ANAESTHETIC AND HYPERGLYCAEMIC PROPERTIES OF HALOTHANE ON THE MICE COMPARED WITH CHLOROFORM AND ETHER

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Halothane, a halogenated derivative of ethane (2-bromo-2-chloro-1:1:1-trifluoroethane), has been introduced recently as a non-inflammable volatile anaesthetic. A series of papers dealing with the clinical properties and uses of this anaesthetic have already been published. Raventos (1956) and Burn *et al.* (1957) described its pharmacological properties in some details.

The method used by Raventos involves a refined apparatus yielding quantitative data on the relative potency of volatile anaesthetics. The aim of the present investigation, however, was to devise a simpler and less expensive apparatus for the purpose of preliminary screening of these anaesthetics and to see how the results obtained by this method compare with those published by Raventos (1956). Consequently, the present study has been extented to the determination of the relative onset and duration of anaesthesia which have not so far been studied. Studies have also been made on blood sugar changes in mice following these anaesthetics, since a rise in blood sugar level is a great drawback of general anaesthetics especially that of chloroform and ether (Goodman and Gilman, 1955).

The preliminary results of these investigations have been reported elsewhere (Chatterjee and Ghosh, 1958; Chatterjee, Seth and Ghosh, 1959).

METHODS

A device, which was adopted for anaesthetic study in the present investigation, has been shown diagrammatically in Fig. 1. The set up consisted of a glass cylinder (2 × 20 cm) with two short side arms opposite each other, one near the top and the other close to the bottom of the cylinder. The cylinder (A) was painted black to avoid undue exposure of the anaesthetic liquid to light. The top of the cylinder was closed tightly by a rubber bung

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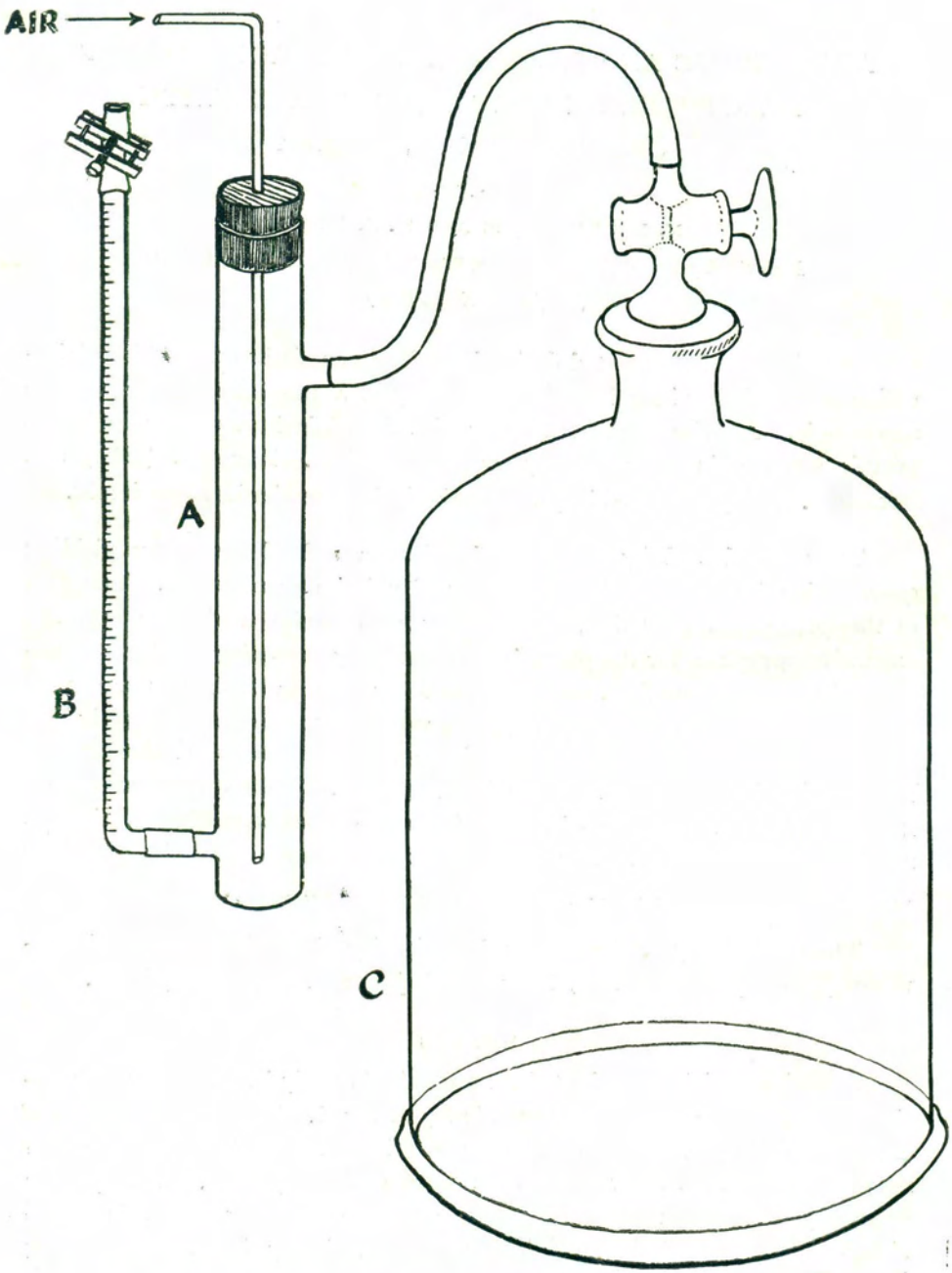

Fig. 1. A set up for testing anaesthetic properties of volatile liquid in small animals.

through which passes a 0.5 cm glass tube to reach almost the bottom of the cylinder. The upper end of this tube, which was bent at right angles was connected with a device by which air was blown through the anaesthetic liquid at a uniform rate (by a principle of water displacement as used in artificial pneumothorax apparatus). The lower side arm was connected to a vertical tube (B) made from 1 ml pipette (graduated to 0.01 ml) so as to enable measurement of a small change in the volume of the anaesthetic placed inside the cylinder. A volume change of 10 ml in cylinder (A) produced a change of 0.16 ml in tube (B), that is, the actual volume of anaesthetic in the cylinder was 62 5 times the volume read off from the tube (B). The upper end of the tube (B) is kept closed by means of a short piece of rubber tube with a screw clip in order to prevent loss of the liquid from volatilisation and only opened to adjust the level as required. The upper side arm was connected by means of a rubber tubing to a bell jar (14.5×25 cm, capacity, 4 litre) having an airtight glass stop-cock. The bell jar (C) was placed on a smooth surface in such a way that there was no appreciable leakage of anaesthetic vapour through the space between the rim of the jar and the surface on which it was placed.

Determination of the anaesthetic properties:—The cylinder was two-third filled up with the anaesthetic liquid and the initial level of the liquid read off from the graduated tube. The mouse was placed inside the bell jar, the stop-cock opened and the air blown through the anaesthetic liquid at a constant rate. The volatilised vapour was led through the upper side arm into the bell jar. As soon as the mouse fell on its side and remained in that position for 4 sec (loss of righting reflex), the air blowing was stopped, the time noted, and the mouse taken out of the jar immediately. The level of the anaesthetic liquid was read off again and its difference from the initial level multiplied by the factor 62.5 recorded as the *volume* of anaesthetic required. The time taken to produce this effect (loss of righting reflex) was also entered as the onset of anaesthesia. The observation was continued until the mouse was able to right itself up (recovery) when the time was noted again. The difference between the time of onset of anaesthesia and that of recovery gave the duration of anaesthesia recorded as the *sleeping time.* Before repeating the test with another mouse, care was taken to see that the bell jar was emptied of any residual anaesthetic vapour.

The mice were starved (excepting water *ad lib.*) on the day and until the end of the experiment. A total of 30 mice weighing between 15 to 30 g (average wt. 23.8 g) were divided into three equal groups and each allocated

to a particular anaesthetic. All the three anaesthetics were tested on the same day. The variations due to the effect of temperature and pressure prevailing at the time of experiment were thus eliminated. The error due to animal variations was also minimised by adopting a repeated cross-over design, so that each group of mice was tested in turn against each anaesthetic on different days.

Estimation of blood sugar in mice—Mice of both sexes were divided into 4 groups, the average weight in each group varying from 23 to 25 g The number of mice in the control was about twice that in each of the treated groups. On any day, a few mice from each of the 4 groups were used and blood sugar estimation made. When all the mice of each group were used up, which took several days, the results were combined and finally analysed. After a preliminary fasting on the day of the experiment, 0.05 ml of blood was withdrawn from the heart of each mouse with a tuberculin syringe, and mixed thoroughly with 1.85 ml of isotonic sodium sulphate-copper sulphate solution in a small conical centrifuge tube. Before taking blood from the treated groups, each mouse was anaesthetised for a period of 15 min with respective anaesthetic. The mice were sacrificed at the end of the experiment and each sample was then analysed for blood sugar by a colorimetric method as described by King (1951).

Materials.—The following anaesthetics were used. Ether Anaestheticus (B. C. P. W. Ltd.), Chloroform, B. P. (Merck & Co.) and Halothane (Fluothane, I. C. I. Ltd.).

RESULTS

The results regarding the amcunt of an anaesthetic required and the duration of anaesthesia are summarised in Table I. Although a total of 30 mice were used to start with, there were some accidental deaths in the course of anaesthesia leading to a small decrease in the number of mice in each group as shown in the Table. It may be mentioned incidentally that the mortality was almost the same with chloroform and halothane but none with ether.

The volume of ether (5.09 ml) required to produce anaesthesia (loss of righting reflex) was about 3.5 times, and of chloroform (1.16 ml) about the same, as that of halothane (1.45 ml). As regards the onset of anaesthesia, it was earliest with halothane (42 sec), intermediate with chloroform (61 sec) and longest with ether (94 sec). The *sleeping time*, however, was least with

ether (24 sec) and longest with chloroform (49 sec), the halothane being intermediate in duration (34 sec).

The significance of these differences either in the volume, or onset of *sleeping time* was tested by the Student's *t* method (Table II). Each anaesthetic was paired in turn with the other two so that there were in all three pairs for the purpose of comparison. It would be seen that the differences between the three anaesthetics were highly significant (P 0.001) for most of the criteria studied.

TABLE I

Anaesthetic properties of Halothane, Chloroform and Ether

Anaesthetic	No. of mice	Volume (ml)	Onset (sec)	Sleeping time (sec)
Halothane	24	1.45 ± 0.09	41.9 ± 1.1	34.4 ± 3.6
		(0.94 — 1.88)	(33 — 54)	(8 — 95)
Chloroform	23	1.16 ± 0.05	61.4 ± 1.7	48.7 ± 6.0
		(0.63 — 1.88)	(45 — 73)	(13 — 123)
Ether	24	5.09 ± 0.20	93.5 ± 5.0	24.0 ± 2.1
		(3.12 — 6.87)	(63 — 148)	(10 — 49)

Values are mean ± standard error. Ranges are given in parenthesis

TABLE II

Comparison of the various anaesthetic properties of Halothane, Chloroform and Ether

Comparison	Volume	Onset	Sleeping time
Halothane *v.*	$t = 3.34^{**}$	$t = 9.65^{**}$	$t = 2.06^{*}$
Chloroform	P 0.01	P 0.001	P 0.05
Halothane *v.*	$t = 16.70^{**}$	$t = 9.90^{**}$	$t = 2.48^{*}$
Ether	P 0.001	P 0.001	P 0.02
Chloroform *v.*	$t = 18.38^{**}$	$t = 5.95^{**}$	$t = 3.98^{**}$
Ether	P 0.001	P 0.001	P 0.001

*Significant : ** Highly significant

A comparison of the relative variations (coefficient of variation, C. V.) of different anaesthetics for various criteria has been made (Table III). On the whole, the criterion for onset was the least variable and the criterion for *sleeping time* the most, indicating that the former was more realiable a criterion than the latter; the criterion for *volume* occupied an intermediate position in this respect. The variations (C. V.) were almost of equal magnitude for different anaesthetics except for the criterion for *onset*; the variation in *onset* for ether was twice that for either halothane or chloroform.

TABLE III

Relative standard deviation or coefficient of variation (C.V.) expressed as percent

Anaesthetic	Volume	Onset	Sleeping time
Halothane	29	13	52
Chlorofrom	22	13	59
Ether	20	26	43

TABLE IV

Blood sugar values of different groups of mice

Group	No. of mice	Body weight (g)	Blood sugar mg/100 ml
Control	49	23.6 (19—32)	157.3 ± 9-4 (57—312)
Halothane	25	24.5 (18—28)	226.9 ± 16.9 (100—500)
Chloroform	26	22.7 (18—27)	195.1 ± 13.3 (64—338)
Ether	24	23.4 (19—28)	197.6 ± 15.9 (100—420)

Values are mean ± standard error. Ranges are given in parenthesis

A summary of the blood sugar values of mice in control and treated groups is presented in Table IV. There was an increase in the blood sugar values in each of the treated groups compared to the control. The increase in the blood sugar following halothane was slightly greater than that following either chloroform or ether. The increase over the control following chloroform or ether was statistically significant (Table V). The relative differences in the blood sugar changes following the administration of the three anaesthetics, however, were not statistically significant.

TABLE V

Significance tests of blood sugar changes in mice

Comparison	*t*	P
Control *v.* Halothane	3.90**	0.001
Control *v.* Chloroform	2.32*	0.05
Control *v.* Ether	2.32*	0.05
Halothane *v.* Chloroform	1.49	0.1
Halothane *v.* Ether	1.03	0.3
Chloroform *v.* Ether	0.38	0.7

* Significant : ** Highly significant

DISCUSSION

The method which has been worked out and adopted in the present investigation (for the comparative evaluation of different anaesthetics) though simple, is not as refined and accurate as that employed by Raventos (1956). Nevertheless, the results obtained by the present method are reproducible and the relative positions of the three anaesthetics as determined by this method agree in general with those observed by Raventos. In Table VI the values obtained with halothane in groups of mice on different days have been presented for comparison as regards the reproducibility of the results. It would be observed that there is good agreement between the different values for respective criteria. Although the mean *sleeping time*

(39.1 sec) for the experiment No. 1. (Table VI) appears to be rather large compared to the two remaining means (30.3 and 32.2 sec, Table VI.), the difference is not statistically significant.

TABLE VI

Reproducibility of the results with Halothane

Nos. of different experiments	No. of Mice	Volume (ml)	Onset (sec)	Sleeping time (sec)
1	10	1.56 ± 0.10	42.3 ± 1.9	39.1 ± 7.8
		(0.94 - 1.88)	(34 - 52)	(8 - 95)
2	8	1.41 ± 0.13	41.2 ± 2.3	30.3 ± 4.0
		(0.94 - 1.88)	(33 - 54)	(13 - 47)
3	6	1.31 ± 0.12	41.7 ± 1.8	32.2 ± 4.0
		(0.94 - 1.88)	(37 - 48)	(18 - 46)

Values are mean ± standard error. Ranges are given in parenthesis

TABLE VII

Relative activities of different anaesthetics for various criteria of measurement

Anaesthetic	Volume	Onset	Sleeping time
Halothane	1	1	1
Chloroform	0.8	1.5	1.4
Ether	3.5	2.2	0.7

According to Raventos (1956), who used the vapour concentrations that anaesthetised 50 per cent of mice (AC 50) as his criterion of measurement, halothane was about 5 times as potent as ether, and about 1.5 times as chloroform. In the present investigation when the relative volumes of anaesthetic were compared as a rough measure of their respective potency, halothane appeared to be about 3.5 times as potent as ether and about equally potent as chloroform (Table VII). Considering enormous variations

in the two experimental conditions (not only in the methods employed but also in the widely seperated situation of the two laboratories with entirely different populations of mice), there is fair agreement between the present results and those obtained by Raventos. However, the absolute difference between the two results may partly be accounted as due to the variation in the speed of diffusion of the anaesthetic fluids in the two methods depending on the volatility of fluids. The plea for adopting the present method is the simplicity with which screening of various anaesthetic fluids could be made.

Attempts have been made to measure by the present method, the individual effective dose as well as the effect regarding onset and duration of anaesthesia on each animal. The information regarding the induction of anaesthesia with halothane, which was rapid in our hands, was also in agreement with that of Raventos. Not only that, it was also possible to evaluate by the present method the relative difference among the various anaesthetics with regard to the duration of anaesthesia. Thus, the induction time (onset of anaesthesia) of ether was roughly twice, and of chloroform about 1.5 times longer than that of halothane (Table VII). No quantitative data are available in the literature to compare the latter findings regarding the onset and duration of anaesthesia in small animals. Following the present method it was possible to evaluate the relative differences of the different anaesthetics.

SUMMARY

A simple method which yields reproducible results is described, for screening volatile anaesthetics in small animals.

The anaesthetic properties of halothane, chloroform and ether were studied with respect to the (a) *volume* of anaesthetic required to produce loss of righting reflex, (b) *onset* of anaesthesia and (c) *sleeping time*.

The *volume* of halothane was about the same as that of chloroform and about three and half times less than that of ether. The *onset* with halothane was about one and half times less than that with chloroform and about half of that with ether. The *sleeping time* of halothane, however, appeared to be about one and half times longer than that with ether and about one and half times shorter than that with chloroform.

All the three anaesthetics produced an increase in the blood sugar level in mice. Their relative differences, however, were not statistically significant.

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