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THE EFFECT OF SOME VOLATILE ANAESTHETICS ON THE TRANSMURALLY STIMULATED GUINEA-PIG ILEUM

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Many volatile anaesthetics have been shown to inhibit intestinal movements during anaesthesia. The inhibition so produced has been attributed to: (1) a direct effect of the anaesthetic on the smooth muscle; (2) an action on the intrinsic nerves of the intestine; (3) an effect on the extrinsic nerve supply; (4) release of some gastrointestinal inhibiting substance into the blood stream; and (5) to hypoxia accompanying the anaesthesia (Miller, 1926; Bhatia & Burn, 1933; Weisel, Youmans & Cassels, 1938; Bisgard & Johnson, 1939; Youmans, Enos, Kastens & Aumann, 1942; Golden & Mann, 1943; Furuse, 1958; Burn & Epstein, 1959; Raventós, 1961; Marshall, Pittinger & Long, 1961). Actions either (1) or (2) seems probable for most if not all volatile anaesthetics as extrinsic denervation, adrenalectomy or a low concentration of inhaled oxygen fails to abolish the depressant effects of those anaesthetics investigated. There have, however, been few attempts, and these only recently, which distinguish between an action on the intrinsic nerves and an action on the muscle itself. These reports are conflicting. Raventós (1961) reported that the response of the cat intestine to acetyl-β-methylcholine—a direct muscle stimulant—was unaffected at a time when halothane had abolished the contraction of the intestine to vagal stimulation. Marshall *et al.* (1961) found, in contrast, that halothane did depress the response of the dog intestine *in situ* to acetyl-β-methylcholine.

The purpose of the present experiments was to see if anaesthetics depressed intestinal movements by an action on the muscle, on the intrinsic nerve supply, or both. The transmurally stimulated guinea-pig isolated ileum preparation (Paton, 1955) was chosen since it provided a cholinergic nerve-muscle preparation which would be sensitive to drugs depressing either nervous tissue or smooth muscle.

METHODS

The methods used were essentially those described by Paton (1955, 1957a). A guinea-pig was stunned by a blow on the head and then bled, and a length of ileum from near the caecum was removed. After the lumen of the ileum was washed out, the aboral end of a suitable length of ileum was tied to a length of polyethylene tubing leading to a reservoir. A platinum electrode was inserted into the lumen of the oral

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end of the gut and the ileum was tied to the polyethylene sleeve of the electrode. The ileum was then placed in an organ-bath (7 ml. capacity) containing Krebs solution and the electrode attached to an auxotonic lever (Paton, 1957b), the pendulum weight of which was kept constant. Contractions were recorded on a smoked drum. A second platinum electrode was placed in the body of the bath. A mixture of 95% oxygen and 5% carbon dioxide was bubbled continuously through the organ-bath, the temperature of which was maintained at 34 to 36° C.

The ileum was stimulated at 1-sec intervals with rectangular pulses of 0.5 or 1 msec duration and of sufficient strength to produce maximal contractions except when chemical stimuli were applied. Supramaximal shocks, 50 to 100% stronger than the maximally effective strength, were used in all experiments.

The various solutions containing anaesthetic were prepared by adding a known amount of anaesthetic to a known volume of Krebs solution. The air space in the reservoir was kept to a minimum (less than 7% of the total volume) in order to reduce loss of anaesthetic from the aqueous phase into the air space. At the required time the ileum was exposed to anaesthetic by washing out (by overflow) the organ-bath with about four bath volumes of the anaesthetic solution. Exchange was complete in less than 10 sec. The anaesthetic solution was then allowed to flow into the organ-bath at a rate of about one bath volume per min throughout the exposure to anaesthetic to compensate for losses of anaesthetic by evaporation. The efficiency of the compensation was tested by rapidly washing out the bath with two or three bath volumes of the anaesthetic solution once the anaesthetic effect was well developed. Such rapid replacement of the solution, which would give little time for evaporation to take place, had only a small additional depressant effect (see Figs. 1, 2, 3, 5 and 6).

Anaesthetic will also escape from solution into the increasing air space in the reservoir as the anaesthetic solution is used. This loss was decreased by reducing the surface of evaporation by placing a thin polyethylene disc, slightly smaller than the internal dimensions of the cylindrical container, on the surface of the solution. The anaesthetic solution was not allowed to come in contact with anything other than glass and the short polyethylene connecting links before reaching the preparation.

Acetylcholine output. One length of ileum was set up (in a 3.5-ml. organ-bath) for transmural stimulation and treated with physostigmine (1 μg/ml.). A maximal output of acetylcholine is obtained when this concentration of physostigmine is used (Paton, unpublished). Another length of ileum, treated with neostigmine (5 μg/l.) and morphine (10 mg/l.) was used to assay the acetylcholine output from the donor preparation. Just before the start of a collection period, the bath was rapidly washed out with four bath volumes of fresh solution; 2 min later 0.4 ml. was removed from the donor bath and assayed immediately. Under these conditions loss of chloroform by evaporation from the donor bath had little effect on the depression of the twitch of a preparation not treated with physostigmine. The twitch depression produced by an ED50 (twitch) concentration of ether did, however, wear off during the 2-min stoppage of the continuous fluid exchange. The twitch remained as depressed for about 1 min, but recovered rapidly during the next minute. In one experiment 35% of the depression had worn off after 2 min. The presence of anaesthetic in the aliquot from the donor bath had no effect on the assay as the 0.4-ml. aliquot was added to a 7-ml. assay bath.

No evidence was found for the release of any stimulating substance other than acetylcholine by transmural stimulation. Thus, the released material stimulated the recipient preparation, an effect which was abolished by treatment of the material with alkali, or by admixture of the material with a sample of dog caudate nucleus extract, and which was inhibited by lachesine to the same extent as acetylcholine-induced contractions of the ileum. Further, the release of this substance was brought about by stimulus strengths close to those required to produce a twitch. The thresholds for eliciting a contraction and for output of acetylcholine were similar and the maximum for both output and twitch height was achieved within the same range of stimulus strengths.

Chemicals. All concentrations of the chemicals are expressed in terms of the salt (w/v) unless stated otherwise. Concentrations of anaesthetic are given in mM. Acetylcholine, histamine or potassium chloride were injected (in volumes of 0.2 to 0.3 ml.) directly into the organ-bath and washed out by overflow.

The drugs used were: acetylcholine bromide, histamine dihydrochloride, morphine sulphate, cocaine hydrochloride, lachesine, hyoscine hydrobromide, hexamethonium bromide, physostigmine sulphate, neostigmine methyl sulphate, anaesthetic ether (Duncan & Flockhart; Hopkin & Williams), trichloroethylene (I.C.I.), halothane (I.C.I.), methoxyflurane (Abbott Laboratories) and chloroform (Analar, B.D.H.).

RESULTS

The striking feature of the present results was the variety of effects on the isolated intestine of the five volatile anaesthetics used—halothane, methoxyflurane, ether, chloroform and trichloroethylene. The only effects shared by all were that all depressed the twitch and the response to a tetanus, all slowed the rate of relaxation of a twitch and all depressed the response to acetylcholine and potassium chloride (Figs. 1 to 7). Doubling the stimulus strength failed to overcome the anaesthetic-induced depression of the twitch. In the majority of experiments (75%) the twitch depression was antagonized less than 10% by doubling the stimulus strength; sometimes no change was detected. These results are summarized in Table 1.

TABLE 1

THE EFFECT OF SOME VOLATILE ANAESTHETICS ON THE RESPONSE OF THE GUINEA-PIG ILEUM TO TRANSMURAL STIMULATION

Figures in parentheses are the numbers of different preparations used for estimating the mean ED50. ED50s are means and standard errors. The twitch was elicited at 6 shocks/min, and antagonisms are of the anaesthetic depression of this twitch. All anaesthetics depressed the response to a tetanus (1 shock/sec for 30 sec)

	Number of		Depressant effect on the twitch		
Anaesthetic	Tests	Preparations	ED50 (mM)	Effect on shape	Antagonism on doubling stimulus (%)
Halothane	36	14	0·54±0·06 (9)	Conc. >ED50 slowed relaxation	3–5
Methoxyflurane	12	8	0·60±0·09 (4)	Conc. >ED50 slowed relaxation	5–11
Ether	31	13	37·9±1·8 (9)	Relaxation slowed	6–18
Chloroform	19	8	0·99±0·16 (4)	Conc. >ED20 slowed relaxation	0–14
Trichloroethylene	20	9	1·52±0·13 (6)	Relaxation slowed	Nil

Effects characteristic to each anaesthetic

Halothane and methoxyflurane. These two anaesthetics were depressants only. Unlike the other anaesthetics, each depressed the response to electrical and chemical stimuli (acetylcholine, histamine and potassium chloride) without sign of stimulation (Figs. 1 and 2). Onset of the depression of the twitch was rapid, as was the recovery, in most experiments, from the induced depression. With halothane some 5 min were needed for the depressant effect to become fully developed for a final twitch depression of about 50% (Fig. 1). Onset was even more rapid when higher concentrations of halothane were used, being virtually complete in 0.5 to 1 min. The depressant effect of methoxyflurane developed as rapidly as that of halothane. For both anaesthetics the time needed for recovery following a 40 to 60% depression of the twitch was independent of the duration of exposure to anaesthetic; 1 to 2.5 min were sufficient for the twitch to return to 90% of its original height following exposures of 12.5 to 33 min duration. However, recovery from a more intense depression of the twitch produced by the higher concentrations of halothane was sometimes slower and once required 40 min before being complete.

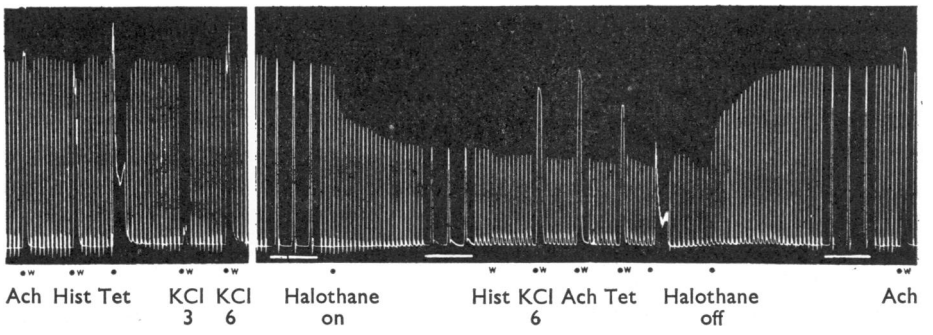

Fig. 1. The effect of halothane (0.47 mM) on the response of the guinea-pig ileum to electrical and chemical stimuli. Acetylcholine (Ach, 60 ng), histamine (Hist, 0.3 μg) and potassium chloride (KCl, 3 and 6 mg) were washed out (at W) after the contraction was fully developed. During exposure to a drug the stimulator was switched off. Otherwise, single shocks at 6 shocks/min or 1 shock/sec (at Tet) were given. A wash not preceded by the addition of a drug was equal in volume to about two bath volumes. The chart speed was increased four-times during the times marked by the horizontal white lines.

Loss of halothane from the bath by evaporation slowed the achievement of full depression of the twitch. A rapid wash with two bath volumes of fresh halothane solution (at W, Fig. 1) caused a further small depression of the twitch, despite the continual replacement of the bath contents at a rate of about one bath volume per min. The error introduced in the estimate of the ED50 (twitch) (Table 1) by evaporation of anaesthetic was reduced by taking the maximal percentage depression of the twitch as that following a rapid wash once the depressant effect was well developed. The ED50 (twitch)—the concentration which depressed the twitch by 50%—was obtained by exposing the gut to two concentrations of anaesthetic; one depressing the twitch by more than 50% and one depressing the twitch by less than 50%. The percentage depression was plotted linearly against the concentration of

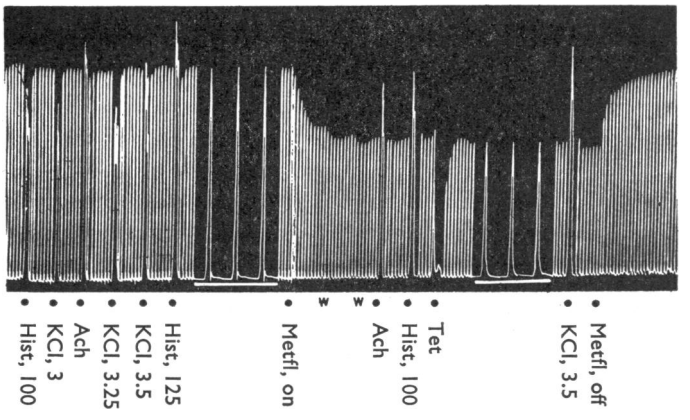

Fig. 2. The effect of methoxyflurane (Metfl, 0.43 mM) on the response of the guinea-pig ileum to histamine (Hist, 100 and 125 ng), acetylcholine (Ach, 7.5 ng) and potassium chloride (KCl, 3, 3.25 and 3.5 mg), and electrical stimulation at 6 shocks/min and 1 shock/sec (at Tet, for 0.5 min). The chart speed was increased eight-times where indicated by the horizontal white lines.

halothane and the ED50 (twitch) read from the graph. Preliminary experiments showed that the relationship between percentage twitch depression and the concentration of anaesthetic was best described by a straight line for twitch depressions of about 30 to 70% (this was the range used in the determination of the ED50 (twitch)).

Ether. The onset of the ether-induced depression of the twitch differed in two ways from those due to halothane and methoxyflurane. In twelve of the thirty-one experiments ether had a slight biphasic effect on the twitch during the first 1 to 2 min of the exposure to ether (Fig. 3). After the first 30 to 60 sec, the twitch recovered slightly from the depression

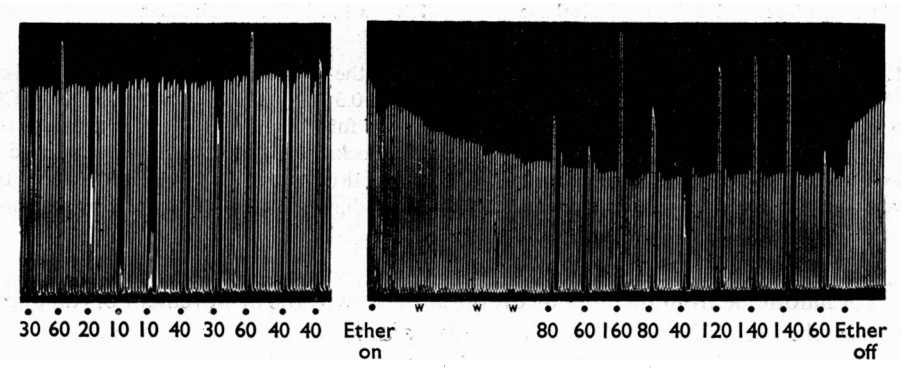

Fig. 3. The effect of ether (38.5 mM) on twitches of the guinea-pig ileum (6 shocks/min) and its response to acetylcholine (doses in ng). Note the slight initial stimulation and the slow onset of the depression of the twitch which was not greatly accelerated by washing (W) with about two bath volumes.

produced, but the recovery was transient and was soon followed (in about 1 min) by a more intense depression of the twitch. This biphasic effect was seen only with ether and trichloroethylene. A small contraction coincided with the initial phase of the biphasic effect (Fig. 3). Secondly, the rate of onset of the ether-induced twitch depression was slower, for the same final percentage depression of the twitch, than with either halothane or methoxyflurane. This can readily be seen by comparing Figs. 1 and 2 with Figs. 3 and 4. The difference could not be attributed solely to differing rates of evaporation of the three anaesthetics from the bath. Rapid washes (at W, Fig. 3) failed to accelerate greatly the onset.

Recovery from the effects of ether was rapid, being complete within 15 min, even after exposure to the highest concentrations used (76 mM). These high concentrations of ether abolished the twitch. As with halothane and methoxyflurane, recovery of the twitch from the effect of ether was independent of the duration of the exposure. Recovery of the twitch to 90% of its original height from depressions of 40 to 60% lasting 14 to 29 min was achieved in 2.5 to 3.25 min.

Chloroform. The effect of chloroform on the transmurally stimulated intestine depended on the concentration used. Concentrations of 0.63 and 1.25 mM simply depressed the twitch without any other effect (Fig. 5,*a*), but increasing the concentration to 2.5 mM not only abolished the twitch but also caused a sustained contraction and induced irregular phasic activity (Fig. 5,*b*). The phasic activity was characterized by slow fluctuations in length which had no obvious periodicity in either the presence or absence of electrical stimulation.

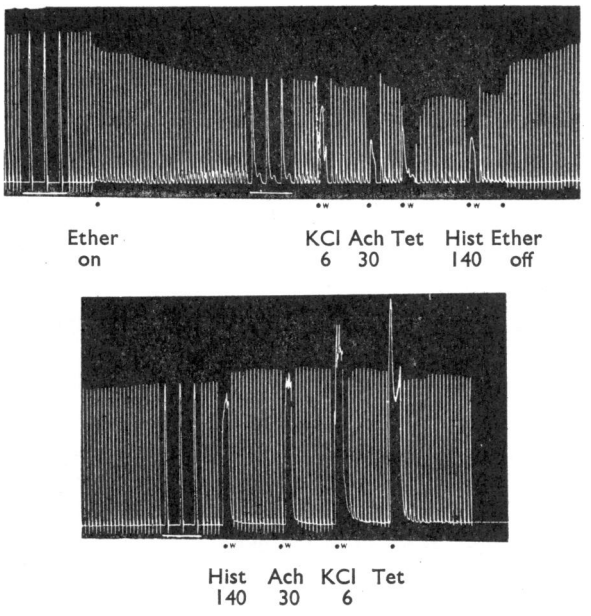

Fig. 4. The effect of ether (38.5 mM) on the responses of the guinea-pig ileum to electrical and chemical stimuli. Abbreviations as in previous Figs. The lower record is a continuation of the upper record.

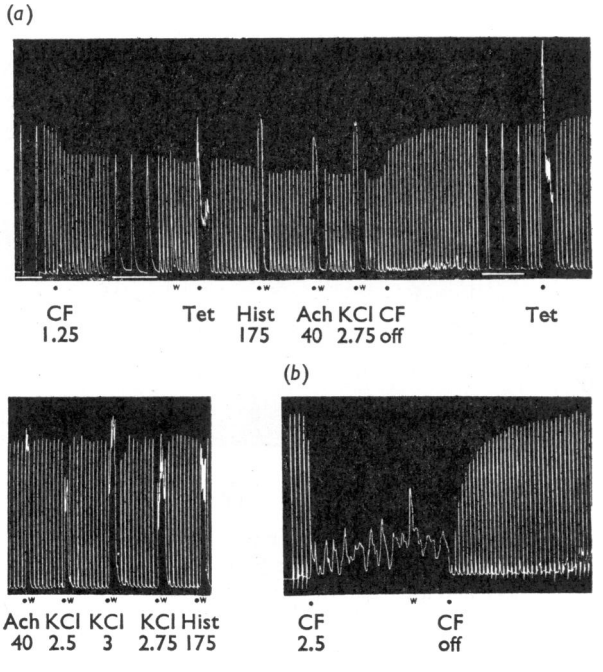

Fig. 5. The effect of chloroform (CF) on the responses of the guinea-pig ileum to electrical and chemical stimuli. (*a*) Exposure to 1.25 mM of chloroform; (*b*) to 2.5 mM of chloroform. Abbreviations as in other Figs. The lower left record is a continuation of the upper record.

Increasing the chloroform concentration to 5 mM abolished all activity. Both onset of and recovery from the effects of chloroform were rapid (Fig. 5) with recovery again being little influenced by the duration of exposure (12 to 32 min) to chloroform.

Chloroform was the only anaesthetic which, instead of impairing the response of the intestine to histamine, sometimes increased it (three out of eleven experiments). The increase (Fig. 5*a*) was small (10 to 18%), but in one experiment occurred at a time when the twitch was depressed by 55%. In five other experiments the response to histamine was unaffected, although in one the twitch was depressed by 71%. Like the other anaesthetics, chloroform impaired the response of the intestine to acetylcholine and potassium chloride (Fig. 5,*a*).

Trichloroethylene. The distinctive feature of the action of trichloroethylene was stimulation. Considerable stimulation occurred when the preparation was first exposed to trichloroethylene and when it was washed out (Figs. 6 and 9). The initial stimulation, best seen in the absence of electrical stimulation (Fig. 9), was invariably present, whereas the stimulation following removal of trichloroethylene was seen only in 60% of the experiments. The stimulation after washing out trichloroethylene was intense for 1 to 5 min, but then declined with periods of quiescence separating bursts of activity. Occasionally these periodic bursts of activity persisted until the end of the experiment.

The twitch began to decline soon after the exposure to trichloroethylene, although sometimes (three out of fourteen experiments) there was a slight recovery in twitch height before the decline set in. Equilibrium was soon attained (1 to 4 min) when the less effective concentrations of trichloroethylene were used (Fig. 6), but the full effect of higher concentrations took longer to develop. In one experiment the intensity of the depression was still increasing after 12 min. Intermittent rapid washes with fresh anaesthetic solution failed to hasten the onset of the depression. A puzzling feature of these washes was that they invariably caused a small contraction (Fig. 6). Presumably trichloroethylene made the ileum more sensitive to the mechanical disturbance associated with the rapid wash as the twitch height was little affected, often unaffected, by the wash.

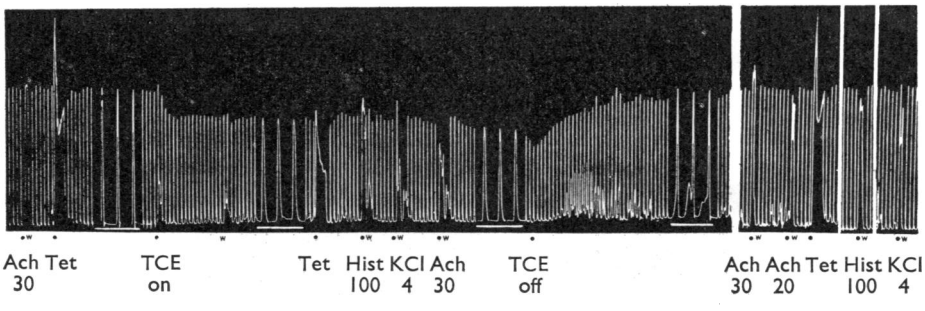

Fig. 6. The effect of trichloroethylene (TCE, 1.1 mM) on the responses of the guinea-pig ileum to electrical and chemical stimuli. Abbreviations as in previous Figs. Note the stimulation both on first exposing the ileum to trichloroethylene and after removal of the anaesthetic.

Analysis of the depressant effect of anaesthetics on the twitch

The depressant effect on muscle. Acetylcholine and histamine elicit a contraction from the guinea-pig ileum by acting mainly, if not completely, on the smooth muscle itself

(Day & Vane, 1963). All the anaesthetics examined depressed the response of the ileum to acetylcholine and usually histamine. Part of the depressant effect of the anaesthetics on the twitch must therefore result from an action on the muscle. However, the depressant effect on the muscle was not the only factor contributing to the depression of the twitch. While each anaesthetic reduced the responsiveness of the muscle to acetylcholine, their relative effect on the twitch and on the contraction due to acetylcholine, matched in height to the twitch, depended on the anaesthetic. This can readily be seen by comparing Figs. 1 to 6. Ether and trichloroethylene invariably depressed the response to acetylcholine to the same extent or more than the twitch; halothane, methoxyflurane and chloroform usually had less effect on the response to acetylcholine than on the twitch. A more precise estimate of the relative effect of the anaesthetics on the acetylcholine sensitivity of the muscle was obtained by determining the acetylcholine dose-ratio (the increase in dose necessary to give a contraction equal to the contraction in the control period which itself was matched in height to the normal twitch) when the twitch was depressed by about 50%. The partial log dose/response curves for acetylcholine in the presence and absence of any of the five anaesthetics were parallel when anaesthetic concentrations which depressed the twitch by about 50% were used. The experiment illustrated in Fig. 3 shows the method used for determining the log dose/response curve presented graphically in Fig. 7. The dose-ratios

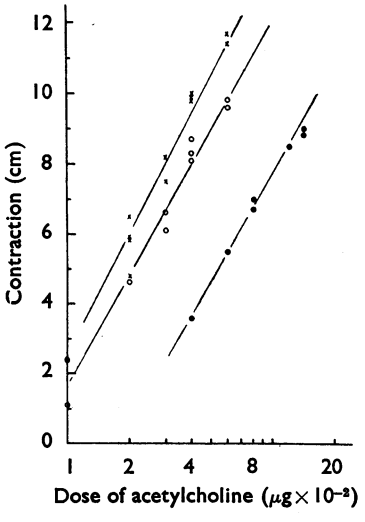

Fig. 7. Acetylcholine dose/response curves before (○), during (●) and after (×) exposure to ether (38.5 mM). Part of the experiment, from which these curves were obtained, is illustrated in Fig. 3.

themselves were determined, once a near equilibrium 40 to 60% depression of the twitch was attained, by increasing the dose until two doses were found which gave contractions just above and just below the height of the control contraction. These doses were plotted linearly against the elicited contraction and the equi-effective dose read from the graph. A log plot was unnecessary because of the close grouping used.

The acetylcholine dose-ratios determined in this way are given in Table 2. The order of increasing dose-ratio was: chloroform, halothane, methoxyflurane, ether and trichloro-

TABLE 2

ACETYLCHOLINE DOSE-RATIOS IN THE PRESENCE OF CONCENTRATIONS OF HALOTHANE, METHOXYFLURANE, ETHER, CHLOROFORM, TRICHLOROETHYLENE, HYOSCINE AND LACHESINE WHICH DEPRESSED THE TWITCH BY 40 TO 60%

Values are means and standard errors. The mean percentage twitch depression is the mean for all experiments. The result for each experiment was also a mean, but in this instance the mean of the twitch height just before addition of all doses of acetylcholine considered. The figures in parentheses are the numbers of observations, each on ilea from different guinea-pigs

Drug	Mean twitch depression (%)	Acetylcholine dose-ratio
Halothane	44·0±2·0 (5)	1·99±0·20
Methoxyflurane	47·5±2·5 (6)	2·12±0·28
Ether	45·9±1·1 (7)	2·95±0·24
Chloroform	50·5±2·0 (4)	1·62±0·33
Trichloroethylene	50·5±3·6 (4)	3·90±0·33
Hyoscine	47·4±3·4 (5)	6·53±1·73
Lachesine	50·2±3·2 (6)	9·29±3·06

ethylene. The acetylcholine dose-ratio for ether was significantly greater than the dose-ratios for chloroform ($P<0.01$), halothane ($P<0.02$) and methoxyflurane ($P<0.05$); and significantly less than the dose-ratio for trichloroethylene. There was no significant difference between the mean percentage twitch depressions produced by the different anaesthetics. This variation in acetylcholine dose-ratios was seen clearly in one experiment where the acetylcholine dose/response curves were determined in the presence and absence of halothane, ether and methoxyflurane. The same piece of gut was exposed to first halothane, then ether and finally methoxyflurane in concentrations which depressed the twitch by means of 43, 45 and 38% respectively. The acetylcholine dose-ratios were 1.60 for halothane, 2.66 for ether and 1.43 for methoxyflurane.

Acetylcholine dose-ratios determined in the same way for two acetylcholine antagonists, lachesine and hyoscine, are also given in Table 2. Different preparations had widely different sensitivities to the acetylcholine antagonists, although for any one preparation the acetylcholine dose-ratio was essentially constant and independent of the antagonist used. With one preparation, for instance, the acetylcholine dose-ratio was 23.6 when the twitch was depressed 54%; in another the dose-ratio was just 3.6, although the twitch was depressed more (58%). It was clear, however, that the average dose-ratio in the presence of equi-effective concentrations of acetylcholine antagonists and anaesthetics was greater for the acetylcholine antagonists (Table 2). The difference was significant ($P<0.05$) for all anaesthetics other than trichloroethylene.

The depressant effect on nervous tissue. The differences between the acetylcholine dose-ratios for the acetylcholine antagonists and the anaesthetics and the differences among the anaesthetics themselves indicate an action of the anaesthetics on intestinal nervous tissue. This possibility was tested by examining the effect of ether and chloroform on the acetylcholine output of the transmurally stimulated guinea-pig ileum in the presence of physostigmine. Results of the experiments are summarized in Table 3 and the record of one assay is presented in Fig. 8. Ether and chloroform reduced the acetylcholine produced at all rates of stimulation with the effect being most dramatic at low rates of stimulation. At the lowest rate of stimulation used, no release of acetylcholine by stimulation could be detected during exposure to anaesthetic. However, a significant release of acetylcholine may have been disguised by the inaccuracy of the assay. An output of less than 0.4 pM/pulse during

TABLE 3

THE EFFECT OF ETHER AND CHLOROFORM ON THE ACETYLCHOLINE OUTPUT OF THE GUINEA-PIG ILEUM AT VARIOUS RATES OF STIMULATION

Output is expressed as pM of cation per shock corrected for resting output. Ether was in a concentration of 38·5 mM, and chloroform of 1·67 mM. Groups (*a*) and (*b*) refer to experiments on different ilea

	Output (pM/shock) for stimulus frequency (shocks/min)				
	(*a*)			(*b*)	
Condition	12	0	60	30	60
Control	1·31	0·91	0·35	1·04	0·33
Treated with ether	0	0·13	0·06	0·13	0·13
Control	1·1	0·80	0·44		
Treated with chloroform	0	0·39	0·20		

stimulation at 12 shocks/sec would remain undetected. In the experiment shown in Fig. 8, 15 pM/0.4 ml. of acetylcholine was released during stimulation at 30 shocks/min. During exposure to ether the stimulated output fell to 7.5 pM/0.4 ml., an effect which was readily reversed by removal of the anaesthetic (Fig. 8,*c*). Subtraction of the resting output of 5 pM/0.4 ml. gives values of 10 and 2.5 pM/0.4 ml. respectively. In this experiment the output from the resting preparation was little affected by ether, but in other experiments the spontaneous output fell by 20 to 70%.

The reduction in acetylcholine output by a near ED50 (twitch) concentration of ether was greater than expected, particularly as the ED50 (twitch) represents a combined action on both nervous tissue and muscle. An estimate of the reduction of acetylcholine output needed to cause a 50% fall in twitch height can be obtained from the relationship between twitch height, frequency of stimulation and acetylcholine output (Paton, 1957a). Increasing the rate of stimulation to 30 shocks/min caused a fall in twitch height of 25.7±3.6%

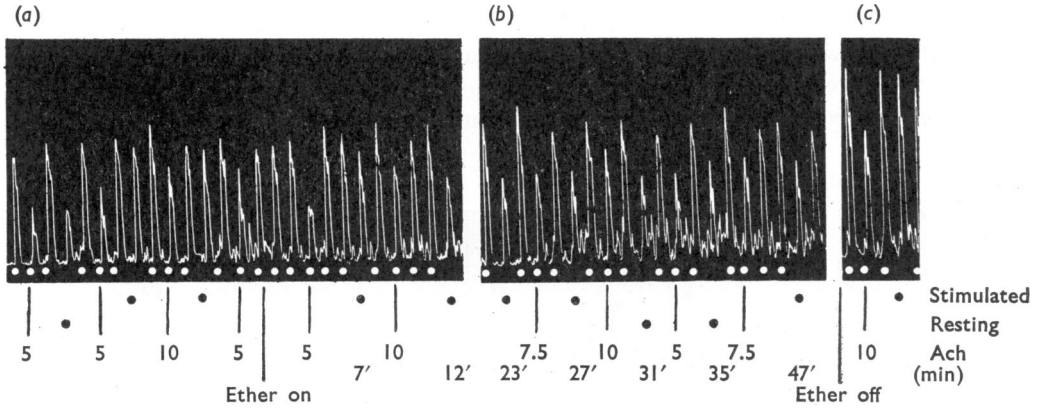

Fig. 8. The reduction by ether (38.5 mM) of acetylcholine (Ach) output from the guinea-pig ileum treated with physostigmine; the assay on another length of ileum. White dots mark the additions of the acetylcholine standards and the black dots the additions of 0.4-ml. samples from the donor bath. Times (in min) refer to the ends of collection periods in relation to the start of exposure to ether. Addition of acetylcholine (15 pM of cation) is indicated by the unlabelled white dots, and smaller doses (pM) are shown for the labelled white dots. Stimulation: 30 shocks/min, 10 V, 0.5 msec. (*a*) and (*b*) samples obtained before and during exposure to ether; (*c*) samples obtained after exposure to ether.

(mean and standard error of eight observations) in the preparation without physostigmine. On further increasing the rate to 60 shocks/min a discrete response to each stimulus was no longer obtained. Instead, a plateau was obtained which was just 19.7±2.4% (nine observations) of the twitch height (transient slow contractions with no frequency relationship to the stimulus frequency were sometimes superimposed on the plateau and these could be of the same magnitude as the twitch, particularly in fresh preparations). From these results and those given in Table 3, it would be expected that a 50% reduction in twitch height would occur when the acetylcholine output had fallen to between 0.35 and 0.91 pM/shock. Instead, no detectable acetylcholine was produced by 12 shocks/sec during exposure to an ED50 (twitch) concentration of ether.

Analysis of the stimulant effect of chloroform and trichloroethylene

Chloroform. Chloroform in a concentration of 2.5 mM abolished the twitch, but at the same time produced a sustained contraction on which were superimposed slow, phasic contractions. The sustained contraction was sometimes slight and occasionally absent. The phasic contractions were unaffected by hyoscine or lachesine in concentrations of 5 to 16×10^{-9} g/ml. (which gave acetylcholine dose-ratios of 20 to 300), or by mepyramine (5×10^{-8} g/ml.). The histamine dose-ratio in the presence of this concentration of mepyramine was 242; the acetylcholine dose-ratio was 2.1. The sustained contraction was either unaffected or slightly reduced by lachesine, hyoscine and mepyramine. These results indicate that 2.5 mM-chloroform stimulates the muscle directly, a conclusion in agreement with that of Rang (1964).

Trichloroethylene. The initial, transient stimulant effect of trichloroethylene was unaffected by hexamethonium (20 and 50 μg/ml.), or mepyramine (5×10^{-8} g/ml.; histamine dose-ratio, 242; acetylcholine dose-ratio, 2.1), but was abolished by morphine (10^{-6} g/ml.), hyoscine and lachesine. The effect of lachesine is shown in Fig. 9. A contraction was elicited with 1.48 mM-trichloroethylene (Fig. 9,*a*) and the stimulator turned on after the anaesthetic was washed out. Contractions produced by acetylcholine and histamine were then matched to the contractions elicited by transmural stimulation, and lachesine (10^{-8} g/ml.) added. When the twitches were virtually abolished, the dose-ratios for acetyl-

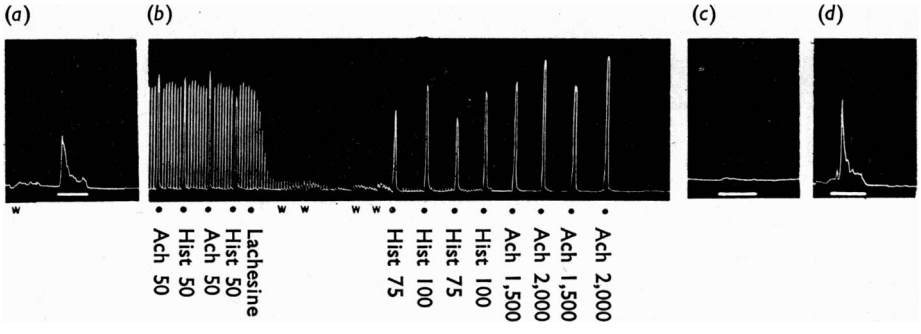

Fig. 9. The effect of lachesine on the initial stimulant effect of trichloroethylene (1.48 mM). Exposures to trichloroethylene are marked by the horizontal white lines. (*a*) Before addition of lachesine; (*b*) and (*c*) lachesine (10^{-8} g/ml.) present; (*d*) after removal of lachesine 5. The chart speed for (*a*), (*c*) and (*d*) was four times faster than in (*b*). Abbreviations as in earlier Figs.

choline (31) and histamine (2) were determined (Fig. 9,*b*) and the stimulator turned off. Trichloroethylene now failed to stimulate the ileum (Fig. 9,*c*), an effect which was readily overcome by the removal of lachesine (Fig. 9,*d*).

The ability of morphine, hyoscine and lachesine to abolish the contraction produced by trichloroethylene (observations consistent with those of Rang, 1964) suggested an action of trichloroethylene on cholinergic nerves. This conclusion was further supported by the effect of trichloroethylene on the spontaneous acetylcholine output of the ileum. A typical result is illustrated in Fig. 10. The spontaneous output and the total output on stimulating

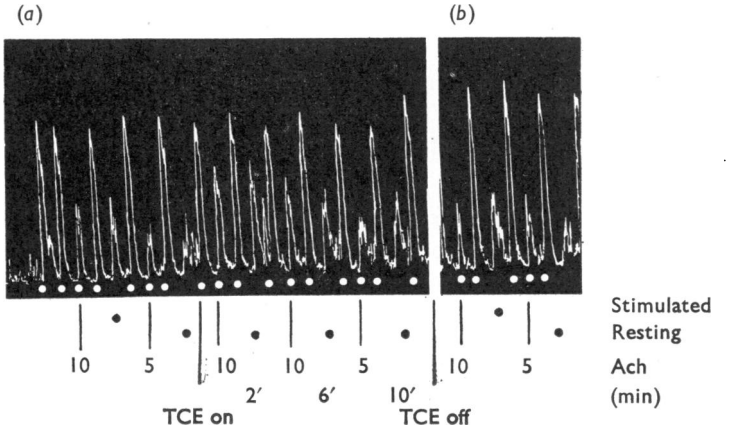

Fig. 10. The increased release of acetylcholine produced by trichloroethylene (1.48 mM) from the " resting " guinea-pig ileum treated with physostigmine; the assay on another length of ileum. The dots have the same meaning as in Fig. 8, the unlabelled white dots indicating the addition of 20 pM of acetylcholine (Ach) cation. (*a*) Samples obtained before and during exposure to trichloroethylene; (*b*) after removal of trichloroethylene.

at a rate of 12 shocks/min were measured just before exposing the donor preparation to trichloroethylene (Fig. 10,*a*). The spontaneous output was less than 5 pM/0.4 ml. But, during the first 2 min of exposure to trichloroethylene it increased to 10 pM/0.4 ml. (Fig. 10,*a*), an output equal to that produced previously by electrical stimulation. Over the next 8 min the spontaneous output declined, although it remained somewhat above control levels. On removal of the trichloroethylene, the spontaneous output again fell to below 5 pM/0.4 ml. (Fig. 10,*c*).

DISCUSSION

Ether often reduced the contraction to acetylcholine, matched in height to the t witch more than the twitch itself; this effect was invariably seen with trichloroethylene. The other three anaesthetics used (halothane, methoxyflurane and chloroform) rarely depressed the matched response to acetylcholine more than the twitch. These results, the first indication that the anaesthetics depressed the twitch by a simultaneous action on postganglionic nerve fibres and on the muscle itself, raise the question of why ether and trichloroethylene should depress the acetylcholine response more than the twitch. There is no reason to suspect the existence of diffusion barriers preventing access of the anaesthetics to the sites

where acetylcholine released by the nerves acts, such as has been postulated for atropine (Dale & Gaddum, 1930). Ether is a smaller molecule than atropine and is un-ionized. Further, there is doubt whether any diffusion barrier exists for even atropine in the guinea-pig ileum as minute amounts of atropine (10^{-8} g/ml.) suffice to abolish the twitch elicited by transmural stimulation (Paton, 1955). A more likely explanation seems to be the different positions of the matched contractions on their respective stimulus/response curves. A contraction equal in height to the twitch produced by a maximal electrical shock was produced by a submaximal dose of acetylcholine. A decrease in acetylcholine sensitivity of the muscle would be expected to exert a greater effect on the matched acetylcholine response because of its position on a steeper part of its stimulus/response curve.

The higher concentrations of anaesthetic, in addition to decreasing the twitch height, also slowed the rate of relaxation of the twitch. The latter effect may well have arisen from a reduced rate of destruction of acetylcholine. In the concentrations used, both ether and chloroform have a weak inhibitory effect on the cholinesterase obtained from cat muscle (Torda, 1943).

Except for ether, the concentrations of anaesthetic in Krebs solution required to depress the twitch by 50% were close to those blood concentrations present during surgical anaesthesia (Table 4). Allowance needs to be made for the differing solubility of anaesthetics in

TABLE 4

COMPARISON OF THE ANAESTHETIC CONCENTRATIONS REQUIRED TO DEPRESS THE TWITCH BY 50% WITH THOSE ARTERIAL BLOOD CONCENTRATIONS ASSOCIATED WITH SURGICAL ANAESTHESIA

The blood concentrations obtained from the literature are for anaesthesia of a variety of species (man, dog, rat, cat and rabbit)

Anaesthetic	ED50 (twitch) (mM)	ED50 (twitch) (mg/100 ml.)	Arterial blood concentration (mg/100 ml.)
Halothane	0·54	10·7	14–22
Methoxyflurane	0·60	9·9	35–40
Chloroform	0·99	11·8	14–50
Ether	37·90	281	75–162
Trichloroethylene	1·52	20	6–17

blood and in water. Halothane, methoxyflurane and chloroform are some two to three times more soluble in human blood than in water (Larsen, Eger & Severinghaus, 1962a,b; Eger & Shargel, 1963), trichloroethylene is some six times as soluble (Powell, 1947) and ether is as soluble in blood as in water (Larsen *et al.*, 1962b; Eger, Shargel & Merkel, 1963). However, multiplying the ED50 (twitch) concentration by the appropriate solubility term still gives methoxyflurane and chloroform concentrations comparable to those which produce surgical anaesthesia. The adjusted halothane concentration corresponds to a blood concentration which causes respiratory arrest in dogs (Raventós, 1956). Only with ether and trichloroethylene did the concentrations far exceed those needed to produce surgical anaesthesia.

The reasonable agreement between the concentrations of halothane effective on the ileum and those producing anaesthesia suggests that *in vivo* halothane may depress intestinal contractions by an action on both nerves and muscle. As such, the present observations offer an explanation for the conflicting results of Raventós (1961) and Marshall *et al.* (1961). Marshall *et al.* reported that the intestinal inhibition produced by halothane anaesthesia

was accompanied by a decreased sensitivity of the muscle to acetyl-β-methylcholine; Raventós found that the sensitivity to the same drug was unaffected by halothane. Marshall *et al.* presumably uncovered the depressant effect of halothane on intestinal muscle by using a higher inhaled concentration of halothane.

The ability of chloroform, trichloroethylene and, to a lesser extent, ether, to stimulate the guinea-pig ileum requires little comment. The present results confirm and extend those recently reported by Rang (1964). However, no initial stimulation was seen with halothane, methoxyflurane and chloroform, although such effects were observed by Rang. The transmural stimulation could well have disguised or suppressed the weak stimulant effects of these three anaesthetics.

The demonstration that some volatile anaesthetics in low concentrations have presynaptic effects at one synapse has its implications. There seems to be no obvious reason why a similar effect should not occur at other synapses.

SUMMARY

1. The effect of ether, halothane, chloroform, methoxyflurane and trichloroethylene on the guinea-pig isolated ileum stimulated electrically or by acetylcholine, histamine and potassium chloride has been studied.

2. All five anaesthetics depressed the twitch evoked by transmural electrical stimulation, slowed the rate of relaxation of a twitch and depressed the response to acetylcholine and potassium chloride. They also showed important individual differences.

3. Halothane and methoxyflurane were the only two which were purely depressant. Ether was mainly depressant, but sometimes caused a slight transient stimulation.

4. Chloroform in high concentrations produced contractions which were resistant to hyoscine and lachesine. The contractions were, however, abolished by even higher concentrations of chloroform. Chloroform in all but high concentrations had only a small effect on the response to chemical stimuli, and sometimes even sensitized the gut to histamine.

5. Trichloroethylene caused a transient contraction, lasting about 10 to 20 sec, before depressing the twitch. This stimulant effect was resistant to mepyramine and hexamethonium, but was readily abolished by hyoscine, lachesine and morphine. Trichloroethylene also initially greatly increased the release of acetylcholine from the resting guinea-pig ileum treated with physostigmine.

6. The acetylcholine dose-ratios in the presence of concentrations of anaesthetic sufficient to depress the twitch by about 50% increased in the order: chloroform, halothane, methoxyflurane, ether and trichloroethylene. The acetylcholine dose-ratios in the presence of hyoscine and lachesine concentrations which also depressed the twitch by about 50% were greater than those in the presence of trichloroethylene.

7. Chloroform and ether inhibited the release of acetylcholine produced by transmural stimulation of the ileum treated with physostigmine.

8. It was concluded that these anaesthetics depressed the twitch by a simultaneous action on muscle and on postganglionic nerve fibres. Trichloroethylene, in addition, stimulated nervous elements before depressing the twitch.

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