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PHARMACOLOGICAL EFFECTS OF SOME DERIVATIVES OF FURAN AND CHOLINE, WITH SPECIAL REFERENCE TO ACTION ON THE BLADDER AND TO TOXICITY

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In their contribution on the structure-action relationships of members of the choline group, Ing, Kordik, and Tudor Williams (1952) give figures for the potency of their compounds on a number of pharmacological preparations. The muscarinic actions studied were those on the cat's blood pressure, the isolated guinea-pig ileum, rabbit auricles, and the isolated perfused frog heart. In confirmation of Fellows and Livingston (1940), a high order of muscarine activity was found in furfuryltrimethylammonium iodide (Formula I), a compound that has already found some clinical application under the name of " Furmethide " (Beaser, Lipton, and Altschule, 1943 ; Owens and Woods, 1946 ; Lawson, 1948 ; Goldberger, Landesman, and Burke, 1949 ; Weinstein, 1949 ; and others). The high muscarinic potency of furmethide is shown by Ing and his co-workers to be excelled, tenfold or more, by that of its 5-methyl analogue, 5-methylfurfuryltrimethylammonium iodide (Formula II), which proved to be as active as, or more active than, acetylcholine on some of the preparations tested.

Since furmethide has achieved some clinical utility, and since the pharmacological properties which underlie this usefulness are so much stronger in its 5-methyl analogue than in furmethide itself, the question might well be asked whether the methyl analogue is not a substance of high therapeutic potentialities. This cannot, of course, be finally answered by pharmacological as distinct from therapeutic observations, and especially not when the observations are made on laboratory animals rather than in man, but it is clearly desirable that any anticipation of therapeutic usefulness should be supported by further pharmacological experiments in laboratory animals. Special attention has therefore been directed towards two aspects of particular significance for the clinical application of substances of this type, namely, activity on the urinary bladder and acute toxicity. Another aspect, that of activity on the pupil of the eye, has been investigated by Grewal (1951), who has shown the miotic power of the methyl derivative when injected intraperitoneally in mice to be more than ten times that of furmethide, and more than twice that of carbachol.

While attention is focused, in the present contribution, on the power of selected compounds to cause contraction of the urinary bladder and on their acute toxicity, several experiments involving an examination of other pharmacological properties

are also described. These are, however, introduced only as corollaries to, or in order to throw light on, features brought out in the main experiments on the bladder or on toxic action.

The substances investigated, in addition to acetylcholine, were the following :

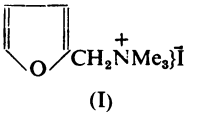
(*a*) Furfuryltrimethylammonium iodide, which we shall continue to designate here as " Furmethide," the name under which it is marketed by Messrs. Smith, Kline, and French. Its A.M.A. name is furtrethonium.

(*b*) The 5-methyl derivative of furmethide (5-methylfurfuryltrimethylammonium iodide), which, for convenience, will here be designated as " Methyl-furmethide." The specimens of (*a*) and (*b*) used in this work were prepared by Dr. H. R. Ing and Mr. D. P. H. Tudor Williams.

(*c*) The formal of 2:3-dihydroxypropyltrimethylammonium iodide, known as " 2249F " (Fourneau, Bovet, Bovet, and Montezin, 1944), and used clinically in France under the name of " Dilvasène " (for reference to clinical use, see Verbeke and De Vleeschouwer, 1950).

(*d*) The acetal corresponding to (*c*), known as " 2268F " (Fourneau *et al.*, 1944). The chemical relationship of 2249F and 2268F to furmethide and methyl-furmethide may be seen by comparing Formulae III and IV with I and II. They were kindly made available by Dr. A. J. Ewins, of Messrs. May and Baker.

(*e*) Carbamylcholine (carbachol).



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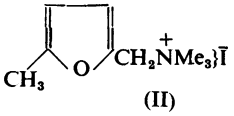

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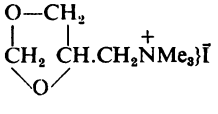

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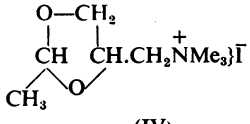

(IV)

Compounds 2249F and 2268F (*c* and *d* above) were included in this study because of their structural analogies to furmethide and methyl-furmethide, respectively, which Ing *et al.* (1952) have correlated with features of pharmacological activity ; in conformity with Ing's " 5-atom rule " (Ing, 1949), the muscarinic properties of both furmethide and 2249F are notably less than those of methyl-furmethide and 2268F. Carbachol was included in the study as a representative parasympathomimetic choline derivative already established in therapeutic practice.

Activity on the Urinary Bladder

Isolated guinea-pig bladders.—The isolated guinea-pig bladder preparation was set up in the following way. A guinea-pig of either sex was used, and the bladder removed after ligation of the ureters. A glass cannula was then tied in at the urethral opening. The cannula consisted of a piece of glass tubing 10 mm. in diameter and about 20 cm. long, narrowed at one end to a diameter of about 3 mm. and slightly flanged to enable it to be inserted into the bladder and firmly held there by the cotton thread ligature. The

cannula was then clamped in an upright position so as to suspend the bladder in an organ bath of about 30 ml. capacity, containing Locke solution at 37° C. Saline or Locke solution (5 to 8 ml., depending on the size of the bladder) was then introduced into the cannula, somewhat distending the bladder, the excess solution remaining as a pressure-column of several inches in the upright cannula. The top end of the cannula was then connected by rubber tubing to a piston-recorder, from which tracings were obtained of the contractions produced by substances introduced into the surrounding Locke solution in the organ bath.

Figs. 1 and 2 show the results obtained in two typical and consecutive experiments, in each of which all the five substances listed above were tested and compared with one another and with acetylcholine. These two experiments differ from one another only in the sequence in which the drugs were tested. It is necessary to vary this sequence in such experiments in order to eliminate the possibility that exposure of the bladder to any one compound might seriously alter its sensitivity, and so give an erroneous impression of the relative activity of two substances under comparison. If, however, the two substances retain the same apparent ratio of activity when tested in other preparations in the reverse sequence, the ratio may confidently be accepted as valid. The two Figures, studied in relation to one another, show that this possible complication of changing sensitivity of the bladder did not seriously arise, and the following conclusions are to be drawn :

(1) The preparation discriminates well and consistently between different concentrations of acetylcholine.

(2) The response to acetylcholine reaches its maximum almost immediately and then usually tends to diminish. To each of the other compounds there may also be a smart initial response, but the maximum effect may not be reached for even as long as 5 min. and shows no tendency to diminish.

(3) If dose-response curves were drawn, those for the three more active substances, methyl-furmethide, 2268F, and carbachol, would evidently be steeper than the curve for acetylcholine.

(4) Because of these differences in the type of response, both qualitative (2 above) and in regard to the slope of the dose-response curve (3 above), unexceptionable acetylcholine equivalents of the substances tested cannot be derived. However, the following approximations (equivalents by weight) are probably permissible :

2268F	0.25–0.5
Methyl-furmethide	0.5 –1.0
Carbachol	1.0
Acetylcholine	1.0
Furmethide	5.0–10.0
2249F	20.0–40.0

Cat bladders in situ.—In repeated trials, methyl-furmethide consistently proved to be more active than carbachol on the isolated bladder, and experiments were therefore performed to see whether this superiority could be demonstrated on the bladder *in situ* after intravenous injection. Fig. 3 shows the results of two such experiments in cats under chloralose anaesthesia, in which the bladder was exposed, the ureters and urethra ligated, and the cannula tied into an incision at the fundus of the bladder. The bladder and cannula were filled with saline to a height of

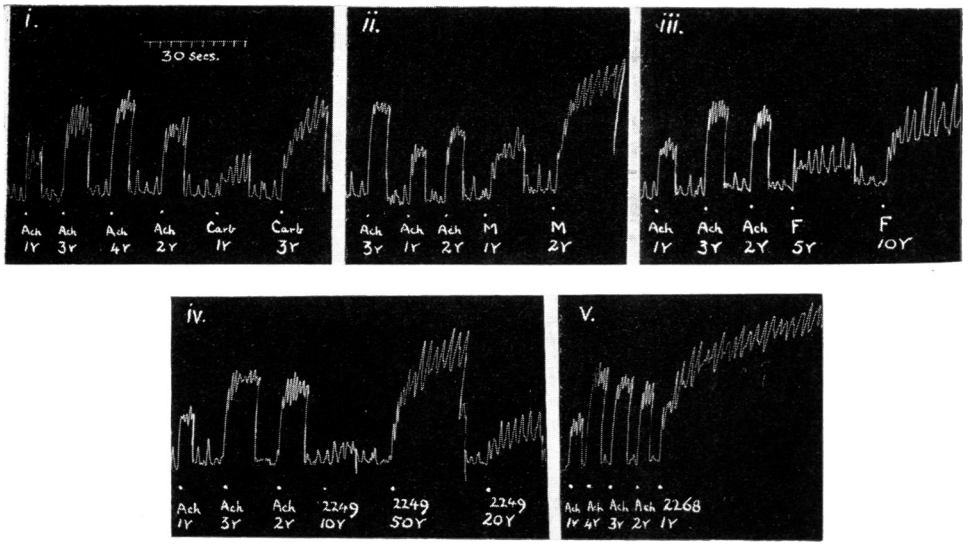

FIG. 1.—Guinea-pig bladder in Locke solution at 37° C. 30 ml. bath. Continuous tracing of a single experiment, each break in the figure representing about 5 min. Ach=acetylcholine. Carb=carbachol. M=methyl-furmethide. F=furmethide. 2249=Fourneau's 2249. 2268=Fourneau's 2268.

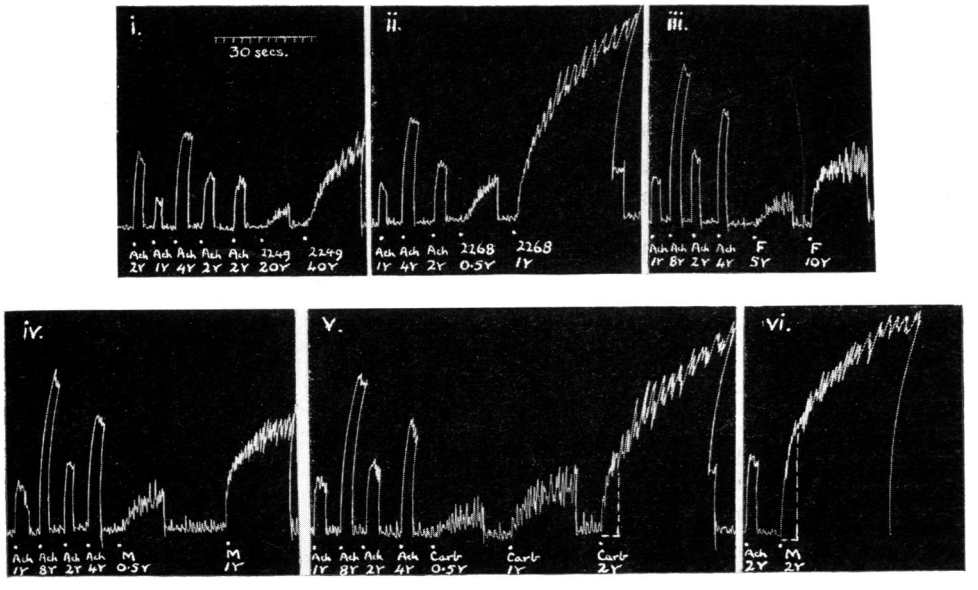

FIG. 2.—Experiment similar to that of Fig. 1 above, but compounds tested in a different sequence. Dotted lines in sections v and vi indicate extent of response 60 sec. after introducing drug.

about five inches, and tracings obtained by means of a piston-recorder, as in the observations on the isolated organ. It may be seen from Fig. 3 that methyl-furmethide caused appreciably greater effects than carbachol, irrespective of the sequence in which they were tested.

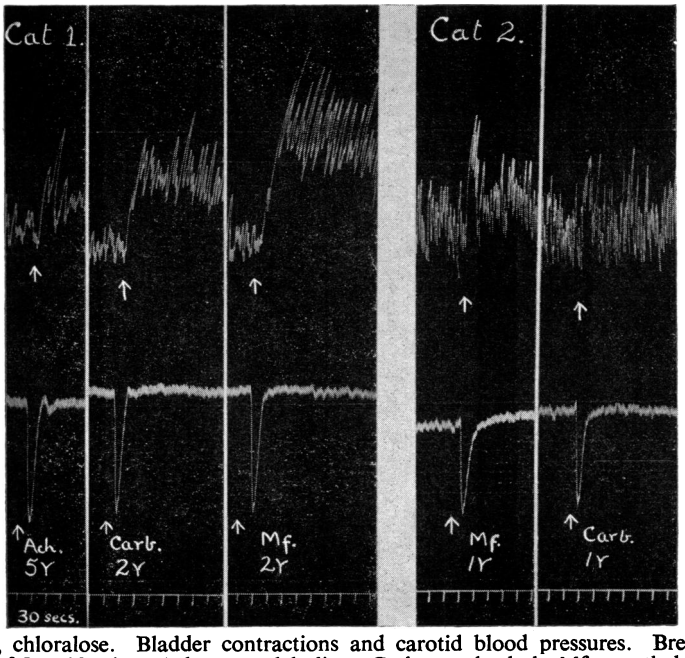

FIG. 3.—Cats, chloralose. Bladder contractions and carotid blood pressures. Breaks represent intervals of 5 to 10 min. Ach. = acetylcholine. Carb. = carbachol. Mf. = methyl-furmethide.

Action on the gut.—The fact, shown by Figs. 1 and 2, that the response of the bladder to acetylcholine reaches its maximum immediately and then usually tends to diminish, while the response to other compounds proceeds more slowly and shows no tendency to diminish, is no doubt related to the immunity of these other compounds to the hydrolytic action of cholinesterase. This stability in the presence of cholinesterase has also been held by Ing *et al.* (1952) to account for the steeper dose-response curves of some of their compounds in comparison with that of acetylcholine, as observed in the pharmacological preparations which they used. Equally it must account for the steeper dose-response curves for bladder activity, evident from inspection of Figs. 1 and 2. Ing and his colleagues have given point to this feature by quoting the decrease in activity compared with acetylcholine of some of their substances when the comparisons were made on rabbit auricles in the presence of eserine, and, in experiments for co-operation in which I am obliged to Dr. J. L. Guimaraes, similar and further observations of this type have been made on isolated rabbit auricles and on the isolated guinea-pig ileum in respect of methyl-furmethide. Our salient findings on both these tissues were (*a*) that when the two compounds were tested in the absence of eserine the dose-response curve for methyl-furmethide was steeper than that for acetylcholine, and (*b*) that in the presence of eserine the dose-

response curve for methyl-furmethide was practically the same as in its absence, while that for acetylcholine was not only shifted to the left (i.e. towards the abscissae for lower concentrations) but it also acquired the steeper inclination characteristic of methyl-furmethide (and of carbachol).

The failure of eserine to potentiate the effects of methyl-furmethide was also unmistakably demonstrated in a cat under chloralose in which observations were made on the blood pressure and on intestinal movements recorded by means of a

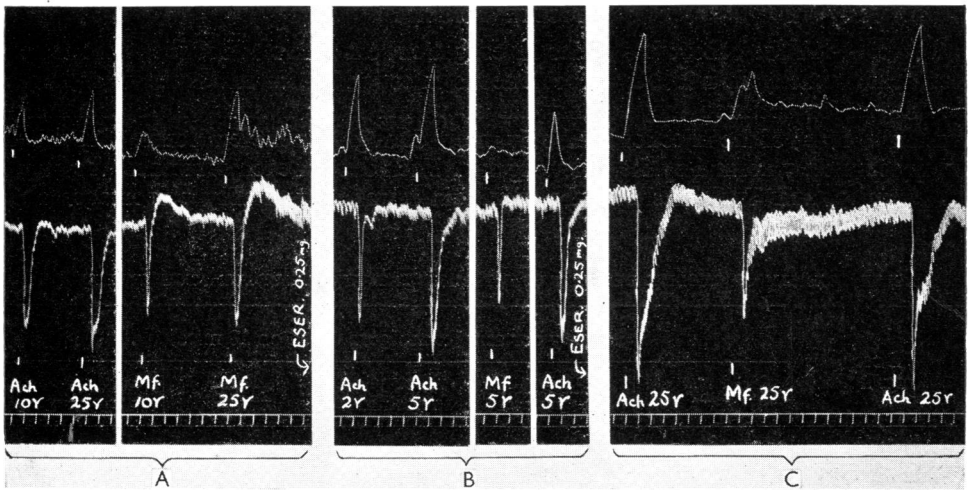

FIG. 4.—Cat, chloralose. Duodenal movements and blood pressure. 30-sec. time-intervals. Between A and B, and between B and C, 20 min. Other breaks represent 6 to 8 min. Ach. = acetylcholine. Mf. = methyl-furmethide. Eser. = eserine.

water-filled balloon tied into the duodenum and leading to a piston-recorder. The results are illustrated in Fig. 4, from which several conclusions may be drawn in support of those derived from the tests on isolated organs. The Figure shows the following :

(1) Before injecting eserine (Section A of the tracing) the responses were, in general, about the same to methyl-furmethide as to acetylcholine. The tracing for duodenal movements in this section again illustrates, however, the difficulty of measuring the potency of a substance of the type of methyl-furmethide in terms of acetylcholine equivalents, since, as had been observed on the isolated organ preparations, the dose-response curve would evidently be steeper for methyl-furmethide than for acetylcholine. Thus, at a dose of 10 μg. the effect of methyl-furmethide was slightly less than that of acetylcholine, but when the dose was raised to 25 μg. the increased response to methyl-furmethide was so much greater than that to acetylcholine that the relationship was reversed and the acetylcholine effect became slightly the smaller of the two. There seemed also to be a tendency for the response to 25 μg. to persist rather longer after methyl-furmethide than after acetylcholine. Incidentally, another feature of this section of the tracing is the slight pressor effect observed immediately after the blood pressure had returned to its original level following a dose of methyl-furmethide ; this may perhaps be related to that compound's slight nicotine-activity, to which Ing *et al.* (1952) have drawn attention.

(2) Failure of eserine to potentiate methyl-furmethide, while considerably potentiating acetylcholine, is evident from the tracings for both the blood pressure and duodenal movements. The effect of eserine was such that responses to 2 or 5 μg. acetylcholine (Section B of the tracing) became greater than those that had been produced by 25 μg. before eserine (Section A). Methyl-furmethide, on the other hand, caused a negligible effect on the duodenum when injected in a dose of 5 μg. after eserine (Section B), and 25 μg. (Section C) did not produce any greater effect on either the blood pressure or the duodenum than it had done before the eserine (Section A).

Acute Toxicity

Mice.—All the substances under investigation, except acetylcholine, were tested for acute toxicity in mice, by subcutaneous injection in 0.4 ml. aqueous solution. When deaths occurred they usually did so within half an hour (more commonly within 15 min.) of the injection, and only rarely during the next few hours. Survivors were completely normal by the following day. Each dose of each substance was given to 20 mice, and Table I shows the results in terms of mortality percentages and

TABLE I

ACUTE TOXICITY OF CHOLINE DERIVATIVES FOR MICE, TREATED BY SUBCUTANEOUS INJECTION

Dose (mg./kg.)	Mortality per cent (each figure based on 20 mice)				
	2268F	Carbachol	Methyl-furmethide	2249F	Furmethide
40.0	—	—	55	60	30
20.0	—	—	25	10	0
10.0	—	—	0	—	—
5.0	100	95	—	—	—
2.5	70	30	—	—	—
1.25	40	10	—	—	—
LD50	1.6	2.8	33.0	34.6	>40.0

estimated median lethal doses (LD50). The LD50 could not be ascertained within close limits of error, from observations on so few mice. It was estimated for each drug by integrating the results (Behrens, 1929) and selecting the two log dose-percentage response values which lay closest to, and one on each side of, the median ; the probits of the two selected percentages were then plotted as ordinates to their corresponding log doses, joined by a straight line, and the LD50 derived from the point corresponding to probit 5 (Gaddum, 1933 ; Bliss, 1934).

The Table shows that 2268F is about twice as toxic to mice as carbachol, and that both these substances are considerably more toxic than any of the others. Perhaps the most striking point emerging is that the toxicity of methyl-furmethide is only about one-tenth that of carbachol.

Rats, guinea-pigs, and rabbits.—Tests for acute toxicity in animals other than mice were limited to a comparison between carbachol and methyl-furmethide, in order to determine whether the much lower toxicity of the latter substance, observed in mice, could be observed also in other species. With compounds of this type it is probably a safe assumption that destruction and elimination are complete in less

than 24 or 48 hours after treatment. Where great precision in the results of acute toxicity tests is not required, considerable economy may therefore be effected by using a relatively small number of animals and treating them at intervals of 48 hours, starting with a small dose and increasing the size of dose on each occasion until the approximate LD50 is indicated by the point where the cumulative total killed amounts to half the original number of animals. Tests were performed in this way in rats, guinea-pigs, and rabbits. The compounds were administered intraperitoneally, and the deaths that occurred did so within a few hours (usually within an hour) of the injection, with the well-known symptoms of acute parasympathetic poisoning. Table II and Fig. 5 show the results of these tests. The LD50 values

TABLE II

ACUTE TOXICITY OF CARBACHOL AND METHYL-FURMETHIDE FOR VARIOUS ANIMAL SPECIES, TREATED BY INTRAPERITONEAL INJECTION EVERY ALTERNATE DAY, THE DOSE BEING DOUBLED ON EACH OCCASION. SHOWING NUMBER DEAD AFTER EACH DOSE (INTEGRATED TOTALS)

Species	Days since start		—	2	4	6	8	10	12	14	16	18	20	22	24	LD50
	Dose (mg./kg.)		0.062	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16	32	64	128	256	
Rats, 10 in each group	No. dead	Carb.	0	0	0	0	0	5	10							2.0
		Methyl-furmeth.	0	0	0	0	0	0	0	0	0	0	7	9	10	32–64
G.-pigs, 8 in each group	No. dead	Carb.	0	5	8											0.06–0.12
		Methyl-furmeth.	0	0	0	0	2	3	6	8						2.0–4.0
Rabbits, 5 in each group	No. dead	Carb.	0	0	0	1	1	4	5							1.0–2.0
		Methyl-furmeth.	0	0	0	0	0	0	1	2	2	4	5			16–32

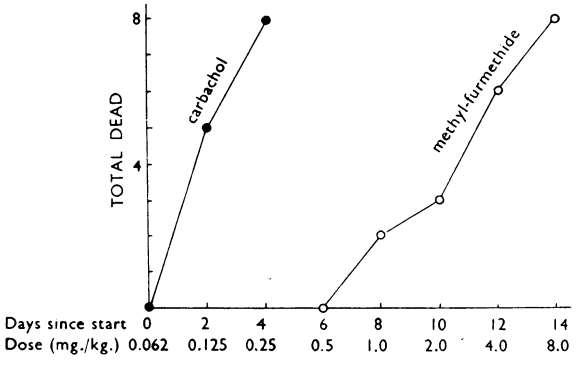

FIG. 5.—Acute toxicity of carbachol and of methyl-furmethide in guinea-pigs.

recorded cannot, of course, claim to be more than rough approximations, but they suffice to show unmistakably that, while there are considerable differences in species-sensitivity (guinea-pigs being much more sensitive than rats or rabbits to both compounds), the lower acute toxicity of methyl-furmethide, about one-tenth

that of carbachol in mice, extends in about the same measure to rats, guinea-pigs, and rabbits.

Broncho-constriction in guinea-pigs.—It is curious that methyl-furmethide should be more potent than carbachol on the bladder and, according to Grewal (1951), on the pupil, and yet be so much less toxic than carbachol. In order to throw more light on this apparent paradox, the two compounds were tested on a system which, unlike the urinary bladder or the pupil of the eye, probably plays an essential rôle

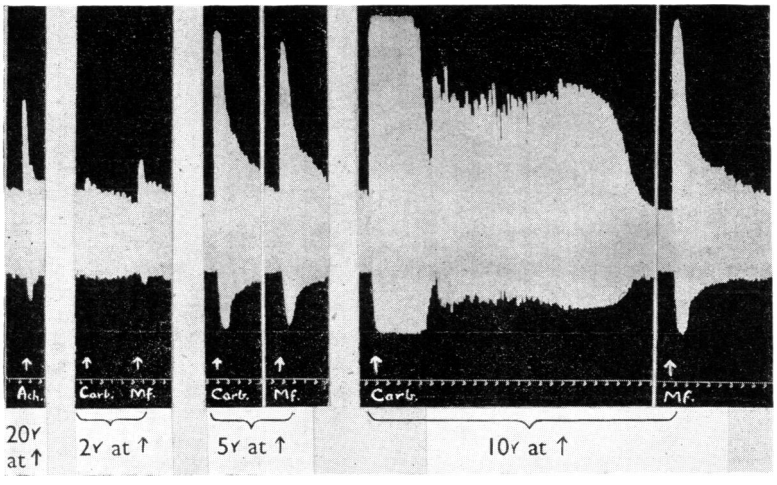

FIG. 6.—Guinea-pig. Urethane anaesthesia. Broncho-constrictor effects. 60-sec. time-intervals. Breaks represent intervals of 4 to 6 min. Ach.= acetylcholine. Carb.= carbachol. Mf.= methyl-furmethide.

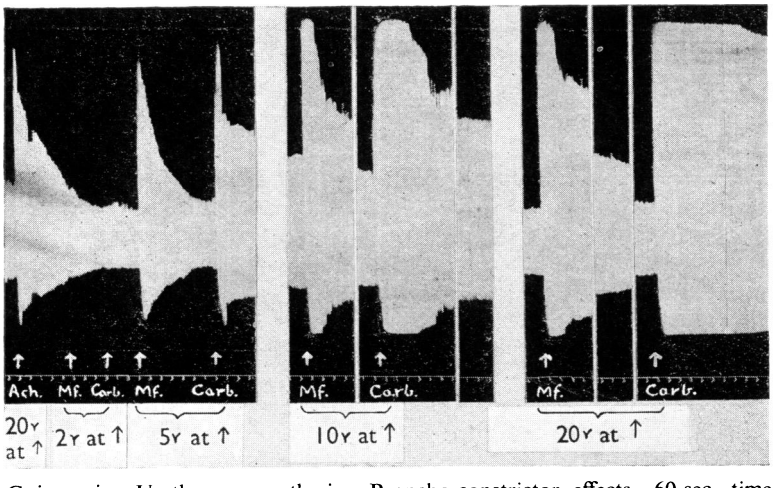

FIG. 7.—Guinea-pig. Urethane anaesthesia. Broncho-constrictor effects. 60-sec. time-intervals. Breaks represent intervals of 10, 30, 20, 30, 20, and 30 min. Ach.= acetylcholine. Mf.= methyl-furmethide. Carb.= carbachol.

in the mechanism which actually kills when the dose is a lethal one ; this is the bronchial tree, and accordingly the capacities of carbachol and methyl-furmethide to cause broncho-constriction on intravenous injection were compared by the technique of Konsett and Rössler (1940). Figs. 6 and 7 show the results of two consecutive experiments, in one of which (Fig. 6) carbachol was tested before methyl-furmethide at each dose level, and in the other (Fig. 7) the sequence was reversed. Studied in relation to one another, the Figures show that in this preparation, as on the isolated bladders, comparison between the effects produced by the two substances is not vitiated by any change in the sensitivity of the preparation produced by the substance initially tested. In both experiments the response was no greater to carbachol than to methyl-furmethide when the dose was less than 5 μg. Indeed, at 2 μg. in the experiment of Fig. 6 methyl-furmethide produced a slightly greater effect than carbachol. When, however, the dose exceeded 5 μg., carbachol caused a much more protracted effect than methyl-furmethide. It is therefore reasonable to associate the higher acute toxicity of carbachol with the fact that in high doses it has a greater effect than methyl-furmethide on the bronchiolar system.

Discussion

This investigation was approached from the point of view that one question arising from the work of Ing *et al.* (1952) was whether methyl-furmethide might not be a potentially useful therapeutic substance, and that any expectations of such usefulness should be supported by further pharmacological observations. Two properties of special significance for the clinical application of compounds of this type were therefore selected for detailed study. These were activity on the bladder and acute toxicity, and in both properties methyl-furmethide appears to be superior to carbachol. It was more active than carbachol on the bladder, both of the guinea-pig in the organ bath and of the cat *in situ,* and it was only about one-tenth as toxic as carbachol to mice, rats, guinea-pigs, and rabbits.

Methyl-furmethide was, however, not the most active of the compounds tested on the bladder. The most active was 2268F, but its high activity on this organ is offset, from the point of view of therapeutic potentialities, by a correspondingly high degree of acute toxicity, which is about twenty times that of methyl-furmethide. The parent substance, furmethide, which has already achieved some clinical repute, exhibited only one-tenth of the activity of its methyl derivative on the bladder, but it has the advantage over the methyl substance of being only about half as toxic (killing 30 per cent of mice at a dose of 40 mg./kg., compared with 25 per cent killed by methyl-furmethide at a dose of 20 mg./kg.). Balancing the tenfold superiority of methyl-furmethide in respect of activity on the bladder against the twofold superiority of furmethide in respect of toxicity, the methyl derivative may therefore be rated, crudely, as having a fivefold advantage over the parent substance.

The facts that methyl-furmethide is, on the one hand, more active than carbachol on the bladder and on the pupil (Grewal, 1951), and, on the other hand, less acutely toxic, might be ascribed on a superficial view to differences between the relative activity of the two compounds on different tissues ; and this seems to be borne out by the experiments which showed that carbachol had a greater effect than methyl-furmethide on the bronchioles in spite of its lower activity on the bladder and the

pupil. The observations on broncho-constriction were, however, not strictly comparable with those on the bladder and with Grewal's observations on the mouse pupil. The greater effect of carbachol than of methyl-furmethide on the bronchioles was observed only in relatively high doses and was manifested by a prolongation of the response rather than by a greater intensity of the effect when it had reached its maximum (see Figs. 6 and 7). If experimental procedures on the bladder or other organs had been devised to take into account the duration of effect at high doses rather than the degree of the immediate response at relatively low doses, they might well have given results similar to those obtained on the bronchioles, especially if the experiments had been done in the whole animal rather than in an organ bath. The much shorter duration of the broncho-constrictor effect of methyl-furmethide than of carbachol suggests that the body is able to break down or eliminate methyl-furmethide much more readily than carbachol. It may be significant in this connexion that, as Ing *et al.* (1952) have mentioned, the methyl group in the furan ring would tend to reduce its stability. They raise this as one of the more unlikely explanations of the high activity of methyl-furmethide ; it is perhaps at least as feasible that the tendency to instability of the ring, instead of accounting for this compound's high activity, may be responsible for curtailing its full effect. The characteristic cause of death with all the compounds of this type is asphyxia, and the shorter duration of the broncho-constrictor effect after methyl-furmethide than after carbachol is probably quite sufficient to account for the fact that it is the less toxic compound of the two.

This investigation does not lose sight of the fact that final judgment on the clinical value of any substance must obviously and necessarily depend on tests on man and not on laboratory animals. Expectation of useful results with methyl-furmethide in man is, however, supported by the variety of animal species (mice, rats, guinea-pigs, cats, and rabbits) in which it has proved to be superior to carbachol in respect of the few properties that have been selected for investigation because of their importance for the clinical application of substances of this type.

Summary

Other workers have shown that furfuryltrimethylammonium iodide (" Furmethide ") is a substance of high muscarinic potency, while its 5-methyl derivative (designated here, for convenience, as " Methyl-furmethide ") is even more active in this respect.

The present communication is a record of studies on both these compounds, together with other choline derivatives, on a number of pharmacological preparations. The high muscarinic activity of methyl-furmethide suggested that it might have useful therapeutic potentialities, and special attention was therefore directed to a comparison of this compound with the others of the present series in respect of features that are important for the clinical application of substances of this type, namely, activity on the urinary bladder and acute toxicity.

Methyl-furmethide proved to be more active than carbachol on the bladder, both in the organ bath and after intravenous injection, and much less toxic than carbachol to mice, rats, guinea-pigs, and rabbits. Its lower toxicity is probably related to

the fact that in high doses it causes a less protracted broncho-constrictor effect than that caused by carbachol.

I am greatly obliged to Professor J. H. Burn and Dr. H. R. Ing for helpful discussions during the course of this work, which was supported by a grant from the Medical Research Council.

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