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STIMULANT ACTIONS OF VOLATILE ANAESTHETICS ON SMOOTH MUSCLE

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A number of volatile anaesthetics, and some compounds synthesized in the search for new anaesthetics, have been tested on guinea-pig intestinal smooth muscle *in vitro*. All the compounds produced a contractile response. This effect did not correlate well with convulsant activity *in vivo* among the compounds tested. Two kinds of stimulant effect were distinguishable: (1) Rapid, transient contractions, abolished by cocaine or lachesine; most of the anaesthetics in clinical use had this action. (2) Slow, sustained contractions, unaffected by cocaine or lachesine; this effect predominated among the fluorinated ring compounds. Hexamethonium and mepyramine did not affect the contractile response to any of the compounds. The first type of effect presumably represents excitation of postganglionic nerve cells, while the second type is a direct action on the muscle cell. The action of perfluorobenzene, which is of the latter kind, was studied further. Adrenaline and lack of calcium diminished the contraction in parallel with the contraction to histamine, which suggests that the cell membrane was the site of action; in contrast to the stimulant action of histamine or acetylcholine, the effect was highly temperature-sensitive, being almost abolished by cooling to 32° C, and enhanced at 40° C. The depressant action of anaesthetics on smooth muscle is affected very little by temperature changes. These findings are discussed in relation to other observations which suggest a stimulant action of volatile anaesthetics on excitable tissues. Protein denaturation is tentatively suggested as a mechanism of action.

The lack of chemical specificity in the narcotic action of organic substances emphasized by the work of Overton (1901) and Ferguson (1939), makes available a very wide range of substances as potential anaesthetics for clinical use. However, very few substances pass the stage of preliminary testing in animals before serious side-effects become apparent. Recent studies have centred on highly fluorinated compounds, since these are often chemically inert and non-inflammable, and one of the most frequently observed side-effects is convulsions in mice. Opisthotonos and excitement are also frequently reported (Struck & Plattner, 1940; Robbins, 1946; Lu, Ling & Krantz, 1953; Poznak & Artusio, 1960a, b; Burns, Hall, Bracken, Gouldstone & Newland, 1961). In these studies a striking feature is the lack of any clear relationship between convulsant properties and chemical structure. This is brought out particularly well by the effect of successive fluorine substitutions in diethyl ether. Substitution with three fluorine atoms at one terminal carbon atom gives a compound with mild anaesthetic activity and no obvious toxicity. Substitution at both terminal carbon atoms gives di(2,2,2-

trifluoroethyl) ether, which is a potent convulsant apparently devoid of any anaesthetic activity (Krantz, Truitt, Speers & Ling, 1957 ; Krantz, Truitt, Ling & Speers, 1957). Complete replacement of the hydrogen atoms by fluorine, however, renders the substance pharmacologically inert (Lu *et al.*, 1953). This uncertainty concerning the chemical factors important in producing anaesthetic activity and toxicity is reflected in the number and diversity of substances at present being produced for testing.

Since excitant effects in the central nervous system are difficult to investigate and measure objectively, it would be helpful if a preparation could be found on which these convulsant compounds exert a stimulant effect the intensity of which parallels their convulsant activity, which might then be more readily analysed.

The present work was done to test whether convulsant activity is related to activity in stimulating smooth muscle. Smooth muscle was chosen because it is easily set up *in vitro*, and because it is readily stimulated by many chemical compounds. In this respect the work was unsuccessful—no correlation was found between convulsant and smooth muscle stimulating activities. However, clear-cut stimulant effects on smooth muscle were found with a variety of compounds, and some preliminary investigation of the mechanisms involved has been carried out.

METHODS

The preparation used was a strip of longitudinal smooth muscle dissected from a length of guinea-pig ileum by a modification of the method described by Ambache (1954) for removing the longitudinal muscle layer from rabbit intestine. The guinea-pig was killed by a blow on the head, and a length of small intestine removed. A 10 to 12 cm length from the mid-region of the ileum was stretched on a glass rod and the mesentery was removed. By stroking tangentially away from the mesenteric attachment at one end of the gut with a wisp of cotton wool, the longitudinal muscle layer was separated from the underlying circular muscle. The longitudinal muscle was tied with a thread and, by gentle tension, stripped from the whole length. Usually the muscle could be removed in this way as an intact sheet, divided at the mesenteric attachment. This preparation, which was devised for another purpose, has for the present experiments no particular advantage over the conventional guinea-pig ileum preparation. Its pharmacological behaviour differs in no important way from that of the intact ileum.

The strip was mounted in a jacketed 3 ml. organ-bath containing bicarbonate-buffered Krebs solution at 37° C and bubbled with 5% carbon dioxide in oxygen. The composition of the Krebs solution was (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and dextrose 11.5. The preparation was stimulated by the automatic injection of histamine every minute, interrupted during the application of the test substances. In some experiments electrical stimulation with 50 cycles/sec a.c. was used. The electrodes consisted of two horizontal platinum rings at top and bottom of the bath, and the stimulus strength was adjusted by a Variac transformer and monitored with an oscilloscope. Stimulation was applied for 5 sec every 30 sec. The preparation was treated with lachesine (10^{-7} g/ml.) to abolish effects mediated by its nerves.

Anaesthetics were applied dissolved in Krebs solution. Effects were produced by replacing the normal Krebs solution in the organ-bath with the solution containing drug, previously warmed to 37° C. The drug solution came into contact only with glass, no rubber, plastic or tap-grease being used, as these substances may extract appreciable quantities of anaesthetics from aqueous solution. Contractions were recorded by a light auxotonic lever writing on a smoked drum.

The compounds tested were ether (diethyl ether; Duncan, Flockhart & Co.), chloroform (B.D.H.), trichlorethylene (I.C.I.), halothane (I.C.I.), 2,2,2-trifluoroethyl vinyl ether (Fluoromar), di(2,2,2-trifluoroethyl) ether (hexafluorodiethyl ether, Indoklon), perfluorobenzene, 2-chloro-1,1,2-trifluoroethyl ethyl ether (Compound 22), 1-methoxynonafluorocyclohexene (Compound 46), 1*H*,4*H*-octafluorocyclohexene (Compound 50) and 4*H*-nonafluorocyclohexene (Compound 53). These substances will, for brevity, be referred to as " anaesthetics," even though some of them do not warrant the term.

RESULTS

All of the compounds tested caused contraction of the smooth muscle preparation.

Fig. 1 shows the effects of ether, halothane, trichlorethylene and di(2,2,2-trifluoroethyl) ether, with responses to histamine for comparison. The contractile response, whether measured directly or expressed as an equivalent dose of histamine, varied widely between different preparations, and is difficult to express quantitatively. The responses in Fig. 1 to two different concentrations (one 50% greater than the other)

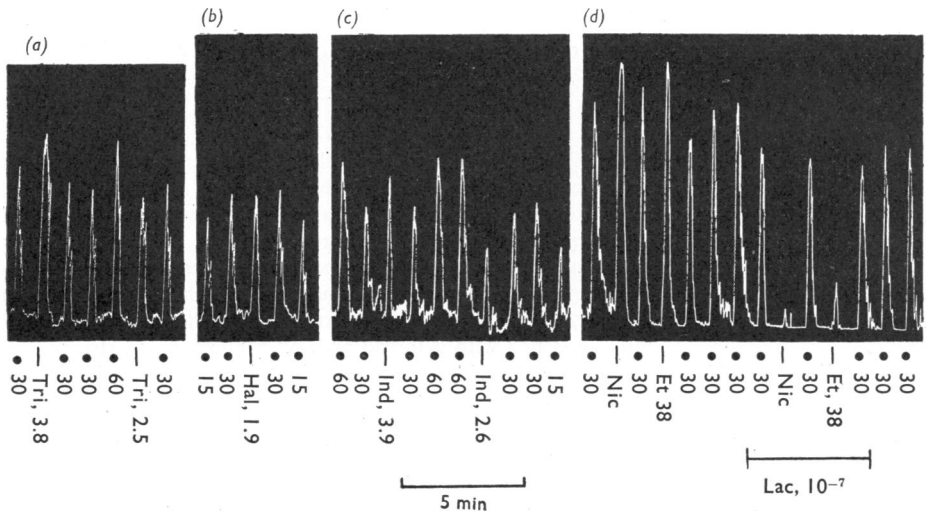

Fig. 1. Contractile responses of guinea-pig ileum longitudinal muscle strip at 37° C to anaesthetics compared with responses to histamine. Anaesthetic concentrations are given in mM. Contractions to histamine are marked •, and the concentrations given in nM. Drugs were added every 60 sec, and left in the organ-bath for 15 sec. (*a*) Trichlorethylene (Tri); (*b*) halothane (Hal); (*c*) di(2,2,2-trifluoroethyl)ether (Ind); and (*d*) ether (Et). In (*d*) the response to nicotine (Nic, 1.7×10^{-6} g/ml.) is shown, and the effect of lachesine (Lac, 10^{-7} g/ml.). The tracings were taken from different preparations.

of trichlorethylene and di(2,2,2-trifluoroethyl) ether show the steepness of the log dose/response curve compared with that for histamine. This was a constant finding. The size of the maximal contraction produced by anaesthetics was usually much smaller than that attainable with histamine. Increasing the concentration of anaesthetic beyond a certain point reduced the contraction, and the effect of a subsequent

dose of histamine was also reduced. The contraction therefore probably represents a balance between stimulant and depressant activity. The nature of the contractions, which were fairly well-sustained when low concentrations of anaesthetic were applied but transient with higher concentrations, also supports this idea.

Effect of blocking agents

Hexamethonium bromide (10^{-5} g/ml.) and mepyramine maleate (10^{-8} g/ml.) did not affect the response to anaesthetics, though matching responses to nicotine and histamine respectively were abolished. Lachesine chloride (10^{-7} g/ml.), however, usually abolished the effect of ether (Fig. 1), halothane, trichlorethylene, 2,2,2-trifluoroethyl vinyl ether and di(2,2,2-trifluoroethyl) ether. Cocaine hydrochloride (3×10^{-6} g/ml.) had the same action. Both of these drugs abolished the effect of nicotine.

The other compounds tested, however, caused contractions which were unaffected by lachesine or cocaine. Chloroform occupied an intermediate position: the contractile response was often diminished, but never by more than half, in the presence of lachesine. Fig. 2 shows responses to two concentrations of chloroform (again

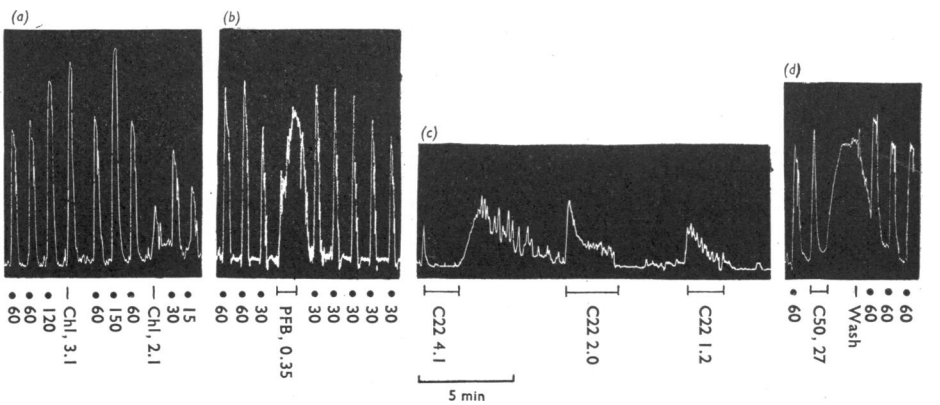

Fig. 2. Contractile responses of guinea-pig ileum longitudinal muscle strip at 37° C to anaesthetics (concentrations in mM), in the presence of lachesine (10^{-7} g/ml.) throughout, compared with responses to histamine ●, concentrations in nM. (*a*) Chloroform (Chl), left in the organ-bath for 15 sec; (*b*) perfluorobenzene (PFB); (*c*) Compound 22 (C22); and (*d*) Compound 50 (C 50).

the steep dose/response curve is apparent), perfluorobenzene, compound 22 and compound 50, in the presence of lachesine (10^{-7} g/ml.). Thus it is likely that these compounds act directly on the muscle, while those of the first group, whose stimulant action is blocked by lachesine, act on the postganglionic nerves. Chloroform may act partly on the nerves, but its predominant action is direct. This conclusion agrees with that of Speden (1963), who tested anaesthetics on the coaxially stimulated guinea-pig ileum, finding stimulant effects with trichlorethylene (blocked by

lachesine) perfluorobenzene, bromopentafluorobenzene and perfluorotoluene (all resistant to lachesine).

Figs. 1 and 2 show the difference in the patterns of response to different compounds. All the compounds acting on the nerves caused rapid, poorly sustained contractions. At higher concentrations the response became smaller, and there was no after-contraction on washing out the drug. Chloroform, although it acts directly on the muscle, showed a similar pattern. Perfluorobenzene on the other hand, caused slow, well-sustained contractions in low concentrations. At higher concentrations, the response was smaller and less well-sustained, but a large after-contraction occurred on washing out (Fig. 3). The action of Compounds 22, 46, 50 and 53

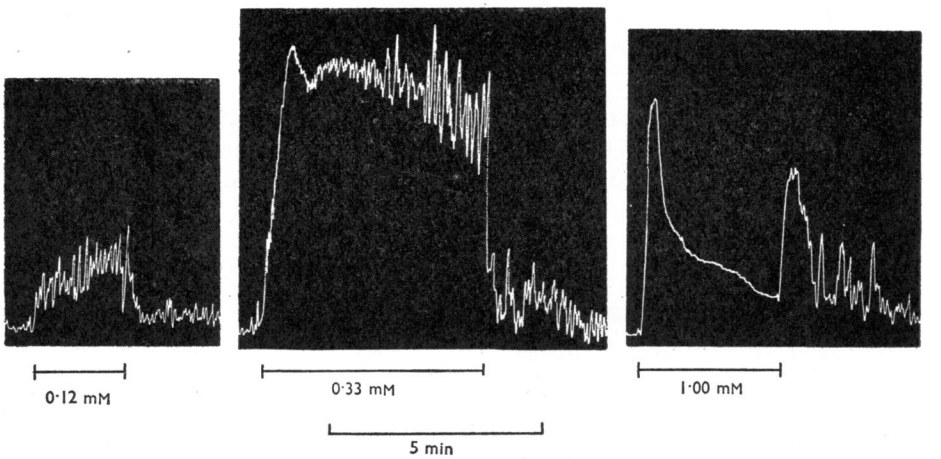

Fig. 3. Contractile responses of guinea-pig ileum longitudinal muscle strip at 37° C to various concentrations of perfluorobenzene. Continuous flows of drug solution (1 to 2 ml./min) were maintained to check losses due to evaporation.

resembled that of perfluorobenzene, but they were incapable of causing such a large response.

The original aim of this study was to see whether smooth muscle stimulating activity *in vitro* was parallel to convulsant activity *in vivo*. Table 1 shows that this is not so. Di(2,2,2-trifluoroethyl) ether, a potent convulsant with little or no anaesthetic action, did not stimulate smooth muscle directly, and its action on postganglionic nerves was no more intense than that of anaesthetics in clinical use. Perfluorobenzene, on the other hand, was the most powerful smooth muscle stimulant among the compounds tested, but appears to be a fairly satisfactory anaesthetic in animal tests, and has no convulsant action. Perfluorotoluene is indistinguishable from perfluorobenzene in its action on smooth muscle (Speden, 1963) but it is toxic in mice. Thus as a means of studying the actions of anaesthestics in relation to their side-effects on the central nervous system, this preparation was singularly unpromising. However, the stimulant effects found with so many anaesthetics might be of interest in themselves, and some further work was done on the stimulant action of perfluorobenzene, to learn more about its mechanism of action.

TABLE 1

EFFECTS OF ANAESTHETICS ON GUINEA-PIG ILEUM LONGITUDINAL MUSCLE PREPARATION

See Methods for constitutions of Compounds 22, 46, 50, and 53. [1] Dundee, Linde & Dripps (1957). [2] Burns, Hall, Bracken & Gouldstone (1961). [3] Adye, Paton & Speden (unpublished). [4] Goodford & Speden (unpublished). [5] Burns, Hall, Bracken & Gouldstone (unpublished). [6] Krantz, Esquibel, Truitt, Ling & Kurland (1958). [7] Speden (1963)

Compound	Status as anaesthetic	References	Stimulation of C.N.S.	Stimulation of smooth muscle: Through nerves	Concentration for maximal effect (mM)	Directly	Concentration for maximal effect (mM)
Ether	In clinical use[1]	1	−	++	50	−	
Chloroform		1	−	+		+++	2·5
Halothane		1	−	+	4	−	
Trichlorethylene		1	−	+++	3	−	
2,2,2-Trifluoroethyl vinyl ether		1	−	++	3	−	
Perfluorobenzene	No serious toxicity in animals	2,3,4	−	?		++++	0·4
Bromopentafluorobenzene		5	−	?		++++	0·4[7]
Compound 22	Toxic in mice	3	+	?		++	2·0
Compound 50		3	+	?		++	2·5
Compound 46		3	+	?		+++	1·5
Compound 53		3	+	?		+	2·5
Perfluorotoluene		5	−	?		++++	0·4[7]
Di(2,2,2-Trifluoroethyl) ether	Convulsant in man	6	+	++	4	−	

Stimulant action of perfluorobenzene

An effect often seen, and evident in Fig. 2, was that the response to histamine was enhanced for a few minutes after the application of perfluorobenzene. Frequently the relaxation after washing out perfluorobenzene was itself delayed, and it seemed likely that the enhancement of the response to histamine simply represented a persistence of the excitatory state produced by perfluorobenzene. This possibility was tested in a muscle stimulated by 50 cycles/sec a.c. in the presence of lachesine (10^{-7} g/ml.).

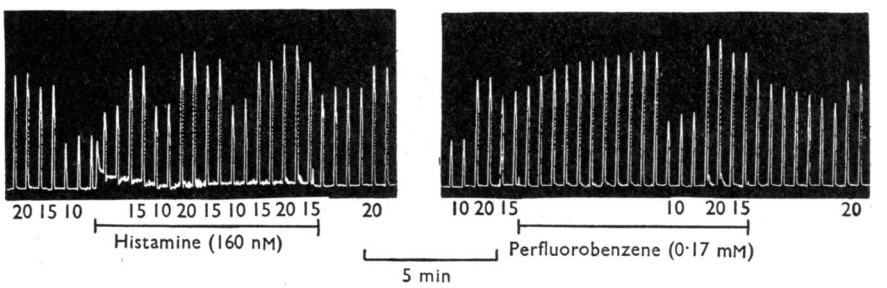

Fig. 4. Contractions of guinea-pig ileum longitudinal muscle strip, stimulated by 50 cycles/sec a.c. for 5 sec every 30 sec. The peak-to-peak stimulus voltage is shown beneath the first of each group of responses recorded at each stimulus strength. Histamine (160 nM) and perfluorobenzene (0.17 mM) were added to the organ-bath during the times indicated.

Fig. 4 illustrates such an experiment, and the effect on the preparation of histamine and perfluorobenzene in concentrations too small to cause an appreciable contraction. Both drugs enhanced the electrically induced responses but, while the action of histamine started and stopped abruptly, that of perfluorobenzene appeared and waned slowly. This result shows that a subliminal concentration of histamine may enhance the size of contractions produced by a different stimulus; that perfluorobenzene can act in the same way; and that the recovery from perfluorobenzene is

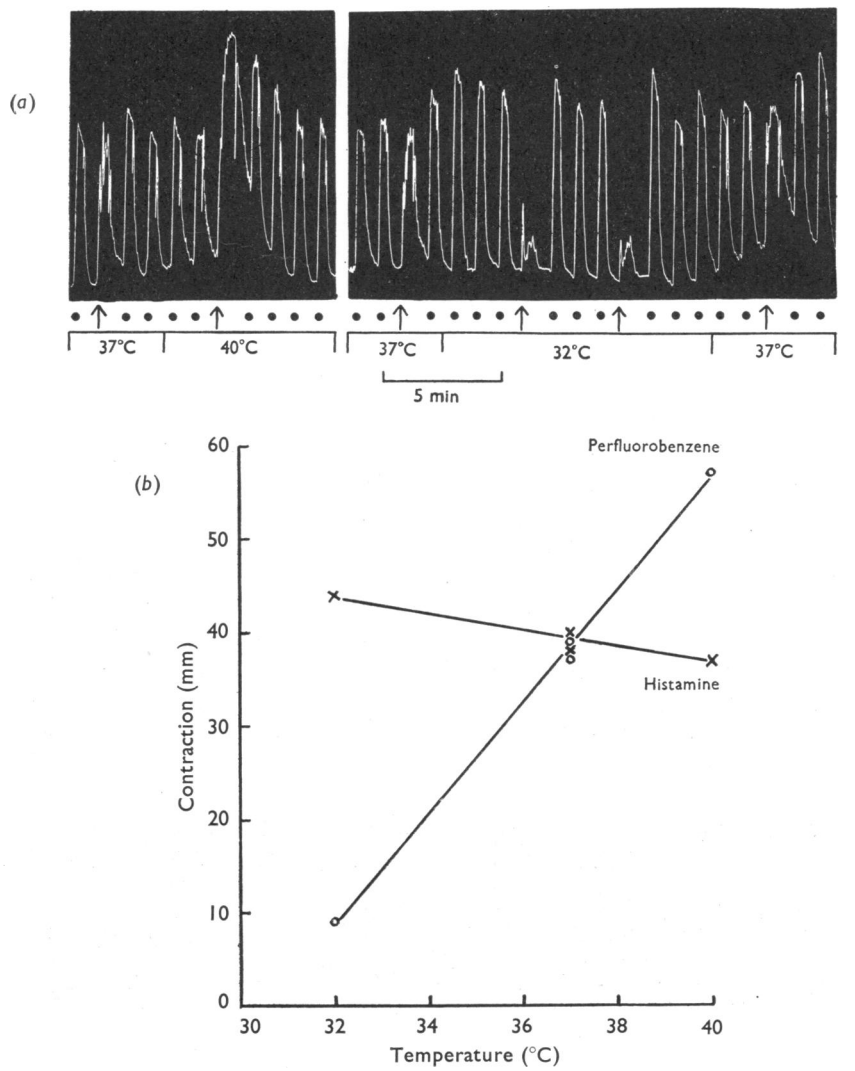

Fig. 5. (*a*) Contractile responses of guinea-pig ileum longitudinal muscle strip to perfluorobenzene (0.17 mM, at arrows) and to histamine (30 nM, at dots). The temperature was changed abruptly at the points indicated. (*b*) The results plotted from a similar experiment in a different preparation, to show relationship between temperature (abscissa) and contractile responses (mm on kymograph) to histamine (x) and perfluorobenzene (o).

slow. The observed enhancement of responses to histamine is therefore likely to be be due to the same mechanism, namely a nonspecific increase in the state of excitation, persisting after the perfluorobenzene is washed out.

Action on other tissues. Perfluorobenzene also contracted strongly the guinea-pig taenia coli and rat uterus preparations, both at 37° C. Frog small intestine and frog rectus abdominis preparations did not respond to perfluorobenzene at room temperature. At 35° C, 0.4 mM-perfluorobenzene caused a small stimulant effect on the intestine, but not on the rectus. Neither preparation retained contractility for long at this temperature.

Effect of temperature. Fig. 5 shows the stimulant effect of 0.17 mM-perfluorobenzene at three different temperatures, with responses to 30 nM-histamine for comparison, which at 37° C matched the effect of perfluorobenzene. The response to perfluorobenzene was strongly influenced by changes in temperature, while the effect of histamine was only slightly affected and in the opposite direction to that of perfluorobenzene. The stimulant effect of chloroform was also enhanced at high temperatures but less strikingly so than that of perfluorobenzene.

In experiments in which the depressant effect of higher concentrations of perfluorobenzene and other anaesthetics on the electrically stimulated preparation was measured, temperature was found to have very little effect.

Effect of adrenaline and calcium lack. Adrenaline hydrochloride (3 and 6×10^{-8} g/ml.) brought about parallel reductions of the responses to histamine and to perfluorobenzene. Similarly, omission of calcium from the bathing solution completely suppressed responses to both drugs, while reduction to one-half and one-quarter of the usual calcium content diminished the responses equally.

DISCUSSION

The idea underlying this work was that the effects of anaesthetics on this guinea-pig ileum preparation might enable predicitions to be made about their effects in whole animals, and that a useful preliminary screening of new compounds might be possible, using only very small amounts. Clearly this is not so: the effects of the substances tested on smooth muscle appear to bear no relation whatever to their uefulness as anaesthetics.

The effects of adrenaline and calcium lack suggest that the site of action of perfluorobenzene is the cell membrane, and there are many other examples of stimulant actions of anaesthetics on excitable membranes. Naess (1950) found that the electrical excitability of the sciatic nerve of rabbits was usually transiently increased during induction of anaesthesia with ether. This effect occurred only during induction: excitability was always reduced when a steady level of ether anaesthesia was maintained. Naess therefore speculated that a concentration gradient across the membrane might be responsible for the increase in excitability. Berney & Posternak (1956) found similar effects with methanol, ethanol, propanol, acetone and chloroform applied to frog sciatic nerve; the increase in excitability was associated in all cases with depolarization. Arvanitaki & Chalazonitis (1951) have found a transient depolarization and increase in excitability of isolated giant axons of Sepia caused by ether and chloroform. Bergmann, Chaimovitz & Wind

(1962) found that a series of nitroparaffins caused a mixture of stimulant and depressant effects in guinea-pig ileum at 37° C. The stimulant actions appeared to be mediated through the nerves, and were most prominent with the short-chain members of the series. The sensitizing action of volatile anaesthetics on pulmonary stretch receptors was demonstrated by Whitteridge & Bülbring (1944). With most anaesthetics, the effect was transient, but nitrous oxide and cyclopropane caused maintained sensitization. A similar sensitization of carotid sinus baroreceptors by anaesthetics was described by Robertson, Swan & Whitteridge (1956). Torda (1943) has found that both chloroform and ether enhance the contracture of the frog rectus abdominis muscle caused by acetylcholine at room temperature. Chloroform produced its maximal effect (enhancement by 64%) at a concentration of 1.2 mM, while ether increased the response more than threefold at a concentration of 48 mM. Increasing the anaesthetic concentration depressed and eventually abolished the response to acetylcholine. Some, but certainly not all, of the effect may have been due to cholinesterase inhibition. Sachdev, Panjwani & Joseph (1963) have shown a similar effect of ethanol on frog rectus muscle, which occurs in preparations fully treated with physostigmine, thus excluding cholinesterase inhibition as a mechanism.

In clinical practice, ether convulsions are a wellknown, though rare, hazard, and the intense irritant action of ether on the bronchial tree is also familiar.

The properties of many of these stimulant actions—their transience and their confinement to the lower members of homologous series (Posternak & Zahnd, 1953)—suggest an explanation in terms of the rate theory of drug action proposed by Paton (1961), by which it might be supposed that the process of interaction of narcotic molecules with the cell membrane results in excitation, while the maintained presence of the drug molecules has a depressant effect. The present results with perfluorobenzene do not support this hypothesis, however. The tracings of Fig. 3 show a response which develops and subsides slowly, while the rate theory demands that the response should fade after an initial peak, and subside quickly on washing out the drug. A renewal of the response on washing out the drug is quite incompatible with the rate theory.

The differential effect of changes in temperature on the stimulant and depressant actions of perfluorobenzene makes it unlikely that a single mechanism can account for both effects. Indeed, qualitative differences between the excitant effects of different substances—for instance, the lack of correlation between stimulation of the central nervous system and of smooth muscle, and the different patterns of the responses of smooth muscle to different agents—may indicate that more than one mechanism is involved in the stimulant action. A possibility raised by the temperature sensitivity of the action of perfluorobenzene is that protein denaturation may be concerned in the mechanism. This was suggested as an explanation of narcosis by Claude Bernard in 1875, and has been sporadically resurrected (Bancroft & Richter, 1931) and refuted (Henderson & Lucas, 1932) since then. It would be interesting to see whether, with more refined physicochemical methods available for detecting changes in protein configuration, a relationship could be established between alteration of protein structure and stimulation by anaesthetics.

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REFERENCES

AMBACHE, N. (1954). Separation of the longitudinal muscle of the rabbit's ileum as a broad sheet. *J. Physiol. (Lond.)*, **125**, 53–54*P*.

ARVANITAKI, A. & CHALAZONITIS, N. (1951). In Mécanisme de la narcose. *Colloq. int. Cent. nat. Rech. sci.*, **26**, 195.

BANCROFT, W. D. & RICHTER, G. H. (1931). The chemistry of anaesthesia. *J. Phys. Chem.*, **35**, 215–268.

BERGMANN, F., CHAIMOVITZ, M. & WIND, E. (1962). Dual action of nitroparaffins on the guinea-pig ileum. *Brit. J. Pharmacol.*, **18**, 381–396.

BERNARD, C. (1875). *Leçons sur les anaesthetiques*. Paris.

BERNEY, J. & POSTERNAK, J. (1956). Modifications de l'excitabilité nerveuse par les narcotiques. *Helv. physiol. pharmacol. Acta*, **14**, *C*5–6.

BURNS, T. H. S., HALL, J. M., BRACKEN, A. & GOULDSTONE, G. (1961). An investigation of new fluorine compounds in anaesthesia (3). *Anaesthesia*, **16**, 333–339.

BURNS, T. H. S., HALL, J. M., BRACKEN, A., GOULDSTONE, G. & NEWLAND, D. S. (1961). An investigation of new fluorine compounds in anaesthesia (1). *Anaesthesia*, **16**, 3–18.

DUNDEE, J. W., LINDE, H. W. & DRIPPS, R. D. (1957). Observations on trifluoroethylvinyl ether. *Anaesthesiology*, **18**, 66–72.

FERGUSON, J. (1939). The use of chemical potentials as indices of toxicity. *Proc. roy. Soc. B.*, **127**, 387–404.

HENDERSON, V. E. & LUCAS, G. H. W. (1932). Claude Bernard's theory of narcosis. *J. Pharmacol. exp. Ther.*, **44**, 253–267.

KRANTZ, J. C., ESQUIBEL, A., TRUITT, E. B., LING, A. S. C. & KURLAND, A. A. (1958). Hexafluorodiethyl ether (indoklon); an inhalant convulsant; its use in psychiatric treatment. *J. Amer. med. Ass.*, **166**, 1555–1562.

KRANTZ, J. C., TRUITT, E. B., LING, A. S. C. & SPEERS, L. (1957). Anaesthesia LV. The pharmacologic response to hexafluorodiethyl ether. *J. Pharmacol. exp. Ther.*, **121**, 362–368.

KRANTZ, J. C., TRUITT, E. B., SPEERS, L. & LING, A. S. C. (1957). New pharmacoconvulsive agent. *Science*, **126**, 353–354.

LU, G. M. D., LING, A. S. C. & KRANTZ, J. C. (1953). Anaesthesia XLI. The anaesthetic properties of certain hydrocarbons and ethers. *Anaesthesiology*, **14**, 466–472.

NAESS, K. (1950). Changes in the excitability curve of the rabbit's motor nerve during ether anaesthesia. *Acta Pharm. Toxicol.*, **6**, 123–136.

OVERTON, E. (1901). *Studien über die Narkose*. Jena: Fischer.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug-receptor combination. *Proc. roy. Soc. B.*, **154**, 21–69.

POSTERNAK, J. & ZAHND, G. (1953). Action de narcotiques sur la conduction par les fibres nerveuses et sur leur potential de membrane. *Helv. physiol. pharmacol. Acta*, **11**, *C*73–74.

POZNAK, A. VAN & ARTUSIO, J. F. (1960a). Anaesthetic properties of a series of fluorinated compounds. I. Fluorinated hydrocarbons. *Toxicol. appl. Pharmacol.*, **2**, 363–373.

POZNAK, A. VAN & ARTUSIO, J. F. (1960b). Anaesthetic properties of a series of fluorinated compounds: II. Fluorinated ethers. *Toxicol. appl. Pharmacol.*, **2**, 374–378.

ROBBINS, B. H. (1946). Preliminary studies of the anaesthetic activity of fluorinated hydrocarbons. *J. Pharmacol. exp. Ther.*, **86**, 197–204.

ROBERTSON, J. D., SWAN, A. A. B. & WHITTERIDGE, D. (1956). Effect of anaesthetics on systemic baroreceptors. *J. Physiol. (Lond.)*, **131**, 463–472.

SACHDEV, K. S., PANJWANI, M. H. & JOSEPH, A. D. (1963). Potentiation on the response to acetylcholine on the frog's rectus abdominis by ethyl alcohol. *Arch. int. Pharmacodyn.*, **145**, 36–43.

SPEDEN, R. N. (1963). *Kinetics and Mechanism of Action of some Volatile Anaesthetics*. Thesis for D.Phil. at the University of Oxford.

STRUCK, H. C. & PLATTNER, E. B. (1940). A study of the pharmacological properties of certain saturated fluorocarbons. *J. Pharmacol. exp. Ther.*, **68**, 217–219.

TORDA, C. (1943). Effect of chloroform and ether on the sensitivity of muscle to acetylcholine. *J. Pharmacol. exp. Ther.*, **77**, 350–356.

WHITTERIDGE, D. & BÜLBRING, E. (1944). Changes in activity of pulmonary receptors in anaesthesia and their influence on respiratory behaviour. *J. Pharmacol. exp. Ther.*, **81**, 340–359.