Comparison of the Effects of Equieffective Concentrations of Anesthetics on the Force of Contraction of Isolated Perfused Rat Hearts:

Correlation with the Equieffective Anesthetizing Partial Pressures

Raymond R. Paradise, Ph.D., and Frank Bibbins †*

Concentrations of chloroform, halothane, methoxyflurane and diethyl ether necessary to depress the force of contraction of the isolated rat heart by 50 per cent in the saline medium perfusing the heart at 29 C were 6.5, 6.9, 9.0 and 328 mg/100 ml, respectively. By making certain assumptions the partial pressures of these anesthetics required to depress the heart to the same extent at 37 C were calculated and were found to be (mm Hg): chloroform, 4.1; halothane, 12; methoxyflurane, 3.25; diethyl ether, 56. In terms of volume per cent, the concentrations required to depress the force of contraction by 50 per cent at 37 C would be 0.54, 1.58, 0.43, and 7.4 per cent, respectively. The calculated partial pressures were divided by the corresponding partial pressures required (1) to prevent response to a painful stimulus in dogs,[1] (2) to prevent a muscular response to skin incision in 50 per cent of a group of patients,[2] and (3) to anesthetize 50 per cent of goldfish,[3] all at 37 C. On the basis of the ratios obtained and the assumptions made, it appears that at a particular level of anesthesia chloroform produces most direct cardiac depression, and the ethers, methoxyflurane and diethyl ether, produce the least, with halothane assuming an intermediate value. To what extent the anesthetic-induced cardiac depression would be modified by compensatory hormonal and neuronal influences *in vivo* is not known. (Key words: Heart; Contractility; Anesthetics; Halothane; Chloroform; Methoxyflurane; Diethyl ether; Partial pressure.)

AN IDEAL ANESTHETIC would produce only anesthesia. Any other action is undesirable. No agent has yet been found to satisfy this criterion. However, intelligent use of anesthetic drugs requires a knowledge of the degrees of undesirable effects to be expected at given levels of anesthesia.

This paper reports an attempt to rate various anesthetics according to the severity of one of these undesirable actions, direct myocardial depression, at a particular level of anesthesia. The partial pressure of anesthetic required to produce a constant depression in myocardial contractility in the isolated perfused rat heart was determined. This was related to the partial pressure necessary to achieve particular levels of anesthesia in the dog,[1] human,[2] and goldfish.[3] On the basis of the relationships seen, it appears that at the same depth of anesthesia there would be more direct myocardial depression with chloroform than with halothane, and still less with methoxyflurane and diethyl ether.

Methods

Male rats weighing 149 to 355 g were killed by decapitation. The hearts were quickly removed and attached to the perfusion apparatus *via* the aorta. The time between sacrifice and start of perfusion was approximately three minutes. The perfusate consisted of a modified Krebs–Henseleit solution[4] bubbled with 95 per cent O_2–5 per cent CO_2. Perfusion height was maintained at 60 cm above the cannula. The temperature of the perfusate was kept constant at 29 C. At this temperature the force of contraction remains relatively constant for several hours. The heart was stimulated electrically at a rate of 180/min through two stainless steel electrodes placed in the perfusate surrounding it. Other details

* Associate Professor.
† Summer research fellow.
Received from the Department of Pharmacology, Indiana University Medical School, Indianapolis, Indiana 46202. Accepted for publication June 19, 1969. Supported in part by research grant PHS HE 07718 and in part with facilities provided by the Heart Research Center Grant H 6308 from the National Heart Institute, United States Public Health Service.

TABLE 1. Concentrations of Anesthetics in Perfusate Required to Produce Approximately 50 Per cent Depression of Force of Contraction of Isolated Perfused Rat Hearts at 29 C

	Experiment No.	First Sample			Second Sample			Third Sample		
		A (min)*	Force of Contraction (per cent)	(mg/100 ml)	B (min)†	Force of Contraction (per cent)	(mg/100 ml)	C (min)‡	Force of Contraction (per cent)	(mg/100 ml)
Halothane	1	48	52	5.8	9	52	5.8	8	51	5.4
	2	25	51	7.8	7	54	8.9	15	50	6.9
	3	46	49	7.0	27	51	6.5	5	49	6.9
	4	63	52	6.7	8	50	7.2	4	46	7.6
MEAN			51	6.9		52	7.1		49	6.7
Chloroform	1	95	56	7.6	14	51	6.5	8	53	7.8
	2	37	50	7.5	8	50	7.6	8	50	7.6
	3	82	50	6.5	5	50	6.2	5	48	6.5
	4	68	50	4.6	7	50	4.6	9	50	5.1
MEAN			52	6.5		50	6.2		50	6.8
Methoxyflurane	1	53	52	6.9	10	51	7.8	7	48	8.1
	2	69	48	9.9	8	50	10.1	7	49	9.9
	3	27	47	8.8	9	54	9.2	11	50	9.6
	4	54	48	9.0	7	48	8.9	5	46	9.1
MEAN			49	8.7		51	9.0		48	9.2
Diethyl ether	1	67	48	337	8	48	348	6	44	355
	2	88	46	296	7	46	303	7	44	309
	3	138	46	276	6	46	272	6	46	274
	4	53	52	379	6	54	394	5	45	388
MEAN			48	322		49	329		47	332

* Time hearts were exposed to anesthetic prior to first sample.
† Time elapsed between first and second samples.
‡ Time elapsed between second and third samples.

TABLE 2. Calculated Partial Pressures of Anesthetics Necessary to Produce a 50 Per cent Decrease in Force of Contraction of Isolated Perfused Rat Heart at 29 C

	No. of Hearts	Concentration in Perfusate at 29 C Causing 50 per cent Decrease in Force of Contraction (mg/100 ml ± SE)	Water/Gas Partition Coefficient at 29 C*	Saline/Gas Partition Coefficient at 29 C†	Concentration in Air in Equilibrium with Saline Necessary for 50 per cent Decrease in Force of Contraction at 29 C‡ (mg/100 ml)	Partial Pressure Necessary for 50 per cent Decrease in Force of Contraction at 29 C§ (mm Hg)
Chloroform	4	6.5 ± 0.6	4.7	4.5	1.4	2.2
Halothane	4	6.9 ± 0.5	1.1	1.0	6.9	6.6
Methoxyflurane	4	9.0 ± 0.5	6.0	5.7	1.6	1.8
Diethyl ether	4	328 ± 24	23.2	22.0	14.9	38.1

* Calculated from data in figure 1.
† Assumed to be 5 per cent less than water/gas partition coefficient. Larson *et al.*[6] reported that the partition coefficient for halothane between saline and gas was 5 per cent less than that between water and gas at 37 C.
‡ Calculated from saline/gas partition coefficient.
§ Calculated from Ideal Gas Law: PV = nRT.

of the perfusing system have been reported previously.[4]

After an equilibration period ranging from 32 to 101 minutes, chloroform, halothane, methoxyflurane or ether was bubbled into the perfusate in sufficient concentration to keep the force of contraction depressed by 50 per cent. The techniques involved have been described previously.[4, 5] Three samples of perfusate were taken from each heart while it was depressed 50 per cent. Four hearts were used with each anesthetic. Following this procedure the hearts were perfused with fresh Krebs–Henseleit medium. Recovery of force of contraction 15 minutes later averaged 93 per cent in the hearts exposed to chloroform, halothane and diethyl ether, and 95 per cent in those exposed to methoxyflurane.

The method for determining the concentration of anesthetic in the perfusate has been reported previously.[4, 5] It involved extraction of the anesthetic from the saline perfusate into an equal amount of tetrachlorethylene, with subsequent analysis using a Barber Coleman thermal conductivity gas chromatograph. Extractions of chloroform, halothane and methoxyflurane were essentially complete. However, only 91 per cent of the ether present in the saline perfusate was extracted by the tetrachlorethylene. Therefore, the ether values obtained were corrected by dividing by 0.91.

The relationships between concentration of chloroform, halothane and methoxyflurane in water at room temperature and their corresponding vapor pressures in the gas phase above the water in a closed system were determined. Various concentrations of anesthetic were mixed with water in a separatory funnel connected to a mercury manometer. Changes in partial pressure on introduction of anesthetic were correlated with anesthetic concentration in the water phase. Analysis of anesthetic concentration in water was the same as described above.

Results

Following an appropriate equilibration period the force of contraction was measured. This value will be called the initial value. Anesthetic was then administered into the perfusate by means of the previously described anesthetistat.[4, 5] Adjustments were made on the anesthetistat until, at a constant setting, the force of contraction was observed to remain constant at approximately 50 per cent of the initial force for a period of 5–10 min. The time required varied from 25 to 138 min (table 1, column A). The force of contraction was then measured, and a sample of perfusate taken for analysis of anesthetic concentration (table 1). Following a period of 5–22 min (table 1, column B), the force of contraction was again recorded and a second sample of perfusate obtained for analysis of anesthetic. During time interval B minor adjustments were made in the anesthetistat when necessary to maintain the force of contraction at approximately 50 per cent depression. Finally, another period of 4–15 min (table 1, column C) was allowed to elapse before the force of contraction was again recorded and a third sample of perfusate analyzed for anesthetic. During time interval C minor adjustments in the anesthetistat were made occasionally to maintain the force of contraction at approximately 50 per cent depression. That the force of contraction and anesthetic concentrations varied little during the three sample periods is taken as evidence for the achievement and attainment of a relatively stable steady state.

The mean concentrations in the saline perfusate of chloroform, halothane, methoxyflurane, and diethyl ether required to depress the force of contraction by 50 per cent were 6.5, 6.9, 9.0 and 328 mg/100 ml, respectively (table 2). To relate the concentrations to those required for achievement of particular levels of anesthesia, it was desirable to convert the values to partial pressures (mm Hg). One can do this if one knows the saline/gas partition coefficient at 29 C (putting the anesthetic into the gas phase, then converting the concentration of anesthetic in the gas phase into mm Hg partial pressure, assuming the anesthetic behaves as an ideal gas and obeys the gas law, $PV = nRT$). Unfortunately, saline/gas partition coefficients at 29 C could not be found. Water/gas partition coefficients, calculated at different temperatures from the data presented by Cherkin and Catchpool,[3] are seen in figure 1. (The ideal

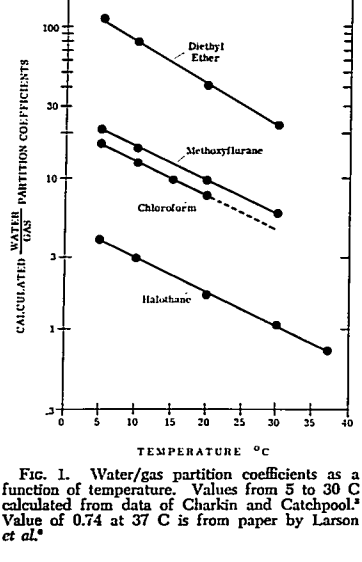

FIG. 1. Water/gas partition coefficients as a function of temperature. Values from 5 to 30 C calculated from data of Charkin and Catchpool.[3] Value of 0.74 at 37 C is from paper by Larson *et al.*[6]

gas law PV = nRT was used to convert the vapor pressures of the anesthetics to concentrations in the gas phase which would be at equilibration with saturated solutions of the anesthetics in water. Partition coefficients were then determined by dividing the concentration of each anesthetic in the aqueous phase by its equilibrium concentration in the gas phase.) As can be seen, there is a linear relation between the logarithm of the partition coefficient and temperature. For these calculations Henry's law was used. One form of this law states that the mass or concentration of gas in solution is related to the partial pressure (or concentration) of the gas above the solution in a closed system at equilibrium. Figure 2 shows that this law holds for chloroform, halothane and methoxyflurane in water at room temperature up to the saturation concentration. Thus, for each of these three anesthetics the water/gas partition coefficients is independent of concentration. Calculations of water/gas partition coefficients from the vapor pressure and solubility data in the paper by Cherkin and Catchpool[3] as depicted in figure 1 therefore should be valid. The situation with diethyl ether is perhaps not so firmly grounded. Eger *et al.*[7] found a small increase in the water/gas partition coefficient at 37 C with high ether concentration. Increasing the ether concentration from 4.1 to 56 per cent increased the partition coefficient from 13.0 to 14.2. An interaction of the unshared electron pairs of the ether oxygen with water molecules may account for this. Thus, the water/gas partition coefficients for ether calculated from the saturation concentration and vapor pressure data from Cherkin and Catchpool[3] may be slightly higher than would be seen at the concentrations of ether used in our experiments. This is probably no greater than 5–7 per cent, however, and was not taken into consideration.

The water/gas partition coefficients at 29 C in table 2 were interpolated from figure 1 for diethyl ether, methoxyflurane and halothane, and extrapolated for chloroform. The partition coefficient for halothane at 37 C (0.74, fig. 1) is that given by Larson *et al.*,[6] and agrees with the expected linear extrapolated

FIG. 2. Relationships of vapor pressures of anesthetics to concentrations in water in a closed system at equilibrium at room temperature (25.1 C for halothane; 24.5 C for chloroform and methoxyflurane).

TABLE 3. Partial Pressures of Anesthetics Required to Produce Equipotent Cardiac Depressant and Anesthetic Effects at 37 C

	Cardiac Depression (rat)* (mm Hg)	Anesthesia (dog)† (mm Hg)	Anesthesia (man)‡ (mm Hg)	Anesthesia (goldfish)§ (mm Hg)
Chloroform	4.1	5.9	—	3.36
Halothane	12	6.6	5.85	4.12
Methoxyflurane	3.25	1.7	1.21	0.67
Diethyl ether	56	23.1	14.59	13.0

* Calculated on the assumption that the slope of the partial pressure curve necessary to produce 50 per cent depression of cardiac contractility as a function of temperature for the isolated perfused rat heart is the same as the slope of the curve relating the partial pressure to anesthetize 50 per cent of goldfish as a function of temperature.[3]

† Data from Eger *et al.*[1-3]

‡ Data from Saidman *et al.*[2]

§ Data from Cherkin and Catchpool.[3]

value of Cherkin and Catchpool. Saline/gas partition coefficients at 29 C for the various anesthetics (table 2) were assumed to be 5 per cent less than the corresponding water/gas values, because Larson *et al.*[6] found the saline/gas partition coefficient for halothane to be 5 per cent less than the water/gas partition coefficient at 37 C.

Using these calculated saline/gas partition coefficients, the partial pressures of chloroform, halothane, methoxyflurane and diethyl ether necessary to cause a 50 per cent decrease in contractile force were 2.2, 6.6, 1.8, and 38.1 mm Hg, respectively (table 2).

Table 3 shows the partial pressures of the anesthetics required to produce equipotent cardiac depressant effects in rats and equipotent anesthetic effects in dogs, humans, and goldfish at 37 C. The partial pressures required to produce 50 per cent depression in cardiac contractility in the rat at 37 C were calculated from the data obtained at 29 C and corrected to the values expected at 37 C, assuming the slopes of the curve relating anesthetic pressure required to anesthetize 50 per cent of goldfish as a function of temperature[3] to be the same as that relating partial pressure to depress cardiac contractility as a function of temperature. The third column in table 3 shows the partial pressures of the anesthetics necessary to prevent response to a painful stimulus in dogs.[1] The next column indicates the partial pressures required to prevent muscular response to a skin incision in 50 per cent of a group of patients.[2] The last column shows the extrapolated partial pressure needed to anesthetize 50 per cent of goldfish at 37 C.

The ratios of the partial pressures of anesthetics required to produce 50 per cent depression of cardiac contractility in the rat to the partial pressures required to produce anesthesia in dog, man and goldfish at 37 C are depicted in table 4. In most cases the ratio is greater than one, indicating that anesthesia can be produced without depressing contractility by 50 per cent. With chloroform in the dog, however, a ratio of less than one indicates depression of contractility greater than 50 per cent at anesthetic levels. The ratio is lowest for chloroform and highest for the two ethers (methoxyflurane and diethyl ether) with halothane having an intermediate value. Providing our previous assumptions are correct, it appears that the least cardiac depression occurs with the ethers, with halothane next and chloroform producing the most cardiac depression at a given level of anesthesia.

Discussion

In a previous paper[5] the concentration of chloroform in the saline medium required to depress the force of contraction of the isolated rat atria at 27 C was found to be 6.1 ± 0.25 mg/100 ml. This is in close agreement with the value of 6.5 ± 0.6 mg/100 ml reported in this paper for the perfused rat heart at 29 C. Likewise, the value of 6.9 ± 0.5 mg/100 ml for halothane reported here is close to the value of approximately 6 mg/100 ml, also at 29 C, previously reported.[4] In the latter pa-

TABLE 4. Ratios of Partial Pressures Required to Produce 50 per cent Depression of Cardiac Contractility in the Rat to Partial Pressures Required to Produce Anesthesia in Dog, Man and Goldfish at 37 C

	Partial Pressure to Depress Rat Heart / Partial Pressure to Anesthetize Dog	Partial Pressure to Depress Rat Heart / Partial Pressure to Anesthetize Man	Partial Pressure to Depress Rat Heart / Partial Pressure to Anesthetize Goldfish
Chloroform	0.69	—	1.22
Halothane	1.82	2.05	2.91
Methoxyflurane	1.91	2.69	4.85
Diethyl ether	2.42	3.84	4.31

per, a blood/saline coefficient of 3.3, calculated at 37 C, was assumed to hold true at 29 C also. Thus, a saline concentration of 6 mg/100 ml would be equivalent to a blood concentration of 19.8 mg/100 ml. Blood levels of 17.9 to 20.3 mg/100 ml were found necessary to anesthetize a dog just sufficiently to produce a loss in the pain reflex of the foot pad.[4] It was concluded, therefore, that "if the dog behaved like the rat, one might conclude that surgical anesthesia with halothane would result in a 50 per cent decrease in contractile force." That the isolated hearts were at 29 C and the dogs at 37 C was not taken into consideration, however. Cherkin and Catchpool[3] have shown that for halothane a ten-degree increase in temperature requires approximately a doubling of the partial pressure required to produce anesthesia in the goldfish. Eger *et al.*[9] found a similar result in the dog. Although not yet tested, it is likely that cardiac depression with halothane is also a function of temperature. In this report it was assumed to follow the same slope as that found by Cherkin and Catchpool in anesthetizing goldfish.[3] Based on that assumption, the partial pressure necessary at 37 C to depress the rat heart by 50 per cent would be 1.82 times greater than that required to prevent movement in response to a painful stimulus in the dog (table 4). Therefore, at a partial pressure of halothane just sufficient to produce this degree of anesthesia in the dog, one would expect less than a 50 per cent decrease in the force of contraction, assuming that rat and the dog responded similarly to the same partial pressure of halothane.

It may be noted that in the last column of table 3 the same figures for this ratio would hold if, instead of calculating the partial pressure to depress the rat heart at 37 C from the slope of the curve relating temperature to anesthetizing partial pressure in the goldfish, as was done, we had used the goldfish data at 29 C for the various anesthetics and divided it into the partial pressure needed to depress the rat heart at 29 C. Thus, insofar as the goldfish at 29 C is concerned, it appears that at equal levels of anesthesia cardiac depression would be most severe with chloroform, least with the ethers, and intermediate with halothane. That the same would hold at 37 C in the goldfish as well as in the other species is subject to further experimentation. Further evidence for this rating may be obtained from the experiments of Robinson *et al.*[10] They reported the following partial pressures of anesthetic required to anesthetize 50 per cent of shrimp larvae at 20 C: chloroform, 1.4 mm Hg; halothane, 2.3 mm Hg; diethyl ether, 6.8 mm Hg. Dividing these partial pressures into the calculated partial pressures of these same anesthetics required to produce 50 per cent depression of cardiac contractility at 29 C gives the following ratios: chloroform, 1.6; halothane, 2.9; diethyl ether, 5.6. Therefore, the same rating applies to the shrimp larvae as to the other species reported above.

In summary, and subject to the assumptions stated above, it appears that in various species production of similar levels of anesthesia is accompanied by similarly varying degrees of inhibition of cardiac contractility, with chloroform producing the most, the ethers, methoxyflurane and diethyl ether the least, and halothane an intermediate amount of cardiac depression. The actual degree of cardiac depression seen *in vivo*, however, would be a

function not only of the partial pressure of anesthetic but also of compensatory humoral and neuronal components. Since these components are absent in the *in vitro* perfused heart we cannot predict the actual degree of *in vivo* cardiac depression from these studies. What we have attempted to identify are the relative degrees of direct cardiac depression to be expected at the same depth of anesthesia with the four anesthetics tested, prior to and in the absence of compensatory adjustments.

Halothane(Fluothane) was supplied by Ayerst Laboratories. Methoxyflurane (Penthrane) was supplied by Abbott Laboratories. The authors are grateful to Mr. Gene Ice, a High School Science Institute student, for technical assistance.

References

1. Eger, E. I., II, Brandstater, B., Saidman, L. J., Regan, M. J., Severinghaus, J. W., and Munson, E. S.: Equipotent alveolar concentrations of methoxyflurane, halothane, diethyl ether, fluroxene, cyclopropane, xenon, and nitrous oxide in the dog, ANESTHESIOLOGY 26: 771, 1965.
2. Saidman, L. J., Eger, E. I., II, Munson, E. S., Babad, A. A., and Muallem, M.: Minimum alveolar concentrations of methoxyflurane, halothane, ether and cyclopropane in man: Correlation with theories of anesthesia, ANESTHESIOLOGY 28: 994, 1967.
3. Cherkin, A., and Catchpool, J. F.: Temperature dependence of anesthesia in goldfish. Science 144: 1460, 1964.
4. Paradise, R. R., and Griffith, L. K.: Electrolyte content of perfused rat ventricles exposed to halothane or anoxia, J. Pharmacol. Exp. Ther. 154: 281, 1966.
5. Paradise, R. R., and Griffith, L. K.: Control of concentration of volatile agents in open *in vitro* systems, ANESTHESIOLOGY 27: 687, 1966.
6. Larson, C. P., Eger, E. I., II, and Severinghaus, J. W.: Solubility of halothane in blood and tissue homogenates, ANESTHESIOLOGY 23: 349, 1962.
7. Eger, E. I., II, Shargel, R., and Merkel, G.: Solubility of diethyl ether in water, blood and oil, ANESTHESIOLOGY 24: 676, 1963.
8. Eger, E. I., II, Lundgren, C., Miller, S. L., and Stevens, W. C.: Anesthetic potencies of sulfur hexofluoride, carbon tetrafluoride, chloroform and Ethrane in dogs: Correlation with the hydrate and lipid theories of anesthetic action, ANESTHESIOLOGY 30: 129, 1969.
9. Eger, E. I., II, Saidman, L. J., and Brandstater, B.: Temperature dependence of halothane and cyclopropane anesthesia in dogs: Correlation with some theories of anesthetic action, ANESTHESIOLOGY 26: 764, 1965.
10. Robinson, A. B., Manly, K. F., Anthony, M. P., Catchpool, J. F., and Pauling, L.: Anesthesia of artemia larvae: Method for quantitative study, Science 149: 1255, 1965.

Drugs

LEVARTERENOL IN INFLAMMATION A sterile inflammation was instituted in dogs by injection of 10 per cent calcium chloride into the muscles of the thigh. Four days later blood flow to that leg was increased and A-V oxygen difference was decreased. There was no change in oxygen consumption. The increased blood flow, through dilated capillary beds or arteriovenous shunts, may contribute to high-output cardiac failure. Levarterenol was given by infusion in an amount sufficient to raise the blood pressure 10 to 20 mm Hg. This reduced the blood flow in the inflamed leg to normal, and brought A-V difference and oxygen consumption toward normal. This study demonstrates that catecholamines may reduce the arteriovenous shunting of blood in inflamed tissues. (*Hopkins, R. W., and others: Effects of Levarterenol on Blood Flow in Inflammation, Arch. Surg. 97: 1032 (Dec.) 1968.*)