ACTIONS OF LOCAL ANÆSTHETICS

By O. Peczenik and G. B. West*

From the Pharmacological Department, School of Pharmacy, University of London

Received August 4, 1950

During recent years, much attention has been drawn to the similarity in the pharmacological properties of quinidine, atropine, pethidine, diphenhydramine and procaine. Dawes[1], for example, pointed out that quinidine and procaine resemble one another in antagonising acetylcholine in all forms of muscle. Dews and Graham[2] showed that the anti-histamine substance mepyramine was quinidine-like on heart muscle and had a local anæsthetic action like procaine on the guinea-pig's skin. Elio[3] examined the actions of atropine, quinidine and procaine on the frog rectus, the rabbit intestine, and the vessels of the rabbit's ear; he found that all these drugs antagonise the action of acetylcholine in all three preparations. Dutta[4] studied the actions of these drugs on adrenalectomised mice and found that they, like procaine (Peczenik[5]), caused a fall of body temperature. Burn and Dutta[6] showed also that they reversed the constrictor action of adrenaline on the perfused vessels of the rabbit's ear. Dutta[7] compared their actions on the perfused superior cervical ganglion of the cat (for curariform activity in the ganglion), on the phrenic nerve-diaphragm preparation of the rat (for curariform activity at the motor end-plate) and on the bronchioles of the guinea-pig (for anti-histamine activity). From these results, it is possible to say that these substances block the receptors to which acetylcholine must attach itself to produce stimulation, and perhaps block other receptors as well.

Dawes[1] pointed out that cinchocaine, unlike procaine, was difficult to assay on the driven rabbit auricle since it caused a profound and prolonged depression of amplitude, as well as a reduction in maximal rate at which the auricle will respond. Feldberg and Lin[8] noted a similar type of response in the rabbit gut; the inhibitory action of cocaine and procaine on the peristaltic reflex was easily reversible, but with cinchocaine it was more prolonged and developed more gradually. In rat diaphragm experiments, de Jalon and West[9] showed that there exists a relationship between the local anæsthetic power of drugs (including the anti-histamine agents, mepyramine, diphenhydramine and antazoline) and their anti-veratrine power; the inhibitory action of procaine on the veratrine response was easily reversible but that of cinchocaine was more persistent. Elio[3] found that, in all concentrations tested, cinchocaine and amethocaine increased the action of acetylcholine on the spontaneously contracting rabbit auricle, whereas procaine inhibited this action. Cocaine was without action. Glaubach and Pick[10] showed that, whereas cocaine caused a rise of body temperature in rabbits and a fall in guinea-pigs, cinchocaine

* Present address: Department of Materia Medica, Medical School, Dundee.

had almost no effect on the temperature of either species; procaine caused a fall of body temperature which was enhanced by adrenalectomy (Peczenik[5]). Cinchocaine and cocaine are known to modify the action of the isolated heart of the cat, and also to augment the action of adrenaline on this preparation (Tripod[11]) whereas procaine has no such effects. Cinchocaine, therefore, provides an example of a local anæsthetic which differs from procaine in reducing neither the action of acetycholine on the rabbit auricle nor the body temperature; in addition its action is more prolonged and it is less easily removed from isolated tissues. This difference in action has been further studied in this paper, with particular reference to the effect on the phrenic nerve-diaphragm preparation of the rat (i.e., on the nicotine action of acetylcholine). A preliminary note on the importance of the potassium ion in regard to this action has already been published (Peczenik and West[12]).

In the assay of parasympatholytic agents using the lachrymation response in rats, Winbury, Schmalgemeier and Hamburger[13] showed that the production of red tears (chromodacryorrhœa response) produced by acetyl-β-methyl choline (methacholine) could be prevented by the previous administration of such an agent. Methacholine chloride is a substance with very feeble nicotine-like actions, and it seemed of interest, therefore, to see if local anæsthetics affected this muscarine action and inhibited lachrymation. Whilst the present work was in progress, Burgen[14] reported that the phenomenon of chromodacryorrhœa in rats, produced by injections of acetylcholine, could be used as a basis for an accurate and quantitative assay of anticholinesterase drugs. We were unable to obtain consistent results with this technique and all our results were obtained with the methocholine test. We expected the test to indicate the anticholinesterase activities of the local anæsthetics, and to assist in the explanation of the difference in activity between procaine on the one hand and amethocaine and cinchocaine on the other.

METHODS

For the work on skeletal muscle, the rat diaphragm method described by Bulbring[15] was used, with slight modifications (West[16]). Tyrode solution containing double the amount of glucose was the bathing fluid, and the 70-ml. bath was maintained at 20°C. and aerated with oxygen containing 5 per cent. of carbon dioxide. The phrenic nerve was stimulated by a pair of platinum electrodes; supramaximal shocks were delivered from a neon-lamp circuit, or from an electronic square-wave stimulator at a desired strength (usually 0·1 V.), frequency (8 per minute), and duration (0·1 msec.). Most of the rats used were normal stock albino animals, but some were adrenalectomised under ether anæsthesia and retained until their body temperature had fallen to 34°C. or lower before being killed.

In the lachrymation test on groups of 12 normal unanæsthetised albino rats (weights 150 to 250 g.) tears were produced by the intraperitoneal

injection of methacholine; male rats have been used throughout since it has been shown that they contain only true cholinesterase (Sawyer and Everett[17]). The local anæsthetics were also injected by the intraperitoneal route at a given time before the methacholine. At fixed times after the methacholine injection (usually every 5 minutes), a spill of filter paper was inserted into the conjunctival sac when the pigment was readily visible on the filter paper. By this procedure, it was possible to assess the inhibiting or potentiating action of the local anæsthetic on the all-or-none response and on the duration of action of methacholine.

The local anæsthetics used were the hydrochlorides of procaine, amylocaine, cocaine, amethocaine and cinchocaine.

Results

A. Experiments with the rat's diaphragm

Actions of Local Anæsthetics per se. Dutta[7] found that procaine in small concentrations increased the contractions of the rat's diaphragm stimulated indirectly at 37°C., but in higher doses it produced inhibition of the contractions. We have confirmed these results at 20°C., although the augmentor action is less easily observed, and the depressor action is more powerful at this temperature. With the other four local anæsthetics, large doses produced block *per se*. The results of contact for 3 minutes are recorded in Table I, and relative activities calculated

TABLE I

Percentage reduction of contraction of rat diaphragm produced by local anæsthetics acting for 3 minutes in a bath of 70 ml. capacity

Drug (as hydrochloride)	Dose (mg.) producing a 50 per cent. reduction	Dose (mg.) producing a 100 per cent. reduction
Procaine	20 to 30	50
Amylocaine	10	20
Cocaine	5 to 15	10 to 30
Amethocaine	1 to 3	—
Cinchocaine	1 to 2	—

on this basis are of the same order as those found for anæsthetic action (Table II). Direct stimulation was effective so that the action was curare-like in nature. Preparations had to be primed with a few large doses

TABLE II

Relative potency of local anæsthetics in terms of procaine

Substance	Frog rectus Elio[3] (anti-acetyl-choline)	Rabbit gut reflex Feldberg & Lin[8] (anti-peristalsis)	Guinea-pig skin anæsthesia Elio[3]	Inhibition of rat diaphragm
Procaine	1·0	1·0	1·0	1·0
Amylocaine	—	—	—	2·5
Cocaine	3·0	0·9	7·4	3·5
Amethocaine	9·0	—	8·0	8·0
Cinchocaine	11·0	11·0	10·0	10·0

of the anæsthetic before consistent responses could be obtained. Amethocaine and cinchocaine, unlike procaine, were difficult to assay since they caused prolonged depression of amplitude; recovery was usually slow.

Action of Local Anæsthetics after Potassium. It has already been shown (West[16]) that small doses of potassium chloride *per se* (used as a 15 per cent. solution) always increase the amplitude of contraction of the normal diaphragm. Doses of amylocaine or amethocaine producing less than a 50 per cent. inhibition in 3 minutes were, however, potentiated by 45 mg. of potassium chloride given half a minute before the anæsthetic (Figure 1). On the other hand, the same dose of potassium chloride

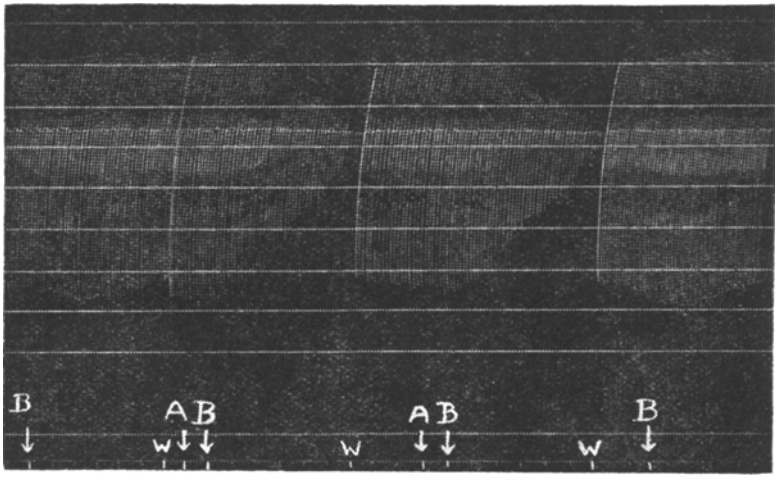

FIG. 1.—A. 45 mg. of potassium chloride added.
B. 8 mg. of amylocaine hydrochloride added.
Isolated diaphragm of rat. Record of muscle contraction in response to supramaximal stimulation of the phrenic nerve. Contraction downwards: rate of stimulation 8 per minute. Time in 30 sec. The effect of 45 mg. of potassium chloride (A) on the response to 8 mg. of amylocaine hydrochloride (B).

had little or no effect on the action of the corresponding dose of procaine. A further difference in action between procaine and other anæsthetics is therefore suggested.

Action of Local Anæsthetics before Potassium. West[16] found that at 20°C., recovery of the preparation after curare can be accelerated by utilising the anti-curare property of potassium ions, provided that the curare is removed from the bath by two changes of Tyrode solution before the potassium chloride is added. This action of potassium ions in the recovery phase was investigated after doses of the anæsthetics. When 45 mg. of potassium chloride was added to the bath for 3 minutes after washing out the anæsthetic, the following results were noted: (1) with all doses of procaine, potassium chloride sometimes aided but never pre-

vented recovery of the contraction (Figure 2); (2) with 10 mg. of amylocaine or cocaine, the effect was variable, sometimes quicker recovery and sometimes slight inhibition of recovery being observed, but after 20 mg. of amylocaine, or 15 mg. or more of cocaine, potassium chloride always produced partial or complete block (Figure 3); (3) with amethocaine and cinchocaine, potassium chloride always prevented recovery and produced block. With the latter 2 drugs, potassium chloride added after complete recovery again produced block. This alteration in the potassium chloride response brings to mind the findings of Elio[3] on the inhibitory effect of acetylcholine on the rabbit's auricle, procaine preventing the response whilst amethocaine and cinchocaine potentiated it.

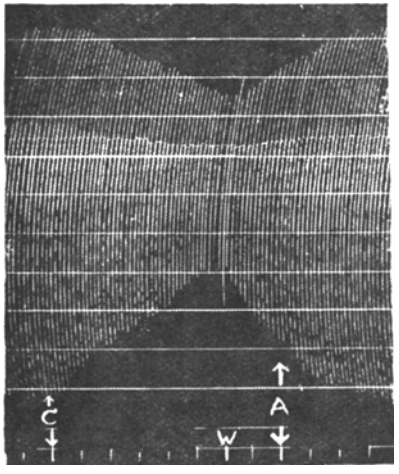

FIG. 2.—C. 20 mg. of procaine hydrochloride added.
A. 45 mg. of potassium chloride added.
Record as Fig. 1. 45 mg. of potassium chloride added (A) after the wash-out (W) aids recovery of the normal preparation previously subjected to 20 mg. of procaine hydrochloride (C).

As the results suggested that K^+ was so important, we repeated the work with potassium chloride-free Tyrode solution. Table III shows the percentage reduction of contraction produced by varying doses of amylocaine. In the absence of K ions, the diaphragm becomes more sensitive to the local anæsthetics and less sensitive to the potassium. The table also shows that if potassium chloride is added during the recovery phase, the curariform response is produced by smaller doses than would normally be the case.

TABLE III

PERCENTAGE REDUCTION OF CONTRACTION OF THE RAT DIAPHRAGM PRODUCED BY AMYLOCAINE, AND THE SUBSEQUENT ACTION OF POTASSIUM

Dose of Amylocaine (mg.)	Percentage reduction			Minimal dose of potassium chloride possessing curariform action (mg.)		
	Tyrode solution	Potassium chloride-free Tyrode solution	Tyrode* solution	Tyrode solution	Potassium chloride-free Tyrode solution	Tyrode* solution
0	—	—	—	82·5	125	64
4	0	26	95	—	75	22·5
8	30	76	100	37·5	75	22·5
20	100	100	100	22·5	64	15

* Diaphragms of adrenalectomised rats in Tyrode solution.

Action of Local Anæsthetics on diaphragms of adrenalectomised animals. The concentration of K^+ is known to be increased in the muscles of completely adrenalectomised rats, and the body temperature of these animals may drop to 34°C. or lower. It seemed to be of interest, therefore, to test the action of local anæsthetics on the diaphragm preparations of such animals. There was increased sensitivity to anæsthetics *per se* (e.g., amylocaine, Table III) and to a subsequent dose of potassium. Potassium chloride in doses of 45 mg. produced block after doses of procaine (Figure 4), an action never seen in normal diaphragms. Preparations of incompletely adrenalectomised rats responded like those of normal animals. It is possible that some of the actions of local anæsthetics are dependent upon the rearrangement of the distribution of potassium ions inside the muscle cell. It is known that adrenalectomy increases the effect of injections of procaine on the fall of body temperature in mice (Peczenik[5]) so that this action may also be connected with the distribution of K^+ and with an effect on the acetylcholine response.

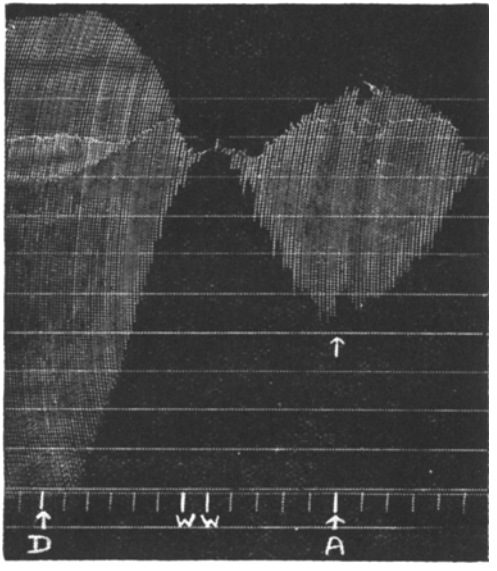

FIG. 3.—D. 25 mg. of cocaine hydrochloride added.

A. 45 mg. of potassium chloride added.

Record as Fig. 1. 45 mg. of potassium chloride added (A) after the wash-out (W) prevents recovery of the normal preparation previously subjected to 25 mg. of cocaine hydrochloride (D) and produces block.

Actions of Local Anæsthetics on the Curare response. As the local anæsthetics were found to possess curariform action, they were tested to see if non-effective doses influenced the response to *d*-tubocurarine. Doses of *d*-tubocurarine chloride producing a 50 per cent. reduction of contraction of the diaphragm in 3 minutes, were potentiated by doses of 2 mg. of procaine, 3 mg. of cocaine or 0·3 mg. of amethocaine given $1\frac{1}{2}$ minutes before the curare (Fig. 5). Longer contact between the local anæsthetic and the muscle resulted in a decreased effect. Small non-effective doses of cinchocaine appeared to possess a weaker augmentor action.

It is known that curare acts on the neuromuscular junction preventing the action of acetylcholine on the receptors in the end-plate. On the rat

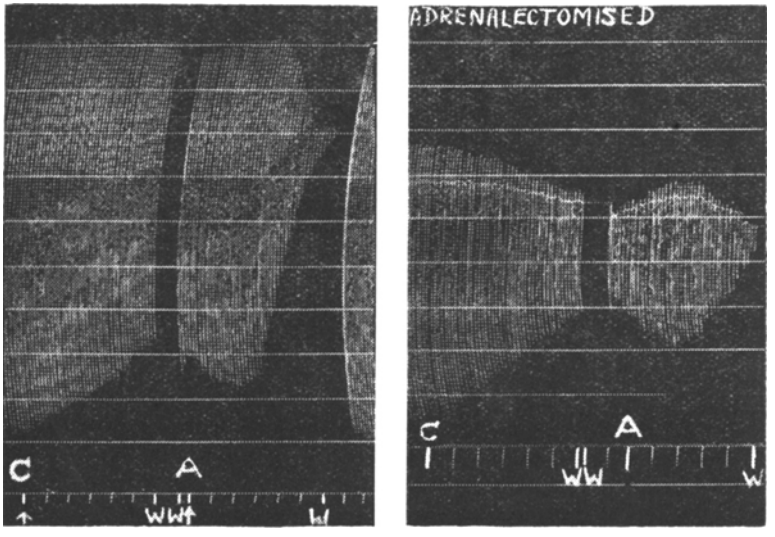

FIG. 4.—C (left). 15 mg. of procaine hydrochloride added.
C (right). 25 mg. of procaine hydrochloride added.
A. 45 mg. of potassium chloride added.

Record as Fig. 1. 45 mg. of potassium chloride added (A) after the wash-out (W) prevents recovery of the adrenalectomised preparation previously subjected to procaine hydrochloride (C).

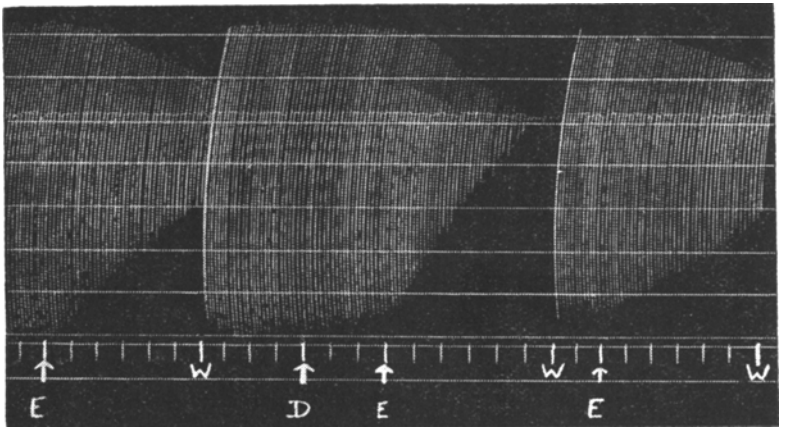

FIG. 5.—E. 50 μg. of *d*-tubocurarine chloride added.
D. 3 mg. of cocaine hydrochloride added.

Record as Fig. 1. The potentiating effects of 3 mg. of cocaine hydrochloride (D) on the response to 50 μg. of *d*-tubocurarine chloride (E).

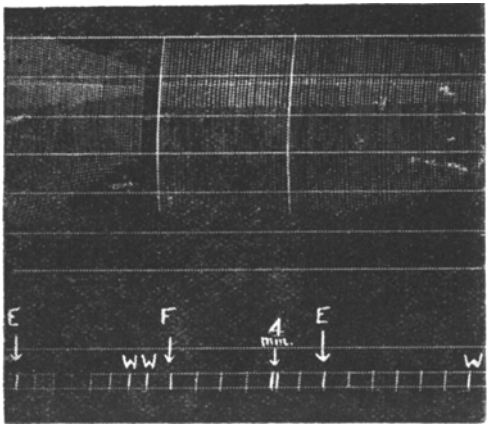

FIG. 6.—E. 50 μg. of *d*-tubocurarine chloride added.
F. 0·5 mg. of neostigmine added.
Record as Fig. 1. The action of 0·5 mg. of neostigmine (F) on the response to 50 μg. of *d*-tubocurarine chloride (E).

diaphragm preparation, the action of *d*-tubocurarine was antagonised by eserine and by neostigmine (Fig. 6). On the other hand the actions of local anæsthetics were potentiated by the same dose of neostigmine (Fig. 7), a result which clearly suggests that local anæsthetics and *d*-tubocurarine act in a different manner, or at different points of the neuromuscular mechanism.

B. THE CHROMODACRYORRHŒA RESPONSE

Winbury *et al.*[13] employed a constant dose of methacholine (10 mg./kg. intraperitoneally) to induce lachrymation in normal untreated rats. This is well above the 100 per cent. effective dose, and it was decided to use lower threshold doses. A positive response of nearly 75 per cent. was

TABLE IV

DOSE-EFFECT RESPONSE OF METHACHOLINE IN NORMAL RATS

Dose of Methacholine (mg./kg.)	No. of positive responses (lachrymations read over 15 minutes)	Percentage responses
10	54/54	100
5	23/24	96
2·5	17/18	94
0·5	31/42	74
0·25	22/54	41

TABLE V

THE EFFECT OF LOCAL ANÆSTHETICS ON THE LACHRYMATION RESPONSE IN GROUPS OF 12 RATS. DOSE OF METHACHOLINE EQUALS 0·5 MG./KG. TIME INTERVAL BETWEEN DOSES OF ANÆSTHETIC AND METHACHOLINE IS 6 MINUTES

Local anæsthetic	Dose (mg./kg.)	Response			Relative anti-cholinesterase activity
		All or none (percentage of positive rats)		Duration	
		Methacholine alone	Methacholine plus anæsthetic		
Procaine	200	74	65	slightly reduced	1
Amylocaine	100	75	69	reduced	0·9
Amethocaine	24·2	72	100	prolonged	104
Cinchocaine	10	62	88	prolonged	90

consistently given by 0·5 mg./kg. (Table IV) and this dose was used in the first experiments. The local anæsthetics were injected intraperiton-

TABLE VI

THE EFFECT OF AMETHOCAINE AND CINCHOCAINE INJECTED AT GIVEN TIMES BEFORE THE METHACHOLINE (0·25 MG./KG.) ON THE LACHRYMATION RESPONSE IN RATS

Local anæsthetic	Dose (mg./kg.)	Time interval between doses in minutes	Percentage of positive rats			
			Mecholyl alone		Mecholyl plus anæsthetic	
			Mean over 30 minutes	At 20 minutes	Mean over 30 minutes	At 20 minutes
Amethocaine	24·2	6	37	25	88	83
	24·2	15	37	17	81	60
Cinchocaine	10	6	36	25	78	50
	10	15	42	25	46	25

eally into groups of 12 rats at set times (6 or 15 minutes) before the dose of methacholine, and permanent records were made on filter paper of the duration and intensity of the lachrymation response. Results for the time interval of 6 minutes are recorded in Table V. Procaine and amylocaine did not significantly affect the total number of positive responses but the duration of response was reduced. Amethocaine and cinchocaine on the other hand increased the all-or-none response (i.e., more rats exhibited the phenomenon) and it also prolonged the duration of the response. Both these latter actions were more pronounced when the dose of methacholine was reduced to 0·25 mg./kg. (Table VI). At this level, with a time interval of 15 minutes, cinchocaine appeared to be ineffective. It is possible that these responses are linked with the relative anticholinesterase properties of the local anæsthetics (Table V), for the doses used were large.

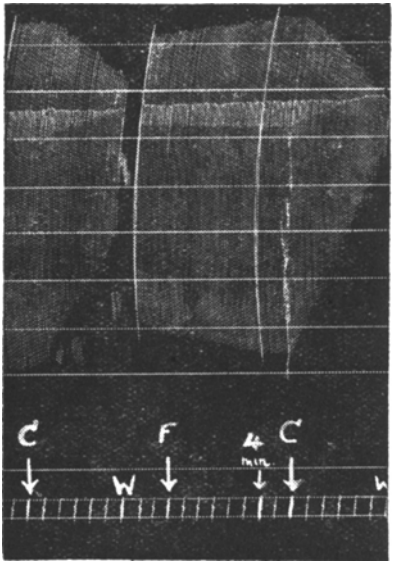

FIG. 7.—C. 30 mg. of procaine hydrochloride added.
F. 0·5 mg. of neostigmine added.
Record as Fig. 1. The action of 0·5 mg. of neostigmine (F) on the response to 30 mg. of procaine hydrochloride (C).

Methacholine is a substance with very feeble nicotinic actions. Its actions are inhibited by atropine so that, in the lachrymation response, the local anæsthetics are affecting the muscarinic effect of acetylcholine as opposed to their nicotinic effects.

Discussion

The relative activities of the five anæsthetics in the normal rat diaphragm preparation are of the same order as those found for anæsthetic and anti-acetylcholine action (Dawes[1], and Elio[3]) and for the inhibition of the peristaltic reflex of the rabbit ileum (Feldberg and Lin[8]), although for this latter test, doses of micrograms (not milligrams) were used. In the experiments with rabbit auricles, both amethocaine and cinchocaine potentiate, instead of inhibiting, the acetylcholine response, whereas cocaine is without effect. In the rat diaphragm experiments, potassium chloride added after washing out amethocaine or cinchocaine always produces block, whereas when added after cocaine or amylocaine, it has either no action or produces block. Procaine inhibits the acetylcholine action on the rabbit auricle and does not affect the normal potassium recovery in the normal rat diaphragm. In preparations from adrenalectomised animals, however, potassium added after washing out procaine produces block. Bein[18] found that the increase of the refractory period of the rabbit auricle produced by procaine is potentiated by potassium chloride. Graham[19] found that relative lack of potassium potentiates the stimulating effect of acetylcholine on the rabbit auricles. The relation of the potassium ion to the acetylcholine action is, therefore, important. According to Jequiér, Plotka and Petergalvi[20], this connection may be in the relation between potassium and degradation of glucose. Local anæsthetics may block the receptors to which acetylcholine must attach itself to produce stimulation (Elio[3]): then stimulatory doses of potassium chloride (which assist conduction in the normal preparation) become toxic in action.

According to Dawes[1], local anæsthetics in general contain a free base (which can penetrate the cell membrane); this base is responsible for the quinidine-like properties. They also contain a cation (which it is believed acts at the cell surface); this gives the curariform and atropine-like properties. Procaine in solution contains both cation and free base in equilibrium. On skeletal muscle, it is said to act at several points: (a) it interferes with the liberation of acetylcholine at the motor nerve endings (Harvey[21]); (b) it depresses the motor end-plate; and (c) it acts directly on the muscle (Macgregor[22]). The position is, therefore, very complex even with regard to procaine, and a study of the chemical constitution of the local anæsthetics as distinct from their physical properties does not provide any assistance in solving the problem.

Summary

1. Large doses of procaine, amylocaine, cocaine, amethocaine, and cinchocaine produce block in the rat phrenic nerve-diaphragm preparations. Relative activities calculated on this basis agree well with those found by previous workers using the anæsthetic action in the guinea-pig skin and on the anti-acetylcholine action in the frog rectus.

2. In the rat diaphragm preparation, small doses of potassium chloride potentiate the depression of contraction caused by some local anæsthetics.

Potassium added after washing out the anæsthetic produces further block in the amethocaine and cinchocaine experiments, but assists recovery in the procaine tests.

3. In diaphragm preparation of adrenalectomised rats, stimulatory doses of potassium chloride added after washing out procaine produce block.

4. Small non-effective doses of five local anæsthetics potentiate the action of *d*-tubocurarine on the rat diaphragm.

5. In the chromodacryorrhœa test, amethocaine and cinchocaine potentiate the action of acetyl-β-methyl choline, whereas procaine and amylocaine inhibit it. This effect may be explained by their relative anticholinesterase activities.

6. The importance of the potassium ion as regards the action of certain local anæsthetics on the acetylcholine response has been discussed.

References

1. Dawes, *Brit. J. Pharmacol.*, 1946, **1**, 90.
2. Dews and Graham, *Brit. J. Pharmacol.*, 1946, **1**, 278.
3. Elio, *Brit. J. Pharmacol.*, 1948, **3**, 108.
4. Dutta, *Brit. J. Pharmacol.*, 1948, **3**, 246.
5. Peczenik, *Proc. roy. Soc.*, 1947, **134B**, 218.
6. Burn and Dutta, *Brit. J. Pharmacol.*, 1948, **3**, 354.
7. Dutta, *Brit. J. Pharmacol.*, 1949, **4**, 197.
8. Feldberg and Lin, *Brit. J. Pharmacol.*, 1949, **4**, 33.
9. Jalon and West, *Farmacoterapia Actual.*, 1948, **5**, 236.
10. Glaubach and Pick, *Arch. exp. Path. Pharmak.*, 1931, **162**, 551.
11. Tripod, *J. Physiol.*, 1940, **97**, 289.
12. Peczenik and West, *Nature*, 1949, **164**, 354.
13. Winbury, Schmalgemeier and Hamburger, *J. Pharmacol.*, 1949, **95**, 53.
14. Burgen, *Brit. J. Pharmacol.*, 1949, **4**, 185.
15. Bulbring, *Brit. J. Pharmacol.*, 1946, **1**, 38.
16. West, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 518.
17. Sawyer and Everett, *Amer. J. Physiol.*, 1947, **148**, 675.
18. Bein, *Brit. J. Pharmacol.*, 1948, **3**, 251.
19. Graham, *Brit. J. Pharmacol.*, 1949, **4**, 308.
20. Jequiér, Plotka and Petergalvi, *C.r. Soc. biol., Paris*, 1949, **143**, 189.
21. Harvey, *J. Physiol.*, 1939, **95**, 45.
22. Macgregor, *J. Pharmacol.*, 1939, **66**, 350.

THE TUBERCULOSTATIC ACTIVITY OF SOME THIOSEMICARBAZONES

By E. M. Bavin, R. J. W. Rees, J. M. Robson, M. Seiler, D. E. Seymour and D. Suddaby

This Journal, 1950, **2**, 764-772

Corrections

Page 767. In Table II, compounds Nos. 7 and 10 were not tested by the corneal test as is indicated in the Table.

Page 771. *Add* One of us (J.M.R.) desires to thank the W. H. Ross Foundation for the Prevention of Blindness (Scotland), who defrayed part of the expenses of this investigation.