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ACTION OF CONDENSED ALKYL PHOSPHATES ON THE NERVE-MUSCLE PREPARATION AND THE CENTRAL NERVOUS SYSTEM OF THE CAT

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A preliminary report on the general pharmacological actions of two condensed alkyl phosphates, hexaethyltetraphosphate ('HETP') and tetraethylpyrophosphate ('TEPP') has already been published (Burgen, Keele, Chennells, del Castillo, Floyd, Slome & Wright, 1947); details about the chemical properties of the drugs were communicated by Topley (1947). In this paper we shall describe the action of these drugs on the nerve-muscle response and on certain reflexes in the cat. The actions of the drugs on other systems are fully described by Burgen, Keele & Slome (1949).

The exact constitution of HETP is at present uncertain. It is known to be a mixture of ester phosphates, polyphosphates and perhaps metaphosphates. On solution in water an immediate hydrolysis occurs and it is possible that triethylpyrophosphate is formed and is the physiologically active molecule. A further slow hydrolysis occurs forming equimolecular proportions of monoethyl phosphoric and diethyl phosphoric acids, which are physiologically inert. Other workers (Hall & Jacobson, 1948) have suggested that the biological activity of HETP is due to a content of 20% TEPP.

METHODS

Cats were used under chloralose anaesthesia (0·08 g./kg. body weight) or after decerebration at the mid-collicular level; in the latter case at least 1 hr. was allowed to elapse to enable the effects of the initial chloroform—ether anaesthesia to wear off. In some experiments the chloralosed or decerebrate cat was subsequently made spinal by a trans-section in the mid-thoracic region. Nerve-muscle and reflex responses were elicited and recorded as described below after appropriate fixation of the limb by means of drills. For nerve stimulation Collison electrodes were sometimes used, but in most experiments open electrodes of silver wire mounted in perspex were employed.

Nerve muscle. The femoral nerve-quadriceps preparation was employed using both mechanical (torsion lever) and electrical recording. Muscle-action potentials were picked up by belly-tendon leads and amplified by a resistance-capacity coupled push-pull amplifier. The action potentials were displayed on a 40 msec. sweep on the cathode ray oscilloscope and photographed so that each sweep appears as an oblique line across the photographic record, with the action potential deflexions parallel to the direction of movement of the paper. An electronic stimulator was used with an air cored coil, giving supra-maximal stimuli which were applied to the motor nerve at rates between 1 in 10 sec. and 100 pulses per sec.

Flexor reflex. Mechanical recording (torsion lever) from the anterior tibial muscle of one side was employed. The central end of the cut ipsilateral popliteal nerve was stimulated with a second electronic stimulator of the type referred to above.

Knee jerk. This was recorded mechanically with a torsion lever in response to the stimulus applied by an automatic knee jerk hammer (Schweitzer & Wright, 1937*a*) which struck the patellar tendon at regular intervals (once in 10 sec.).

Crossed extensor reflex. Movements of the distal part of the leg employed for the knee jerk were recorded in response to stimulation of the central end of the contralateral popliteal nerve as described for the flexor reflex (see above).

Other responses were studied occasionally, e.g. ipsilateral reflex response of m. peroneus longus, or reflex response of various muscles to table-banging.

The drugs used were injected intravenously (into the central end of the jugular vein), intra-arterially (into the central end of the inferior mesenteric or iliac arteries (Wilson & Wright, 1936–7), or intrathecally (Calma & Wright, 1947) approximately at the level of the seventh lumbar vertebra. The HETP and TEPP were diluted with saline just before being injected; in some experiments the dilutions were made from the original liquid, and in others from a 1% stock solution of the drug in propylene glycol.

RESULTS

In the cat, HETP and TEPP have, generally speaking, qualitatively similar effects as judged by the responses of the nerve muscle preparation and the reflexes; TEPP is, however, about five to ten times as powerful as HETP. The effects make their appearance after a latency which varies inversely with the dose employed. With large doses the maximal effects appear within a few seconds to a few minutes; with threshold doses the delay may be up to 5–10 min. The drugs initially potentiate the nerve-muscle response and enhance the reflexes; with larger doses depression usually develops. The effect on the reflexes is in part due to a direct action on the central nervous system (spinal cord), for intrathecal injections modify the reflexes at a time when the drug has produced no changes in peripheral structures.

Action on the nerve-muscle preparation

Response to single supramaximal motor-nerve stimuli. Typical mechanical responses of the femoral nerve-quadriceps preparation in the cat under chloralose anaesthesia are shown in Figs. 1 and 2. Records on the slow drum illustrate changes in contraction tension. Records on the fast drum show the changes in the shape of the contraction curve. In Fig. 1, ½–1 min. after an intravenous injection of 1 mg. of HETP, there was an increase in the tension of the muscle response which rose to a maximum of 40% above the control level within 2 min. The tension was still 20% above the control level after 24 min. The fast records show a considerable prolongation of the response. Very slight fibrillar twitching developed. A second intravenous injection of 1 mg. of HETP produced a trivial further increase in contraction tension and an increase in the size and extent of the fibrillar twitching which was soon followed by a marked depression in the height of the nerve-muscle response. Some recovery took place after 15 min. but the tension was then still below the control level.

A further dose of 5 mg. of HETP injected 70 min. after the first and 46 min. after the second injection produced a profound and lasting depression of the response accompanied by an intensification of the twitching.

Fig. 2 illustrates similar changes produced by TEPP. In this experiment, at the peak of the response, the duration of the muscle curve was 2 sec.

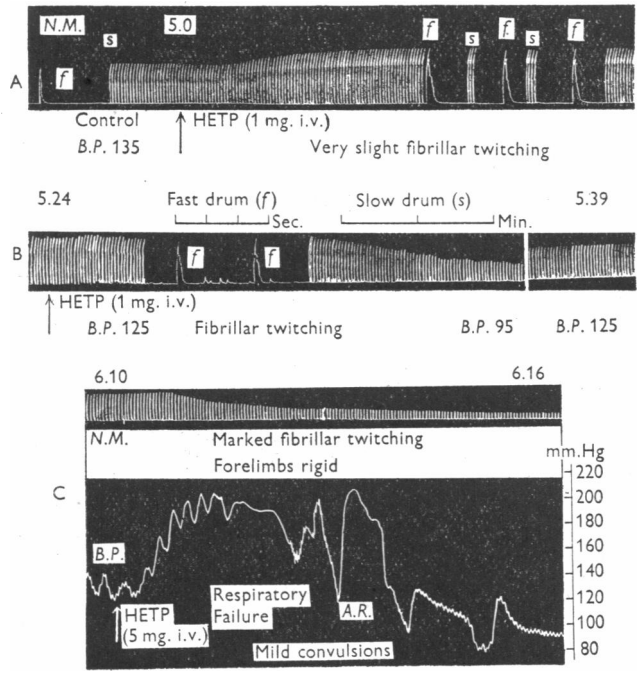

Fig. 1. Cat. 2·3 kg. Chloralose. A, B. Isometric myogram of quadriceps muscle, in response to femoral nerve stimulation at rate of 1 in 2 sec. Records are taken on a slow drum except when labelled *f* (=fast drum). In A when the drum was alternately run fast and slow, the slow records are labelled *s*. Time scales for fast drum and slow drum shown in B. C. Records from above downwards: contraction of quadriceps (as in A, B); arterial blood pressure (*B.P.*). Respiratory failure developed in C and at *A.R.* artificial respiration was applied. Injections of HETP given as signalled by arrows. (See text.)

Electrical studies show that the increase in the tension, and the delayed relaxation of the muscular response to a single stimulus applied to the motor nerve, are due to repetitive asynchronous firing of the muscle fibres. This response can be attributed to the persistence of the acetylcholine transmitter at the motor end-plates owing to the anti-cholinesterase action of the condensed alkyl phosphates. In other words, the muscle response to a single nerve stimulus is now not a twitch but an irregular diminishing tetanus. Close intra-arterial injection of acetyl choline likewise produces not a twitch but a brief asynchronous tetanus (Brown, Dale & Feldberg, 1936; Brown, 1937*a*).

With belly-tendon leads (hypodermic needles introduced into the muscle) the muscle action potential recorded by us in response to a single maximal nerve volley resembled the somewhat irregular diphasic wave, sometimes of complex character, obtained by Eccles & O'Connor (1939) from the soleus and anterior tibial muscles of the cat. The degree of complexity of the diphasic wave can be modified by adjustment of the position of the belly lead, and we have always moved the needle so as to give as simple a diphasic wave pattern as possible. Under these latter conditions the total duration of the action potential is 15–20 msec. We shall call the two phases of the 'elementary'

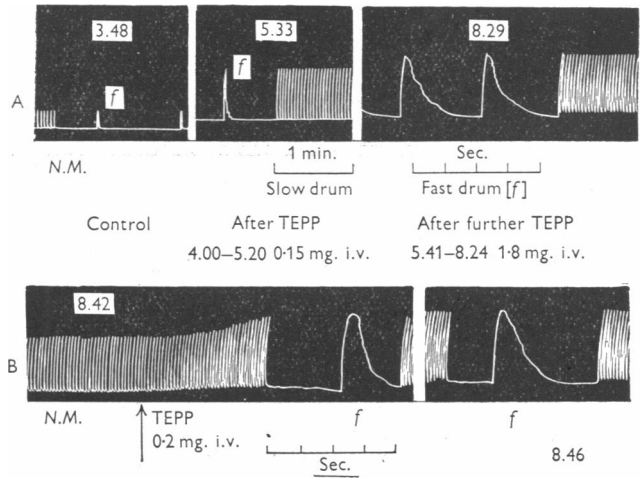

Fig. 2. Cat. 2·3 kg. Chloralose. A, B. Isometric myogram of quadriceps muscle in response to stimulation of femoral nerve at rate of 1 in 2 sec. Records on slow drum unless indicated by *f* (=fast drum); time scales in A and B. Effect of intravenous injection of TEPP. (See text.)

diphasic wave the first and second phase respectively. It is not always possible, however, to find a position of the electrode in which the whole action potential is complete in less than 20 msec., and the return stroke of the second phase of the action potential wave may be prolonged.

The first changes observed in the muscle action potential produced by HETP and TEPP are successively: (i) prolongation of the descending limb of the second phase; (ii) appearance of slight irregularities in this prolonged limb; (iii) these irregularities gradually become larger and take the form of repetitive waves; (iv) at this stage the descending limb of the second phase (of the initial diphasic wave) descends more rapidly so that the total duration of the initial diphasic variation may be shorter than in the control records.

In Fig. 3 are seen the muscle (belly-tendon) action potentials set up in response to a single maximal motor nerve volley in an experiment in which the total dose of HETP used was 20 mg. (intravenously). The first two sweeps at 12.50 and

3.57 p.m. show almost identical control records consisting only of a slightly irregular diphasic wave. After the injection of 5 mg. of HETP during 20 min. the electrical response (4.13 p.m.) showed what might be called 'peripheral afterdischarge', i.e. small high-frequency waves following on an initial diphasic wave which may be of control magnitude and duration or slightly shortened in duration as mentioned above; the peripheral afterdischarge lasted for about 150 msec. At 5.59 p.m., the initial diphasic response is followed by two larger

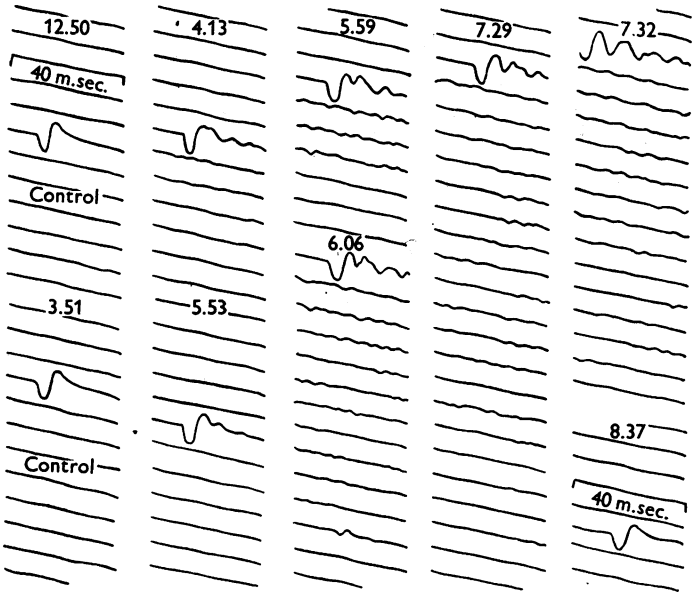

Fig. 3. Cat. 4·3 kg. Chloralose. Action potentials (belly-tendon leads) of quadriceps muscle in response to femoral nerve stimulation at rate 1 in 10 sec., showing successive stages in the development of peripheral afterdischarge, and its decline, due to the action of HETP. Each record reads from above downwards and from left to right. One sweep = 40 msec. 12.50 and 3.51 p.m., controls. HETP was then given as follows: 3.53–4.13 p.m., 5 mg. by intravenous infusion; 4.30 p.m., 1 mg. i.v.; 5.54 p.m., 1 mg. i.v.; 6.03 p.m., 1 mg. i.v.; 7.28 p.m., 1 mg. i.v.; 7.53–8.35 p.m., 11 mg. i.v. in divided doses. The figures heading each record are the times at which the records were taken. (See text.)

waves doubtless representing the synchronous firing of many muscle fibres. A further 1 mg. of HETP was injected at 6.03 p.m.; the maximum effect of this dose appeared at 6.06 p.m. The two secondary waves were now almost as large as the initial diphasic response; the small wave discharge lasted about 500 msec. Towards the end of the response silent periods became more numerous and longer in duration. The discharge thus develops a very irregular appearance. At 7.29 p.m. after another dose of 1 mg. the repetitive electrical response persisted for about 800 msec. By 7.32 p.m. the duration of the response was beginning to decline. Further doses totalling 8·5 mg. produced progressive

extinction of the peripheral afterdischarge and at 8.37 p.m. the action potential had been reduced to a simple diphasic wave without repetition and with an amplitude only 80% of the original control responses.

In Fig. 4 the mechanical and electrical responses are compared before and after the injection of 2·5 mg. of HETP over 80 min. in the decerebrate cat. The prolongation of relaxation in the mechanical record is evidently due to the repetitive firing of the muscle fibres which is clearly seen in the electrical record.

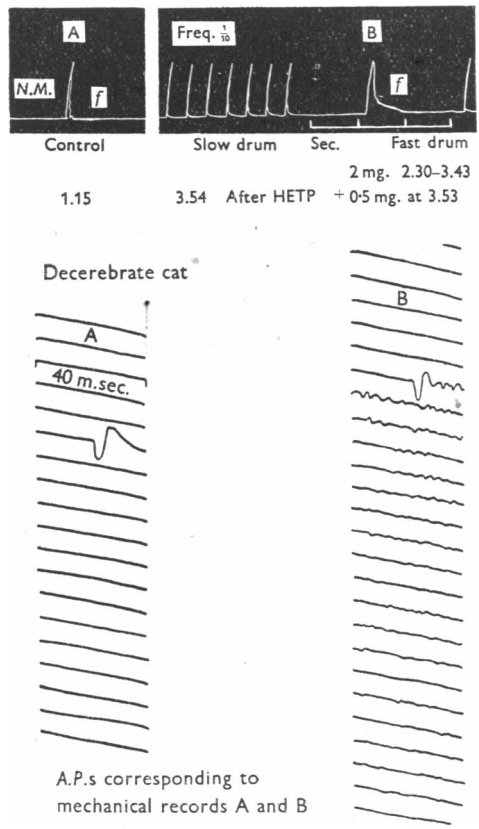

Fig. 4. Cat. 3·8 kg. Decerebrate. Upper records: isometric myogram of quadriceps (*N.M.*) in response to femoral nerve stimulation at rate 1 in 10 sec. Lower records: action potentials (*A.P.*) of same muscle—using belly-tendon leads. A, controls; B, showing effects of intravenous injection of 2·5 mg. HETP in divided doses. (See text.)

We should mention that in conformity with the results of Eccles & O'Connor (1939) we have sometimes observed in the decerebrate cat (but not under chloralose anaesthesia) repetitive action potentials in response to single maximal nerve volleys as a variation of the normal pattern. The repetition

produced by the drugs is, however, more marked and more prolonged than that ever noted in untreated decerebrated animals.

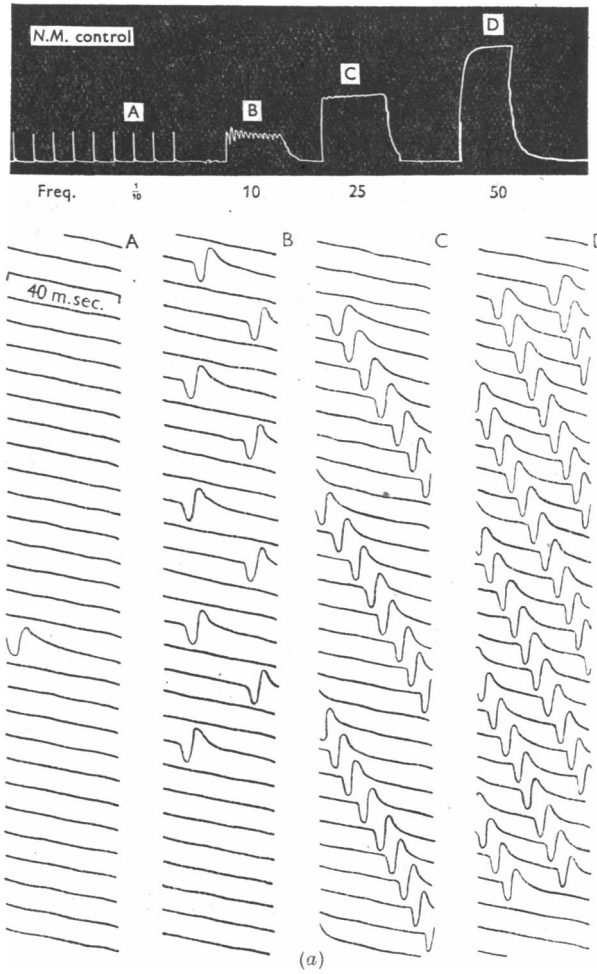

Fig. 5. Cat. 4·3 kg. Chloralose. Upper records: isometric myogram of quadriceps (*N.M.*) in response to femoral nerve stimulation at nominal rates: A, 1 in 10 sec.; B, 10; C, 25; and D, 50 per sec. Lower records: corresponding muscle action potentials. (*a*) Controls. (*b*) (See p. 382.) Showing effects of HETP after the following doses: 3.53–4.13 p.m., 5 mg. by intravenous infusion; 4.30 p.m., 1 mg. i.v.; 5.54 p.m., 1 mg. i.v.; 6.03 p.m., 1 mg. i.v. Records taken at 6.07 p.m. (See text.)

Response to supramaximal repetitive motor-nerve stimulation. The muscle responses to repetitive supramaximal stimuli at frequencies ranging from 1 in 10 sec. to 50 per sec. were studied. Fig. 5*a* shows a control series and Fig. 5*b* the responses of the muscle after the administration of 8 mg. of HETP over

a period of 2 hr. The mechanical response to stimulation at once in 10 sec. is recorded on a slow drum; the response to higher rates of stimulation (10, 25, 50 per sec.) on a fast drum.

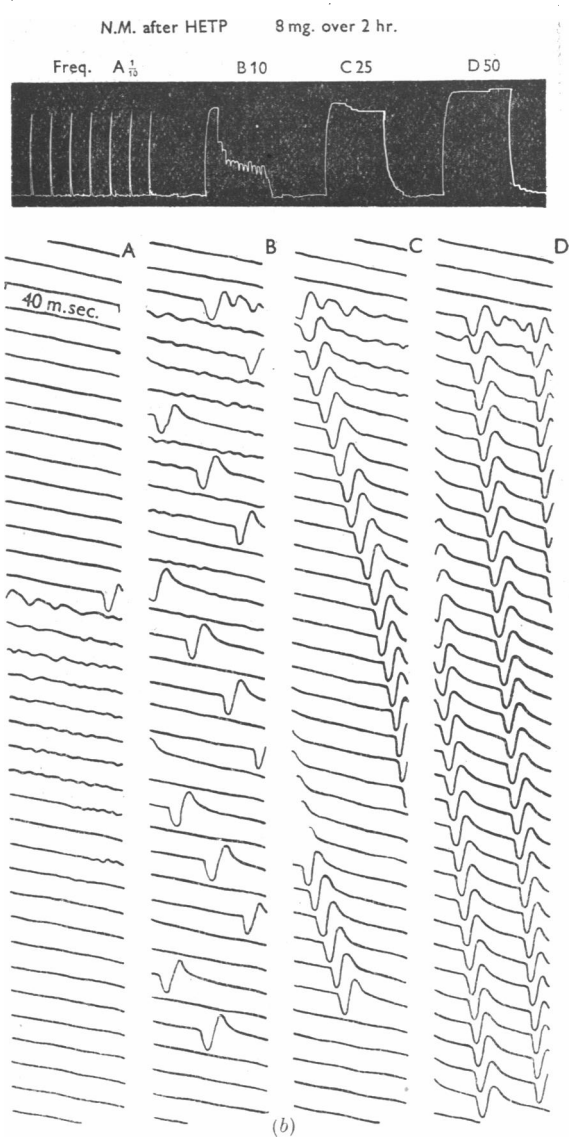

Fig. 5. For legend see p. 381.

After HETP the mechanical responses were modified as follows. Single twitch responses (1 in 10 sec.) showed in this experiment an increase of 160% in the tension. At stimulus frequency 10 per sec. the response showed a greater

initial tension than in the control but the tension rapidly declined, as stimulation was continued, to the same level as in the control curve. At stimulus frequency 25 per sec. the initial tension was some 40% greater than in the control: it declined slightly, whereas the tension of the control rose slightly over the same period of stimulation. The final tension was 20% greater than the final tension in the control. At stimulus frequency 50 per sec. the tension was diminished by about 10% throughout. Our general experience has been that the mechanical responses to higher stimulus frequencies are less affected by HETP than those to lower frequencies. With doses of HETP which produce enhancement of the single twitch response (frequency 1 in 10 sec.) the mechanical response to frequencies 10 and 25 per sec. is at times increased and at others diminished.

The electrical records in the control experiments at all stimulus frequencies show diphasic responses which faithfully follow in frequency the rate of nerve stimulation; with the higher stimulus rates there is a slight progressive reduction in the duration of individual diphasic responses, but there is no decrease in response amplitude and no peripheral afterdischarge occurs.

After HETP the electrical responses were modified as follows. At stimulus frequency 1 in 10 sec. the muscle-action potential showed some 500 msec. of repetition like that described above. The electrical responses to the more rapid rates of stimulation show characteristically rapid extinction of the peripheral afterdischarge. Thus at stimulus frequency 10 per sec., associated with the initial potentiation of tension, the first responses show peripheral afterdischarge. In the first electrical response there are several large secondary waves after the initial diphasic response, followed by small-amplitude high-frequency waves which fill the entire stimulus interval; the large secondary waves are no longer present in the second electrical response though the smaller waves still appear; in the eighth and subsequent responses no peripheral afterdischarge is present. At the higher stimulus rates (25, 50 per sec.) a similar train of events occurred. The first electrical response shows large secondary waves only. In the second, third and fourth responses the large secondary waves are absent, but the interval is filled with small-amplitude waves. In subsequent responses the peripheral afterdischarge completely disappears and the simple diphasic responses to each stimulus closely resemble those obtained in the control period.

Anti-curari action of HETP. The anti-curari action of HETP demonstrated by Burgen *et al.* (1949) on the rat-isolated phrenic nerve-diaphragm preparation can readily be shown in cat muscle receiving its normal blood supply. In Fig. 6 supramaximal stimuli were applied to the motor nerve at a rate of 1 in 2 sec. Intocostrin (Squibb) was used for curarization and was injected intra-arterially (into the central end of the opposite iliac artery with the medial sacral artery tied) in two doses totalling 0·6 rabbit head-drop units. The mechanical response rapidly declined to a minimum representing 10% of the control tension; the

peak depression lasted about 5 min.; recovery then set in and was complete in a further 10–12 min. A second curarizing dose was given which produced the same initial depressing effect as before; 2 mg. of HETP were injected intravenously when the depression was at its maximum. Recovery set in immediately, developed rapidly and was almost complete in under 3 min.

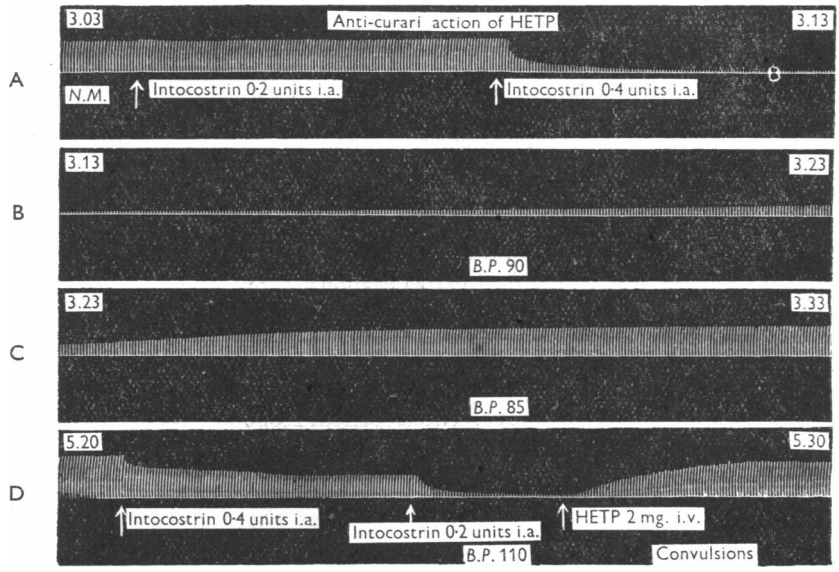

Fig. 6. Cat. 1·9 kg. Chloralose. Isometric myogram of quadriceps (*N.M.*) in response to femoral nerve stimulation at rate 1 in 2 sec. Records (A, B, C, D) read from left to right and from above downwards. Time is shown above records. In A: inject 0·2, 0·4 units of Intocostrin intra-arterially. In D: inject successively 0·4, 0·2 units of Intocostrin intra-arterially, 2 mg. of HETP intravenously. (See text.)

Action of curari on peripheral afterdischarge produced by HETP. Suitable doses of curari abolish the peripheral afterdischarge produced by HETP. Fig. 7*a* shows a control muscle action potential in response to a single maximal nerve volley. After intravenous injection of 4·75 mg. of HETP in divided doses characteristic peripheral afterdischarge, lasting about 40 msec., developed (Fig. 7*b*). One minute after an intra-arterial injection of Intocostrin (Squibb) partial extinction of the afterdischarge occurred (Fig. 7*c*). After further doses of Intocostrin the afterdischarge declined further and was finally extinguished (Fig. 7*d*, *e*), the action potential now closely resembling the control record. Partial recovery occurred as the effect of the curari wore off (Fig. 7*f*, *g*). Intravenous injection of further doses of HETP totalling 4·75 mg. caused a return and progressive increase of the peripheral afterdischarge (Fig. 7*h*, *i*). In Fig. 7*i* the afterdischarge lasted about 110 msec. It was again extinguished

by a further dose of Intocostrin (Fig. 7*j*), and the action potential once more resembled the original control. The condensed alkyl phosphates thus not only have an anti-curari action, but their action on muscle is likewise antagonized by curari.

Effect of HETP on denervated muscle. In three cats the femoral nerve on one side was cut and the animals allowed to survive for 2–5 weeks. Under chloralose anaesthesia, using supramaximal stimulation, the twitch responses of the normal quadriceps muscle, stimulated through its nerve, and of the chronically

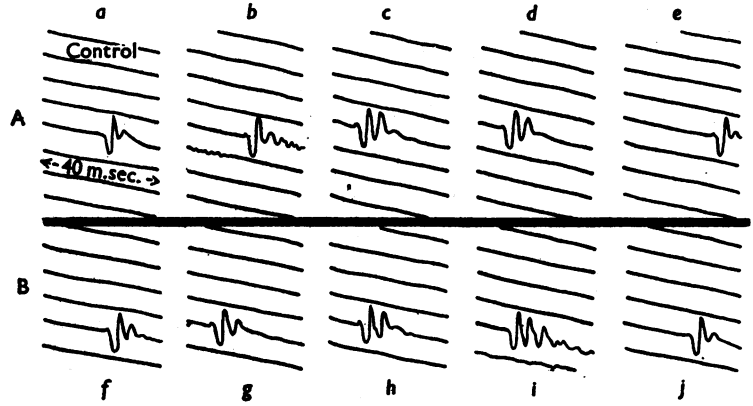

Fig. 7. Cat. 3·8 kg. Chloralose. Action potentials (belly-tendon leads) of quadriceps muscle in response to femoral nerve stimulation at rate 1 in 5 sec. A (*a*) control 4.33 p.m.; (*b*) 5.21 p.m. after injection of 4·75 mg. of HETP in divided doses; (*c*) 5.23 p.m. 1 min. after intra-arterial injection of 1 rabbit head-drop unit of Intocostrin (Squibb); (*d*) 5.25 p.m. after further dose of 0·5 unit of Intocostrin; (*e*) 5.29 p.m. after further dose of 1 unit of Intocostrin. B. (*f*) 5.42 p.m. and (*g*) 6.02 p.m. partial recovery; (*h*) 6.56 p.m. and (*i*) 7.01 p.m. after injection of 4·75 mg. of HETP in divided doses; (*j*) 7.04 p.m. after injection of 1 unit of Intocostrin. (See text.)

denervated muscle, stimulated directly, were compared before and after injection of suitable doses of HETP. The normal muscle after HETP showed characteristic peripheral afterdischarges: in the chronically denervated muscle no repetitive action potentials were observed.

Action on reflexes

Intact animal under chloralose anaesthesia. Fig. 8 shows the characteristic changes in the reflexes produced by HETP. In the experiment illustrated the flexor reflex was recorded in one hindlimb and the knee jerk in the other. After intravenous injection of 3·5 mg. of HETP in divided doses in 90 min. the flexor reflex remained unaffected, but the extensor reflexes were facilitated; the height of the knee jerk was about doubled and the crossed extensor reflex, which was initially absent, made its appearance. A further injection of 0·4 mg. produced a significant increase in the flexor reflex. The stimulus for the knee

jerk was discontinued to enable the crossed extensor in that limb to be studied in an uncomplicated manner. The crossed extensor reflex was now found to be still further enhanced and spontaneous extension movements of the limb began to appear. A further dose of 1 mg. produced more violent 'spontaneous' movements sufficiently general to merit the appellation of 'convulsions'. The 'convulsions' and the crossed extensor responses in the limb became so intermingled that they could no longer be readily differentiated. The flexor responses,

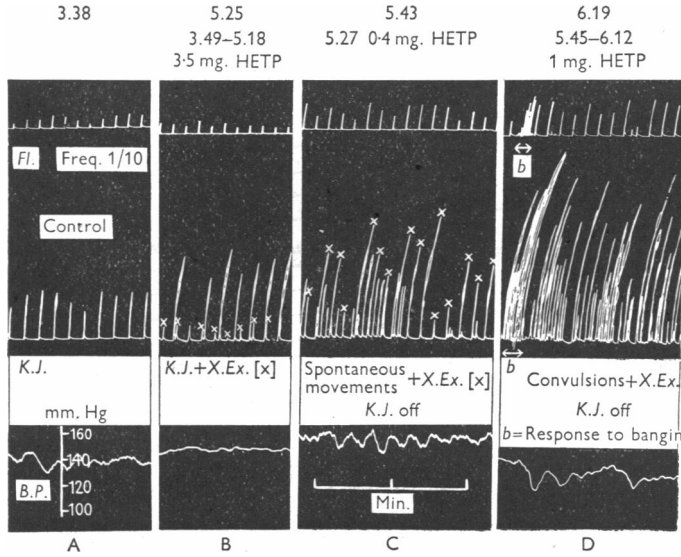

Fig. 8. Cat. 5·2 kg. Chloralose. Upper record: isometric recording of flexor reflex (*Fl.*) (anterior tibial muscle) in response to ipsilateral popliteal nerve stimulation (central end) at rate of 1 in 10 sec. Middle record: knee jerk (*K.J.*) and crossed extensor reflex (*X.Ex.*), marked with a × in B and C in response to contralateral popliteal stimulation at 1 in 10 sec. Lower record: blood pressure (*B.P.*). The knee jerk was not elicited in C and D. The records show the potentiating action of HETP on *Fl.* and *K.J.* responses and on genesis of *X.Ex.* response, responses to table banging (during period marked *b*) and spontaneous activity. (See text.)

unlike the extensor, showed little further potentiation. The stimulus of table-banging, which was without effect during the control period, produced at this stage regular reflex responses not less notably in the flexor muscles than in the extensor.

As the level of arterial blood pressure changed very little throughout the experiment the reflex changes described are entirely independent of alterations in the circulation.

Small doses of the drug have been noted to stimulate respiration and larger doses to cause respiratory failure. This, when remedied by artificial respiration, was not accompanied by circulatory collapse. From previous work (Schweitzer & Wright, 1937*a*) it is clear that these changes in breathing do not affect

somatic reflexes. Terminally, the blood pressure may fall to a very low level; the reflexes then tend to decline or disappear as a secondary effect.

The question must be carefully considered as to what extent the changes recorded in the reflexes are due to the demonstrated changes in the nerve-muscle responses and to what extent to an action on the central nervous system. It will be proved later, using the technique of intrathecal injection, that the condensed alkyl phosphates have a direct stimulating action on the nerve centres (spinal cord). But at this stage some suggestive evidence may be mentioned: (1) there is no quantitative relationship between the magnitude of the potentiation of the nerve-muscle response and of the reflexes. (2) The effect

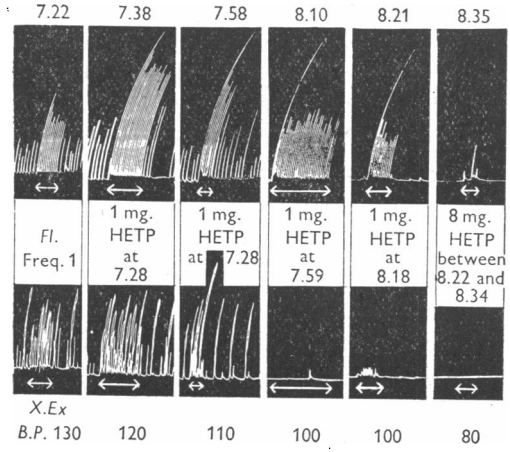

Fig. 9. Cat. 4·3 kg. Chloralose. Upper record: isometric recording of flexor reflex (*Fl.*) (anterior tibial muscle) in response to ipsilateral popliteal nerve stimulation (central end) at rate of 1 per sec. Lower record: crossed extensor response (*X.Ex.*) to same stimulation. Period of stimulation indicated by double arrow heads. Blood pressure (*B.P.*) in mm. Hg. The records show effect of HETP on these reflexes and on the background of spontaneous activity initially present. (See text.)

on the nerve muscle and on the reflexes do not necessarily follow the same temporal course; e.g. after the nerve-muscle potentiation has reached its peak the reflex responses may go on growing in size, or depression of one may coincide with enhancement of the other. (3) The changes in different reflexes are not quantitatively similar (Fig. 8). (4) The appearance of reflexes that were initially absent, and especially the onset of 'spontaneous' convulsions, can only be due to central effects of the drug.

Fig. 9 illustrates some further points. The animal had received intravenously 8 mg. of HETP in divided doses over a period of 2 hr. One hour had elapsed after the last injection before the first record in this figure was taken. The record opens with a background of fairly regular 'spontaneous' contractions

in both flexor and extensor muscles. Stimulation of the central end of the popliteal nerve at a frequency of 1 per sec. elicited an ipsilateral flexor reflex and a contralateral extensor reflex. The initial and the immediately following flexor response in any one series of stimuli were considerably larger than the later responses. Another 1 mg. of HETP was injected intravenously; 10 min. later the 'spontaneous' movements in the flexor muscles were enhanced; the flexor reflex elicited by ipsilateral popliteal nerve stimulation was markedly increased (by 100%) both as regards the large initial contractions and the succeeding somewhat smaller reactions. The crossed extensor reflex showed a smaller increase. Further injections of HETP led to depression of all the reactions, the 'spontaneous' movements and crossed extensor reflex disappearing first; at this stage the flexor reflex began to decline. It was almost completely abolished after further injections totalling 9 mg. In this experiment the arterial blood pressure fell from over 130 to 80 mm. Hg, but the latter level is of course quite adequate to maintain normal reflex reactions.

In Fig. 10 the effect of TEPP on a number of reflexes was studied simultaneously, namely reflex responses of peroneus longus (an evertor) and tibialis anticus (a flexor), elicited by stimulation of the central end of the ipsilateral nerve, and the crossed extensor reflex of the quadriceps of the opposite side. The effects of different frequencies of afferent stimulation were specially examined. The insets *a*, *b*, *c*, in Fig. 10 show the reflex responses of the muscles mentioned to short periods of stimulation at frequencies of 1 in 2 sec. and 1, 2, 4, 10, 25 and 50 per sec.; peroneus longus gave small responses at all stimulus frequencies up to 25 per sec., reflex fusion occurring at 25 per sec.; there was no response at 50 per sec. Tibialis anticus responded somewhat differently; a contraction followed each stimulus at 1, 2 and 4 per sec., there was a single initial contraction in response to 10 per sec., the succeeding volleys arousing no reaction, and there was no response to the higher stimulus frequencies of 25 and 50 per sec. In chloralosed cats we have frequently noted this lack of response or rapid dying down of the response in the flexor reflex at these stimulus frequencies. The control crossed extensor reflex only responded to stimulation at 4 and 10 per sec.; at 4 per sec. the response was tremulous, at 10 per sec. there was a small initial response only. Less than 1 min. after an intravenous injection of 0·05 mg. of TEPP the crossed extensor reflex, initially absent to stimulation at 1 in 2 sec., made its appearance and progressively increased in amplitude. Extensor tone also increased. In the case of the peroneus longus reflex, at each frequency at which responses had previously been elicited, larger responses were obtained; stimulus frequency 50 per sec. which was previously ineffective produced a tetanic response maintained somewhat below the initial peak value. With the tibialis anticus reflex, the previously ineffective rates of 25 and 50 per sec. now produced responses and the response to 10 per sec. was repetitive instead of initial only. The changes were most dramatic with the

crossed extensor reflex. Stimulus frequency of 1 in 2 sec. which in the control period was ineffective now gave large regular responses to each volley, the peak tension rising as stimulation was continued, representing recruitment; relaxation was incomplete between the volleys, representing central afterdischarge;

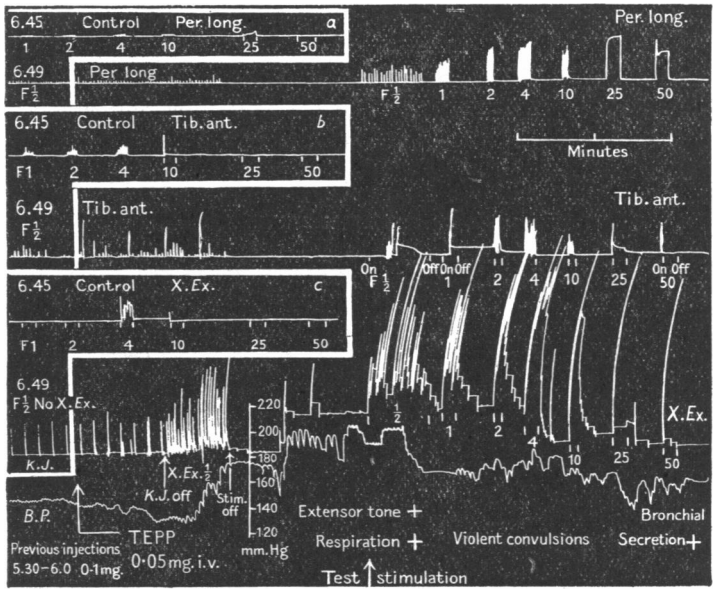

Fig. 10. Cat. 3·7 kg. Chloralose. Inset *a*, *b*, *c* are control responses: *a* of m. peroneus longus (per. long.); *b* of m. tibialis anticus (tib.ant.) to ipsilateral, and *c* of m. quadriceps (crossed extensor reflex–*X.Ex.*) to contralateral, popliteal nerve stimulation at rates of 1, 2, 4, 10, 25, 50 per sec. (F1–F50) and 1 in 2 sec. (F½). The lower part of inset *c* shows the knee jerk elicited by stimulating at 1 in 12 sec., as well as absence of *X.Ex.* to F½. The main records from above downwards: response of m. peroneus longus; of m. tibialis anticus; of m. quadriceps; blood pressure (*B.P.*). At arrow inject 0·05 mg. of TEPP intravenously (after previous injection of 0·1 mg.). When the crossed extensor reflex began to appear on the quadriceps record the knee jerk hammer was switched off (second arrow). The quadriceps record now shows the crossed extensor reflex only. At third arrow all nerve stimulation was temporarily stopped and the drum was run for 2 min. At arrow labelled 'Test stimulation' the responses of m. peroneus longus, m. tibialis anticus and m. quadriceps were tested to bursts of stimuli (duration indicated by 'on','off') at 1 in 2 sec., 1, 2, 4, 10, 25 and 50 sec. (See text.)

towards the end of the bout of stimulation the responses declined, but some central afterdischarge was still noticeable on cessation of stimulation. At 1 per sec., relaxation between the volleys was even less complete and there was considerable central afterdischarge. At 2 and 4 per sec. more complete reflex fusion was occurring, while at 10 per sec., reflex fusion was complete at a level somewhat below the opening contraction. Stimuli of 25 and 50 per sec. only produced initial responses.

It is interesting to note that the detailed changes in the two ipsilateral reflexes studied were not identical, illustrating the danger of generalizing about the central action of these drugs from a study of too few reflex types. The crossed extensor reflex brings out well some features not so well shown by the flexors, namely, how the drug facilitates recruitment and central afterdischarge, as well as facilitating responses previously not elicitable. Presumably reflex fields previously subliminally stimulated are brought up to discharge level of central excitation. The results described were accompanied by a rise and subsequent decline of blood pressure and stimulation of breathing, but not to an extent that might be expected to modify the reflex reactions significantly (Schweitzer & Wright, 1937*a*).

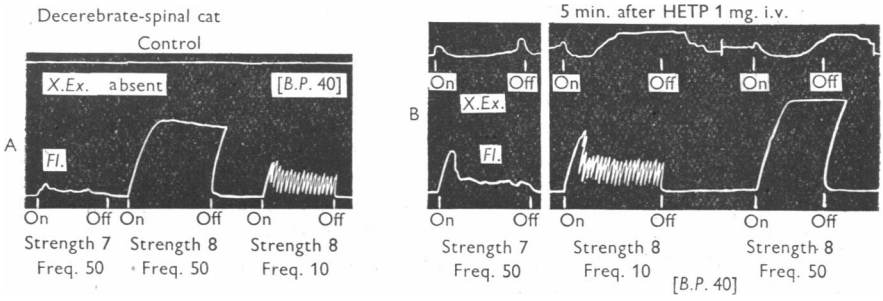

Fig. 11. Cat. 3·0 kg. Decerebrate-spinal. A, B. Lower record: isometric recording of flexor reflex (*Fl.*) (anterior tibial muscle) in response to ipsilateral popliteal nerve stimulation. Upper record: crossed extensor response (*X.Ex.*) to same stimulation. A, control records—three bursts of stimulation were employed at strengths (arbitrary units) and frequencies indicated. B, action of HETP in potentiating flexor and facilitating crossed extensor responses. (See text.)

Decerebrate animal made spinal. To eliminate the action of the chloralose anaesthesia and the influence of supraspinal levels, studies were carried out in decerebrate animals made spinal. In the experiment illustrated in Fig. 11 stimulation was carried out at various strengths (expressed in arbitrary units) and frequencies. The crossed extensor reflex could not be elicited at any of the strengths or frequencies employed. The flexor reflex showed a feeble irregular response at strength 7, frequency 50; a powerful well-sustained tetanus at strength 8, frequency 50; and a weaker repetitive response to stimulation at strength 8, frequency 10. Five minutes after the injection of 1 mg. of HETP the crossed extensor reflex appeared in a complex form with a well-marked afterdischarge. Both responses at strength 8 showed evidence of an inhibitory component in the afferent nerve which was gradually overcome by afferent excitation. The potentiation of the flexor reflex was not so dramatic, but at strength 7, frequency 50, the opening reflex contraction was markedly increased and it was better sustained; at strength 8, frequency 10, the opening response was doubled; and at strength 8, frequency 50, it was increased by 15%.

Intrathecal injection of alkyl phosphates

Conclusive evidence about the central action of the alkyl phosphates is provided by experiments in which injections were made intrathecally (Calma & Wright, 1947) generally at the level of the seventh lumbar vertebra. In the experiment illustrated by Figs. 12 and 13 mechanical and electrical records* of the nerve-muscle responses showed no change whatever, demonstrating that the drug had not entered the general circulation in significant doses. The blood pressure and respiration likewise remained unaltered till nearly the end of the experiment. The reflexes studied were the knee jerk, flexor reflex and crossed extensor reflex, the two latter in response to bursts of repetitive stimulation at frequencies of 1 in 10 sec. and 1, 2, 5, 10, 25, 50 and 100 per sec. The controls (Fig. 12*a*) showed very small flexor responses at stimulus frequency of 1 and 2 per sec.; fused responses (sometimes with rising tension) at 5 and 10 per sec.; a fused response rising rapidly to a peak and then declining, at 25 per sec.; a similar initial reaction but with rapid decline of tension almost to zero in spite of persistent stimulation, at 50 and 100 per sec. The crossed extensor could not be elicited. Control intrathecal injections of propylene glycol gave the following results: 0·1 c.c. of 1% propylene glycol in saline was without effect; 0·1 c.c. of 10% propylene glycol in saline gave a sharp rise of blood pressure which declined slowly and a very transient increase in the knee jerk; 0·1 c.c. of 100% propylene glycol, which produced a bigger and more rapid rise of blood pressure with a still slower recovery, temporarily depressed the knee jerk on the first injection and produced transient extensor spasm after the second injection. There were no persistent effects. The doses of TEPP used were such that, on dilution with saline from a 1% solution in propylene glycol, the vehicle for injection was a solution of propylene glycol ranging from 1 to 10%, usually 5%. It can be safely concluded that the changes in the reflexes, described below as following the intrathecal injection of TEPP, are due to the drug and not to the vehicle.

Intrathecal injection of 0·01 mg. of TEPP produced a transient rise of blood pressure but no changes in the somatic reflexes. Repeated injections of 0·05 mg. of TEPP amounting in all to 0·4 mg. over 135 min. produced the changes illustrated in Figs. 12 and 13.

Knee jerk (Fig. 13*a*). There is a progressive increase in the tension of the response, delayed relaxation and the development of secondary contractions. The increased tension can be attributed to central facilitation, the latter changes to enhanced and prolonged afterdischarge.

Flexor reflex. The initial change was an increased response to stimulus frequencies 1 and 2 per sec. (Fig. 12*b*). Later the level or descending plateau

* The action potentials were recorded with both high and low amplification, the former giving overloading on the initial diphasic response, but enabling easier detection of the onset of repetition.

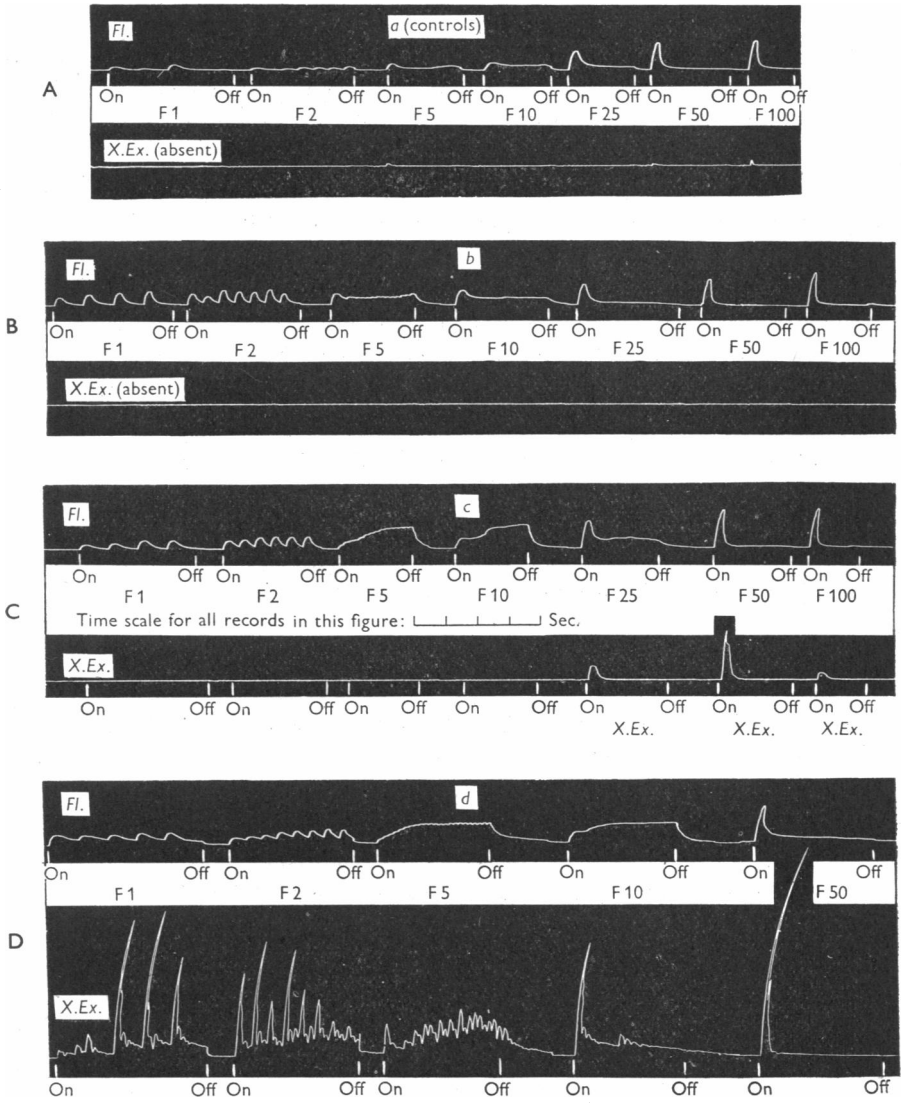

Fig. 12. Cat. 2·8 kg. Chloralose. A, B, C, D. Upper record: flexor reflex (*Fl.*) (anterior tibial muscle) to ipsilateral stimulation; lower record: crossed extensor reflex (*X.Ex.*) of quadriceps muscle to contralateral stimulation, of central popliteal nerve at rates of 1, 2, 5, 10, 25, 50 and 100 per sec. for periods indicated by 'on', 'off'. A. Control records—crossed extensor response absent. B. 6.57 p.m. after total of 0·12 mg. of TEPP injected *intrathecally* in divided doses. Crossed extensor reflex still absent. C. 7.52 p.m. after further 0·1 mg. of TEPP *intrathecally* in two doses. D. 8.40 p.m. after further 0·2 mg. of TEPP *intrathecally* in divided doses. (See text.)

of the fused response to stimulus frequencies 5 and 10 per sec. was converted into a smoothly or irregularly rising tension attaining a greater maximum than previously. The response to 25 per sec. showed a slightly enhanced opening contraction which was somewhat better sustained throughout the period of afferent stimulation than before (Fig. 12*c*). The responses to higher frequencies were slightly increased but otherwise unaltered.

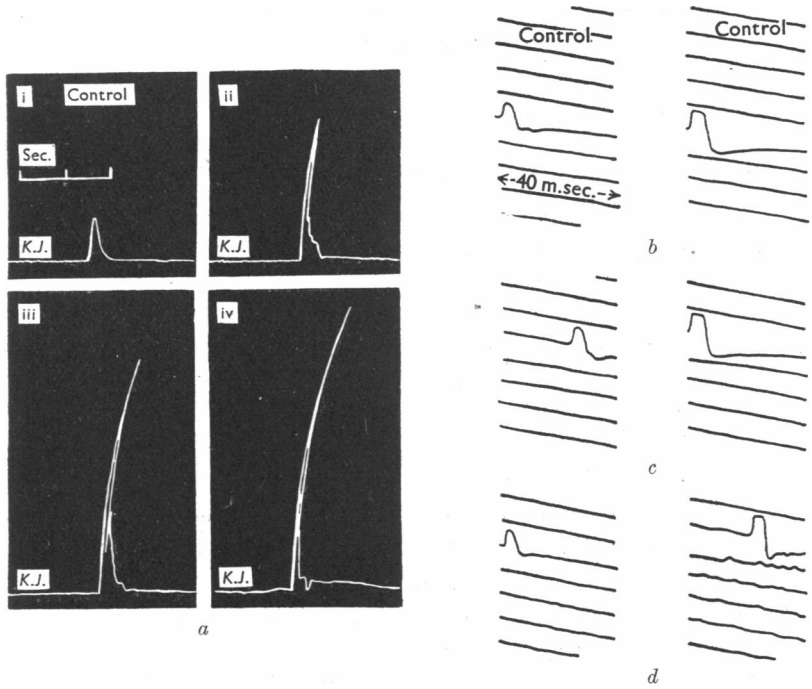

Fig. 13. Cat. 2·8 kg. Chloralose (same experiment as in Fig. 12) *a*, i–iv knee jerk (*K.J.*) on fast drum, showing potentiation with successive *intrathecal* injections of TEPP. i, control; ii, 6.55 p.m., after four injections of TEPP totalling 0·12 mg. between 6.30 and 6.51 p.m.; iii, 7.51 p.m. after two further injections of 0·05 mg. of TEPP; iv, 9.04 p.m. after six injections totalling 0·4 mg. *b*, *c* and *d* are action potentials (belly-tendon leads) of quadriceps muscle in response to femoral nerve stimulation at rate 1 in 5 sec. Left-hand record in each case is at low amplification and right-hand record at high amplification. *b*, is control. *c*, is record taken at 10.0 p.m. at peak of reflex potentiation when convulsions had set in, showing no 'peripheral afterdischarge'. *d*, is record taken at 10.10 p.m. after an *intravenous* injection of 4 mg. TEPP, peripheral afterdischarge now developing. (See text.)

Crossed extensor reflex. This reflex which was initially absent began to appear 60 min. after the first injection. At this time (Fig. 12*c*) there was a response to stimulus frequency 25 per sec. but no reaction to lower frequencies. There was a bigger response to stimulus frequency 50 per sec. and a very small one to 100 per sec. The responses consisted of 'opening' contractions dying down rapidly in spite of persistence of afferent stimulation. Fig. 12*d* illustrates the

reactions near the height of the TEPP effect. At stimulus frequency of 1 per sec., each succeeding stimulus produced for a time contractions of increasing magnitude. The relaxation curve of each reflex response was slow and irregular and displayed secondary contractions like those seen on the knee jerk. There was clear evidence of the onset of recruitment and of marked central after-discharge. With frequencies of 10 and 50 per sec. there were extremely powerful 'opening' contractions which declined irregularly and with varying speed to base-line in spite of sustained stimulation.

As the reflex changes described occurred without any alteration in the mechanical and electrical responses of the nerve muscle preparation (Fig. 13*b*, *c*) or changes in blood pressure or respiration, they must be due to a direct action of TEPP on the central nervous system.

Finally, 'spontaneous' movements appeared, and after a further 0·2 mg. of TEPP intrathecally they built up slowly to convulsions of increasing violence. As there was still no change in the nerve-muscle response these convulsions must likewise be of central origin. An intravenous injection of TEPP at this stage produced the usual changes in the mechanical and electrical response of the nerve muscle preparation (Fig. 13*d*), and fibrillar twitching made its appearance for the first time.

Action of atropine. The observations on HETP were generally carried out after intravenous administration of atropine (0·65 mg.). The dose of atropine needed to annul the effects of stimulation of para-sympathetic nerves may depress somatic reflex reactions (Schweitzer & Wright, 1937*b*). After a dose of 0·65 mg. the central stimulating action of HETP develops quite well. Similar observations have been made in the case of the central action of eserine, prostigmine and related methyl carbamic ester anticholinesterases (Schweitzer & Wright, 1937*b*; Calma & Wright, 1944) and of diisopropylfluorophosphonate (Chennells & Wright, 1947).

DISCUSSION

The effects of the condensed alkyl phosphates on the mechanical and electrical responses of the nerve-muscle preparation closely resemble those of eserine or prostigmine (Brown 1937*a*, *b*). Diisopropylfluorophosphonate produces similar effects but with larger doses (Brown, Burns & Feldberg, 1947). As with eserine the potentiating effect of the condensed alkyl phosphates is abolished after chronic denervation indicating that its action depends on the integrity of the end-plate mechanisms.

The *central* excitant action of the condensed alkyl phosphates resembles that of eserine and of diisopropylfluorophosphonate. In a number of studies (Schweitzer & Wright, 1937*b–d*, 1938; Schweitzer, Stedman & Wright, 1939; Calma & Wright, 1944, 1947; Kremer, Pearson & Wright, 1937; Kremer, 1942) it has been shown that the carbamic ester group of anticholinesterases can be divided into two classes with respect to their central action: eserine and other

tertiary compounds (e.g. methyl carbamic ester of hordenine hydrochloride) are central excitants, while the corresponding quaternary compounds like prostigmine and the methyl carbamic ester of hordenine methiodide are central depressants. Schweitzer *et al.* (1939) drew attention to the difference in the physical properties of these two classes of compounds, the convulsant tertiary compounds giving rise to lipoid-soluble derivatives, while the quaternary compounds give rise to water-soluble but lipoid-insoluble derivatives. It was suggested that these differences in solubility might determine the direction of the action on the central nervous system. As pointed out by Topley (1947) both HETP and TEPP are lipoid soluble. When HETP is dissolved in water it immediately decomposes into simpler products which are presumably the pharmacologically active principles. These decomposition products are water soluble but *lipoid-insoluble*. It seems, therefore, that with the condensed alkyl phosphates, differences in lipoid solubility do *not* modify the direction of the action on the central nervous system, both TEPP and the active products of HETP being convulsants.

Earlier studies (Schweitzer *et al.* 1939) on eserine and prostigmine showed that the central excitant action of the former and the central depressant action of the latter could be attributed to their specific anticholinesterase action. The most suggestive evidence was the fact that the central convulsant action of the tertiary group and the central depressant action of the quaternary group increased directly with their anticholinesterase activity. Furthermore, when the carbamic ester grouping was removed from the tertiary anticholinesterases both their anticholinesterase action and their central excitant action were abolished. It is natural to argue that as the condensed alkyl phosphates are convulsants and are also anticholinesterases, and as certain anticholinesterases are convulsants, that the convulsant action of the alkyl phosphates is due to their anticholinesterase activity. But too little is known for certain about the humoral mechanisms, if any, involved in central transmission to make the above argument more than merely very plausible. It would be necessary to study many other members of the alkyl phosphate series to determine whether or not the central excitant and the anticholinesterase action follow a parallel course before the two actions could be correlated as effect and cause. It should be pointed out, however, that the central excitant action of HETP and TEPP is roughly directly proportional to their anticholinesterase action, TEPP being a more powerful anticholinesterase and a more potent convulsant.

From the evidence available there is no reason to doubt that the peripheral neuro-muscular effects of the condensed alkyl phosphates are attributable to their anticholinesterase action.

SUMMARY

1. HETP and TEPP potentiate the mechanical response of the nerve-muscle preparation in the cat to single motor volleys. Using belly-tendon leads, the electrical response is characteristically modified by the addition, after the initial diphasic wave, of further waves referred to by us as 'peripheral afterdischarge' which may be repeated for a period up to 1 sec. The electrical changes are described in detail.

2. The effects of HETP and TEPP on the response to repetitive stimulation at frequencies of 10–50 per sec. are more complex. The outstanding feature of the electrical response is the disappearance of the peripheral afterdischarge after the first few responses.

3. HETP and TEPP antagonize the peripheral action of curari on the nerve-muscle preparation. Curari, injected after potentiation has been produced by HETP and TEPP, rapidly extinguishes the peripheral afterdischarge to single motor-nerve volleys.

4. The characteristic effects of HETP and TEPP on the nerve-muscle response are abolished after chronic motor denervation of the muscle.

5. HETP and TEPP facilitate various reflexes (knee jerk; flexor, peroneus, crossed extensor and jar reflex) and induce convulsions. Central afterdischarge is enhanced and prolonged. The effects described are independent of changes in the nerve-muscle response or in circulation and respiration, and are due to a direct action on the central nervous system. The central actions are obtained on intravenous and intrathecal injection in animals under chloralose anaesthesia or decerebrated, and after spinal section.

6. Attention is drawn to the similarity in the central action of the condensed alkyl phosphates to that of eserine and the tertiary carbamic ester anticholinesterases, and to that of diisopropylfluorophosphonate.

7. The peripheral neuromuscular action of HETP and TEPP can be confidently attributed to their anticholinesterase action. The mechanism of the central excitant action cannot yet be ascribed with certainty to this property of the drugs.

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