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A METHOD FOR TESTING MIOTICS ON THE MOUSE PUPIL

BY

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At present there is no good method available for testing the activity of substances which constrict the pupil. Aeschlimann and Reinert (1931), in their investigation of analogues of physostigmine, tested substances by instillation into the eye of the cat, comparing the pupil of the eye with that of the other eye. Fellows and Livingston (1940) made similar observations on furfurylammonium iodides using rabbits.

Pulewka (1932) described a quantitative method for testing mydriatics on the pupil of the mouse, in which he determined the mean effect on the diameter of the pupil in groups of mice. This method was used extensively by Ing, Dawes, and Wajda (1945) in the work which led to the introduction of lachesine as a mydriatic. It seemed possible that a method for testing miotics might be based on their ability to antagonize the action of atropine when Pulewka's procedure was followed. If different groups of mice were injected with a given dose of atropine so as to produce a moderate mydriasis, then miotic substances might be compared by finding the dose required to reduce the size of the pupil to the same extent.

METHOD

In Pulewka's method, as it has been used in this laboratory, groups of five mice are taken. They are not kept without food beforehand. The pupil of each mouse is examined under a binocular microscope ($\times$ 10) which has a scale in the eyepiece. A small lamp is attached to the microscope so as to shine on the object, and the diameter of the pupil when thus illuminated is measured on the scale. The mean figure for the pupil of normal mice is about two divisions (7.7 divisions = 1 mm.). If mice are given an intraperitoneal injection of atropine sulphate and are examined about 15 min. later, the mean diameter of the pupil increases in linear proportion to the log-dose. Thus 2 μg. atropine increases the mean size to about 4 divisions, 4 μg. increases it to about 8 divisions, and 8 μg. increases it to about 14 divisions (Ing, Dawes, and Wajda, 1945).

In the present investigation groups of mice were taken, in any experiment mice of the same range in weight being used. Mice from 15–20 g. are more sensitive to atropine than heavier ones. The solution of the miotic was mixed with a solution of atropine sulphate, and a volume of the mixture was injected intraperitoneally which contained 4 μg. atropine sulphate and a certain weight of the miotic in 0.2 ml. The pupil of the mouse was examined after 15 min.

Substances investigated.—The substances tested were in the first place compounds prepared here by Dr. H. R. Ing and Mr. D. P. H. Tudor Williams, namely:

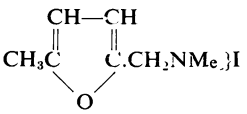
(*a*) 5-Methyl-furfuryltrimethylammonium iodide (I), referred to as the methyl-furfuryl compound.

(*b*) Furfuryltrimethyl ammonium iodide (II), referred to as the furfuryl compound; this substance is marketed as Furmethide (Smith, Kline and French); it was previously examined by Fellows and Livingston (1940).

Through the kindness of Dr. A. J. Ewins, F.R.S., of Messrs. May & Baker, there were available:

(*c*) Compound 2268F (Fourneau, Bovet, Bovet, and Montezin, 1944), the acetal of 2 : 3-dihydroxypropyltrimethylammonium iodide (III), referred to as the acetal compound; and

(*d*) Compound 2249F (Fourneau *et al.*, 1944), the corresponding formal derivative (IV), referred to as the formal compound.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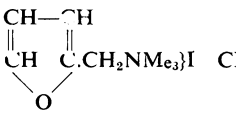
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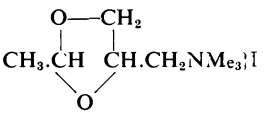
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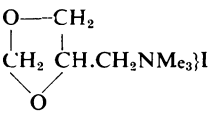
IV

The following substances were also examined:

(*e*) Carbamylcholine (carbachol).

(*f*) Eserine sulphate.

(*g*) Neostigmine methylsulphate.

RESULTS

Carrying out the test.—In preliminary tests, groups of five mice were injected with varying doses of each miotic in order to determine the range of activity. A dose which reduced the effect of atropine to about half was selected together with doses smaller and larger. The results of one experiment with the methyl-furfuryl compound are given in Table I.

The final observations with the different compounds are recorded in Table II. When the mean results were plotted against the log dose as in Fig. 1, a linear relation between dose and pupil diameter was obtained. The lines for eserine, the methyl-

TABLE I

Diameter of pupil (in scale divisions) after injection with mixture containing 4 μg. atropine and the stated amount of the methyl-furfuryl compound

Group 1 5 μg.	Group 2 10 μg.	Group 3 20 μg.	Group 4 40 μg.
4.0	7.0	2.5	1.0
7.5	2.5	5.0	2.5
4.0	4.0	6.0	1.5
3.5	3.5	2.0	2.0
5.5	3.5	2.0	4.0
5.0	4.5	4.5	2.5
2.5	3.5	2.0	2.5
5.0	2.5	2.0	2.0
8.5	5.0	4.0	4.0
4.5	4.5	5.0	1.5
Mean 5.0	4.05	3.5	2.35

TABLE II

Mean results with different doses, each dose being given together with 4 μg. atropine; size of pupil in scale divisions

Compound	No. of mice	Dose μg.	Size of pupil	Dose μg.	Size of pupil	Dose μg.	Size of pupil	Dose μg.	Size of pupil
Methyl-furfuryl ..	20	5	5.5	10	4.4	20	3.6	40	2.7
Furfuryl	30	40	5.9	80	4.5	160	4.05	—	—
Acetal	30	5	6.15	10	5.1	20	3.55	40	2.4
Formal	40	40	6.0	80	5.7	160	5.4	—	—
Carbachol	30	10	5.75	20	4.3	40	3.7	80	2.45
Eserine	25	—	—	2.5	3.6	5.0	2.85	10	2.0
Neostigmine	20	0.625	4.2	1.25	2.8	2.5	1.3	—	—

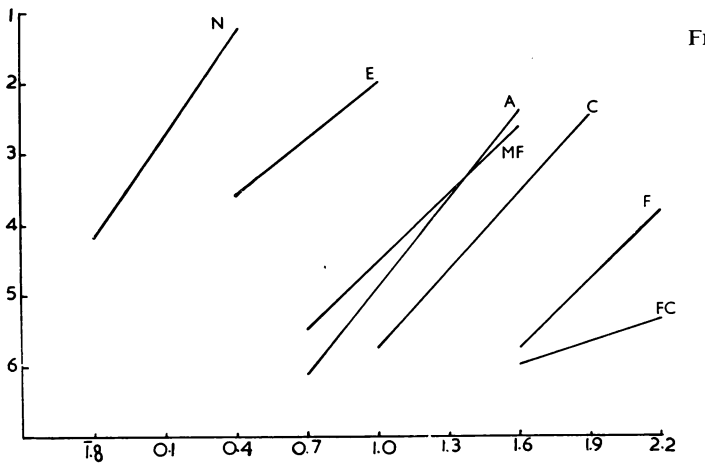

FIG. 1.—To show the relation between log dose of the miotic (abscissae) and the effect measured in scale divisions (ordinates). Since each dose is given together with a standard dose of atropine, the greater the dose of miotic the smaller the pupil becomes. Hence the ordinate is marked in descending order of magnitude. N = neostigmine, E = eserine, A = acetal compound, MF = methyl-furfuryl compound, C = carbamylcholine, F = furfuryl compound, and FC = formal compound.

furfuryl compound, the acetal compound, carbachol, and the furfuryl compound were approximately parallel. The line for neostigmine was steeper than the others, and the line for the formal compound was less steep than the others.

Neostigmine appears as the most potent miotic, and eserine is the next. The methyl-furfuryl compound and the acetal compound are again weaker and approximately equal, being stronger than carbachol, which is in turn stronger than the furfuryl compound. The formal compound shows only traces of activity.

Since the slope of the line for eserine is very nearly parallel to that of four of the other compounds, their activity has been expressed in relation to that of eserine taken as =100. The figures are given in Table III, in which an average figure for neostigmine is also included.

DISCUSSION

The evidence shows that substances with a muscarine-like action are not such strong miotics as substances which are powerful inhibitors of cholinesterase. It will be seen from Table III that neostigmine and eserine are far stronger than carbachol or the methyl-furfuryl compound or the acetal compound. Neostigmine, being more

TABLE III

RELATIVE MIOTIC ACTIVITY

Neostigmine	350
Eserine	100
Methyl-furfuryl compound	13.5
Acetal compound	13.5
Carbachol	5.0
Furfuryl compound	1.0
Formal compound	very weak

stable than eserine, is a powerful miotic. Aeschlimann and Reinert (1931) found neostigmine weaker than eserine on the eye of the cat, but, in a clinical trial in acute and chronic glaucoma, Clarke (1939) found neostigmine to be better than eserine in that it did not deteriorate and had a stronger and more prolonged action. He did not, however, test the two substances in the same concentration. It is probable that eserine is absorbed more readily than neostigmine from the conjunctival sac, and therefore when these substances are given by instillation the greater potency of neostigmine observed in the present experiments is not apparent.

Additional evidence that the anticholinesterases are more potent miotics than the muscarine-like compounds is provided by the well-known activity of di-*iso*propyl-fluorophosphonate, which continues for 6–27 hr. on the human eye (Leopold and Comroe, 1946).

SUMMARY

1. A method is described for measuring miotic activity on the pupil of the mouse in which the miotic is given by intraperitoneal injection, together with a standard dose of atropine. The miotic then antagonizes the dilator action of atropine to an extent which is proportional to the log dose of the miotic.

2. Anticholinesterases are more powerful as miotics than muscarine-like substances ; thus eserine is 20 times as potent as carbamylcholine, and neostigmine is 3–4 times as potent as eserine.

3. In addition to carbachol, the following substances have been tested : (*a*) the Fourneau compound 2268, the acetal of dihydroxypropyltrimethylammonium iodide ; (*b*) F2249, which is the corresponding formal ; (*c*) furfuryltrimethyl-ammonium iodide (Furmethide) ; (*d*) 5-methyl-furfuryltrimethylammonium iodide.

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