THE ABSORPTION AND ELIMINATION

OF TRICHLOROETHYLENE

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FOR MY PARENTS

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ABSTRACT

THE ABSORPTION AND ELIMINATION OF TRICHLOROETHYLENE

Trichloroethylene is an inhalation anaesthetic which undergoes biotransformation in the body. The application of gas chromatography to the investigation of uptake, distribution and elimination of trichloroethylene permits a very sensitive analysis, with the detection of the vapour in much lower concentrations than was previously possible.

A study was carried out on 36 patients during clinical anaesthesia in the operating room of the Winnipeg General Hospital. Different inspired concentrations of trichloroethylene were administered for various durations in order to determine if these factors have an effect on the proportion of the drug which is excreted through the lungs unchanged and the proportion which is metabolized.

Analysis of inspired and expired vapours was carried out to examine to what extent the agent was absorbed in the body and to follow its elimination almost to completion.

A statistical analysis was carried out to determine what factors influenced the absorption and elimination of trichloroethylene in the human organism. It was found that minute volume, body weight, age and sex were not significant. The duration of exposure to trichloroethylene as well as the inspired concentration of trichloroethylene administered were found to be of importance.

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INTRODUCTION

The purpose of the present investigation was to study the uptake, distribution and elimination of trichloroethylene in the human body. Trichloroethylene is an inhalation anaesthetic with a narcotic effect and notable, analgesic properties. Trichloroethylene was the first inhalation anaesthetic known to undergo appreciable metabolism and it has been only recently that metabolism has been demonstrated in other inhalation anaesthetics. Trichloroethylene is also widely known for its use in industry as a solvent and degreaser of metal parts.

The toxic properties of the drug have been studied on factory workers chronically exposed to low concentrations of the vapour for long periods of time. The metabolism of the drug has also been studied in animals but little work has been carried out concerning exposures for relatively short time periods during clinical anaesthesia.

Previous methods of investigation were based on the Fujiwara pyridene reaction and the Conway microdiffusion technique. These methods, although reliable, are less sensitive, more time consuming and less specific than the present technique. This study was designed to make use of gas chromatography in order to learn more about the metabolism of trichloroethylene in the body.

Known concentrations of inspired trichloroethylene were administered during clinical anaesthesia for varying periods of time. The factors affecting the absorption and elimination were studied to ascertain their relationship to the clinically observed effect of trichloroethylene.

REVIEW OF THE LITERATURE

RICHLOROETHYLENE

istory:

The history of trichloroethylene dates back to 1864, when it was discovered by mil Fischer as a byproduct in the preparation of tetrachlorethane. Its usefulness as n organic solvent and a degreaser for metal parts soon became apparent, and it was mployed extensively in heavy industry in Germany and Great Britain. Plessner (1915) rst reported the toxic properties of the drug, notably its anaesthetic effect in the istribution of the trigeminal nerve. Further research was carried out by Plessner in ie treatment of trigeminal neuralgia. However the value of trichloroethylene in this ondition was not substantiated. Oljenick (1928) and Glaser (1931) also endeavored to reat trigeminal neuralgia in the same manner but both were unsuccessful. Their bservations of the narcotic properties of trichloroethylene were in agreement with Lehman, /ho in anaesthetic experiments in 1911 had also noted tachypnoea and decomposition of richloroethylene with temperature.

Animal experiments involving trichloroethylene were performed in the United tates by Jackson (1933) and Hertzberg (1934). Hertzberg's work demonstrated that iurified trichloroethylene could be used safely as an anaesthetic agent. As a result of his work, a clinical study made up of three hundred cases of anaesthesia and analgesia, /as carried out (Striker et al, 1935). In 1936, the report of the A.M.A. Council of ?harmacology and Chemistry concluded that the evidence did not justify the use of richloroethylene as a general anaesthetic. The consequence of the report was a declining nterest of trichloroethylene in clinical use in the United States.

In 1939, however, the British Government authorized the Joint Anaesthetic Committee of the Medical Research Council and the Royal Society of Medicine to find a iafe, non flammable, inexpensive anaesthetic agent satisfactory for wartime application. iubsequently Hewer and Hadfield, members of the Joint Committee reviewed the iterature on trichloroethylene and in 1940 Hewer carried out a clinical trial involving ine hundred and twenty-seven cases (Hewer and Hadfield, 1941). Humphrey and VlcClelland, in 1944, only three years after the introduction of trichloroethylene, reported

irteen cases of cranial nerve palsey, in a rebreathing system, in which soda lime had

een in contact with the anaesthetic agent. The poor quality of the soda lime, resulting

1 high temperatures, may have contributed to these misfortunes. Earlier, as noted,

ehman (1911) had pointed out the danger of the drug when it decomposed. A study on

1e effects of the drug was carried out by Waters et al (1943). Various adverse effects

n cardiac rhythmn were attributed to trichloroethylene. However these effects could

ave been due to high concentrations of the drug administered from unquantitated

pparatus.

The tenor of these studies, in conjunction with the widespread use of the

ircle carbon dioxide absorption system has restricted the use of trichloroethylene in

anada and the United States as a general anaesthetic. However, it is still popular in

ngland and in underdeveloped countries where cost is a significant factor in the choice

f the anaesthetic agent.

Physical and Chemical Characteristics:

The chemical formula of trichloroethylene is:

$$\begin{matrix} Cl \\ Cl \end{matrix} C = C \begin{matrix} H \\ Cl \end{matrix}$$
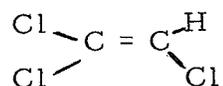
t is a colorless, substituted aliphatic hydrocarbon, liquid at usual temperatures.

Trichloroethylene has a boiling point of 86 degrees centigrade, a melting point of

- 87.1 degrees centigrade, and a specific gravity of 1.47 at 15.4 degrees centigrade.

At room temperature the liquid is readily volatile since its vapour pressure is high and

ts latent heat is low. Its vapour is heavier than air, and its fruity odor resembles

chloroform without its pungency.

"Trilene" consists of purified trichloroethylene, with 1:10,000 thymol which

retards decomposition, and 1:200,000 waxoline blue as a coloring agent to distinguish

t from chloroform. The United States Pharmacopocia states that trichloroethylene must

not be less than 99.5% of the pure product, that the thymol content, if any is used,

should be between 0.010 and 0.012 per cent and that it must be sold with a warning

against use with soda lime. The commercial product is checked for three groups of impurities: excess of acids, presence of chlorine, and the presence of chloride ions (Delfalque, 1961).

The industrial preparation of trichloroethylene is rather inexpensive. It is produced in quantity by the controlled chlorination of acetylene which gives tetrachlorethane. This is treated with lime slurry to form trichloroethylene, which is purified by distillation

$$C_2H_2 + 2\ Cl_2 \longrightarrow C_2H_2Cl_4$$
$$2\ C_2H_2Cl_4 + Ca(OH)_2 \longrightarrow CaCl_2 + 2\ H_2O + 2\ C_2HCl_3$$
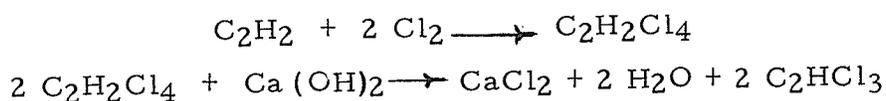
It is also produced by the action of nascent hydrogen on carbon hexachloride and in the laboratory from isometric tetrachlorethane, alcoholic potassium or from anhydrous chloral (Ostlere, 1953).

A disadvantage is its lack of stability in the presence of strong light and heat. The compound, in the presence of air and light decomposes with the formation of dichloracetylene, hydrochloric acid, chlorine, carbon monoxide and phosgene (Ostlere, 1953). In industry, the presence of catalysts such as finely divided aluminum or exposure to ultra-violet light at temperatures in the range of 120 degrees centigrade may readily cause thermo-decomposition (Smith, 1966). Decomposition due to strong light may be prevented by storage in the dark, in tinted bottles, or in cans. Humphrey and McClelland (1944) demonstrated that at a temperature of 37 degrees centigrade, trichloroethylene decomposes readily in carbon dioxide absorbing cannisters containing soda lime. Dichloracetylene is produced which oxidizes readily to phosgene and carbon dioxide. By employing an open circuit system, free of soda lime, this may be prevented.

Trichloroethylene is non-flammable in air. It will become combustible only at high temperatures (410 degrees centigrade) or at lower temperatures in oxygen enriched atmospheres greater than 24% (Jones and Scott, 1943).

Powell (1947) investigated the solubility properties of trichloroethylene upon which its initial absorption and transference in the body depend. The distribution

coefficient of trichloroethylene between water and air is 3 at 20 degrees centigrade and 1.6 at 37 degrees centigrade for concentrations of trichloroethylene in the air between 0.26 and 15.8 mgm/100 ml. The distribution coefficient between blood and air is 18-22 at 20 degrees centigrade and 8-10 at 37 degrees centigrade (Powell, 1947). Powell also demonstrated that the absorption of trichloroethylene by plasma is correlated with its fat and protein content. The fat/gas partition coefficient is given by Mapleson (1963) as 960 at 37 degrees centigrade. Trichloroethylene is practically insoluble in water but is freely miscible in a wide range of solvents, dissolves plastics, gums, various fats and oils, and mixes readily with ether and chloroform without chemical change. No azeotropic mixture of trichloroethylene and any other commonly used liquid anaesthetic agent is known (Parkhouse, 1965).

Uses of Trichloroethylene:

After its discovery trichloroethylene was profitably employed in industry as a metal degreaser. It was used in Germany for this purpose during the First World War; and as a preliminary to plating, anodizing, and painting, replacing the combustible benzol, previously utilized (Weitbrecht, 1965). It has been found to be functional in many industries, namely in the production of printing inks, paints, lacquers, varnishes, gas and tar purification, as a vehicle for adhesives, and for drugs, chemicals and perfume manufacture. It is suitable for fat extraction from cotton and for impregnation, because it takes up substances of high molecular weight without reacting with them. The fact that trichloroethylene has a stable viscosity has made it useful in low temperature research. Its bactericidal properties have been used in egg preservation, insecticides and hair washes (Imperial Chemical Industries).

A major advantage in utilizing trichloroethylene in industry is its lack of flammability. It only reacts to form combustible or explosive mixtures with air at high temperatures (410 degrees centigrade) or in oxygen enriched atmospheres at lower temperatures (Jones and Scott, 1943). Its versatility, relative chemical stability and poor solubility in water make trichloroethylene a valuable solvent.

On the other hand, there are many disadvantages involved in the application

of trichloroethylene in industry. Decomposition, due to strong light and heat under industrial conditions, results in the formation of highly toxic products. Therefore, many accidents can be expected to occur when poor hygienic conditions exist. Also the inhalation of a sufficient amount of trichloroethylene over a period of time will result in various toxic effects (Bardodej and Viskocyl, 1956).

Trichloroethylene is distributed for medical usage under the name Trilene in Great Britain and Canada, and Trethylene in the United States. Trilene is utilized in general surgery in combination with oxygen and nitrous oxide, where it is a convenient supplement when light anaesthesia is required in a patient who breathes spontaneously. Hence it is useful in plastic surgery, opthamology and orthopedics where little muscular relaxation is required.

As an analgesic it is most commonly used in obstetrics as a 0.5% mixture with air as recommended by the Medical Research Council, 1954 (British Pharmaceutical Codex, 1963). Its use involves less risk of maternal and foetal hypoxia and appears to be more effective than nitrous oxide and air (Seward, 1949).

Trichloroethylene has also been advocated in dental anaesthesia as a supplement to oxygen and nitrous oxide (Boston, 1956). Dillon (1965) described the use of trichloroethylene inhalations to relieve the pain of intractable malignant disease and Ellis and Bryce Smith (1965) used it to relieve the pain of post operative breathing exercises. Trumper et al (1936) advocated its use as a wound cleanser in preference to spirit and ether. It has also been utilized in narcoanalysis, veterinary anaesthesia and in various chronic painful conditions.

A major advantage of using trichloroethylene is its cheapness and availability, making it ideal for use under field conditions and in developing countries. Also its non-flammability allows its use with electrical cautery. It is effective when used in small amounts and causes little post operative disturbance. It is relatively pleasant to inhale, and is a non-irritant and unlikely to cause coughing during induction (Ostlere, 1950). Ostlere (1948) reported, in a review of 40,000 cases, that trichloroethylene demonstrated its low toxicity compared with chloroform, which is

said to produce a similar type of anaesthesia (Hewer, 1942).

A major disadvantage of trichloroethylene, is that it has poor muscular relaxation properties, and it easily produces tachypnoea in anaesthesia and analgesia. Cardiac arrhythmias are uncommon but may occur if the concentration is greater than 2.5 per cent. It may cause a delayed recovery with drowsiness lasting 24 hours. Also its unsuitability for use in a closed circuit apparatus due to its reaction with soda lime has limited its use with controlled ventilation.

Toxicity of Trichloroethylene:

The problem of trichloroethylene toxicity occurs in both medicine and industry. In industry contact with liquid trichloroethylene or with its concentrated fumes may result in eczema, dermatitis, conjunctivitis, as well as skin burns. Internal ingestion of liquid trichloroethylene causes intoxication, vomiting and diarrhea, and may cause death with pulmonary oedema and hepatic or renal necrosis. Recovery may occur, but there may be residual amnesia, psychosis or hemiparesis. In industrial plants with poor hygienic conditions, the extent of toxicity is a function of the trichloroethylene vapour concentration in the atmosphere. These conditions result mainly in neurological disturbances. A large range of toxic symptoms due to chronic exposure is discussed by Delfalque (1961). Some of the most common effects are neurological upsets, restlessness, fatigue, anorexia, autonomic disturbances, cranial nerve lesions, cerebellar symptoms, peripheral neuropathy, dyspnoea, anginal pain, alcohol intolerance, leucopaenia and other blood changes.

Pure trichloroethylene or trichloroethylene in combination with impure products easily breaks down into highly toxic products. In 1944, both the British Medical Journal and Lancet warned of using trichloroethylene with soda lime during or shortly before anaesthesia. Humphrey and McClelland (1944) had confirmed that at temperatures over 130 degrees centigrade in the presence of potassium or sodium hydroxide, trichloroethylene decomposes into dichloracetylene and hydrochloric acid. Dichloracetylene may then decompose into phosgene and carbon monoxide, or in the

presence of water, produce dichloracetyl chloride and trichloracetyl chloride. All these decomposition products are highly toxic. Firth and Stuckey (1945) demonstrated that several factors affect the rate of decomposition. Moisture, size and alkalinity of soda lime, as well as the length of time that trichloroethylene remained in contact with the soda lime were shown to be related to the decomposition of trichloroethylene. As the temperature of the soda lime rose above 45 degrees centigrade, decomposition began to increase and accelerated rapidly at temperatures above 60 degrees centigrade. Therefore the use of a circle CO_2 absorption system associated with trichloroethylene is prohibited, even though the quality of soda lime has improved, and the possibility of high temperatures has been reduced.

The toxic effects of trichloroethylene decomposition are characterized by cranial nerve palsy. Damage may occur in the trigeminal nerve and sometimes in the oculomotor, auditory and facial nerves.

Studies of Metabolism:

Trichloroethylene has been considered unique in being the only known inhalation anaesthetic which is metabolized, instead of being excreted unchanged. There is evidence, now, that other inhalation anaesthetics, particularly halothane are metabolized to some extent (Stier, 1964). The route of uptake plays a significant role in the degree of metabolism. The most significant route of absorption by far is the lungs because metabolism can take place in the lungs themselves. Lande et al (1939) and Malkinson (1960) regard absorption through the skin as negligible.

Studies of the metabolism of trichloroethylene have been undertaken because of the importance of trichloroethylene to industry and the frequent occurrence of acute and chronic poisoning by this solvent. Most of these studies were carried out with a small number of subjects being exposed to trichloroethylene for long periods, up to five hours, at concentrations ranging from 50-1300 mgm/1 of trichloroethylene vapour (Teisinger, 1960). This type of experiment was performed utilizing a single exposure rather than several successive exposures. The results of such experiments

ndicate the percentage of retained trichloroethylene to be in the range of 50-65%.
These findings have been in agreement with those of Teisinger (1960); Bardodez and
Viskocyl (1956); Soucek and Vlachova (1960); Bartonicek (1962) and Ahlmark and
Forssman (1951). However, Grandjean et al (1955) found a retention of 70% which
was in contrast to the previous findings. Teisinger (1960) reported that for
administration ranging from 50-1300 mgm/l in 25 patients, the lungs absorbed an
average of 56% of the inhaled vapours. From this amount, the lungs subsequently
eliminated 20% of the trichloroethylene with 80% being metabolized in the body, which
produces 20% trichloracetic acid, 50% trichlorethanol and 10% unknown metabolites.

Powell (1945, 1947) carried out equilibrium experiments with trichloroethylene
air mixtures in whole blood, plasma, washed cell suspensions, haemoglobin solution and
water. The greater percentage of trichloroethylene uptake in the blood was found to
be by the haemoglobin in the erythrocytes. Powell concluded that the absorption of
trichlorethylene by the plasma depends not only on the fat but also on its protein content.
Thus when a lipoid soluble anaesthetic agent, such as trichloroethylene is used for
anaesthesia, it is transported in the blood by both the plasma and the blood corpuscles.

Genevois (1936) performed uptake experiments on mice. These experiments
suggested that the uptake of trichloroethylene followed the general principle that when
uptake is dependent on tissue water concentration its rate is proportional to molecular
weight. Helliwell and Hutton (1950) demonstrated transplacental movement of
trichloroethylene in pregnant sheep and goats. Barrett et al (1936) found that in dogs,
fat was the tissue which retained trichloroethylene the longest, whereas Fabre and
Truhaut (1952) concluded that duration of exposure influenced the distribution of
trichloroethylene in animal tissues. The lungs contained the most after acute
exposures, whereas organs such as the gonads and the spleen contained a larger
proportion after chronic exposures.

Teisinger (1960) has expressed reservations about the relevance of animal
research on trichloroethylene uptake and metabolism, to the problems of human
exposure, because animals metabolize the drug differently; in particular, metabolism

the lungs is more notable in many species than in man. He suggested that a large number of carefully made human observations would be more relevant for studies involving chemicals that have been in use medically and in industry for several years.

The excretion of unchanged trichloroethylene through the lungs after exposure was found to begin at maximum levels and be complete within 14-48 hours (Powell, 1945; Sutton and Helliwell, 1950; Bartonicek, 1962; Ahlmark and Forssman, 1951). The pulmonary excretion may be increased by substances which inhibit its metabolism, such as disulfiram (Bartonicek and Teisinger, 1962). The urinary excretion of unchanged trichloroethylene in man is very slight (Powell, 1945; Teisinger, 1961) and likewise in animals (Fabre and Truhaut, 1952; Forssman and Holmquist, 1953).

Metabolism of trichloroethylene is influenced by intravenous glucose and insulin, which increases the metabolism of trichloroethylene, while fructose and sodium lactate increase the proportion of trichloracetic acid derived from the metabolism of trichloroethylene. On the other hand disulfiram (Antabuse) retards the metabolism of trichloroethylene (Bartonicek and Teisinger, 1962). It is thought that the action of disulfiram blocks the metabolism of trichloroethylene at the stage where the aldehyde of trichloroethylene is oxidized to a substituted acetic acid, resulting in a build up of aldehydes and interfering with the production of trichlorethanol (Fig. 1) (Forssman, Owe, Larsson and Skog, 1955). Therefore, the above factors may influence the toxicity of trichloroethylene in proportion to the toxicity of its metabolites.

Metabolites:

The metabolism of trichloroethylene results in the production of trichloracetic acid (TCA), monochloracetic acid (MCA) and trichlorethanol (TCE). Chloral hydrate (CH) may play a role in the pathway of metabolism as an intermediary (Bardodej and Viskocyl, 1956).

Trichloracetic acid was first identified as a metabolite of trichloroethylene by Bruning and Schnetka (1933) and was isolated in the urine of dogs by Barrett and Johnston (1939) after trichloroethylene anaesthesia. Powell (1945) isolated TCA in the urine of

FIGURE 1

Metabolism of Trichloroethylene
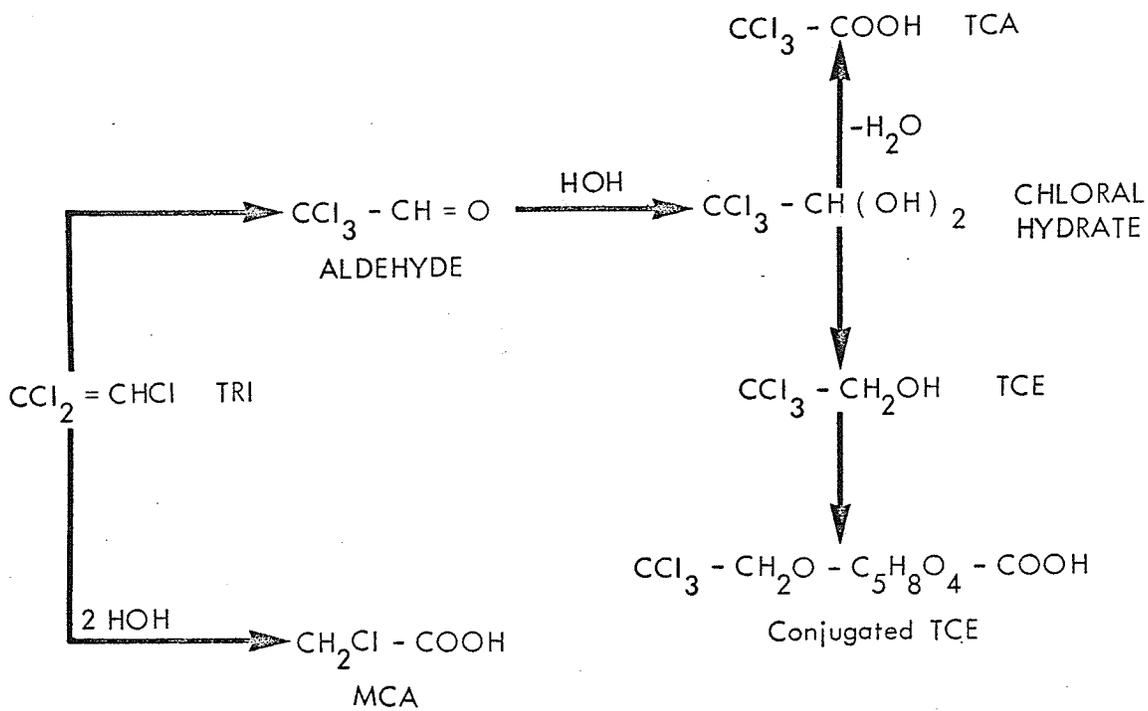
Postulated Metabolism of TCE

logs after trichloroethylene administration.

The narcotic effect of TCA was first noted by Leibreich (1869). This was confirmed by Paykoc and Powell (1945) when sodium trichloracetate was administered intravenously to human volunteers. TCA first appears in the blood within 3-4 hours after administration of trichloroethylene in animals in amounts proportional to the quantity of uptake. Elimination of TCA in the urine was found to reach a maximum level within 48 hours (Barrett et al, 1936; Powell, 1945; Soucek and Vlachova, 1960) and then to decline exponentially for 10-15 days (Bartonicek, 1962; Powell, 1945). The site of transformation according to experiments in rodents and dogs by Fabre and Truhaut (1951, 1952) may be the lungs, and perhaps the spleen. The extent of metabolism is believed to be related to several factors: duration and intensity of exposure, age, weight, drugs, and whether the administration is in animals or human subjects. Butler (1949), after administration of trichloroethylene to dogs, found trichlorethanol in small quantities in the plasma and conjugated TCE in the urine. Trichlorethanol is produced in far greater amounts during trichloroethylene anaesthesia than TCA (Butler, 1949; Soucek and Vlachova, 1960). TCE is excreted through the lungs for about four days after exposure (Bartonicek, 1962).

The postulation of monochloracetic acid as a metabolite of trichloroethylene was first made by Jones and Scott (1943) and it was isolated in human urine by Soucek and Vlachova (1954). MCA appears in the urine a few minutes after exposure to trichloroethylene vapour and reaches a maximum immediately after exposure. An exponential fall in MCA concentration occurs for an average of 112 hours after exposure. MCA is produced in much smaller quantities than TCA and TCE. However MCA is the most toxic of the metabolites (Soucek and Vlachova, 1960). Therefore, it must play a significant role in the toxicity of trichloroethylene on the human organism.

UPTAKE, DISTRIBUTION AND ELIMINATION

Factors Affecting Uptake, Distribution and Elimination:

In considering the uptake and distribution of any anaesthetic drug there are many factors which must be taken into account. They can be broadly classified into three categories. Firstly, there are the physiochemical factors, those which affect the drug itself. Secondly, there are the respiratory factors which determine the uptake into the lungs. Thirdly, there are the circulation factors which are the factors determining the actual distribution of the drug throughout the body and its delivery to various organs and tissues.

The solubility of an anaesthetic agent in blood and body tissues is one of the most significant physiochemical factors affecting uptake and distribution. The solubility of an inert gas in matter can be expressed as an absolute quantity of a gas in a known quantity of matter. The Bunsen absorption coefficient is the volume of gas, which dissolves in a given volume of solvent at S.T.P. The Ostwald solubility coefficient is a distribution ratio of the volume of absorbed gas, expressed at the temperature of the experiment, to a unit volume of liquid with a partial pressure of dissolved gas equal to 760 mm.Hg. Gas solubility can also be expressed as a comparative distribution between two phases. The ratio of the amount of gas in equal volumes of two phases at a stated temperature, independent of pressure when the two phases are in equilibrium is known as a partition or distribution coefficient.

The solubilities of gases in solutions are influenced by temperature, pressure and by additional substances dissolved in the solution. Henrey's Law is obeyed over a wide range of pressures, in that gas solubility increases proportionately to pressure. On the other hand gas solubility is decreased by the addition of electrolytes to a solution or by increasing the temperature.

Diffusion, another physiochemical factor, is the exchange of chemically inert gases through cell boundaries and tissue membranes. The present evidence indicates that inert gas exchange in the human body occurs by diffusion only. Thus exchange by diffusion takes place between capillary blood and alveolar gas in the lungs and between capillary blood and tissues in the periphery. The diffusion coefficient is dependent upon the partial pressure of the dissolved gas and is proportional to the solubility of the gas in the medium.

Drugs acting on membranes hamper the transport of solutes across biological membranes by disrupting the diffusion process. The lipid solubility of the membrane and its equivalent pore radius play an important role in allowing the passage of molecules into the cell.

The ionization of organic compounds when placed in aqueous solution influences the uptake and distribution of some drugs. The ratio of ionized and non-ionized forms of a drug, which behave quite differently, determines the passage of the drug across body membranes, the pharmacologic activity, physiologic distribution and chemical reactivity. However anaesthetics do not ionize and therefore do not vary in behavior.

The various <u>respiratory factors</u> which directly influence the lung also control uptake and distribution. The effect of the lung is to delay the time required for equilibrium between inspired anaesthetic gases and arterial blood.

The first practical consideration in looking at lung uptake is knowledge of the inspired concentration. When an anaesthetic is introduced into a circle system the inspired concentration will not be the same as the concentration delivered by the vaporizer and significant delay of uptake may result. With a non-rebreathing of "open" system of administration, using a reliable quantitative vaporizer, the inspired concentration will be as indicated by the vaporizer control setting.

When an anaesthetic gas is inhaled, it is diluted by gases already present in the lungs and its partial pressure is further reduced by the addition of water vapour, with which the respiratory passages are saturated. With continuing ventilation, the alveolar tension increases until eventually equilibration is achieved between the inspired tension and that in the lung alveoli.

In general, alveolar concentration approached that inspired most rapidly with the less soluble agents. With any given agent, the higher the inspired concentration, the more rapid is the approach of alveolar concentration to that inspired (Eger, 1963). Increasing the alveolar minute ventilation, by facilitating "lung washout" will accelerate the uptake of the anaesthetic. Changes in alveolar ventilation have less effect

on relatively insoluble gases than on soluble gases. Uneven distribution of ventilation and perfusion within the lungs results in delayed equilibration between inspired and alveolar concentrations.

Blood solubility influences the amount of anaesthetic agent lost from the alveolar gas to the pulmonary capillary blood. Blood uptake decreases the lung gas volume and the alveolar partial pressure of the anaesthetic gas.

Cardiac output, which is the product of the volume of blood ejected from each ventricle per beat and the number of beats per minute, has a considerable effect on uptake, depending on the solubility of the agent in the blood. High cardiac output causes a slower rise in alveolar tension, due to the higher clearance of anaesthetic by the circulating pulmonary blood. However, insoluble gases are not greatly affected by cardiac output, because a relatively small proportion is taken up. Doubling the cardiac output reduces the arterial tension by not more than 30% of normal for highly soluble agents such as ether and halothane and not more than 15% for the less soluble nitrous oxide (Mapleson, 1963). Thus fluctuations in cardiac output can play an important role in the uptake and distribution of inhalation anaesthetics.

Severinghaus (1963) has also employed electronic analogues to predict the effect of various factors on the rate of uptake of anaesthetics. It was shown how differences between inspired, alveolar and tissue tensions- that is, failure to reach equilibrium in a given time - could be related to anaesthetic solubility, ventilation, inspired concentration, cardiac output and blood supply to individual organs.

The presence of an effective circulation is essential in order to distribute an anaesthetic agent to the various parts of the body. The distribution of any agent depends upon many factors, since the tissues of the body are perfused at widely different rates. The availability of any drug to be taken up by a tissue, in relation to that available to other tissues depends directly upon the blood flow in the tissue expressed as a fraction of total blood flow- that is cardiac output. The relationship between blood flow and uptake depends on the affinity of the tissue for the agent and

thus the fat content of the tissue.

The arterial and venous blood concentrations reflect the degree of body saturation with an anesthetic agent. At the beginning of inhalation the effective pulmonary ventilation permits diffusion of the anaesthetic into the pulmonary capillary blood, where equilibrium is reached. The difference in the arterial and venous blood concentration decreases as the tension in the tissues progressively rises, approaching full equilibrium of the body. The anaesthetic content of arterial blood rises rapidly after the beginning of inhalation, whereas total body uptake continues over a longer period of time. The tissue tension rises gradually from zero, approximating the anaesthetic tension of the arterial blood at full saturation. The relationship between the anaesthetic content of alveolar gas and arterial blood, at equilibrium, is defined by the gas/blood partition coefficient; the concentration relationship between blood and fatty tissue, at equilibrium, is determined by the blood/fat partition coefficient. For practical purposes, blood may be regarded as a watery solution, and the oil/water distribution coefficient is taken as an index.

Theoretical Considerations

Mapleson (1964) has attacked the problem of uptake, distribution and elimination of inhaled gases and vapours by setting up mathematical models and electrical analogues. He argues that the theory of uptake and distribution is now capable of predicting whole body rates of uptake and alveolar tensions which are in agreement with experimental data (Mapleson, 1962, 1963; Eger, 1963). He points out the need for the theoretician as well as the experimentalist, working together, in order to interpret the problems. He states that given measurements or good estimates of the variables involved, the making certain assumptions, it is possible to calculate the way in which tension in, and rate of uptake by any tissue or organ changes during the administration of the anaesthetic and during recovery.

The theoretical predictions of Mapleson (1963), Egar (1963) and Severinghaus (1963) and other workers postulate that the body is divided into two, three or several components. This may not be a sufficiently true representation of the actual situation.

Various assumptions such as constant inspired tension and constant cardiac output tend to over-simplify the problem. More reliable scientific data are required before these postulates may be substantiated.

For most theoretical purposes the body has been regarded as a number of components: the central nervous system, the viscera, the lean muscle mass and the fat or adipose tissue. In 1951, Kety predicted that the higher the blood solubility coefficient of the anaesthetic agent, the slower will be the saturation of the arterial blood.

Mapleson (1963) discusses the use of the analogue, based on the observed agreement between computed and experimental rates of uptake, in order to predict the behavior of variables for various anaesthetics. The inspired concentration, the ventilation and the cardiac output are assumed to remain constant throughout the administration when such analogues are utilized. He pointed out that the effect of high fat solubility would be to retard even further the time for equilibrium, and demonstrated that with an agent with a high fat solubility and a low blood solubility, whole body saturation is reached more slowly than with an agent with a high blood solubility and a low fat solubility.

Several anaesthetics are compared in terms of alveolar or arterial tension as a percentage of inspired tension against time. The whole body rate of uptake curve would be a mirror image of the alveolar tension curve, and therefore the latter is a measure of the former. However the degree of fat solubility will affect the rate of saturation. (Table 1. Fig.2). This is illustrated by cyclopropane and nitrous oxide, which have similar blood gas coefficients but cyclopropane has a higher fat gas coefficient and thus requires more than 24 hours longer to reach equilibrium. On the other hand, ether, which has a higher blood gas coefficient than chloroform and halothane reaches saturation sooner because its fat gas coefficient is considerably smaller.

In the case of trichloroethylene, Mapleson has made predictions based on the experimental data of Clayton and Parkhouse (1962). The high blood-gas coefficient (960) (Powell, 1945; Soucek, 1955) of trichloroethylene, partially explain why

TABLE 1

Ostwald Partition Coefficients at 37 degrees C.		
Anaesthetic	Blood-Gas	Fat-Gas
Ethylene	0.14	1.28
Cyclopropane	0.46	9.20
Nitrous Oxide	0.47	1.41
Halothane	2.30	138.0
Chloroform	7.30	500.0
Trichloroethylene	9.00	960.0
Diethyl Ether	15.00	50.0
Methoxyflurane	20.00	1800.0

FIGURE 2

Alveolar or Arterial Tension as Percentage of Inspired Tension
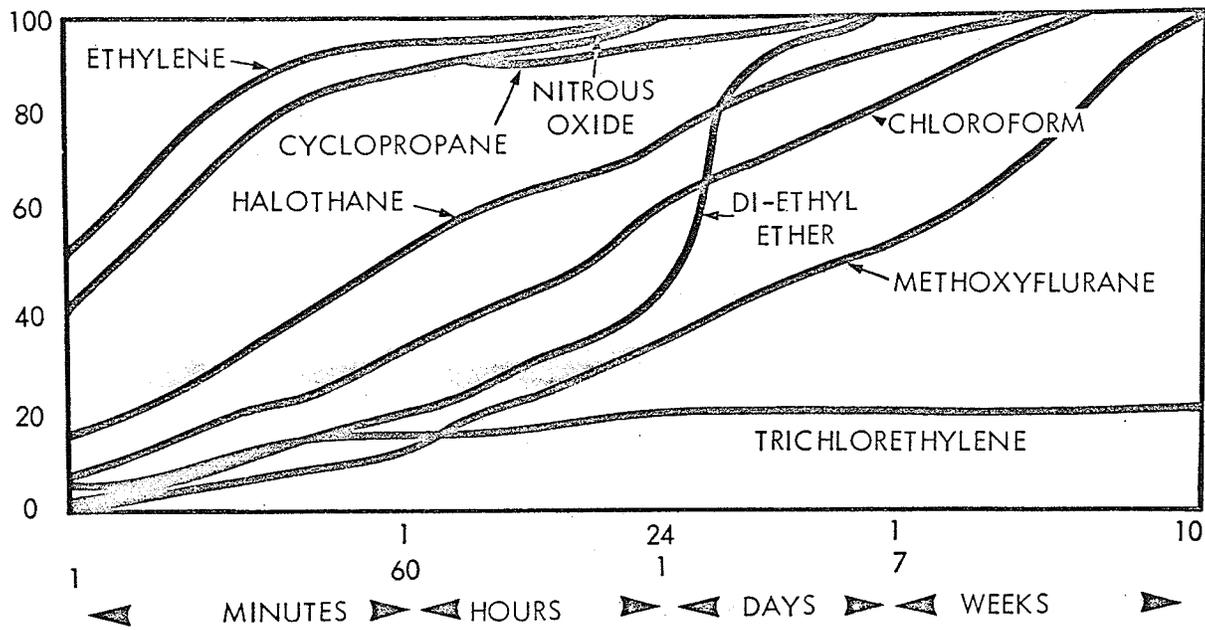
saturation is not achieved (Fig. 1). Parkhouse and Clayton found no evidence of equilibrium after three hours of anaesthesia and the failure to reach equilibrium may be explained by the metabolism of the drug.

In his model, Mapleson assumed that one quarter of the cardiac output was cleared of trichloroethylene to obtain agreement between the computed values and the observed tensions of Parkhouse and Clayton. Mapleson concluded from his model, that due to the breakdown of trichloroethylene, the arterial tension was less than 20% of the inspired tension.

For highly fat soluble agents the relationship between blood flow and fat content in a tissue is a very significant factor in uptake. The blood supply will carry the agent to the tissue, but the uptake of the drug then depends upon the fat content of the tissue. The greater the fat content, the more the agent will be absorbed. The outstanding example of a body tissue which has both a high blood flow and a high lipid content is the central nervous system: hence the preferential distribution of anaesthetic agents to the brain and the clinical feasibility of maintaining unconciousness without undue toxic effects on other organs and tissues.

PREVIOUS STUDIES

Anaesthetic gases and vapours which are not altered or destroyed when inhaled in the body are termed "non-reactive" anaesthetics. In 1924, Haggard published a classic series of papers on the absorption, distribution and elimination of ethyl ether. The methods available at this time made such work toilsome. These ether experiments were performed on a small series of animals. Haggard's work and his conclusions laid the foundations for future studies on uptake and distribution and the need to apply these principles to study on human subjects. Earlier, Nicloux (1908) had studied the equilibration of anaesthetics by administering ether and chloroform to animals for long periods of time. The time required for equilibration was determined by killing the animals and estimating the concentrations of the agents in various tissues and organs.

The purpose of Haggard's experiments was to demonstrate the fate of ether

in the body and the relationship of the total amount of ether absorbed and its inhalation concentrations. The compiled data indicated that ether, which was absorbed into the body, was in no manner transformed or modified. In a number of administrations, ranging from 21-241 minutes of duration, 87% of the absorbed ether was eliminated through the expired air unchanged. The failure to recover a higher percentage of the inhaled ether may be accounted for by elimination through the urine, perspiration, exposed serous surfaces, and by experimental error. At conditions of full saturation, an index of the total ether absorbed was the content in the arterial blood multiplied by the body weight. A ratio of 1:1 was assumed between the arterial blood and the tissues as a whole, per unit mass, for any tension of inhaled ether. Calculation of whole body uptake, from this assumption was shown to agree with experimentally measured uptake. The principles that Haggard defined may pertain in general, to any gas or vapour which, like ether, is absorbed and eliminated unchanged. Thus, under similar conditions of respiration and circulation, the rate of absorption of ether or any vapour is in direct proportion to the concentrations inhaled. The time required to obtain full equilibrium or any given percentage of whole body uptake is identical for all concentrations administered. However, absorption of any given mass is inversely proportional to time, with absorption increasing to a greater degree as the concentrations become greater. The rate of absorption and elimination varies almost directly with the volume of the air breathed. The influence of the volume of air breathed is dependent on the solubility coefficient of the inhalation anaesthetic in question.

It has been stated previously that the concentration of a volatile agent in the blood is an approximate index of how the agent is distributed throughout the body as a whole. This fact does not apply to any individual organ or tissue but is an expression of the integration of all tissues in the body; some of which take up more and some less than others. Haggard demonstrated that there was a relationship between the concentration of ether or any similar volatile substance in the central nervous system and the concentration in the arterial blood.

Haggard proved experimentally that the partial pressure of ether in the tissue of the central nervous system determines its anaesthetic action. In effect the agent, in this case ether, after absorption, is distributed between a small mass, the central nervous system, upon which it acts physiologically, and a large mass, the remainder of the body, upon which it has an insignificant effect. Thus, Haggard described a "buffer" effect of the body which may delay induction of anaesthesia and later the elimination of the agent depending on the tension of the anaesthetic in the arterial blood.

Powell (1945) performed a detailed study of the absorption and elimination of trichloroethylene in human subjects, as opposed to experimental animals. The study was principally concerned with the metabolism of the drug. The elimination of trichloroethylene and its metabolite trichloracetic acid, from the body was carried out by an analysis of blood, urine, and expired air in four patients after anaesthesia. However, a methodical study of blood trichloroethylene concentration changes was not made and the standardization of the patient's minute volume was not undertaken.

Smith and Barker (1948) studied the quantitative estimation of ether in the newborn infant. They demonstrated that the average concentration of ether in the blood of the umbilical vein at birth was proportional to the average concentration in the venous blood of the mother. They concluded that a gradient between the maternal arterial blood and the blood in the umbilical artery does exist, and that the concentration of the anaesthetic in the foetal circulation is less than in the maternal circulation.

Helliwell and Hutton (1950) studied the distribution of trichloroethylene in the foetal and maternal circulation of pregnant sheep and goats. Their findings were in contrast to those of Smith and Barker, for ether, in that they measured higher concentrations in the foetal than in the maternal blood. They noted that the drug passed rapidly into the foetal circulation. The differences from the findings of Helliwell and Hutton were explained by the proportionately greater amount of erythrocyte envelope and lipoid material in the foetal circulation which would be sufficient to account for the increased mass of trichloroethylene under conditions approaching equilibrium.

Clayton and Parkhouse (1962) studied the uptake and distribution of trichloroethylene under controlled conditions and their results were expanded upon by Mapleson (1963) as previously discussed.

Clayton and Parkhouse investigated the concentrations of trichloroethylene in the arterial and venous blood during anaesthesia and the early postoperative period. Constant predetermined concentrations of trichloroethylene in air were delivered to patients at a known and constant respiratory rate.

They found that a continuous administration of low concentrations (0.5%) required at least one hour before the venous blood trichloroethylene was at a steady level. The absorption rate at various concentrations was also illustrated by the comparison of arterial and venous blood trichloroethylene concentrations for administrations of .5% and 1% respectively.

In studying the mean venous trichloroethylene concentrations, it was shown that by maintaining the minute volume and increasing the inspired concentration, the mean venous trichloroethylene concentrations were appreciably increased, and alternatively by maintaining the inspired concentrations and increasing the minute volume, that the mean concentrations in the venous blood increased accordingly. They concluded, by comparison of blood solubility concentration figures for trichloroethylene in blood, that equilibrium was not achieved. This fact was probably due to the breakdown of trichloroethylene.

A comparison of venous blood samples from the back of the hand, which contains relatively little fat tissue, and the anticubital fossa, which contains venous blood that has drained through fatty tissue in the arm on its pathway to the anticubital fossa, was made. Thus, in obese subjects, a large proportion of trichloroethylene could be extracted from the venous blood between the hand and the elbow. It was established that the venous blood concentrations from the back of the hand approximated more closely to the arterial blood concentrations than the samples from the anticubital fossa. In slim subjects, the venous blood from the anticubital fossa began to approximat the arterial blood concentrations after 30 minutes. The obese subjects required at least one hour before even approximately the same trichloroethylene content as

arterial blood, while the venous blood collected from the back of the hand resembled arterial blood concentrations more closely and rapidly. Thus by measuring differences in arterial and venous blood with time, they concluded that even after two or three hours of anaesthesia, equilibrium is not complete. This illustrates the importance of the very high fat solubility of trichloroethylene.

Onchi and Asao (1961) endeavored to repeat Haggard's classic study of the absorption, distribution and elimination of diethyl ether, using human subjects rather than experimental animals. The application of gas chromatography to this study was of major importance, since it indicated the pronounced sensitivity of this method as an analytical tool. The detection of a vapour in expired air as long as twenty hours after the termination of anaesthesia, and the determination of ether concentrations with a five microliter gas sample from a 10, 000 microliter volume was now possible. The results varied a great deal from those of Haggard, but the discrepancies may be explained by the fact that so few experiments were carried out in this study. Onchi and Asao concluded that the elimination of ether from the body could be represented by a combination of three exponential curves with different time constants, each exponential representing the clearance of a different compartment of the body. The most rapid clearance of the anaesthetic being from the lungs, the next most rapid clearance being from the blood stream, and finally a slower clearance from the tissues.

Experimental Methods:

Haggard (1923) utilized a train of iodine pentoxide as a means of analyzing ethyl ether in air and blood. The ether passes over iodine and is oxidized liberating iodine. The iodine is collected and determined by titration with thiosulfate, using starch as an indicator (Haggard, 1923). This method may also be used for many other hydrocarbon vapours, which are oxidized by hot iodine pentoxide, liberating iodine.

Onchi and Asao (1961) utilized rubber bags in order to administer known concentrations of ether through a non-return valve to healthy human subjects. The elimination was studied by collecting the expired air in the same manner. Measured volumes of ether vapour were converted to S.T.P. and analyzed quantitatively in a

gas chromatograph.

Stewart and Erley (1963) used saran bags for the collection and sampling of expired air, because they had proven to be superior to rubber, synthetic or polyethylene bags. The saran was found to be resistant to vapour diffusion and very few volatile compounds were found to react chemically with or be soluble in it. Stewart et al (1959, 1962, 1963) used infrared analysis of the expired breath and found it to be effective. Characteristic "decay" over a time period, of the concentration of volatile compounds in the expired breath was observed. The concentration of the expired air was proportional to the blood concentration which, in turn, was related to the length of exposure and the inhaled concentration. The collected samples were analyzed for identification of specific compounds by scanning the infrared spectrum. After identification, the absorbance of the compound was measured at the appropriate wave length to determine the concentration of the vapour. Infrared analysis offers the advantages of specificty, sensitivity, speed and simplicity in the detection and quantification of inspired and expired gases and vapours.

Other techniques of gas and vapour analysis have been used in anaesthetic studies, though not necessarily with trichloroethylene. For instance, some anaesthetics for example halothane, have a specific absorption band in the ultra-violet range and this form of detector, although slow in response, is useful. The effect of a vapour on the speed of conduction of sound may be used to construct a sonic analyzer, or the alteration in thermoconductivity may be used quantitatively as a katharometer. Binary mixtures may be analyzed by means of a Raleigh refractometer, and for sophisticated studies a mass spectrograph may be used.

ESTIMATION OF TRICHLOROETHYLENE

Estimation in Air:

The production of a quantitative color reaction with alkaline pyridine is the basis for the estimation of trichloroethylene (Fujiwara, 1914). Trichloroethylene has been estimated in expired air after absorption in toluene (Powell, 1945; Bartonicek, 1962; Bartonicek and Teisinger, 1962). The absorption apparatus employed in these experiments was designed to create a minimum resistance to expiration; the subjects expired into a mask fitted with inspiratory and expiratory valves, by way of igelite tubing, through a gas meter and into a series of collection flasks. The method of Soucek and Frankova (1952) was employed in the analysis and colorimetric determination of the trichloroethylene content. Analysis was carried out by spectrophotometric methods.

Forssman and Holmquist (1953) in a series of experiments using rats, studied the relationship between inhaled and exhaled trichloroethylene. The rats were placed in exposure chambers and trichloroethylene was administered for varying time periods. A current of air containing known concentrations of trichloroethylene was passed into the chamber and the expired air leaving the chamber was analyzed. The expired air was collected in flasks containing silica gel in order to absorb the trichloroethylene vapour. The silica gel was washed with ethanol several times to extract the trichloroethylene and the ethanol was burned in a combustion chamber. The gases formed during combustion were collected in wash bottles and the amount of chloride in the wash bottles was determined by the modified Volhard method (Caldwell and Mayer, 1935). The large amount of unavoidable experimental error in this procedure permitted the investigators to draw only limited conclusions about the conversion of trichloroethylene in the organism.

Estimation in Blood:

Steam distillation has been employed for the estimation of trichloroethylene in blood. The aqueous distillate was extracted with toluene, and an aliquot of the toluene extract was treated with pyridine and alkali (Habgood and Powell, 1945; Powell, 1945; Bruning and Schnetka, 1953). The trichloroethylene was removed from

diluted blood, tissue or urine solution by the passage of a stream of air through it
(Powell, 1945; Fabre and Truhaut, 1951, 1952; Julharni, 1954). The trichloroethylene
was then absorbed in toluene and subjected to colorimetric analysis. The Conway
microduffusion technique (Conway, 1957) was employed by Clayton and Parkhouse (1962)
in order to extract trichloroethylene from blood. A conway cell is a circular glass
dish with a concentric inner wall approximately five mm. lower than the main outer
wall. The system is sealed off from the atmosphere by a glass lid resting on the
outer wall. Toluene, which has a partial pressure close to zero, is placed in the
central chamber and the blood containing trichloroethylene in the outer circle. The
trichloroethylene diffuses through the five mm. wide gap into the central chamber. The
estimation of the toluene-trichloroethylene was accomplished by the pyridine reaction
(Powell, 1945; Brain and Helliwell, 1950). The main disadvantage of this technique
is that 15 to 20 hours are required for all of the trichloroethylene to diffuse from the
outer chamber to the central compartments.

Estimation of Metabolites:

Trichloracetic acid may be separated from protein in blood and tissues by
precipitation with sodium tungstate. The supernatant is filtered off and a pyridine
reaction is used to determine the trichloracetic acid content. A similar analysis is
used for identifying trichloracetic acid in urine (Soucek and Vlachova, 1960; Ahlmark
and Forssman, 1951; Powell, 1945; Butler, 1948).

Conjugated trichlorethanol is isolated in urine by first hydrolyzing with acid
and then oxidizing to trichloracetic acid (Butler, 1949; Soucek and Vlachova, 1960).
In order to estimate TCE glucuronide in plasma, heptane is used as an extracting
agent and the same procedure is carried out (Butler, 1948).

Soucek and Vlachova (1960) developed a method to isolate and identify
monochloracetic acid. After acidifying the urine, and extracting with ether, the
extracts were mixed with pyridine and descending paper chromatography was used to
isolate MCA. The MCA was then quantitatively ascertained colorimetrically on the
basis of the organically bound chlorine.

Gas Chromatography:

Gas chromatography is a technique for separating volatile substances based on the distribution of a sample between two phases. The separation of a mixture of gases and vapours by chromatography was first employed by Ramsey (1905) utilizing selective absorption on solid absorbents, such as active charcoal. Following the suggestion of Martin and Synge (1941) in a study for which they were later awarded the Nobel Prize, James and Martin (1952) introduced gas liquid chromatography. The sensitivity, speed, accuracy, and simplicity of this method for the separation, identification, and quantitative estimation of volatile compounds has resulted in its various applications as an analytical tool in research and industry.

Basically the gas chromatograph is composed of an analytical column, a detector and a recorder. The simplest concept of a chromatograph is to regard it as a molecular filter which selectively impedes the passage of molecules according to various physical characteristics such as shape, size, weight, and boiling point. Thus a mixture of gases and/or vapours is swept into a stream of carrier gas, which transports the mixture through a column packed with an inert material impregnated with a liquid of low volatility. The column consists of two phases, a moving carrier gas phase, and a stationary phase which is a heavy non-volatile fluid. The liquid presents a large surface area to the mixture flowing through it. This liquid, which is absorbed in fine particles of ground firebrick packed in the column, presents a large surface area to the mixture flowing through it. By the time the mixture emerges from the distil end of the column, it has separated into its components. This order will depend upon various factors including type of column, length of column, temperature of column and the number of carbon atoms in the compound. The detector, which may be of various types depending on the type of analysis, transmits a signal to the recorder for each component, which varies in its intensity according to the amount present. The recorder, in turn, transcribes the signal onto paper in the form of a peak or curve. The area under the curve, which may be accurately determined by integration, is directly proportional to the concentration

of the component.

The sample enters into the column through the injection apparatus which is proximal to the chromatographic column. The injection apparatus may take the form of a simple diaphram, through which the needle of a microlitre syringe is inserted, or a blood/gas extracting device, which provides a technique for extracting dissolved gases from blood and injecting them into the column. Another device is a gas sample loop, which allows a known fixed quantity of a gas sample to be injected into the system at atmospheric pressure. The injection chamber, as well as the detector and the column oven are thermostatically controlled, thus providing a wide variety of controlled conditions for various analyses. (Fig. 3).

Applications of Gas Chromatography in Anaesthesia:

Butler and Hill (1961) described a method for the estimation of volatile anaesthetics in tissues, employing gas chromatography. Butler and Freeman (1962) advocated gas chromatography for the estimation of anaesthetic vapours in blood (Wolfson et, 1966). Variations of the technique appear in the literature with regard to preparation of the sample for analysis. Onchi and Asao (1961) employed gas chromatography in studying the uptake, distribution and elimination of diethyl ether. The application of gas chromatography allows the anaesthesiologist to follow the exponential processes of uptake, distribution and elimination much farther towards finality than ever before. Gas chromatography can be used to study new anaesthetic agents and to test the accuracy of inhalers and vaporizers. The determination of the relative activity coefficients for volatile anaesthetics was proposed by Butler and Freeman (1962). The rate of uptake of an anaesthetic may be calculated by the determination of the difference in content of anaesthetic in arterial and mixed venous blood.

FIGURE 3
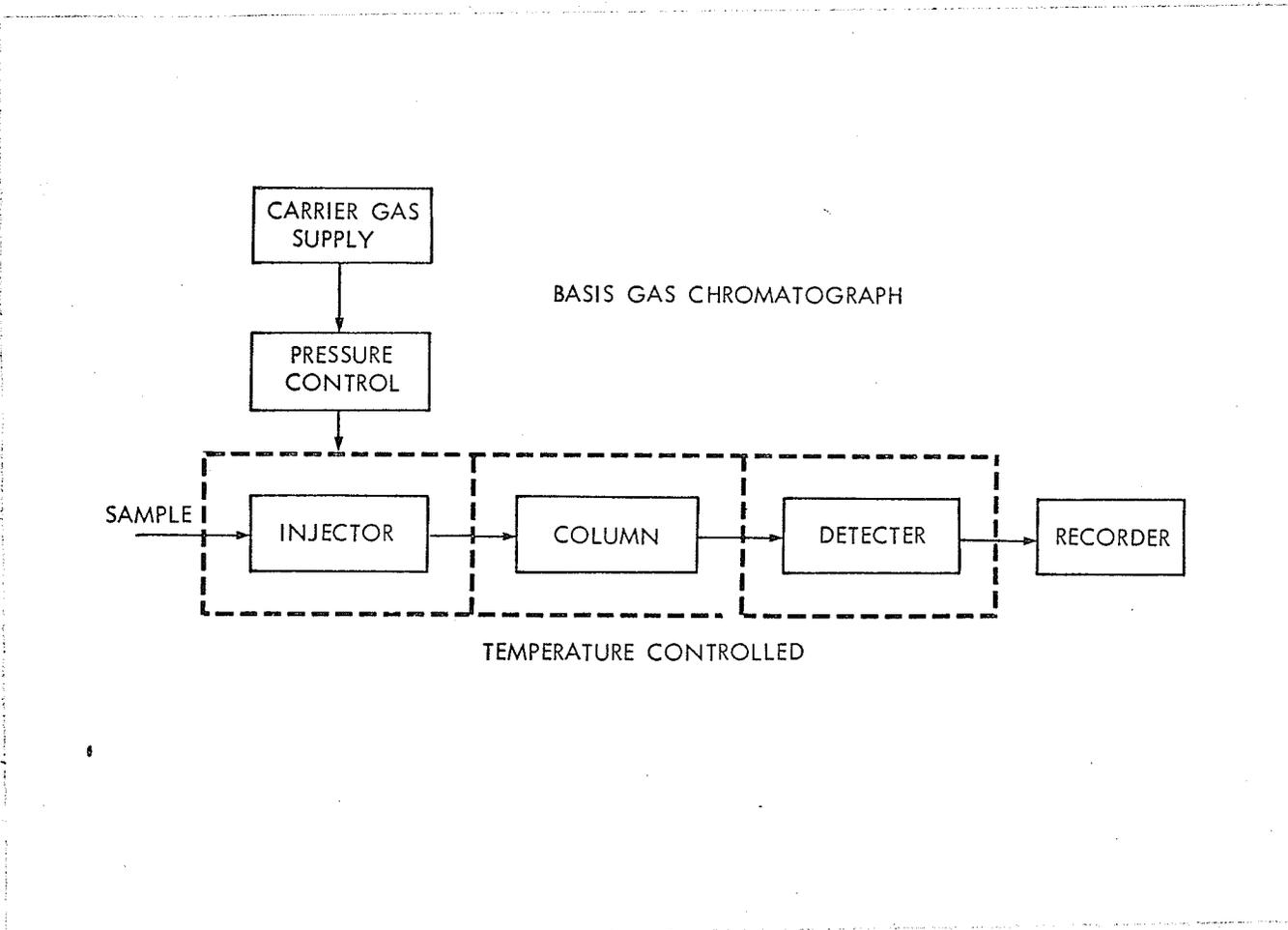
PART 11

CONCEPT OF INVESTIGATION

In studies reported in the literature, the differencebetween the amount of anaesthetic inhaled and the amount exhaled <u>during administration</u> is termed the amount (or percentage) <u>retained.</u> After the administration is terminated, the amount of agent excreted through the lungs is characterized as the amount (or percentage) <u>recovered.</u> If the intake and output of the anaesthetic are followed to finality, and a discrepancy is observed between the amount inhaled and the <u>total</u> amount exhaled, both during and after administration, this must be accounted for in terms of metabolism or non-pulmonary excretion. Mass or amount may be designated M, and the situation can be expressed as $M_I - M_E = M_M$, that is, in the case of trichloroethylene the sum of all the trichloroethylene inspired minus the sum of all the trichloroethylene expired equals the total amount metabolized, neglecting skin loss etc.

In practice it may be desirable to study the situation only up to a certain stage of the total process. The concept of uptake, as viewed at some moment during administration, is then introduced. The amount of anaesthetic in the body at any given moment can be determined by subtracting the mass inhaled from the mass exhaled up to that time. This amount of anaesthetic must be in the blood stream or the tissues, neglecting lung gas content; otherwise it has been metabolized. By measuring and subtracting the amount of agent in the blood, an estimation of the amount presently in the tissues or broken down can be made. An approximate indication of tissue uptake can thus be made if the metabolites can be isolated and measured quantitatively. Thus:

TRICHLOROETHYLENE IN minus TRICHLORETHYLENE OUT equals
TOTAL UPTAKE

TOTAL UPTAKE minus BLOOD (lung gas) CONTENT equals TISSUE UPTAKE
AND METABOLISM

TISSUE UPTAKE and METABOLISM minus METABOLITE ACCUMULATION
equals TISSUE UPTAKE

The Fick principle allows one to derive the quantity of a substance extracted

from an organ over a time period, if the concentration of the substance in the fluid entering and leaving the organ is known and if the total volume of fluid passing through the organ per unit time is known. In the case of respiration, the fluid being air and the organ being lung, the appropriate statement would be $\dot{V}(\bar{F}_I - \bar{F}_E) = $ average minute uptake through the lung, assuming a respiratory ratio of one. $\bar{F}_I$ and $\bar{F}_E$ signify the average inspired and expired concentrations respectively of the anaesthetic for the minute volume, $\dot{V}$. The difference in the average concentrations between inspired and expired gases multiplied by the total flow yields the actual quantity absorbed during the time period considered. The total uptake, up to the time considered, can be calculated by summing all of the quantal uptakes for all time intervals. It may then be expressed as

$$\sum_{o}^{i} \bar{F}_I \dot{V}_I - \sum_{o}^{i} \bar{F}_E \dot{V}_E = \sum_{o}^{i} M_U \qquad \text{(where } U = \text{total uptake)}$$

$\bar{F}_I \qquad \bar{M}_U$

to the ith minute of administration.

After the administration is terminated, the inspired concentration falls to zero. The amount of agent recovered can be determined by measuring the volume and concentration of the expired air. Thus with suitably sensitive methods, an agent in expired air can be followed up to any time after the end of the administration.

By comparing inspired and expired gases in this way, conclusions may be drawn about the total amount of trichloroethylene in the body. This total uptake will include all the trichloroethylene in the respiratory passages, the lung gases, the blood stream, and the tissues, all the trichloroethylene excreted through the skin and all the trichloroethylene that has been metabolized. Other studies of "uptake" have been based upon arterial-venous differences of an anaesthetic agent. Such studies give an indication of the loss from the blood stream into the tissues, but do not take into account the quantity of anaesthetic currently contained in the lung gases and respiratory passages, or in the blood stream itself.

Once administration is complete, and expiratory loss of anaesthetic has been followed to negligible levels, it should be possible, by isolating and measuring quantitatively the metabolites in the blood and urine to account for all the inhaled

trichloroethylene.

The present study is concerned only with the total uptake of trichloroethylene. Analysis of inspired and expired vapours have been carried out to examine to what extent the agent has been absorbed in the body and to follow the expiration of this absorbed amount. Different inspired concentrations were administered for various durations in order to determine if these factors have an effect on the proportion of the drug which is excreted through the lungs unchanged and the proportion which is metabolized.

METHODS AND MATERIALS

Measurement of Trichloroethylene Concentrations:

Inspired and expired gas samples were analyzed for trichloroethylene with a Varian Aerograph Model 1520B Gas Chromatograph. The contents of the sample syringe were injected onto the column by means of a gas sample valve, which enabled a known constant volume of the gas sample to be introduced onto the column at atmospheric pressure. The retention time for trichloroethylene was two and one-half minutes, with an 8-foot long- 1/8 inch diameter column composed of 20% Carbowax on 20M 60/80 Chromosorb W at a temperature of 125 degrees centigrade, using a carrier gas flow rate of 22.5 ml./min. nitrogen. A flame ionization detector, sensitive to one part per million, was used. The temperature of the detector was 170 degrees centigrade and the injection chamber was at 174 degrees centigrade. Gas concentrations, which are directly proportional to peak areas, were estimated by means of a disc integrator and duo-digital counter. The consistency and repeatability of this method is demonstrated in Fig. 4. This shows successive peaks obtained from the repeated injection of samples from the same trichloroethylene/air mixture. The peaks show a mean area of 364.6 with a standard deviation of $\pm$ 2.5.

A Rayleigh interferometer (Carl Zeiss) was calibrated for trichloroethylene from a specially prepared cylinder of 3.12% vapour in air (Matheson Co.). An inter-ferometer is an absolute instrument based on the change in refractive index of a gas mixture caused by the introduction of an additional component. The change is linearly related to concentration and with sufficient knowledge of the components of the mixture, the instrument can be calibrated from first principles. It will not vary from day to day providing corrections for temperature and barometric pressure are made.

The interferometer is a valuable instrument because after correcting for temperature and barometric pressure, it yields a constant measurement whereas the gas chromatograph response shows slight day to day fluctuations due to various factors such as slight changes in carrier gas flow and detector sensitivity. However, after the interferometer has been calibrated for a gas or vapour mixture, the presence of other vapours, gases or water vapour will alter the correct reading. On the other hand,

FIGURE 4

Repeatability of Gas Chromatography
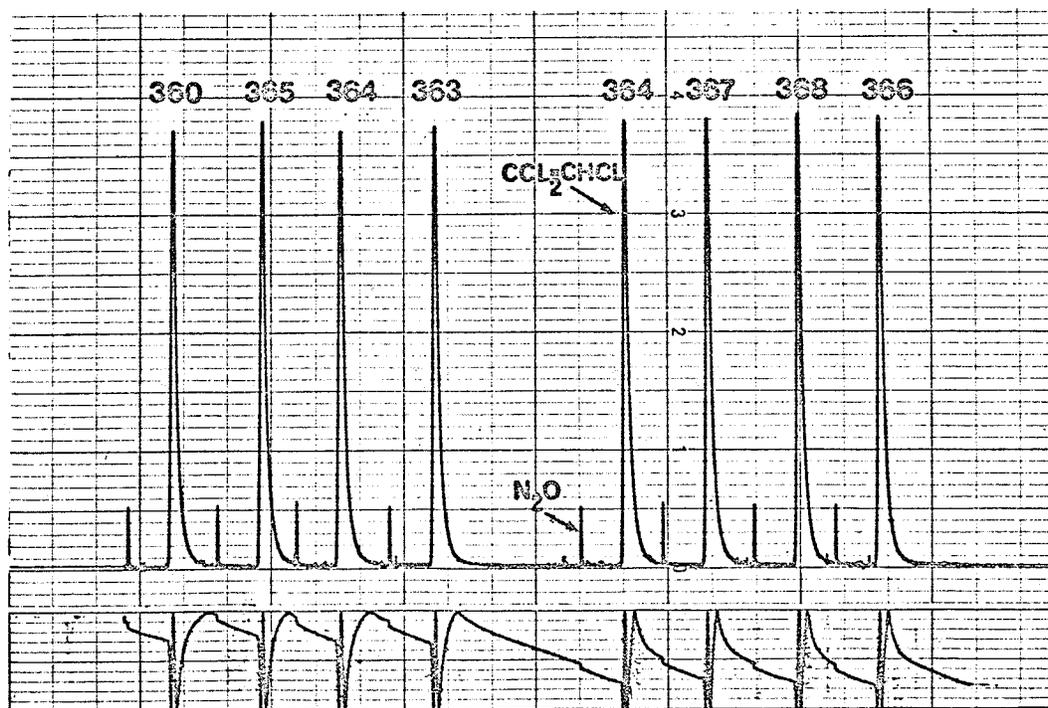
Successive peaks obtained from injections of
samples from the same vapour mixture containing
trichloroethylene. Numbers above peaks are peak
areas; small peaks represent nitrous oxide. See
text.

the gas chromatograph separates other gases and vapours allowing identification of the desired substance by its retention time at a specified temperature. The response, which is directly proportional to concentration is not affected by other gases or water vapour. In addition the separation of components may be achieved using the gas chromatograph by altering the temperature and carrier gas flow rates. Thus the gas chromatograph may be used for qualitative and quantitative measurement while the interferometer may be only used quantitatively for a mixture with two components and is therefore unsuitable for measuring expired gas samples.

Each day during the investigation split samples of a trichloroethylene/air mixture drawn from a specially calibrated EMO inhaler were simultaneously analyzed by interferometry and gas chromatography and thus, from the known calibration of the interferometer, and with the appropriate corrections for temperature and pressure the gas chromatograph was calibrated.

Inspired gases were dry, and were sampled at operating room temperature and ambient barometric pressure. They were analyzed at laboratory temperature. Expired gases were presumably saturated with water vapour and were also collected and measured at operating room temperature and ambient barometric pressure. All samples were injected into the gas sample valve through a calcium chloride drying tube, so that analysis of expired gas samples represented dry gas percentages; this was also at laboratory temperature.

Clinical Apparatus:

A study involving 36 patients carried out during clinical anaesthesia utilized the following apparatus, as illustrated in Fig. 5.2

A Boyle's anaesthetic machine was fitted with an accurately calibrated "tritec" vaporizer. Because of the absorption of trichloroethylene by corrugated rubber tubing, the apparatus was connected to the patient by way of a specially constructed reservoir bag and tube of nylon. A non-rebreathing system was employed utilizing a large diameter low resistance respiratory non-return valve assembly (Warren Collins*).

* Warren E. Collins Inc. Cat. #P306.

CLINICAL EXPERIMENTAL APPARATUS

FIGURE 1
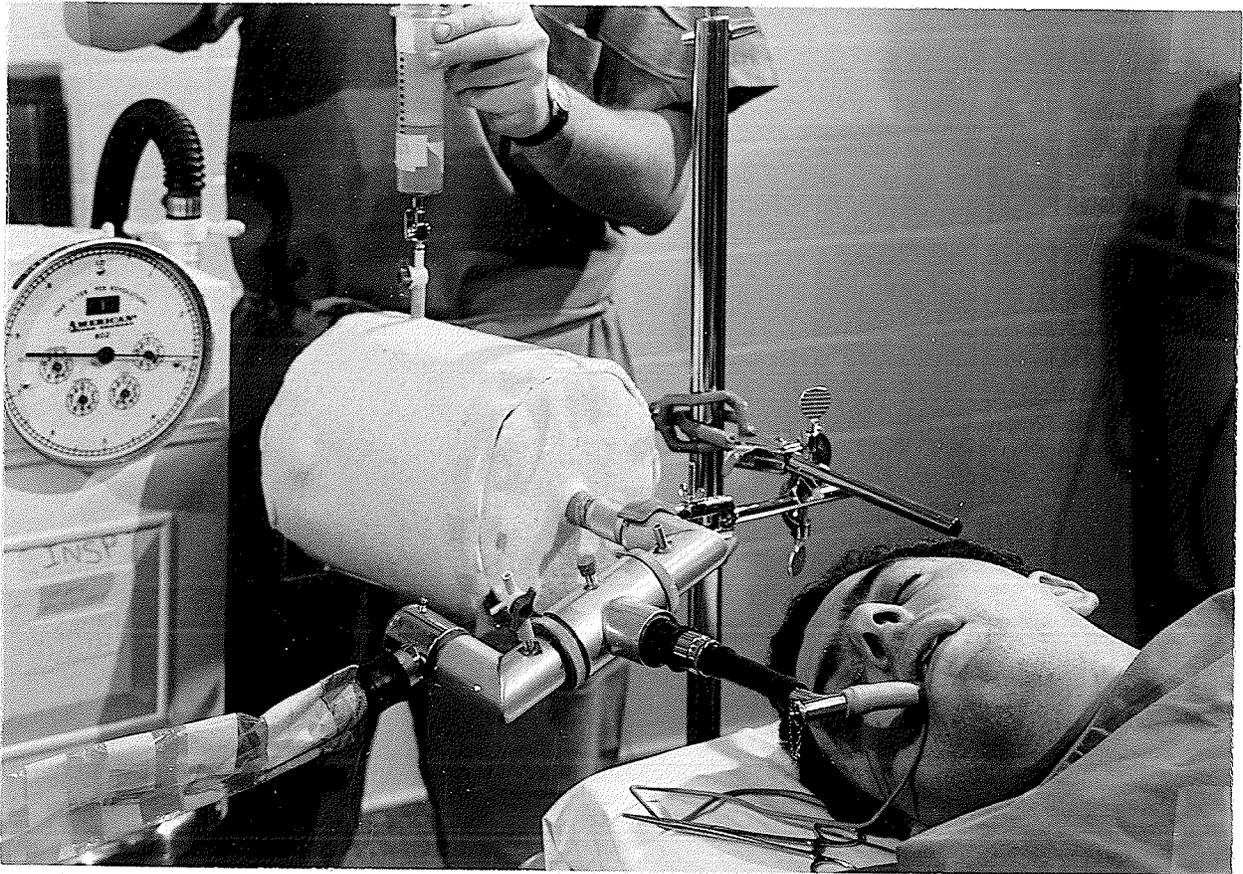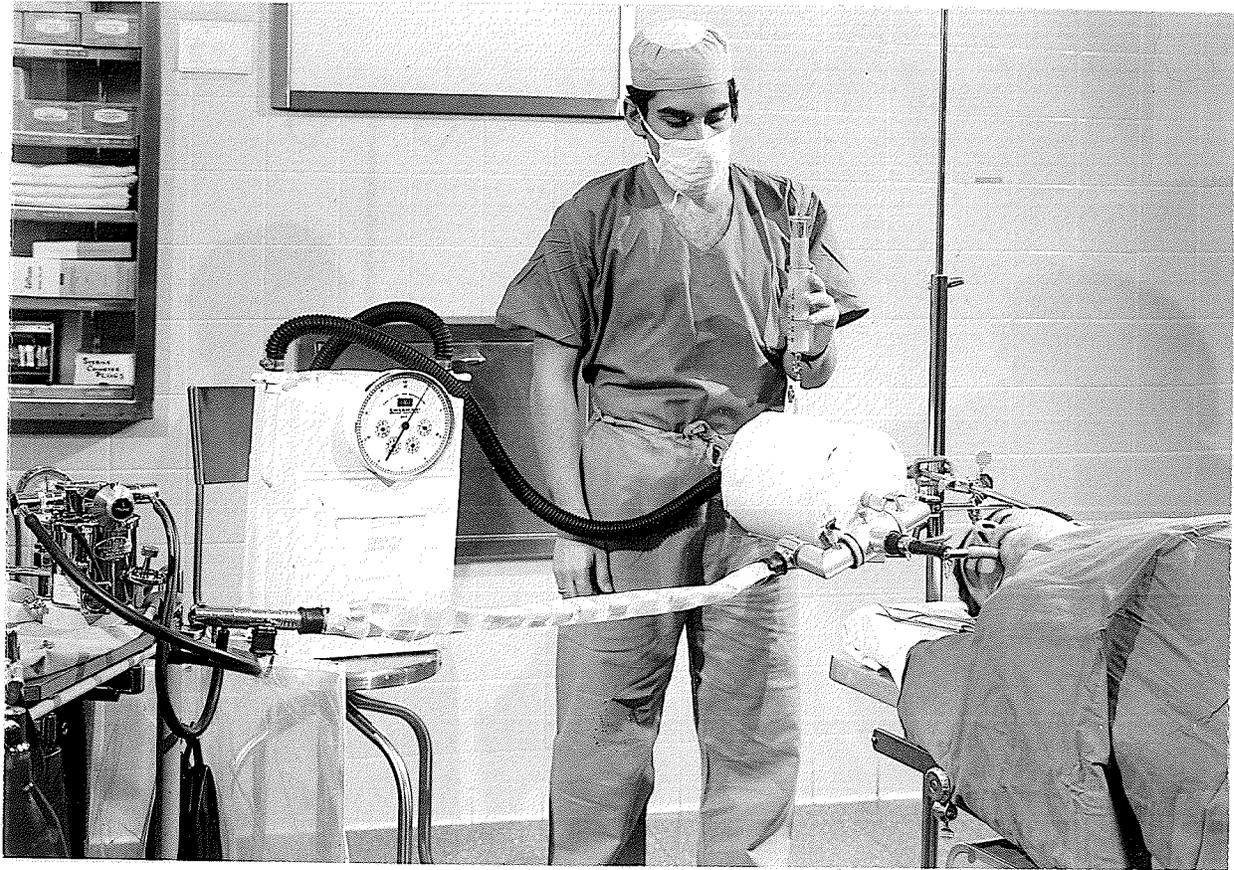
A Wright respirometer was included in the system, immediately on the inspiratory side of the valve assembly in order to measure and monitor inspired volumes. A sample port fitted with a plastic two-way stopcock immediately before the inspiratory valve permitted sampling and analysis of the inspired trichloroethylene. The non-return valve was connected to the patient by means of a hard rubber union, an endotracheal tube and a metal connector.

Expired gas from the valve assembly was passed into an air tight insulated metal container, of approximately four litre capacity, which was connected directly to the valve assembly by means of a short metal union. Mixing of the expired gas occurred in this chamber and a sample port fitted with a plastic two-way stopcock permitted regular sampling and analysis of expired gas.

The distil end of the container was connected by corrugated tubing to a laboratory type dry gas meter (American Meter Co.) so that expired gas volume could be continuously and cumulatively measured. As described above, the inspiratory volume was measured in some cases by a Wright respirometer. However readings did not always correspond with those of the dry gas meter on the expiratory side, and laboratory comparison of the two meters confirmed this discrepancy. It was not possible to use a dry gas meter on the inspiratory side because this would have absorbed a variable amount of trichloroethylene vapour. It was felt, however, that the dry gas meter was considerably more accurate than the Wright respirometer and in most cases measurements were accordingly confined to expired gas volumes.

The non-return valve and mixing chamber were fastened to a movable intravenous stand by means of clamps. The clamps allowed adjustment of the apparatus to the desired height, and the portability of the stand facilitated connection of the non-return valve to the endotracheal tube connector.

The samples were collected in gas tight 50 ml. all glass syringes fitted with metal two-way stopcocks.

Clinical Procedure:

Studies were confined to surgical operations occupying a sufficient length of time to permit the excretion of trichloroethylene after exposure to be followed for a reasonable time. Studies were confined to operations in which spontaneous respiration could be maintained throughout, and to cases not requiring muscular relaxation with the risk of concomitant respiratory depression. All patients had undergone the usual preoperative fast, and an intravenous infusion of 5% dextrose was maintained at a slow rate.

The apparatus was assembled as previously described, without connecting the non-return valve to the patient. The patient was induced and the pharynx was anaesthetized with a xylocaine spray. After intubation the patients lungs were ventilated with a mixture of oxygen and nitrous oxide using the closed circuit of the Boyle's apparatus until spontaneous breathing returned and conditions were stabilized. Spontaneous breathing was required in order to facilitate proper operation of the non-return valve.

Anaesthesia was maintained with the closed system until the experiment was ready to begin. A change was then made to the experimental apparatus connected to the open circuit. This enabled the inspired and expired gases to be separated and analyzed independently, and prevented trichloroethylene from coming into contact with soda lime. Making sure that there were no leaks in the system, the "tritec" inhaler was adjusted to the desired setting to administer a constant inspired concentration for a pre-determined time period.

After the experimental exposure to trichloroethylene, the "tritec" was turned off and removed from the circuit, together with the rubber connecting hose on the outlet side. The nylon reservoir bag and tubing were replaced by a fresh trichloroethylene-free nylon tube and bag. Inspiratory sampling showed with these arrangements the inspired trichloroethylene concentration rapidly became negligible, being of the order of 0.05% during the first ten minutes after discontinuation and falling thereafter to the order of 0.001%.

Sampling of inspired and expired gas continued until the conclusion of surgery. In practice, exhalation of trichloroethylene was followed for between 14 and 62 minutes after the end of exposure; by this time the concentrations were very low.

The sampling technique involved flusing the syringe connected to the sampling ports by means of stopcocks several times in order to promote mixing. Gas meter readings for expiratory volume determinations were taken periodically, and expiratory gas samples were collected midway between these readings. Thus, the total inhalation and exhalation of trichloroethylene was divided for experimental purposes, into a series of time periods, with a concentration reading for the mid-point of each period. During times of rapid change, for example at the start of administration and immediately after exposure, samples and readings were taken every minute: at other stages two and four minute intervals were used.

With the methods described, results were obtained from 36 patients who received .5% and 1% trichloroethylene for durations of time varying from 15-60 minutes. Three experiments were performed on the inhalation of lower concentrations in the range of 0.1 - 0.3%, in two volunteers and one patient. Data was also obtained from three patients who received 0.5% halothane for 16 minutes.

LABORATORY EXPERIMENTS

Apparatus and Procedure:

Laboratory experiments were carried out on volunteers using ether, halothane, and trichloroethylene vapours, previous to the clinical experiments.

Analysis of the inspired and expired vapours and the sampling technique was similar to the clinical experiments. In some cases EMO vaporizers were utilized for the administration of ether and trichloroethylene, and an OMV (Oxford Miniature Vaporizer) was used to administer halothane. In other cases, a known volume and concentration of an anaesthetic agent was administered from a pre-filled nylon bag. The experimental arrangement is illustrated in Fig.6.

FIGURE 6

Laboratory Experimental Apparatus

Nylon material found to be non-permeable to anaesthetic vapours was used in constructing gas tight bags. The openings into the bags were made and necks were constructed from tracheostomy tubes sealed to rubber flanges. The flanges were sealed to the nylon using plastic glue and reinforced with autoclave tape. Sampling was accomplished by threading a plastic catheter through a metal endotracheal tube connector into the centre of the bag and the connector was sealed with a rubber stopper. A #19 needle with a stopcock was attached to the end of the catheter to stop leakage and allow sampling with a 50 ml. glass syringe. The expired samples were collected in total in nylon bags and the volume of gases in the bag were measured with a laboratory type dry gas meter.

A non-rebreathing system utilizing a Ruben non-return valve was used with a rubber mouthpiece and noseclip. Further laboratory experiments were carried out using the apparatus and procedure described for the clinical studies.

Data was obtained from five volunteers who inspired .5% trichloroethylene for time periods of 1.5 minutes to 10 minute durations. Two experiments were performed on the inhalation of .5% halothane for 10 minutes, and once for 20 minutes. Two experiments, administering .5% ether for periods of 16 and 30 minutes and two experiments administering 1% ether for 30 minute time periods were also carried out.

RESULTS

Clinical Experiments:

It was recognized that many factors might influence the metabolism of trichloroethylene, and hence the percentage recovery. It was felt that two of the most important factors might be the inspired concentration and the duration of exposure. Cases were therefore studied in groups of varying lengths of exposure from 15 to 60 minutes, and within these groups there were exposures to 0.5% and 1% trichloroethylene. Table 11 presents a summary of the factors studied and how they were calculated. An analysis of the details of these 36 cases is given in Table 111. This table also shows for each inspired concentration and each duration of exposure the means and their standard erros for total percentage recovery of inhaled trichloro-ethylene, percentage of inhaled trichloroethylene absorbed and percentage recovery of trichloroethylene absorbed. A typical case is illustrated in Fig. 7.

The behavior of the "tritec" vaporizer was fairly consistent, although sampling of the inspired mixture at 10 minute intervals disclosed some fluctuations. In occasional cases there was a fairly marked transient fall in inspired concentration which seemed to be related to change in ventilation; it is unlikely that this was an experimental error in analysis since it was mirrored by a fall in expired concentration. When marked fluctuations in inspired concentration were observed the data were rejected.

A comparison was made of 0.5% administration and 1% administration on the basis of the total amount of trichloroethylene exhaled as a percentage of the total amount inhaled (total percentage recovery). Approximately half the patients showed a recovery of over 75% and this was accordingly taken as a dividing line; a χ^2 analysis shows a statistically significant difference, the percentage recovery of unchanged trichloroethylen being lower after 1% administration than after 0.5% ($P < 0.025$). But in view of the fact that the administration of 1% trichloroethylene was limited to 30 minutes, while some of the 0.5% administrations extended over 60 minutes (see below) a further comparison was made in which the only cases included were those receiving trichloro-ethylene for 30 minutes or less. Here there was no significant difference, although

TABLE 11

	Percentage Recovery	Percentage absorbed of the mass of the anaesthetic inspired in the body	Percentage Recovery of the mass of the anaesthetic absorbed in the body
$\dot{V}$ = minute volume $\bar{F}_I$ = average insp. concentration $\bar{F}_E$ = average exp. concentration MI- is the sum of the masses of insp. anaesthetic up to the time considered where $M_I = \dot{V}\,F_I$ ME- is the sum of the masses of exp. anaesthetic taken up to the time considered where ME $= \dot{V}\bar{F}_E$ t_1 = duration of exposure t_2 = duration of experiment	$\sum_{0}^{t_1} MI$ = Mass Inspired during administration $\sum_{0}^{t_1} ME$ = Mass expired during and following administration. % Recovery= $\dfrac{\sum_{0}^{t_2} ME}{\sum_{0}^{t_1} MI} \times 100$	% Absorbed= $\dfrac{\sum_{0}^{t_1} MI - \sum_{0}^{t_1} ME}{\sum_{0}^{t_1} MI} \times 100$	Total Uptake = $\sum_{0}^{t_1} MI - \sum_{0}^{t_1} ME$ Recovery of the amount of trichloroethylene absorbed after administration equals $\sum_{0}^{t_2} ME - \sum_{0}^{t_1} ME = \sum_{t_1}^{t_2} ME$ % Recovery of the amount of trichloroethylene absorbed in the body equals $\dfrac{\sum_{t_1}^{t_2} ME}{\sum_{0}^{t_1} MI - \sum_{0}^{t_1} ME} \times 100$

TABLE 111

Inspired Conc. %	Minute of Admin.	Minute Vent.	Wt.	Age	Sex	% Recovery	% Absorbed	% Recovered From Absorb.	$\bar{X} \pm SE$ % Recovery	$\bar{X} \pm SE$ % Absorbed	$\bar{X} \pm SE$ % Recovered From Absorbed
0.5	15	7.2	127	52	F	83.8	22.9	29.2	77.7 ± 5.6	29.5 ± 6.1	26.2 ± 3.2
0.5	16	4.9	142	49	F	66.5	41.7	19.8			
0.5	16	6.2	109	49	F	82.8	24.0	29.5			
0.5	20	10.4	156	49	F	77.2	29.3	22.1	76.5 ± 1.4	30.0 ± 5.1	21.6 ± 2.8
0.5	20	10.6	132	52	F	78.2	26.3	16.9			
0.5	20	10.6	100	88	M	78.4	26.1	17.2			
0.5	20	6.4	99	26	F	77.5	30.3	32.3			
0.5	20	7.0	160	31	M	71.0	35.8	19.5			
0.5	30	5.6	150	49	F	71.0	34.4	15.2	67.6 ± 3.2	37.2 ± 2.8	14.7 ± 3.3
0.5	30	4.6	97	16	F	69.6	37.6	22.2			
0.5	30	7.1	130	48	F	71.6	32.1	15.1			
0.5	30	11.7	121	50	F	58.0	44.8	6.2			
0.5	40	11.5	290	22	F	61.5	41.6	7.4	76.2 ± 5.3	27.2 ± 5.1	16 ± 3.3
0.5	40	8.1	216	63	M	85.0	18.0	17.0			
0.5	40	4.8	145	83	M	82.7	22.5	23.5			
0.5	40	8.0	130	62	F	77.4	26.8	16.1			
0.5	45	7.2	165	38	F	84	21.8	26.6	83.3 ± 0.73	22.5 ± 1.0	25.7 ± 1.7
0.5	46	6.5	121	22	F	84.2	20.2	21.8			
0.5	46	11.5	160	28	M	81.1	25.0	24.4			
0.5	46	5.0	126	21	F	83.8	23.0	29.9			
0.5	60	7.0	100	52	F	74.6	30.2	15.8	77.8 ± 4.2	26.3 ± 4.5	17.1 ± 2.6
0.5	60	12.4	200	23	M	90.0	13.1	24.6			
0.5	60	6.8	190	27	M	70.2	33.6	13.2			
0.5	60	9.8	111	25	F	75.8	28.3	14.7			
1.0	16	6.7	112	26	F	74.2	32.5	20.8	72.3 ± 1.1	34.4 ± 1.8	19.1 ± 1.9
1.0	15	6.3	175	15	M	74.1	30.2	14.0			
1.0	16	8.0	132	23	M	70.4	36.3	18.6			
1.0	16	7.2	138	48	M	70.3	38.4	22.9			
1.0	20	6.1	110	74	F	70.3	35.6	16.6	69.4 ± 1.4	36.0 ± 1.7	15 ± 2.0
1.0	20	8.5	174	46	F	67.5	40.0	18.8			
1.0	20	9.5	125	48	F	73.0	31.8	15.3			
1.0	20	6.1	198	57	M	66.9	36.5	9.4			
1.0	30	5.8	114	24	F	67.2	36.4	10.1	72.4 ± 2.9	31.7 ± 2.9	13.2 ± 1.4
1.0	30	6.9	142	65	F	70.0	34.6	13.4			
1.0	30	5.9	119	58	F	71.6	32.5	12.5			
1.0	30	7.6	150	83	F	80.7	23.2	17.0			

FIGURE 7

Percentage Inspired and Expired for 0.5% Administration of 60 Minutes
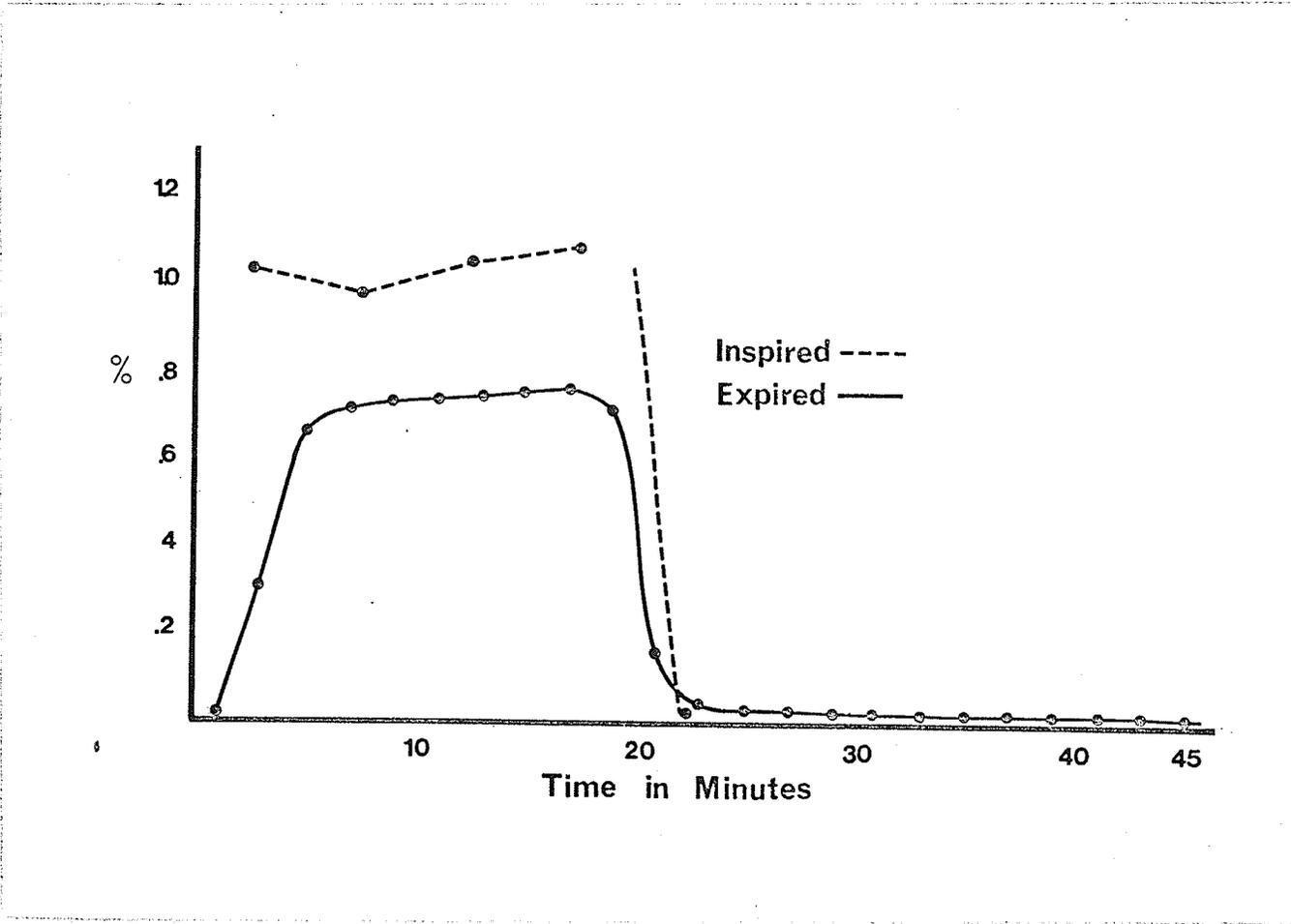
Inspired and expired trichloroethylene concentrations during
and after the clinical administration of a nominal 1% trichloro-
ethylene for 20 minutes.

only 24 cases were available for comparison. Similarly a χ^2 analysis for the percentage of inhaled trichlorethylene absorbed and the percentage recovery of trichloroethylene absorbed was carried out. A summary of these results are illustrated in Table 1V.

Fig. 8 shows the mean percentages of total recovery with standard errors of the means for the groups of patients who received 0.5% trichloroethylene for varying periods of time. Similarly Fig.9 shows the mean percentage of absorption for a 0.5% administration. The lowest percentage recovery was noted after 30 minutes exposure, higher recoveries apparently being obtained with longer exposures. The highest percentage absorption occurred, as might be expected at 30 minutes. Although there was considerable variation between individuals, as evidenced by the standard errors of the means, and although the numbers of cases in each group was small, the differences in recovery rate between 20 minutes and 30 minutes of administration and between 30 minutes and 45 minutes administration were significant, also the difference in the percentage absorption between 30 minutes and 45 minutes administration was significant. The mean values and standard errors for the total percentage recovery and for percentage absorption of trichloroethylene for 0.5% administrations of all durations are summarized in Table V and Table V1 respectively. The significant differences between these mean values are illustrated in Table V11 and Table V111.

The comparable results for a 1% administration showed a fall in percentage recovery from 16 minutes administration (72.3) to 20 minutes administration (69.4%), the mean percentage recovery for 30 minutes being again higher (72.4%). For these 1% data, however, none of the differences was statistically significant.

A χ^2 analysis, illustrated in Table 1X, demonstrates that there was no significant influence of age, body weight and minute volume. A t-test performed on the differences in mean values for males and females was not significant.

TABLE 1V

Chi Square Analysis: Inhaled Concentrations vs. Percentage Recovery, Percentage Absorbed, Percentage Recovered from Absorbed.

|		% Recovery	% Absorbed	% Recovery from amount absorbed
		Below 75 vs. Above 75	Below 30 vs. Above 30	Below 19 vs. Above 19
Up to 60 Min.	1% and .5%	$\chi^2 = 6.3$ * n = 36	$\chi^2 = 6.3$ ** n = 36	$\chi^2 = 6.12$ ** n = 36
Up to 30 Min.	1% and .5%	$\chi^2 = 2.0$ n = 24	$\chi^2 = 2.00$ n = 24	$\chi^2 = 2.844$ n = 24

* =   < 0.05

** =   < 0.025

FIGURE 8

Percentage Recovery for 0.5% Administration: $\bar{X} \pm SE$
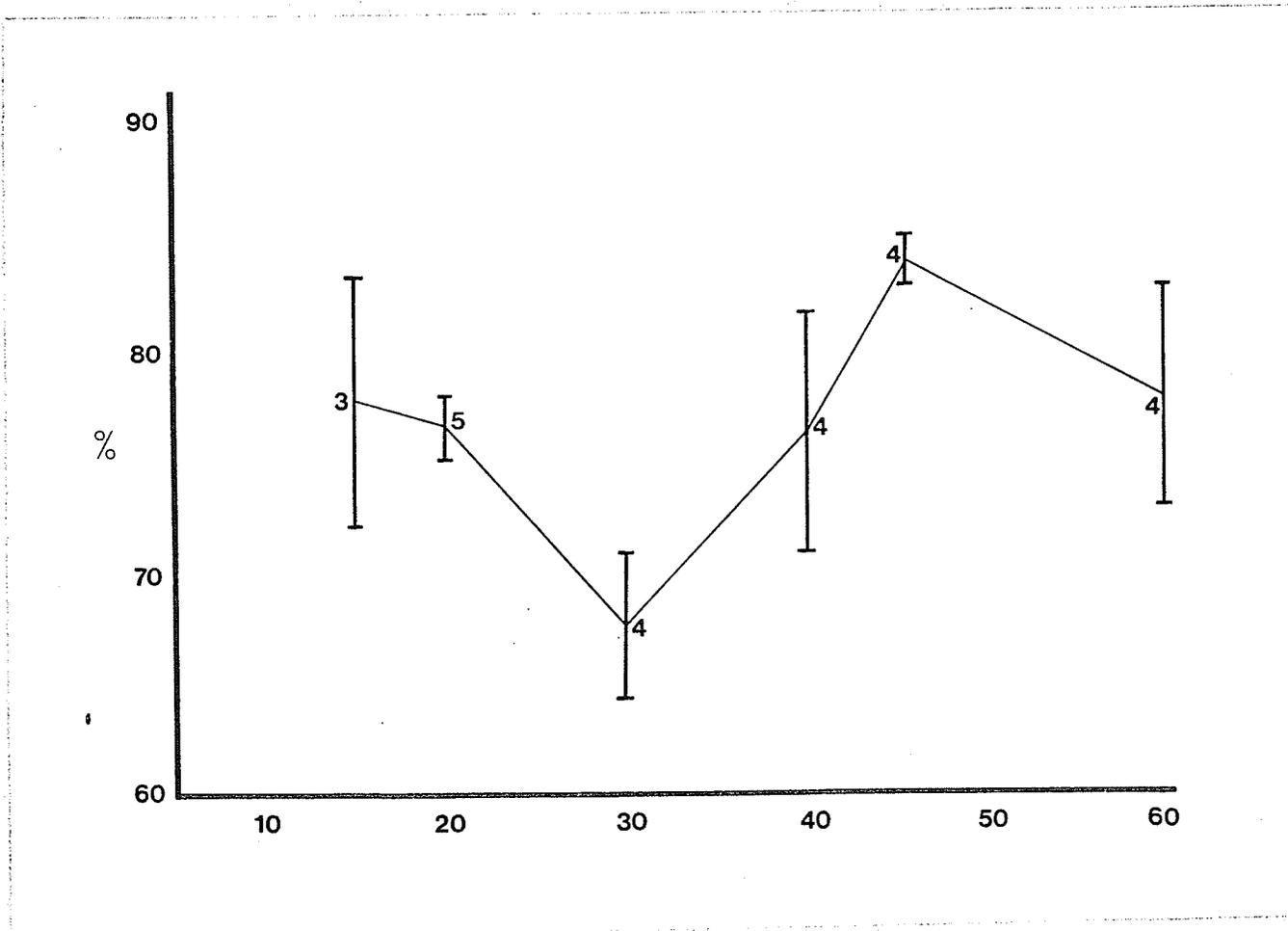
Mean percentages of total recovery of trichloroethylene, with
standard errors of the means, for administration of 0.5%.
Numbers beside points indicate number of patients in each group.
The horizontal axis indicates duration of administration in
minutes. (See text).

FIGURE 9

Percentage Absorbed for 0.5% Administration: $\bar{X} \pm SE$
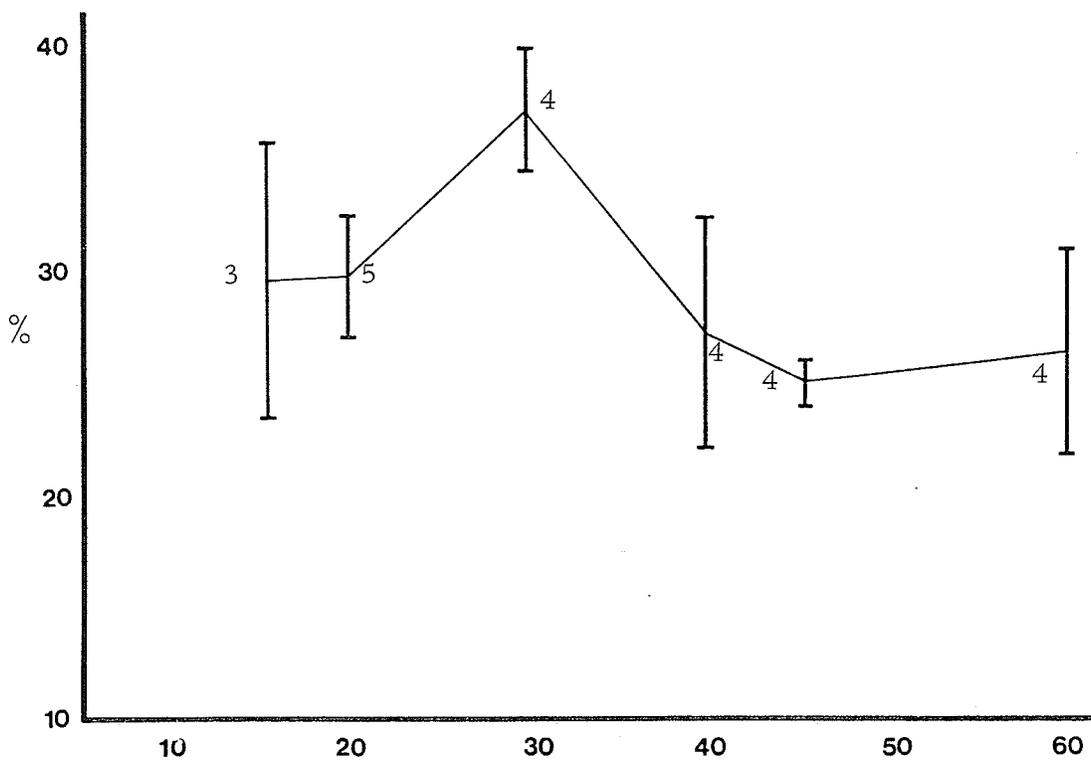
Mean percentages of absorbed trichloroethylene with standard errors of the means, for administration of 0.5%. Numbers beside points indicate number of patients in each group. The horizontal axis indicates duration of administration in minutes. (See text).

TABLE V

Mean Values and Standard Errors of the Means for % Recovery

Inspired Conc.	Minutes Duration	n	$\bar{X}$	SE
.5%	16	3	$\bar{X}_1 = 77.7$	5.61
.5%	20	5	$\bar{X}_2 = 76.5$	1.38
.5%	30	4	$\bar{X}_3 = 67.6$	3.20
.5%	40	4	$\bar{X}_4 = 76.2$	5.29
.5%	45	4	$\bar{X}_5 = 83.3$	.73
.5%	60	4	$\bar{X}_6 = 77.8$	4.20

TABLE VI

Mean Values and SE of the Means for % Absorbed

Inspired Conc.	Duration	n	$\bar{X}$	SE
.5 %	16	3	$\bar{X}_1 = 29.5$	6.09
.5%	20	5	$\bar{X}_2 = 29.97$	5.12
.5%	30	4	$\bar{X}_3 = 37.2$	2.77
.5%	40	4	$\bar{X}_4 = 27.2$	5.12
.5%	45	4	$\bar{X}_5 = 22.5$	1.01
.5%	60	4	$\bar{X}_6 = 26.3$	4.53

TABLE V11

Significant Differences of Mean Values for % Recovery

Diff. between $\bar{X}_i - \bar{X}_j$	df	$df^t 0.05$	Student t Value
$\bar{X}_1 - \bar{X}_3$	5	2.57	1.67
$\bar{X}_2 - \bar{X}_3$	7	2.37	2.76 *
$\bar{X}_4 - \bar{X}_3$	6	2.45	1.38
$\bar{X}_5 - \bar{X}_3$	6	2.45	4.83 **
$\bar{X}_6 - \bar{X}_3$	6	2.45	1.93

TABLE V111

Significant Differences of Mean Values for % Absorbed

Diff. between $\bar{X}_i - \bar{X}_j$	df	$df^t 0.05$	Student t Value
$\bar{X}_1 - \bar{X}_3$	5	2.57	- 1.27
$\bar{X}_2 - \bar{X}_3$	7	2.37	- 2.04
$\bar{X}_4 - \bar{X}_3$	6	2.45	- 1.72
$\bar{X}_5 - \bar{X}_3$	6	2.45	- 4.99 **
$\bar{X}_6 - \bar{X}_3$	6	2.45	- 2.05

$* \ = \ < 0.05$

$** = \ < 0.025$

TABLE IX

Chi Square Analysis: Effect of Body, Weight, Age, Minute Volume

		% Recovery		% Absorbed		% Recovered from Absorbed	
		Below 75% vs. Above 75%		Below 30% vs. Above 30%		Below 19 vs. Above 19%	
W E I G H T	Up to 140 lb. vs. Over 140 lb.	$\chi^2 = 0.013$ n = 36	$\chi^2 = .388$ n = 24	$\chi^2 = 0.013$ n = 36	$\chi^2 = 0.0047$ n = 24	$\chi^2 = 0.013$ n = 36	$\chi^2 = 0.198$ n = 24
	Minute Ventilation up to 6.9 L. vs. over 6.9	$\chi^2 = .63$ n = 36	$\chi^2 = 0.069$ n = 24	$\chi^2 = 2.173$ n = 36	$\chi^2 = 0.41$ n = 24	$\chi^2 = 0.32$ n = 36	$\chi^2 = 1.89$ n = 24
A G E	Below 40 vs. Above 40	$\chi^2 = 0.013$ n = 36	$\chi^2 = .171$ n = 24	$\chi^2 = 0.013$ n = 36	$\chi^2 = 0.171$ n = 24	$\chi^2 = 3.72$ n = 36	$\chi^2 = 2.69$ n = 24

$$* \ = < \ 0.05$$

$$** \ = < \ 0.01$$

Low concentrations of trichloroethylene in the range of 0.1 to 0.3% were administered to a healthy volunteer on two occasions in the laboratory, and to one patient during anaesthesia. Each of these exposures was for 16 minutes and the procedure was the same as for the experiments reported above.

In the experimental subject the percentage recovery of inhaled trichloroethylene was 82.3% on one occasion and 76.5% on the other. In this latter case, however, there was a small loss of expired trichloroethylene due to slipping of the mouthpiece at the end of exposure. In the anaesthetized patient recovery was 80.7%. The mean recovery for these three exposures was 79.8%, which compares to a mean of 77.7% for the same length of exposure to 0.5% trichloroethylene and 72.3% for the same length of exposure to 1%.

In three anaesthetized patients the experiment was reproduced with the introduction of 0.5% halothane instead of trichloroethylene for 16 minutes. The percentage recovery of inhaled halothane ranged from 73.9% to 86.5% with a mean of 80.5% for the three cases.

Pre-Clinical Laboratory Experiments:

In the laboratory .5% trichloroethylene was administered to four healthy subjects for three minutes and to one for 1.5 minutes and the expired air was analyzed for 30 minutes. The recovery of inhaled trichloroethylene ranged from 50.6 to 59.6% with a mean recovery of 53.4% for the three minute administration and 59% for the 1.5 minute administration.

Two subjects who had inhaled .5% halothane for 10 minutes had percentage recoveries of 67% and 71% while a 20 minute administration showed a 70% recovery.

Two subjects were given 0.5% ether for 16 and 30 minute periods with expired air being analyzed for 30 minutes after administration. The percentage recovery was 56.4% and 71.8% respectively.

Two other subjects inhaled 1% ether for 30 minutes and had percentage recoveries of 45.4% and 73.1%.

The mean recovery of 53.4% in the laboratory experiments for trichloro-ethylene as compared to much higher percentage recoveries in the clinical experiments and the wide variations observed in similar experiments for ether and halothane indicates a larger experimental error in the laboratory apparatus.

DISCUSSION

Various methods are available for the comparison of inspired and expired quantities of an inhalation anaesthetic agent. A known concentration of the agent can be prepared in a large bag, and the inhaled volume of this can be measured. Likewise, the expired air can be collected in suitable bags, over appropriate periods of time, and these can then be analyzed and measured for volume. We made preliminary studies with a number of bags and found most materials to be unsuitable; rubber, latex, neoprene, canvas (the conventional Douglas bag), and PVC were all found to be freely permeable to trichloroethylene. The only suitable material readily available was nylon, and a number of bags were constructed from large rolls of nylon film. It proved possible to make gas-tight bags of this nature which would retain trichloroethylene, and some studies were performed in the laboratory in this manner on volunteers. For clinical investigation, however, it is difficult to prepare a sufficiently large volume of gas to permit the careful study of a reasonably prolonged exposure. Furthermore, although concentrations can be measured accurately in this way there is likely to be an error in the measurement of volume; inspired volumes cannot easily be measured without the risk of trichloroethylene uptake, and if expired gas is collected in bags there is a risk of volume loss during the changeover from one bag to another and the precise measurement of the gas content of the bags is more difficult than the continuous use of a dry gas meter on the expired side.

Continuous sampling of inspired and end-tidal trichloroethylene concentrations is possible with an infrared analyzer. Suitable apparatus was not available when these studies began, and although a cell and detector for trichloroethylene were later obtained we have some reservations about the speed of response of this instrument with trichloroethylene vapour. Continuous sampling of mixed expired trichloroethylene concentration is possible in this way, but this has no advantage over gas chromatography. The freezing out of volatile agents from expired gas has also been used in studies of other agents.

Studies were restricted to spontaneous respiration, in order to avoid the complications of gas leakage from the system during raised inspiratory pressure, pressure effects on the output of the "tritec" vaporizer, and errors due to blow-through or rebreathing with the available non-return valves for controlled ventilation. Furthermore, the fact that no ventilator was available which would not absorb trichloroethylene vapour would have meant that controlled ventilation must be carried out by hand, using a nylon reservoir bag, in which case it would have been difficult to maintain a constant rate and tidal volume.

Sources of Error:

An investigation of this kind is subject to experimental error for a variety of reasons.

Inspired concentration is liable to vary, even with a calibrated quantitative vaporizer. Various devices were tested for administering trichloroethylene and the "tritec" proved the most suitable and reliable. Inspired samples were taken at no longer than 10 minute intervals in each experiment, and the inspired amount of trichloroethylene was calculated as the respiratory volume, over the time period concerned, multiplied by the inspired concentration at the mid-point of the time period. This does not rule out the possibility of transient fluctuations; the effect of these on the total calculated input would probably be small unless a transient fall coincided with a concentration reading, in which case substantial underestimation would result.

Inspired gas volume was deduced in these experiments from expired gas measurements. Expired gas was saturated with water vapour at room temperature, and its volume would have to be converted to a dry gas volume in order to obtain a "true" inspired volume. A small correction for the respiratory exchange ratio would then have to be made, for strict accuracy. In these studies it was felt that the error introduced by neglecting R would be negligible in proportion to other errors and this was done. Wet and dry gas volumes are further discussed below. As much care was taken as possible, in each experiment, to ensure that there was no leakage in the system at any point.

The inspired amount of trichloroethylene was derived from the inspired concentration and the inspired volume. There remains the problem of residual trichloroethylene, from the apparatus, which is inhaled after the supposed termination of exposure. The experiments were arranged in such a way as to reduce this factor to a minimum, and for the purpose of calculation it has been assumed that no trichloroethylene was inhaled after the "tritec" was removed from the system. The small amounts which were afterwards detected in inspiratory samples almost certainly originated from the rubber disc valves at the mouth. In calculating the percentage of trichloroethylene absorbed, during the course of administration, and in calculating the percentage of this absorbed amount which is recovered in the expired air after exposure, it is clearly reasonable to regard the administration as having finished completely when the vaporizer is removed. In calculating total percentage recovery, however, by subtracting all the trichloroethylene exhaled from all the trichloroethylene inhaled, it would be slightly more accurate, from the inspired point of view, to include the traces inhaled after exposure had "ended". In the results presented, as stated above, inspired amounts were calculated up to the moment when the "tritec"was removed from the system.

Leakage in the non-return valve system, and blowing back of expired gas into the inspiratory limb are possible sources of error. With controlled ventilation, the blow-across of inspired gases may vitiate the expired sample. Only spontaneous breathing was used for these studies and the valve assembly was carefully tested in the laboratory before experiments began and proved to be virtually free from leak and blow-back.

The small uptake of trichloroethylene by the rubber components of the valve assembly, and its subsequent release into the passing gas stream has been mentioned above. These were the only rubber parts that it was impossible to eliminate from the system. This source of error was ignored.

Expired gas volume was measured at room temperature, saturated with water vapour. Expired concentration was measured dry, having passed through

calcium chloride before entering the gas chromatograph. To obtain a true expired amount of trichloroethylene, therefore, the measured expired dry gas concentration for each time period should be multiplied by the dry gas volume for the corresponding period. However, the calculations in this experiment involved the subtraction of an inspired amount of trichloroethylene from an expired amount. Since both inspired volume (see above) and expired volume were taken from the same gas meter reading, and since both inspired and expired trichloroethylene concentrations were measured dry, no corrections were in fact applied to either inspired or expired gas volumes. This should be borne in mind in interpreting the results presented. If true inspired or expired amounts were required, rather than merely the difference between them, they would have to be derived from corrected gas volumes.

Estimation of the expired amount of trichloroethylene depends on a number of factors in addition to those mentioned above. With regard to sampling technique, the gas loss and overall error from syringes was regarded as negligible, since it was shown that when a 50 ml. syringe was charged with a sample delay in analysis did not materially affect the repeatability of results.

The use of a mixing chamber on the expiratory side, while facilitating mixing of expired gases, raises the obvious possibility of a time lag. Preliminary studies on mixing were carried out by using an infrared CO_2 analyzer. Fig. 10 shows that when a normal subject expired through the mixing chamber there was a negligible respiratory swing in CO_2 concentration within the box; hyperventilation was followed by a fairly prompt change in CO_2 concentration within the chamber. Further laboratory experiments were carried out by passing a flow of 10 litres/min. nitrous oxide and oxygen from a standard anaesthetic machine through a nylon tube to the expiratory chamber. Trichloroethylene was then introduced from the "tritec" vaporizer and samples were taken simultaneously from the inlet end of the nylon tube and the chamber. Fig. 11 shows the chromatographic peaks thus obtained: the "inspired" concentration fell slightly with time and was closely followed by the chamber concentration; reduction of the "inspired" concentration

from 1% and 0.5% resulted in a corresponding fall in chamber concentration within 2 to 3 minutes. Raising the "inspired" concentration again to 1% resulted in a comparable rise in chamber concentration with about the same time lag. For this test samples were drawn from the chamber in the same manner as for the experiments described, and the representativeness of a sample taken in this way can be deduced from the evidence presented.

The expired amount of trichloroethylene must in some way be calculated from the data obtained. The two ways of doing this are to plot the expired concentrations as measured at various times, join the points with the curve of best fit, and calculate the area under the curve; or to take each expired concentration as the average for the gas volume in the time-period concerned and calculate the total expired amount as the sum of a number of rectangular areas obtained in this way. The latter method was used in calculating the results presented in this paper. Fig. 12 and Fig. 13 show two representative washout curves, from a 20 minute administration and a 60 minute administration, from which it can be seen that there is a rapid initial phase followed by a long, exponential washout. The deviation of the points from a straight line is not to be attributed entirely to experimental error, but rather to random variations in minute volume. Fig. 14 and Fig. 15 show the washout curves for the same two cases plotted on a linear scale and compared with the rectangular areas obtained by calculating output from mean concentration multiplied by time. It is apparent that with the exception of the first two minutes of washout the difference between the two methods of calculation is small.

During the first two minutes of washout the expired trichloroethylene concentration changes very rapidly, and an average mixed expired concentration can hardly be said to exist during this period. The "buffering" effect of the expiratory mixing chamber would tend to minimize error during this phase particularly if output is estimated by the "rectangular" method of calculation. We found this error hard to estimate from first principles and therefore a further laboratory experiment

was performed: a gas flow of 6L/min. was passed through a nylon tube into the chamber, then through a Wright respirometer into a nylon bag of 70 litres capacity (Fig. 16). Volume readings were taken at 1 minute intervals and samples were drawn from the chamber midway between consecutive readings. At 1 minute from the start, 1% trichloroethylene was introduced; at 5 minutes this was reduced to 0.5% and at 9 minutes it was turned off. Gas flow was stopped at 12 minutes and a final volume meter reading was taken. It was now assumed that all the gas and all the trichloroethylene which had entered the nylon bag had passed through the chamber. Two calculations of the amount of trichloroethylene were made. Firstly, the concentration in the nylon bag was determined and multiplied by the gas volume in the bag. Secondly, the concentrations in samples drawn from the chamber were multiplied by the volumes of gas flow for the corresponding time periods. This test represented a rough imitation of the clinical experiments, in that the chamber was exposed firstly to a trichloroethylene-free gas flow, then to differing concentrations of trichloroethylene; finally, washout from the chamber was followed for some time, but not until the last traces of trichloroethylene had gone. Calculations showed that in this test the total amount of trichloroethylene estimated from the chamber was 92.8% of the amount in the nylon bag at the conclusion of the last time period.

Fig. 14 and Fig. 15 also give an indication of the amount of trichloroethylene excretion which was missed in these experiments through failute to follow washout for a prolonged period of time. The decision to do this was deliberate, since at the conclusion of surgery the patients were extubated and ventilation often became irregular and variable in volume. These and other complicating factors would probably have made the pursuit of the final traces of trichloroethylene too inaccurate to be worthwhile. Inspection of Figures 14 and 15 would suggest, from extrapolation, that about an additional 10% of the estimated amount of trichloroethylene washout would have resulted from following excretion to virtual finality. This refers, of course, to an experimental loss of 10% of the trichloroethylene exhaled after exposure was concluded; as a percentage of the total amount of trichloroethylene exhaled <u>during and after</u> the

administration the tail would represent a very much smaller amount, and would thus introduce a relatively small error into the calculation of total percentage recovery. These experimental limitations should be borne in mind in interpreting the results presented.

FIGURE 10

Mixing Chamber: Average Expired CO_2 Concentration
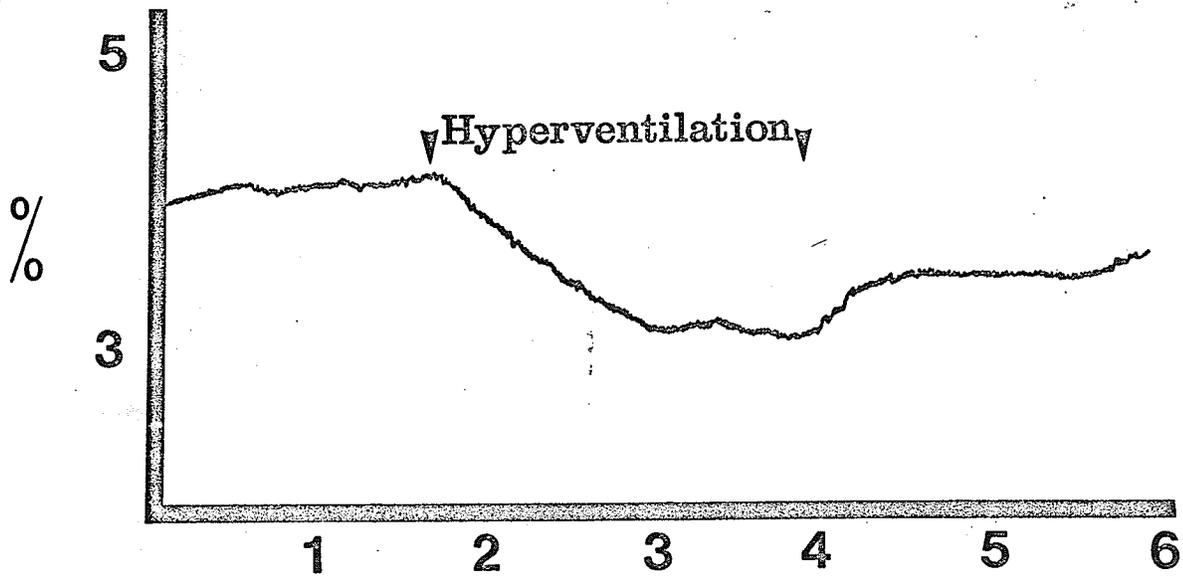
Results of sampling from expiratory mixing chamber with infrared CO_2 analyzer during spontaneous breathing in a normal subject. Carbon dioxide percentage on vertical axis; time in minutes on horizontal axis. The mixing of expired gas during respiratory cycles is evident, ·and the response to hyperventilation is shown. (See text).

FIGURE 11

Mixing Chamber: Time Lag
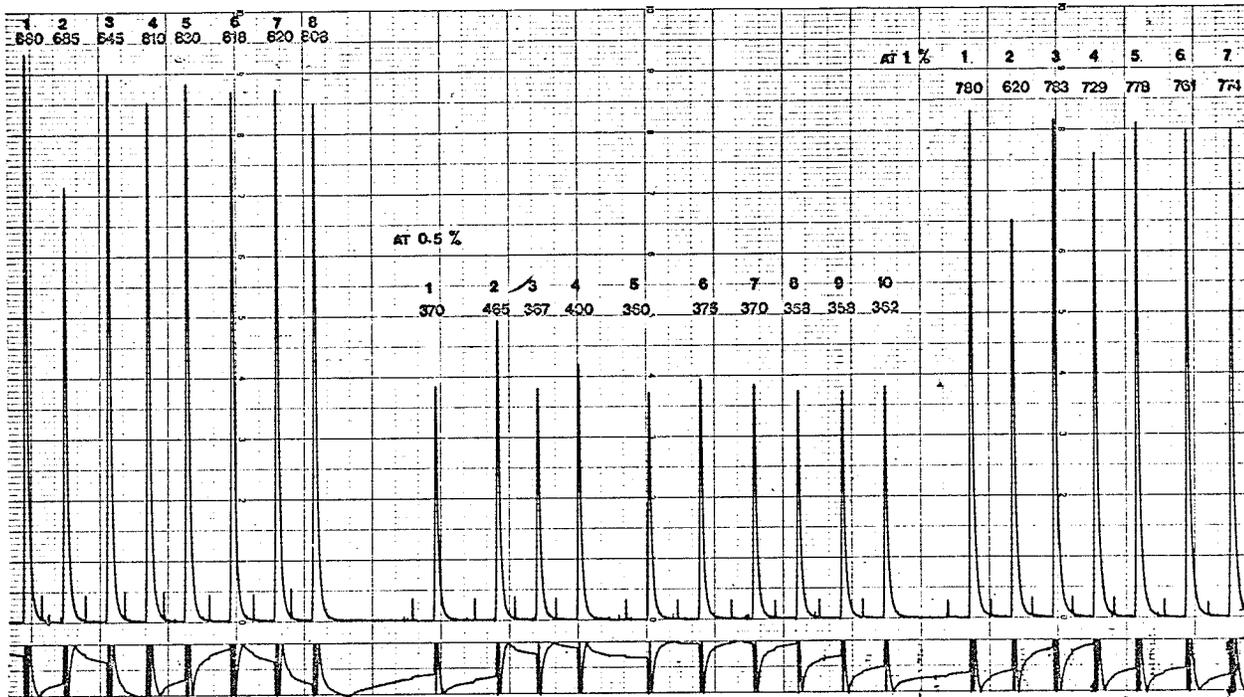
Chromatographic peaks from laboratory test of expiratory chamber time-lag, described in text. Odd numbered peaks represent "inspired" concentrations, showing a slight fall with time, followed by a reduction from 1% to 0.5% followed by a return to 1% and a further slight fall with time. Even numbered peaks show concentrations from the chamber, in samples drawn at the same moment as those analyzed in the immediately preceding "inspiratory" peaks. For example, peaks 5 and 6 represent samples taken at the same moment. Samples were taken at 1 minute intervals; the chamber concentration is seen to approximate to the "inspired" concentration in 2 to 3 minutes. (See text).

FIGURE 12

Percentage Expired for a 20 minute administration of 0.5%
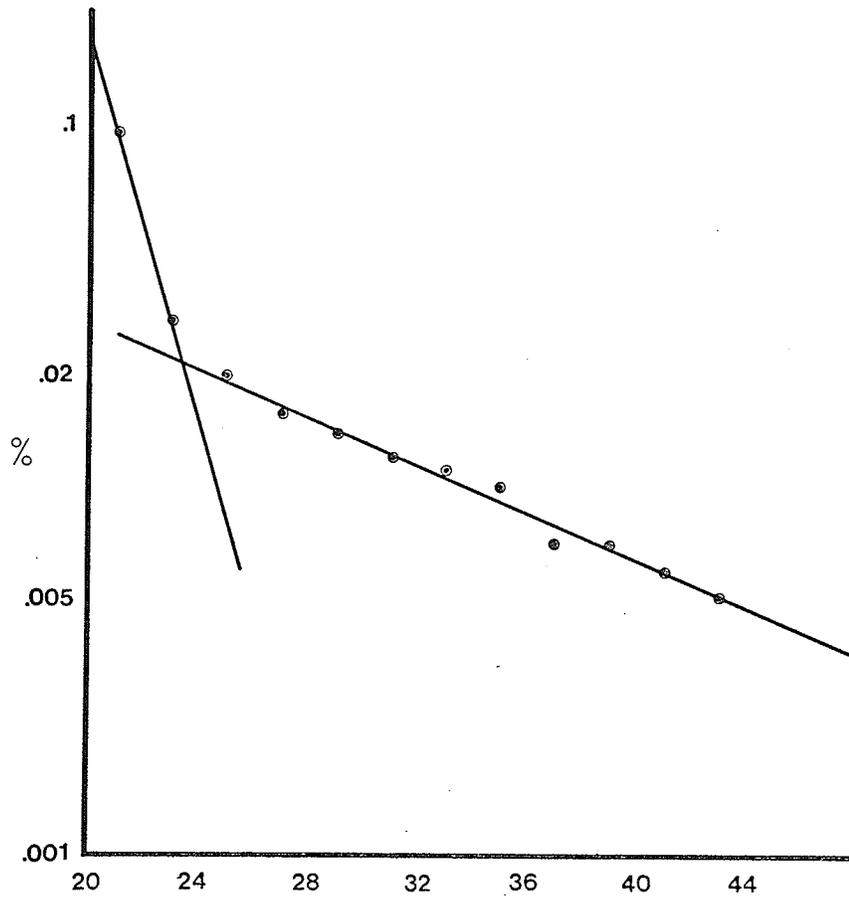
Expired trichloroethylene concentrations plotted
logarithmically against time in minutes
(horizontal axis) to show exponential washout
after a 20 minute administration of 0.5%
trichloroethylene. (See text).

FIGURE 13

Percentage Expired for a 60 minute administration of 0.5%
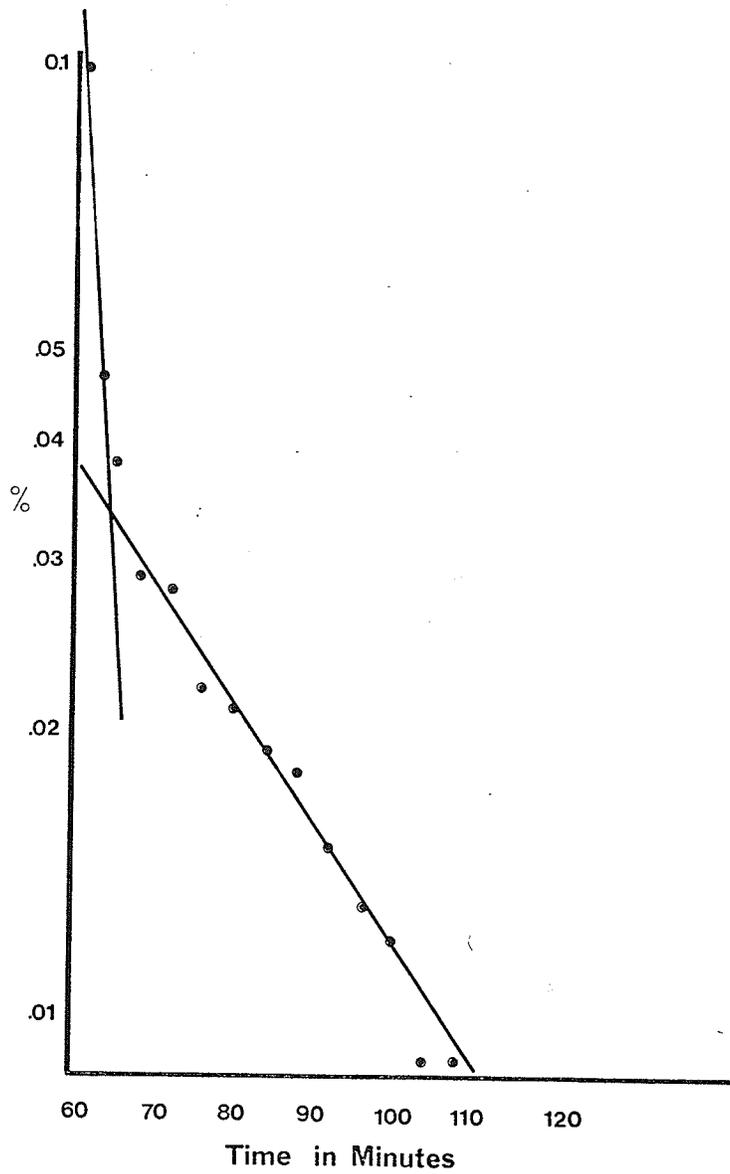
Expired trichloroethylene concentrations
plotted logarithmically against time in
minutes (horizontal axis) to show exponential
washout after a 60 minute administration of
0.5% trichloroethylene. (See text).

FIGURE 14

Percentage Expired for a 20 minute administration of 0.5%
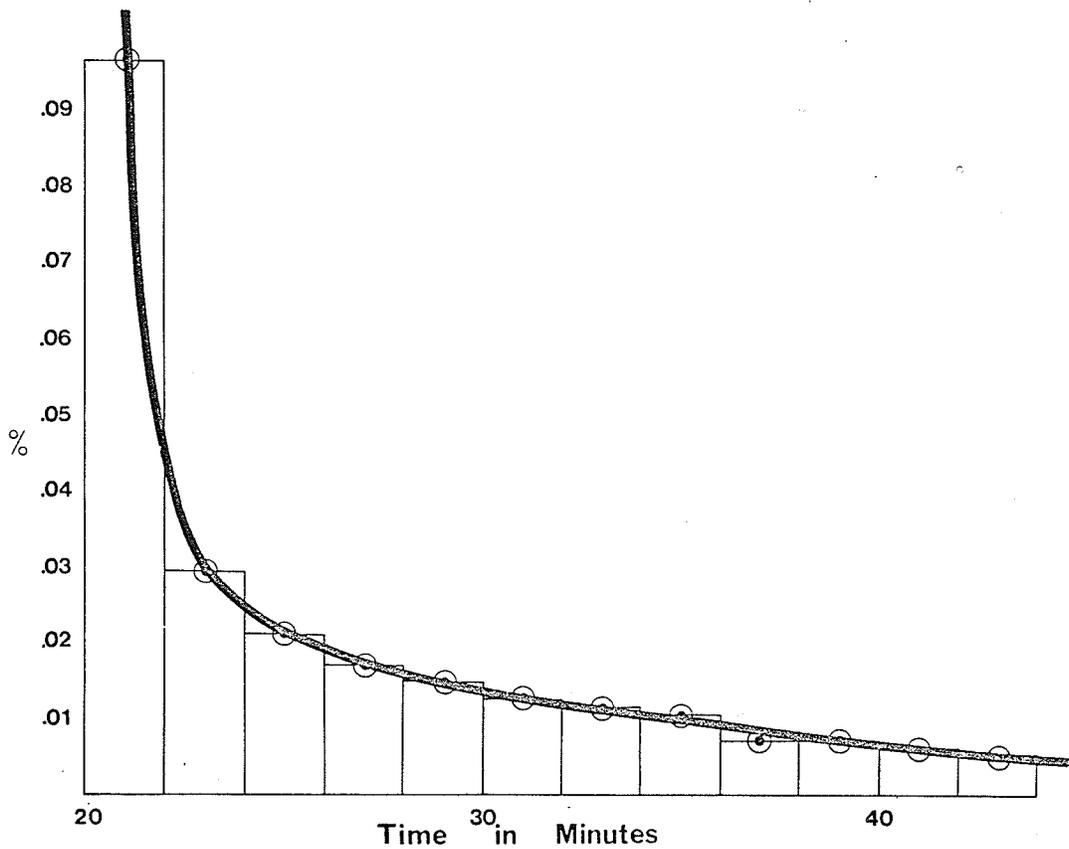
Expired trichloroethylene concentrations plotted linearly against time in minutes to show relationship of washout curve to average concentrations for each time-period (see text). 20 minute administration of 0.5% trichloroethylene.

FIGURE 15

Percentage Expired for a 60 minute administration of 0.5%
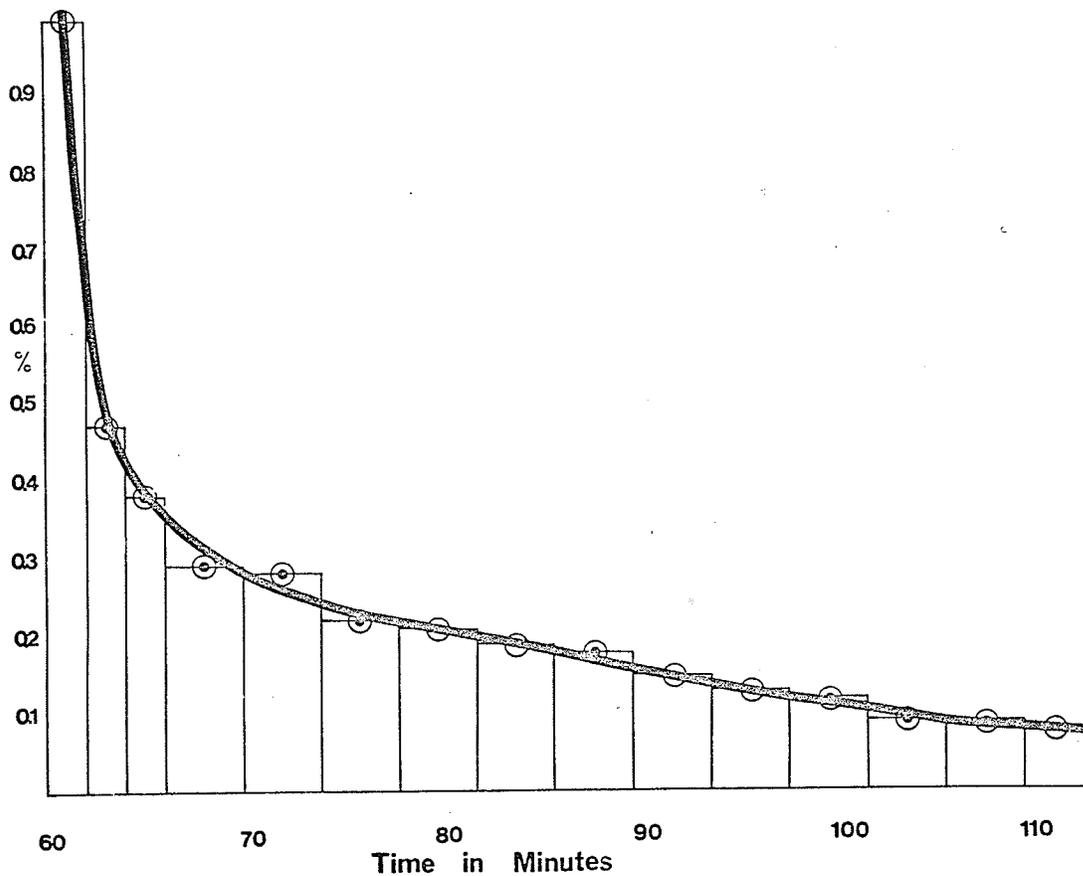
Expired trichloroethylene concentrations plotted linearly against time in minutes to show relationship of washout curve to average concentrations for each time-period (see text). 60 minute administration of 0.5% trichloroethylene.

FIGURE 16

Mixing Chamber: Comparison with Nylon Bag
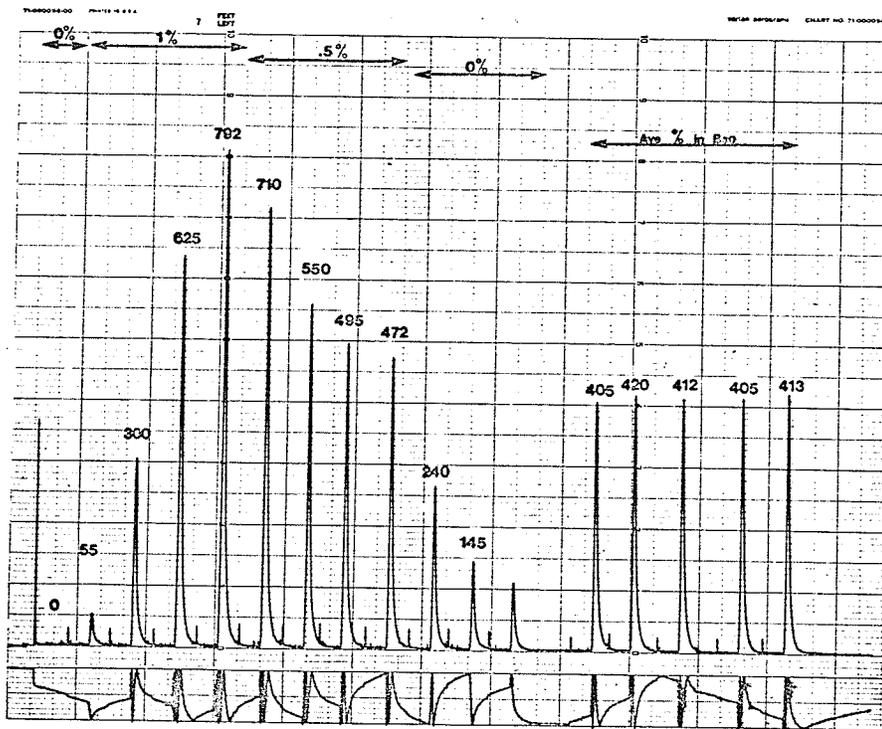
Chromatographic peaks from laboratory test of expiratory chamber and nylon bag. The first set of peaks represent expired concentrations from the expiratory chamber when the inspired concentration changes from 0% to 1% followed by a reduction to .5% and from .5% to 0%. The second set of peaks represent the average expired concentrations in the nylon bag.

Clayton and Parkhouse (1962) found that a continuous administration with low concentrations required at least one hour before the venous blood trichloroethylene was at a steady level. They found no evidence of equilibrium after three hours of anaesthesia, and attributed this failure to reach equilibrium to the metabolism of the drug.

In the present study the administration of trichloroethylene under clinical conditions was too short to even approach equilibrium. This was reflected in the differences between the inspired and expired levels of trichloroethylene during administration as illustrated in Fig. 7. This difference is a measure of the rate of uptake and metabolism, which declines as equilibrium is approached but never reaches zero since there is a constant loss due to metabolism.

Industrial studies carried out by Teisinger (1960), Bartonicek (1962), Soucek and Vlachova (1960) etc. indicate the percentage of trichloroethylene absorbed in the body to be in the range of 50 to 60%. In the present study the percentage of absorbed trichloroethylene was found to be in the range of 13% to 45% with a mean percentage absorbed of 30.5%.

The differences in these findings may be attributed to the fact that the industrial studies relate to the breathing of very low concentrations over long periods of time while under clinical conditions, trichloroethylene was administered for short periods of time ranging from 15 to 60 minutes at considerably higher concentrations than those found under industrial circumstances.

Implications for other Inhalation Anaesthetics:

The metabolism of trichloroethylene has been known since Bruning and Schnetka identified trichloracetic acid as a metabolite in 1933. All other inhalation anaesthetics had been regarded as chemically stable compounds. Recent studies, however, have demonstrated that many inhalation anaesthetics including diethyl ether, chloroform, methoxyflurane and halothane do in fact undergo some degree of biotransformation in the body.

Duncan and Raventos (1959) investigated the breakdown of halothane using various analytical procedures. Their results were negative and this has been attributed to the lack of sensitivity of the analytical methods in use at that time. However, Stier (1964) reported the identification of trifluoracetic acid in the urine of rats and anaesthetized with halothane.

In the present investigation ether uptake was studied in the laboratory and halothane uptake was studied under clinical conditions as well as in the laboratory. The mean percentage recovery for ether was 61.1% compared to 53.4 % for trichloroethylene for different exposures on volunteers in the laboratory. The mean percentage recovery of halothane for three experiments in the laboratory is 69.3%. Under clinical conditions a mean percentage recovery of 80.5% was found for a 0.5% administration of halothane compared to 77.5% for a 0.5% administration of trichloroethylene for the same length of exposure. It is interesting to note that trichloroethylene was found to antagonize the breakdown of halothane and vice versa (Stier and Gerth, 1965) and therefore combination of the two agents during surgery was avoided.

In all cases the mean percentage recovery for halothane and ether were higher than the mean percentage recovery for trichloroethylene. This would suggest that biotransformation has occurred for ether and halothane, but not to the same extent as it did for trichloroethylene. However the significance of these comparisons is limited because of the small sample size for agents other than trichloroethylene.

Indications for Further Studies:

The present study has given some insight into the metabolism of trichloroethylene with respect to the amount absorbed in the body in the circumstances of clinical anaesthesia. A method and procedure for the sampling and measurement of inspired and expired gas samples has been developed. The use of gas chromatograph has proven to be extremely sensitive and its usefulness may now be applied to the identification and quantification of trichloroethylene and its metabolites in the blood, the urine and in the tissues.

Further investigation may throw some light on the pathway of trichloroethylene metabolism.

The use of radioactive tracers employing a scintillation counter in conjunction with gas chromatography, could conceivably aid in the determination of the exact pathway.

The application of an infrared analyzer, with a cell and detector for trichloroethylene offers the advantage of continuous monitor of the inspired and expired concentrations. In addition, this would speed up the analytical procedure and decrease the potential error from the storage and analysis of gas samples in syringes.

SUMMARY

A study involving 36 patients was carried out during clinical anaesthesia, administering known concentrations of trichloroethylene for various durations. The inspired and expired vapours were analyzed by means of gas chromatography. Statistical analysis demonstrated that body weight, minute ventilation, age and sex had no effect on the total percentage recovery of inhaled trichloroethylene, percentage of inhaled trichloroethylene absorbed, and the percentage recovery of trichloroethylene absorbed.

The length of administration of 0.5% trichloroethylene was found to be significant. Comparison of mean values for the various time durations for the percentage recovery showed that differences between 20 minutes and 30 minutes administration and between 30 minutes and 45 minutes administration were significant; the difference in the percentage absorption between 30 minutes and 45 minutes administration was also significant.

Further analysis showed a significant difference for the administration of 0.5% and 1.0% administration of trichloroethylene with regard to the total percentage recovery, which was lower after 1% administration than after 0.5% administration ($P<0.025$). However, when the comparison was confined to administrations of comparable duration for the two concentrations there was no significant difference between the 0.5% and 1% administration. Thus on the evidence obtained from this study it would appear that the length of duration is the most important factor.

The administration of 0.5% halothane resulted in a mean percentage recovery of 80.5% compared to 77.7% for the same length of exposure to 0.5% trichloroethylene.

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