	-0.038	NS	0.009	NS	0.089	NS	0.266	NS

* =pless than 0.05

** =pless than 0.01

t-test BCF AM - PM

Range	t	
X10	1.023	NS
X20	0.660	NS
X40	0.347	NS
X100	0.745	NS
X200	1.551	NS
X400	0.351	NS

temperature at the five per cent level.

The t-test revealed no significant difference between the morning and afternoon results on any range.

The possibility of predicting the box calibration factor from measurements of atmospheric parameters was examined. To do this, an equation for oscilloscope reading was used as shown below:

$$\text{reading} = \frac{BCF}{R_i} \left(\frac{p}{760}\right)^{\alpha} \left(\frac{t}{70}\right)^{\beta} \left(\frac{h}{50}\right)^{\gamma} V^{\delta}$$

where R_i = Range setting on oscilloscope

p = Barometric pressure

t = Temperature

h = Humidity

V = Volume pumped

An initial analysis of covariance using a general linear hypothesis produced a call for more data. It was decided to repeat the experiment and acquire more readings over a greater range of temperatures. On half the days the temperature was reduced in the mornings by leaving a window open overnight with the heating turned off and then warming up the room for the afternoon readings. On alternate days the sequence was reversed. The data obtained from

the second series was of better quality with smaller residuals. The results of this analysis are shown in Table 10.8.

Conclusion.

From three hundred and twentyfive estimations of the box calibration factor it has been possible to obtain the true box calibration factor (i.e. for attenuation range x 1 , not provided on the oscilloscope.) Also the relative values of the six ranges of attenuation using the smallest range (x10) as reference.

To answer the question "how frequently is it necessary to change the box calibration factor?" the answer could be "never" since the corrections due to atmospheric conditions are small. However a useful procedure could be to recalibrate the box at frequent intervals and compare the value obtained with the predicted value. Any systematic departure from the predicted value could be an early indication of fault in the equipment.

Part 3.

3. Mixing Efficiency in Breathing System.

The addition of a further wire mesh baffle within the glass breathing tube was investigated by

TABLE 10.8.

ESTIMATES OF ATTENUATION RANGES AND CONSTANTS OF MODEL EMPLOYED IN BOX CALIBRATION FACTOR EXPERIMENT.

Parameter	Estimate	Standard Error
BCF	10.18	0.3
R_1 (not estimated)	10.0	-
R_2 (x 20)	17.6	0.4
R_3 (x 40)	44.8	0.9
R_4 (x 100)	93.0	2.9
R_5 (x 200)	186.0	4.7
R_6 (x 400)	373.0	7.1
α	0.53	1.0
β	-0.103	0.069
γ	-0.02	0.14
δ	0.9930	0.0076

placing a stainless steel sampling tube through the mouth breathing point 30mm downstream from the additional baffle and feeding the sample to a gas liquid chromatograph. The sampling tube was inserted through a rubber septum which sealed the system. The sampling tube, horizontal and perpendicular to the flow sampled at fixed points across the diameter of the tube. In addition, selected other sampling points were sampled above and below the horizontal plane.

Results.

Table 10.9 shows the peak heights from the chromatograms sampled at different points across the breathing tube. Fig 10.2 is a typical example of the chromatograms obtained. Table 10.10 gives the analysis of variance for the readings obtained. This may be abstracted as follows:

Analysis 1.

(1) values at positions a, b, c, d, e, f, g, h.

$F = 7.4225 \ (p < .01)$

(2) positions a and h

$F = 0.0004$ NS

Analysis 2.

(This analysis treated a.m. and p.m. values separately).

placing a stainless steel sampling tube through the mouth breathing point 80mm downstream from the additional baffle and feeding the sample to a gas liquid chromatograph. The sampling tube was inserted through a rubber septum which sealed the system. The sampling tube, horizontal and perpendicular to the flow sampled at fixed points across the diameter of the tube. In addition, selected other sampling points were sampled above and below the horizontal plane.

Results.

Table 10.9 shows the peak heights from the chromatograms sampled at different points across the breathing tube. Fig. 10.2 is a typical example of the chromatograms obtained. Table 10.10 gives the analysis of variance for the readings obtained. This may be abstracted as follows:

Analysis 1.

(A) values at positions a, b, c, d, e, f, g, h.

$F = 7.1825$ $(p < .01)$

(B) positions x and y

$F = 0.0004$ NS

Analysis 2.

(This analysis treated a.m. and p.m. values separately).

Table 10.9. CHROMATOGRAM PEAK HEIGHTS (ARBITRARY UNITS) AT DIFFERENT SAMPLING POINTS.

a		b	c	d	e	f	g	h	x	y
38	40	41	41	40	41	43.5	39.5	41.5	45	42
38	42	41	43	40	41	38.5	39	43.5	43.5	42.5
40	40	40	44	40	39.5	36	39	44	41.5	43
41	42	44	43	38	39	35.5	42	42.5	42	42
42	39.5	46	48	41	38	42		43.5	43	44
39	42	45.5	47	39	39	39		37	43	
44	40	42.5	44.5	40.5		40		46	41	
36	43.5	42	48	42						
40	41.5	43		38						
43	41			38.5						
41	42.5			38						
42	39									
40	42.5									
44	43									
44	43.5									
42										
Mean	41.2	42.8	44.8	39.6	39.6	39.2	39.9	42.6	42.7	42.7
Position	centre	wall	centre	breathg. point	3/4 way	1/4 way	centre	centre & up	wall	centre

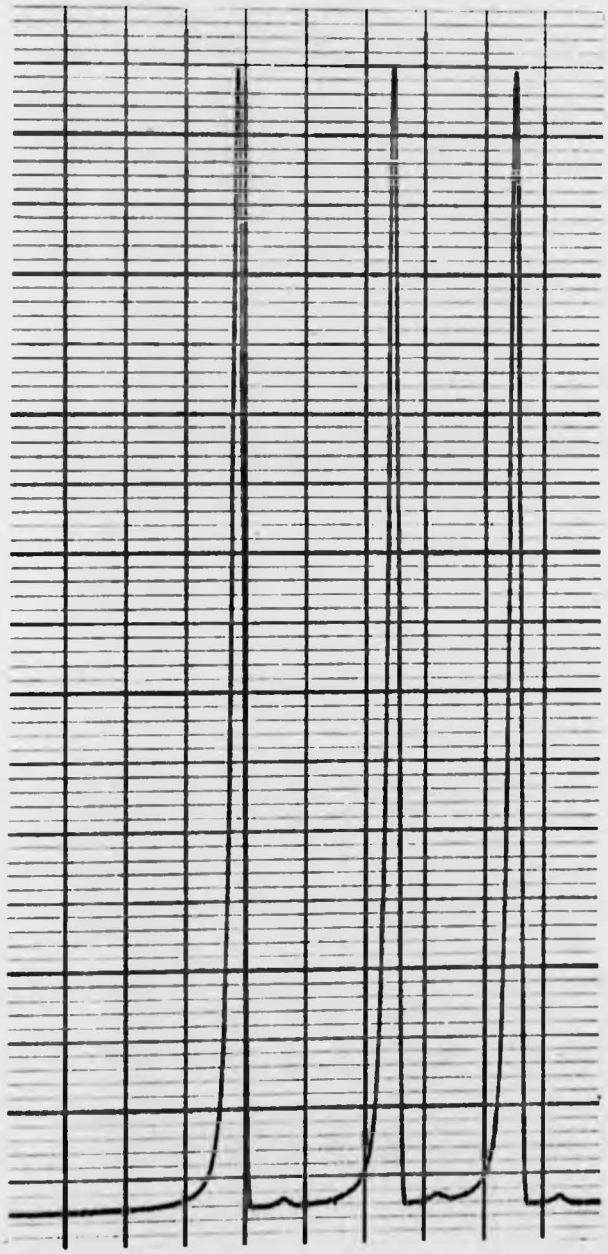


Fig.10.2. TYPICAL CHROMATOGRAM.

Table 10.10. ANALYSIS OF VARIANCE TABLE : VAPOUR CONCENTRATION ACROSS BREATHING TUBE.

Source	Total sum of squares	Between Groups D.F.	Between Groups Mean Sq.	Within Groups D.F.	Within Groups Mean Sq.	F	p
1(A)	548.69	7	31.46	75	4.38	7.18	<.01
1(B)	13.73	1	0.00	10	1.37	0.00	NS
2(A)	287.99	2	45.14	45	4.39	10.27	<.01
2(B)	184.17	4	13.35	30	4.36	3.06	<.05
2(C)	84.24	3	0.40	24	3.46	0.11	NS
(D)	269.65	2	49.90	40	4.25	11.75	<.01

(A) a.m. values i.e. a, b, and c positions.

$F = 10.2734$ $(p < .01)$

(B) values at positions d, e, f, g, h. (p.m. values)

$F = 3.0630$ $(.05 < p < .01)$

(C) values d, e, f, g only

$F = 0.1145$ NS

Conclusions.

1. The effect of position has been confounded with that of time i.e. cannot distinguish between effect of time and position as both measured by the means of the peak heights.

2. There is a suggestion of a time trend and this would appear to be the most plausible explanation of the significant "F" factors.

3. Since the effect due to time cannot be eliminated we cannot make exact statements about the effect of position except to say that it does not appear to be very important as readings at the centre are associated with relatively very high and very low values. Thus it would seem safe to conclude that the mixing efficiency of the baffle is very high.

4. Taking all the "centre" values there is a significant difference ($p < 0.01$) between runs at the centre. This indicates that the effect is due to time.

REFERENCES

Adrian, E.D. (1933). Afferent impulses in the vagus and their effect on respiration. J. Physiol. 79, 332-358.

Altshuller, A.P., Wartburg, A.F., Cohen, I.R., and Sleva, S.F. (1962), Int.J.Air Wat.Polln., 6, 75-81.

Amdur, M.O., Silverman, L., and Drinker, P. (1953). Lancet 10, 758.

Amdur, M.O. (1966). Respiratory absorption data and dose response curves. Arch. Environ. Health. 12, 729.

Amdur, M.O. (1971). Aerosols formed by oxidation of sulphur dioxide. Arch. Environ. Health. 23, 459-468.

Amer. Conf. Govtl. Ind. Hygienists. (1973). Documentation of the Threshold Limit Values.

Andersen, I., Lundqvist, G.R., Jensen, P.L., and Proctor, D.F. Human response to control levels of sulphur dioxide. Arch. Environ. Health. 28, 31-39.

Anderson, A. (1950). Possible Long Term Effects of Exposure to Sulphur Dioxide. Brit.J. Industr. Med. 7, 82-86.

Atkins, D.H.F., Cox, R.A., and Eggleton, A.E.J. Photochemical Ozone and Sulphuric Acid Aerosol Formation in the Atmosphere over Southern England. (1972). Nature 235, 372-376.

Autrum. H. (1959). Nonphotic receptors in lower forms. In: Handbook of Physiology, Section 1. Neurophysiology, vol.1, 369-385. Amer. Physiol. Soc.

Baker, R.A., and Doerr, R.C. (1959). Int. J. Air Wat. Pollut., 2, 142-158.

Beidler, L.M. (1957). Chemistry of Natural Food Flavors. Adv. Board on Quartermaster R. and D. Washington D.C.

Beidler, L.M. (1961). The chemical senses. Ann. Rev. Psychol. 12, 363-388.

Burnett, M.G., and Swaboda, P.A.T. (1962). Analyt. Chem. 34, 1162-3.

Challen, P.J.R., Hickish, D.E., and Bedford, J. (1958). An Investigation of Some Health Hazards in an Inert Gas Tungsten-Arc Welding Shop. Br.J. Ind. Med. 15, 276.

Cole, L.W. (1910). Reactions of frogs to chlorides of ammonia, potassium, sodium and lithium. J. Comp. Neurol. 20, 601-614.

Corn, M., and Burton, G. (1967). The Irritant Potential of Pollutants in the Atmosphere. Arch. Environ. Health. 14, 54-61.

Crozier, W.J. (1916). Regarding the existence of the common chemical sense in vertebrates. J. Comp. Neurol. 26, 1-8.

Cumming, G. (1961). The body plethsmograph. Postgrad. Med. J. 37, 257-258.

Dethier, V.G. (1955). The Physiology and histology of the contact chemoreceptors of the blowfly. Quart. Rev. Biol. 30, 348-371.

Dubois, A.B., Bothelho, S.Y., and Comroe, J.H., Jr., (1954). Resistance to airflow through the tracheo-bronchial tree as measured by means of a body plethysmograph. J. Clin. Invest. 33, 929.

Dubois, A.B., Bothelho, S.Y., Bedell, G.N., Marshall, R., and Comroe, J.H., Jr. (1956(a)). A rapid plehysmographic method for measuring thoracic gas volume : a comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. J. Clin. Invest. 35, 322.

Dubois, A.B., Bothelho, S.Y., and Comroe, J.H., Jr. (1956(b)). A new method for measuring airway resistance in man using a body plethysmograph : values in normal subjects and in patients with respiratory disease. J. Clin. Invest. 35, 329.

Fairchild, E.J. (1967). Tolerance Mechanisms : Determinants of Lung Responses to Injurious Agents. Arch. Environ. Health. 14, 111.

Ferris, B.G., Burgess, W.A., and Worcester, J. (1967). Prevalence of Chronic Respiratory Disease in a Pulp Mill in the United States. Brit.J. Industr. Med. 24, 26-37.

Fowlis, I.A., and Scott, R.P.W. (1963). J. Chromat. 11, 1-10.

Frank, N.R., Amdur, M.O., Worcester, J., and Whittenberger, J.L. (1962). Effects of acute controlled exposure to SO_2 on respiratory mechanics in healthy male adults. J. Appl.Physiol. 17(2). 252-258.

Glogowska, M., and Widdicombe, J.G. (1973). Respiratory and Circulatory Responses to Phenyl Diguanide in Rabbits. Arch. Internat. de Pharmacodynamie et de Therapie. 203 (2). 313-322.

Guz, A., Noble, M.I., Widdicombe, J.G. (1966) The role of vagal and glossopharyngeal afferent nerves in respiratory sensation, control of breathing and arterial pressure regulation in conscious man. Clin. Sci. 30, 161-170

Guz, A., Widdicombe, J.G. (1968). Reflexes respiratoires provenant des poumons de l'homme. Poumon. Coeur. 24. 1033-1057.

Harris, W. (1952). Fifth and seventh cranial nerves in relation to nervous mechanism of taste sensation; new approach. Brit. Med. J. 1, 831-836.

Hatch, T.F. (1971). Thresholds - do they exist? Arch. Environ. Health. 22, 687.

Hatch, T.F. (1972). The role of permissible limits of airborne hazardous substances at the workplace in the prevention of occupational diseases. Bull World Hlth. Org.

Hauser, T.R., and Cummins, R.L. (1964). Increasing Sensitivity of 3-Methyl-2-Benzothiazolone Hydrazone Test for Analysis of Aliphatic Aldehydes in Air. Anal. Chem. 36, 679-681.

Henderson, Y., and Haggard, H.W. (1943). Noxious Gases. Amer. Chem. Soc. Monograph. Pub. Reinhold.

Henschler, D. (1962). Reactions of the Lung to Repeated and Long-term Exposures to Respiratory Irritants. Gordon Res. Conf. on Toxicol. (1962) Cited by Roe, F.J.C., in Modern Trends in Toxicology, Eds. Boyland and Goulding, Butterworths (1968).

Hersch, P.A. (1969). Controlled Addition of Experimental Pollutants to Air. J. Air. Polln. Control Assn. 19(3), 164-172.

Hill, D.W. (1961). Brit. J. Appl. Phys. 12, 410-413.

Hill, D.W., and Newell, H.A. (1965). Nature. 206, 708-709.

Hodgson, E.S. (1955). Problems in invertebrate chemoreception. Quart. Rev. Biol. 30, 331-347.

Jancso, N. (1958). Die Desensibilisierung des Organismus gegenüber reizenden und entzündungserreizenden chemischen Wirkungen. Acta physiol. Hung. Suppl. 12, 15.

Jancso, N., and Gabor, A. (1959). Dauerausschaltung der chemischen Schmerzempfindlichkeit durch Capsaicin. Arch. exp. Path. Pharmak. 236, 142-145.

Jancso, N. (1960). Role of the nerve terminals in the mechanism of inflammatory reactions. Bull. Millard. Fillemore Hosp., Buffalo, N.Y. 7, 53-77.

Jancso, N., Gabor, A., and Takats, I. (1961). Pain

Jeffery, P. and Lynne Reid. (1973). Intra-epithelial nerves in normal rat airways: a quantitative electron microscope study. J. Anat. 114(1), 35-45.

Kasl, S.V. (1964). Some Phychological Factors Associated with Illness Behaviour and Selected Illnesses. J. Chron. Diseases. 17, 325-345.

Keele, C.A. (1964). and Armstrong, D. Substances producing pain and itch. Physiol. Soc. Monograph.

Kehoe, R.A., Machle, W.F., Kitzmiller, K.,and Le Blanc, T.J. (1932). J. Industr. Hyg. 14, 159.

Kleinfeld, M., and Giel, C.P. (1956). Clinical Manifestation of Ozone Poisoning. Amer. J. Med. Sci. 231, 638.

Lashley and Sperry. (1943). Amer. J. Physiol. 139, 446-450.

Lawther, P.J. (1952). Lancet. 21, 745.

Hersch, P.A. (1969). Controlled Addition of Experimental Pollutants to Air. J. Air. Polln. Control Assn. 19(3). 164-172.

Hill, D.W. (1961). Brit. J. Appl. Phys. 12, 410-413.

Hill, D.W., and Newell, H.A. (1965). Nature. 206, 708-709.

Hodgson, E.S. (1955). Problems in invertebrate chemoreception. Quart. Rev. Biol. 30, 331-347.

Jancso, N. (1958). Die Desensibilisierung des Organismus gegenüber reizenden und entzündungserreizenden chemischen Wirkungen. Acta physiol. Hung. Suppl. 12, 15.

Jancso, N., and Gabor, A. (1959). Dauerausschaltung der chemischen Schmerzempfindlichkeit durch Capsaicin. Arch. exp. Path. Pharmak. 236. 142-145.

Jancso, N. (1960). Role of the nerve terminals in the mechanism of inflammatory reactions. Bull. Millard. Fillemore Hosp., Buffalo, N.Y. 7, 53-77.

Jancso, N., Gabor, A., and Takats, I. (1961). Pain and inflammation induced by nicotine, acetylcholine and structurally related compounds and their prevention by desensitizing agents. Arch. physiol. Acad. Scient. Hungar. 19, 113-132.

Kagan, E. (1924). Arch. Hyg. 94, 41-53.

Kamburoff, P.L. (1969). Ph.D. Thesis, London University.

Kasl, S.V. (1964). Some Phychological Factors Associated with Illness Behaviour and Selected Illnesses. J. Chron. Diseases. 17, 325-345.

Keele, C.A. (1964). and Armstrong, D. Substances producing pain and itch. Physiol. Soc. Monograph.

Kehoe, R.A., Machle, W.F., Kitzmiller, K.,and Le Blanc, T.J. (1932). J. Industr. Hyg. 14, 159.

Kleinfeld, M., and Giel, C.P. (1956). Clinical Manifestation of Ozone Poisoning. Amer. J. Med. Sci. 231, 638.

Lashley and Sperry. (1943). Amer. J. Physiol. 139, 446-450.

Lawther, P.J. (1952). Lancet. 21, 745.

Pepys, J. "Hypersensitivity Diseases of the Lungs Due to Fungi and Organic Dusts" Pub. S.Karger, Basel, 1969.

Lovelock, J.E. (1961). Analyt. Chem. 33, 162-178.

Lewis, T., and Hess, W. (1933). Pain derived from the skin and the mechanism of its production. Clin. Sci. 1, 39-61.

Lewis, T. (1942). Pain, New York: Macmillian.

Lloyd, T.C., Jr., and Wright, G.W. (1963). Evaluation of methods used in detecting changes of airway resistance in man. Amer. rev. resp. dis. 87, 529-537.

Moncrieff, R.W. (1967). The Chemical Senses, 3rd ed., Leonard Hill Ltd. pub.

Nadel, J.A. (1973). Aerosol Effects on Smooth Muscle and Airways Visualization Technique, Arch. Int. Med. 131. 83-87.

Nelson, K.W., Edge, J.F., Ross, M., Woodman, L.E., and Silverman, L. (1943). Sensory Response to Certain Industrial Solvent Vapours. J. Ind. Hyg. & Toxicol. 25(7). 282-285.

Paintal, . (1970). The Mechanism of Excitation of Type J Receptors and the J Reflex. Herring-Brewer Centenary Symposium, p. 59-71. CIBA. Pub. Churchill.

Parker, G.H. (1910). Olfactory reactions in fishes. J. exper. Zool. 8, 535-542.

Parker, G.H. (1922). Smell. Taste and Allied Senses in the Vertebrates. Philadelphia: J.B. Lippincott Co.

Pate, J.B., Lodge, J.P., and Neary, M.P. (1963). Analytica Chim. Acta. 28, 341-348.

Patty, (1967). Industrial Hygiene and Toxicology Vol. 2. pub. Interscience N.Y.

Pelzer, A.M. (1965). Ph.D. Thesis. London University.

Pelzer, A.M., and Thomson, M.L.T. (1969). Body plethysmographic Measurements of Airway Conductance in obstructive pulmonary disease. Amer. Rev. Resp. Dis. 99,194.

Pride, N. (1970). Brit. J. Dis. Chest. 65, 135-169.

Ramazzini, B. (1713). Diseases of Workers. Republished 1964, Hafner pub. Co.

Roach.S.A.(1970). Hygiene Standards for Asbestos Ann. Occ. Hygiene 13, 7-15.

Sellick, H. and Widdicombe, J.G. (1970). Vagal deflation and inflation reflexes mediated by lung irritant receptors. Q. Jl. exp. Physiol..55 153-163.

Stransky, A., Malgorzata Szereda-Przestaszewska and Widdicombe, J.G. (1973). The effects of lung reflexes on laryngeal resistance and motoneurone discharge. J. Physiol. 231 417-438.

Malgorzata Szereda-Przestaszewska and Widdicombe J.G. (1973). Reflex effects of chemical irritation of the upper airways on the laryngeal lumen in cats. Respiration Physiology 18, 107-115.

Sawicki, E., Hauser, T.R., Stanley, T.W., and Elbert, W. (1961). Anal, Chem. 33(1). 93-96.

Sellick, H., and Widdicombe, J.G. (1971). Stimulation of Lung Irritant Receptors by cigarette smoke, carbon dust and histamine aerosol. J. Appl. Physiol. 31, 15-19.

Sheldon, R.E. (1909). The reactions of the dogfish to chemical stimuli. J. comp. Neurol. 19, 273-311.

Silverman, L., Schulter, H.F., First. M.W. (1946). Further Studies on Sensory Response to Certain Industrial Solvents. J. Ind. Hyg. & Toxicol. 28(6). 262-266.

Sim, V.M. and Pattle, R. (1957). J. Amer. Med. Assn. 165. 1908.

Sixth Report joint ICO/WHO Committee on Occ. Health, (1969).

Speizer, F.E. and Frank, N.R. (1966). The Uptake and release of SO_2 by the human nose. Arch. Environ. Health. 12, 725.

Stokinger, H.E. (1969). Introduction to threshold limit values for 1969 Am. Confr. Govt. Ind. Hygienists, Chicago,

Silverman, P. (1974). Behavioural Toxicology. New Scientist. 61, 255-257.

Strandberg, L.G. (1964). Absorption in the Respiratory Tract. Arch. Environ. Health. 9, 160.

Symposium on Assessment of Exposure and Risk (St. Bartholomew's Hospital), (1972). Ed. B.W. Duck, Soc. Occ. Med., R.C.P. London.

U.S. Public Health Service (1969). Air quality Criteria for Sulphur Oxides.

Widdicombe, J.G., Kent, D.C., Nadel, J.A. (1962). Mechanism of bronchoconstriction during inhalation of dust. J. Appl. Physiol. 17, 613-616.

Sawicki, E., Hauser, T.R., Stanley, T.W., and Elbert, W. (1961). Anal, Chem. 33(1). 93-96.

Sellick, H., and Widdicombe, J.G. (1971). Stimulation of Lung Irritant Receptors by cigarette smoke, carbon dust and histamine aerosol. J. Appl. Physiol. 31, 15-19.

Sheldon, R.E. (1909). The reactions of the dogfish to chemical stimuli. J. comp. Neurol. 19, 273-311.

Silverman, L., Schulter, H.F., First. M.W. (1946). Further Studies on Sensory Response to Certain Industrial Solvents. J. Ind. Hyg. & Toxicol. 28(6). 262-266.

Sellick, H. and Widdicombe, J.G. (1970). Vagal deflation and inflation reflexes mediated by lung irritant receptors. Q. Jl. exp. Physiol..55 153-163.

Stransky, A., Malgorzata Szereda-Przestaszewska and Widdicombe, J.G. (1973). The effects of lung reflexes on laryngeal resistance and motoneurone discharge. J. Physiol. 231 417-438.

Malgorzata Szereda-Przestaszewska and Widdicombe J.G. (1973). Reflex effects of chemical irritation of the upper airways on the laryngeal lumen in cats. Respiration Physiology 18, 107-115.

Widdicombe, J.G., Kent,D.C., Nadel, J.A. (1962). Mechanism of bronchoconstriction during inhalation of dust. J. Appl. Physiol. 17, 613-616.

Widdicombe, J.G. (1964). Respiratory Reflexes. In: Handbook of Physiology, section 3: Respiration, 1, 583-630, Washington D.C. Amer. Physiol. Soc.

Widdicombe, J.G., and Sterling, G.M. (1970). The Autonomic nervous system and breathing. Archs. Intern. Med. 126, 311.

APPENDIX A

INVESTIGATION OF ADEQUACY OF SIMPLE BUBBLER FOR PRODUCING SATURATED VAPOURS IN AIR.

The trilene experiment referred to in chapter 3 contained discrepancies between the nominal values of concentration based on the saturation vapour pressure at 20°C and the actual values as measured by Kitagawa gas detector tube (see Fig. A.1 and Table A.1).

Experiments were performed in which a light polythene bottle was filled with trilene and weighed. The bottles was thermostated at 20°C and a steady flow of air was bubbled through for thirty minutes after which the bottle was reweighed. This produced an estimated vapour pressure of about 32 mm hg (much less than the s.v.p. of 58 mm hg obtained by interpolation between known points)(Fig A.4).

Turning to the original values of concentration obtained in the trilene experiment the nominal values were based on a variety of different bubbler and diluting flows. The absolute discrepancies between

nominal and estimated concentration were plotted against flow through the bubbler (Fig. A.2). The correlation (r = 0.8540) is better than in Fig. A.1 (r = 0.7944).

Let "X" be the number of ccs. of trilene vapour theoretically removed per minute and "x(X)" the actual number.

Let the flow of air through the bubbler be $\dot{v}$ cc min^{-1} and the diluting air stream $\dot{V}$ cc min^{-1} .

Define "F" by the relationship:

$$x(X) = F.X$$

$$X = \frac{58}{760}\dot{v}$$

True concentration "K" = $\frac{x(X)}{\dot{V}}$ 10^6 ppm.

$$F = \frac{\dot{V}\,K}{\dot{v}\,10^6\,58/760}$$

The best available estimate of "K" is the Kitagawa reading.

$$F = \frac{\text{Kitagawa reading}}{\text{Nominal value}}$$

That "F" is a function of $\dot{v}$ is implicit in x(X) , since $X = c\dot{v}$

Fig. A.3 shows F plotted versus $\dot{v}$.

With the exception of the points labelled "A" and "B" there is a trend showing falling values of "F" with increasing bubbler flow. Within the data recorded a straight line is appropriate, although the approach to the abscissa is likely to become increasingly asymptotic at flows greater than 200 cc min^{-1}. The interesting feature of the points "A" and "B" is that they are derived from the three lowest Kitagawa readings. The two points at "A" were both ten ppm. : the claimed lower limit of measurement. The evidence suggests that the accuracy of measurement is greatly reduced in this region.

Excluding the points "A" and "B", the correlation obtained was $r = -0.9766$. The least squares regression line was computed and used as a correction to the nominal values.

Conclusion.

The proportional saturation of the stream through the bubbler falls with increasing flow. A chain of condensing bottles is proposed as a method of obtaining one hundred percent saturation.

TABLE A.1.

CONCENTRATIONS OF TRILENE (PPM) PRODUCED AT DIFFERENT FLOWS USING A SIMPLE BUBBLER.

Bubbler flow ($cc\ min^{-1}$)	Concentration Nominal	Concentration Kita-gawa	Absolute error	F	Concentration corrected value
50	70	70	0	0.92	70
60	152	130	22	0.86	137
90	229	170	59	0.74	177
50	70	50	20	0.66	70
150	573	250	323	0.44	287
50	38	10	28	0.26	36
50	38	10	28	0.26	36
200	764	180	584	0.24	229
150	229	120	109	0.53	115
150	382	220	162	0.58	191
150	382	220	162	0.58	191
50	127	120	7	0.95	118

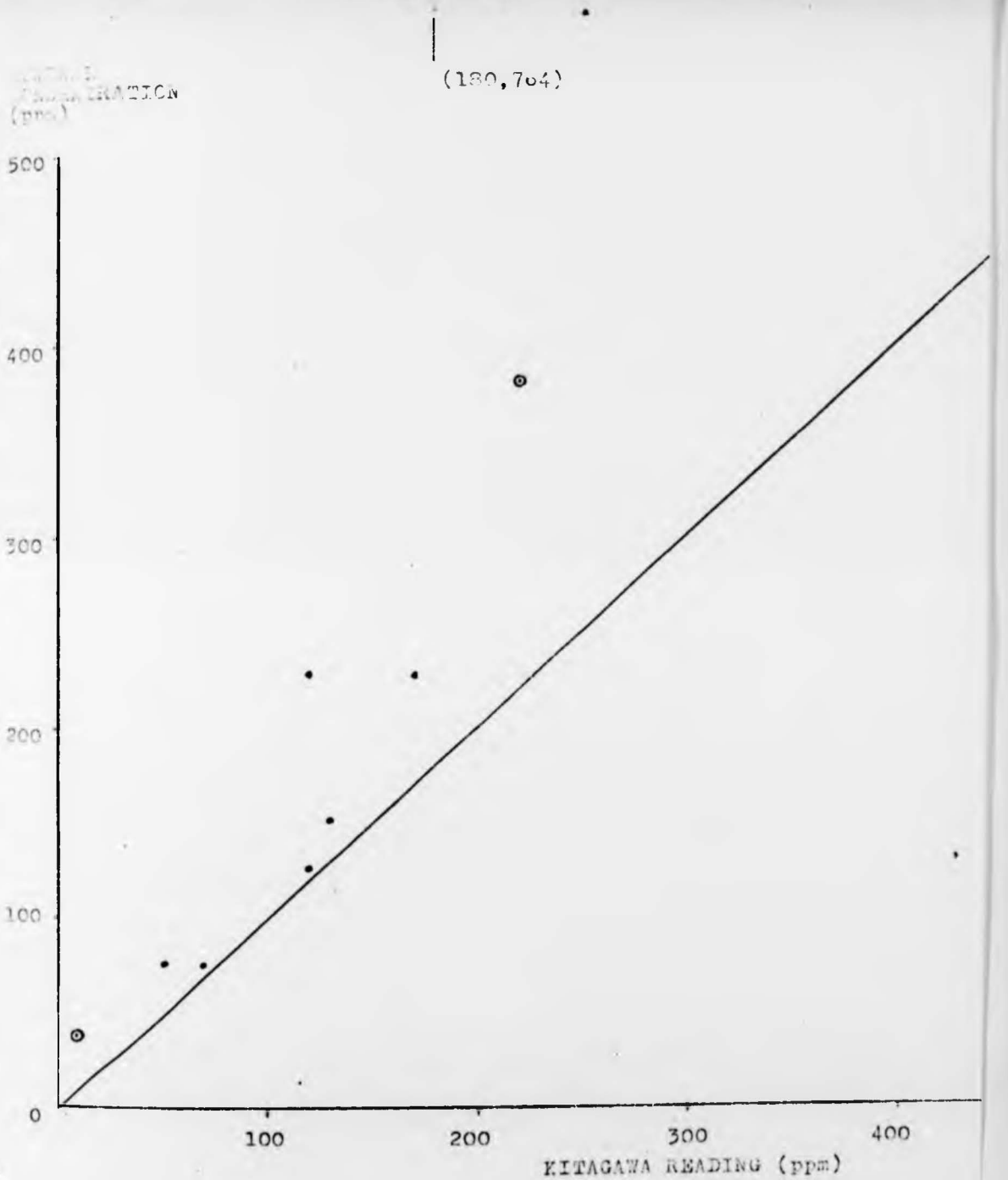


Fig. A.1. Nominal values of concentration against actual.

⊙ TWO POINTS COINCIDENT.

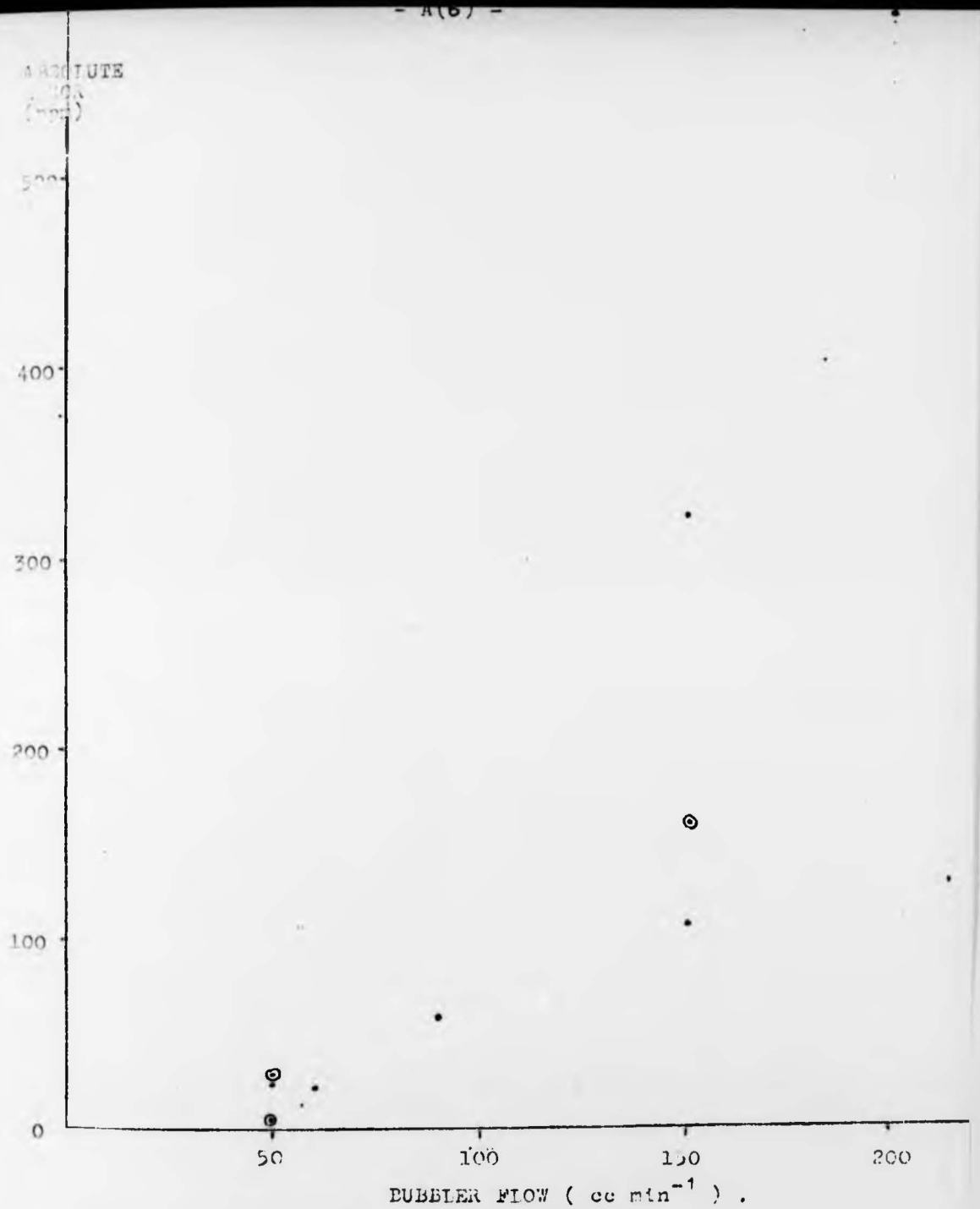


Fig. A.2. Absolute error against bubbler flow.

⊙ TWO POINTS COINCIDENT.

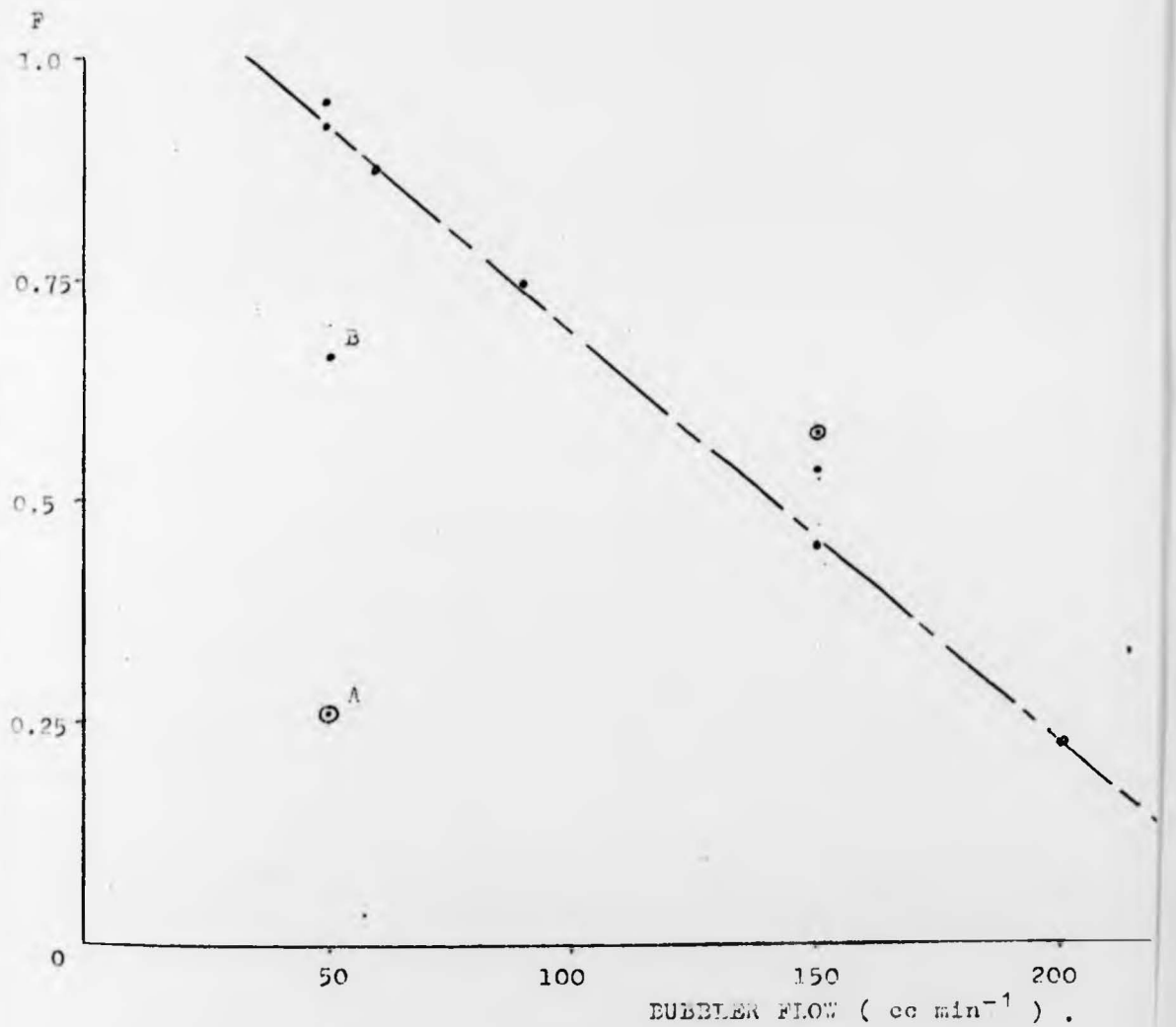


Fig. A.3. Fractional saturation (F) against bubbler flow.

⊙ TWO POINTS COINCIDENT.

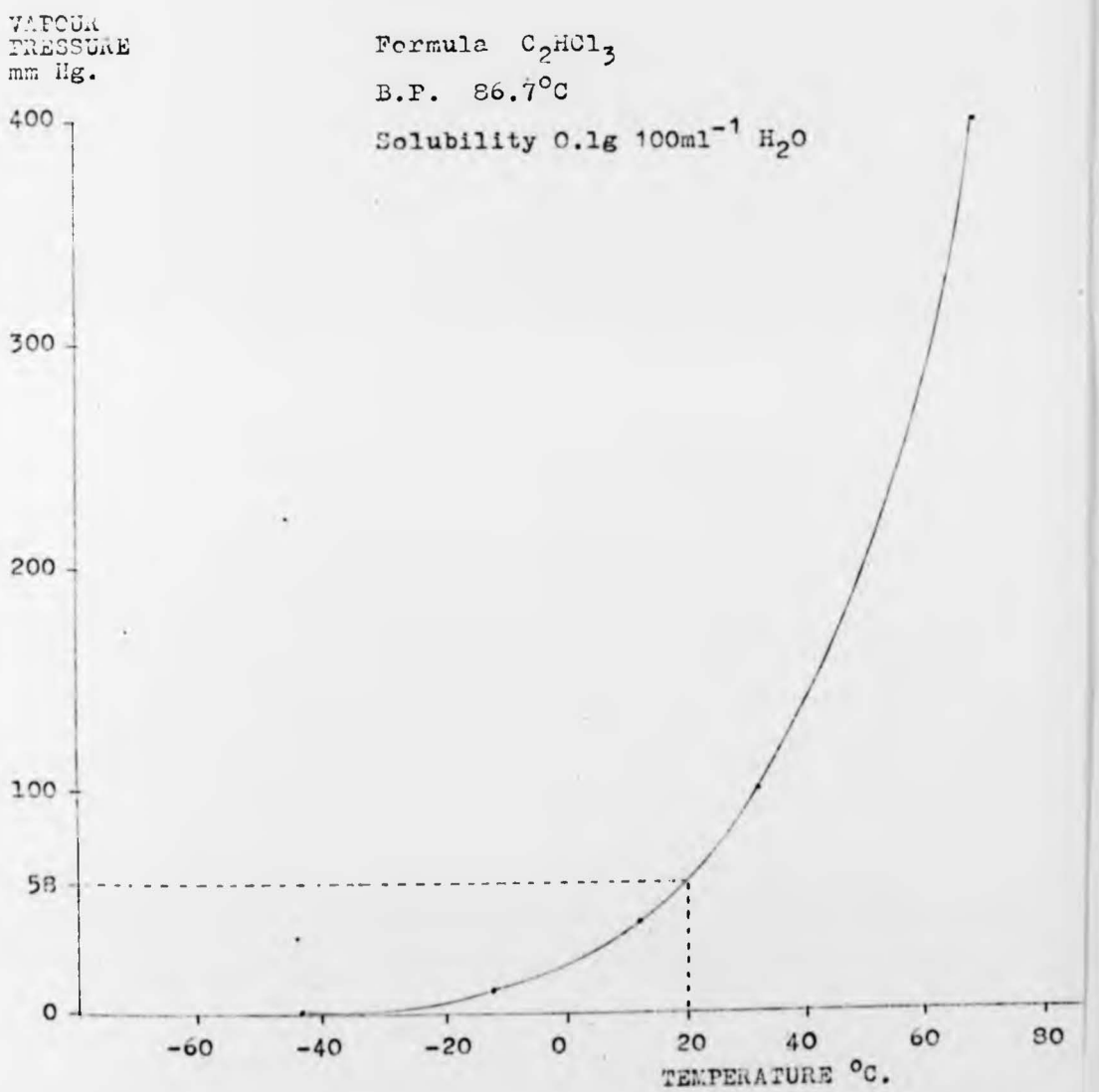


Fig.A.4. Saturated Vapour Pressure Curve Trichlorethylene.

APPENDIX B.

ATOPY AND SMOKING.

Prick tests for three common allergens: grass pollen (group B2), house dust and aspergillus fumigatus, were carried out on twentynine subjects, and the results are summarised in Table B.1. Of these, four subjects were sensitive to grass pollen only, three subjects were sensitive to house dust only and three subjects were sensitive to both. No subjects were sensitive to aspergillus. Subjects responding to one or more allergens were defined as atopic. It was observed during the experiments that some subjects were more sensitive to irritants than others but it was not possible to associate this with atopy or smoking. The number of smokers * (thirteen/fortyfive) was small and while the number of atopics ($\simeq$ 30%) was on the large side it contained several anomolies. For instance MT and PB were both regarded as relatively insensitive subjects to irritants yet both responded strongly to grass pollen. Neither suffered from hay fever yet PB had an identical twin who suffered from hay fever and MT (a South African) had children who suffered from hay fever.

* Range 448 - 11,607 pack years.

TABLE B.1.

PRICK TEST RESULTS

Subject	Grass pollen	House dust	Aspergillus	Comments
P.B.	+++(4)	-	-	No history - but twin sister hay-fever.
C.B.	-	-	-	
J.C.(a)	-	-	-	
D.C.	-	-	-	
J.C.(b)	-	-	-	
J.C.(c)	-	-	-	
G.C.	+++(4)	+	-	Asthma in family.
G.D.	-	-	-	
R.D.	-	-	-	
N.F.	-	+++(4)	-	Allergy to house-hold dust.
S.F.	++(3)	-	-	Hay fever.
P.G.				
A.H.	-	-	-	
M.H.	-	-	-	
S.H.	-	-	-	
D.H.	-	-	-	
P.K.	+	-	-	No history.
P.M.	-	-	-	
E.O.	-	-	-	
D.P.	-	++(3)	-	
H.R.	+++(4)	+++(4)	-	History of allergy to feathers.
H.S.	+++(5)	+++(5)	-	History of asthma.

PRICK TEST RESULTS (Cont'd.)

Subject	Grass pollen	House dust	Aspergillus	Comments
M.T.	+++(5)	-	-	
M.L.T.	-	-	-	
S.W.	-	-	-	
A.W.	-	-	-	
M.L.	-	-	-	Allergic to penicillin.
P.C.	-	-	-	
C.C.	-	+++(6)	-	

APPENDIX C.

Results for SO_2 , NH_3 and Trichloroethylene reworked using all five replicates in each set.

The numbering is sequential as follows:

	Subject	week	day	
Thus	1	01	01	
		to		SO_2
	12	03	04	
	17	05	01	
		to		NH_3
	28	07	04	
	29	08	01	
		to		Trichloroethylene.
	33	08	05	

SERIAL NUMBER 1011

DATE	29/ 6/70	NUMBER	1	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	68	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	761

	R	V	G	SG
CONTROL 1	1.2356	5.3873	.8283	.1532
CONTROL 2	1.3635	5.3656	.7414	.1381
CONTROL MEAN	1.2995	5.3764	.7848	.1456
POST 1	1.3946	5.4755	.7337	.1335
POST 2	1.2644	5.8129	.8132	.1394
POST MEAN	1.3295	5.6442	.7735	.1364

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	7.32	1.84	-8.35	3.5	-0
POST 2	-2.70	8.12	-4.31	8.3	-0

SERIAL NUMBER 1012

DATE	30/ 6/70	NUMBER	1	MOUTH PRESSURE	.7700
GAS	SO2	WEIGHT	68	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	758

	R	V	G	SG
CONTROL 1	.7221	3.0161	1.4054	.4712
CONTROL 2	.7712	2.7093	1.3145	.4843
CONTROL MEAN	.7466	2.8627	1.3599	.4778
POST 1	1.0315	2.7629	.9751	.3526
POST 2	.9840	2.7629	1.0316	.3733
POST MEAN	1.0078	2.7629	1.0034	.3630

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	38.15	-3.49	-26.20	3.5	5.0
POST 2	31.60	-3.49	-21.86	7.5	9.0

SERIAL NUMBER 1013

DATE	1/ 7/70	NUMBER	1	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	68	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	752

	R	V	G	SG
CONTROL 1	1.1697	5.4075	.8577	.1592
CONTROL 2	1.0512	6.0817	.9695	.1595
CONTROL MEAN	1.1105	5.7446	.9136	.1593
POST 1	1.3286	7.3022	.7602	.1048
POST 2	1.3814	6.2031	.7462	.1196
POST MEAN	1.3550	6.7526	.7532	.1122

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	19.85	27.11	-34.24	2.5	4.0
POST 2	24.40	7.98	-24.96	9.5	11.0

SERIAL NUMBER 1014

DATE	2/ 7/70	NUMBER	1	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	68	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	754

	R	V	G	SG
CONTROL 1	1.1587	5.9772	.8692	.1456
CONTROL 2	.4578	6.4346	1.0731	.1658
CONTROL MEAN	1.0572	6.2059	.9711	.1557
POST 1	1.4382	11.2627	.7167	.0646
POST 2	1.3748	7.2089	.7564	.1044
POST 3	1.4429	8.0368	.7271	.0895
POST MEAN	1.4186	8.8362	.7334	.0862

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	36.03	81.48	-58.51	2.5	4.0
POST 2	30.04	16.16	-32.97	6.0	7.5
POST 3	36.48	29.50	-42.53	9.5	10.7

SERIAL NUMBER 2011

DATE	29/ 6/70	NUMBER	2	MOUTH PRESSURE	.7700
GAS	SO2	WEIGHT	62	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	761

	R	V	G	SG
CONTROL 1	1.0640	5.1614	.9832	.1888
CONTROL 2	1.0903	5.1072	.9211	.1808
CONTROL MEAN	1.0771	5.1343	.9522	.1848
POST 1	1.3836	4.9378	.7293	.1480
POST 2	1.0838	4.9413	.9488	.1928
POST MEAN	1.2338	4.9395	.8390	.1704

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	29.47	-3.83	-19.93	2.5	3.7
POST 2	.62	-3.76	4.31	8.5	9.7

SERIAL NUMBER [illegible]

DATE	30/ 6/70	NUMBER	2	MOUTH PRESSURE	.7700
GAS	502	HEIGHT	62	NOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	758

	R	V	G	SG
CONTROL 1	.9883	1.8692	1.1652	.6135
CONTROL 2	1.1337	1.5332	.8898	.5823
CONTROL MEAN	1.0610	1.7012	1.0275	.5979
POST 1	1.2887	1.5811	.7926	.5018
POST 2	1.2894	1.5584	.7914	.5059
POST MEAN	1.2892	1.5708	.7920	.5038

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	23.79	-7.06	-16.07	2.5	3.8
POST 2	23.90	-8.37	-15.39	6.5	8.0

SERIAL NUMBER 2013

DATE	1/ 7/70	NUMBER	2	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	62	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	752

	R	V	G	SG
CONTROL 1	1.1166	4.9387	.9270	.1869
CONTROL 2	1.1274	5.5422	.8900	.1607
CONTROL MEAN	1.1220	5.2405	.9085	.1738
POST 1	1.2342	5.3995	.8445	.1568
POST 2	1.0060	5.9549	.9971	.1674
POST MEAN	1.1201	5.6772	.9208	.1621

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES	
				START	FINISH
POST 1	10.00	3.04	-9.80	2.5	3.7
POST 2	-10.34	13.63	-3.68	9.0	10.3

SERIAL NUMBER 2014

DATE	2/ 7/70	NUMBER	2	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	62	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	754

	R	V	G	SG
CONTROL 1	1.0619	5.2993	.9724	.1828
CONTROL 2	.9047	5.7915	1.1280	.1940
CONTROL MEAN	.9833	5.5454	1.0502	.1884
POST 1	1.1747	6.9270	.9929	.1418
POST 2	1.0856	5.7167	.9378	.1635
POST 3	.9701	5.3650	1.0771	.1995
POST MEAN	1.0768	6.0029	1.0026	.1683

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	19.46	24.91	-24.73	2.3	3.3
POST 2	10.41	3.09	-13.20	5.5	6.8
POST 3	-1.34	-3.25	5.90	8.5	9.8

SERIAL NUMBER 3011

DATE	29/ 6/70	NUMBER	3	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	54	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	761

	R	V	G	SG
CONTROL 1	1.1301	4.2617	.8951	.2114
CONTROL 2	1.1702	3.8729	.8700	.2241
CONTROL MEAN	1.1502	4.0673	.8825	.2177
POST 1	1.5552	3.7857	.6481	.1717
POST 2	1.1297	4.1074	.9055	.2197
POST MEAN	1.3424	3.9465	.7768	.1957

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	35.21	-6.92	-21.16	3.0	4.3
POST 2	-1.78	.99	.90	7.0	8.3

SERIAL NUMBER 3012

DATE	30/ 6/70	NUMBER	3	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	54	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	758

	R	V	G	SG
CONTROL 1	1.6338	1.0971	.6152	.5772
CONTROL 2	1.2299	3.2324	.8578	.3212
CONTROL MEAN	1.4319	2.1648	.7365	.4492
POST 1	1.3713	4.0122	.7459	.1886
POST 2	1.3382	3.6082	.7710	.2129
POST MEAN	1.3548	3.8102	.7584	.2008

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-4.23	85.34	-58.01	2.0	3.3
POST 2	-6.54	66.68	-52.60	8.0	10.0

SERIAL NUMBER 3013

DATE	1/ 7/70	NUMBER	3	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	54	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	752

	R	V	G	SG
CONTROL 1	1.5361	4.2405	.6722	.1616
CONTROL 2	1.2297	4.0278	.8308	.2055
CONTROL MEAN	1.3829	4.1342	.7515	.1836
POST 1	1.7889	4.3141	.5840	.1346
POST 2	1.5566	4.2445	.6455	.1522
POST MEAN	1.6728	4.2793	.6148	.1434

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	29.36	4.35	-26.68	2.5	4.0
POST 2	12.56	2.67	-17.08	6.0	7.3

SERIAL NUMBER 3014

DATE	2/ 7/70	NUMBER	3	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	54	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	754

	R	V	G	SG
CONTROL 1	1.3714	4.0390	.7343	.1824
CONTROL 2	1.2180	3.7151	.8224	.2214
CONTROL MEAN	1.2947	3.8771	.7784	.2019
POST 1	1.7178	3.8583	.6619	.1680
POST 2	1.1692	3.7248	.8621	.2317
POST 3	1.3236	3.7729	.7604	.2023
POST MEAN	1.4035	3.7853	.7615	.2007

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	32.68	-.48	-16.81	2.3	3.5
POST 2	-9.70	-3.93	14.75	6.0	7.3
POST 3	2.23	-2.69	.19	9.5	10.8

SERIAL NUMBER 4011

DATE	29/ 6/70	NUMBER	4	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	61	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	761

	R	V	G	SG
CONTROL 1	1.5152	4.7058	.6644	.1418
CONTROL 2	1.5543	4.3979	.6453	.1484
CONTROL MEAN	1.5348	4.5518	.6549	.1451
POST 1	1.4997	5.6923	.6796	.1201
POST 2	1.7731	4.8700	.5713	.1171
POST MEAN	1.6364	5.2811	.6255	.1186

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-2.29	25.05	-17.26	2.8	4.0
POST 2	15.53	6.99	-19.30	13.0	14.5

SERIAL NUMBER 4012

DATE	30/ 6/70	NUMBER	4	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	61	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	758

	R	V	G	SG
CONTROL 1	1.3281	4.5162	.8305	.1798
CONTROL 2	1.3719	3.9858	.7327	.1853
CONTROL MEAN	1.3500	4.2510	.7816	.1826
POST 1	1.5533	4.1077	.6550	.1621
POST 2	1.7909	3.8749	.5657	.1517
POST MEAN	1.6721	3.9913	.6104	.1569

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	15.06	-3.37	-11.18	2.0	3.3
POST 2	32.66	-8.85	-16.89	8.0	-0

SERIAL NUMBER 4013

DATE	1/ 7/70	NUMBER	4	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	61	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	752

	R	V	G	SG
CONTROL 1	1.6471	4.4962	.6188	.1382
CONTROL 2	1.4587	4.8488	.6903	.1426
CONTROL MEAN	1.5529	4.6725	.6545	.1404
POST 1	1.6847	5.2793	.5942	.1133
POST 2	1.6809	5.0968	.6073	.1189
POST MEAN	1.6828	5.1881	.6007	.1161

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	8.49	12.99	-19.27	2.0	3.5
POST 2	8.24	9.08	-15.34	5.5	-0

SERIAL NUMBER 4014

DATE	2/ 7/70	NUMBER	4	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	61	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	754

	R	V	G	SG
CONTROL 1	1.8542	4.6255	.6418	.1337
CONTROL 2	1.1086	4.9106	.9651	.1938
CONTROL MEAN	1.4814	4.7681	.8034	.1637
POST 1	1.3759	5.5667	.7378	.1328
POST 2	1.2883	4.9546	.8005	.1610
POST 3	1.2905	4.8742	.8054	.1674
POST MEAN	1.3182	5.1319	.7813	.1537

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-7.12	16.75	-18.93	2.0	3.3
POST 2	-13.04	3.91	-1.69	6.3	7.5
POST 3	-12.89	2.23	2.24	11.3	12.8

SERIAL NUMBER 5021

DATE	6/ 7/70	NUMBER	5	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	51	BOX PRESSURE	4.7500
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	765

	R	V	G	SG
CONTROL 1	1.1176	5.3657	.9329	.1724
CONTROL 2	1.2299	4.9577	.8206	.1657
CONTROL MEAN	1.1737	5.1617	.8768	.1690
POST 1	1.4255	4.6210	.7427	.1595
POST 2	1.1940	4.5472	.8393	.1854
POST MEAN	1.3098	4.5841	.7910	.1724

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	21.45	-10.48	-5.66	2.0	3.3
POST 2	1.73	-11.90	9.70	5.5	6.8

SERIAL NUMBER 5022

DATE	7/ 7/70	NUMBER	5	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	51	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	759

	R	V	G	SG
CONTROL 1	1.2225	3.7646	.8287	.2212
CONTROL 2	1.4044	3.8502	.7160	.1859
CONTROL MEAN	1.3134	3.8074	.7723	.2036
POST 1	1.5436	4.7375	.6498	.1373
POST 2	1.5480	5.1845	.6491	.1252
POST MEAN	1.5458	4.9610	.6495	.1312

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	17.52	24.43	-32.55	2.3	3.5
POST 2	17.86	36.17	-38.51	6.0	7.0

SERIAL NUMBER 5023

DATE	8/ 7/70	NUMBER	5	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	51	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	753

	R	V	G	SG
CONTROL 1	1.3008	3.8038	.7908	.2072
CONTROL 2	1.2059	3.7558	.8332	.2216
CONTROL MEAN	1.2533	3.7798	.8120	.2144
POST 1	1.5497	3.9660	.6497	.1644
POST 2	1.3362	3.9602	.7584	.1919
POST MEAN	1.4429	3.9631	.7040	.1781

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	23.65	4.93	-23.34	3.0	4.3
POST 2	6.61	4.77	-10.48	6.0	7.0

SERIAL NUMBER 5024

DATE	9/ 7/70	NUMBER	5	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	51	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	755

	R	V	G	SG
CONTROL 1	1.0422	3.7790	.9820	.2590
CONTROL 2	.9607	3.4843	1.0488	.3014
CONTROL MEAN	1.0015	3.6317	1.0154	.2802
POST 1	1.3554	3.3928	.7399	.2188
POST 2	1.0238	3.8026	.9806	.2587
POST MEAN	1.1896	3.5977	.8602	.2387

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	35.34	-6.58	-21.93	2.3	3.3
POST 2	2.23	4.71	-7.69	6.3	7.5

SERIAL NUMBER 6021

DATE	6/ 7/70	NUMBER	6	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	53	BOX PRESSURE	4.7500
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	766

	R	V	G	SG
CONTROL 1	1.5606	6.2614	.6420	.1026
CONTROL 2	1.6189	5.9150	.6185	.1075
CONTROL MEAN	1.5898	6.0882	.6302	.1050
POST 1	1.6832	5.8140	.5950	.1038
POST 2	1.8456	4.7338	.5547	.1174
POST MEAN	1.7644	5.2739	.5749	.1106

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	5.88	-4.50	-1.13	2.3	3.5
POST 2	16.09	-22.25	11.75	6.8	8.3

SERIAL NUMBER 6022

DATE	7/ 7/70	NUMBER	6	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	53	BOX PRESSURE	4.7500
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.7118	4.2799	.5899	.1387
CONTROL 2	2.1282	3.6166	.4741	.1334
CONTROL MEAN	1.9200	3.9482	.5320	.1360
POST 1	2.0432	3.4914	.5269	.1510
POST 2	2.0292	3.4513	.4968	.1468
POST MEAN	2.0362	3.4714	.5118	.1489

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	6.42	-11.57	10.97	2.3	3.5
POST 2	5.69	-12.59	7.92	7.0	8.5

SERIAL NUMBER 6023

DATE	8/ 7/70	NUMBER	6	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	53	BOX PRESSURE	4.7500
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	753

	R	V	G	SG
CONTROL 1	1.6868	4.3750	.6165	.1444
CONTROL 2	1.5377	4.6123	.6585	.1428
CONTROL MEAN	1.6123	4.4937	.6375	.1436
POST 1	1.5493	4.5682	.6508	.1443
POST 2	1.6434	4.4562	.6094	.1375
POST MEAN	1.5963	4.5122	.6301	.1409

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-3.91	1.66	.51	2.5	3.8
POST 2	1.93	-.83	-4.25	7.3	-0

SERIAL NUMBER 6024

DATE	9/ 7/70	NUMBER	6	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	53	BOX PRESSURE	4.7500
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.7349	3.6533	.5988	.1637
CONTROL 2	1.4731	3.8275	.6831	.1799
CONTROL MEAN	1.6040	3.7404	.6410	.1718
POST 1	1.7781	3.8248	.5661	.1479
POST 2	1.7945	3.9780	.5960	.1481
POST MEAN	1.7863	3.9014	.5810	.1480

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

					CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	10.85	2.26	-13.91		2.0	3.3
POST 2	11.88	6.35	-13.80		6.0	7.3

SERIAL NUMBER 7021

DATE	6/ 7/70	NUMBER	7	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	63	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	765

	R	V	G	SG
CONTROL 1	1.2124	4.0031	.8307	.2078
CONTROL 2	1.2124	4.0031	.8307	.2078
CONTROL MEAN	1.2124	4.0031	.8307	.2078
POST 1	1.4584	4.9348	.6996	.1436
POST 2	1.5325	4.3851	.6658	.1517
POST MEAN	1.4955	4.6599	.6827	.1477

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	20.29	23.27	-30.90	2.3	3.5
POST 2	26.40	9.54	-26.98	6.3	8.0

SERIAL NUMBER 7022

DATE	7/ 7/70	NUMBER	7	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	63	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	759

	R	V	G	SG
CONTROL 1	1.3782	3.0835	.7385	.2395
CONTROL 2	1.3906	4.3175	.7244	.1683
CONTROL MEAN	1.3844	3.7005	.7315	.2039
POST 1	1.5553	4.4521	.6495	.1459
POST 2	1.2596	4.4760	.8038	.1795
POST MEAN	1.4074	4.4640	.7267	.1627

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	12.35	20.31	-28.43	2.8	4.0
POST 2	-9.02	20.96	-11.95	8.5	9.5

SERIAL NUMBER 7023

DATE	8/ 7/70	NUMBER	7	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	63	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.2044	3.7427	.8323	.2223
CONTROL 2	1.3153	3.5585	.7674	.2155
CONTROL MEAN	1.2599	3.6506	.7998	.2189
POST 1	1.6495	3.6145	.6390	.1759
POST 2	1.9004	3.6652	.5356	.1466
POST MEAN	1.7750	3.6399	.5873	.1612

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	30.93	-.99	-19.65	2.8	3.8
POST 2	50.85	.40	-33.01	7.0	8.3

SERIAL NUMBER 7024

DATE	9/ 7/70	NUMBER	7	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	63	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.7539	3.5306	.5975	.1690
CONTROL 2	1.4077	3.7253	.7151	.1934
CONTROL MEAN	1.5808	3.6279	.6563	.1812
POST 1	1.5998	3.5363	.6254	.1774
POST 2	1.7809	4.1183	.5816	.1409
POST MEAN	1.6904	3.8273	.6035	.1591

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	1.20	-2.52	-2.11	2.0	3.0
POST 2	12.66	13.52	-22.28	5.8	7.0

SERIAL NUMBER 8021

DATE	6/ 7/70	NUMBER	8	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.2786	4.8126	.7888	.1639
CONTROL 2	1.1088	6.7833	.9106	.1356
CONTROL MEAN	1.1937	5.7980	.8497	.1497
POST 1	1.1412	6.1938	.8856	.1431
POST 2	1.2228	6.1274	.8309	.1362
POST MEAN	1.1820	6.1606	.8582	.1396

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-4.40	6.83	-4.42	2.0	3.3
POST 2	2.44	5.68	-9.08	7.0	9.3

SERIAL NUMBER 8022

DATE	7/ 7/70	NUMBER	8	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	4.7500
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.2125	7.6029	.8490	.1133
CONTROL 2	1.2600	8.6862	.7939	.0920
CONTROL MEAN	1.2363	8.1445	.8214	.1026
POST 1	1.3785	7.7454	.7394	.0951
POST 2	1.4785	8.0183	.6872	.0861
POST MEAN	1.4285	7.8818	.7133	.0906

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	11.51	-4.90	-7.37	2.0	3.0
POST 2	19.60	-1.55	-16.06	5.0	6.0

SERIAL NUMBER 8023

DATE	8/ 7/70	NUMBER	8	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	4.7500
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.3733	7.6190	.7386	.1017
CONTROL 2	1.3733	7.6190	.7386	.1017
CONTROL MEAN	1.3733	7.6190	.7386	.1017
POST 1	1.3466	6.5566	.7513	.1145
POST 2	1.4964	6.8163	.6692	.0983
POST MEAN	1.4215	6.6864	.7102	.1064

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-1.94	-13.95	12.59	2.0	3.3
POST 2	8.97	-10.54	-3.37	7.5	8.8

SERIAL NUMBER 8024

DATE	14/ 7/70	NUMBER	8	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	4.7500
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	755

	R	V	G	SG
CONTROL 1	1.1229	6.8462	.8955	.1310
CONTROL 2	1.4158	5.8699	.7108	.1213
CONTROL MEAN	1.2694	6.3580	.8031	.1261
POST 1	1.9929	7.3285	.5104	.0695
POST 2	1.8025	7.2723	.5635	.0774
POST MEAN	1.8977	7.3004	.5371	.0735

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	57.00	15.26	-44.91	2.0	3.3
POST 2	41.99	14.38	-38.64	6.0	7.3

SERIAL NUMBER 9031

DATE	14/ 7/70	NUMBER	9	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.3054	3.0123	.7707	.2557
CONTROL 2	1.1333	3.6275	.8933	.2459
CONTROL MEAN	1.2193	3.3199	.8320	.2508
POST 1	1.3225	3.6275	.7605	.2099
POST 2	1.1533	4.0940	.8891	.2178
POST MEAN	1.2379	3.8607	.8248	.2138

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	8.46	9.27	-16.32	2.3	3.5
POST 2	-5.42	23.32	-13.17	6.0	7.3

SERIAL NUMBER 9032

DATE	15/ 7/70	NUMBER	9	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0964	3.7095	.9270	.2493
CONTROL 2	1.0877	3.9451	.9315	.2355
CONTROL MEAN	1.0920	3.8273	.9292	.2424
POST 1	1.2055	3.9898	.8624	.2162
POST 2	1.5569	3.3219	.6441	.1939
POST MEAN	1.3812	3.6558	.7532	.2051

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	10.39	4.25	-10.82	2.8	4.3
POST 2	42.57	-13.21	-20.02	7.8	8.8

SERIAL NUMBER 9033

DATE	16/ 7/70	NUMBER	9	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0644	4.1540	.9406	.2264
CONTROL 2	1.4506	3.3509	.6913	.2087
CONTROL MEAN	1.2575	3.7525	.8159	.2176
POST 1	1.4854	3.1261	.6772	.2189
POST 2	1.5458	2.8049	.6490	.2322
POST MEAN	1.5156	2.9655	.6631	.2256

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	18.13	-16.69	.60	1.8	3.0
POST 2	22.93	-25.25	6.73	6.0	7.3

SERIAL NUMBER 9034

DATE	17/ 7/70	NUMBER	9	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	85	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.4940	3.4263	.6703	.1963
CONTROL 2	1.4147	3.1851	.7153	.2259
CONTROL MEAN	1.4543	3.3057	.6928	.2111
POST 1	1.3843	3.2814	.7267	.2214
POST 2	1.3025	3.1916	.7708	.2423
POST MEAN	1.3434	3.2365	.7488	.2319

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-4.82	-.74	4.87	2.3	3.3
POST 2	-10.44	-3.45	14.77	7.3	8.3

SERIAL NUMBER 10031

DATE	14/ 7/70	NUMBER	10	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	59	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	754

	R	V	G	SG
CONTROL 1	1.2247	2.9913	.8188	.2737
CONTROL 2	1.2907	3.1255	.7775	.2495
CONTROL MEAN	1.2577	3.0584	.7981	.2616
POST 1	2.6638	3.8856	.3793	.0979
POST 2	1.7711	3.8500	.5720	.1489
POST MEAN	2.2175	3.8678	.4757	.1234

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	111.80	27.05	-62.60	2.5	3.8
POST 2	40.82	25.88	-43.10	8.0	9.5

SERIAL NUMBER 10032

DATE	15/ 7/70	NUMBER	10	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	59	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.1378	3.3571	.9551	.2805
CONTROL 2	1.2424	3.0448	.8238	.2718
CONTROL MEAN	1.1901	3.2010	.8895	.2762
POST 1	4.7829	2.1697	.2364	.1090
POST 2	6.3027	2.0544	.1700	.0817
POST MEAN	5.5428	2.1121	.2032	.0953

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	301.90	-32.22	-60.55	2.0	3.5
POST 2	429.60	-35.82	-70.41	6.0	7.5

SERIAL NUMBER 10033

DATE	16/ 7/70	NUMBER	10	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	59	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.3384	2.8569	.7491	.2675
CONTROL 2	1.3981	2.9059	.7180	.2495
CONTROL MEAN	1.3683	2.8814	.7335	.2585
POST 1	2.2062	2.8091	.4554	.1647
POST 2	1.4517	2.8400	.7040	.2466
POST MEAN	1.8289	2.8245	.5797	.2057

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	61.24	-2.51	-36.27	2.0	3.3
POST 2	6.10	-1.44	-4.58	5.8	7.0

SERIAL NUMBER 10034

DATE	17/ 7/70	NUMBER	10	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	59	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.3189	2.9591	.7670	.2612
CONTROL 2	1.7064	3.6639	.6132	.1661
CONTROL MEAN	1.5126	3.3115	.6901	.2137
POST 1	7.0022	1.0390	.1525	.1505
POST 2	10.0564	.8108	.1069	.1307
POST MEAN	8.5293	.9249	.1297	.1406

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	362.91	-68.62	-29.56	2.5	3.5
POST 2	564.82	-75.52	-38.84	7.3	8.5

SERIAL NUMBER 11031

DATE	14/ 7/70	NUMBER	11	MOUTH PRESSURE	.7700
GAS	502	#FIGHT	62	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	754

	R	V	G	SG
CONTROL 1	1.7161	3.0408	.5832	.1920
CONTROL 2	1.6043	3.3354	.6254	.1876
CONTROL MEAN	1.6602	3.1881	.6043	.1898
POST 1	1.6866	3.2494	.5984	.1841
POST 2	1.6494	3.5530	.6109	.1727
POST MEAN	1.6680	3.4012	.6047	.1784

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	1.59	1.93	-3.02	2.3	3.5
POST 2	-.65	11.45	-8.98	5.8	6.8

SERIAL NUMBER 11032

DATE	15/ 7/70	NUMBER	11	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	62	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	764

	R	V	G	SG
CONTROL 1	1.3174	3.2463	.7628	.2357
CONTROL 2	1.4003	3.1317	.7166	.2292
CONTROL MEAN	1.3588	3.1890	.7397	.2325
POST 1	3.0871	1.8479	.3250	.1765
POST 2	3.6191	1.6616	.2809	.1706
POST MEAN	3.3531	1.7548	.3030	.1736

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	127.19	-42.05	-24.08	2.5	4.0
POST 2	166.34	-47.90	-26.60	6.3	7.5

SERIAL NUMBER 11033

DATE	16/ 7/70	NUMBER	11	MOUTH PRESSURE	.7700
GAS	SO2	WEIGHT	62	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.4598	3.1664	.6927	.2186
CONTROL 2	1.4695	3.4158	.6841	.2007
CONTROL MEAN	1.4646	3.2911	.6884	.2096
POST 1	1.9218	3.2410	.5224	.1623
POST 2	1.7600	3.0516	.5788	.1892
POST MEAN	1.8409	3.1463	.5506	.1757

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	31.21	-1.52	-22.56	2.0	3.0
POST 2	20.17	-7.28	-9.76	6.0	7.3

SERIAL NUMBER 11034

DATE	17/ 7/70	NUMBER	11	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	62	BOX PRESSURE	2.3000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.4185	2.8794	.7133	.2477
CONTROL 2	1.5298	2.6478	.6567	.2485
CONTROL MEAN	1.4742	2.7636	.6850	.2481
POST 1	1.9459	1.8524	.5189	.2808
POST 2	1.9459	1.8524	.5189	.2808
POST MEAN	1.9459	1.8524	.5189	.2808

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	32.00	-32.97	13.18	2.3	3.8
POST 2	32.00	-32.97	13.18	7.5	8.8

SERIAL NUMBER 12031

DATE	14/ 7/70	NUMBER	12	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	56	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	747

	R	V	G	SG
CONTROL 1	1.1229	3.6433	.9010	.2468
CONTROL 2	.9974	4.1438	1.0334	.2478
CONTROL MEAN	1.0601	3.8935	.9672	.2473
POST 1	1.2204	3.7203	.8277	.2224
POST 2	.8406	4.4673	1.2720	.2796
POST MEAN	1.0305	4.0938	1.0498	.2510

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	15.12	-4.45	-10.05	2.0	3.5
POST 2	-20.71	14.74	13.08	5.0	6.5

SERIAL NUMBER 12032

DATE	15/ 7/70	NUMBER	12	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	56	BOX PRESSURE	2.3000
DOSE	5	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	763

	R	V	G	SG
CONTROL 1	.9970	3.8451	1.0509	.2719
CONTROL 2	.7547	4.2942	1.3520	.3145
CONTROL MEAN	.8759	4.0697	1.2014	.2932
POST 1	.7810	4.2594	1.2833	.3020
POST 2	.8898	3.5315	1.1579	.3394
POST MEAN	.8354	3.8955	1.2206	.3207

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-10.83	4.66	3.02	2.5	4.0
POST 2	1.59	-13.22	15.78	5.5	6.8

SERIAL NUMBER 12033

DATE	16/ 7/70	NUMBER	12	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	56	BOX PRESSURE	2.3000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	769

	R	V	G	SG
CONTROL 1	.8944	3.5995	1.1288	.3152
CONTROL 2	.9710	3.7314	1.0894	.2896
CONTROL MEAN	.9327	3.6655	1.1091	.3024
POST 1	1.2821	3.4449	.7938	.2318
POST 2	1.0273	3.6601	.9798	.2683
POST MEAN	1.1547	3.5525	.8868	.2501

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	37.46	-6.02	-23.33	2.0	3.0
POST 2	10.14	-.15	-11.29	5.5	6.5

SERIAL NUMBER 12034

DATE	17/ 7/70	NUMBER	12	MOUTH PRESSURE	.7700
GAS	502	WEIGHT	56	BOX PRESSURE	2.3000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.3100
				ATMOSPHERIC PRESSURE	769

	R	V	G	SG
CONTROL 1	.8762	3.5032	1.2170	.3437
CONTROL 2	.9351	3.5830	1.0702	.3008
CONTROL MEAN	.9057	3.5431	1.1436	.3223
POST 1	1.3596	3.4785	.7883	.2243
POST 2	1.0300	3.7002	.9848	.2660
POST MEAN	1.1948	3.5893	.8865	.2452

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	50.12	-1.82	-30.39	2.0	3.5
POST 2	13.73	4.43	-17.46	6.3	7.3

SERIAL NUMBER 17051

DATE	2/11/70	NUMBER	17	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	51	BOX PRESSURE	1.8300
DOSE	60	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.8752	3.1408	1.2443	.4055
CONTROL 2	.8364	3.3963	1.2664	.3679
CONTROL MEAN	.8558	3.2685	1.2553	.3867
POST 1	.8704	3.6729	1.1666	.3185
POST 2	.8788	3.3134	1.1529	.3484
POST 3	.7583	3.6272	1.3989	.3819
POST 4	.6083	3.7011	1.7195	.4649
POST MEAN	.7789	3.5786	1.3595	.3784

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	1.71	12.37	-17.63	3.0	4.0
POST 2	2.69	1.37	-9.91	7.8	8.8
POST 3	-11.40	10.98	-1.24	11.0	12.3
POST 4	-28.92	13.23	20.21	18.0	19.0

SERIAL NUMBER 17052

DATE	3/11/70	NUMBER	17	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	51	BOX PRESSURE	4.7000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.1075	3.8366	.9346	.2460
CONTROL 2	.9586	7.6415	1.0745	.1399
CONTROL MEAN	1.0330	5.7391	1.0046	.1929
POST 1	1.3210	7.2509	.7638	.1054
POST 2	1.2884	7.1132	.7988	.1122
POST 3	1.2655	7.3037	.8163	.1124
POST 4	.9624	7.1997	1.0463	.1453
POST MEAN	1.2093	7.2169	.8563	.1189

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	27.88	26.34	-45.34	4.0	5.0
POST 2	24.72	23.94	-41.82	10.8	11.8
POST 3	22.50	27.26	-41.72	13.3	14.3
POST 4	-6.84	25.45	-24.67	19.8	20.5

SERIAL NUMBER 17053

DATE	4/11/70	NUMBER	17	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	51	BOX PRESSURE	1.8300
DOSE	180	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.1877	2.8979	.8454	.2960
CONTROL 2	1.0935	2.1602	.9550	.4394
CONTROL MEAN	1.1406	2.5291	.9002	.3677
POST 1	1.2297	2.8033	.8256	.2945
POST 2	1.1250	2.5698	.8953	.3535
POST 3	1.0298	2.8052	.9777	.3495
POST 4	1.0419	2.7461	.9689	.3535
POST MEAN	1.1066	2.7311	.9169	.3378

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	7.81	10.84	-19.90	2.0	3.0
POST 2	-1.37	1.61	-3.85	5.3	6.0
POST 3	-9.72	10.92	-4.96	10.8	11.8
POST 4	-8.66	8.58	-3.86	16.0	16.8

SERIAL NUMBER 17054

DATE	5/11/70	NUMBER	17	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	51	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0642	2.3350	.9396	.4029
CONTROL 2	1.0549	2.2038	.9484	.4307
CONTROL MEAN	1.0596	2.2694	.9440	.4168
POST 1	1.1679	2.2748	.8695	.3863
POST 2	1.0953	2.0497	.9156	.4489
POST 3	1.1236	2.2046	.9122	.4124
POST 4	1.1823	2.1568	.8575	.3972
POST MEAN	1.1423	2.1715	.8887	.4112

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES	
				START	FINISH
POST 1	10.23	.24	-7.31	2.5	3.3
POST 2	3.37	-9.68	7.70	6.0	6.8
POST 3	6.04	-2.85	-1.05	8.5	9.3
POST 4	11.58	-4.96	-4.69	11.5	12.3

SERIAL NUMBER 18051

DATE	2/11/70	NUMBER	18	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	63	BOX PRESSURE	1.8300
DOSE	150	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.8244	3.3482	1.3164	.3876
CONTROL 2	.7993	3.3493	1.2799	.3823
CONTROL MEAN	.8119	3.3488	1.2981	.3850
POST 1	1.1426	3.0335	.8856	.2927
POST 2	1.4093	2.9401	.7221	.2546
POST 3	1.2838	2.8168	.7871	.2832
POST MEAN	1.2786	2.9301	.7983	.2768

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	40.73	-9.41	-23.96	3.3	4.8
POST 2	73.59	-12.20	-33.86	7.5	8.8
POST 3	58.13	-15.88	-26.44	12.5	14.0

SERIAL NUMBER 18052

DATE	3/11/70	NUMBER	18	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	63	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.2174	2.1188	.8413	.4052
CONTROL 2	1.1505	2.2068	.8727	.3969
CONTROL MEAN	1.1840	2.1628	.8570	.4010
POST 1	.9733	2.4269	1.0308	.4290
POST 2	1.1385	2.2560	.8835	.3936
POST MEAN	1.0559	2.3414	.9571	.4113

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-17.80	12.21	6.98	2.3	3.3
POST 2	-3.84	4.31	-1.85	6.0	6.8

SERIAL NUMBER 18053

DATE	4/11/70	NUMBER	18	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	63	BOX PRESSURE	1.8300
DOSE	70	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.9839	2.3736	1.0171	.4309
CONTROL 2	1.0536	2.5091	.9574	.3813
CONTROL MEAN	1.0188	2.4414	.9873	.4061
POST 1	1.0071	2.5623	.9944	.3938
POST 2	1.0905	2.3092	.9241	.4044
POST MEAN	1.0488	2.4358	.9593	.3991

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-1.15	4.96	-3.03	2.3	3.3
POST 2	7.04	-5.41	-.43	6.0	7.0

SERIAL NUMBER 18054

DATE	5/11/70	NUMBER	18	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	63	BOX PRESSURE	1.8300
DOSE	20	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.1241	2.4622	.8938	.3637
CONTROL 2	1.1696	2.0555	.8597	.4203
CONTROL MEAN	1.1468	2.2589	.8768	.3920
POST 1	1.2295	2.8566	.8233	.2891
POST 2	1.1995	2.9488	.8340	.2835
POST MEAN	1.2145	2.9027	.8286	.2863

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	7.21	26.46	-26.23	2.0	2.8
POST 2	4.59	30.54	-27.68	5.0	6.0

SERIAL NUMBER 19051

DATE	2/11/70	NUMBER	19	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	4.7000
DOSE	15	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.2054	4.5709	.8458	.1894
CONTROL 2	1.0516	4.8902	.9887	.2042
CONTROL MEAN	1.1285	4.7306	.9172	.1968
POST 1	1.2654	4.2710	.8035	.1881
POST 2	1.1955	4.8902	.9008	.1833
POST MEAN	1.2304	4.5806	.8521	.1857

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	12.13	-9.71	-4.44	4.3	5.3
POST 2	5.93	3.38	-6.86	11.8	14.5

SERIAL NUMBER 19052

DATE	3/11/70	NUMBER	19	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	4.7000
DOSE	60	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0321	4.6520	.9704	.2143
CONTROL 2	1.1158	4.7780	.8990	.1907
CONTROL MEAN	1.0740	4.7150	.9347	.2025
POST 1	1.3739	4.5856	.7754	.1689
POST 2	1.3333	4.2365	.7615	.1808
POST MEAN	1.3536	4.4111	.7684	.1749

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	27.92	-2.75	-16.60	-0.0	-0.0
POST 2	24.14	-10.15	-10.71	-0.0	-0.0

SERIAL NUMBER 19053

DATE	4/11/70	NUMBER	19	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	4.7000
DOSE	45	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0581	4.5505	.9475	.2087
CONTROL 2	.9750	4.6291	1.0355	.2252
CONTROL MEAN	1.0166	4.5898	.9915	.2170
POST 1	1.1574	5.4721	.8718	.1611
POST 2	1.0168	5.0682	.9941	.1972
POST MEAN	1.0871	5.2701	.9330	.1792

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	13.85	19.22	-25.72	1.5	2.5
POST 2	.03	10.42	-9.09	5.5	6.5

SERIAL NUMBER 19054

DATE	5/11/70	NUMBER	19	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	1.8300
DOSE	180	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0550	1.8021	.9507	.5353
CONTROL 2	1.1003	1.7323	.9140	.5288
CONTROL MEAN	1.0777	1.7672	.9324	.5321
POST 1	1.1966	1.9992	.8412	.4213
POST 2	1.0593	1.7196	.9469	.5588
POST 3	1.1384	1.6912	.8792	.5225
POST MEAN	1.1314	1.8033	.8891	.5009

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	11.03	13.12	-20.82	2.0	3.0
POST 2	-1.70	-2.69	5.02	6.3	7.5
POST 3	5.63	-4.30	-1.79	10.3	11.3

SERIAL NUMBER 20051

DATE	2/11/70	NUMBER	20	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	53	BOX PRESSURE	4.7000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.9949	4.5306	1.0275	.2325
CONTROL 2	1.0604	4.4365	.9495	.2162
CONTROL MEAN	1.0276	4.4836	.9885	.2243
POST 1	1.4026	4.5816	.7149	.1570
POST 2	1.2666	4.7862	.7945	.1685
POST MEAN	1.3346	4.6839	.7547	.1628

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	36.49	2.19	-30.01	3.0	4.0
POST 2	23.25	6.75	-24.88	5.5	6.5

SERIAL NUMBER 20052

DATE	3/11/70	NUMBER	20	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	53	BOX PRESSURE	4.7000
DOSE	170	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.1377	3.7835	.8945	.2396
CONTROL 2	1.1018	6.7023	.9091	.1403
CONTROL MEAN	1.1198	5.2429	.9018	.1899
POST 1	1.1555	7.5948	.8800	.1159
POST 2	1.2956	8.2785	.7879	.0948
POST MEAN	1.2256	7.9366	.8339	.1054

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES	
				START	FINISH
POST 1	3.19	44.86	-38.97	2.0	3.5
POST 2	15.70	57.90	-50.07	7.5	8.5

SERIAL NUMBER 20053

DATE	4/11/70	NUMBER	20	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	53	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0656	2.8879	.9397	.3337
CONTROL 2	1.2238	2.8562	.8410	.2949
CONTROL MEAN	1.1447	2.8721	.8904	.3143
POST 1	1.1220	3.1905	.9109	.2856
POST 2	1.0929	3.3734	.9197	.2761
POST MEAN	1.1074	3.2819	.9153	.2809

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-1.98	11.09	-9.14	2.0	3.3
POST 2	-4.53	17.46	-12.17	4.8	5.5

SERIAL NUMBER 20054

DATE	5/11/70	NUMBER	20	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	53	BOX PRESSURE	1.8300
DOSE	90	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	1.0732	2.8777	.9527	.3415
CONTROL 2	1.2603	2.5362	.7947	.3140
CONTROL MEAN	1.1667	2.7070	.8737	.3277
POST 1	1.1132	1.7297	.9008	.5249
POST 2	1.1428	1.3860	.8852	.6399
POST 3	1.2554	2.7033	.8086	.3011
POST MEAN	1.1705	1.9397	.8649	.4886

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-4.59	-36.10	60.17	3.0	4.0
POST 2	-2.06	-48.80	95.23	5.8	6.5
POST 3	7.60	-.14	-8.14	6.5	7.8

SERIAL NUMBER 21061

DATE	9/11/70	NUMBER	21	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	60	BOX PRESSURE	1.8300
DOSE	20	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.3271	4.2996	3.1437	.7344
CONTROL 2	.3433	4.5088	2.9493	.6547
CONTROL MEAN	.3352	4.4042	3.0465	.6946
POST 1	.4174	4.1315	2.5955	.6198
POST 2	.4037	3.7193	2.5883	.7032
POST MEAN	.4105	3.9254	2.5919	.6615

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	24.52	-6.19	-10.77	2.0	3.0
POST 2	20.46	-15.55	1.25	4.8	5.8

SERIAL NUMBER 21062

DATE	10/11/70	NUMBER	21	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	60	BOX PRESSURE	1.8300
DOSE	170	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.2203	3.9650	4.6535	1.1711
CONTROL 2	.2932	3.6946	3.7561	1.0047
CONTROL MEAN	.2568	3.8298	4.2048	1.0879
POST 1	.4653	4.0954	2.2499	.5542
POST 2	.4126	3.7427	2.5648	.6839
POST MEAN	.4390	3.9191	2.4074	.6190

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	81.22	6.94	-49.06	2.0	3.0
POST 2	60.69	-2.27	-37.14	5.3	6.5

SERIAL NUMBER 21063

DATE	11/11/70	NUMBER	21	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	60	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.2899	4.1224	3.8745	.9306
CONTROL 2	.4196	3.9822	2.8855	.6861
CONTROL MEAN	.3547	4.0523	3.3800	.8083
POST 1	.5472	3.6876	1.8721	.5075
POST 2	.4512	3.7759	2.2226	.5901
POST MEAN	.4992	3.7317	2.0474	.5488

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	54.27	-9.00	-37.22	2.0	3.0
POST 2	27.19	-6.82	-27.00	4.5	5.5

SERIAL NUMBER 21064

DATE	12/11/70	NUMBER	21	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	60	BOX PRESSURE	1.8300
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.4038	4.1489	2.4921	.6031
CONTROL 2	.3724	4.6471	2.7069	.5850
CONTROL MEAN	.3881	4.3980	2.5995	.5940
POST 1	.6326	3.8176	1.8838	.4822
POST 2	.6150	3.9640	1.7157	.4303
POST 3	.1563	4.2459	6.9144	1.6330
POST 4	.2177	4.0030	6.1558	1.5251
POST MEAN	.4054	4.0076	4.1674	1.0176

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	63.00	-13.20	-18.83	2.3	3.5
POST 2	58.47	-9.87	-27.56	4.8	5.8
POST 3	-59.71	-3.46	174.89	-0.0	-0.0
POST 4	-43.92	-8.98	156.74	-0.0	-0.0

SERIAL NUMBER 22061

DATE	9/11/70	NUMBER	22	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5600	2.9419	1.8679	.6290
CONTROL 2	.6045	2.7701	1.6801	.6073
CONTROL MEAN	.5822	2.8560	1.7740	.6181
POST 1	.8553	3.4904	1.2232	.3471
POST 2	.6655	3.3665	1.5206	.4548
POST MEAN	.7604	3.4284	1.3719	.4009

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	46.89	22.21	-43.85	2.5	3.8
POST 2	14.29	17.88	-26.43	5.8	7.5

SERIAL NUMBER 22062

DATE	10/11/70	NUMBER	22	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	70	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6575	2.6245	1.5545	.5902
CONTROL 2	.7005	2.6163	1.4405	.5529
CONTROL MEAN	.6790	2.6204	1.4975	.5715
POST 1	1.2417	2.8975	.8111	.2824
POST 2	1.0877	2.4798	.9221	.3761
POST MEAN	1.1647	2.6886	.8666	.3292

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	82.87	10.57	-50.60	2.8	4.0
POST 2	60.19	-5.37	-34.20	6.3	7.3

SERIAL NUMBER 22063

DATE	11/11/70	NUMBER	22	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	20	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5081	3.0135	2.0084	.6654
CONTROL 2	.4878	2.8566	2.2457	.7756
CONTROL MEAN	.4980	2.9351	2.1271	.7205
POST 1	.6926	3.2696	1.4679	.4659
POST 2	.6563	2.7011	1.5372	.5695
POST MEAN	.6744	2.9853	1.5025	.5177

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	39.08	11.40	-35.34	2.0	2.8
POST 2	31.79	-7.97	-20.96	4.8	6.0

SERIAL NUMBER 22064

DATE	12/11/70	NUMBER	22	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	200	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5673	2.9256	1.9049	.6634
CONTROL 2	.6422	2.3869	1.5743	.6616
CONTROL MEAN	.6048	2.6562	1.7396	.6625
POST 1	1.1322	3.0616	.9695	.3108
POST 2	.7381	3.0800	1.3580	.4435
POST 3	.5585	2.6187	1.8031	.6897
POST 4	.6101	2.5874	1.6517	.6399
POST MEAN	.7597	2.8369	1.4456	.5210

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	87.21	15.26	-53.08	2.8	4.3
POST 2	22.04	15.95	-33.06	6.0	6.8
POST 3	-7.64	-1.41	4.11	10.3	11.3
POST 4	.87	-2.59	-3.41	13.0	14.0

SERIAL NUMBER 23061

DATE	9/11/70	NUMBER	23	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	4.7000
DOSE	110	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5555	6.7231	1.9678	.2900
CONTROL 2	.6138	6.5401	1.6670	.2539
CONTROL MEAN	.5847	6.6316	1.8174	.2719
POST 1	.9194	5.6617	1.1289	.1982
POST 2	.8174	5.7505	1.2781	.2202
POST MEAN	.8684	5.7061	1.2035	.2092

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	57.25	-14.63	-27.12	2.3	3.3
POST 2	39.80	-13.29	-19.03	5.0	5.8

SERIAL NUMBER 23062

DATE	10/11/70	NUMBER	23	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	4.7000
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5264	6.2081	1.9770	.3176
CONTROL 2	.6177	5.8194	1.6830	.2903
CONTROL MEAN	.5721	6.0137	1.8300	.3040
POST 1	.5877	5.8618	1.7187	.2945
POST 2	.7178	6.1168	1.3994	.2295
POST MEAN	.6528	5.9893	1.5590	.2620

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	2.73	-2.53	-3.13	2.3	3.0
POST 2	25.47	1.71	-24.49	4.8	5.5

SERIAL NUMBER 23063

DATE	11/11/70	NUMBER	23	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	4.7000
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6637	6.7056	1.5140	.2279
CONTROL 2	.6873	5.5784	1.4638	.2624
CONTROL MEAN	.6755	6.1420	1.4889	.2451
POST 1	.7276	6.3895	1.3806	.2181
POST 2	.6866	5.6969	1.4575	.2567
POST MEAN	.7071	6.0432	1.4191	.2374

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	7.70	4.03	-11.03	2.3	3.0
POST 2	1.64	-7.25	4.71	5.0	6.0

SERIAL NUMBER 23064

DATE	12/11/70	NUMBER	23	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	4.7000
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6302	6.4807	1.6159	.2503
CONTROL 2	.6617	5.9969	1.5202	.2542
CONTROL MEAN	.6459	6.2388	1.5680	.2522
POST 1	.6615	6.1231	1.5253	.2515
POST 2	.6342	6.1650	1.5789	.2576
POST MEAN	.6479	6.1441	1.5521	.2545

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	2.41	-1.85	-.29	2.0	2.8
POST 2	-1.82	-1.18	2.12	5.8	6.5

SERIAL NUMBER 24061

DATE	9/11/70	NUMBER	24	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	1.8300
DOSE	100	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.2799	3.8826	3.6430	.9424
CONTROL 2	.5640	3.2685	1.7994	.5528
CONTROL MEAN	.4219	3.5756	2.7212	.7476
POST 1	.6744	2.9075	1.4896	.5139
POST 2	.7037	2.8221	1.4508	.5180
POST MEAN	.6890	2.8648	1.4702	.5159

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	59.83	-18.68	-31.26	2.0	3.0
POST 2	66.77	-21.07	-30.71	6.0	6.8

SERIAL NUMBER 24062

DATE	10/11/70	NUMBER	24	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	1.8300
DOSE	20	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5811	2.9232	1.7538	.6025
CONTROL 2	.6653	3.1370	1.5120	.4872
CONTROL MEAN	.6232	3.0301	1.6329	.5449
POST 1	.7843	3.0049	1.3143	.4437
POST 2	.6397	3.0226	1.5731	.5201
POST MEAN	.7120	3.0137	1.4437	.4819

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	25.85	-.83	-18.57	2.0	2.8
POST 2	2.65	-.25	-4.54	4.8	5.3

SERIAL NUMBER 24063

DATE	11/11/70	NUMBER	24	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	1.8300
DOSE	200	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6003	3.4268	1.7972	.5178
CONTROL 2	.6486	2.8026	1.5608	.5590
CONTROL MEAN	.6245	3.1147	1.6790	.5384
POST 1	.7094	3.1389	1.4105	.4534
POST 2	.6897	3.4459	1.4534	.4253
POST MEAN	.6996	3.2924	1.4319	.4394

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	13.60	.78	-15.79	2.0	3.0
POST 2	10.45	10.64	-21.01	5.3	6.3

SERIAL NUMBER 24064

DATE	12/11/70	NUMBER	24	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	85	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.4708	3.7806	2.3270	.6034
CONTROL 2	.5961	3.9099	1.6809	.4419
CONTROL MEAN	.5334	3.8453	2.0040	.5227
POST 1	.5414	3.9871	1.9038	.4784
POST 2	.4932	3.9226	2.0546	.5265
POST MEAN	.5173	3.9549	1.9792	.5025

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	1.49	3.69	-8.47	2.3	3.3
POST 2	-7.54	2.01	.73	5.8	6.8

SERIAL NUMBER 25071

DATE	16/11/70	NUMBER	25	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	72	BOX PRESSURE	1.8300
DOSE	130	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.2745	4.4557	3.9734	.8820
CONTROL 2	.4889	3.9919	2.1713	.5359
CONTROL MEAN	.3817	4.2238	3.0723	.7089
POST 1	.5860	4.2646	1.7319	.4060
POST 2	.5478	3.2228	1.8447	.5724
POST MEAN	.5669	3.7437	1.7883	.4892

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	53.54	.96	-42.74	3.5	4.3
POST 2	43.53	-23.70	-19.26	8.0	8.8

SERIAL NUMBER 25072

DATE	17/11/70	NUMBER	25	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	72	BOX PRESSURE	1.8300
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5871	3.2165	1.7098	.5344
CONTROL 2	.5370	3.5930	1.8771	.5238
CONTROL MEAN	.5620	3.4047	1.7934	.5291
POST 1	.5172	4.2646	2.3175	.5333
POST 2	.5956	3.5722	1.7108	.4807
POST MEAN	.5564	3.9184	2.0141	.5070

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-7.98	25.25	.80	3.0	4.0
POST 2	5.98	4.92	-9.14	6.5	7.5

SERIAL NUMBER 25073

DATE	18/11/70	NUMBER	25	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	72	BOX PRESSURE	1.8300
DOSE	200	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5219	3.5162	1.9321	.5489
CONTROL 2	.4753	3.5875	2.1186	.5904
CONTROL MEAN	.4986	3.5519	2.0254	.5697
POST 1	.6230	4.0447	1.6166	.4013
POST 2	.6718	3.4532	1.4901	.4353
POST MEAN	.6474	3.7490	1.5533	.4183

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	24.95	13.88	-29.55	2.8	3.8
POST 2	34.74	-2.78	-23.59	6.8	7.5

SERIAL NUMBER 25074

DATE	19/11/70	NUMBER	25	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	72	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.3915	4.0312	2.6110	.6475
CONTROL 2	.5218	3.5714	1.9409	.5430
CONTROL MEAN	.4567	3.8013	2.2759	.5953
POST 1	.5546	4.0981	1.8315	.4509
POST 2	.6174	3.7501	1.6242	.4341
POST MEAN	.5860	3.9241	1.7279	.4425

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	21.45	7.81	-24.26	3.0	3.8
POST 2	35.20	-1.35	-27.08	6.5	7.5

SERIAL NUMBER 26071

DATE 16/11/70
GAS NH3
DOSE 200

NUMBER 26
WEIGHT 61
NO.READINGS PER SET 5

MOUTH PRESSURE .5811
BOX PRESSURE 1.8300
FLOW CALIBRATION .4022
ATMOSPHERIC PRESSURE 760

	R	V	G	SG
CONTROL 1	.6211	2.6459	1.6327	.6158
CONTROL 2	.6062	2.6888	1.6976	.6279
CONTROL MEAN	.6136	2.6673	1.6652	.6219
POST 1	.6296	2.6354	1.6081	.6112
POST 2	.5953	2.7444	1.6946	.6177
POST MEAN	.6124	2.6899	1.6514	.6145

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	2.60	-1.20	-1.71	1.8	2.8
POST 2	-2.99	2.89	-.67	4.0	4.8

SERIAL NUMBER 26072

DATE	17/11/70	NUMBER	26	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	1.8300
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6846	2.7457	1.4872	.5477
CONTROL 2	.7161	2.8193	1.5048	.5304
CONTROL MEAN	.7004	2.7825	1.4960	.5391
POST 1	.6633	2.5930	1.5141	.5857
POST 2	.7210	2.4854	1.4122	.5663
POST MEAN	.6922	2.5392	1.4632	.5760

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-5.29	-6.81	8.66	2.0	3.0
POST 2	2.95	-10.68	5.05	4.8	5.5

SERIAL NUMBER 26073

DATE	18/11/70	NUMBER	26	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	1.8300
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5878	3.4023	1.7101	.5024
CONTROL 2	.6233	3.1326	1.6287	.5204
CONTROL MEAN	.6056	3.2675	1.6694	.5114
POST 1	.8037	3.1755	1.2943	.4078
POST 2	.6829	2.9400	1.4659	.4996
POST MEAN	.7433	3.0578	1.3801	.4537

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	32.71	-2.81	-20.26	2.0	3.0
POST 2	12.77	-10.02	-2.30	4.8	5.8

SERIAL NUMBER 26074

DATE	19/11/70	NUMBER	26	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	61	BOX PRESSURE	1.8300
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5570	3.0881	1.8268	.5898
CONTROL 2	.5874	3.0709	1.7112	.5627
CONTROL MEAN	.5722	3.0795	1.7690	.5763
POST 1	.6480	2.8602	1.5584	.5484
POST 2	.5944	2.7186	1.6915	.6224
POST MEAN	.6212	2.7894	1.6249	.5854

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	13.25	-7.12	-4.84	2.0	3.0
POST 2	3.88	-11.72	8.01	5.8	6.5

SERIAL NUMBER 27071

DATE	16/11/70	NUMBER	27	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	62	BOX PRESSURE	4.7000
DOSE	10	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5689	5.8233	1.8332	.3165
CONTROL 2	.4345	8.9895	.7225	.2444
CONTROL MEAN	.5017	7.4064	1.2778	.2805
POST 1	.6652	5.5649	1.5076	.2716
POST 2	.5915	5.1641	1.7099	.3331
POST MEAN	.6283	5.3645	1.6088	.3024

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	32.58	-24.86	-3.15	2.5	3.3
POST 2	17.89	-30.27	18.77	6.3	7.3

SERIAL NUMBER 27072

DATE	17/11/70	NUMBER	27	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	62	BOX PRESSURE	1.8300
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.4838	4.1652	2.1111	.5062
CONTROL 2	.5515	4.2030	2.2299	.5117
CONTROL MEAN	.5177	4.1841	2.1705	.5090
POST 1	.5220	4.6356	1.9261	.4163
POST 2	.6291	4.9440	1.6046	.3289
POST MEAN	.5756	4.7898	1.7653	.3726

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	.84	10.79	-18.21	2.0	3.0
POST 2	21.53	18.16	-35.37	5.8	6.5

SERIAL NUMBER 27073

DATE	18/11/70	NUMBER	27	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	62	BOX PRESSURE	1.8300
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.5253	5.0135	2.1949	.4210
CONTROL 2	.5587	4.0709	1.8314	.4486
CONTROL MEAN	.5420	4.5422	2.0131	.4348
POST 1	.5233	4.1156	1.9296	.4708
POST 2	.5992	4.5732	1.6879	.3695
POST MEAN	.5612	4.3444	1.8088	.4201

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-3.45	-9.39	8.27	2.3	3.0
POST 2	10.55	.68	-15.02	6.8	7.5

SERIAL NUMBER 27074

DATE	19/11/70	NUMBER	27	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	62	BOX PRESSURE	1.8300
DOSE	320	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6297	4.1428	1.6579	.3984
CONTROL 2	.5768	3.9288	1.7698	.4503
CONTROL MEAN	.6033	4.0358	1.7138	.4243
POST 1	.6779	3.9653	1.4826	.3749
POST 2	.6067	3.4766	1.7124	.4903
POST MEAN	.6423	3.7209	1.5975	.4326

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	12.37	-1.75	-11.66	2.0	3.0
POST 2	.57	-13.86	15.55	6.0	7.0

SERIAL NUMBER 28071

DATE	16/11/70	NUMBER	28	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	58	BOX PRESSURE	1.8300
DOSE	30	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.4846	3.5289	2.3717	.6596
CONTROL 2	.5929	3.7592	1.6923	.4505
CONTROL MEAN	.5387	3.6440	2.0320	.5551
POST 1	.6353	3.7706	1.5825	.4209
POST 2	.7087	3.6261	1.4169	.3915
POST MEAN	.6720	3.6984	1.4997	.4062

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	17.92	3.47	-24.18	2.8	3.8
POST 2	31.55	-.49	-29.46	5.8	6.5

SERIAL NUMBER 28072

DATE	17/11/70	NUMBER	28	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	58	BOX PRESSURE	1.8300
DOSE	200	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6106	3.6815	1.6442	.4484
CONTROL 2	.5063	3.6003	1.9881	.5520
CONTROL MEAN	.5584	3.6409	1.8162	.5002
POST 1	.7631	4.2452	1.4414	.3342
POST 2	.5783	4.2554	1.7354	.4078
POST MEAN	.6707	4.2503	1.5884	.3710

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	36.65	16.60	-33.17	3.3	4.5
POST 2	3.56	16.88	-18.47	6.3	7.3

SERIAL NUMBER 28073

DATE	18/11/70	NUMBER	28	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	58	BOX PRESSURE	1.8300
DOSE	20	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6141	3.3102	1.6517	.5016
CONTROL 2	.5404	3.3397	1.8917	.5660
CONTROL MEAN	.5773	3.3250	1.7717	.5338
POST 1	.5458	3.3951	1.8428	.5465
POST 2	.5707	3.5031	1.7667	.5067
POST MEAN	.5583	3.4491	1.8048	.5266

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-5.45	2.11	2.37	3.0	3.8
POST 2	-1.14	5.36	-5.07	6.3	7.0

SERIAL NUMBER 28074

DATE	19/11/70	NUMBER	28	MOUTH PRESSURE	.5811
GAS	NH3	WEIGHT	58	BOX PRESSURE	1.8300
DOSE	80	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	760

	R	V	G	SG
CONTROL 1	.6418	3.3973	1.6719	.4857
CONTROL 2	.5438	3.8098	1.8493	.4859
CONTROL MEAN	.5928	3.6035	1.7606	.4858
POST 1	.5233	3.2449	1.9300	.5961
POST 2	.5270	3.1046	1.9798	.6350
POST MEAN	.5251	3.1748	1.9549	.6155

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-11.73	-9.95	22.70	4.8	5.8
POST 2	-11.10	-13.84	30.70	9.5	10.5

SERIAL NUMBER 29081

DATE	15/ 3/71	NUMBER	29	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	57	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	743

	R	V	G	SG
CONTROL 1	.3537	3.3092	2.8858	.8738
CONTROL 2	.2539	3.3993	4.6726	1.3576
CONTROL MEAN	.3038	3.3542	3.7792	1.1157
POST 1	.3486	3.2802	3.0197	.9137
POST 2	.4467	3.2618	2.2800	.7023
POST MEAN	.3977	3.2710	2.6498	.8080

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	14.75	-2.21	-18.10	2.2	3.7
POST 2	47.05	-2.76	-37.05	6.5	7.5

SERIAL NUMBER 29082

DATE	16/ 3/71	NUMBER	29	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	57	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	747

	R	V	G	SG
CONTROL 1	.3529	3.0985	2.9958	.9692
CONTROL 2	.3459	3.1482	2.9673	.9370
CONTROL MEAN	.3494	3.1233	2.9815	.9531
POST 1	.3192	3.1200	4.3867	1.3806
POST 2	.3614	3.1741	2.8846	.9020
POST MEAN	.3403	3.1471	3.6357	1.1413

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-8.65	-.11	44.86	2.3	3.7
POST 2	3.43	1.63	-5.37	7.0	8.3

SERIAL NUMBER 29083

DATE	17/ 3/71	NUMBER	29	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	57	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	744

	R	V	G	SG
CONTROL 1	.3391	2.6470	3.7783	1.3626
CONTROL 2	.3596	2.7170	2.9441	1.0768
CONTROL MEAN	.3494	2.6820	3.3612	1.2197
POST 1	.5049	2.7903	2.0353	.7349
POST 2	.3749	3.0125	2.8663	.9443
POST MEAN	.4399	2.9014	2.4508	.8396

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	44.53	4.04	-39.75	2.5	4.0
POST 2	7.30	12.32	-22.58	6.5	7.8

SERIAL NUMBER 29084

DATE	18/ 3/71	NUMBER	29	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	57	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	733

	R	V	G	SG
CONTROL 1	.1138	3.3074	40.3890	10.9182
CONTROL 2	.1458	3.6491	7.3674	2.0061
CONTROL MEAN	.1298	3.4782	23.8782	6.4622
POST 1	.3795	2.9815	2.8820	.9822
POST 2	.3857	2.9908	2.6061	.8759
POST MEAN	.3826	2.9862	2.7441	.9290

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	192.34	-14.28	-84.80	3.0	4.5
POST 2	197.18	-14.01	-86.45	6.7	8.0

SERIAL NUMBER 29085

DATE	19/ 3/71	NUMBER	29	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	57	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	735

	R	V	G	SG
CONTROL 1	.3578	2.9652	2.8609	.9610
CONTROL 2	.2897	3.1944	3.7852	1.1736
CONTROL MEAN	.3237	3.0798	3.3230	1.0673
POST 1	.4484	3.1739	2.3166	.7309
POST 2	.4170	3.1916	2.4054	.7539
POST MEAN	.4327	3.1828	2.3610	.7424

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	38.49	3.06	-31.52	4.5	5.6
POST 2	28.80	3.63	-29.36	8.0	9.3

SERIAL NUMBER 300ei

DATE	11/ 3/71	NUMBER	30	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	69	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	766

	R	V	G	SG
CONTROL 1	.5048	3.8967	2.0471	.5212
CONTROL 2	.6195	4.3537	1.6308	.3745
CONTROL MEAN	.5621	4.1252	1.8390	.4478
POST 1	.6305	4.7106	1.5878	.3388
POST 2	.6106	4.3931	1.6567	.3789
POST MEAN	.6206	4.5518	1.6223	.3588

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	12.16	14.19	-24.35	1.5	2.5
POST 2	8.62	6.49	-15.40	5.0	6.0

SERIAL NUMBER 300H2

DATE	12/ 3/71	NUMBER	30	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	69	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	767

	R	V	G	SG
CONTROL 1	.4330	5.2121	2.4144	.4617
CONTROL 2	.5423	5.3328	1.8851	.3552
CONTROL MEAN	.4876	5.2724	2.1497	.4084
POST 1	.5634	4.7075	1.7990	.3846
POST 2	.4707	4.8904	2.1731	.4438
POST MEAN	.5171	4.7990	1.9861	.4142

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	15.55	-10.72	-5.85	2.0	3.3
POST 2	-3.47	-7.25	8.66	6.5	8.0

SERIAL NUMBER 30083

DATE	15/ 3/71	NUMBER	30	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	69	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	743

	R	V	G	SG
CONTROL 1	.4277	4.6324	2.4199	.5224
CONTROL 2	.3921	4.8395	2.5590	.5289
CONTROL MEAN	.4099	4.7359	2.4894	.5256
POST 1	.4082	4.4448	2.4543	.5534
POST 2	.4090	4.2525	2.5808	.6045
POST MEAN	.4086	4.3487	2.5175	.5789

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-.42	-6.15	5.28	2.3	3.5
POST 2	-.22	-10.21	15.00	7.0	8.5

SERIAL NUMBER 30084

DATE	16/ 3/71	NUMBER	30	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	69	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	747

	R	V	G	SG
CONTROL 1	.4028	4.0789	2.5756	.6465
CONTROL 2	.3239	5.1377	3.1403	.6118
CONTROL MEAN	.3633	4.6083	2.8579	.6292
POST 1	.3901	4.7001	2.6379	.5593
POST 2	.4270	4.2958	2.4466	.5685
POST MEAN	.4086	4.4979	2.5423	.5639

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	7.37	1.99	-11.10	2.3	3.5
POST 2	17.53	-6.78	-9.65	6.3	7.3

SERIAL NUMBER 30085

DATE	17/ 3/71	NUMBER	30	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	69	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	744

	R	V	G	SG
CONTROL 1	.2884	4.5384	3.6272	.7949
CONTROL 2	.4371	4.5651	2.3482	.5257
CONTROL MEAN	.3627	4.5517	2.9877	.6603
POST 1	.4383	4.0359	2.2977	.5720
POST 2	.3673	3.7126	2.8100	.7577
POST MEAN	.4028	3.8743	2.5538	.6648

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	20.84	-11.33	-13.37	2.5	3.8
POST 2	1.26	-18.44	14.75	6.5	7.5

SERIAL NUMBER 31081

DATE	15/ 3/71	NUMBER	31	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	71	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	743

	R	V	G	SG
CONTROL 1	.4102	4.4737	2.5808	.5691
CONTROL 2	.4714	4.2535	2.1366	.5020
CONTROL MEAN	.4408	4.3636	2.3587	.5356
POST 1	.5057	4.8226	1.9871	.4124
POST 2	.4463	4.7623	2.2888	.4828
POST MEAN	.4760	4.7924	2.1379	.4476

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	14.72	10.52	-22.99	3.0	4.2
POST 2	1.24	9.14	-9.85	7.0	8.5

SERIAL NUMBER 31082

DATE	16/ 3/71	NUMBER	31	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	71	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	747

	R	V	G	SG
CONTROL 1	.4660	4.5701	2.1840	.4795
CONTROL 2	.4440	4.8623	2.2889	.4717
CONTROL MEAN	.4550	4.7162	2.2364	.4756
POST 1	.5030	4.6427	2.0066	.4364
POST 2	.5112	4.1325	1.9705	.4824
POST MEAN	.5071	4.3876	1.9885	.4594

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	10.55	-1.56	-8.24	2.5	3.8
POST 2	12.35	-12.38	1.43	6.5	7.5

SERIAL NUMBER 31083

DATE	17/ 3/71	NUMBER	31	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	71	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	744

	R	V	G	SG
CONTROL 1	.4764	4.0760	2.1697	.5316
CONTROL 2	.4194	4.5820	2.4001	.5243
CONTROL MEAN	.4479	4.3290	2.2849	.5280
POST 1	.5517	4.3021	1.8659	.4319
POST 2	.5002	3.9839	2.0272	.5101
POST MEAN	.5259	4.1430	1.9465	.4710

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	23.18	-.62	-18.20	2.8	4.0
POST 2	11.68	-7.97	-3.39	6.3	7.3

SERIAL NUMBER 31084

DATE 18/ 3/71
GAS TRICHLOR
DOSE -0

NUMBER 31
WEIGHT 71
NO.READINGS PER SET 5

MOUTH PRESSURE .5811
BOX PRESSURE 1.8300
FLOW CALIBRATION .4022
ATMOSPHERIC PRESSURE 733

	R	V	G	SG
CONTROL 1	.4914	4.6543	2.0463	.4400
CONTROL 2	.5117	4.5577	1.9939	.4450
CONTROL MEAN	.5016	4.6060	2.0201	.4425
POST 1	.4899	5.0337	2.0616	.4107
POST 2	.4497	4.8751	2.2929	.4689
POST MEAN	.4698	4.9544	2.1772	.4398

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-2.33	9.29	-7.19	2.5	3.7
POST 2	-10.34	5.84	5.95	6.5	8.5

SERIAL NUMBER 31085

DATE	19/ 3/71	NUMBER	31	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	71	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	735

	R	V	G	SG
CONTROL 1	.4889	4.7171	2.2290	.4662
CONTROL 2	.3163	5.7315	3.2318	.5616
CONTROL MEAN	.4026	5.2243	2.7304	.5139
POST 1	.3972	5.4302	2.5919	.4777
POST 2	.3828	5.1163	2.6352	.5191
POST MEAN	.3900	5.2742	2.6135	.4984

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-1.33	3.94	-7.04	2.8	4.0
POST 2	-4.91	-2.03	1.01	7.0	8.2

SERIAL NUMBER 32081

DATE	15/ 3/71	NUMBER	32	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	743

	R	V	G	SG
CONTROL 1	.5170	3.1115	1.9765	.6345
CONTROL 2	.5133	3.5672	1.9640	.5524
CONTROL MEAN	.5152	3.3393	1.9702	.5935
POST 1	.5817	4.0569	1.7464	.4311
POST 2	.6019	3.8216	1.6913	.4500
POST MEAN	.5918	3.9393	1.7189	.4405

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	12.92	21.49	-27.36	2.5	4.0
POST 2	16.82	14.44	-24.17	5.8	7.0

SERIAL NUMBER 32082

DATE 16/ 3/71
GAS TRICHLOR
DOSE -0

NUMBER 32
WEIGHT 59
NO.READINGS PER SET 5

MOUTH PRESSURE .5811
BOX PRESSURE 1.8300
FLOW CALIBRATION .4022
ATMOSPHERIC PRESSURE 747

	R	V	G	SG
CONTROL 1	.5420	3.8626	1.8653	.4839
CONTROL 2	.6943	3.4993	1.4917	.4235
CONTROL MEAN	.6182	3.6809	1.6785	.4537
POST 1	.5628	3.7262	1.8344	.4965
POST 2	.4314	3.5747	2.3418	.6570
POST MEAN	.4971	3.6504	2.0881	.5767

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-8.96	1.23	9.43	2.5	3.8
POST 2	-30.22	-2.89	44.80	5.8	7.0

SERIAL NUMBER 32084

DATE	18/ 3/71	NUMBER	32	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	733

	R	V	G	SG
CONTROL 1	.5921	4.3008	1.7048	.3987
CONTROL 2	.5846	4.6607	1.7157	.3718
CONTROL MEAN	.5884	4.4808	1.7103	.3853
POST 1	.7257	4.0998	1.3937	.3441
POST 2	.6812	4.6681	1.4967	.3214
POST MEAN	.7034	4.3840	1.4452	.3328

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	23.33	-8.50	-10.69	2.7	4.3
POST 2	15.78	4.18	-16.57	7.5	8.8

SERIAL NUMBER 32085

DATE	19/ 3/71	NUMBER	32	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	59	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	735

	R	V	G	SG
CONTROL 1	.7570	4.8582	1.3475	.2765
CONTROL 2	.7078	4.8348	1.4408	.2972
CONTROL MEAN	.7324	4.8465	1.3941	.2869
POST 1	.8371	4.3976	1.2036	.2751
POST 2	.6940	4.0884	1.4529	.3564
POST MEAN	.7656	4.2430	1.3283	.3158

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	14.30	-9.26	-4.10	4.0	5.3
POST 2	-5.25	-15.64	24.22	9.0	10.0

SERIAL NUMBER 33081

DATE	15/ 3/71	NUMBER	33	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	65	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	743

	R	V	G	SG
CONTROL 1	.4672	2.5360	2.1991	.8764
CONTROL 2	.4138	2.3306	2.5070	1.0669
CONTROL MEAN	.4405	2.4333	2.3530	.9716
POST 1	.5855	2.1399	1.8347	.8711
POST 2	.4184	2.3233	2.4017	1.0344
POST MEAN	.5019	2.2316	2.1182	.9527

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	32.91	-12.06	-10.35	2.7	4.0
POST 2	-5.01	-4.52	6.46	7.5	8.7

SERIAL NUMBER 33082

DATE	16/ 3/71	NUMBER	33	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	65	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	747

	R	V	G	SG
CONTROL 1	.6478	2.1303	1.5672	.7367
CONTROL 2	.6015	2.1621	1.6898	.7839
CONTROL MEAN	.6247	2.1462	1.6285	.7603
POST 1	.5909	2.7784	1.8071	.6519
POST 2	.6081	2.1149	1.6492	.7819
POST MEAN	.5995	2.4466	1.7282	.7169

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	-5.41	29.45	-14.25	2.3	3.7
POST 2	-2.65	-1.46	2.84	7.8	9.5

SERIAL NUMBER 33083

DATE	17/ 3/71	NUMBER	33	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	65	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	744

	R	V	G	SG
CONTROL 1	.5628	2.9310	1.8169	.6244
CONTROL 2	.6723	2.4622	1.4992	.6137
CONTROL MEAN	.6175	2.6966	1.6580	.6191
POST 1	.7072	2.5072	1.4174	.5807
POST 2	.6996	2.4805	1.4343	.5798
POST MEAN	.7034	2.4938	1.4259	.5802

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	14.52	-7.02	-6.20	3.5	5.5
POST 2	13.29	-8.01	-6.35	9.0	10.0

SERIAL NUMBER 33084

DATE	18/ 3/71	NUMBER	33	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	65	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	733

	R	V	G	SG
CONTROL 1	.6831	2.8758	1.4705	.5165
CONTROL 2	.6747	2.7940	1.4942	.5396
CONTROL MEAN	.6789	2.8349	1.4823	.5280
POST 1	.7009	2.9197	1.4318	.4943
POST 2	.7291	2.7497	1.3775	.5034
POST MEAN	.7150	2.8347	1.4046	.4988

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	3.24	2.99	-6.40	2.8	4.2
POST 2	7.40	-3.00	-4.66	8.8	10.0

SERIAL NUMBER 33085

DATE	19/ 3/71	NUMBER	33	MOUTH PRESSURE	.5811
GAS	TRICHLOR	WEIGHT	65	BOX PRESSURE	1.8300
DOSE	-0	NO.READINGS PER SET	5	FLOW CALIBRATION	.4022
				ATMOSPHERIC PRESSURE	735

	R	V	G	SG
CONTROL 1	.6823	2.5009	1.4829	.6026
CONTROL 2	.6824	2.5014	1.4799	.5943
CONTROL MEAN	.6823	2.5011	1.4814	.5985
POST 1	.7405	2.7363	1.6007	.5729
POST 2	.7263	2.3475	1.3930	.6085
POST MEAN	.7334	2.5419	1.4968	.5907

PERCENTAGE CHANGE : (POST GAS VALUE - CONTROL MEAN)/CONTROL MEAN

				CLOCK TIMES START	CLOCK TIMES FINISH
POST 1	8.53	9.40	-4.27	2.8	4.2
POST 2	6.44	-6.14	1.68	7.2	8.5

ACKNOWLEDGEMENTS.

I wish to thank Dr. M. L. Thomson for introducing me to the field of pulmonary physiology, for supervising my research and for interesting discussions.

My gratitude is due:

to Professor R. S. F. Schilling, Director of the Institute for his co-operation and interest in my work;

to Mrs. S. M. Forsey for assistance and for kindly allowing me to use her values of residual volume obtained from helium dilution;

to Dr. S. Pocock of the State University of Buffalo, N.Y., Mr. H. Shannon, Miss J. Walford and Mr. A. Westlake for statistical advice;

to Miss J. Bailey and Miss M. Chandler for advice on preparation of data for computerization;

to Dr. C. N. Davies, University of Essex.

Dr. D. Dennison Davies, Central Middx. Hosp.
Mr. J. Few, Medical Research Council.
Dr. P. Heaf, U.C.H. Medical School.

Dr. P. Kamburoff, Inst. Diseases of the Chest, Brompton Hospital.

Prof. C. A. Keele, Middx. Hospital. Medical School.

Dr. J. McKEllison, Dept. of Health and Social Security.

Mr. D. Pavia, L.S.H.T.M.

Prof. J. G. Widdicombe, St. Georges Hospital. Medical School.

for helpful discussions;

to members of the school glassblowing and photographic depts. and workshops for skilled help;

to all other members of the institute and school both past and present who from time to time have helped with valuable discussions and especially also the volunteer subjects without whom the work would not have been possible;

to my wife who typed this thesis over many long evenings.

Bronchodilatation Induced by Methoxyflurane

SIR,—Methoxyflurane (Penthrane) is a self-administered analgesic used in childbirth. We have investigated the bronchoactivity of methoxyflurane in seven adults with normal ventilatory indices by means of a whole body plethysmograph. It is usual practice to express resistance of the airways as specific airways conductance, which is conductance divided by lung volume. After control values had been obtained the subject remained seated in the plethysmograph and inhaled from a Cyprane Cardiff inhaler through the standard face mask. The inhaler delivers methoxyflurane at a fixed concentration of 0·35% v/v. The exhalate was voided externally to avoid contamination of the plethysmograph. Breathing was at tidal volume for 1½ minutes, following which the specific conductance was remeasured immediately.

Six subjects showed a rise in specific conductance (range 7·2% to 33·6%; mean 20·3%). Bronchodilatation also occurred in the seventh subject, but this was overridden by a larger change in lung volume.

Thus we feel that consideration should be given to favouring this analgesic for women presenting with airways obstruction or with a history of asthma.—We are, etc.,

R. B. DOUGLAS
S. M. FORSEY

T.U.C. Centenary Institute,
London School of Hygiene and
Tropical Medicine,
London W.C.1

Printed in Great Britain by William Lewis (Printers) Ltd, Cardiff

New method of mounting filters in all-glass systems

In experiments on human volunteers, known low concentrations of pollutants were generated by bubbling air through a Dreschel bottle at constant temperature. It was necessary to remove entrained aerosol from the airstream to avoid artefact, and the incorporation of a filter for this purpose proved to be difficult in our glass and stainless-steel system.

The difficulty was overcome by sealing a glass fibre filter to a cone as follows. Firstly, a 'B-34' socket was added to the top of a Dreschel bottle. The rim of the corresponding cone was then heated in an oxygen and coal gas flame to 'orange heat' and immediately applied firmly to a filter paper (100% glass fibre, Reeve Angel Ltd), of diameter greater than that of the cone, with a rolling action which cut away any redundant filter and sealed a disc across the cone (figure 1). Microscopic

Figure 1 Modified Dreschel bottle with cone and glass fibre filter

examination revealed complete fusion between the glass fibres and the rim of the cone. Pressure testing showed that the seal was stronger than the filter paper.

R B Douglas
J Turner
London School of Hygiene and Tropical Medicine
London WC1E 7HT

3 December 1973

Journal of Physics E: Scientific Instruments 1974 Volume 7

† On leave from the Smithsonian Astrophysical Observatory.