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THE ACTION OF SYNTHETIC CURARIZING COMPOUNDS ON SKELETAL MUSCLE AND SYMPATHETIC GANGLIA BOTH NORMAL AND DENERVATED

BY

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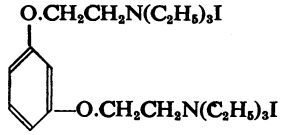
A recently synthesized substance F2559 with curarizing properties was shown by Bovet, Depierre, and de Lestrange (1947) to differ from *d*-tubocurarine in its effect on the blood pressure. An intravenous dose of tubocurarine chloride which caused muscular paralysis in the dog lasting several hours also caused a fall in general blood pressure, but a dose of the synthetic compound with the same curarizing action did not depress the blood pressure. The fall of blood pressure after an injection of tubocurarine may be caused in two ways. Firstly, it may be due to the release of histamine which was first observed by Anrep and his co-workers (Alam *et al.*, 1939). However, Grob, Lilienthal, and Harvey (1947) showed that in man tubocurarine produced vascular changes only after intra-arterial injection and not when given intravenously. The second mechanism by which tubocurarine may lower the blood pressure is by its action on sympathetic ganglia. When a comparison was made of the activity of the mono-, di-, and tri-quaternary ammonium salts shown below, of which the substance mentioned above is a member, it was found that the compound with the strongest action on the blood pressure had the weakest curarizing action on skeletal muscle, whereas the compound with the strongest action on the muscle had the weakest action on the blood pressure. Moreover, Depierre (1947) showed that the ratio of doses of these compounds for blocking transmission in the superior cervical ganglion was similar to that for lowering blood pressure. It appeared interesting to make a more detailed investigation of the action of these three compounds on the normal and denervated sympathetic ganglion and to compare it with their action on normal and denervated skeletal muscle.

F2512

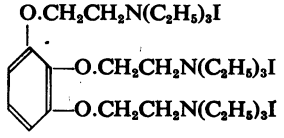
β-diethylaminoethoxybenzene ethiodide

F2557



1 : 3-bis-(β-diethylaminoethoxy)benzene diethiodide

F2559



1 : 2 : 3-tri-(β-diethylaminoethoxy)benzene triethiodide

EXPERIMENTAL RESULTS

(1) *Effect on the response of the nictitating membrane to preganglionic sympathetic nerve stimulation in the perfused superior cervical ganglion*

Method.—Cats under chloralose were used. The ganglion was perfused with Locke's solution according to Kibjakow's (1933) method, modified by Feldberg and Vartiainen (1934). The preganglionic nerve was stimulated maximally at a rate of 16 per sec. for 15 sec. every 4 min. The compounds were injected into the arterial cannula one minute before each stimulation.

Results.—Depierre's finding was confirmed that F2559 was very weak in depressing synaptic transmission, the lowest dose having some effect being 1 mg. in one experiment whereas in another as much as 10 mg. was required. On the other hand, F2512 was found to be nearly as strong as *d*-tubocurarine chloride, acting in doses from 40 to 200 μg. Doses of F2557 causing synaptic depression were intermediate between those of the two other compounds. In six perfusions the average doses depressing synaptic transmission were 5 mg.

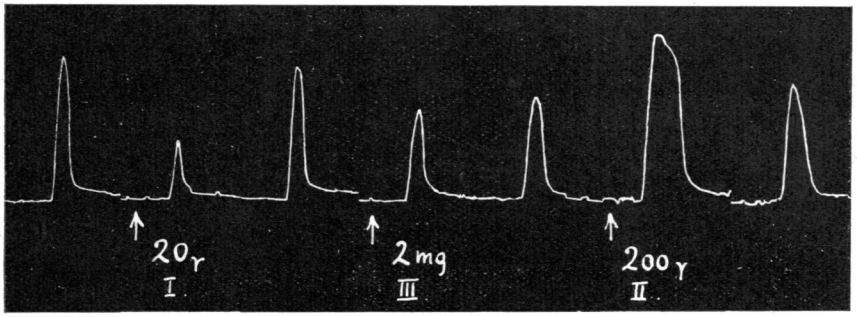


FIG. 1.—Cat, chloralose. Perfused superior cervical ganglion. Record of contractions of the nictitating membrane to intra-arterial doses of 50 μg. acetylcholine. I = F2512, III = F2559, II = F2557.

F2559, 500 μg. F2557, and 100 μg. F2512 If these doses are compared with those injected intra-arterially to depress neuromuscular transmission (Section (4)) it will be seen that they are of similar magnitude but in the reverse order, the ratio being 50 : 5 : 1.

(2) *Effect on the contractions of the nictitating membrane induced by the injection of acetylcholine into the perfused superior cervical ganglion*

Method.—The method was the same as that in Section (1).

Results.—Fig. 1 shows the effect of the three compounds given in succession. A dose as small as 20 μg. F2512 reduced the response to acetylcholine by 50 per cent; it recovered very quickly. As much as 2 mg. F2559 were required to reduce the response by about 25 per cent, but recovery was slow. When now 200 μg. F2557 were injected the response was greatly augmented. About 400–500 μg. F2557 produced depression from which the ganglion did not recover. The ratio of paralysing doses was thus 100 : 20 : 1.

The injection of the three compounds had no action by itself except that F2512, in a dose of 50–100 μg., sometimes caused a contraction of the nictitating membrane just as *d*-tubocurarine occasionally does.

The surprising observation in these experiments was the sensitizing action of F2557 on the ganglion to the injection of acetylcholine. Probably this action is related to the inhibition of cholinesterase by this compound which will be discussed below.

(3) *Effect on the contractions of the nictitating membrane induced by the injection of acetylcholine into the denervated ganglion*

(*a*) Administration of doses into the lingual artery.

Method.—Seven to eight days after the cervical sympathetic nerve had been cut the cats were anaesthetized with chloralose. A loop was put round the external carotid artery, which was temporarily occluded while injections were made through a cannula inserted into the lingual artery and pointing backwards to the common carotid.

Results.—A slight peripheral action on the nictitating membrane itself, producing a slow small contraction, was observed after the injection of acetylcholine even after removal of the ganglion at the end of the experiment. However, the contractions of the membrane produced by the ganglionic action of acetylcholine were immediate and much larger, and the following results were obtained: No dose of F2559 up to 10 mg. depressed the response to acetylcholine. Doses of F2557 up to 5 mg. caused sensitization like that seen in Fig. 1; larger doses, up to 10 mg., caused some depression. The most active substance of the three was F2512, which depressed the acetylcholine response in a dose of about 100 μg.; in this dose it was once observed to cause a contraction by itself. The ratio of paralysing doses was thus > 100 : 100 : 1.

(*b*) Experiments on the perfused denervated superior cervical ganglion.

Method.—This was the same as in (1).

Results.—Again it was found that F2559 in doses up to 10 mg. had no action, 5 mg. F2557 caused sensitization and 10 mg. depression; 100 μg. F2512 caused a big contraction by itself (as 40 μg. tubocurarine chloride did in this same experiment) and depressed the response to acetylcholine by 50 per cent. The ratio of paralysing doses was again > 100 : 100 : 1.

Thus the difference between the responses of normal and denervated ganglia to acetylcholine was that about five times the amount of the curarizing agent was required to depress the action of acetylcholine in denervated ganglia. The activities

of the three substances remained in the same order but the ratios were very much greater.

(4) *Effect on the response of skeletal muscle to stimulation of the motor nerve*

Method.—Cats anaesthetized with chloralose were used. The contractions of the gastrocnemius to maximal single shocks (15 per min.) applied to the sciatic nerve were recorded. The compounds were injected intra-arterially through a cannula inserted into the opposite iliac artery and pointing towards the bifurcation of the aorta.

Results.—The ratio of intra-arterial doses required to reduce muscular contractions to about 50 per cent was found to be the same as that of intravenous doses. The curarizing intravenous doses were 500–750 μg. of F2559, 1–2 mg. of F2557, and 20 mg. of F2512 per kg. respectively (Depierre, 1947). By the arterial route they were 200 μg., 400 μg., and 4 mg. per kg. The ratio of curarizing doses was thus 1 : 2–4 : 20–40.

(5) *Effect on the response of skeletal muscle to close arterial injections of acetylcholine*

Method.—The tibialis anterior muscle preparation was used as described by Brown (1938) and acetylcholine was injected into the tibial artery. The compounds, however, were injected intra-

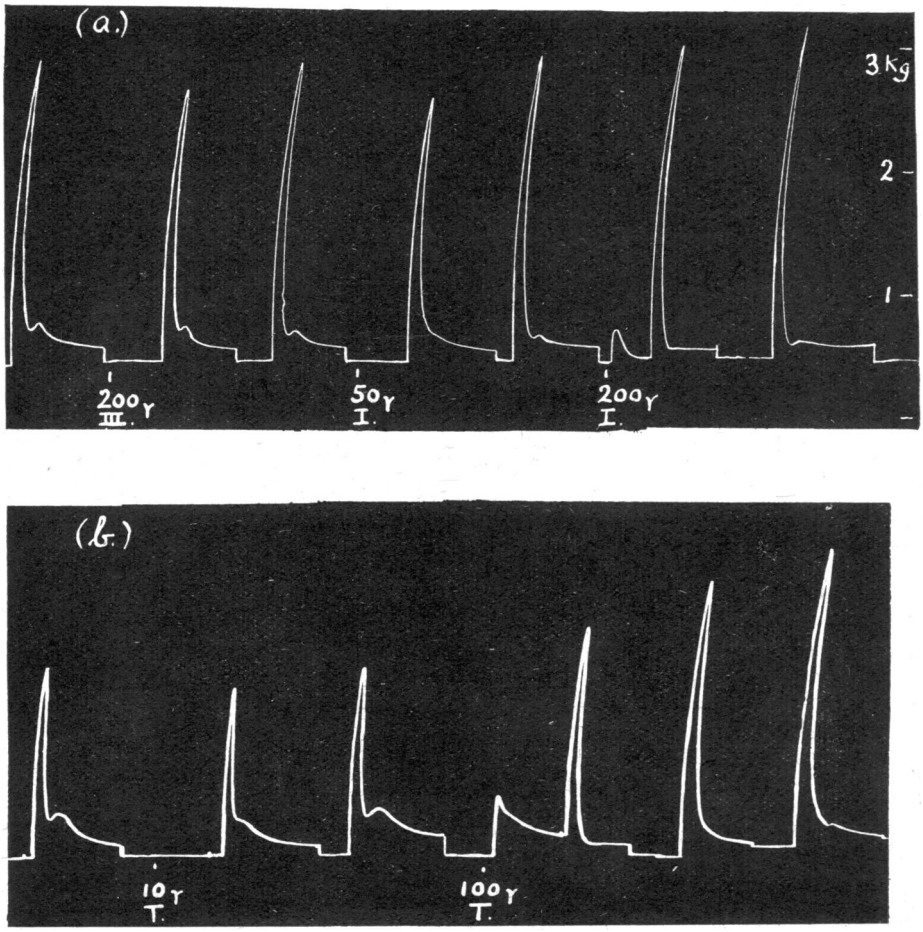


FIG. 2.—Cat, chloralose. Record of contractions of denervated gastrocnemius evoked by intra-arterial injections of acetylcholine. (*a*) Exp. No. 12, muscle denervated for 99 days, dose of acetylcholine = 30 μg. (*b*) Exp. No. 2, muscle denervated for 9 days, dose of acetylcholine = 100 μg. I = F2512, III = F2559. T = *d*-tubocurarine chloride. Note that I and T depress the response in small doses and augment it in large doses.

venously because it was found that the muscle did not recover if they were given by close arterial injection.

Results.—The doses required to produce a depression of the muscle contraction of 20 per cent were 0.4 mg. F2559, 1.5 mg. F2557, and 5 mg. F2512. For a depression of 75 per cent the doses required were 0.75 mg., 2 mg., and 100 mg. respectively. Thus the ratio was found to be about 1 : 4 : 12.

(6) *Effect on the response of denervated muscle to the distant arterial injection of acetylcholine*

Method.—The sciatic nerve was cut 6–30 days before the experiment. The cats were anaesthetized with chloralose. The contractions of the gastrocnemius were recorded, and the injections of acetylcholine, as well as of the curarizing compounds, were made into the iliac artery as in (4).

Results.—The action of the three compounds on denervated muscle was found to be very complex. F2559 was not so potent as F2512 when given in small doses, but as the dose of F2559 was increased the paralysing effect on the muscle response became greater. On the other hand, F2512 depressed the response of denervated muscle to acetylcholine in small doses only; as the dose of F2512 was increased its paralysing effect became less and less, and when given in large doses it actually increased the response to acetylcholine (Fig. 2*a*). F2557 was found to depress the muscle response to

TABLE I

PERCENTAGE CHANGES IN THE RESPONSE OF DENERVATED MUSCLE TO ACETYLCHOLINE PRODUCED BY DIFFERENT DOSES OF F2559 AND F2512

F2559

No. of exp.	Dose of ACh. μg.	20 μg.	50 μg.	100 μg.	200 μg.	400–500 μg.	1 mg.	2 mg.	4 mg.	Days denervated
2	100		−16		−40					9
3	20				−10	−17				11
4	100					+5	−50			11
5	50		−10		−20					14
6	20						−12	−10	+30	14
8	35		+8	−20						20
8	50					0	−44			20
10	20		0			−62				20
11	50			+2		−16	−19			21
12	30		+10	+5	−12	−14				29
Mean			−2	−4	−20	−17	−31	−10	+30	

F2512

No. of exp.	Dose of ACh μg.	20 μg.	40–50 μg.	80–100 μg.	150–200 μg.	400–500 μg.	600–800 μg.	1–1.2 mg.	Days denervated
1	20		−31		−40	−14		+4	6
2	100					−10	+22		9
4	100			+16		+29			11
5	50				0			+20	14
6	20	−13							14
7	30		−35		0				15
8	35		−11	−33					20
8	50				−11			−8	20
9	50			0	−4	−28	−5	+18	20
10	20	−62	0						20
11	50			−12		+2		+10	21
12	30		−13	0	+7	+5			29
Mean		−37	−18	−6	−8	−4	+8	+9	

acetylcholine in relatively large doses only (1–2 mg.). No augmentation of the muscle response was observed (though this was regularly seen in the sympathetic ganglion), but as this compound possesses some anticholinesterase activity (see Section (8)), which would naturally interfere with its action, it was not examined so thoroughly as the other two compounds.

The results of 12 experiments in which the effects of several doses of F2559 and F2512 were compared are given in Table I. In order to make such a comparison doses of acetylcholine were given at intervals of 15 min. One minute before the injection of acetylcholine the curarizing compound was given, and care was taken not to cause too large a depression, as otherwise the previous muscle response to acetylcholine would not return; for this reason the percentage changes of the muscle response are mostly small, but in each experiment it will be seen that the depressing action of F2559 increased with increasing doses, whereas that of F2512 diminished when larger doses were used. The latter compound had a stimulating action itself; in doses of 100 μg. or more it caused a muscle contraction, and it was in this range, from 0.1 to 1.0 mg., that it often did not diminish but increased the subsequent response to acetylcholine. F2559 in doses of about 1 mg. caused a considerable depression. In one experiment only had F2559 a stimulant action itself; 1 mg. caused a small muscle contraction (see Fig. 3 (*a*)) and depressed the subsequent response to acetylcholine, whereas 4 mg. caused a large contraction and sensitized the muscle to a subsequent injection of acetylcholine (Exp. 6 in Table I)..

If the percentage changes shown in Table I are taken as an index of the activities of the two compounds it will be seen that F2512 appears to have a much stronger paralysing action than F2559 in the dosage range 20–50 μg., whereas in the range 200–500 μg. this ratio of potency is completely reversed.

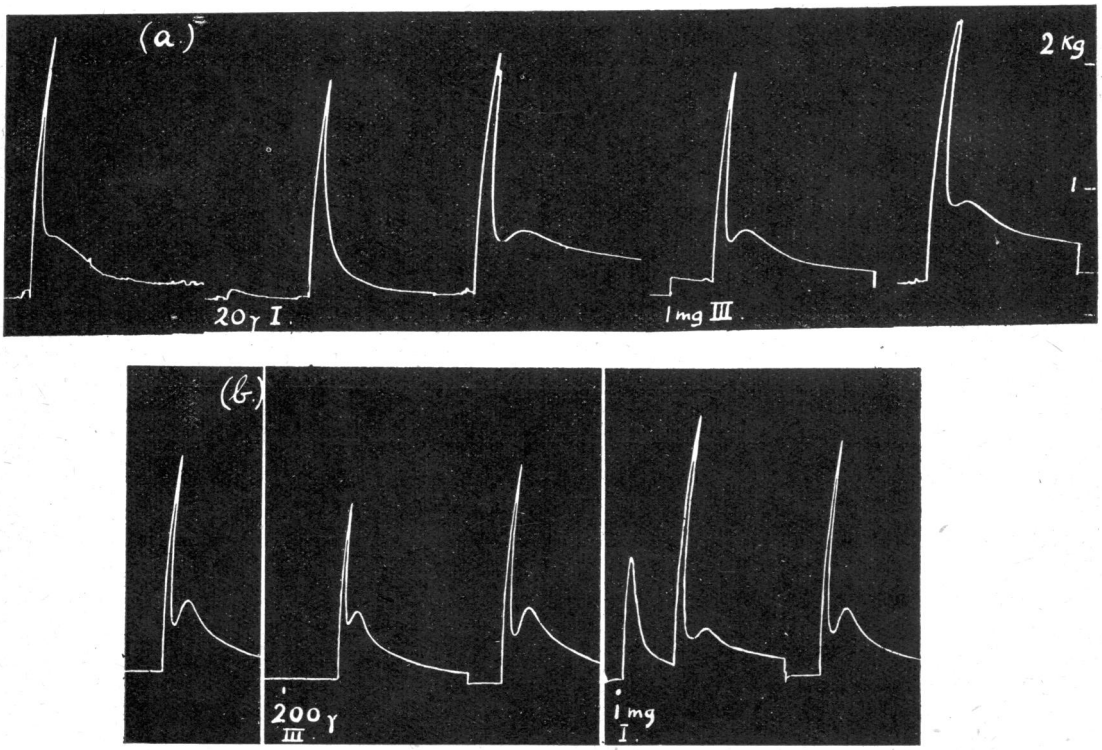


FIG. 3.—Records as in Fig. 2. (*a*) Exp. No. 6, muscle denervated for 14 days, dose of acetylcholine = 20 μg. (*b*) Exp. No. 5, muscle denervated for 14 days, dose of acetylcholine = 50 μg. I = F2512, III = F2559. Note that 20 μg. I depress, 1 mg. I augments quick phase, both depress slow phase, while III depresses quick phase only.

The difficulties encountered in assessing the relative activities of the two compounds may be seen in Fig. 2 (*a*). In this experiment 200 μg. F2559 and 50 μg. F2512 were found to be equiactive doses. However, when the dose of F2512 was increased to 200 μg. it produced not more depression but instead an augmentation of the muscle response to acetylcholine. This sensitizing action was still seen in the response to acetylcholine 15 min. later. A similar effect obtained with *d*-tubocurarine chloride is shown in Fig. 2 (*b*). A small dose, i.e., 10 μg., caused some depression (as 50 μg. F2512 in (*a*)), but a larger dose, 100 μg., not only caused a muscle contraction by itself (as 200 μg. F2512 in (*a*)) but increased the three subsequent responses to acetylcholine. It may also be noted that whereas the first quick phase of the contraction was increased the second slow phase was depressed. Again, F2512 usually, though not always, depressed the slow phase more than the quick phase, whereas F2559 depressed the quick phase and usually left the second phase unchanged. An example of this is given in Fig. 3.

The sensitizing action of F2512 was clearly related to its own stimulating action, as it mostly occurred after the compound itself had elicited a muscle contraction ; thus, in comparing its paralysing activity with that of F2559 the results obtained with small doses are the more important ones. In the experiments Nos. 1, 8, and 9 it was possible to observe a progressive depression with increasing doses and then in Nos. 1 and 9 a change to augmentation. In the remaining experiments the threshold was obviously below the smallest dose employed.

Table I also shows that the action of the two compounds was independent both of the number of days the muscle had been denervated and of the dose of acetylcholine used to elicit the muscle contraction.

(7) *Effect on the response of the isolated diaphragm to stimulation of the phrenic nerve*

Method.—The preparation was that described by Bülbring (1946) and the test was carried out as described by Chou (1947). Rats were used as well as a newborn kitten.

Results.—The ratio of potency of the three compounds on the rat's isolated muscle was different from that on the kitten's muscle, which was, however, found to be similar to that on the cat's muscle *in situ*. This species difference was first pointed out to us in a personal communication from Dr. J. W. Trevan and has recently been reported by Wien (1948). On the rat's diaphragm a 50 per cent depression of maximal muscle contractions was produced by 6–8 mg. F2559, 15–22 mg. F2557, and 3–7 mg. F2512 in a 50 cc. bath, which gives a ratio of 1 : 2 : 0.5–1 ; thus on the rat's muscle F2559 and F2512 were of about equal strength, but on the kitten's muscle F2559 was about 10 times stronger than F2512, which is a similar result to those obtained on the cat's muscle *in situ*.

(8) *Effect on cholinesterase*

Method.—The details of the method used are described by Bülbring and Chou (1947). Only the inhibition of " true " cholinesterase (dog's caudate nucleus suspension) has been studied. Mrs. P. Holton very kindly carried out these experiments for us.

Results.—It was found that F2559 and F2512 had no anti-cholinesterase activity in a concentration of $10^{-3}M$. F2557 was found to have a weak inhibitory action. The results are given in Table II. The concentration of acetylcholine used in these experiments was $6 \times 10^{-3}M$.

TABLE II

INHIBITION OF CHOLINESTERASE (DOG'S CAUDATE NUCLEUS) BY F2557

Experiment	Percentage inhibition at			
	$10^{-4}M$	$3 \times 10^{-4}M$	$10^{-3}M$	$3 \times 10^{-3}M$
1	10	—	50	—
2	7	—	47	—
3	15	26	41	64

DISCUSSION

The compounds investigated are of special interest because they show a remarkable discrepancy between their paralysing action on neuromuscular transmission and their blocking action on synaptic transmission in the sympathetic ganglion. From the doses required to cause a given depression of the response Table III has been constructed, which gives the relative activities of the three compounds on various preparations.

The ratio of activity on denervated muscle could not be assessed, since it varied with the dose employed. Thus, if small doses of the curarizing substance, e.g., 20–100 μg., were used, F2512 was found to be 10–20 times stronger than F2559. When larger doses were given, e.g., 200 μg.–500 μg., the ratio was reversed and F2559 was found to be 2–5 times stronger than F2512. When still larger doses were used the stimulant action of F2512 itself and its sensitizing effect on the

TABLE III

Preparation	Relative activities		
	F2512	F2557	F2559
Sympathetic ganglion stimulated through preganglionic nerve	100	20	2
Sympathetic ganglion stimulated by intra-arterial acetylcholine	100	5	1
Denervated sympathetic ganglion stimulated by i.a. acetylcholine	100	1	1
Skeletal muscle stimulated through motor nerve ..	2–5	25–50	100
Skeletal muscle stimulated by intra-arterial acetylcholine ..	8	25	100

response to acetylcholine became prominent and the comparison of the two compounds became impossible. F2557, which depressed the muscle response to acetylcholine only in large doses of about 1 mg., was again intermediate in potency between the two other compounds. However, this substance has some anti-cholinesterase activity and this was probably responsible for the augmentation of the action of acetylcholine in the sympathetic ganglion. On the muscle we never observed this augmentor action, but we did not include F2557 in the detailed comparison on denervated muscle.

A striking similarity was found to exist between the inhibitory effect of F2512 and F2559 on the action of acetylcholine in the normal sympathetic ganglion and in the denervated skeletal muscle. This was evident in a certain range of doses, in which the stimulant action of the compounds themselves was too small to interfere. In the normal sympathetic ganglion the potency of F2512 was found by Depierre (1947) to be very similar to that of *d*-tubocurarine, though the effect of F2512 is short-lasting. In denervated muscle we observed the same similarity. Not only were the doses of the two substances required to depress the action of acetylcholine of the same magnitude, but *d*-tubocurarine was also found to have a stimulant action itself and to sensitize the denervated muscle to subsequent injections of acetylcholine. This observation supplements that of McIntyre and King (1943), who found that in the dog stimulant doses of *d*-tubocurarine rendered the muscle unresponsive to previously effective quantities of acetylcholine. It may be that the doses of *d*-tubocurarine used by McIntyre were larger than those we employed. We found that stimulant doses of both substances do not necessarily depress the subsequent muscle response to acetylcholine. Both *d*-tubocurarine and F2512 depress in small doses; with larger doses they sensitize; and with still larger doses they depress once more.

Summary

1. The action of β-diethylaminoethoxybenzene ethiodide (F2512), 1 : 3-bis-(β-diethylaminoethoxy)-benzene diethiodide (F2557), and 1 : 2 : 3 - tri - (β - diethylaminoethoxy)benzene triethiodide (F2559) was studied on skeletal muscle and the sympathetic ganglion, both normal and denervated.

2. On normal skeletal muscle F2559 has a strong curarizing action both when the muscle is stimulated through its nerve and when it is exposed to intra-arterial injections of acetylcholine; F2512 is 12 to 50 times weaker. This ratio of activity is reversed on the sympathetic ganglion whether it is stimulated through its preganglionic nerve or by intra-arterial injection of acetylcholine, F2512 being 50 to 100 times stronger than F2559.

3. On denervated skeletal muscle, stimulated by intra-arterial injection of acetylcholine, the ratio of activities of F2512 and F2559 is the reverse of that in normal muscle, provided that the doses of the curarizing agents are small. F2512 is then, as in the normal sympathetic ganglion, 10–20 times stronger than F2559.

4. Large doses of F2512 have a sensitizing action on denervated muscle; as the dose is increased the diminution of the muscle response to acetylcholine gives way to an augmentation. This effect has also been observed with *d*-tubocurarine.

5. F2512 and *d*-tubocurarine both have a stimulant action by themselves on the normal as well as on the denervated sympathetic ganglion and on the denervated muscle.

6. F2557 causes a 50 per cent inhibition of "true" cholinesterase (dog's caudate nucleus suspension) in a concentration $10^{-3}M$. F2512 and F2559 do not inhibit cholinesterase in this concentration.

References

Alam, M., Anrep, G. V., Barsoum, G. S., Talaat, M., and Wieninger, E. (1939). *J. Physiol.*, **95**, 148.
Bovet, D., Depierre, F., and de Lestrange, Y. (1947). *C. R. Acad. Sci., Paris*, **225**, 74.
Brown, G. L. (1938). *J. Physiol.*, **92**, 22P.
Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.
Bülbring, E., and Chou, T. C. (1947). *Brit. J. Pharmacol.*, **2**, 5.
Chou, T. C. (1947). *Brit. J. Pharmacol.*, **2**, 1.
Depierre, F. (1947). *C. R. Acad. Sci., Paris*, **225**, 956.
Feldberg, E., and Vartia inen, A. (1934). *J. Physiol.*, **83**, 103.
Grob, L., Lilienthal, J. L., and Harvey, A. M. (1947). *Bull. Johns Hopk. Hosp.*, **80**, 299.
Kibjakow, A. W. (1933). *Pflüg. Arch. ges. Physiol.*, **232**, 432.
McIntyre, A. A., and King, R. E. (1943). *Science*, **97**, 516.
Wien, R. (1948). *J. Physiol.*, **107**, 44P.