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AN ANALYSIS OF THE KINETICS OF ANAESTHESIA OF MICE

BY

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A number of mathematical theories have been developed in an attempt to describe quantitatively the rate of uptake of anaesthetics by the body. Most involve one, two or more exponential processes (see Kety, 1951; Mapleson, 1962; Eger, 1963; Mapleson, 1963). These same theories have also been used to explain differences between the speeds of induction of anaesthesia by the various anaesthetics as well as the rapidity of recovery from anaesthesia (Kety, 1951; Butler, 1958; Adriani, 1963). However, it is not known whether induction of, or recovery from, anaesthesia follows the single or multiple exponential time-course predicted by these theories. Onset of anaesthesia and recovery from anaesthesia have been described as fast or slow, nothing more. The object of the present experiments was to determine the rates (that is, the kinetics) of anaesthesia more quantitatively. To do so, a simple theory has been formulated such that the existence of one or more exponential components in the onset of anaesthesia could be detected. Three volatile anaesthetics possessing markedly different solubilities in fat and blood were selected for study. These were halothane, ether and methoxyflurane.

THEORETICAL CONSIDERATIONS

Onset of anaesthesia

The simplest description of the uptake of anaesthetic at its sites of action in the brain is for the situation where uptake is determined by a single exponential process:

$$x = aC(1-e^{-kt}) \qquad (1)$$

where x is the concentration of anaesthetic at its sites of action in the brain at a time, t, after exposure to a constant vapour concentration, C, of anaesthetic; k is a rate constant and a is a proportionality constant which includes the blood/air partition coefficient of the anaesthetic.

In a series of experiments using different inhaled concentrations of anaesthetic and different times of exposure, we can identify a standard pharmacological effect of the anaesthetic. Let it be assumed that equal concentrations of the same anaesthetic exist at its site of action in the brain when a standard anaesthetic effect is observed, regardless of how these concentrations are achieved. We then have:

$$aC(1-e^{-kt}) = a'C_e(1-e^{-kt'}) \qquad (2)$$

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where the times t and t' are now the times at which the standard anaesthetic effect is produced.

If C_e is the inhaled concentration which, at equilibrium, just produces the standard effect, and if the proportionality constants a and a' are the same, then equation (2) reduces to:

$$C(1-e^{-kt}) = C_e$$

which can be re-arranged to

$$\ln [(C-C_e)/C] = -kt \qquad (3)$$

The validity of equation (3) can be tested by timing the onset of the effect when various values of C are used and plotting $(C-C_e)/C$ on the log ordinate of semilog paper against these measured times. The points should fall on a straight line which should intersect the ordinate at a value of one.

If the plotted points give a curve instead of a straight line, then an analysis for two or more exponentials can be carried out. For example, the equation for the sum of two exponential events is:

$$x = aC(1-B_1e^{-k1t}-B_2e^{-k2t}) \qquad (4)$$

where B_1 and B_2 are constants and k_1 and k_2 are rate constants. After some interval of time one exponential term should become negligible and equation (4) can again be written in the same form as equation (3). Deviations from simple theory (equation (1)) should therefore be exponential if the event is determined by the sum of the two exponential processes. The presence of an exponential deviation can readily be detected (see Defares & Sneddon, 1960).

An important property of the plot of log $[(C-C_e)/C]$ against t is that the resulting curve should be identical to the curve obtained from exponential analysis of the uptake of anaesthetic at the sites where it acts when the anaesthetic tissue concentration is expressed as a fraction of the final equilibrium concentration of anaesthetic. This consequence can readily be seen by choosing arbitrary values for the constants in either equation (1) or (4) and plotting the concentration of anaesthetic (x) against time of exposure (t) to the various inhaled concentrations (C) of anaesthetic. The time taken to reach the same tissue concentration ($x=aC_e$) can be obtained from these curves and plotted against log $[(C-C_e)/C]$. The resulting curve will be identical to that obtained from exponential analysis of the chosen equation for any given inhaled concentration of anaesthetic. As the rate of onset of anaesthesia should depend essentially on the rate of saturation of the brain sites where the anaesthetic acts, the experimental curves have been called onset of anaesthesia curves, and the study has been called kinetics of anaesthesia.

Recovery from anaesthesia

The corresponding equations for recovery from anaesthesia are:

$$x' = xe^{-k3t} \qquad (5)$$

where x' is the concentration of anaesthetic at its sites of action in the brain at a time t after the end of exposure to anaesthetic and x is the tissue concentration achieved during the anaesthesia. And

$$\ln (C_e/C) = -k_3t$$

METHODS

Two considerations influenced the choice of animal. These were: the presence of a clear indication of a standard anaesthetic effect which was preferably a sign of light anaesthesia; and the ease of obtaining large numbers of observations. Mice fulfilled these requirements best.

Groups of ten albino mice of either sex (the mean weight of a mouse in each group of ten varied from 18 to 25.5 g) were exposed to anaesthetic using a method based on that described by Raventos (1956) and Burn, Epstein & Goodford (1959). The anaesthetic vapour mixtures were prepared by injecting liquid anaesthetic at a known rate into a stream of air which was also flowing at a constant rate. The air itself was first passed through a column of activated charcoal before being allowed to enter the vaporizing chamber. Liquid anaesthetic was introduced near the outlet of the vaporizing chamber and fell in drops on to the wall of the chamber. The drops flowed down the wall into the body of the chamber (a spirally wound length of glass tubing kept in a thermostat) and volatilized before reaching the bottom. The anaesthetic vapour so produced was passed into the mouse chamber (a desiccator of about 9 l. capacity).

A constant flow of liquid anaesthetic was produced in the following way. The liquid anaesthetic was placed in a conical flask which possessed an inlet and an outlet capillary tube. Clean mercury was introduced through the inlet capillary tube to displace the liquid anaesthetic into the vaporizing chamber. The constant flow of mercury was obtained by displacing the mercury from a second container by means of a constant inflow of either distilled water or glycerol produced by a Palmer slow-injection apparatus.

The concentration of anaesthetic in the air (%, v/v) was calculated using the laws for ideal gases. These laws are strictly applicable only to dilute real gases, a condition fulfilled by the anaesthetic vapour concentrations required for anaesthesia (Haggard, 1924a; Larsen, Eger & Severinghaus, 1962). The rate of flow of liquid anaesthetic was usually chosen first and the air flow was adjusted to give the required vapour concentration. The equation used was:

$$V = \frac{d V_M}{M} \cdot \frac{100 - x}{x} \cdot V_L$$

where V is the air flow in ml./min; d, the density of the liquid at the room temperature; V_M, the volume occupied by one mole of anaesthetic gas at the existing temperature and pressure; M, the molecular weight; x, the required percentage v/v; and V_L, the volume in ml. of liquid anaesthetic vaporized per min. The volume of air flow was usually between 1.5 and 3.0 l./min. The estimated relative error in the anaesthetic vapour concentrations given in the text is $\pm 2.5\%$ of the stated concentration.

Three holes were cut in the glass lid of the mouse chamber. One was for the inlet and outlet of the anaesthetic vapour (two glass tubes inserted into the rubber bung); another was used for placing the mice in the chamber; and the small third hole allowed a glass rod to be inserted into the chamber. These last two holes were kept covered except when in use. Before placing the mice in the mouse chamber, the air in the chamber was replaced by the anaesthetic vapour. A group of ten mice, which had earlier been placed in a 500-ml. measuring cylinder, was then slid rapidly (less than 10 sec) into the chamber by inverting the cylinder over the appropriate hole in the lid. The mice occupied the upper part of the chamber which was separated from the lower part by a perforated platform. The lower part contained soda lime. Anaesthetic in the chamber was replaced at a constant rate, throughout the exposure of the mice to anaesthetic. The half-time for the replacement varied from 1.5 to 3 min, depending on the concentration used.

Respiration of a mouse was measured using a body plethysmograph method. The mouse, excluding its head, was enclosed in a Perspex box possessing internal dimensions of 76.3, 44.5 and 31.8 mm (3, 3.75 and 1.25 in.). The neck of the animal was clamped in a stock which was close fitting but did not obstruct breathing of the animal. Plasticine was packed around the animal, apart from around the chest, to restrict its movements further, and petroleum jelly was smeared liberally around the neck and all other joins in the box to prevent leaks. Inset into one wall of this box, and held horizontally, was a 1-ml. pipette with the tip cut off. A small drop of water containing detergent (Teepol) and a colouring agent (Nigrosine, B.D.H.) was injected into the lumen of this pipette in such a way that a film was formed across the lumen. Rate of respiration was measured by photographing the changes in output of a photocell (displayed on an oscilloscope screen) produced by movements of the film across the surface of the photocell. The tidal volume was measured, by eye, from the excursion of the film.

The blood/air partition coefficient of methoxyflurane was determined in the following way. A known volume of citrated human blood, contained in a flask of known volume, was warmed to 37.5° C. A known amount of methoxyflurane was then placed in the distal end of a hollow U-shaped glass stopper which was immediately pushed into the neck of the flask. The flask was gently shaken until evaporation was complete. The methoxyflurane was extracted from the blood with carbon tetrachloride and the concentration present in the extract estimated from the height of the infrared peak at 1,070 cm^{-1}. The mean partition coefficient, after correction for losses during the extraction, was 12.3 (11.2, 12.1, 13.4 and 12.4). This value agrees well with that of 13.0±0.5 (mean and standard deviation) which has since been reported by Eger & Shargel (1963).

Hypothermia, in addition to the effect on the animal itself, alters the uptake and elimination of anaesthetic (Duncan & Raventos, 1959) and as such would make any interpretation of the kinetics of anaesthesia more difficult. All experiments were therefore carried out at an environmental temperature of about 30° C (29 to 32° C). At this environmental temperature, the fall in mean rectal temperature (measured by inserting a copper-constantan thermocouple 1.5 cm into the rectum of a mouse) was only about 1.5° C after 1 hr of ether (4%, v/v) or methoxyflurane (1.5%, v/v) anaesthesia. At normal room temperatures (18 to 20° C) the fall was 8° C. Although it is not the immediate concern of this paper, it may be noted that we found that ether was more toxic at room temperatures of 35 to 38° C than at 20° C; and it is likely that the high death rate with 4.57% ether described later is linked with the maintenance of the body temperature near normal levels.

The anaesthetics used were anaesthetic diethyl ether (Duncan & Flockhart, Hopkins & Williams), halothane (Fluothane, I.C.I.) and methoxyflurane (Penthrane, Abbott Laboratories). All concentrations are given as v/v.

RESULTS AND ANALYSIS OF RESULTS

The selection of a standard anaesthetic effect

The loss of righting reflex by a mouse was chosen as the sign of a standard depth of anaesthesia because this was the first clear indication of light anaesthesia. The righting reflex was considered absent when the animal failed to right itself after being gently rolled on to its side. The time taken for loss of righting reflex was chosen as that time t_A, when five out of ten mice had lost their righting reflex. This time was obtained by plotting the number of mice anaesthetized to loss of righting reflex against the time of exposure to anaesthetic, drawing a curve through the points and then reading the time, t_A, from the graph. The arithmetic mean of the times of onset was not used because in a number of experiments some mice were still unanaesthetized at the end of exposure to anaesthetic.

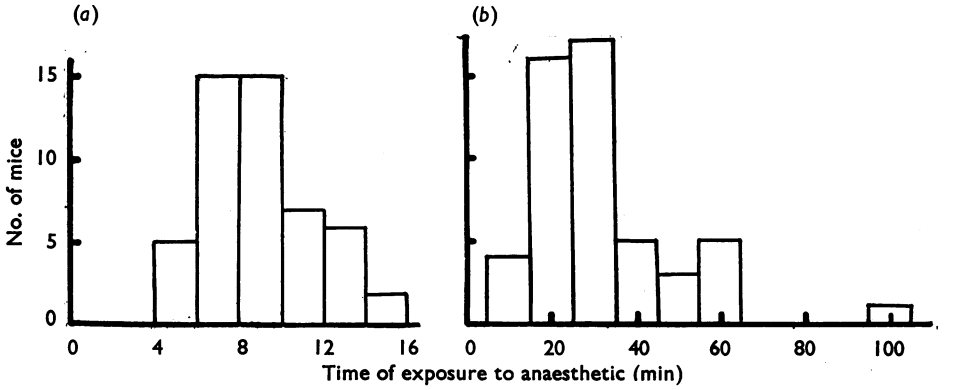

Fig. 1. Histograms showing the distribution of times at which lightly anaesthetized mice lost their righting reflex when exposed to 4.57% v/v ether (*a*) and 0.494% v/v methoxyflurane (*b*).

Moreover, the arithmetic mean was not necessarily the best estimate of the median for a group of mice. The histograms given in Fig. 1 show that the distribution of onset times tended to be positively skew. The skewness was significant for methoxyflurane anaesthesia ($P<0.001$). This distribution can be normalized by plotting the time logarithmically, but this complicates the analysis without gaining any significant advantage. Thus, for anaesthesia with 0.49% methoxyflurane, the arithmetic mean for all mice was 31.1 min, the geometric mean 27.1 min and the median obtained graphically 25.5±2 min (standard error for five groups of ten mice each).

The estimation of C_e

C_e, the concentration which, at equilibrium, just anaesthetizes five out of ten mice, was determined by finding two anaesthetic concentrations one of which just anaesthetized more than five mice and the other which just failed to anaesthetize five mice. C_e was then taken as the anaesthetic concentration midway between the two values. These two concentrations were determined in two ways, both of which gave similar values for C_e. In the first method mice were exposed to a constant vapour concentration of anaesthetic for periods up to 3 to 5 hr according to the anaesthetic (Tables 2 and 3). The values obtained for C_e were 0.69, 2.93 and 0.196% for halothane, ether and methoxyflurane respectively.

A possible criticism of this method of determining C_e is that the effective concentration of anaesthetic in the brain might not approach sufficiently closely to equilibrium during the exposure to anaesthetic. (Equilibration with the body as a whole would certainly take far longer than the exposure times used.) An alternative method of determining C_e was therefore used in which mice were anaesthetized to a stage of respiratory depression with a high concentration of anaesthetic before decreasing the inhaled concentration. Thus, the concentration of anaesthetic just necessary to produce the standard anaesthetic effect was exceeded and then approached in the opposite direction. Haggard (1924c), Robbins (1935)

TABLE 1

ESTIMATION OF THE INHALED CONCENTRATION (C_e) OF HALOTHANE WHICH JUST CAUSED FIVE OUT OF TEN MICE TO LOSE THEIR RIGHTING REFLEX

The presence of the righting reflex was tested just before altering the inhaled concentration (v/v) of anaesthetic

Inhaled concentration (%)	Time (min)	No. of mice with righting reflex abolished	Respiration
1·97	0	0	
	3	10	
	10	10	
0·59	15	10	Some breathing slowly
	30	5	(less than 100/min)
1·97	40	2	
	50	10	Depressed
0·79	55	10	
	75	10	
	115	10	
1·97	150	10	Severely depressed
0·59	160	10	
	190	10	
	215	6	
	235	4	
	275	2	

TABLE 2

THE TIMES REQUIRED FOR ONSET OF, AND RECOVERY FROM, ETHER ANAESTHESIA

Observations were on groups of ten mice. A is the number of mice anaesthetized to loss of righting reflex; D, the number dead in this time; t_A, the time taken for five out of ten mice to lose their righting reflex; and t_R the time taken for five out of ten mice to walk after end of exposure to anaesthetic

Inhaled concentration (%)	Time of exposure (min)	A	D	t_A (min)	t_R (min)
2·54	240	0	0	—	—
2·84	240	4	0	—	—
	120	3	0	—	—
3·03	120	7	0	50	0·5
	60	6	0	52	0·5
	60	10	0	42·5	0·5
	60	5	0	52·5	0·25
3·35	120	10	1	25	2
	120	10	0	19·5	4·75
	120	10	0	20	5·25
	120	9	0	20·5	4
	60	10	0	16·5	2
	60	10	0	18·5	1·75
3·80	60	10	0	18	3
	70	10	2	16	3·25
	70	10	0	20	3·75
4·06	60	10	2	10	2
	60	10	1	11·5	5
	60	10	1	10	3·25
4·57	60	10	3	8	7·75
	60	10	5	6·5	11
	60	10	5	8·5	—
	60	10	6	8	—
	60	10	5	7·5	11·5
5·08	60	10	10	6·5	—
	60	10	10	6·5	—
	21	10	0	6·25	—
7·09	15	10	1	2·5	3·5
	15	10	1	2·5	3·5
	15	10	3	2·5	4
12·9	5	10	0	0·75	3
	5	10	0	0·75	2·75

TABLE 3

THE TIMES REQUIRED FOR ONSET OF, AND RECOVERY FROM, METHOXYFLURANE AND HALOTHANE ANAESTHESIA

The symbols have the same meaning as in Table 2

Methoxyflurane						Halothane					
Inhaled concentration (%)	Duration of exposure (min)	A	D	t_A (min)	t_R (min)	Inhaled concentration (%)	Duration of exposure (min)	A	D	t_A (min)	t_R (min)
0·177	300	2	0	—	—	0·59	180	0	0	—	—
0·216	245	8	0	165	1·5	0·79	90	7	0	72	0·25
0·246	120	3	0	—	—		90	6	0	75	0·5
	185	7	0	138	—		120	7	0	90	0·5
0·346	120	10	0	70	2	0·89	60	7	0	40	0·25
	90	7	0	64	3		60	7	0	45	0·5
	90	7	0	60	1·5		85	6	0	65	0·5
0·494	120	10	0	28	—	0·98	30	8	0	10	1·25
	120	10	0	20	63		30	8	0	12	0·75
	120	10	0	28	34		60	10	0	11·5	1·5
	60	10	0	21·5	7·5	1·47	60	10	0	3·75	2·25
	60	10	0	30	1		60	10	0	4	1·75
0·592	80	10	0	17	21	1·97	15	10	0	1·5	—
	60	10	1	16	10		15	10	0	1·5	1·75
0·737	60	10	0	7·5	16·5		15	10	0	1·25	1·75
	60	10	0	8	14		30	10	0	1·25	1·75
	60	10	0	5	16·5		30	10	0	1·5	2
0·98	60	10	1	2·25	21·5		60	10	0	1·75	5·5
	60	10	0	2·25	22		60	10	0	1·5	6·5
	60	10	2	2·75	27		60	10	0	1·75	6·25
1·48	60	10	1	0·75	47						
	60	10	0	1·5	43						
	60	10	3	1·25	40						

and Raventos (1956) have shown that a short preliminary exposure to a high concentration of anaesthetic hastens the achievement of equilibrium to a subsequent lower inhaled concentration of anaesthetic. The results of one such experiment are summarized in Table 1. In this experiment ten mice were first anaesthetized deeply with 1.97% halothane before decreasing the concentration to 0.59%. This low concentration failed to maintain anaesthesia as, 25 min later, all but two mice were active. The inhaled concentration was then increased to 1.97% and, as soon as the respiration became depressed, replaced by an inhaled concentration of 0.79%. The anaesthesia became lighter during the exposure to 0.79% halothane, but a light anaesthesia was still maintained at the end of 95 min. Then the mice were re-exposed to 1.97% until the respiration became severely depressed. Eight of the ten mice subsequently regained their righting reflex during the following exposure to 0.59% halothane, but recovery was slower than previously. Nearly 2 hr were needed for eight out of ten mice to recover; whereas earlier only 25 min were needed. This slower recovery could have been due to a greater saturation of the " buffering " capacity of the body (see Haggard, 1924b; Duncan & Raventos, 1959), or to deleterious effects of a prolonged and sometimes profound anaesthesia. A similar effect was seen after repeated exposure of mice to a variety of ether or methoxyflurane vapour concentrations. The values obtained for C_e using this method were 0.69% for halothane, 2.93% for ether and 0.197% for methoxyflurane, results virtually identical with those obtained by the first method.

The onset of anaesthesia

The rates of onset of anaesthesia, analysed on the assumption that onset is governed by a single exponential process, are presented in Fig. 2. The results on which Fig. 2 is based are given in Tables 2 and 3. The three curves obtained for ether, halothane and methoxy-

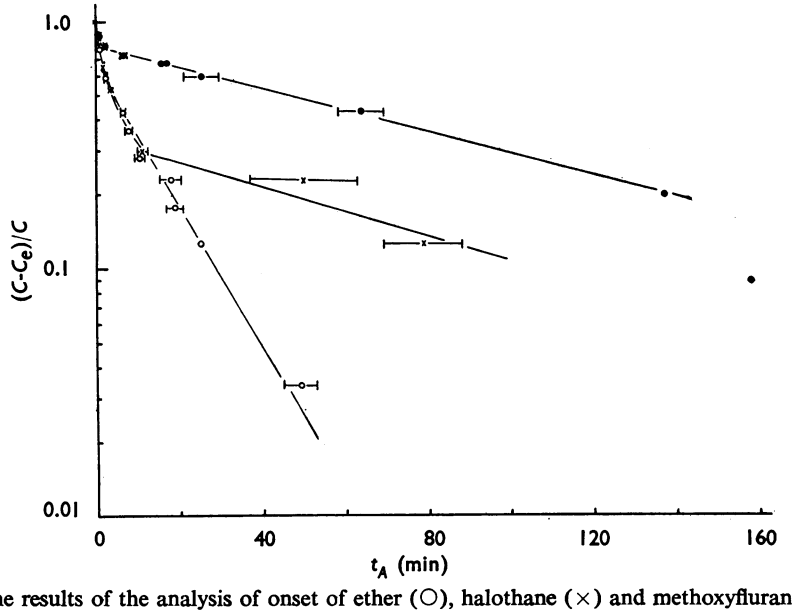

Fig. 2. The results of the analysis of onset of ether (○), halothane (×) and methoxyflurane (●) anaesthesias of mice. When three or more observations were made, using the same inhaled concentration, then the mean of the observations has been used with the standard deviation as a horizontal line. The other points each represent a single observation.

flurane differed markedly (Fig. 2). Moreover, the points did not fall on a straight line as would be expected if onset of anaesthesia were governed only by a single exponential process. But the curves for ether and methoxyflurane anaesthesias were still simple, consisting essentially of two components—an initial fast component followed by a slower component. For ether, the points fell on a straight line, possessing a half-time of about 11 min, after the first 3 min. The slow component for methoxyflurane anaesthesia also followed an exponential time course after the first few minutes, but one with a half-time of about 70 min. The onset curve for halothane was more complex, and could be analysed into three components. Initially, the curve for halothane anaesthesia followed a similar time course to that of ether, but after 10 min became slower and by the end of 1 hr was only about 83% complete. In the same time, the curve for ether anaesthesia was over 98% complete.

In interpreting these curves it is useful to obtain some idea of the way in which variations in C_e influence the shape of the curve. The curve given in Fig. 2 indicates that the anaesthetic effect of ether is more than 98% complete in 1 hr. Consequently the variations in the number of mice anaesthetized by different low concentrations of ether, for exposures exceeding 1 hr, should reflect mainly differences in C_e among mice. A value for the C_e of each mouse in a group of ten can be obtained by plotting the number of mice anaesthetized after 1 hr against the inhaled concentration. The C_e was $2.96 \pm 0.067\%$ (mean and standard error). In Fig. 3 the ether results have been replotted using values of C_e+2 s.e.m., C_e-2 s.e.m. and C_e (when $C_e=2.96\%$). The three straight lines have only slightly different slopes and the intercept on the ordinate is little altered. The half-times for the three slow components were 9, 10.5 and 14 min. These values undoubtedly exaggerate the magnitude

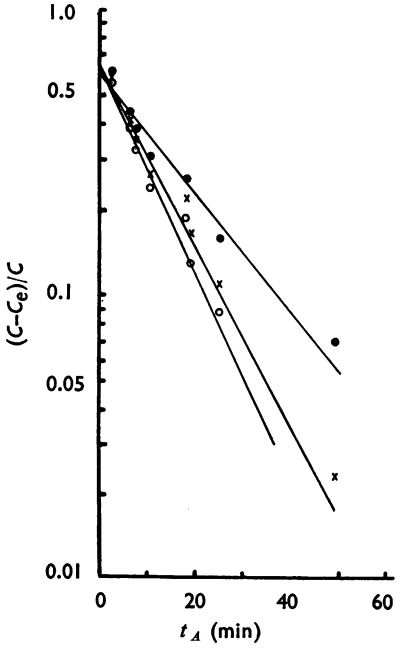

Fig. 3. The effects of variations in C_e on the shape of the onset of ether anaesthesia curve. The ordinate scale refers to $(C-C_e)/C$ (×), and to the same term with C_e replaced by C_e- two standard errors (●) and C_e+two standard errors (○).

of the possible error in the half-time arising from variations in C_e among mice. Part of the scatter in C_e has already been accounted for by using t_A values based on five out of ten mice. For instance, C_e+2 s.e.m. exceeds 3.03%, a concentration which was found to anaesthetize more than five mice in all four experiments (Table 2).

The initial deviation of the onset curves from linearity was, within the limits of the accuracy of the experiment, exponential. However, the rapidity of the first phase makes it difficult to exclude the existence of other components. The method of analysis is illustrated for ether in Fig. 4. A line (*b*) was drawn through the slowest linear part of the curve and

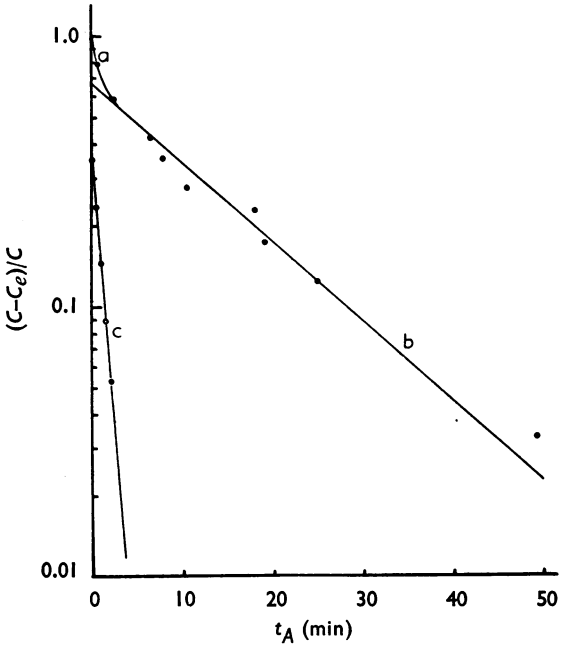

Fig. 4. Further analysis of the onset of ether anaesthesia curve. Details of the analysis are given in the text.

extrapolated to intersect the ordinate. The difference, at various times of exposure, between the straight line (*b*) and the curve (*a*) was determined and plotted on the same graph (*c*). With each of the anaesthetics the points for the initial rapid component fell on a straight line which had a half-time of about 0.5, 1 and 1.5 min for halothane, methoxyflurane and ether respectively. From the halothane curve, second and third components were derived, with half-times of 3 to 4 and 60 to 70 min respectively. The analysis of the halothane curve is less precise than for either the ether or methoxyflurane curves because of the scatter of points after the first 15 min. The half-times for the components of the halothane curve can therefore only be considered a rough estimate. The second component for methoxyflurane had a half-time of about 70 min.

The effect of ether on the respiration of a mouse

To attribute differences in the onset of anaesthesia solely to differences in the solubility of the three anaesthetics in blood or fat necessitates assuming that they have either a similar

or a slight effect on blood flow and respiration. For respiration, this assumption was obviously questionable, in the light of previous work. Halothane and methoxyflurane have little effect on the minute volume of dogs during light anaesthesia or depress it slightly (Raventos, 1956; Hall & Norris, 1958; D'Arcy, Holmdahl & Payne, 1959; Dobkin & Fedoruk, 1961; North, Knox, Vartanian & Stephen, 1961), but ether has a marked stimulant effect on the respiration of dogs, cats and rabbits, an effect often pronounced during light anaesthesia (Haggard, 1924d; Schmidt, 1938; Dripps & Dumke, 1943; Gordh, 1945; Albers, Brendel & Usinger, 1955; Katz & Ngai, 1962). As ether is relatively soluble in blood, an increase in minute volume would be expected to accelerate the uptake of ether and hence speed the induction of anaesthesia.

However, no sign of respiratory stimulation was seen during ether anaesthesia in mice. Concentrations of ether from 2.5 to 6% invariably depressed respiration. Results of a

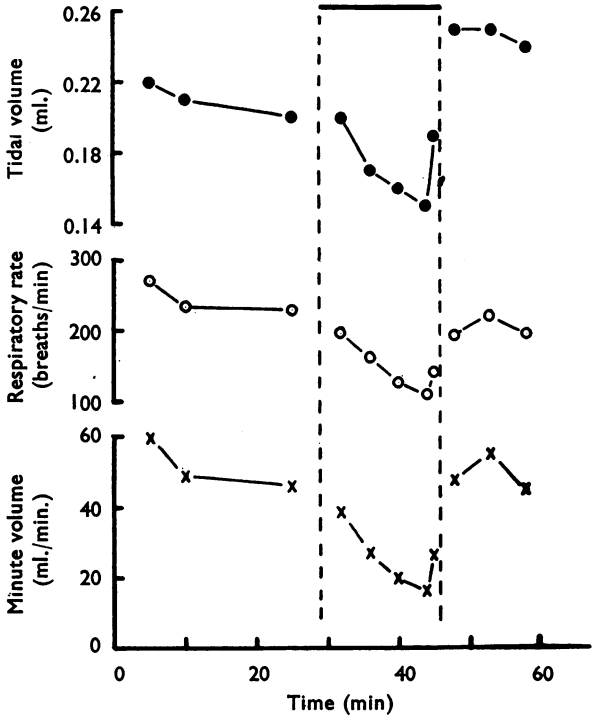

Fig. 5. The effect of 4% v/v ether (between the vertical interrupted lines) on the respiratory rate, tidal volume and minute volume of a 22-g mouse.

typical experiment are shown in Fig. 5. After 3 min exposure to ether the respiratory rate had fallen slightly and, as the exposure continued, the rate became progressively slower. Tidal volume also fell. Both respiratory rate and tidal volume recovered promptly when administration of the anaesthetic was stopped. The respiratory rate of conscious mice was 224 ± 19 per min; the tidal volume, 0.193 ± 0.036 ml.; and the respiratory minute volume, 43.3 ± 9.1 ml./min (means and standard deviations for seven mice).

Recovery from anaesthesia

The equation for recovery from anaesthesia, $\ln (C_e/C) = -k_3 t$, is valid only when equilibrium concentrations of anaesthetic have been attained in the brain. Allowance can be made for non-achievement of equilibrium by inserting a correction factor, y, into the equation:

$$\ln (C_e/yC) = -k_3 t$$

where y is the fraction of the equilibrium concentration achieved by the end of the anaesthesia. This correction factor can be obtained from the onset of anaesthesia curves and is valid provided that three conditions are fulfilled. These are: (1) that the onset of anaesthesia curve is identical to the curve for uptake of anaesthetic by the whole brain; (2) that the differing amounts of anaesthetic contained in the various tissues, as a result of their differing rates of uptake of anaesthetic, have no effect on the elimination of anaesthetic from the brain; and (3) that the respiratory and blood flow characteristics of the mice are not much different during and at the end of anaesthesia of differing depth and duration. All three assumptions are certainly unjustified and this is borne out by the results of the analysis given in Fig. 6. The analysis of recovery from anaesthesia based on brief administrations revealed a more rapid rate of recovery than did the analysis using recoveries from longer anaesthesia. One contributing factor was undoubtedly the respiratory depression produced by the higher anaesthetic concentrations; these had to be used so that the critical endpoint occurred during recovery. Thus, with 4.57% ether the respiratory depression was such that about half the mice died (Table 2). Respiratory depression would also

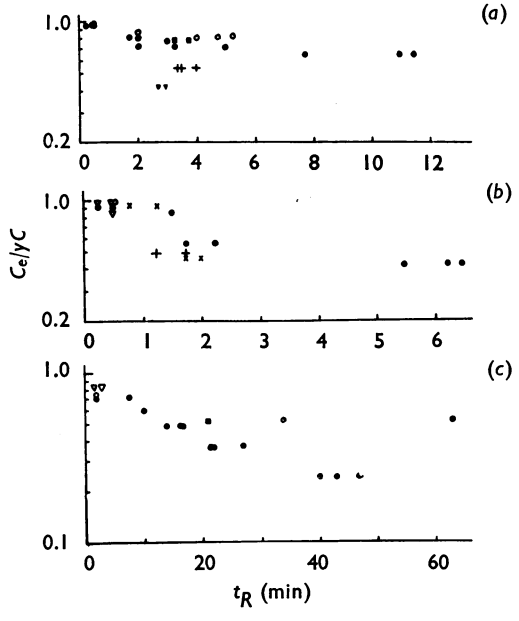

Fig. 6. Results of the analysis of the recovery of mice from ether (*a*), halothane (*b*) and methoxyflurane (*c*) anaesthesia. Each point represents the time taken for five out of ten mice to walk. The times of exposure to anaesthetic were 5 min (▼), 15 min (+), 30 min (×), 60 min (●), 70 to 80 min (■), 85 to 90 min (▽) and 120 min (○). The room temperature was 28 to 31° C.

slow the uptake of anaesthetic, particularly of ether and methoxyflurane, and the uptake curve could no longer be expected to remain the same as in the experiments in which the onset of anaesthesia curve was determined.

Despite the respiratory depression, a reasonably linear relationship was obtained from methoxyflurane administrations of the same duration but of different depths (Fig. 6,*c*). This was true also for ether anaesthesia (Fig. 6,*a*), but was less obvious for halothane anaesthesia (Fig. 6,*b*).

DISCUSSION

In analysing the kinetics of the onset of anaesthesia, two important assumptions were made. The first was that the rate constant(s) were independent of the inhaled concentration of anaesthetic. This assumption can only be approximately true during induction of anaesthesia while anaesthesia is still light. Deeper anaesthesia is well known to have profound effects on both respiration and blood flow, and hence on uptake of anaesthetic. For this reason, the first clear sign of unconsciousness of mice was chosen as the standard anaesthetic effect. This choice was vindicated by the relative simplicity of the onset of anaesthesia curves. The second assumption was that equal concentrations of the same anaesthetic exist at its sites of action in the brain when a standard anaesthetic effect is observed, irrespective of the way in which this concentration is achieved. This assumption also appeared to be valid under the present experimental conditions. The concentration, C_e, was the same irrespective of whether it was determined by using constant low concentrations of anaesthetic or by first exceeding C_e and then approaching it from the opposite direction by lowering the inhaled concentration.

The experimentally determined curve of log $(C-C_e/C)$ against t_A should theoretically be identical to the curve obtained from exponential analysis of the uptake of the same anaesthetic at its sites of action in the brain. In practice this would be expected only when the inhaled concentrations are low so that respiratory and blood flow changes are absent or minimal. This does not mean that the two curves need be identical to the curve for the uptake of anaesthetic by the brain as a whole for the anaesthetic may not be equally soluble in all parts of the brain and, further, the blood flow to different parts of the brain differs (see Sokoloff, 1959).

Of the factors influencing uptake of anaesthetic by the brain, only one can apparently give rise to a short-lasting effect. This is lung dilution, the initial dilution of the inhaled anaesthetic vapour by the gas already present in the lungs and by the pulmonary blood flow. However, it seems unlikely that lung dilution is rate-limiting because of the large minute volume of a mouse relative to its size. Bucher (1949) has measured the tidal volume, respiratory rate and functional residual lung capacity of a mouse. He reported a mean respiratory rate of 228 per min, a mean tidal volume of 0.22 ml. and a mean residual lung capacity of 0.45 ml. for ether-anaesthetized mice. From these values the half-time for lung washout—the initial diluting effect of the gas already in the lungs on the new inhaled mixture—can approximately be calculated using the equation of Darling, Cournand & Richards (1944). The calculated half-time is about 0.6 sec, assuming a dead space equal to 25% of the tidal volume, a value which suggests that lung washout is too brief to be rate-limiting. Even if the dead space is as much as 40% of the tidal volume, the half-time is still short (about 0.7 sec). Bucher's values are higher than would be expected from the

present observations, particularly as ether was found to depress the respiration of a mouse. We found that a mouse exposed to 4% ether for some 18 min had a respiratory rate of 158 per min and a tidal volume of 0.17 ml. Irrespective of these differences, the above argument remains valid. Thus the calculated half-time for washout of the lungs of a mouse taking breaths of 0.14 ml. at a rate of 180 per min and possessing a respiratory dead space of 25% of the tidal volume is only 1.1 sec. The equation of Darling *et al.* (1944) is an approximation since it is assumed that the lungs function as perfect bellows. The introduced error is, however, small (see Fowler, 1952) and seems unlikely to account for the more than forty-fold difference between the calculated half-time and the half-times for the first rapid phase of onset of anaesthesia.

The delay introduced by the pulmonary blood flow component of the lung dilution phase also seems negligible. Values of 7.5 to 40 ml. for the respiratory minute volume, 5 to 20 ml./min for the minute blood flow and 0.45 to 0.75 ml. for the alveolar volume, were inserted into equation 55 derived by Kety (1951) and the half-time for the first component was calculated for ether and halothane. In no instance did the half-time exceed 2 sec. Kety's equation 55 is an approximation, but again it is doubtful if the introduced error is sufficient to account for the difference between the calculated and observed values.

A more likely rate-limiting factor seems to be that controlling transfer of anaesthetic from the lungs to the brain, that is, cerebral blood flow. This could explain the similar half-times found for the first component of the three onset curves. But it does not readily explain the similar contribution of the first components to their respective onset curves. The first component would be expected to contribute more to the curve for halothane than to the curves for either ether or methoxyflurane because of the smaller blood/air partition coefficient of halothane (see Kety, 1951). Instead, the contribution was 30% for halothane, 34% for ether and 24% for methoxyflurane. An idea of the expected difference can readily be obtained from Kety's equation 55 by inserting values for the variables such that the predicted curve is similar to that obtained for the onset of ether anaesthesia. The values chosen were: respiratory minute volume, 30 ml.; pulmonary blood flow, 5 ml./min; alveolar volume, 0.55 ml.; volume of body, 20 ml.; and a blood/air partition coefficient of 15. The calculated contribution of the first component to the total onset curve was 28.5%. On repeating the calculation using the blood/air partition coefficient of halothane (2.3), but leaving all other values unchanged, the first component made up 72% of the total calculated curve.

A possible explanation for this discrepancy is that the three anaesthetics were having markedly different effects on respiration and/or blood flow. There is no reason to suspect the validity of the implicit assumption that diffusion of anaesthetic from air to blood and from blood to tissue is almost instantaneous. This assumption has theoretical justification (Kety, 1951) and, moreover, has been shown to be valid for some gases (Jones, 1950). A likely possibility seemed to be a respiratory stimulant effect of ether. Such an effect of ether could explain the relatively large contribution of the first component to the total onset curve of ether. An attempt was made to see if ether had a respiratory stimulant effect on mice, but the results were inconclusive. Ether failed to stimulate respiration of the mice, but the existence of respiratory stimulation, under more normal conditions, could not be excluded because the measurement of the minute volume subjected the mice to some unavoidable stress.

TABLE 4

THE BLOOD/AIR AND OIL/WATER PARTITION COEFFICIENTS FOR ETHER, HALOTHANE AND METHOXYFLURANE

The figures in parentheses refer to the following references: (1) Shaffer & Ronzoni, 1923. (2) Jones, Baldes & Faulconer, 1950. (3) Haggard, 1923. (4) Robbins, 1935. (5) Eger, Shargel & Merkel, 1963. (6) Orcutt & Seevers, 1937. (7) Raventos, 1956. (8) Duncan & Raventos, 1959. (9) Larsen, Eger & Severinghaus, 1962. (10) This paper (see Methods). (11) Eger & Shargel, 1963. (12) Abbott Laboratories; personal communication

	Partition coefficients (at 37 to 38° C)	
Anaesthetic	Blood/air	Oil/water
Ether	14·9 (1)	3·2 (6)
	14·4 (2)	15·5 (7)
	15·2 (3)	5·3 (5)
	14·4 (4)	
	12·1 (5)	
Halothane	3·6 (8)	330 (7)
	2·3 (9)	300 (9)
Methoxyflurane	12·3 (10)	390–400 (12)
	13·0 (11)	183 (11)

Despite this complication, there were differences in the subsequent development of the three onset curves which were clearly attributable to the differing solubilities of the anaesthetics in blood and in fat. The second component of the halothane curve was the most rapid as would be expected for the anaesthetic having the smallest blood/air partition coefficient (Table 4). The onset curves for halothane anaesthesia also possessed a third component, a finding consistent with the relatively high solubility of halothane in fat (Duncan & Raventos, 1959). Ether is relatively insoluble in fat (Nicloux, 1907; Dybing & Skovland, 1957) and there was no third component in the ether onset curve. Further, methoxyflurane is the anaesthetic most soluble in both blood and fat (Table 4) and would be expected to induce anaesthesia the least rapidly. This was what was found. In the methoxyflurane curve only two components could be recognized. The last 10% of the curve, which might be illuminating, could not be determined because anaesthetic vapour concentrations differing only by 0.005% could not be produced. It is possible that the second component for this anaesthetic, in Fig. 2, in fact represents two components, a watery component merging into a fatty component too slowly for the two to be discriminated.

These results provide further support for the view that the kinetics of anaesthesia is open to prediction from mathematical models describing the uptake of anaesthetic. They also emphasize that allowance needs to be made for any effect of the anaesthetic on respiration or blood flow.

SUMMARY

1. A simple theory has been formulated with the object of studying the kinetics of halothane, methoxyflurane and ether anaesthesia of mice.

2. At an environmental temperature of about 30° C, groups of ten mice were exposed to different vapour concentrations of anaesthetic and the time taken for five out of the ten mice to lose their righting reflex was determined.

3. An analysis of the results, based on the theory, gave relatively simple curves for the onset of anaesthesia. The first component of the curve was rapid, being virtually complete in 3 to 5 min irrespective of the anaesthetic used. This component was thought to arise

from the initial dilution of the anaesthetic vapour during lung washout, but cerebral blood flow was considered to be the rate-limiting step. The time course of the second component, which was exponential, was greatly influenced by the anaesthetic used. The half-times were about 4, 11 and 70 min for halothane, ether and methoxyflurane respectively. The curve for halothane anaesthesia also possessed a third component.

4. It was concluded that some, but not all, of the differences between the onset curves for the three anaesthetics could be explained by their differing solubilities in blood and fat.

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