J. Physiol. (1939) 96, 302–336 612.833.94:577.153.9:547.439

CENTRAL ACTION OF ANTICHOLINESTERASES

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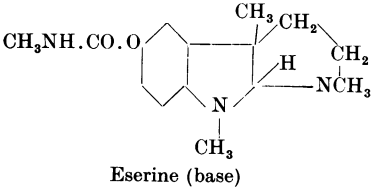
(*Received* 22 *April* 1939)

Our previous studies [Schweitzer & Wright, 1937*c*, *d*, *e*, 1938*a*] on the action of various autonomic drugs on the central nervous system of the cat have led to the following main conclusions. (1) Acetylcholine and certain other more stable choline esters (doryl, mecholin) have a direct inhibitory action on the spinal cord. (2) Prostigmine and certain other anticholinesterases such as methyl carbamic ester of *m*-hydroxyphenyl-trimethyl ammonium iodide (Stedman's "meta" methiodide compound), methyl carbamic ester of *m*-hydroxyphenyl trimethyl ammonium methyl sulphate (substance 13),[1] and methyl phenyl carbamic ester of *m*-hydroxy-phenyl trimethyl ammonium methyl sulphate (substance 36),[1] also exert a direct inhibitory action on the spinal cord. In experiments on man, Kremer, Pearson & Wright [1937] found that prostigmine injected into the lumbar spinal fluid diminished or abolished normal or pathologically increased muscle tone and spinal reflexes by a direct action on the spinal cord. We suggested that the central inhibitory action of this group of substances was related to their anticholinesterase activity which Stedman [1926] has shown to be due to the presence in the molecule of the urethane grouping. (3) The action of the best known of the anticholinesterases, namely, eserine sulphate, differed radically from that of the other members of the group mentioned above. The main action of eserine sulphate on the spinal cord is excitatory, leading to an increase in reflex activity and the production of convulsions. It was noted, however, that an initial inhibitory effect might precede the characteristic stimulating action.

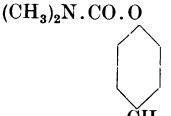
[1] These figures correspond to preparations of Aeschlimann and Reinert's series of anticholinesterases [1931].

To explain these apparently conflicting results we initially put forward [Schweitzer & Wright, 1937*c*] the following tentative suggestion. The anticholinesterases of the prostigmine group acted on the spinal cord by inhibiting the cholinesterase which is present in the central nervous system. These substances thus act qualitatively like injected acetylcholine, and depress spinal reflexes. The chemical composition of eserine, however, differs considerably from that of the other known anticholinesterases. We thought that the exceptional action of eserine sulphate, which caused an increase of the patellar reflex and convulsions, might be attributed to the presence in the molecule of two groupings with antagonistic pharmacological actions: (1) the urethane anticholinesterase group to which we ascribed the initial inhibitory action of the drug, and (2) an unidentified (hypothetical) convulsant group which antagonized and usually overcame the inhibitory action of the former, thus giving rise to increased reflex activity and convulsions.

Further experiments [Schweitzer & Wright, 1938*a*], however, indicated that this hypothesis was inadequate to explain the difference between the action of eserine sulphate and the other anticholinesterases that had been studied. We examined the action of two further substances, namely, the dimethyl carbamic ester of hordenine hydrochloride and the dimethyl carbamic ester of hordenine methiodide (or methyl sulphate). These compounds have an identical anticholinesterase action *in vitro* [Stedman, unpublished results] and an identical action, qualitatively and probably quantitatively, on the mammalian nerve-muscle preparation and on the circulation. The first compound was proved to have a central convulsant action like eserine sulphate, and the second a central inhibitory action like prostigmine. These results have since been confirmed by Briscoe [1938]. The formulae of eserine base and of the dimethyl carbamic ester of hordenine HCl are set out below for comparison:



Eserine (base)



Dimethyl carbamic ester of hordenine hydrochloride (Substance 41)

The main structural feature common to the two substances is the urethane grouping attached to the benzene ring. The complex ring system of eserine is absent in the convulsant hordenine derivative. When the urethane anticholinesterase grouping was removed from the dimethyl

carbamic ester of hordenine hydrochloride to leave hordenine hydrochloride, the convulsant action of the drug disappeared. It seemed to us, therefore, more probable that members of the anticholinesterase group of drugs, by virtue of their anticholinesterase activity, might produce diametrically opposite actions on the spinal cord: eserine sulphate and the dimethyl carbamic ester of hordenine hydrochloride being convulsant, while prostigmine, substances 13 and 36, and the dimethyl carbamic ester of hordenine methiodide are depressant. We have made a more extensive examination of the actions of a number of other anticholinesterases and related substances to test this hypothesis more thoroughly. Some of the results to be described below have been the subject of preliminary communications [Schweitzer, Stedman & Wright, 1938; Schweitzer & Wright, 1938*c*].

METHODS

The experiments were performed on cats under chloralose anaesthesia (0·05–0·08 g./kg. body weight). The hindlimbs were firmly fixed in clamps by means of drills through the femur and ischial tuberosity. The right patellar reflex was recorded as follows. The limb was flexed at the knee joint and the patellar tendon was regularly tapped at the same place at intervals of 6 or 10 sec. with an electrically operated automatic hammer which has previously been fully described [Schweitzer & Wright, 1937*a*]. The extension movements of the limb were recorded on smoked paper by means of a tension lever myograph. In the left limb the response of the gastrocnemius muscle to submaximal or maximal break-shock stimulation of its motor nerve was recorded with another tension lever myograph. The nerve was stimulated with a fluid electrode of the type described by Collison [1933] at the same rate as the knee jerk was elicited. In some instances, when the patellar reflex was feeble because of the depth of anaesthesia or for other reasons, strychnine in doses of 0·05–0·1 mg./kg. body weight was injected into the jugular vein to raise the reflex excitability of the spinal cord.

To determine the extent to which the drugs under examination produced their effects by a peripheral action on the motor nerve or the skeletal muscle, or by an effect on the central nervous system, we used the ischaemic hindlimb technique which has previously been fully described [Schweitzer & Wright, 1937*d*, *e*]. The abdominal aorta and the inferior vena cava were exposed, and all branches of the aorta below the diaphragm, with the exception of both common iliacs, were carefully dissected out and cut between ligatures. The internal mammary arteries

were tied on both sides in the second intercostal space. In many experiments a T section was made at a high lumbar level through both sides of the abdominal wall down to the muscles of the back. We have previously demonstrated that obstruction of the abdominal aorta and the inferior vena cava with rubber-protected strong clamps prevents, under these experimental conditions, any significant amount of blood from reaching the hindlimbs. A control period of ischaemia of 5–10 min. usually showed that the force of contraction of the knee jerk and of the gastrocnemius of the opposite side was little changed. The clamps were then removed from the vessels and time was allowed for recovery. The aorta and inferior vena cava were clamped again, and the drug under examination was then injected into the jugular vein. Any changes in reflex activity caused by such an injection during the period of ischaemia cannot be attributed to a peripheral action of the substance on the nerve and muscle, but must be due to an effect on the central nervous system. The response of the gastrocnemius muscle to motor-nerve stimulation during the period of ischaemia served as an additional safeguard for the strict control of the ischaemia, as any leakage into the hindlimbs of the intrajugularly injected drugs with a considerable peripheral action would have been immediately detected.

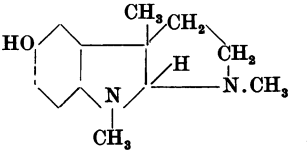
Many of the experiments referred to below were carried out in atropinized cats in order to exclude the depressant actions of the drugs on the cardio-vascular system and to allow larger doses of the drug to be given. Atropine was given in doses sufficient to block the transmission of parasympathetic impulses (0·5–1·0 mg./kg. body weight). All injections were made, in equal volumes of fluid, into the jugular vein. The drugs were dissolved in acid saline at *p*H 4·0. Artificial respiration by means of a pump was used in most of the experiments in order to avoid any asphyxiation of the animals from the depressant action on breathing of many of the substances tested.

Results

Action of eseroline hydrochloride

Our original theory, that the seemingly exceptional central action of eserine sulphate is due to a conflict between a special convulsant grouping and a depressant (anticholinesterase) grouping, can be tested by examining the pharmacological actions of eseroline hydrochloride. Eseroline is the substance derived from eserine by the removal of the urethane grouping, which is responsible for practically all the anti-

cholinesterase activity of the substance. Its structural formula is shown below:



Eseroline (base)

Eseroline has a negligible anticholinesterase activity *in vitro*. In a concentration of 5×10^{-4} *M*, eseroline hydrochloride showed a very small effect, but eserine (sulphate) was active in a concentration of 10^{-8} *M*. If our original views are correct, eseroline should have a much greater convulsant action than eserine, as the competing urethane grouping, which was thought to be responsible for the depressant action on the central nervous system, is no longer present. The experimental results obtained, however, show that this is not so.

Intravenous injections of eseroline hydrochloride in doses of 0·5–2 mg. (which, in the case of eserine sulphate, produce violent convulsions) have, as a rule, no effect on the knee jerk or on the response of skeletal muscle to submaximal or maximal stimulation of its motor nerve. In unatropinized animals, doses of 5–20 mg. produce a fall of blood pressure, which is usually gradual and progressive and sometimes considerable, e.g. from 140 to 60 mm. Hg. Depression of respiration commonly occurs. After administration of atropine in doses sufficient to annul the effects of stimulation of parasympathetic nerves, injection of similar doses (5–20 mg.) of eseroline hydrochloride produces a less marked or negligible fall of blood pressure. With these bigger doses the response of the nerve-muscle preparation is usually slightly enhanced, the knee jerk may be increased in amplitude, and sometimes weak convulsions occur. In the experiment from which Fig. 1 is taken, two successive injections of 10 mg. of eseroline hydrochloride produced no change in the knee jerk but occasional slight convulsive movements. Intravenous injection of doses of eseroline hydrochloride of 25–50 mg. inhibits respiration, so that artificial respiration is necessary. The knee jerk may be increased and definite convulsions appear more regularly, but not invariably. The convulsions are usually feeble in character. The convulsions and the general increase in reflex activity are due mainly to a direct effect on the spinal cord, as is shown by experiments performed on "ischaemic" preparations in which the action of the drug on the nerve and muscle is excluded. Very many such experiments were performed; Fig. 2 is

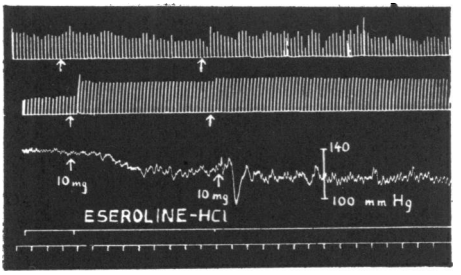


Fig. 1. Cat, chloralose. 1 mg. atropine intravenously per kg. body wt. Records from above downward are: knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, signa line, time in 30 sec. Two successive intrajugular injections of 10 mg. each of eseroline hydrochloride produce no change in the knee jerk, except occasional slight convulsive movements. The neuro-muscular responses are increased.

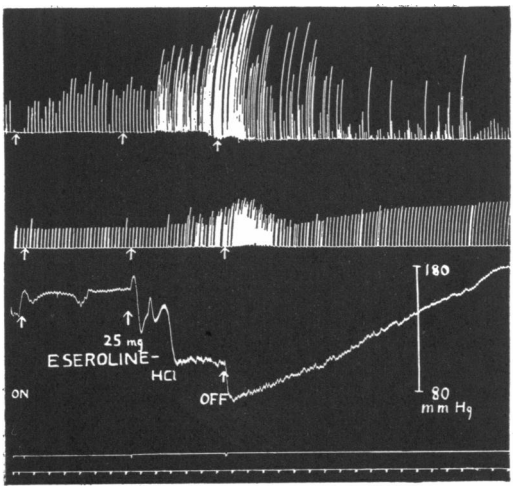


Fig. 2. Cat, chloralose. 0·5 mg. atropine per kg. body wt., "ischaemic" preparation. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, signal line, time in 30 sec. At "on" clamp abdominal aorta and inferior vena cava. Note temporary abolition of the knee jerk whilst manipulating the intestines and putting the clamps in position. At second arrow inject into jugular vein 25 mg. of eseroline hydrochloride. The knee jerk increases and convulsions appear. On "off" release clamps; some potentiation of neuro-muscular responses occurs. (The convulsive movements mechanically modified the record of the gastrocnemius contractions.)

taken from the experiment (an atypical one), in which the convulsions were most marked. The increase in reflex activity after intrajugular injection of 25 mg. of eseroline hydrochloride was, however, short-lived, and was soon replaced, on restoring the circulation to the hindlimbs, by a considerable depression, which coincided, however, with the appearance of peripheral neuro-muscular potentiation.

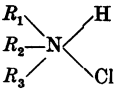
It must be recalled that doses of eserine sulphate of 1 mg., injected under similar conditions, regularly produce striking evidence of central excitation. Doses of eseroline hydrochloride, on the other hand, which are 25–50 times as great, only produce much feebler and much less consistent effects. Thus, the removal of the anticholinesterase (urethane) grouping from eserine sulphate, far from enhancing its convulsant action, almost completely abolishes it. It must be concluded, therefore, that *the convulsant action of eserine is associated with the presence of the urethane grouping*, and probably, therefore, with its specific anticholinesterase activity.

Difference in action of tertiary and quaternary ammonium anticholinesterases

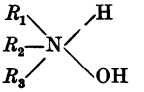
The peripheral parasympathomimetic action of the urethanes examined in this series of investigations has been shown [Stedman, 1926; White & Stedman, 1931] to depend on the presence in the molecule of both a urethane and a basic grouping. It appears to be immaterial as regards the qualitative peripheral effect whether the basic group is of a tertiary or quaternary nature or whether, as in eserine itself, it forms part of a heterocyclic ring. When, however, the central action is concerned, the nature of the basic group seems to be of fundamental importance.

Examination of the structure of the anticholinesterases which have been proved to have a central inhibitory action shows that they have a structural resemblance to one another in respect of the fact that their basic nitrogen atoms are present in the form of quaternary ammonium groups. The two convulsant anticholinesterases previously examined (eserine sulphate; dimethyl carbamic ester of hordenine hydrochloride) resemble one another in being salts of tertiary ammonium bases.

We have given considerable thought to the question of nomenclature of this group of drugs. The use of the term "tertiary ammonium base" is unusual, and "tertiary amine" is more customary. It would, of course, be wrong to apply the term "tertiary ammonium base" to the *free* tertiary base; but when one is dealing with salts, e.g.



one is really dealing with a salt of the hypothetical base



and this must be regarded as a substituted ammonium hydroxide.

We examined further members of the anticholinesterase group to determine whether this striking difference in constitution bore a regular relationship to the difference in their action on the spinal cord. The additional tertiary ammonium compounds of the anticholinesterase group studied were methyl carbamic ester of *m*-hydroxyphenyl dimethylamine hydrochloride (Stedman's "meta"-hydrochloride compound), miotine hydrochloride, and dimethyl carbamic ester of *m*-hydroxyphenyl dimethylamine hydrochloride (Roche liquid, substance 31). The additional quaternary ammonium compounds with anticholinesterase activity which were examined were eserine methiodide, dimethyl carbamic ester of *m*-hydroxyphenyl diethyl methyl ammonium iodide (preparation 3393 Roche) and miotine methiodide.

Action of eserine methiodide

Eserine methiodide is the quaternary ammonium compound corresponding to the tertiary ammonium compound eserine sulphate (or hydrochloride) which is a powerful convulsant. Eserine methiodide has an anticholinesterase action *in vitro* which is less than that of prostigmine or eserine sulphate. The central action of the methiodide would form a crucial test of the hypothesis just set out above. The results showed that in fact eserine methiodide has a central inhibitory action.

Intravenous injection of 1 mg. of eserine methiodide produces peripheral potentiation of the response of skeletal muscle to submaximal or maximal stimulation of its motor nerve, and also generalized muscular twitching. With the onset of this peripheral potentiation, and almost certainly because of it, there may be an initial increase in the knee jerk, but this is rapidly followed by its decline or disappearance (Fig. 3). The blood pressure, in unatropinized animals, shows a slow transient fall, and the heart rate is markedly slowed. With larger doses (2 mg.) there is a more marked initial fall of blood pressure. Inhibition of the knee jerk may persist for 30 min. or longer, though, when recovery ultimately sets in, occasional large isolated contractions may occur. In atropinized animals changes similar to those just described are produced

in the response of the nerve-muscle preparation and the knee jerk, but the depressant action on the circulation of small doses of eserine methiodide is annulled, and that of large doses is diminished.

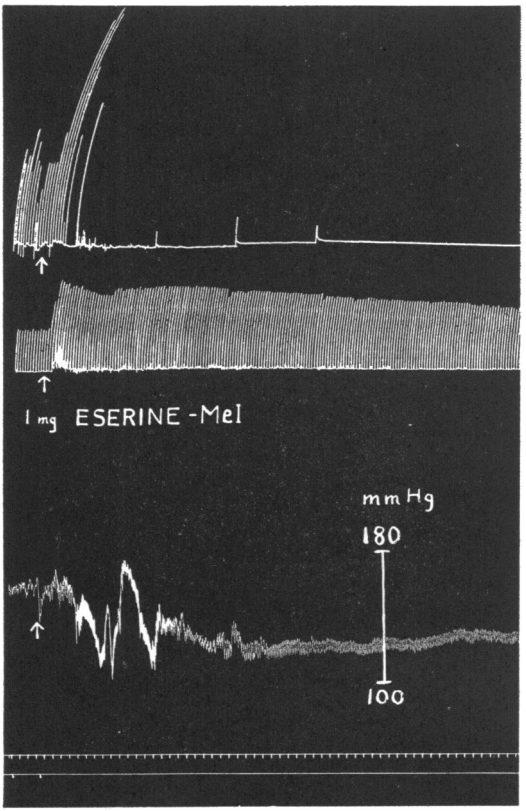


Fig. 3. Cat, chloralose, no atropine. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. At arrow inject into jugular vein 1 mg. of eserine methiodide. Patellar reflex abolished after initial short-lived increase; increases of the responses of the gastrocnemius to motor-nerve stimulation.

Experiments on the "ischaemic preparation" show that eserine methiodide has a central depressant action. Fig. 4 illustrates this central depressant action of eserine methiodide in spite of a previously high degree of reflex excitability, while larger doses completely abolished the knee jerk. As these large doses inhibit breathing, the experiments were

performed under artificial respiration. Eserine methiodide therefore resembles in its central action on the spinal cord the other quaternary ammonium anticholinesterases, e.g. prostigmine, and has a diametrically opposite action to that of eserine sulphate. The only obvious difference between the methiodide and sulphate of eserine is that the former is a quaternary and the latter a tertiary ammonium compound.

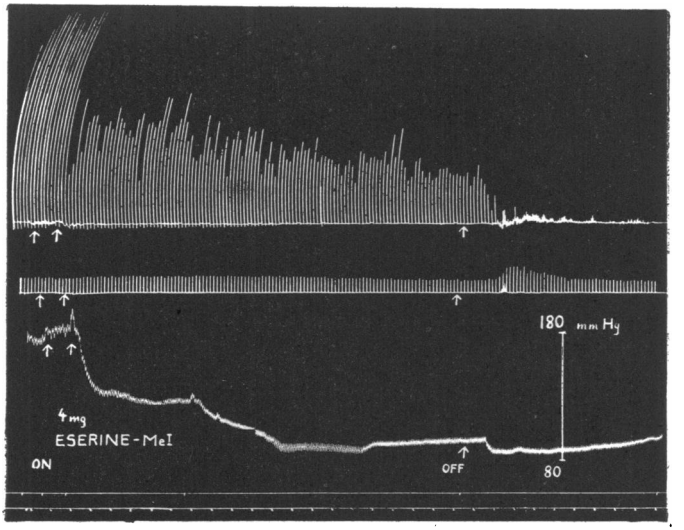
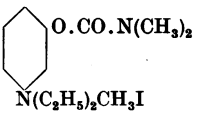


Fig. 4. Cat, light chloralose anaesthesia, 1 mg. atropine per kg. body wt.; "ischaemic" preparation. Records from above downwards are: knee jerk (right side), contractions of gastrocnemius (left side), stimulated through its motor nerve, carotid blood pressure, signal line, time in 30 sec. At "on" clamp abdominal aorta and inferior vena cava; at second arrow inject into jugular vein 4 mg. of eserine methiodide. Knee jerk considerably reduced. At "off" release clamps; marked fibrillary twitchings. Knee jerk abolished; potentiation of gastrocnemius contractions.

Action of dimethyl carbamic ester of m-*hydroxyphenyl diethyl methyl ammonium iodide* (*Preparation* 3393 *Roche*)



This compound, which is a quaternary ammonium compound, behaves like a typical member of the "prostigmine group". When doses of 0·2 mg. are given intravenously (Fig. 5), there is sustained peripheral potentiation of the responses of the gastocnemius to motor nerve stimulation with

slight contracture and transient intense fibrillary twitching of the skeletal muscles. The knee jerk, after an initial increase which runs parallel with, and is presumably due to, the peripheral potentiation, is abolished for varying periods of time. The responses to the tap on the patellar tendon return gradually and irregularly, with the appearance of occasional large

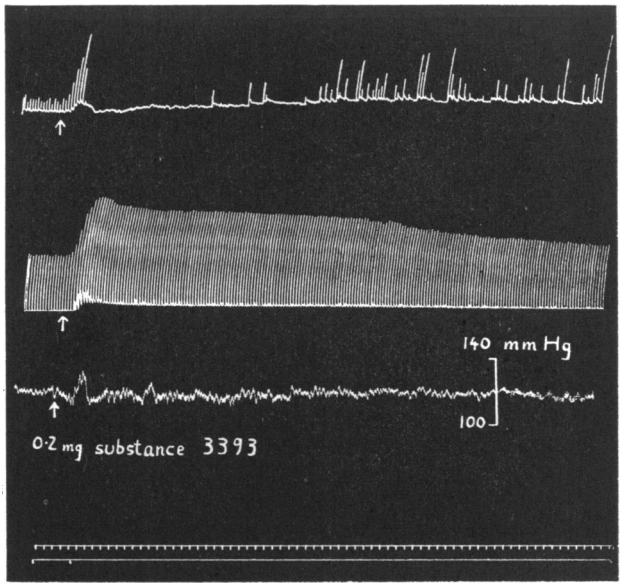


Fig. 5. Cat, chloralose, 1 mg. atropine per kg. body wt. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. At arrow inject into jugular vein 0·2 mg. of substance 3393. Initial short-lived increase in knee jerk, coinciding with increase in the responses of the gastrocnemius to motor-nerve stimulation; knee jerk subsequently abolished; incomplete recovery.

contractions. In atropinized animals similar alterations in the reflexes are obtained without any associated changes in blood pressure. This substance readily diminishes or abolishes convulsions produced by previous intravenous injection of strychnine (Fig. 6). The convulsions subsequently return to their full amplitude, though not to their full frequency. By means of experiments on the "ischaemic" preparation it can be readily shown that the depressant effect on the patellar reflex is due to an inhibitory action of the drug on the central nervous system (Fig. 7).

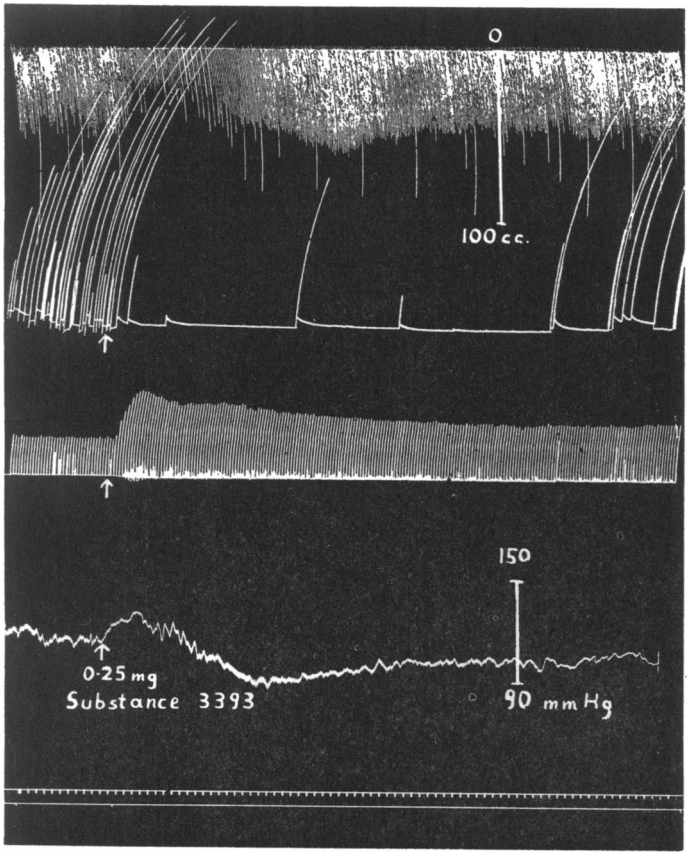


Fig. 6.

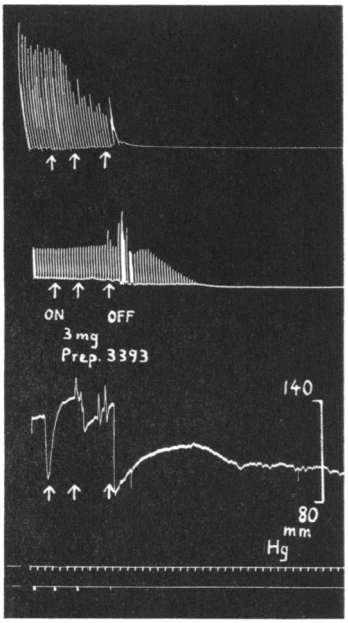


Fig. 7.

Fig. 6. Cat, chloralose, 0·5 atropine per kg. body wt. Records from above downwards are: respiration recorded with Wright's [1934] quantitative method (inspiration downwards), knee jerk (right side), contractions of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec. signal line. 0·25 mg. of strychnine were intravenously injected in the beginning of the experiment. At arrow inject into the jugular vein 0·25 mg. of substance 3393. Strychnine convulsions and knee jerk are abolished. The responses of the gastrocnemius to motor-nerve stimulation are increased. Respiration is initially decreased in rate and amplitude; note, however, that breathing is increased in rate and amplitude while the knee jerk is absent.

Fig. 7. Cat, chloralose, 1 mg. atropine per kg. body wt., "ischaemic" preparation. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. At "on" clamp abdominal aorta and inferior vena cava. At second arrow inject into jugular vein 3 mg. of preparation 3393; knee jerk considerably reduced in size. At "off" release clamps; marked fibrillary twitching and contracture occur, knee jerk abolished; gastrocnemius responses to motor-nerve stimulation disappear gradually.

Action of miotine methiodide

This quaternary ammonium compound has a strong anticholinesterase activity *in vitro*, and its pharmacological actions in the cat resemble qualitatively those produced by other quaternary anticholinesterases, e.g. prostigmine. Miotine methiodide was intravenously injected in doses of 1–2 mg. into the unatropinized chloralosed cat (Fig. 8). Arterial

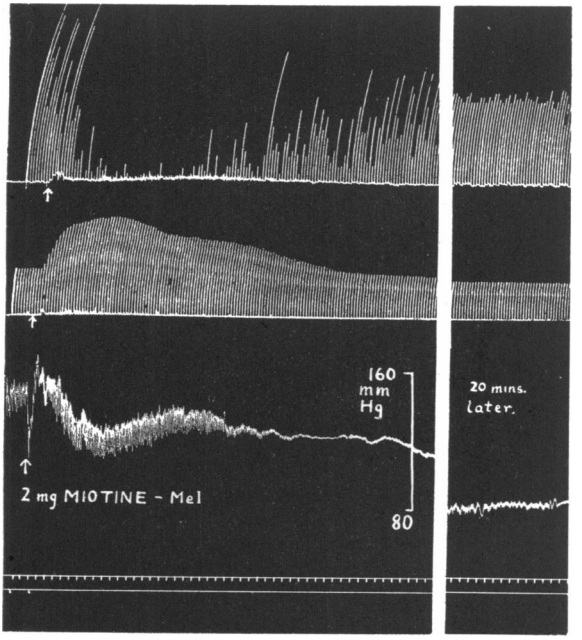


Fig. 8. Cat, chloralose, no atropine. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side), stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. At arrow inject into jugular vein 2 mg. of miotine methiodide. Knee jerk abolished, subsequent recovery; potentiation of the contractions of the gastrocnemius muscle to motor-nerve stimulation. Second part of record taken after interval of 20 min.

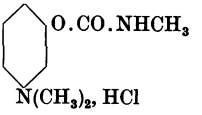
blood pressure is temporarily lowered and the rate of the heart is diminished. Marked fibrillary twitchings occur in the skeletal muscles. The response of the gastrocnemius muscle to submaximal or maximal stimulation of its motor nerve is potentiated, while the knee jerk is depressed or may disappear. Larger doses of the drug depress the responses of the skeletal muscle to motor-nerve stimulation. Respiration may cease, partly perhaps because of paralysis of the respiratory muscles.

The cardio-vascular depression produced by miotine methiodide can, at least partially, be prevented by previous atropinization of the animal. The actions of the drug on the nerve-muscle preparation and on the knee jerk are not, however, influenced by previous injections of atropine.

As miotine methiodide produces very marked effects on peripheral transmission processes, it was again necessary to employ strict precautions when the central action was tested. We used our routine "ischaemic" technique, thus preventing the drug (on intrajugular injection) from reaching the hindlimbs of the animal. It was found that intrajugular injection of miotine methiodide, in doses of 5–10 mg., in strictly controlled "ischaemic" experiments under artificial respiration, depresses or abolishes the knee jerk. The nerve-muscle preparation on the contralateral side, which served as additional evidence that ischaemia was complete, did not show any alterations in its responses. On restoring the circulation to the hindlimbs, violent fibrillary twitching occurred in the muscles of both legs, and the responses of the gastrocnemius to motor-nerve stimulation were markedly depressed after an initial short-lived increase. Recovery set in slowly.

We conclude, therefore, that the depression of the knee jerk produced by intrajugular injections of miotine methiodide is partly due to an inhibitory action on the central nervous system.

Action of methyl carbamic ester of m-*hydroxyphenyl dimethyl ammonium hydrochloride* (*Stedman's "meta"-hydrochloride*)



Stedman's "meta" methiodide compound is the most potent anticholinesterase so far prepared, and as we have previously shown [Schweitzer & Wright, 1937*c*], its central action is powerfully inhibitory. On the analogy of eserine sulphate and dimethyl carbamic ester of hordenine hydrochloride, we thought that the "meta" hydrochloride, which is a tertiary ammonium compound, might prove convulsant in action. The results obtained were in conformity with this view. In atropinized animals, intravenous injection of 10–15 mg. of "meta" hydrochloride produces small changes in blood pressure. There is usually a considerable increase in the responses of the gastrocnemius muscle to submaximal or maximal motor-nerve stimulation (Fig. 9). The knee jerk (sometimes after a transient phase of initial inhibition) may be

considerably increased. Quadriceps tone may rise (Fig. 10), and spontaneous convulsions occur. It can be readily shown that the changes in reflex activity are, in the main, central in origin: (1) there is no regular

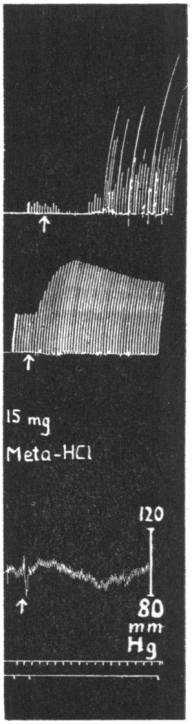


Fig. 9.

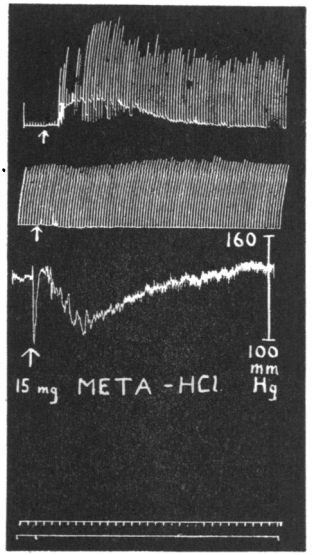


Fig. 10.

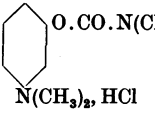
Fig. 9. Cat, deep chloralose anaesthesia, 1 mg. per kg. body wt. Records from above downwards are: knee jerk (right side), response of gastrocnemius to motor-nerve stimulation (left side) carotid blood pressure, time in 30 sec., signal line. At arrow inject into jugular vein 15 mg. of Stedman's "meta"-hydrochloride. Note marked potentiation of nerve muscle responses. There is an initial depression of the knee jerk followed by a marked sustained increase accompanied by violent spontaneous convulsions.

Fig. 10. Cat, deep chloralose anaesthesia, no atropine. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. At arrow inject intravenously 15 mg. of Stedman's "meta"-hydrochloride. Note increase in muscle tone and reflex activity. Initial convulsive movements.

time relationship between the increase in the knee jerk and that of the nerve-muscle preparation; (2) the increase in the knee jerk may occur without any associated change in the nerve-muscle response; (3) the "spontaneous" and generalized convulsions which occur cannot,

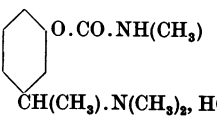
of course, be attributed to peripheral changes, but must be due to heightened central excitability; (4) in "ischaemic" preparations, injection of the drug into the jugular vein produced the same changes in the reflexes as those seen in the intact animal. Prolonged convulsions of great force can be elicited under such experimental conditions.

Action of dimethyl carbamic ester of m-*hydroxyphenyl dimethylamine hydrochloride* (*Substance* 31)



This anticholinesterase, which is a tertiary ammonium derivative, has a weak convulsant action. The great toxicity of the substance made a thorough examination of its central action difficult. In unatropinized animals 0·5 c.c. (0·1 g.) practically stopped the heart. The blood pressure subsequently returned gradually to the normal level. There was potentiation of the gastrocnemius response to motor-nerve stimulation. The knee jerk was increased and slight generalized convulsions appeared. These changes are gradual in onset, but may last for a considerable time, e.g. 40 min. In atropinized animals doses up to 2 c.c. were intravenously injected. There was always initially a profound fall of blood pressure, which might be depressed (e.g. to 70 mm. Hg) for 30–60 min. An increase of the knee jerk and convulsions were readily observed.

Action of miotine hydrochloride



Miotine hydrochloride is a tertiary ammonium compound with considerable anticholinesterase activity *in vitro*. Its action in the atropinized animal proved to be complex. Intrajugular injection of 20 mg. produced only small changes of blood pressure, and slowing of the heart rate was sometimes observed. Violent and generalized fibrillary muscle twitchings occurred. The response of the gastrocnemius muscle to submaximal or maximal motor-nerve stimulation was greatly potentiated; an increase in the height of contraction of over 100 % from the pre-injection level was often observed. The knee jerk usually disappeared, but the quiescent reflex background was disturbed at intervals by violent jerks in response to the tap on the tendon or by spontaneous convulsions of great force

which appeared in the intervals between the stimulation of the tendon. These heightened responses occurred either singly or in bouts, but, as explained, they were separated by intervals during which no reflex reactions took place (Fig. 11). The results obtained in ischaemic preparations are very similar to those recorded in intact animals. Intrajugular

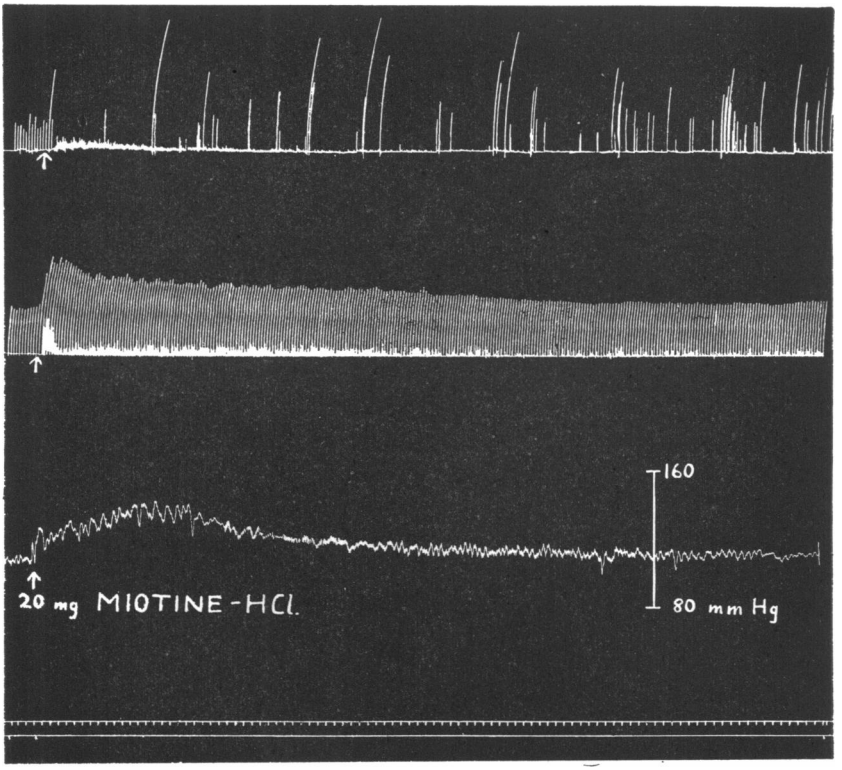


Fig. 11. Cat, chloralose, 1 mg. atropine per kg. body wt. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side) stimulated through its motor nerve, carotid blood pressure, time in 30 sec., signal line. At arrow, inject 20 mg. of miotine hydrochloride. There is marked fibrillation and potentiation of the nerve muscle responses. Bouts of convulsive movements occur against a background of depression of the knee jerk.

injection of 10–30 mg. of miotine hydrochloride produced a complex picture of depression of the knee jerk, interrupted from time to time by exaggerated jerks or bouts of spontaneous convulsions. The results obtained were contrary to expectations, as miotine hydrochloride is a tertiary ammonium cholinesterase compound, and we thought that

it would prove to be predominantly convulsant in action. It is true, of course, that preceding the increase in reflex excitability, an initial phase of inhibition not uncommonly occurs with the other tertiary compounds examined, e.g. eserine sulphate, "meta"-hydrochloride. In some instances this inhibition may last as long as 30 min., e.g. eserine sulphate. But undoubtedly the usual and common effect of the tertiary

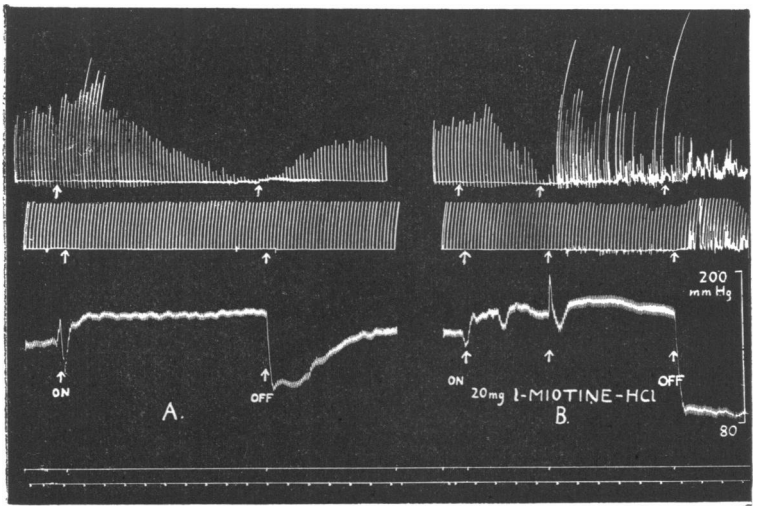


Fig. 12. Cat, chloralose, 1 mg. atropine per kg. body wt., "ischaemic" preparation. Records from above downwards are: knee jerk (right side), contraction of gastrocnemius (left side), stimulated through its motor nerve, carotid blood pressure, signal line, time in 30 sec. A. Between "on" and "off", control period of ischaemia; there is an initial increase in the knee jerk followed by a secondary decline which is probably due to anaemia of the spinal cord; recovery occurs when the blood supply is restored. B. At "on", clamp abdominal aorta and inferior vena cava; knee jerk rapidly declines as in control. At second arrow inject into jugular vein 20 mg. of *l*-miotine hydrochloride; note onset of frequent and violent convulsions. At "off" release clamps; fibrillary twitchings and contractures set in.

ammonium anticholinesterase compounds is to produce increased reflex excitability. Miotine hydrochloride, on the other hand, produced mainly a depressant effect, interrupted by transient periods of increased excitability. Further experiments were carried out to try and solve this difficulty.

The results previously recorded were obtained with a racemic compound of miotine hydrochloride. It was thought desirable to test separately the central action of the dextro- and laevorotary components, as the complex effects of the racemic compound might be due to conflicting

properties of the two isomers. A large number of experiments were performed on ischaemic preparations, and rather irregular results were obtained. Intrajugular injections of 25 mg. of *d*-miotine hydrochloride appeared to have predominantly a central inhibitory effect on the knee jerk. Doses of the same order of *l*-miotine hydrochloride often had a definite excitatory action on the central nervous system, producing a marked increase of the knee jerk and violent spontaneous convulsions. Fig. 12 illustrates such a response.

It is possible, therefore, that the complex action of the racemic miotine hydrochloride is partly the resultant of the differences in the action of the dextro- and laevorotary compounds. We do not think, however, that the results of our experiments were uniform enough to allow us to come dogmatically to any such conclusion, and they merely suggest that this may be one of the factors concerned. It must be remembered, also, that though laevo- and dextro-isomers often differ quantitatively in their pharmacological action, we cannot recall any examples in which the isomers differed qualitatively. If we regard miotine hydrochloride as having a mixed inhibitory and excitatory action, it may be supposed that the laevorotary compound has more of the excitatory and the dextrorotary substance more of the inhibitory activity. It must be mentioned, however, that the laevorotary compound has about three times the anticholinesterase activity of the dextro isomer, and possibly had we used larger doses of the latter a more predominant convulsant action might have been produced. We will consider these points again in the discussion.

Relationship of anticholinesterase activity of the quaternary ammonium compounds to their central inhibitory action. Comparison of in vivo *and* in vitro *activities*

The results recorded previously [Schweitzer & Wright 1937*c*, *d*, *e*, 1938*a*] and in an earlier section of this paper show that all the quaternary anticholinesterases examined have an inhibitory action on the spinal cord. We have now to consider whether, or to what extent, this central inhibitory action is due to the fact that these drugs can inhibit cholinesterase. It is first necessary to compare the degree of anticholinesterase potency shown by the various members of the group *in vitro* with the intensity of their central inhibitory action when injected. This has been done by examining their inhibitory action on somewhat, but not highly, purified preparations of cholinesterase obtained from horse serum by the method of Stedman & Stedman [1935]. The enzyme was diluted to

a suitable concentration with $NaHCO_3/CO_2$ buffer of *p*H 7·4, mixed with the urethane under investigation, and the hydrolytic activity of the mixture towards butylcholine measured after various intervals of time by the continuous titration method described by Stedman, Stedman & White [1933], using bromothymol blue as indicator. It was not technically possible to make a direct comparison of the inhibitory actions of all the substances on one and the same specimen of cholinesterase. The results are, nevertheless, directly comparable with a fair degree of accuracy, for, as shown by Easson & Stedman [1936] the inhibitory activities of urethanes of the nature of those under consideration are, within wide limits, independent of the degree of purity of the preparation of cholinesterase. The results obtained are shown in the following Tables I and II.

TABLE I. Quaternary bases

Compound	Concentration (molar)	% inhibition in 1 hr.	3 hr.	3½ hr.	4½ hr.
Prostigmine	5×10^{-8}	35	—	—	72
"Meta"-MeI	5×10^{-8}	67	—	—	55
36	5×10^{-8}	15	—	—	30
Dimethylcarbamic ester of hordenine methyl methyl sulphate	$2{\cdot}5 \times 10^{-6}$	34	—	67	—
Prostigmine	$2{\cdot}5 \times 10^{-8}$	—	38	—	—
Dimethylcarbamic ester of *m*-hydroxyphenyl diethyl methyl-ammonium iodide	$2{\cdot}5 \times 10^{-8}$	—	65	—	—
Prostigmine	$2{\cdot}5 \times 10^{-8}$	25·25	48	—	—
Eserine MeI	$2{\cdot}5 \times 10^{-8}$	20·5	35	—	—

TABLE II. Tertiary bases

Compound	Concentration (molar)	% inhibition in 1 hr.	3 hr.	3½ hr.	4½ hr.
Eserine sulphate	5×10^{-8}	58	—	—	53
37	5×10^{-8}	6	—	—	11
Dimethyl urethane of hordenine HCl (41)	$2{\cdot}5 \times 10^{-6}$	36	—	—	71
"Meta"-HCl	$2{\cdot}5 \times 10^{-7}$	—	26	—	8
Miotine HCl	5×10^{-8}	7	—	22	—

In addition to comparing the *in vitro* inhibitory activities of the urethanes mentioned in the above table, we have also examined for anticholinesterase activity certain of the phenols derived from these urethanes by removal of the carbamic ester grouping. As the following results show, when used in a concentration of 5×10^{-4} *M*, i.e. in a concentration one thousand times greater than that of the eserine employed in the above experiments, the inhibitory activities are almost negligible.

Compound	Concentration (molar)	% inhibition in 1 hr.	2 hr.	3 hr.	23½ hr.
Eseroline MeI	5×10^{-4}	8	—	11	12
Eseroline HCl	5×10^{-4}	—	15	—	13
Hordenine HCl	5×10^{-4}	—	1	—	19
Hordenine MeI	5×10^{-4}	—	0	—	0

It is, for the following reasons, a matter of some difficulty to arrange the above urethanes in the precise order of their *in vitro* activities. (1) Owing to the slight variation in the concentration of the cholinesterase in the various preparations employed the results are not comparable to a high degree of accuracy. That the magnitude of the error involved is, however, not large is indicated by the results obtained with prostigmine, using these different preparations of cholinesterase. (2) As demonstrated by Easson & Stedman [1936], most of the urethanes are themselves slowly decomposed by cholinesterase. This decomposition, moreover, occurs at widely different rates for the various members. In general, the esters of dimethylcarbamic acid are very much more stable than those of methylcarbamic acid. With the former, decomposition is inappreciable during the first few hours, at any rate at room temperature, with the latter it may be large under these conditions. (3) Under the conditions of our experiments the maximum inhibitory activity in the absence of appreciable decomposition is not attained until some 9 hr. after mixture with the enzyme.

Taking all these factors into account we believe that, on the basis of the above results, the quaternary compounds can be arranged in the following order of their *in vitro* anticholinesterase potencies:

Meta-methiodide compound$\geqslant$dimethyl carbamic ester of *m*-hydroxyphenyl diethylmethyl ammonium iodide (preparation 3393 Roche) > prostigmine > eserine methiodide > methylphenyl carbamic ester of *m*-hydroxyphenyl trimethyl ammonium methyl sulphate (substance 36) > dimethyl carbamic ester of hordenine methyl sulphate. Most of the above compounds are active *in vitro* in concentrations of 10^{-8} *M*. The exception is the hordenine derivative, of which a concentration of 10^{-6} *M* is necessary.

The *in vivo* results must now be considered. We used as a basis for comparison the dose of an anticholinesterase necessary to produce, on intravenous injection, a definite depression of the patellar reflex in ischaemic preparations. The *in vivo* results with regard to the activity of these substances on the spinal cord show that the "meta"-methiodide and preparation 3393 Roche are much the most potent, and are about equally active. Prostigmine came next, followed at an interval by

substance 36, which was about equal or perhaps somewhat more potent than eserine methiodide. The hordenine derivative is much the weakest in the *in vivo* series. These results are shortly summarized in Table III.

TABLE III. Order of potency of quaternary anticholinesterases

	In vitro	*In vivo*
"Meta"-methiodide Preparation 3393	1st	1st
Prostigmine	3rd	3rd
Eserine methiodide	4th	5th
Substance 36	5th	4th
Dimethyl carbamic ester of hordenine methiodide	6th	6th

Taking into account the obvious difficulties and uncertainties involved in this comparison, e.g. the difficulty of producing the same depth of anaesthesia in each animal, it can be said that the degree of general agreement obtained between *in vitro* and *in vivo* activity of the substances is almost surprisingly good.

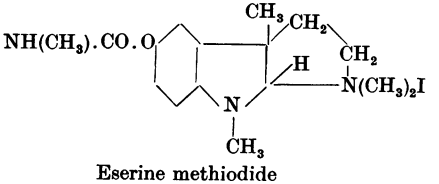
One discrepancy, however, must be mentioned. Substance 13 is the methyl sulphate corresponding to the "meta"-methiodide, and has the same activity *in vitro* as the "meta"-methiodide compound. *In vivo*, however, it comes lower down on the list and is about as active as prostigmine.

Action of quaternary ammonium compounds after removal of the urethane grouping

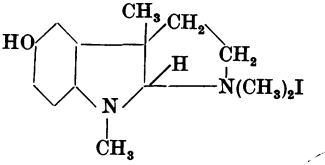
As has been shown by one of us [Stedman, 1926; White & Stedman, 1931], removal of the urethane grouping almost completely abolishes the anticholinesterase potency *in vitro* (cf. pp. 302, 308 above). We have studied the *in vivo* action of two such derivatives: eseroline methiodide, corresponding to eserine methiodide, and *m*-hydroxyphenyl trimethyl ammonium iodide, corresponding both to "meta"-methiodide and to prostigmine. We have previously recorded our results with (the urethane-free) hordenine methiodide, corresponding to dimethyl carbamic ester of hordenine methiodide [Schweitzer & Wright, 1938*a*].

Action of eseroline methiodide

The formulae of eserine methiodide and of eseroline methiodide are set out below:



Eserine methiodide

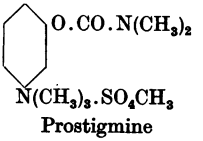


Eseroline methiodide

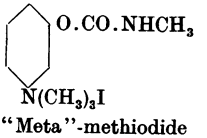
The anticholinesterase activity *in vitro* of eseroline methiodide is very feeble and is of the same order of magnitude as the corresponding hydrochloride, the *in vivo* action of which was discussed above. Intravenous injection of eseroline methiodide in doses of 1–2 mg. gives rise to an increase in the knee jerk which can, in considerable measure, be attributed to the associated potentiation of the muscle responses to motor-nerve stimulation. In atropinized animals there was little effect on blood pressure. Larger doses of this drug, 5, 10 or 20 mg., in atropinized animals produced, in some cases, a slight increase in the general excitability of the animal, considerable peripheral neuro-muscular potentiation, and an increase in the knee jerk. In most of our experiments on "ischaemic" preparations, 15–25 mg. of the drug injected into the jugular vein had no effect on the knee jerk. In two experiments only it appeared, however, that such doses diminished strychnine convulsions. It is thus quite clear that eseroline methiodide is much less active on the central nervous system than eserine methiodide. It can also be concluded that the marked central depressant action which is characteristic of eserine methiodide is, in large measure, if not wholly, associated with its urethane grouping and presumably with its anticholinesterase properties.

Action of m-*hydroxyphenyl trimethyl ammonium iodide*

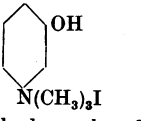
This substance, which is the phenolic base corresponding to both prostigmine and the "meta"-methodide (see formulae below) has a small anticholinesterase effect *in vitro* in concentrations of 5×10^{-4} *M*, compared with an appreciable activity by the urethane derivatives of the substance in concentrations of 10^{-8} *M*.



Prostigmine



"Meta"-methiodide



m-hydroxyphenyl trimethyl ammonium iodide

In the intact animal, intravenous injection of doses of 2·5 mg. or over of this substance produces violent muscular twitching and abolishes the knee jerk. With small doses (0·5 mg.) the response of the gastrocnemius to break shocks, at a rate of one in 10 sec., is slightly potentiated, but with larger doses or with repeated small doses, it is depressed. The response of the muscle to tetanic stimulation may be temporarily abolished. Breathing is also depressed or stopped and artificial respiration has to be employed.

This peripheral depressant effect on neuro-muscular transmission is to be expected from a quaternary ammonium salt [cf. Ing, 1936]. Special precautions must therefore be taken to determine whether the changes in the reflexes are due wholly to this peripheral curari-like action, or whether there is any central depressant action as well. In experiments carried out under rigidly controlled "ischaemic" conditions it was found that the intrajugular injection of large doses of the drug, e.g. 14 mg. in divided doses, abolished the knee jerk. Removal of the urethane grouping from prostigmine or from the "meta"-methiodide compound thus considerably weakens but does not abolish the central depressant action of the remaining phenolic substance.

Discussion

The experiments recorded in this paper have provided further evidence that the anticholinesterase group of drugs have a direct action on the spinal cord, which may be of an excitatory or inhibitory nature. All the quaternary ammonium anticholinesterase compounds examined have a central inhibitory action. All the tertiary ammonium compounds (with the partial exception of miotine hydrochloride) are excitatory. The results are summarized in Table IV.

Table IV

Central convulsants	Central depressants
Eserine (44)	Eserine methiodide (45)
Dimethylcarbamic ester of hordenine hydrochloride (41)	Dimethylcarbamic ester of hordenine methiodide (42)
Stedman's "meta"-hydrochloride	Stedman's "meta"-methiodide
Miotine hydrochloride (28)	Miotine methiodide
Dimethylcarbamic ester of *m*-dimethylaminophenol hydrochloride (31)	Dimethylcarbamic ester of methyl hordenine methylsulphate
Dimethylcarbamic ester of 8-hydroxyquinoline hydrochloride (37)	Dimethylcarbamic ester of *m*-hydroxyphenyldiethylmethyl ammonium iodide
	Prostigmine
	Methylcarbamic ester of 3-oxyphenyltrimethyl ammonium methylsulphate (13)
	Methylphenylcarbamic ester of 3-oxyphenyltrimethyl ammonium methylsulphate (36)

(The numbers in brackets refer to Aeschlimann & Reinert's [1931] series.)

These results are, in part, supported by experimental evidence obtained by other workers. Miller [1937] applied eserine sulphate in 1 % and 10 % solution (40–100 μg.) by means of small squares of filter paper on to the cerebral motor cortex of cats under dial anaesthesia.

The contralateral fore- or hindleg showed tremor, powerful clonus and generalized rigidity after a latency of about 6 sec. The threshold to faradic stimulation was notably lowered, and the responses were augmented and accompanied by powerful clonus and prolonged after-discharges. Normal responses to electrical stimulation were obtained from the non-eserinized cortex. Bremer & Kleyntjens [1937] and Bonnet & Bremer [1937] showed that eserine (sulphate) increases the reflex sensitivity of the spinal cord in frogs, and produces prolonged after-discharge. Injection of small doses of acetylcholine (0·02–1 μg. in 0·1 c.c.) into the right aortic arch had an augmentatory effect on the after-discharge "without necessarily increasing (on the contrary, sometimes depressing) the height of the twitch to which it is appended" [Bonnet & Bremer, 1937]. Larger doses of acetylcholine (1·0–5·0 μg.) have only a depressing effect on the spinal centres. Lefèbre & Minz [1936] and Bonvallet & Minz [1938] have demonstrated that acetylcholine diminishes the excitability of the spinal cord in frogs, and in spinal cats and dogs. Eserine increases the excitability.

Therman [1938] studied the action of acetylcholine and eserine on the action potentials of the excised frog's retina. He found that eserine sometimes increased, but, in most cases, diminished the potentials. Acetylcholine in all cases depressed retinal excitability.

Thus it appears that general agreement exists with regard to the excitatory action of (tertiary) eserine sulphate on the central nervous system. Therman [1938] explains the depressant action of eserine on retinal action potentials in frogs on the basis of an increased concentration of acetylcholine in the retinal tissue, from which considerable amounts of acetylcholine can be extracted, thus aligning his findings with regard to the eserine effect with the action of acetylcholine on retinal potentials. It is difficult to explain Bonnet & Bremer's [1937] finding of an augmentary effect of small doses of acetylcholine on after-discharge. It may be argued, however, that there are differences of reaction in various species, especially as Bonnet & Bremer [1937] also found a spontaneous and long lasting discharge of the spinal centres in frogs after administration of 1–10 μg. of nicotine, although nicotine has proved to be a very strong depressant of spinal reflexes in cats [Schweitzer & Wright, 1938*b*].

Briscoe [1938] confirmed recently the central excitatory effect of dimethyl carbamic ester of hordenine hydrochloride in cats. The enhancement of the patellar reflex was coincident with depression of neuromuscular transmission on the contra-lateral side. The dimethyl carbamic ester of hordenine methyl sulphate caused depression or temporary abolition of the knee jerk.

Interesting observations on the action of acetylcholine and eserine and other anticholinesterases on the central nervous system in man have recently been made by Kremer [1939]. Kremer has compared the action of acetylcholine, eserine (sulphate), prostigmine and the dimethyl carbamic ester of hordenine hydrochloride and of hordenine methiodide on the spinal cord in man by injecting the drugs directly into the lumbar spinal fluid. He has confirmed the previous observations of Kremer *et al.* [1937] that prostigmine is a central depressant: tone and voluntary power may be diminished without any change in blood pressure, pulse rate, respiration or sensation. Eserine (sulphate) (1 mg.), after an initial period of inhibition, acted as a central stimulant, increasing tone and reflexes and producing spontaneous pain. Dimethyl carbamic ester of hordenine methiodide acted like prostigmine, while the dimethyl carbamic ester of hordenine hydrochloride acted like eserine. The relative potency of eserine (sulphate) and the hordenine hydrochloride compound was 50 : 1, and that of prostigmine and the hordenine methiodide compound was 50 or 100 : 1; both results agreed very well with our animal experiments. Thus, when the action of these drugs is studied directly on the human spinal cord there is also a close agreement between the *in vitro* and the *in vivo* activity. Acetylcholine injected intrathecally, even in doses of 500 mg., was inactive, but given together with small doses of prostigmine (e.g. 0·1 mg.) which are themselves inactive, it becomes potentiated and may produce central inhibition in doses of 10 mg. There is thus close general agreement between the clinical and experimental findings. The central action of none of these substances was intensified or annulled by repeated intravenous injection of 2 mg. of atropine.

We have carefully considered the possible causes of the remarkable qualitative difference in the central activity of the two classes of anticholinesterase compounds (tertiary and quaternary compounds) that have identical actions *in vitro* and on all other tissues so far examined (e.g. skeletal muscle, heart, blood vessels). The difference in the nature of the basic group would presumably cause differences in the distribution of the drug in the tissues. The quaternary ammonium compounds are, as such, necessarily soluble in water and insoluble in lipoid. The salts of the tertiary bases, however, undergo some hydrolytic dissociation in solution, giving rise to an equilibrium mixture of basic ions and free base, and the latter will be soluble in lipoids. The difference in this respect may perhaps account for the different kind of action which these substances exert on the spinal centres.

The probable dissociation processes taking place are indicated diagrammatically below in Tables V and VI.

TABLE V. Quaternary ammonium compounds

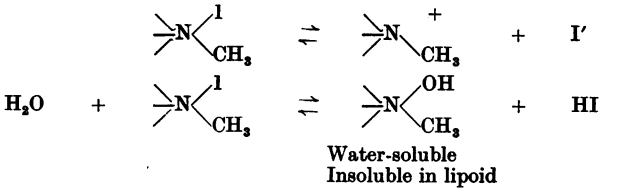
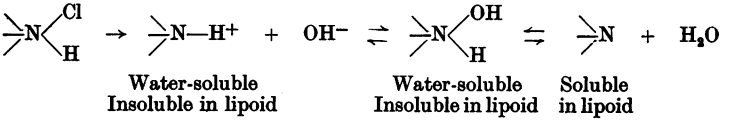


TABLE VI. Tertiary ammonium compounds



The anticholinesterases influence transmission processes in the central nervous system presumably by a direct action on nervous elements. It is most unlikely that they act merely by altering the local blood supply. Their central action is totally unrelated to changes in the general level of blood pressure: further, both classes of anticholinesterases act similarly on the blood vessels, and so it would be impossible to account for the qualitative differences described if this were their sole mode of action. As the drugs in the doses used have no action on peripheral nerve fibres, they must be supposed to act either on the surface membrane of the nerve cell, its dendrites and its related synaptic terminals, or in the interior of the nerve cell itself.

The quaternary ammonium cholinesterase compounds and their derivatives, being lipoid insoluble, may be regarded as unable to penetrate the lipoid envelopes of the nerve cells and so be limited to the "outside" of these cells. The tertiary ammonium compounds are present both as cations and free base. The cation, being water soluble only, can likewise be supposed to be unable to enter the cells; the tertiary base, however, being lipoid soluble, may perhaps dissolve in the lipoid material of the cell surface and enter the cell (where it may act as the free base or as the cation). We venture to suggest that an anticholinesterase which penetrates into the cell will act as a convulsant, while a member of the group which is unable so to penetrate will be a central depressant.

This suggestion explains an important anomaly in connection with the physiological activity of the tertiary group, namely that they often exhibit an *initial* inhibitory action, which is followed after an interval

of longer or shorter duration by the characteristic convulsant action. This is to be expected from the method of dissociation of these drugs, which leads to the formation of a water-soluble cation which remains *ex hypothesi* "outside" the cells, and so has an inhibitory action. The tertiary compounds would, therefore, tend to exhibit a mixed effect, though usually the convulsant effect predominates.

These considerations may help to explain the somewhat irregular behaviour of miotine hydrochloride which (in the racemic form) produces a predominantly central depressant action interrupted by occasional bursts of exaggerated central activity. The experiments with the laevorotary compound showed conclusively that miotine hydrochloride *can* produce convulsions under certain circumstances. It may be supposed that the ionization of this substance proceeds in such a manner that it gives rise commonly to a preponderance of concentration of the inhibiting cation at the cell surfaces. As Dr Danielli has reminded us, the *p*H of the blood would modify the dissociation of the tertiary compounds. At a more acid *p*H more of the cation would be formed and so the inhibitory action should be enhanced: at a more alkaline *p*H more free base would be present to penetrate into the interior of the cells and so intensify the excitatory effect. We tested the point experimentally with miotine hydrochloride by attempting to increase the *p*H by means of overventilation. We were not able to satisfy ourselves that the convulsant action of the drug was thereby enhanced. But the change in *p*H that can be produced by such means in the body is most probably too small to influence the extent of dissociation to a significant degree.

The tertiary salts do not hydrolyse much at *p*H 7, and it is therefore a matter for comment that the free base enters the cells so rapidly, judging by the physiological effect. Danielli [1937], however, has demonstrated that the dissociation curve of a base is displaced towards the acid side when the base is adsorbed at a surface. Thus a base which is only 5 % in the form of a free base in blood may be 50 % or more as a free base at a surface in contact with the blood. This factor may help to account for the minor discrepancies that have been recorded.

Mode of action of convulsant tertiary anticholinesterases

The essential question to be decided is whether these substances produce their effects on the central nervous system because they inhibit the action of cholinesterase or whether their effects are quite unrelated to this property. The following observations may assist in arriving at a conclusion. (1) There is a high concentration of cholinesterase present

in the grey matter of the central nervous system. The presence of the enzyme in the central nervous system was first demonstrated by Plattner & Hintner [1930]. Stedman & Stedman [1935] found that basal ganglia contain twice as much cholinesterase as cortex. Recently, Nachmansohn [1937, 1938] compared the enzyme concentration in grey and white matter in various regions and found a much higher amount of cholinesterase in the former, the largest concentrations of enzyme, calculated from the amount of acetylcholine hydrolysed by 100 mg. tissue in 60 min., being demonstrated in nuclei rich in synapses.

(2) We have compared the inhibitory activity of the various members of this group *in vitro* on cholinesterase with their potency as convulsants *in vivo*. The probable *in vitro* order of activity of the members of this group (experiments of E.S.) is: Eserine (sulphate) > miotine hydrochloride) > "meta"-hydrochloride > dimethyl carbamic ester of hordenine hydrochloride. In arranging these in order of potency *in vivo* consideration must be paid to the fact that all these substances have some initial inhibitory action on the central nervous system, which tends to counteract their dominant convulsant action. The *in vivo* comparative activity of eserine sulphate, "meta"-hydrochloride and the dimethyl carbamic ester of hordenine hydrochloride is approximately 50, 10, 1. The miotine compound cannot be placed in this series because of its very marked inhibitory component. There is therefore, making proper allowance for the inherent difficulty of such a comparison, a considerable degree of correlation between anticholinesterase activity *in vitro* and convulsant action *in vivo*. The results are summarized in Table VII below.

TABLE VII. Order of potency of tertiary anticholinesterases

	In vitro	*In vivo*
Eserine sulphate	1st	1st
"Meta"-HCl	2nd	2nd
Dimethyl carbamic ester of hordenine HCl	3rd	3rd

(3) Our experiments have shown that the central excitatory action of the members of this group of substances is diminished, abolished or converted into a central inhibitory action when the urethane grouping, which is responsible for anticholinesterase activity, is removed. Thus the central excitatory effect of eseroline hydrochloride is approximately $\frac{1}{50}$th of that of eserine sulphate; if the urethane grouping is removed from the dimethyl carbamic ester of hordenine hydrochloride (a substance with a strong convulsant action), the resultant compound, hordenine hydrochloride, has a central inhibitory effect.

These two lines of evidence, (i) the quantitative parallelism of *in vitro* anticholinesterase activity and *in vivo* action on the central nervous system, and (ii) the dependence of the central excitatory action on the presence in the molecule of the carbamic ester grouping, make it probable that the group of tertiary ammonium compounds are convulsant *by virtue of their anticholinesterase properties* and to the degree to which they inhibit this enzyme in the central nervous system.

It is reasonable to suggest that the esterase is present at the site of action of the tertiary convulsants and this may well be, in consequence of their lipoid solubility, in the interior of the nerve cells. It seems to follow, too, that acetylcholine must be available there normally to be acted upon by the enzyme, and that its local concentration can influence transmission processes. Though it is suggested by Threthewie [1938] that synthesis of acetylcholine in the presence of glucose and eserine occurs in extracts of brain tissue the work of Mann, Tennenbaum & Quastel [1938] casts doubt on the validity of this claim. It seems more probable that the enzyme is concerned only with the hydrolysis of acetylcholine in nervous tissue. The inhibition of the enzyme by the anticholinesterase drugs would thus increase the concentration of acetylcholine at its site of action. It seems proper to conclude that the tertiary ammonium anticholinesterases facilitate central transmission processes by modifying (most probably increasing) the concentration of acetylcholine within the nerve cells of the spinal cord.

Mode of action of quaternary anticholinesterases

It has already been shown that there is a high degree of correlation between the strength of anticholinesterase activity of the members of this group *in vitro* and the intensity of their inhibitory activity on the spinal cord *in vivo* (p. 323, Table III).

The second line of approach to the problem, namely to study the central action of these drugs after removal of their urethane group (and anticholinesterase activity), leads to somewhat contradictory results. The substance so obtained from eserine methiodide (eseroline methiodide) has practically no inhibitory action on the central nervous system; that derived from prostigmine and "meta"-methiodide (*m*-hydroxyphenyl dimethyl ammonium iodide) has a much weaker central action. In these instances it seems clear that the anticholinesterase grouping is responsible wholly or largely for the central inhibitory action.

The substance derived from the dimethyl carbamic ester of hordenine methiodide, namely hordenine methiodide itself, has, however, a central

inhibitory activity *some fifty times as great* as that of the parent body and is of the same order of magnitude as prostigmine, and yet hordenine methiodide has a negligible anticholinesterase action *in vitro*. Again, the tertiary hordenine hydrochloride has a definite, though weaker, central inhibitory action. It is clear, from these and other examples like that of adrenaline and nicotine [Schweitzer & Wright, 1937*b*, 1938*b*], that central inhibitory action is not restricted to any particular class of chemical compounds. There is nothing surprising in that; eserine and pilocarpine for instance both have a parasympathetico-mimetic activity, but for different reasons.

We have to decide whether the facts set out in the preceding paragraph compel us to conclude that the central inhibitory activity of the quaternary anticholinesterases is entirely non-specific in the sense that it bears no relationship to its effect on cholinesterase. The balance of the evidence seems to us, however, to point in the opposite direction:

(1) As already mentioned, there are high concentrations of cholinesterase present in the grey matter of the central nervous system (p. 330).

(2) Acetylcholine and other choline esters injected intravenously, in the cat, have a central inhibitory action and this action is potentiated by the anticholinesterases [Schweitzer & Wright, 1937*c*]. In the animal experiments this potentiation might be attributed to inhibition of cholinesterase in the blood, protecting acetylcholine from destruction and so allowing higher concentrations of acetylcholine to reach the central nervous system. Kremer [1939], however, has shown in man that intrathecally injected prostigmine in subthreshold doses potentiates the action of intrathecally injected subthreshold doses of acetylcholine. It seems, therefore, reasonable to suppose that the prostigmine inhibited the cholinesterase in the spinal cord and thus permitted the injected acetylcholine to act more effectively.

(3) There is a remarkably close relationship between *in vitro* potency and central inhibitory activity (Table III).

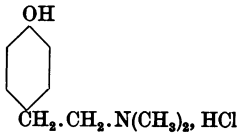
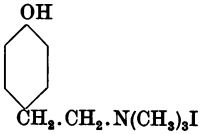
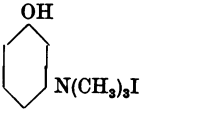
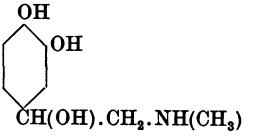
(4) The central inhibitory action of some quaternary ammonium anticholinesterases is diminished or even abolished by removal of the grouping in the molecule which is responsible for their anticholinesterase activity.

These arguments incline us to adopt the view that the central inhibitory action of these quaternary anticholinesterases is due largely, and perhaps in some instance wholly, to their action on the cholinesterase of the nervous system.

The activity of the derived phenolic bases may perhaps be related to the presence of the free hydroxyl grouping attached to the benzene ring. When the hydroxyl is replaced by a urethane grouping the inhibitory action due to hydroxyl (which we suggest is exerted by a different mechanism), is perhaps diminished or lost, and the specific anticholinesterase inhibitory action makes its appearance. This latter may be stronger, as with prostigmine or "meta"-methiodide, or weaker, as with the dimethyl carbamic ester of hordenine methiodide, than that of the phenolic base.

Examination of the phenolic bases derived from the anticholinesterases studied (Table VIII) shows that they resemble adrenaline, which is also

TABLE VIII

Hordenine HCl	
Hordenine MeI	
m-hydroxyphenyl trimethyl ammonium iodide	
Adrenaline	

a central inhibitor [Schweitzer & Wright, 1937*b*]. If our arguments about the mode of action of the quaternary anticholinesterases are justified, certain conclusions seem to follow. Cholinesterase must be present at the site of action of this group, which site, we suggest, is outside the nerve cells, e.g. on the surface membrane, or at the synaptic terminals. The enzyme must be modifying the local acetylcholine concentration probably by hydrolysing it (see p. 330). Our pharmacological experiments suggest that an alteration in the acetylcholine concentration in this locality can depress transmission processes and inhibit reflex activity.

Our results lead us to the general conclusion that the central action of both the convulsant and depressant anticholinesterases may be due to modification of the acetylcholine concentration at specific regions of the grey matter. The difference between the two groups may depend on the site of their activity in relation to the surface membranes of the nerve cell or its related synaptic terminals. The quaternary lipoid insoluble anticholinesterases may influence the acetylcholine concentration outside these membranes and so produce inhibition. The tertiary anticholinesterases, which give rise to lipoid soluble free base, can also modify the acetylcholine concentration deep to these membranes, and thus facilitate transmission processes or initiate a discharge of motor impulses from the spinal cord. Acetylcholine present in the central nervous system could thus act either as an inhibitory or as an excitatory agent, according to its relative site of action with regard to the nerve cell. Injected acetylcholine acts only as an inhibitory substance because, as a quaternary ammonium compound, it is not lipoid soluble and so acts in the same locality as the quaternary anticholinesterases. Naturally formed acetylcholine might, however, appear in regions to which the artificially introduced substance could not penetrate.

It is not our intention to discuss the mechanism of normal transmission of impulses in the central nervous system of the intact organism. The processes are more complex than in autonomic ganglia, as both central inhibition and excitation can occur. Suffice it to point out that our results suggest that acetylcholine could act both as a central excitatory and central inhibitory agent within the spinal cord, and that the level of activity of anterior horn cells may depend on the relative concentrations of acetylcholine within and without these cells.

Summary

1. The action on the reflex activity of the spinal cord of various anticholinesterases and the derived phenolic bases, after removal of the urethane grouping, was examined in cats under chloralose anaesthesia. The potency of the action on the spinal cord *in vivo* and the inhibitory action on cholinesterase *in vitro* were compared.

2. Eseroline (the phenolic base derived from eserine hydrochloride) has a negligible anticholinesterase action *in vitro* and a very feeble stimulating action on spinal reflexes *in vivo*, approximately $\frac{1}{50}$th that of eserine.

3. Eserine methiodide is a central depressant, in contrast to eserine hydrochloride or sulphate which is a powerful central excitant.

4. Preparation 3393 and miotine methiodide are central depressants.

5. Stedman's "meta"-hydrochloride is a central convulsant, in contrast to the corresponding "meta"-methiodide which is a central depressant.

6. Miotine hydrochloride has a mixed depressant and excitatory action on the spinal cord.

7. Eseroline methiodide (derived from eserine methiodide) has a weak anticholinesterase activity *in vitro*. It has very little action on spinal reflexes.

8. *m*-hydroxyphenyl trimethyl ammonium iodide (derived from either prostigmine or "meta"-methiodide) has a much weaker anticholinesterase activity *in vitro* and a much feebler central inhibitory action *in vivo* than the parent substances.

9. The central excitatory anticholinesterases are tertiary ammonium compounds; the central inhibitory anticholinesterases are quaternary ammonium compounds.

10. It is suggested that the difference in the central action of the tertiary and quaternary ammonium anticholinesterases respectively may be due to differences in their physical properties. The tertiary compounds give rise in solution to lipoid soluble base which may penetrate to regions e.g. the interior of the nerve cell, which the water soluble derivatives of the quaternary compounds in solution cannot reach.

11. There is a close quantitative relationship between anticholinesterase activity *in vitro* and action on the spinal cord *in vivo* in the case of both the tertiary and quaternary substances.

12. Arguments are advanced to support the view that the central action of both the excitatory and depressant anticholinesterases is due wholly or in part to their inhibitory action on cholinesterase in the spinal cord. It follows that variations in the concentration of acetylcholine in various regions of the grey matter of the central nervous system may influence transmission processes, producing either excitation or inhibition.

The expenses of this research have been partially defrayed from grants through the Government Research Committee of the Royal Society and from the Thomas Smythe Hughes Medical Research Fund of the University of London.

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