Brit. J. Pharmacol. (1956), **11**, 394.

THE ACTION OF FLUOTHANE*—A NEW VOLATILE ANAESTHETIC

BY

J. RAVENTÓS

From the Research Department, Imperial Chemical (Pharmaceuticals) Ltd., Hexagon House, Blackley, Manchester, 9

WITH AN APPENDIX BY R. R. GOODALL

(RECEIVED MAY 31, 1956)

During the search for non-explosive inhalation anaesthetics some authors have examined large numbers of fluoro derivatives of hydrocarbons and ethers. Robbins (1946) found that some fluorohydrocarbons had anaesthetic properties with therapeutic ratios greater than those of ether and chloroform. These compounds, however, produced intense hypotension, particularly in deep anaesthesia, with abnormal cardiac rhythms and sometimes with ventricular fibrillation. More recently Lu, Ling and Krantz (1953) examined some fluorinated hydrocarbons and ethers. Their most promising compound, trifluoroethylvinyl ether (Fluoromar), had, in monkeys, an activity similar to that of ether, but its therapeutic ratio was slightly larger than that of ether. Although this compound seems to be fairly promising, it is explosive when mixed with oxygen in concentrations above 3.0% (Krantz, Carr, Lu, and Bell, 1953). A short clinical report on Fluoromar was published by Orth and Dornette in 1955.

A series of fluorinated hydrocarbons was synthesized by Dr. C. W. Suckling of the General Chemicals Division of I.C.I. and examined in these laboratories (Raventós and Suckling, unpublished). Among them $CF_3CHClBr$ (Fluothane) was found to be a non-inflammable volatile liquid with outstanding anaesthetic properties. The results of experiments on its anaesthetic action and on some of its pharmacological effects are summarized in this article; for the sake of convenience, it is referred to by its trade name.

Physical Characteristics of Fluothane

The physical data on fluothane have been determined by Dr. Pryce, of these laboratories, and by the Physics Section of the Research Laboratories of the General Chemicals Division of Imperial Chemical Industries Limited, Widnes. These data are compared with those of some other anaesthetics in Table I. Fluothane is a liquid with an S.G. of 1.86 at 20° C. and its boiling point is 50.2° C. at 760 mm. Hg. Its vapour pressure at 20° C. is 243 mm. Hg. It has a characteristic but not unpleasant odour which is difficult to define. Like most heavily fluorinated hydrocarbons, fluothane is not inflammable and its vapours mixed with O_2 in proportions from 0.5% to 50% (v/v) are not explosive. Fluothane decomposes slowly with the formation of volatile acids when exposed to light; it is stable, however, if stored in amber-coloured bottles. Thymol in the proportion of 0.01% w/w added to fluothane stabilizes the anaesthetic to the action of light.

Fluothane is stable when in contact with soda lime. When its vapours, mixed with 5% CO_2 + 95% O_2 saturated with water, were recirculated for 2 hr. in a closed system containing soda lime kept at 50° C., taking care to replace the CO_2 absorbed by the soda lime by further additions of this gas, there was only 0.02% decomposition as indicated by the increase in the halide of the soda lime (Suckling, personal communication). Its decomposition has been also studied in experiments on anaesthetized dogs using a close-circuit method. Samples of the vapour mixture were taken from the rebreathing bag at the beginning and after 1, 2, and 3 hr. of anaesthesia. The mass spectra of these samples were not different from that of pure fluothane and the halide content of a sample of the soda lime of the canister taken at the end of the experiment was the same as that of a control sample. These two sets of experiments show that fluothane is stable in contact with soda lime and that it is possible to use it in close-circuit methods of anaesthesia without the formation of toxic decomposition products.

METHODS

Vapour mixtures of fluothane of known concentrations were prepared using a modified Kochmann (1912) apparatus. It can be used for the study of the actions of any volatile liquid.

For the experiments with mice and rats the apparatus shown in Fig. 1 was devised. It consists

* Patent protection pending; registered trade mark of Imperial Chemical (Pharmaceuticals) Ltd.

TABLE I

PHYSICAL CHARACTERISTICS OF SOME INHALATION ANAESTHETICS

	Mol. Wt.	Boiling Point (°C.)	Specific Gravity		Solubility in 100 Parts Water	Oil/ Water Solubility	Oil/ Blood Solubility	Inflammability Limits (%)		Vapour Tension at 20°C. (mm. Hg)
			Gas or Vapour (Air = 1)	Liquid (Water = 1)				Air	O_2	
Chloroform	119·39	61·26	4·12	1·49	0·822	100		—	—	160·5
Cyclopropane	42·08	−34·4	1·42	—	33 ml.	34·3	15·3	2·4–10·3	2·45	
Ether	74·12	34·6	2·6	0·713	7·5	15·46 3·2	15·08 3·3	1·85–36·5	2·10–82·0	439
Divinyl ether	70·09	28·3	2·2	0·774	4·0	41·3		1·7–27·0	1·85–85·0	
Ethyl chloride	64·52	12·2	2·28	0·921	0·574			4·0–14·8		
Ethylene	28·05	−103	0·978	—	25 ml. at 0°C. 10 ml. at 25°C.	14·4	9·3	3·5–15	1·5–85·0	
Nitrous oxide	44·2	−181·0	1·527	1·226	150 ml.	3·2	3·0	—	—	49·4
Fluoromar.. .. .. .. $CF_3—CH_2—O—CH=CH_2$	126	42·7	—	1·13	0·4	94		3·0		
$CF_3—CHClBr$ .. .. Fluothane	197·39	50·2	—	1·86	0·345	330	—	Not inflammable in O_2 0·5 to 50·0		243

of a vaporization chamber, an exposure chamber and a recovery tower.

The vaporization chamber (1) is a test-tube (1½ by 8 in.) kept in a thermostatically controlled bath at the boiling point of fluothane. The end is closed with a rubber bung through which pass two ⅛ in. glass tubes and one capillary. One of the larger tubes has a glass jacket around it and almost reaches the bottom of the chamber. Air or O_2 is introduced by this tube at a constant rate measured by a rotameter. The volatile liquid is forced into the chamber through the capillary tube and falls in drops on the glass jacket. The liquid spreads over the jacket and volatilizes before it reaches the bottom of the chamber; the vapour produced is mixed with air or O_2 and passes out by the second ⅛ in. tube.

The rate of introduction of fluothane into the chamber is regulated as follows. The volatile liquid is contained in a cylindrical glass vessel (2) connected to two capillary tubes. One protrudes about ¼ in. into the upper quarter of the vessel and is fixed horizontally within it (3); the other capillary is fixed vertically, and at its upper end has a three-way tap. The vertical arm of the tap is fitted with a small funnel, which is used for filling the vessel with the volatile liquid; the horizontal arm (4) is the outlet of the vessel. At the bottom of the vessel there is another tap for emptying it. Mercury is introduced into the vessel through the capillary tube (3) at a constant rate by means of a continuous injection apparatus (5), and displaces the volatile liquid into the vaporization chamber.

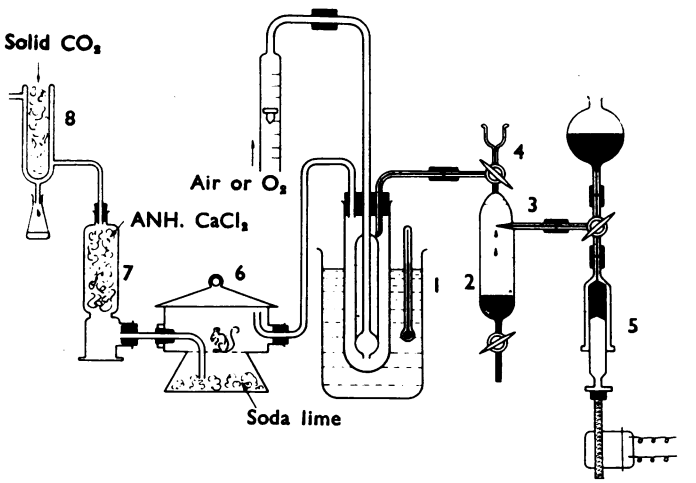

FIG. 1.—Diagram (not to scale) of apparatus for exposing rats and mice to known concentrations of fluothane—see text for description.

Rats and mice are exposed to the vapour on a wire mesh in the upper part of a desiccator of about 10 l. capacity (6). The lower part contains soda lime. The outlet of the exposure chamber is connected to an anhydrous $CaCl_2$ tower (7), where the moisture of the vapour mixture is removed. Finally, the mixture passes into a solid CO_2 condenser (8), where the anaesthetic is condensed. The recovered material can be used again after distillation.

For experiments on rabbits, dogs, cats, and monkeys it was necessary to modify this apparatus (Fig. 2). The outlet of the vaporization chamber is connected to a series of polythene bags

where the vapour mixtures are collected (1). The mouths of these bags are tied around large rubber bungs fitted with inlet and outlet tubes. The outlet tube is connected to a wide-bore T-piece; one of its branches can be closed or left open to the air, and the other branch, which contains a short glass tube acting as a light gas resistance, is connected to the damping chamber of a modified Gaddum's (1941) apparatus for recording respiratory movements (3). Its outlet is connected by a rubber tube to the inspiration side of a set of unidirectional respiratory valves (4). The expiratory valve is connected to the recovery system.

The tubes and connexions in this apparatus must have an internal diameter sufficiently large not to produce excessive resistance to the flow of the gas mixtures. For experiments on dogs it was found that the best size was 1 in., and that polythene bags of approximately 50 l. capacity were sufficiently large for most experiments. The damping chamber was a 3 l. round-bottomed flask fitted with a rubber balloon of 4 in. diameter. For experiments on cats, rabbits, and monkeys the diameters of the tubes and sizes of the bags were reduced to one quarter.

The gas laws were used to calculate the concentrations of the vapour mixtures. The volume of vapour produced is given by the following formula:

$$\frac{V \times S.G.}{M.W.} \times 22.4 = \text{l. vapour/min. at S.T.P.}$$

where M.W. is the molecular weight of the compound, S.G. its specific gravity, and V the volume of liquid vaporized/min. The % concentration of vapour in air or O_2 was calculated in terms of v/v, and is expressed thus unless otherwise stated.

Rats and Mice.—After flushing the exposure chamber with the vapour mixture for at least 5 min., batches of 10 mice or 4 rats were introduced into the chamber as quickly as possible (in not more than 30 sec.). The animals were observed at regular intervals and the incidence of anaesthesia (side position) and death were recorded. The vapour concentrations that anaesthetize (AC50) or kill (LC50) half the animals after 30 min. exposure were calculated. This time of exposure was chosen because it was thought that the concentration of the anaesthetic in the blood would then be virtually in equilibrium with that of the vapour mixture.

Dogs and Cats.—In all, 48 dogs—beagles of 10–14 kg. body weight—were used. As a rule, they were premedicated with 2–3 mg./kg. morphine sulphate and 0.05 mg./kg. atropine sulphate, both given subcutaneously about 1 hr. before the experiments. The inguinal region was anaesthetized with 2% procaine hydrochloride. The femoral artery was connected to a mercury manometer. An airtight mask was fixed on the dog's face and connected to the inhalation apparatus, with the mixture bags by-passed. Control records of the respiration and blood pressure were taken for about 5 min. before starting inhalation of the anaesthetic mixture.

In some dogs, anaesthesia was induced with thialbarbitone, 40–50 mg./kg. i.v. Control tracings of the blood pressure and respiration were taken until the dog showed the first signs of recovery from the effects of thialbarbitone. As soon as these appeared, inhalation of fluothane in concentrations between 2% and 4% was begun until full surgical anaesthesia was obtained, usually in about 3–8 min. The trachea was then intubated, using an endotracheal tube with an inflatable cuff, and the inhalation of the anaesthetic in high concentrations was continued for a few min. more, after which lower concentrations could be used. No dissection was carried out in unpremedicated dogs until they were fully anaesthetized with fluothane.

Other observations were carried out on dogs and cats anaesthetized with chloralose, 60–80 mg./kg. i.v. A total of 19 dogs and 17 cats were used in these experiments.

The cardiac output of two dogs, premedicated with morphine, was measured before and during anaesthesia by the Evans-blue dilution method. The cardiac output and cardiac index were calculated by the method of Lewis (1953).

The concentration of fluothane in the blood of dogs was estimated during anaesthesia by the method of Goodall described in the appendix.

Monkeys.—Eight rhesus monkeys were anaesthetized with thialbarbitone (30–40 mg./kg. i.v., or more if necessary). Sometimes a mask was placed on the face and connected to

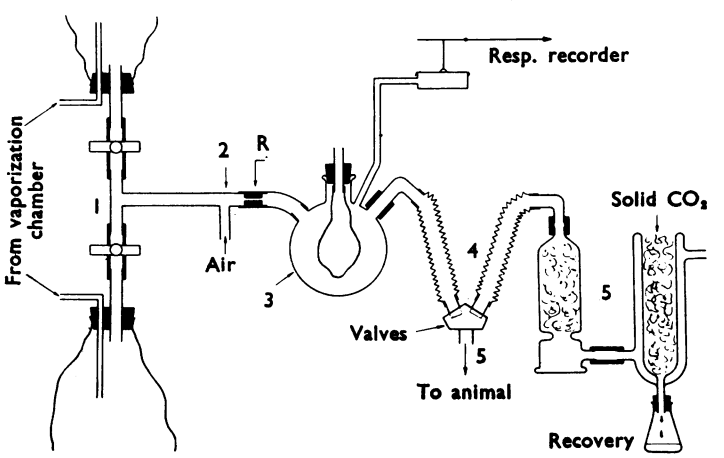

FIG. 2.—Diagram of apparatus for exposing larger animals to known concentrations of fluothane—see text for description.

the inhalation apparatus. Otherwise an endotracheal tube with an inflatable cuff was inserted after spraying the larynx with a solution of cocaine hydrochloride, while the monkey was still under the influence of thialbarbitone. Inhalation of fluothane was not started until the monkey showed the first signs of recovery. The respiration and the ECG were recorded.

Isolated Tortoise Atrium.—The tortoise *T. groeca* was decapitated, its plastron removed, and strips of both atria containing the sinus were suspended in a 10 ml. bath of Clark's frog Ringer at room temperature. The contractions were recorded on a smoked drum, using a light isotonic lever. The Ringer solutions, saturated with ether, chloroform, and fluothane, were prepared by shaking, for approximately 20 min., an excess of the volatile liquids in Ringer solution and letting them stand until complete separation of the two phases occurred. Saturated solutions of cyclopropane were prepared by bubbling the gas through Ringer solution for at least ½ hr. The final concentration of each anaesthetic in the bath was calculated from its solubility in water, and no correction was made for any change of solubility due to the salts of the Ringer solution. The height of the contraction of the strips was measured before and after the addition of the anaesthetic solutions, and the degree of inhibition produced by the anaesthetic was calculated as a percentage of the normal contraction of the strip.

Hepatic and Renal Function Tests.—The hepatic function of dogs anaesthetized with fluothane was estimated by the bromosulphalein method of Reinhold and Hutchinson (1954), and in rats by the hippuric acid excretion method of Quick (1940).

The renal function of rats and dogs anaesthetized with fluothane was examined by the phenol red excretion test (Hawk, 1954). The water diuresis test was also used. Rats were starved overnight and given 5 ml./100 g. of tepid tap water by mouth; urine was then collected at ½ hourly intervals for 3 hr.

Electro-encephalography.—Records of the EEG of rabbits with electrodes implanted in their skull were taken during consciousness and at different levels of anaesthesia. The electrodes consisted of a $\frac{1}{16}$ in. silver rod embedded in a perspex sleeve. One end of the sleeve was screwed in a trephine hole made in the skull and the other was outside the skin. One end of the silver electrode made contact with the dura and the other could be connected to the recording apparatus.

Histopathology.—Animals anaesthetized with fluothane for different times were allowed to recover and kept under observation for at least 2 days. They were then killed and specimens of their tissues were obtained immediately after death and fixed with formol-saline or with acetic acid-Zenker solutions. The microscopical sections of these specimens, obtained by standard methods, were examined by my colleague, Dr. Paget.

Definition of Surgical Anaesthesia.—The term "surgical anaesthesia" is used in this paper to describe a level of anaesthesia similar to that of Stage III, plane II–III, of Guedel's classification (1951), i.e., anaesthesia with loss of corneal reflex, predominantly abdominal respiration and muscular relaxation.

Results

Mice.—The anaesthesia produced in mice with fluothane is characterized by the rapidity of its onset. In experiments with 1.0 to 2.0% fluothane most mice were anaesthetized in less than 5 min. Recovery from anaesthesia was equally fast, and mice that had been anaesthetized for over 1 hr. recovered fully in 5–6 min. after their removal from the exposure chamber.

The AC50 and LC50 of fluothane and other inhalation anaesthetics are summarized in Table II, which shows that fluothane is about 1.5 times as potent as chloroform and about five times as potent as diethyl ether. Its AC50 is lower than that of all the anaesthetics of Table II with the exception of trichloroethylene which, under the conditions of these experiments, seems excellent. The ratio $\frac{LC50}{AC50}$ of fluothane, 3.3, is about twice that of ether, 1.7, and of chloroform, 1.5.

TABLE II

EFFECT OF INHALATION OF ANAESTHETICS ON MICE AFTER 30 MIN. EXPOSURE

Numerals in parentheses indicate 95% confidence limits

Compound	AC50 (Vapour, %)	LC50 (Vapour, %)	Ratio LC50/AC50
Chloroform ..	1·3 (±0·13)	2·0 (±0·1)	1·5 (±0·16)
Diethyl ether	4·3 (±0·215)	7·3 (±0·51)	1·7 (±0·15)
Trichloroethylene ..	0·82 (±0·041)	4·9 (±0·29)	5·0 (±0·35)
Cyclopropane	17·4 (±1·57)	25·8 (±2·06)	1·5 (±0·18)
Ethyl chloride	4·7 (±0·28)	8·2 (±0·74)	1·7 (±0·19)
Fluothane ..	0·86 (±0·069)	2·8 (±0·34)	3·3 (±0·46)

Dogs and Monkeys.—Concentrations of fluothane between 2% and 4% in air or in O_2 were sufficient for induction of anaesthesia, and there was scarcely any excitement. Full surgical anaesthesia was obtained after 2–5 min. whether the dogs were premedicated or not. At this point, both corneal and laryngeal reflexes were abolished, and the trachea could be intubated without provoking coughing (Fig. 3a). As the apparatus used in these experiments had a fairly large dead space, the times of onset of anaesthesia were more or less the same with any concentration of fluothane within the limits mentioned above.

When surgical anaesthesia had been obtained, the concentration of fluothane could be decreased to a minimum which maintained anaesthesia at a steady level for several hours. This concentration

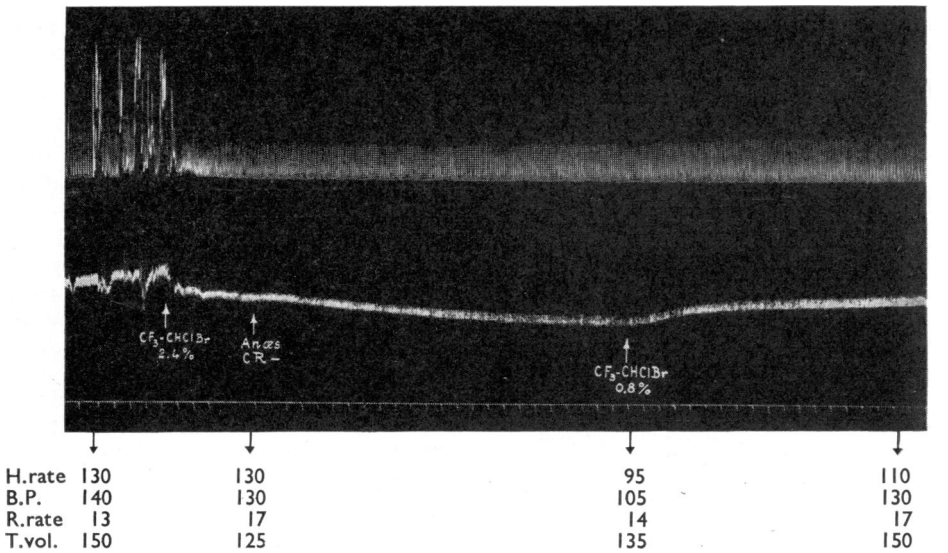

FIG. 3a.—Dog 14½ kg. Induction, 30 mg./kg. Na thialbarbitone i.v. In the control period the dog was recovering from the effects of thialbarbitone. At the first arrow, beginning of the inhalation of 2.4% fluothane. Surgical anaesthesia with loss of corneal reflex was observed at the second arrow. Third arrow, change over to maintenance concentration, 0.8%, of fluothane. Upper tracing, respiration; middle tracing, blood pressure; and lower tracing, time in 30 sec. Heart rate, blood pressure, respiratory rate, and tidal volume are given below the tracing.

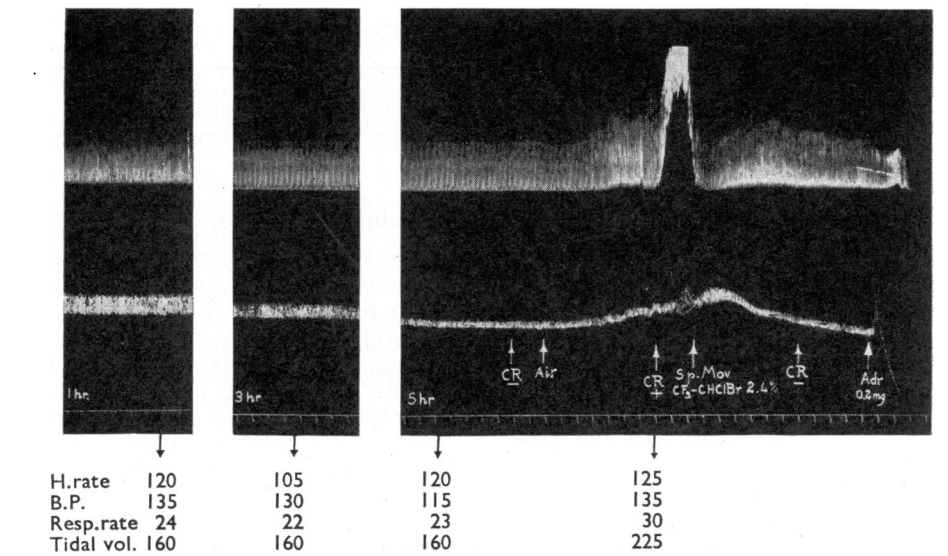

FIG. 3b.—Continuation of Fig. 3a. Records obtained 1, 3, and 5 hr. after the beginning of anaesthesia with fluothane. At 5 hr.: first arrow, negative corneal reflex; second arrow, cessation of anaesthesia; third arrow, positive corneal reflex; fourth arrow, first stage of recovery and spontaneous movements. Inhalation of 2.4% fluothane: fifth arrow, negative corneal reflex; sixth arrow, i.v. injection of 0.2 mg. of adrenaline and ventricular fibrillation.

varied from dog to dog and also depended on whether the dogs had been premedicated. In about 5% of the dogs which had received morphine or thialbarbitone, it was possible to maintain anaesthesia by the inhalation of fluothane in concentrations as low as 0.4–0.5%. More usually, anaesthesia was maintained with 0.8% fluothane (Fig. 3a and 3b). This was not sufficient in unpremedicated dogs, which required about 1.0–1.2% of fluothane for the maintenance of anaesthesia.

In similar experiments on premedicated dogs, it was found that the maintenance concentrations of ether and chloroform were at least 3.0% and 1.5% respectively. If the maintenance concentrations of the anaesthetics are taken as a basis of comparison of their relative potency, fluothane appears to be about twice as potent as chloroform and four times as potent as ether, a result very similar to that obtained in mice.

The concentrations of fluothane needed for the induction and maintenance of anaesthesia in monkeys pretreated with thialbarbitone were approximately the same as those found effective in premedicated dogs. As all the monkeys used in this series were pretreated with thialbarbitone, it is not possible to say whether the concentrations active in the unpremedicated dogs mentioned above would also be active in monkeys under the same conditions.

Recovery from anaesthesia with fluothane was equally fast and free from excitement in all the animal species in which it has been tried. All animals recovered completely in about 10–20 min., even from periods of anaesthesia lasting 5–6 hr., and they were then able to walk normally. The first signs of recovery appeared sooner, as is shown in Fig. 3b, where the corneal reflex became positive in 2 min. and spontaneous movements were observed 5 min. after the cessation of the inhalation of fluothane.

Other Methods of Administration and Surgical Interventions.—Besides the experimental method used in this series, which can be considered as an open-circuit method, some clinical methods of anaesthesia were tried on animals. The open mask method was tried successfully in dogs, cats, and rabbits, but the level of anaesthesia was somewhat difficult to control, and relatively small changes in the concentration of the agent markedly influenced the depth of anaesthesia. As the action of the substance is characterized by rapidity of induction and recovery, it is not surprising that the open mask method of anaesthesia should be difficult to control. Unpremedicated dogs and cats have been anaesthetized by this method without any undue excitement during induction. Recovery was as rapid as after anaesthesia by the experimental method. Fluothane has also been used in close-circuit methods, and dogs have been anaesthetized for as long as 4 hr. using a Boyle's or a Gillies apparatus.

In operations carried out in dogs anaesthetized with fluothane, the muscular relaxation produced was sufficient for abdominal interventions, such as the preparation of stomach pouches. Another striking observation was the scarce capillary bleeding of both skin and muscle incisions. The operative field was therefore free from blood.

Respiration.—Both the amplitude and frequency of respiratory movements were decreased during anaesthesia with fluothane. The reduction in the respiratory rate was, as a rule, more than the reduction in amplitude. In our dogs, weighing 10–14 kg. at the level of surgical anaesthesia, the rate could be as low as 15–25/min. with a tidal volume of about 125–250 ml. (Figs. 3a and 3b).

As with other anaesthetics, the thoracic respiratory movements were abolished earlier than the diaphragmatic so that the latter became predominant as the level of surgical anaesthesia was reached. The inhalation of fluothane in concentrations of 3.0% or lower for 1 hr. did not stop respiration. Higher concentrations stopped respiration in a time inversely proportional to the concentration. In five experiments the inhalation of 3.6% fluothane was continued until respiratory arrest was produced, in an average of 60 min. from the begining of the experiment. In Table III the times given by Guedel (1951) for the production of respiratory arrest with induction concentrations of some anaesthetics are compared with those observed in experiments with different concentrations of fluothane.

When the respiration ceased, the blood pressure was still reasonably high, between 60–80 mm. Hg, and the heart continued beating for about 9 to 10 min. This time-lag between respiratory and

TABLE III

TIMES NEEDED FOR THE PRODUCTION OF RESPIRATORY ARREST WITH INDUCTION CONCENTRATIONS OF SOME ANAESTHETICS

Anaesthetic	Time (min.)	Authority
Ether	10–30	Guedel (1951)
Cyclopropane	½–3	
Ethylene	1–4	
Chloroform	4–12	
Nitrous oxide	1–4	
Ethyl chloride	1–4	
Fluothane 3·0%	No paralysis in 1 hr.	This paper
,, 3·5%	45–60	
,, 4·0%	20–30	

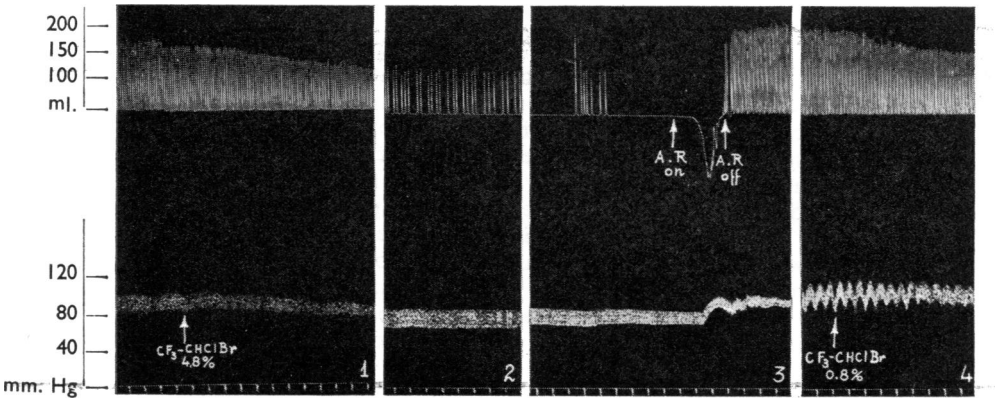

FIG. 4.—Respiratory arrest in a dog after 4 hr. anaesthesia, produced by increasing the concentration of fluothane. The dog inhaled 0.8% fluothane for 4 hr., and at the first arrow the concentration was increased to 4.8%. The inhalation of this concentration was continued for 35 min. until respiratory arrest (3). Artificial respiration (A.R.) and return of spontaneous respiratory movements 3 min. later.

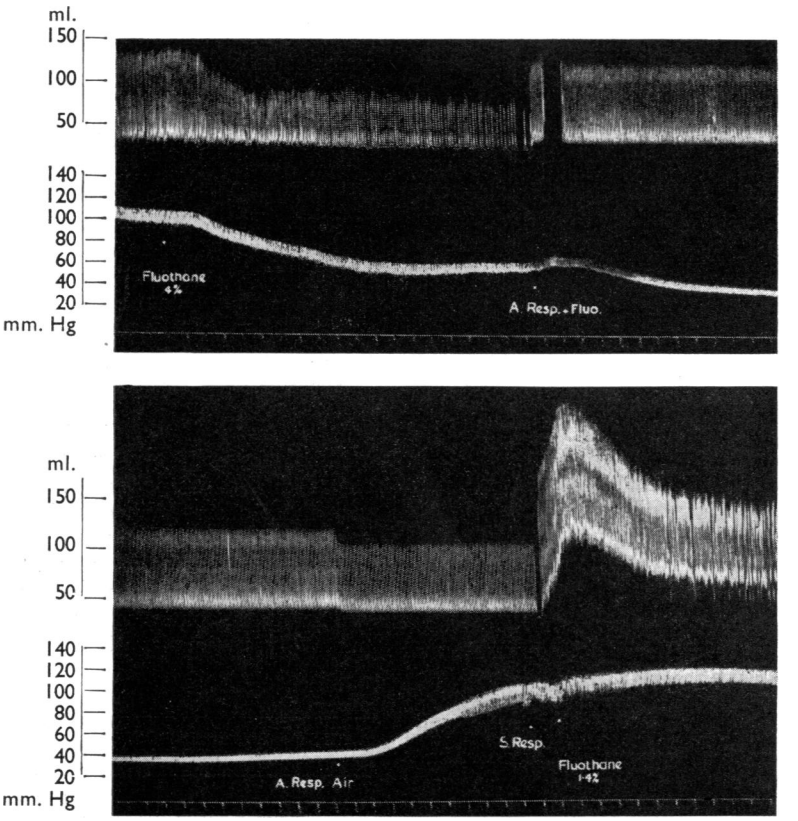

FIG. 5.—Dog 11 kg. Induction, fluothane 4.0%. Maintenance 1.2% fluothane in O_2. Upper record: The dog had inhaled 1.2% fluothane for 1 hr. when the anaesthetic concentration was increased to 4.0%. Respiratory arrest 9 min. later and start of administration of the same anaesthetic mixture by means of an artificial respiration pump (A.Resp. + Fluo). Lower record: After 90 min. of administration of 4.0% fluothane by means of artificial respiration pump. A.Resp.Air, start of artificial respiration with air. S.Resp., spontaneous respiratory movements. Fluothane 1.4% was given at the end of the experiments. In both records the top tracing is the respiratory movement and the lower tracing is the blood pressure. Time, 30 sec. See Fig. 12 for ECG records of this experiment.

cardiac arrest is fairly long and provides plenty of warning when a dangerous level of anaesthesia has been reached.

The respiratory arrest produced by fluothane was easily reversible and normal respiratory movements were usually present after a few minutes of artificial respiration. Even when this arrest was produced deliberately at the end of a long anaesthesia, by increasing the concentration of the anaesthetic, it was found that the apnoea could be reversed by artificial respiration with air or O_2 (Fig. 4). In three experiments the administration of these high concentrations of fluothane was continued for 1½ hr. after respiratory arrest by means of an artificial respiration pump. The apnoea produced in these experiments was reversed with the same ease when artificial respiration was continued with air instead of the anaesthetic mixture (Fig. 5), and the recovery was not appreciably longer than from anaesthesia of long duration where no apnoea was produced.

In other experiments, once the dogs were anaesthetized with fluothane, their respiratory movements were arrested by means of (+)-tubocurarine and the administration of the anaesthetic was continued with a Starling respiration pump. In the experiment of Fig. 6 a first dose of 5 mg. of (+)-tubocurarine was administered and 6 further doses of 2 mg. were given when necessary, i.e., as soon as spontaneous respiratory movements occurred. The first dose of (+)-tubocurarine produced a fall of blood pressure, but once its effects had disappeared the pressure remained at the same level during the experiment, which lasted for 4 hr.

Blood Pressure.—The inhalation of fluothane produced a fall of blood pressure, roughly proportional to the concentration of the vapour. This effect was larger during the induction period when high concentrations of 2.0–4.0% were administered, and reached its maximum about 30–40 min. after the beginning of the inhalation, or 23–35 min. after the production of surgical anaesthesia. The hypotension was relatively small in dogs, in which the pressure fell from 120–140 mm. Hg to 90 mm. Hg; it was larger, however, in cats and rabbits. A comparison of Figs. 3a and 3b, taken from an experiment on a dog, with Figs. 7 and 8 from experiments on cats, shows this difference.

When full surgical anaesthesia had been obtained, the hypotension was not great, and the blood pressure rose as soon as the dogs were put on to the maintenance concentration (0.8% to 1.0%). It remained between 100–130 mm. Hg for the rest of the experiments, provided the concentration of the vapour mixture was kept constant (Figs. 3a and 3b).

Blood pressures between 60–80 mm. Hg were recorded when the respiration was stopped by the

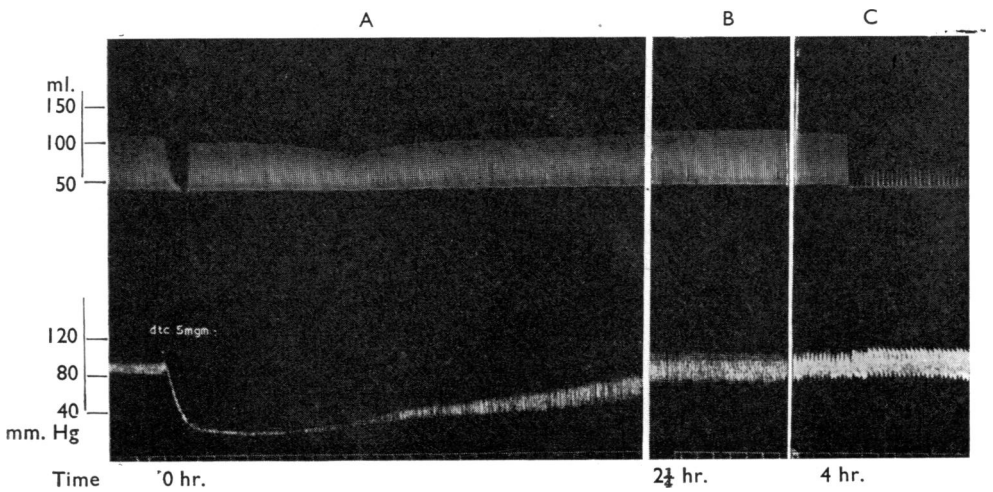

FIG. 6.—Dog 12.5 kg. Premedication, morphine+atropine; induction, fluothane 2.4%; maintenance, fluothane 1.2%. A, 1 hr. after induction. At 0 hr. 5 mg. of (+)-tubocurarine (TC) i.v. and beginning of the inhalation of the anaesthetic mixture (1.2%) by means of an artificial respiration pump. B, 2¼ hr. after the first dose of TC. Five further doses of 2 mg. TC were given between A and B. C, 4 hr. after the first dose of TC. The seventh and last dose of 2 mg. TC was given 45 min. before C. Administration of fluothane stopped, artificial respiration and return of spontaneous respiration. Top tracing, respiration; lower tracing, blood pressure. Time, 30 sec. See Fig. 13 for ECG records of this experiment.

inhalation of high concentrations of fluothane, and fell to 40–50 mm. Hg if the administration of these vapour mixtures was continued by means of an artificial respiration pump. However, the blood pressure returned to normal levels soon after starting artificial respiration with air or O_2 (Fig. 5).

The cause of the hypotension produced by fluothane has been studied by several methods. It was found that plethysmographic records of the spleen (3 expt.) and intestine of cats (4 expt.) anaesthetized with chloralose showed an increase in the volume of these organs (Fig. 7). Under the same conditions, the volume of the leg (4 expt.) was not altered and there was a reduction in the volume of the kidney (Fig. 8).

During the inhalation of fluothane in cats and dogs anaesthetized with chloralose, there was a block of conduction in the sympathetic ganglia. This was observed in the following experiments: (*a*) the contraction of the nictitating membrane produced by the preganglionic stimulation of the cervical sympathetic nerve was reduced, (*b*) the hypertension produced by large doses of acetylcholine in atropinized animals was abolished,

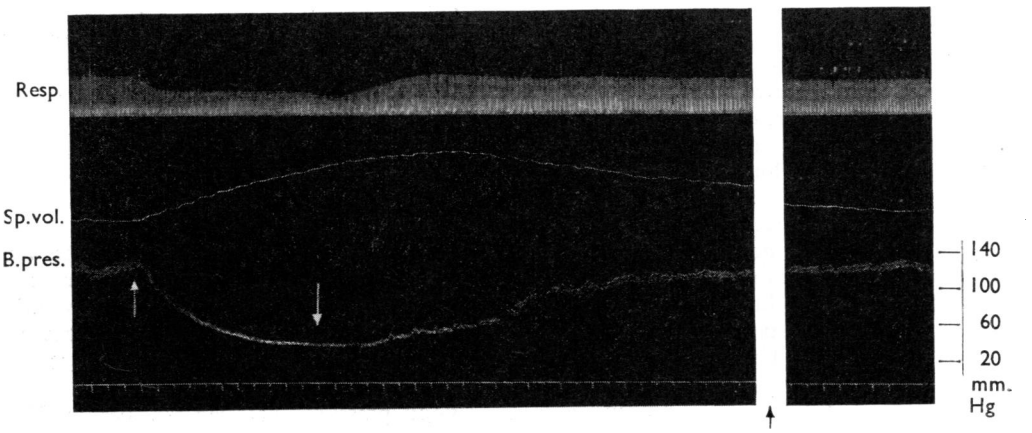

FIG. 7.—Action of fluothane on the spleen volume of a cat anaesthetized with chloralose (70 mg./kg. i.v.). Fluothane (2.4%) was inhaled between the two arrows. Resp., respiration; sp.vol., plethysmographic record of the spleen volume, increase of spleen volume upwards; B.pres., blood pressure. Time, 30 sec.

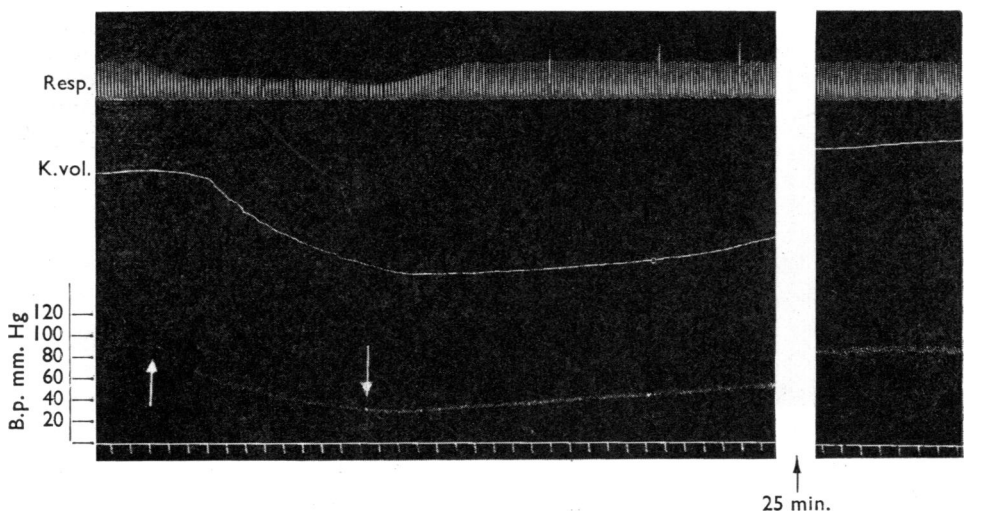

FIG. 8.—Action of fluothane on the kidney volume of a cat anaesthetized with chloralose (70 mg./kg. i.v.). Fluothane (2.4%) was inhaled between the two arrows. Resp., respiration; K.vol., plethysmographic record of kidney volume, decrease of volume downwards; B.p., blood pressure. Time, 30 sec.

(*c*) the hypertension produced by stimulation of the splanchnic nerve, or (*d*) by occlusion of the carotid arteries was reduced (Figs. 9, 10, and 11). All these effects were reversible and disappeared shortly after inhalation of the anaesthetic had ceased. There is, however, some specificity in the action of fluothane on different ganglia, and from the preliminary results reported here it seems likely that the mesenteric ganglion is more sensitive to its action than the superior cervical ganglion.

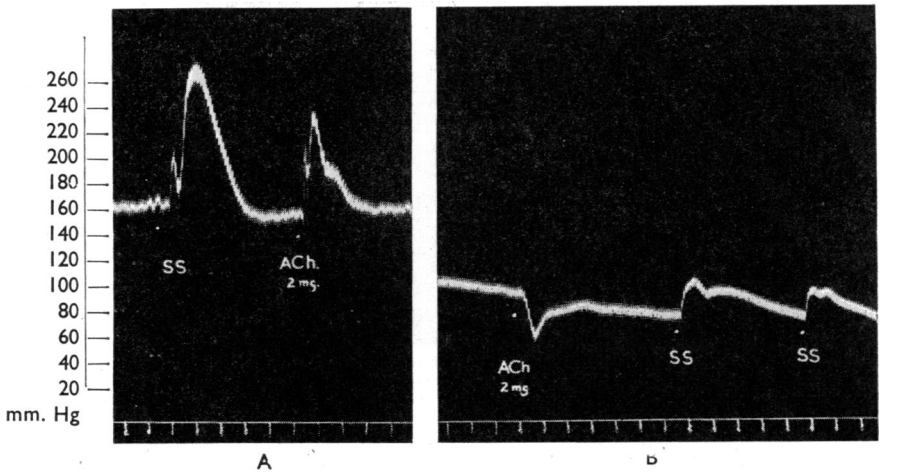

FIG. 9.—Dog 11.1 kg. Chloralose 80 mg./kg. i.v. Atropine 5 mg. i.v. Effects of the stimulation of the splanchnic nerve (s.s.) and of 2 mg. acetylcholine (ACh) on the blood pressure of a dog before (A) and during (B) the inhalation of 2.4% fluothane. Time, 30 sec.

In experiments, such as that of Fig. 9, the inhalation of 2.4% fluothane for 10–15 min. abolished the nicotinic action of large doses of acetylcholine and it produced a considerable decrease of the effects of the stimulation of the splanchnic nerve. On the other hand, this concentration of fluothane produced only between 25–50% decrease in the height of the contraction of the nictitating membrane of 6 cats caused by maximal preganglionic stimulation of the cervical sympathetic nerve (Fig. 11).

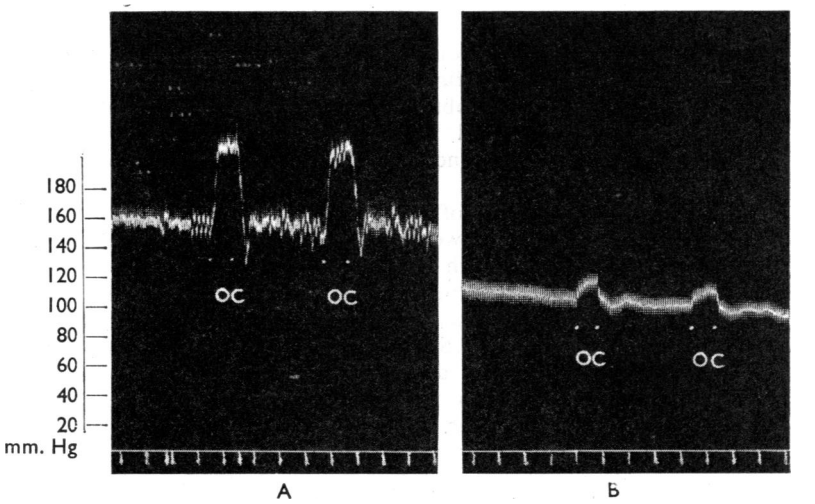

FIG. 10.—Dog 12.3 kg. Chloralose 80 mg./kg. i.v. Effects of the occlusion of the carotids (OC) on the blood pressure of a dog before (A) and during (B) the inhalation of 2.4% fluothane. Time, 30 sec.

These results cannot be due to a reduction of the peripheral action of adrenaline or noradrenaline because the pressor effects of the i.v. administration of small doses of these amines is not modified by the inhalation of the fluothane. The ganglion-blocking properties of other anaesthetics have been described by Larrabee, García Ramos, and Bülbring (1952).

In two dogs premedicated with morphine, no significant difference was found between their cardiac output and cardiac index measured before and during the inhalation of maintenance concentrations of fluothane. Since the cardiac output was not altered during anaesthesia with fluothane it can be assumed that vasodilatation in the splanchnic area, probably due to a sympathetic ganglion block, is the main cause of the hypotension.

Heart Rate and EEG.—A decrease in the heart rate was observed in all animals under the action of fluothane. During the induction period in dogs, a reduction from 130–170 to 80–100 beats/min. was recorded, but the heart rate rose again to about 120–140 beats/min. shortly after the beginning of inhalation of the maintenance con-

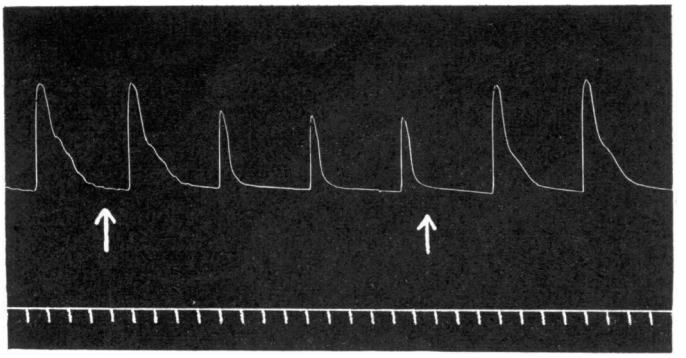

FIG. 11.—Cat 3.2 kg. Chloralose 70 mg./kg. i.v. Contraction of the nictitating membrane produced by supramaximal preganglionic stimulation (20 stim/sec. for 12 sec. every 2 min.). Fluothane (2.4%) was administered between the arrows.

centration ; it remained within these limits as long as the low concentration of fluothane was inhaled (Fig. 3a and 3b).

The electrocardiograms, lead II, of dogs and monkeys anaesthetized with fluothane did not differ from those taken in the same animals during the control periods. No obvious irregularities were recorded during either the induction or maintenance of anaesthesia, a finding that compares favourably with the results obtained with cyclopropane (Seevers, Meek, and Rovenstine, 1934 ; Robbins and Baxter, 1937) and with chloroform (Hill, 1932a and b).

A decrease in the voltage of the whole QRST complex but no irregularities were observed when apnoea was produced by high concentrations of fluothane (4.0%) and the administration of the same anaesthetic mixture was continued for a further 90 min. by means of a Starling pump (Fig. 12). This effect cannot be due to stretch or chemical pulmonary reflexes produced by the forced administration of the anaesthetic mixture, because it was not observed in other experiments where the respiratory movements had been stopped with (+)-tubocurarine and maintenance concentrations of the anaesthetic were administered by the same method for periods up to 4 hr. The only possible explanation of this result is the difference in the concentration of anaesthetic in the blood in the two types of experiments.

Cardiac Irregularities Produced by Adrenaline.—Like some other anaesthetics, fluothane was found to increase the sensitivity of the heart to adrenaline. When adrenaline was injected intravenously during fluothane anaesthesia, ventricular fibrillation sometimes occurred. A comparison was made in 13 dogs of the adrenaline hypersensitivity produced by fluothane, chloroform, and cyclopropane. Each dog was anaesthetized to the level of surgical anaesthesia, and increasing doses of adrenaline were injected intravenously at 20 min. intervals. Great care was taken to inject the adrenaline solutions at a constant rate, since in preliminary experiments it was found that the speed of injection was an important factor in the production of heart irregularities. Each dose was therefore dissolved in 5 ml. normal saline and was injected at a rate of 1.0 ml./10 sec. The injections of adrenaline were continued until a dose was found that produced ventricular tachycardia. Sometimes fatal ventricular fibrillation occurred. The dogs that survived the first experiment were allowed to recover and kept under observation for a week. This procedure was then repeated with another anaesthetic, until each dog had been anaesthetized with the three agents. In a few experiments, the dogs were premedicated with morphine or with chlorpromazine. ECG records, lead II, were taken during these experiments.

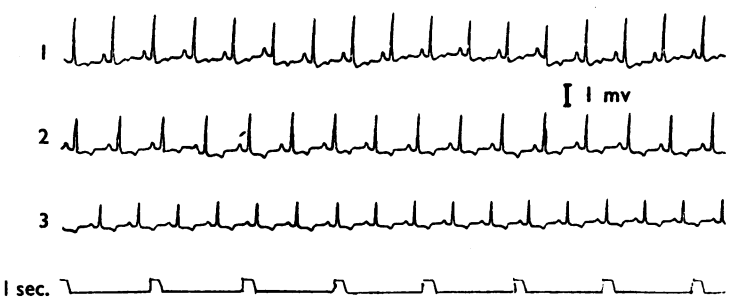

FIG. 12.—ECG's of a dog recorded during the forced administration of high concentration of fluothane. Records obtained in the experiment of Fig. 6. 1, ECG at the end of 90 min. of inhalation of the maintenance concentration of fluothane 1.2%. 2, ECG at respiratory arrest after 9 min. of inhalation of 4.0% fluothane. 3, ECG taken after 90 min. of respiratory arrest. Fluothane (4.0%) was administered during this time by means of an artificial respiration pump.

This method is essentially that of Meek, Hathaway, and Orth (1937), who assessed their results chiefly on the duration of the ventricular tachycardia produced by a dose of 10 μg./kg. adrenaline. We found it was difficult to use this criterion because our dogs often had more than one period of ventricular tachycardia following the adrenaline injection, and there was a great variation in the doses of adrenaline that produced this

TABLE IV

MEAN DOSES OF ADRENALINE THAT PRODUCE VENTRICULAR TACHYCARDIA IN ANAESTHETIZED DOGS

Numerals in parentheses indicate 95% confidence limits

Anaesthetic	No. of Dogs	Adrenaline (μg./kg.)	Deaths	Remarks
Controls—no anaesthesia	8	17·5 (8–38)		Large variation in the controls; range from 4 to 32 μg./kg.
Fluothane ..	13	8·8 (7–11)	1	
Chloroform ..	9	13·3 (10–17)	1	Unpremedicated
Cyclopropane ..	12	4·3 (3·3–5·4)	2	
Fluothane ..	6	10·1 (7–14)	0	Morphine 2 mg./kg. s.c.
Fluothane ..	4	15·5 (10–23)	0	Chlorpromazine 0·5 mg./kg. i.v.

effect. It was thought better to assess the hypersensitivity by estimating the dose of adrenaline that produced ventricular tachycardia in each dog. A mean dose of 88 μg./kg. (−)-adrenaline base injected intravenously produced ventricular tachycardia in unpremedicated dogs anaesthetized with fluothane. The same effect was obtained with 4.3 μg./kg. adrenaline, under cyclopropane, and with 13.3 μg./kg. under chloroform anaesthesia (Table IV). The sensitivity of the heart to adrenaline is therefore increased to a lesser degree by fluothane than it is by cyclopropane, but to a greater degree than by chloroform. If adrenaline was given intramuscularly or subcutaneously, it could be administered in very large doses without producing disturbances of the heart rate or of the conduction mechanism. In some dogs, as much as 200 ml. of 1:100,000 adrenaline was injected subcutaneously in different parts of the body without marked effects. In others, up to 5 ml. of 1:1,000 adrenaline solution was injected intramuscularly into the four limbs, and produced only a slight rise in blood pressure, which was not associated with any cardiac irregularities. Adrenaline is probably absorbed slowly by these routes, and the blood concentration of the amine is never sufficiently high to produce ventricular tachycardia.

The effect of premedication with morphine, and chlorpromazine, was also investigated (Table IV). Morphine did not modify the hypersensitivity to intravenous adrenaline produced by fluothane. On the other hand, chlorpromazine, administered intravenously shortly before the induction, decreased adrenaline hypersensitivity, but it did not protect the dogs completely from the effects of adrenaline.

Equipressor doses of noradrenaline and adrenaline were equally active in producing ventricular tachycardia. This means that the production of ventricular tachycardia and fibrillation in hypersensitive hearts is closely related to hypertension. On the other hand, the intravenous administration of other sympathomimetic amines, such as mephentermine (Wyamine) or methoxamine (Vasoxine) produced rises in the blood pressure comparable to those obtained with adrenaline or with noradrenaline, but without ventricular fibrillation or tachycardia.

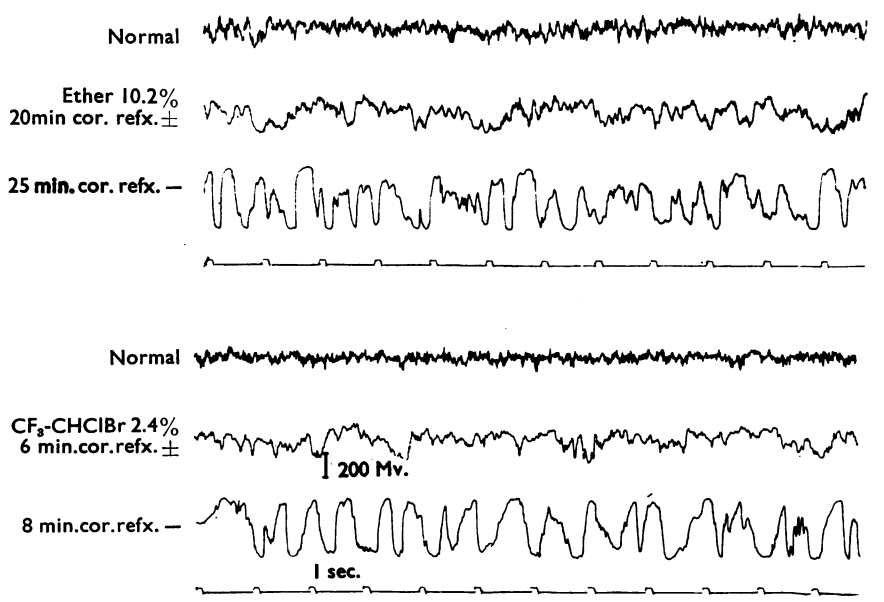

FIG. 13.—Electroencephalograms of a rabbit obtained before and during anaesthesia with ether (top) and with fluothane (bottom).

Electro-encephalography.—Records of the EEG of rabbits with electrodes implanted in the skulls were taken during consciousness, and at different levels of anaesthesia with fluothane. The EEG changes recorded in these experiments were practically the same as those observed in the same animals when anaes-

thetized with ether some days later (Fig. 13).

Salivation, Mucous Secretion, Vomiting and Irritation.—The mouth and trachea of animals anaesthetized with fluothane were always remarkably dry and free from secretion. This is probably due to the ganglion-blocking action of the anaesthetic.

None of the dogs and monkeys anaesthetized with fluothane vomited either during or after its administration. This result contrasts with the high incidence of vomiting, about 50%, found in our laboratories in dogs and monkeys after cyclopropane anaesthesia.

Fluothane applied to the skin did not produce the burning sensation felt after the application of ether or chloroform. Irritation of the mucosa of the mouth and trachea has never been observed during or after the inhalation of its vapours. Its action on the eye has been examined by applying some drops of the liquid in the conjunctival sac and leaving it to evaporate. This produced a fairly intense conjunctivitis and corneal lesions that could be revealed with fluorescine. These lesions, however, were not permanent and no corneal ulcers could be seen two or three days later.

Concentration of Fluothane in the Blood During Anaesthesia.—The concentration of fluothane was estimated in samples of the arterial blood of two dogs and three rabbits taken at different levels of anaesthesia. During induction with 2.4% fluothane in O_2 the concentration of the anaesthetic rose progressively, and in 16 min. had reached an equilibrium at 17.0–22.0 mg./100 ml. (Fig. 14). This blood concentration produced a level of anaesthesia equivalent to Stage II, plane IV, of Guedel's classification. If, at this point, the animals were put on to the maintenance concentration, 0.8%, the concentration of anaesthetic in the blood decreased to about 14.0 mg./100 ml. and remained at this level for as long as the maintenance mixture of fluothane was inhaled. The level of anaesthesia produced by this concentration was equivalent to Stage III, plane II. In some experiments, the concentration of fluothane in the inhaled mixture was increased so as to produce respiratory arrest. The samples of blood taken at this time contained 28–35 mg./100 ml. of the anaesthetic. During recovery from anaesthesia the concentration of fluothane in the blood decreased very rapidly. When the corneal reflex had returned to normal, about 10 min. after the inhalation of the anaesthetic had ceased, its concentration was 7–9 mg./100 ml. Spontaneous movements were observed as a rule 5 min. later, when the concentration had fallen to 4–6 mg./100 ml. No estimations were carried out on samples of blood taken after full recovery.

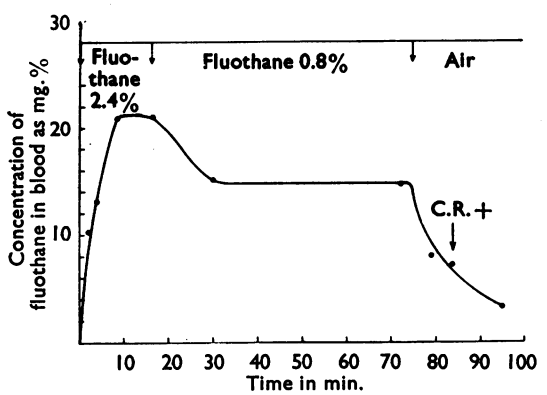

FIG. 14.—Concentration of fluothane in the blood of one dog during anaesthesia. Ordinate, fluothane as mg./100 ml. Abscissa, time in min.

Action of Fluothane on the Isolated Atrium of the Tortoise.—The action of different concentrations of fluothane on the tortoise atrium was compared with the action of ether, chloroform and cyclopropane. An inhibition of the contraction of atrial strips was produced when Ringer solution saturated with these substances was added to the bath.

The average concentrations of the anaesthetics that produced 50% inhibition of the contraction of the tortoise atrium in 8 experiments were: ether, 217 mg./100 ml.; chloroform, 23.7 mg./100 ml.; cyclopropane, 14.3 mg./100 ml.; and fluothane, 38.3 mg./100 ml. It is evident that both ether and fluothane inhibit the heart only when their concentration in the bath is higher than that found in dogs during anaesthesia so deep as to produce respiratory arrest, i.e., 30 mg.% and 150 mg.% respectively.

Action of Fluothane on the Liver.—Although in the experiments described above no delayed deaths attributable to the action of fluothane were observed, two liver function tests were undertaken. Two batches of 4 rats each were anaesthetized for 1 hr. with fluothane every day for 5 consecutive days, and the hippuric acid excretion test was carried out before the first, and immediately after the last, period of anaesthesia. The amount of hippuric acid excreted in the urine was the same before and after repeated anaesthesia.

A similar negative result was found in a dog by the bromosulphalein method. In the tests carried out before and after repeated periods of anaesthesia with fluothane, no significant differences

were observed either in the retention of the dye in the blood or in the rate of its disappearance. These results suggest that hepatic function is not affected by anaesthesia with fluothane.

Renal Function Tests.—The renal function of rats was examined by the water diuresis and phenol red excretion tests. Seven batches of 4 rats each were anaesthetized 5 times with fluothane for 1 hr. on consecutive days and both tests were carried out, in different batches of animals, before and immediately after the periods of anaesthesia. No significant difference was found between the results of the control tests and those carried out after anaesthesia. The renal function of one dog was examined by the phenol red excretion method once before the animal was anaesthetized (twice for 3 hr. on two consecutive days) and again 24 hr. after the second period of anaesthesia. The excretion of phenol red was unaltered.

Histopathology. — Histopathological observations were made of the tissues of 20 untreated rats, 6 rats anaesthetized with ether, and 30 rats anaesthetized with fluothane for 45 min. on each of 5 consecutive days; 2 dogs anaesthetized with fluothane for 6 hr., and one dog anaesthetized for 1½ hr. with a concentration that produced respiratory arrest; and 1 untreated monkey, 1 monkey anaesthetized for 2 hr. with chloroform, and 4 monkeys anaesthetized with fluothane once for periods up to 6 hr. and one monkey anaesthetized 6 times for 3 hr. on alternate days.

Dr. G. E. Paget, who examined the sections of the specimens from these animals, reported: "The most consistent histopathological change seen in animals treated with fluothane occurs in the kidney. It consists of a dilatation of the proximal convoluted tubules and is associated with slight cytological changes in the cells of these tubules (Fig. 15).

"The changes are more prominent in animals subjected to repeated prolonged anaesthesia with this agent, but it can be seen in animals killed after only one period of anaesthesia. No necrosis of renal tissue was seen in any of the animals. These changes must be taken to indicate mild damage to the proximal convoluted tubule cells. In order to assess the significance of these changes, histochemical studies were made on kidneys from animals treated with fluothane. These showed that no significant change occurs in the quantity and distribution of alkaline phosphatase or of non-specific esterase in the dilated tubules. In view of this and of the absence of necrosis, it seems probable that the changes do not indicate any serious or irreversible damage to the kidney. However,

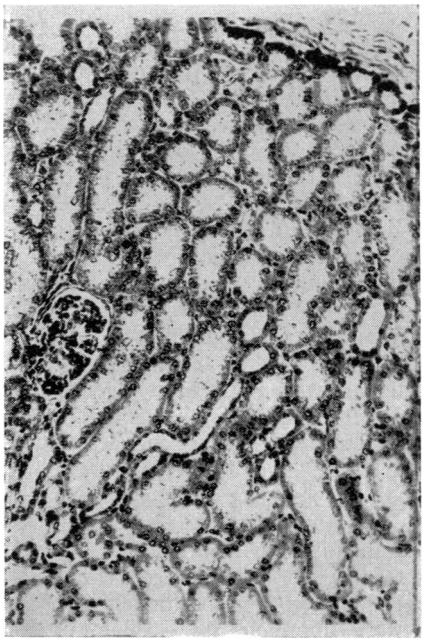

FIG. 15A

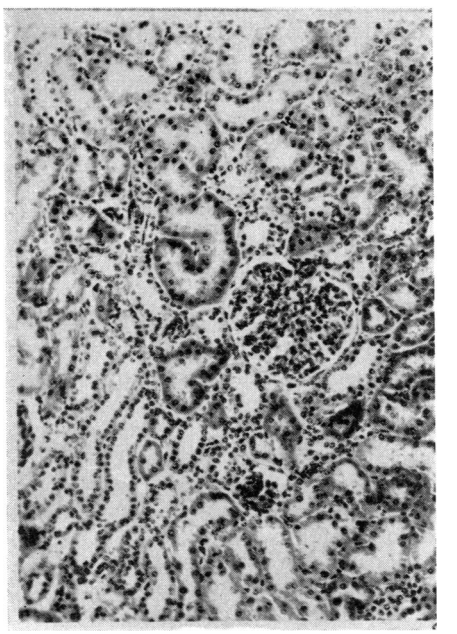

FIG. 15B

FIG. 15.—A = Section of the kidney of a monkey two days following one period of anaesthesia with fluothane. B = Section of kidney of a normal monkey. H. & E., × 120.

until something is known of the action of this agent in man, it would be wise to avoid its use in the presence of severely impaired renal function.

"There are also minor changes in the liver. These are of trivial extent and degree compared with those seen in a monkey anaesthetized on one occasion with chloroform or with the changes known to occur in man after chloroform anaesthesia.

"The lungs of all rats show varying degrees of naturally occurring chronic interstitial pneumonia. No consistent changes attributable to the administration of the anaesthetic can be detected in the lungs of rats or other animals.

"No changes due to administration of this anaesthetic agent were seen in any other organ examined. It thus seems that, apart from mild renal changes, this agent produces little toxic damage to tissues, and, apart from the previously-mentioned proviso concerning impaired renal function, there is, from this evidence, no contra-indication to its use in man."

From this report it is clear that the only obvious pathological change found in the tissues of animals treated with fluothane is a dilatation of the proximal convoluted renal tubules and slight cytological changes in their cells. It is probable that this mild lesion is of no great importance because (1) renal necrosis has never been observed in these animals, (2) the change is not associated with any alteration in the amounts and distribution of two enzymes of the tubules, and (3) the renal functions, as measured by the phenol-red excretion and water-diuresis tests, were not altered even immediately after repeated anaesthesia.

On the other hand, Eschenbrenner (1944) and Eschenbrenner and Miller (1945) found extensive tubular necrosis in the kidneys of adult male mice anaesthetized for long periods with chloroform. Hewitt (1956) has been able to produce this lesion in some strains (CBA) of adult male mice kept for 2 hours in concentrations of chloroform (4 mg./l. =0.078%) too low to produce any signs of anaesthesia. No lesions were found in the kidneys of female mice of these sensitive strains after the same treatment with chloroform. The production of renal necrosis with sub-anaesthetic concentrations of chloroform has been confirmed in our laboratories, but we have been unable to observe similar results in the kidneys of male mice after they have been exposed for 2 and 4 hours to similar concentrations of fluothane (0.075%).

Microscopical examination of the livers of animals anaesthetized for long periods, or repeatedly, with fluothane showed only minor changes, a result that has been corroborated by biochemical tests. In no experiment was fatty degeneration of the liver found, a very frequent result of chloroform and divinyl ether anaesthesia. The fatty degeneration produced by chloroform anaesthesia, which is a classic example of delayed toxic action, can be obtained with very low concentrations of the agent. Hewitt (1956), and ourselves, have found it in mice, males and females, that had been exposed to chloroform in concentrations of about 0.078% for 2 hr. No hepatic lesions have been found in mice after being exposed to similar concentrations of fluothane.

Summary

1. Fluothane ($CF_3CHClBr$) is a volatile liquid with a b.p. of 50.2° C. and an S.G. of 1.86. It is not inflammable when mixed with O_2 in concentrations from 0.5 to 50% (v/v). It is stable over soda lime.

2. It is an inhalation anaesthetic more potent than ether and choloroform on experimental animals. Its therapeutic ratio is about twice that of ether. Induction of anaesthesia and recovery are both rapid and free from excitement. It produces good muscular relaxation. It does not cause salivation or vomiting.

3. With the exception of hypotension, it does not produce any serious functional disturbances. It does not produce cardiac irregularities, but increases the sensitivity of the heart to adrenaline. It does not increase capillary bleeding.

4. The inhalation of high concentrations (3.5%) stops the respiration, but this apnoea is easily reversible.

5. The only pathological lesion found in animals after its use is a mild dilatation of the proximal tubules of the kidney. This lesion is not associated with alteration of the renal function.

Clinical trials on fluothane are being carried out by Dr. M. Johnstone. An account of his results on 500 patients has been published (Johnstone, 1956) since this paper was submitted for publication.

The author is grateful to Dr. C. W. Suckling and other members of the staff of the Research Laboratories of the General Chemicals Division of I.C.I. at Widnes for preparing the fluothane used in these experiments. He would like also to thank his colleagues, Dr. R. R. Goodall, for developing the method for the estimation of fluothane in blood, Dr. J. M. Pryce, for the estimation of some of its physical constants, Mr. J. M. Thorp, for the measurements of the cardiac outputs, and Dr. G. E. Paget, for the pathological examination of animals anaesthetized with fluothane. Finally, he would like to acknowledge the technical help given by Mr. J. Dee.

REFERENCES

Eschenbrenner, A. B. (1944). *J. Nat. Cancer. Inst.*, **5**, 21.
—— and Miller, E. (1945). *Science*, **102**, 302.
Gaddum, J. H. (1941). *J. Physiol.*, **99**, 257.
Guedel, A. E. (1951). *Inhalation Anaesthesia*, p. 10. New York: Macmillan.
Hawk, P. B. (1954). *Practical Physiological Chemistry*, 13th ed., p. 966. London: Churchill.
Hewitt, H. B. (1956). *Brit. J. exp. Path.*, **37**, 37.
Hill, I. G. W. (1932a). *Edinb. med. J.*, **39**, 533.
—— (1932b). *Lancet*, **1**, 1139.
Johnstone, M. (1956). *Brit. J. Anaesth.*, **28**, 392.
Kochmann, M. (1912). *Arch. Int. Pharmacodyn.*, **22**, 487.
Krantz, J. C., Carr, C. J., Lu, G., and Bell, F. K. (1953). *J. Pharmacol.*, **108**, 488.
Larrabee, M. G., García Ramos, J., and Bülbring, E. (1952). *J. cell. comp. Physiol.*, **40**, 461.
Lewis, A. E. (1953). *J. appl. Physiol.*, **6**, 93.
Lu, G., Ling, S. L., and Krantz, J. C. (1953). *Anaesthesiol.*, **14**, 466.
Meek, W. J., Hathaway, H. R., and Orth, O. S. (1937). *J. Pharmacol.*, **61**, 240.
Orth, O. S., and Dornette, W. H. L. (1955). *Fed. Proc.*, **14**, 376.
Quick, A. J. (1940). *Amer. J. clin. Path.*, **10**, 222.
Reinhold and Hutchinson (1954). Cited in Hawk (1954), p. 598.
Robbins, B. H. (1946). *J. Pharmacol.*, **86**, 197.
—— and Baxter, H. (1937). Ibid., **51**, 162.
Seevers, M. H., Meek, W. J., and Rovenstine, E. A. (1934). Ibid., **51**, 1.

APPENDIX

ESTIMATION OF FLUOTHANE IN BLOOD

By R. R. GOODALL

For the estimation of fluothane in blood, extracts in petroleum ether were heated in sealed ampoules with sodium amoxide. Bromide was released and determined nephelometrically as silver bromide. The hydrolytic reaction was limited to 1½ hr., since prolonged heating caused solution of chloride from the glass. It was essential to hydrolyse samples and standards together in the same pressure vessels, as the reaction was incomplete (about 60% of theory).

Special Reagents

Sodium in Amyl Alcohol.—Place 50 ml. of amyl alcohol (A.R. quality) in a stainless steel beaker and add 1.1 ±0.1 g. of clean sodium (free from halide) in small pieces. Cover the beaker with a petri dish during solution and warm if the last small pieces of sodium are slow to dissolve.

Standard Solution of $CF_3CHBrCl$.—Place 100 ml. of distilled water in a 4 oz. liquid bottle with a well-fitting glass stopper. Chill the bottle and contents in an ice-bath. Weigh accurately anaesthetic (90–110 mg.) in a tared capillary pipette having a rubber teat at the top. Deliver the contents of the pipette under the surface of the water in the bottle and rinse the pipette several times by pressing and releasing the rubber teat. Leave the contents of the bottle unshaken in the ice-bath until just before use. Then shake for one minute to dissolve the droplets of $CF_3CHBrCl$. Anaesthetic vapour is lost very readily from aqueous solution, and it is essential to use a bottle that is almost full of liquid. It is now known that fluothane is less readily lost from petroleum ether, and standards might advantageously be prepared in this solvent.

Extraction

Standards.—Into 10 ml. tubes (B14 stoppered) place 4 ml. of petroleum ether (b.p. 100–120° C.). In successive tubes place 5, 4.5, 4.0 and 3.5 ml. of water and then 0, 0.5, 1.0 and 1.50 ml. of the standard aqueous anaesthetic solution, delivered from 0.50 and 1.00 ml. Folin–Ostwald pipettes filled by immersion and *not* by suction. Stopper each tube and shake for 2 min. Allow the layers to separate. Then from each tube withdraw a 3.5 ml. aliquot of the petrol layer and place in a 10 ml. ampoule into which 2.0 ml. of the sodium amoxide solution has been introduced. Seal each ampoule.

Prepare in duplicate three tubes each containing petroleum ether (4 ml.) and normal blood (4 ml.). In successive tubes, place water (1, 0.5, and 0 ml.) followed by standard anaesthetic solution (0, 0.5, and 1.0 ml. from Folin–Ostwald pipettes). Extract by shaking for 5 min., centrifuge at 2,000 rev./min. for 2 min. with stoppers removed; then remove a 3.5 ml. aliquot of each petrol layer and continue as described above.

Tests.—For each test, place 4 ml. of petroleum ether and 1 ml. of water in an appropriate 10 ml. tube. Place 4 ml. of the blood sample (or less if the concentration of $CF_3CHBrCl$ is expected to exceed 25 mg. % w/v) under the surface of the petroleum. If required, add more blood from an untreated animal to the tube until the total blood volume is 4.0 ml. Extract, centrifuge, and transfer 3.5 ml. aliquots to ampoules containing sodium amoxide and seal as described above.

Hydrolysis

The ampoules are heated in a pressure cooker at 15 lb./sq. in. for 1½ hr.

Determination of Halide as Silver Halide.—Open each ampoule and transfer the contents by shaking into a 6 in. by ⅝ in. test-tube. Rinse each ampoule with N-sulphuric acid (volume, 0.5 ml. more than that required to neutralize the sodium amoxide) followed by sufficient water to make the total aqueous layer 8 ml., and mix well. After settling, remove the lower (aqueous layer) to a clean tube, add 2 ml. 0.01N-silver nitrate, stir momentarily, and transfer immediately to a dark cupboard for 10 min. Then read quickly the optical density of each colloidal suspension of silver halide in a 1 cm. cell at 520 mμ against water, using a Unicam S.P. Spectrophotometer. (Note: After adding the silver nitrate

avoid unnecessary exposure to bright light.) Plot the optical density of the aqueous and blood standards against the known concentration. From the observed optical density of each test obtain the concentration on the aqueous standard graph and multiply the result by the ratio of aqueous/blood slopes.

Optical densities of extracts of aqueous and blood standards are exemplified in the following table:

Mg.% w/v in Original Solution			d_1 cm.	at 520 mμ
Aqueous	..	0	0·10	0·11
,,	..	13·3	0·30	0·31
,,	..	26·5	0·47	0·51
,,	..	39·8	0·76	0·77
Blood	..	0	0·08	0·09
,,	..	13·3	0·25	0·26
,,	..	26·5	0·43	0·46

Note added in proof: An improved method, using reduction by lithium aluminium hydride to release halide from fluothane, is being developed.