RESEARCH PAPERS

PHARMACOLOGICAL ACTIONS OF HEMLOCK (*CONIUM MACULATUM*) ALKALOIDS

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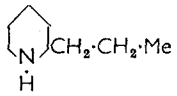
The actions of three hemlock alkaloids—coniine, *N*-methylconiine and γ-coniceine—have been examined on isolated tissues, on anaesthetised cats and hens and on conscious mice and young chicks. The most pronounced action of the alkaloids was to block spinal reflexes by an action exerted in the spinal cord. Their peripheral actions on autonomically innervated structures were mainly a consequence of an initial stimulant and a secondary depressant action on autonomic ganglia. Large doses of the alkaloids stimulated skeletal muscle and subsequently caused neuromuscular block. This blocking action differed in many respects from that produced by decamethonium or tubocurarine.

MOST of the pharmacological studies to date have been either with the juice of hemlock or with coniine (I); studies of the activity of γ-coniceine (II) and conhydrine (III) have largely been restricted to toxicity tests, while *N*-methylconiine (IV) does not appear to have been examined.



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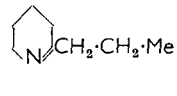

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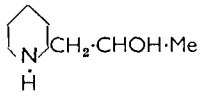

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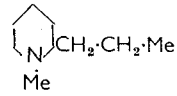

(IV)

According to Sollmann (1957), "the peripheral actions of coniine are similar to those of nicotine, but it produces more pronounced paralysis of the central nervous system and of the skeletal muscle nerve endings". However, de Boer (1950) concluded that the central actions of coniine resembled those of strychnine.

The dried leaf and juice of *Conium maculatum* were official in the London and Edinburgh pharmacopoeias from 1864 to 1898 and the last official recognition of the medicinal use of hemlock in Great Britain appeared in the B.P.C. of 1934. One of the reasons for the discontinuance of the use of hemlock appears to have been the fact that different preparations varied widely in their potency. A possible explanation of the differing potency was recently provided by Fairbairn and Challen (1959).

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Using extracts of *Conium maculatum* these authors found that the alkaloidal content and composition differed widely according to the climatic conditions and even according to the time of day at which the plants were collected. It was therefore considered worthwhile to study the pharmacological actions of the individual alkaloids. Of the four main alkaloids, conhydrine occurs in the smallest proportions and, according to the limited information available, has the weakest pharmacological actions (von Oettingen, 1936). In the present experiments the actions of the remaining three alkaloids have been compared.

Methods

*LD*50. The acute toxicity of each of the alkaloids after oral, intravenous and subcutaneous administration was determined in albino mice (A.R.C. strain) weighing between 19 and 21 g. In each test the animals were randomly distributed into 5 groups of 10 or 20 and a different dose of the alkaloid under study was administered to each group. The LD50 for each alkaloid administered by each route was calculated by the method of Litchfield and Wilcoxon (1949).

Intestinal muscle. Isolated segments of guinea-pig ileum or rabbit duodenum were suspended in continuously aerated Tyrode solution at 32° and 37° respectively. The longitudinal contractions were recorded on smoked paper. Peristalsis was recorded in isolated segments of guinea-pig ileum bathed in Tyrode solution at 32° by the method of Trendelenburg as described by Burn (1952).

Heart. Isolated rabbit hearts were perfused with McEwen's (1956) solution at 37° (gassed with 95 per cent oxygen and 5 per cent carbon dioxide) by the method described by Langendorff (see Burn, 1952).

Local anaesthetic activity. Local anaesthetic activity in small animals was studied by the guinea-pig wheal (Bülbring and Wajda, 1945), the rabbit cornea (Koppanyi and Karczmar, 1958) and the frog plexus (Burn, 1952) methods. Local anaesthetic activity in cats under chloralose anaesthesia was measured by a method similar to that described by Withrington and Zaimis (1961). Sensory impulses, initiated by regularly tapping the skin of the ankle, were recorded on a cathode ray oscilloscope by means of bipolar platinum electrodes placed on the saphenous nerve. The ability of the drugs, injected subcutaneously or intra-arterially, to abolish the sensory discharge was determined. Intra-arterial injections were made retrogradely through a needle cannula tied into the central end of the cut popliteal artery below the saphenous branch. At the moment of injection, the flow in the femoral artery was occluded, the injected fluid thereby being forced down the saphenous artery.

Skeletal muscle. Cats and hens were anaesthetised with chloralose (8 ml./kg. of a 1 per cent solution) to which pentobarbitone sodium (6 mg./kg.) was added and this mixture was injected into a subcutaneous vein of the fore-limb or into a wing vein respectively. Maximal twitches and tetani of the tibialis anterior and soleus muscles of cats and of the

lateral head of the gastrocnemius muscle of hens were elicited with rectangular pulses of 50–100 μsec. duration and supra-maximal strength applied to the peripheral end of the cut sciatic nerves; they were recorded on a kymograph. When the muscles were stimulated directly, supra-maximal shocks of 0·5 msec. duration were applied between the tendons and the steel drill supporting the femur.

Nerve action potentials were recorded in cats by means of bipolar platinum electrodes placed on the peripheral end of the common peroneal nerve, the nerve being crushed between the recording electrodes and the muscle. Muscle action potentials were recorded from the tibialis anterior muscle by means of belly-tendon leads or concentric-needle electrodes. After differential amplification by a Tektronix (Type 122) battery driven pre-amplifier, action potentials were displayed on a Tektronix (Type 502) double beam oscilloscope and photographed on 35 mm. film. In some experiments isometric muscle tension was recorded electrically by means of a RCA 5734 mechano-electric transducer. Close-arterial injections to the tibialis anterior muscles of cats or the gastrocnemius muscles of hens were made by the methods described by Brown (1938) and Brown and Harvey (1938) respectively.

Isolated rectus abdominis muscles of frogs were suspended in aerated frog Ringer solution at room temperature and their contractions recorded on smoked paper according to the method of Chang and Gaddum (1933).

Respiration. Respiration was recorded in chloralosed cats by means of a piston recorder connected through valves to a cannula in the trachea. Only expired air moved the piston. Intra-arterial injections were made through a needle cannula tied into the cut thyroid or superior lingual artery so that the tip pointed towards the right common carotid artery.

Blood flow. The venous outflow from the skeletal muscles was recorded in the femoral vein of anaesthetised cats after ligating the skin branches. The method was similar to that described by Bowman and Zaimis (1958), except that the combined flow from all of the muscles of the lower hind-limb was recorded. When skin flow was recorded a similar method was used except that the flow in the femoral vein was restricted to that which entered it from the saphenous vein. Drugs were injected intra-venously or intra-arterially through a needle cannula tied into the central end of the gracilis branch of the femoral artery. The maximum volume administered intra-arterially was 0·01 ml. delivered from a micro-syringe. The drop chamber used was that described by Hilton (1952) and the rate of flow from muscle or skin was recorded by Gaddum or Thorpe impulse counters.

Nictitating membrane. Contractions of the nictitating membrane of cats under chloralose anaesthesia were elicited by pre- and post-ganglionic stimulation of the cervical sympathetic after sectioning the nerve centrally to the pre-ganglionic electrodes. The nerve was stimulated at a frequency of 5 or 10 per sec. for 20 or 30 sec. every 2 or 3 min. For pre-ganglionic stimulation rectangular pulses of 0·2 msec. duration and 2 V strength were used. For stimulation of the non-myelinated post-ganglionic fibres

the pulse width was increased to 1 msec. and the strength to 10 V. Intra-arterial injections were made through a needle cannula in the cut thyroid artery.

Spinal reflexes. Reflex contractions of the quadriceps femoris or of the tibialis anterior muscles were recorded kymographically in cats under chloralose anesthesia and in cats previously decerebrated or spinalised under ether anaesthesia. In some experiments, contractions of the same quadriceps were elicited by alternately tapping the patellar tendon (Palmer automatic knee jerk hammer) and by stimulating the central end of the cut contra-lateral sciatic nerve with rectangular pulses of 1 msec. duration and strength 1–3 V at a frequency of 5–10/sec. for 1 sec. The method was similar to that described by Schweitzer and Wright (1938). In other experiments, patellar reflex contractions were recorded from the right hind leg once every 10 sec., as described above, and flexor reflex contractions of the tibialis anterior muscle were recorded from the opposite leg. Flexor contractions were elicited every 10 sec. by stimulation of the ipsilateral musculo-cutaneous branch of the peroneal nerve with single rectangular pulses of 0·5 msec. duration and 2–3 V strength. Drugs were injected intravenously, sometimes after occluding the circulation to the hind-limbs by clamping the iliac arteries (Schweitzer and Wright, 1938).

In all experiments on cats, blood pressure was recorded by means of a mercury manometer attached to a cannula in a common carotid artery or in a femoral artery. Intravenous injections were made through a cannula in a jugular or a femoral vein.

The hemlock alkaloids were extracted from the dried leaves and fruit of *Conium maculatum* by Professor J. W. Fairbairn and Mr. C. Lavender of the Department of Pharmacognosy of this School. The method of extraction for coniine and *N*-methylconiine was as described by Cromwell (1956) and that for γ-coniceine was as described by Fairbairn and Challen (1959). The alkaloids were supplied as hydrochlorides for this investigation. The optical rotations of natural coniine base and of natural *N*-methylconiine base are $[\alpha]_D^{19} = +16°$ and $[\alpha]_D^{24} = +81°$ respectively (Merck Index). The optical rotation of the coniine base used in these experiments was $[\alpha]_D^{20} = +3{\cdot}6°$ showing that racemization had occurred during extraction. The *N*-methylconiine base used showed an optical rotation of $[\alpha]_D^{20} = 86{\cdot}4°$ showing that it was almost entirely the (+) form. γ-Coniceine is not optically active. The doses of acetylcholine, cocaine, nicotine and the hemlock alkaloids quoted in the text, refer to the cations. Those of the other drugs refer to the salts.

RESULTS

Acute toxicity. The acute toxicity of the three hemlock alkaloids, coniine, *N*-methylconiine and γ-coniceine, after oral, intravenous and subcutaneous administration was determined in mice. The results obtained, expressed as the LD50, are presented in Table I which also shows their relative toxicities compared to coniine and the approximate

times of death after administration. The toxic symptoms were similar to those described for coniine by de Boer (1950). They included fasciculations of skeletal muscles, clonic and tonic contractions of separate limbs and convulsions of the whole animal. These signs of excessive activity were interspersed with periods of quietness, the final stage before death being one of paralysis. Respiration was first stimulated, particularly with coniine and γ-coniceine, and then depressed, the animals finally becoming cyanosed and dying apparently of respiratory failure. Micturition was frequently observed and the eyes protruded from the head.

TABLE I

ACUTE TOXICITY IN MICE

Alkaloid		Route of administration		
		I.V.	Subcut.	Oral
Coniine	LD50 (mg./kg.)	19 (16–22)	80 (72–88)	100 (97–103)
	Time of death	30 sec.	15 min.	10 min.
	Dose ratio Coniine = 1	1	1	1
N-Methylconiine	LD50 (mg./kg.)	27·5 (24·75–30·25)	150·5 (146·2–154·8)	204·5 (176–233)
	Time of death	30 sec.	16 min.	12 min.
	Dose ratio Coniine = 1	1·5	1·9	2
γ-Coniceine	LD50 (mg./kg.)	2·6 (1·95–3·25)	12 (11–13)	12 (10·4–13·5)
	Time of death	30 sec.	12 min.	8 min.
	Dose ratio Coniine = 1	0·14	0·15	0·12

The figures in brackets are the 95 per cent fiducial limits.

Smooth muscle of the intestine. Coniine (15–50 μg./ml.) and γ-coniceine (5–15 μg./ml.) caused contraction of the isolated guinea-pig ileum and rabbit duodenum. This result with coniine confirms that of others (Tamba, 1921; Hamet, 1931). The addition of atropine (1 μg./ml.) or hexamethonium (25 μg./ml.) to the bath fluid prevented this action of the alkaloids suggesting that it arose through stimulation of parasympathetic ganglia. *N*-methylconiine occasionally caused a weak contraction but more usually was without effect in this respect in concentrations up to the maximum used (250 μg./ml.). In concentrations of 100 μg./ml., *N*-methylconiine prevented the response of the smooth muscle to subsequently added nicotine but not to acetylcholine. It was not possible to demonstrate any ability of γ-coniceine or coniine to inhibit the effect of nicotine specifically, although high concentrations (200 μg./ml.) of all three hemlock alkaloids possessed a non-specific depressant effect and even after washing the tissue several times, the responses to stimulating agents such as acetylcholine, histamine, nicotine and to the alkaloids themselves, were reduced. Such concentrations also depressed the spontaneous pendular movements of the rabbit intestine. Fig. 1*a*

illustrates an experiment in which the potency of coniine was compared with that of nicotine on the guinea-pig ileum and Fig. 1*b* shows the effects of γ-coniceine, nicotine and *N*-methylconiine on the rabbit duodenum.

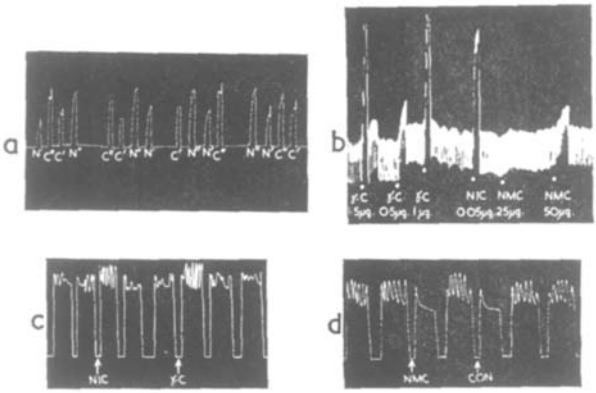

FIG. 1. *a*. Isolated guinea-pig ileum. At N′ and N″, 1 and 2 μg./ml. nicotine; at C′ and C″, 15 and 30 μg./ml. coniine. Contact time, 30 sec. Dose interval, 5 min. *b*. Isolated rabbit duodenum. At γ-C, γ-coniceine; at NIC, nicotine and at NMC, *N*-methylconiine. Doses in μg./ml. *c* and *d*. Peristalsis in isolated guinea-pig ileum. At NIC, 0·5 μg./ml. nicotine. At γ-C, 25 μg./ml. γ-coniceine; at NMC, 10 μg./ml. *N*-methylconiine and at CON, 15 μg./ml. coniine. (The lower tracings have been retouched.)

N-Methylconiine (2·5–5 μg./ml.) and coniine (15 μg./ml.) abolished the peristaltic reflex in isolated segments of guinea-pig ileum after being in contact with the tissue for 90 sec. (Fig. 1*d*). Neither substance accentuated peristalsis in any concentration. Weak concentrations of γ-coniceine (5–25 μg./ml.) and of nicotine (0·1–0·5 μg./ml.) augmented peristalsis (Fig. 1*c*) and larger concentrations depressed it.

Cardiovascular system. Minimal effective intravenous doses of coniine (0·5–2 mg./kg.) and *N*-methylconiine (1–4 mg./kg.) caused a small and short-lasting fall in blood pressure which was prevented by previous atropinisation (1 mg./kg.). γ-Coniceine (0·2–0·5 mg./kg.) only occasionally produced a fall in blood pressure and this was always followed by a rise. Doses of coniine slightly larger than about 2 mg./kg. produced a similar biphasic response but with very large doses both of coniine (5–10 mg./kg.) and of γ-coniceine (2–5 mg./kg.) the initial fall was absent and only a rise in blood pressure was produced. With large doses of *N*-methylconiine (6–8 mg./kg. and above), the initial short-lasting fall in blood pressure was often followed by a more slowly developing and longer lasting fall during which the pressor response to nicotine was reduced or abolished. When the background level of blood pressure was low, as for example in the spinal animal, the depressor response to all three alkaloids was absent and under these conditions *N*-methylconiine also caused a small rise in blood pressure. The pressor response to the alkaloids was prevented by the previous intravenous administration of

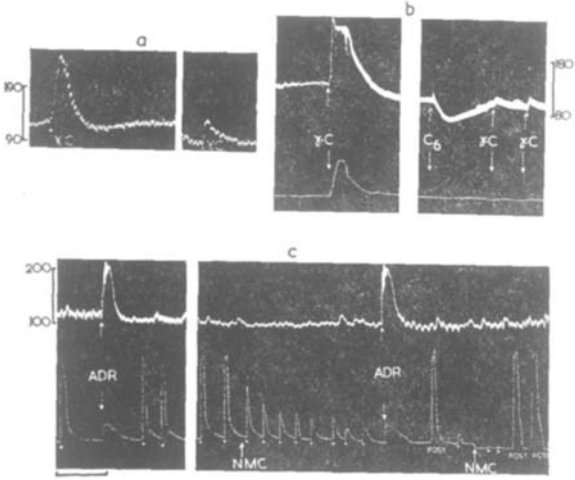

FIG. 2. Cats, chloralose anaesthesia. *a*. Arterial blood pressure responses to 3 mg./kg. γ-coniceine intravenously before and after ligating the adrenal glands. *b*. Blood pressure and nictitating membrane. At γ-C, 2 mg./kg. γ-coniceine and at C_6, 2 mg./kg. hexamethonium intravenously. *c*. Blood pressure and nictitating membrane. At the white dots, pre-ganglionic stimulation and at POST, post-ganglionic stimulation of cervical sympathetic (10/sec. for 20 sec.). At ADR, 5 μg./kg. adrenaline intravenously and at NMC, 2 mg. *N*-methylconiine intra-arterially. Time calibration for whole Fig., 5 min. Blood pressure in mm. Hg.

3 mg./kg. hexamethonium (Fig. 2*b*) and was markedly reduced but not abolished when the adrenal glands were excluded from the circulation (Fig. 2*a*). All three alkaloids in large doses (γ-coniceine 2 mg./kg., coniine 5 mg./kg., *N*-methylconiine 4 mg./kg.) administered intravenously, blocked or reduced the depressor response to stimulation of the left vagus (Fig. 3*b*).

In the isolated perfused rabbit heart all three alkaloids in large amounts (γ-coniceine 0·2 mg., coniine 2 mg., *N*-methylconiine 4 mg.) caused a decrease in the force of the beat, the rate of beating being unaffected. These results with coniine on the rabbit's heart confirm those of de Boer (1950) who used frog hearts.

The only effect of the hemlock alkaloids on skeletal muscle blood flow, whether injected intra-arterially (γ-coniceine, 100 μg.; coniine and *N*-methylconiine, 1 mg.) or intravenously (γ-coniceine, 0·5 mg./kg. and above; coniine, 2 mg./kg. and above; *N*-methylconiine, 4 mg./kg. and above) was a small increase in venous outflow (Figs. 3*a*, *b* and *c*). With intravenous injection, the pressor effects of γ-coniceine and coniine added to the local dilator action by forcing more blood through the muscle vessels. The venous outflow from the skin vessels was also increased by intra-arterial injection of the same doses of the alkaloids (Fig. 3*d*) but when administered intravenously, the pressor effects of γ-coniceine and coniine were accompanied by vasoconstriction in the skin (Fig. 3*d*). *N*-Methylconiine which did not usually cause a rise in blood pressure, produced only a slight vasodilatation in the skin vessels.

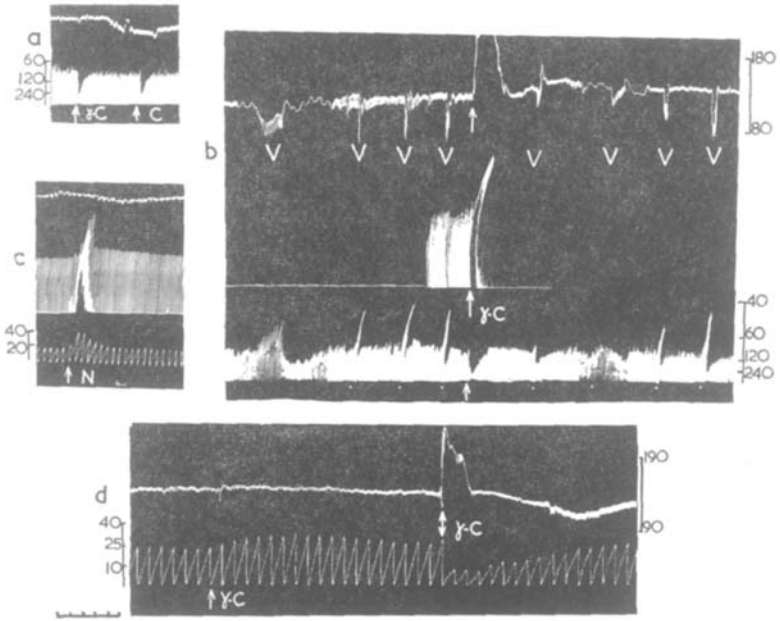

FIG. 3. Cats, chloralose anaesthesia. *a.* Blood pressure and venous outflow from hind-limb muscles. At γ-C, 200 μg. γ-coniceine and at C, 1 mg. coniine injected intra-arterially. *b.* Blood pressure, respiration and venous outflow from hind-limb muscles. Respiration was recorded only for the period shown. Artificial respiration was applied after γ-coniceine had depressed respiration. At V, the left cervical vagus was stimulated (5/sec. for 10 sec.), the kymograph speed being increased during the first and seventh period of vagal stimulation. At γ-C, 2·5 mg./kg. γ-coniceine intravenously. *c.* Blood pressure, maximal twitches of the tibialis anterior muscle elicited indirectly 1/10 sec. and venous outflow from the hind-limb muscles. At N, 0·2 mg. *N*-methylconiine intra-arterially. *d.* Blood pressure and venous outflow from the skin of the hind-limb. At γ-C, γ-coniceine 100 μg. intra-arterially and 1 mg./kg. intravenously. Time calibration in min. Note that in *a* and *b*, a Gaddum impulse counter was used to record blood flow and an increase in the height of the record means a decrease in flow. In *c* and *d*, a Thorpe impulse counter was used and an increase in the height of the record means an increase in flow. Blood flow calibration, drops/min. Blood pressure in mm. Hg.

Nictitating membrane. γ-Coniceine (50–100 μg. i.a. and 0·5–2 mg./kg. i.v.) and coniine (2 mg. i.a. and 10–15 mg./kg. i.v.) caused contraction of the nictitating membrane of the cat but *N*-methylconiine was without this effect in doses up to 5 mg. i.a. The effect of coniine and γ-coniceine was prevented by the previous administration of hexamethonium (3 mg./kg. i.v.). All three alkaloids, γ-coniceine (0·3 mg. i.a., 2 mg./kg. i.v.), *N*-methylconiine (1–2 mg. i.a., 5–8 mg./kg. i.v.) and coniine (1–2 mg. i.a., 10 mg./kg. i.v.) blocked or reduced the contractions of the nictitating membrane elicited by pre-ganglionic stimulation. During this effect, the response of the membrane to adrenaline and to post-ganglionic stimulation was unaffected. These results show that the site of both the stimulant and the blocking action of the alkaloids was the superior cervical ganglion. Fig. 2*b* and *c* illustrate some of these effects of γ-coniceine and *N*-methylconiine on the nictitating membrane.

Respiration. Small doses of coniine (1–4 mg./kg.) and γ-coniceine (0·3–1 mg./kg.) administered intravenously, stimulated respiration but larger doses depressed it after an initial abrupt stimulation. Fig. 3*b* illustrates stimulation and block of respiration produced by intravenously injected γ-coniceine. *N*-Methylconiine did not stimulate respiration and depressed it only in doses large enough to cause neuromuscular block (see later).

When injected into the carotid artery so that they passed through the carotid sinus, small doses of coniine (20–30 μg.) and γ-coniceine (10–20 μg.) caused a slowly developing increase in respiratory rate and depth. In contrast to the hemlock alkaloids, small doses of nicotine (1–2 μg.) injected by the same route, caused an abrupt and short-lasting increase in respiration. This well known action of nicotine on chemoreceptors in the carotid body (Heymans, Bouckaert and Dautrebande, 1931) did not occur with intra-arterial injection of the hemlock alkaloids, possibly because their rate of reaction with these receptors is too slow. By this route of injection the hemlock alkaloids therefore appeared to act only directly upon the respiratory centres. Larger doses of γ-coniceine and coniine injected into the carotid artery depressed respiration and eventually caused respiratory failure. *N*-Methylconiine in doses up to 100 μg. was without effect on respiration when administered into the carotid artery. Fig. 4 compares the effects of intra-carotid injection of nicotine and of coniine on respiration.

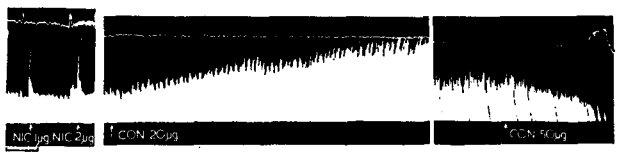

FIG. 4. Cat chloralose anaesthesia. Blood pressure and respiration. At NIC, nicotine and at CON, coniine injected retrogradely into the thyroid artery. 10 min. elapsed between the 1st and 2nd panels and 20 min. between the 2nd and 3rd panels. The second dose of coniine was lethal. Time calibration, 5 min.

Local anaesthetic action. With concentrations of the hemlock alkaloids up to 10 mg./ml. no local anaesthetic action could be demonstrated using the rabbit cornea or frog plexus tests. In these experiments, cocaine was used for comparison. In the guinea-pig wheal test, a weak local anaesthetic action of coniine and *N*-methylconiine was demonstrable. In this test approximately equi-potent doses by intra-dermal injection were: cocaine 150 μg., coniine 2·5 mg. and *N*-methylconiine 6 mg. γ-Coniceine was less active than coniine in this respect and no local anaesthetic potency was evident with doses up to 2·5 mg. Larger doses of this alkaloid were lethal.

Cocaine, by intra-arterial or subcutaneous injection, was markedly effective in abolishing the sensory discharge in the cat saphenous nerve elicited by tapping the skin. The hemlock alkaloids showed only a very weak effect, however, in doses up to the maximum administered (0·2 ml.

of a 10 mg./ml. solution). Fig. 5 illustrates an experiment in which the effect of γ-coniceine was compared with that of cocaine.

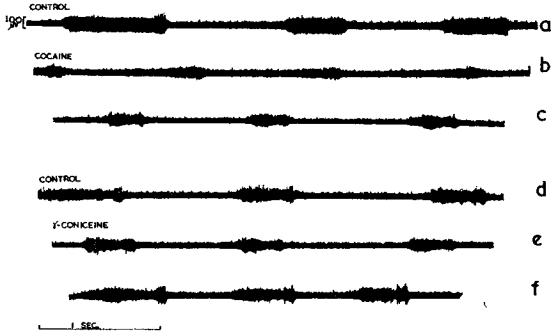

FIG. 5. Cat, chloralose anaesthesia. Oscilloscope recording of sensory discharges evoked in the saphenous nerve by tapping the same area of skin near the ankle about once every sec. *a.* Control responses at start of experiment. *b.* 10 min. after injection of cocaine (0·3 mg. in 0·2 ml.) beneath the skin at the site of stimulation. *c.* 30 min. later. *d.* Control responses 2 hr. later. *e.* 10 min. after subcutaneous injection of γ-coniceine (2 mg. in 0·2 ml.). *f.* 30 min. later. Control injections of 0·2 ml. saline were without effect on the sensory discharge.

Frog. skeletal muscle. Coniine (0·1–0·5 mg./ml.) and γ-coniceine (5–10 μg./ml.) caused contracture of the rectus abdominis muscle of the frog but *N*-methylconiine was without effect in concentrations up to the maximum used (1 mg./ml.). This effect of coniine and γ-coniceine was prevented by the presence of tubocurarine (1–2 μg./ml.). Providing that they were given time to act, all three alkaloids reversibly blocked or reduced the response of the muscle to acetylcholine. This effect occurred with concentrations of the alkaloids below those which caused contracture of the muscle. Thus coniine (5–10 μg./ml.), γ-coniceine (1–2 μg./ml.) and *N*-methylconiine (3–6 μg./ml.) blocked the response of the muscle to acetylcholine after being in contact with the tissue for 90–120 sec. After washing the tissue two or three times with fresh Ringer solution, the response to acetylcholine returned to normal. Similar small concentrations of the alkaloids prevented the contractural response to larger concentrations added subsequently.

Mammalian skeletal muscle. On close-arterial injection into the tibialis anterior muscle of the cat, all three hemlock alkaloids (coniine, 2–3 mg., γ-coniceine 25–100 μg., *N*-methylconiine 0·5–1 mg.) caused a quick contraction of the muscle similar to that produced by depolarising drugs such as acetylcholine, suxamethonium or nicotine (Fig. 7). Mammalian muscle therefore differed from frog muscle in which *N*-methylconiine was without a stimulant action in the concentrations used. Electrical recording from the tibialis anterior muscle showed that the contraction produced by the alkaloids was accompanied by an asynchronous burst of action potentials similar to that following close-arterial injection of acetylcholine (Fig. 6).

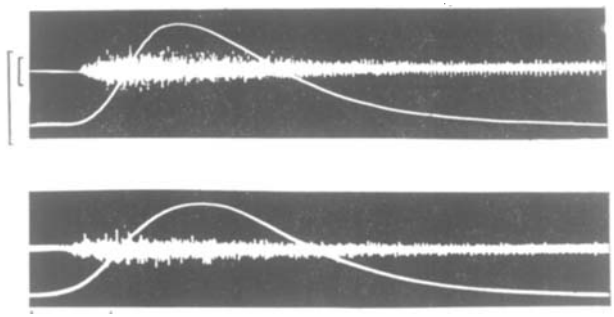

FIG. 6. Cat chloralose anaesthesia. Electromyogram (concentric needle electrode) and isometric myogram (RCA 5734 transducer valve) recorded from tibialis anterior muscle. Upper panel: response to 5 μg. acetylcholine close-arterially. Lower panel: response to 50 μg. γ-coniceine close-arterially. Voltage calibration for electromyogram, 0·5 mV. Tension calibration, 0·5 kg. Time calibration, 200 msec.

The contraction produced by the first close-arterial injection of the hemlock alkaloids was usually followed by a very slight and transient depression of the maximal twitches. This depression was followed by potentiation of the twitches but this then gradually faded and changed to a slowly developing and more pronounced reduction in twitch tension. Fig. 7*c* illustrates these changes produced by coniine. In striking contrast to the effect of the hemlock alkaloids, the block produced by close-arterial doses of the depolarising blocking drugs, suxamethonium (Fig. 7*a*) and decamethonium, was present immediately after the initial contraction which they produced. This is to be expected with these drugs since both the contraction and the block are believed to be a consequence of the

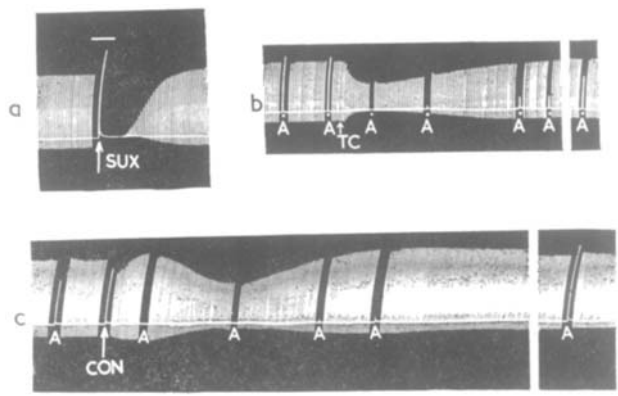

FIG. 7. Cats, chloralose anaesthesia. Maximal twitches of tibialis anterior muscles elicited indirectly once every 10 sec. except during close-arterial injections when electrical stimulation was stopped. *a*. At SUX, 10 μg. suxamethonium close-arterially. The horizontal bar shows the period during which electrical stimulation was stopped. *b*. At A, 10 μg. acetylcholine close-arterially and at TC, 0·3 mg./kg. tubocurarine intravenously. The last response to acetylcholine was recorded 40 min. after the previous one. *c*. At A, 5 μg. acetylcholine and at CON, 3 mg. coniine close-arterially. The last response to acetylcholine was recorded 20 min. after the previous one.

same mechanism, namely, depolarisation of the motor end-plates, and the overall depolarisation must be greatest during the drug-induced contraction. In view of these considerations, it seems likely that only the initial transient and slight depression of the twitches immediately following the contraction produced by the hemlock alkaloids, was a consequence of depolarization block. With the second and subsequent injections of the hemlock alkaloids the initial contraction was smaller and the onset of depression of the twitches followed more rapidly. In fact with the third and fourth dose, the initial contraction was absent and block ensued immediately. Successive doses of γ-coniceine and coniine showed a cumulative paralyzing effect when administered at intervals of 2–3 hr. or less but with *N*-methylconiine, tachyphylaxis was evident.

Distant arterial injection of sub-blocking doses of the alkaloids did not cause contraction of the non-stimulated muscle but produced powerful fasciculations and potentiation of the maximal twitch. This effect of *N*-methylconiine is illustrated in Fig. 3*c*.

Intravenous administration of the alkaloids (coniine 15 mg./kg., *N*-methylconiine 10 mg./kg., γ-coniceine 1 mg./kg.) caused an initial small potentiation of the indirectly excited maximal twitches of the

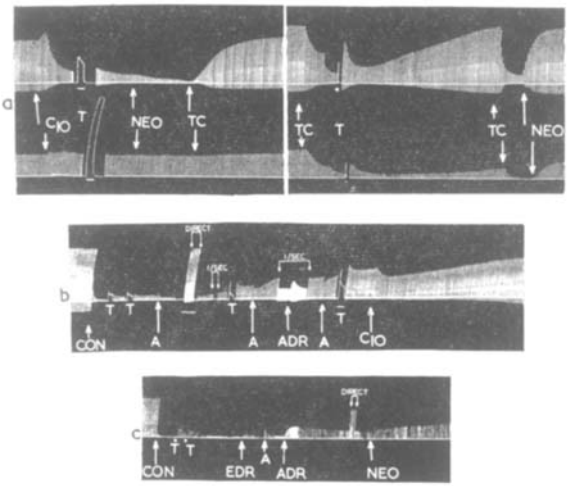

FIG. 8. Cats chloralose anaesthesia. Maximal twitches of muscles elicited indirectly once every 10 sec. except where otherwise stated. *a*. Tibialis anterior (upper record) and soleus muscles (lower record). At C_{10}, 35 μg./kg. decamethonium and at NEO, 50 μg./kg. neostigmine. At TC in the left-hand panel, 100 μg./kg. and in the right-hand panel 0·4 and 0·3 mg./kg. tubocurarine injected intravenously. *b*. Tibialis anterior muscle. At CON, 2 mg. coniine and at A, 200 μg. acetylcholine close-arterially. At ADR, 10 μg./kg. adrenaline and at C_{10}, 10 μg./kg. decamethonium intravenously. *c*. Tibialis anterior muscle. At CON, 5 mg. coniine, at A, 200 μg. acetylcholine and at EDR, 5 μg. edrophonium close-arterially. At ADR, 20 μg./kg. adrenaline and at NEO, 100 μg./kg. neostigmine intravenously. At T in all experiments, a tetanus was elicited by stimulation of the motor nerve (50/sec. for 10 sec.). At DIRECT, the muscle was stimulated directly. Similar responses were obtained in other experiments when the effects of each drug were studied separately.

the tibialis anterior and soleus muscles of the cat which was followed by a slowly developing reduction in twitch tension to about 50 per cent of the original level. Larger doses caused more complete paralysis. The paralysis of the soleus muscle was at least as great and sometimes slightly greater than that of the tibialis anterior muscle. The sensitivity of the two muscles to the blocking action of the alkaloids therefore resembled that to tubocurarine rather than that to decamethonium (Paton and Zaimis, 1952 and see Fig. 8*a*). During complete paralysis of the indirectly excited maximal twitches produced by the alkaloids, the muscle responded normally to direct electrical stimulation (Fig. 8*b* and *c*) and the shape and size of action potentials recorded from the nerve remained unchanged. These results therefore locate the site of the blocking action at the neuromuscular junction, either on the motor end-plates or at the fine nerve terminals.

After the administration of a blocking dose of one of the alkaloids, the twitch-like response to close-arterially injected acetylcholine was reduced or abolished and this occurred even during the latent period between injection and the onset of the depression of the twitches (Fig. 7*c*) showing that even at this stage the sensitivity of the motor end plates was depressed. At the height of the block, the response to acetylcholine was completely abolished even when the dose of alkaloid was such that the maximal twitches were only partially depressed (Fig. 7*c*). These results show that a depression of motor end-plate sensitivity plays a large part in the blocking action of the alkaloids. However, the response to injected acetylcholine reappeared sooner than it does after a comparable degree of block produced by tubocurarine (Fig. 7*b*). In fact, after recovery from block produced by coniine, the response to acetylcholine was often greater than the control responses before coniine (Fig. 7*c*). The block produced by the hemlock alkaloids differed both from that produced by curare-like drugs such as tubocurarine and gallamine, and from that produced by depolarising drugs such as decamethonium or suxamethonium. Curare-like drugs and depolarising drugs can be shown to be mutually antagonistic in the tibialis anterior muscle of the cat (Paton and Zaimis, 1952; Hutter and Pascoe, 1951; Dellamagne and Phillipot, 1952; and see Fig. 8*a*). However, the blocking action of the hemlock alkaloids added to that of both types of neuromuscular blocking drug and this occurred no matter in what order the drugs were administered. Thus a small dose of a hemlock alkaloid, administered at the height of a partial block produced by tubocurarine or decamethonium, caused a further increase in the paralysis. Similarly, decamethonium or tubocurarine increased the block when administered during the effect of the hemlock alkaloids. Experiments were carried out in which maximal twitches of both tibialis anterior muscles were recorded simultaneously. A constant dose of tubocurarine was injected intravenously at intervals of 90 min. During one of the intervals between tubocurarine injections, a single dose of a hemlock alkaloid was injected close-arterially to one muscle only. The block produced in this muscle by the subsequent intravenous dose of tubocurarine was much greater than that produced in the control muscle

of the opposite leg which merely showed the normal cumulative effect of tubocurarine (Fig. 9*b*). A similar potentiation of decamethonium block was obtained when this drug was used in place of tubocurarine (Fig. 9*a*).

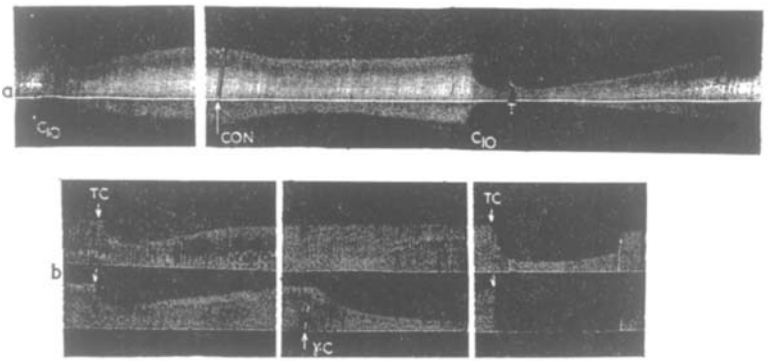

FIG. 9. Cats, chloralose anaesthesia. Maximal twitches of tibialis anterior muscles elicited indirectly once every 10 sec. *a*. Right tibialis only. At C_{10}, 25 μg./kg. decamethonium intravenously (90 min. between each dose). At CON, 2 mg. coniine close-arterially. The block produced by decamethonium was greater after coniine. *b*. Left (upper) and right (lower) tibialis muscles. At TC, 0·3 mg./kg. tubocurarine intravenously (150 min. between doses). At γ-C, 25 μg. γ-coniceine close-arterially to right tibialis only. The block produced by tubocurarine was greater in the right tibialis after γ-coniceine.

A motor nerve tetanus, edrophonium, neostigmine, acetylcholine and adrenaline all possess marked decurarising actions but are without effect or slightly increase block produced by decamethonium in the tibialis anterior muscle of the cat (Paton and Zaimis, 1952). During a partial block produced by the hemlock alkaloids these tests produced responses which were intermediate between those described above. Tetanic tension was less well sustained than it is during decamethonium block but was better sustained than the brief twitch-like response characteristic of tubocurarine paralysis. The post-tetanic twitches were slightly increased in tension. Edrophonium and neostigmine produced only weak antagonistic actions and the effect of close-arterially injected acetylcholine was variable. Sometimes a small antagonistic action resulted while on other occasions the block was increased. Adrenaline exerted an antagonistic action which was roughly equal to its anti-curare effect; it was a more powerful antagonist than neostigmine or edrophonium of block produced by hemlock alkaloids. Fig. 8 illustrates experiments carried out under the same conditions, in which some of these tests were applied during blocks produced by tubocurarine, coniine and decamethonium.

Avian skeletal muscle. On intravenous injection into the conscious chick, the smallest effective doses of coniine (70–80 μg./10 g.) often, but not invariably, caused flaccid paralysis similar in appearance to that produced by tubocurarine. The effect differed from that produced by tubocurarine, however, in that pinching the foot still evoked a flexor reflex. Minimal effective doses of nicotine (1–2 μg./10 g.), produced flaccid paralysis in all chicks tested. In the same chicks, and in all

others tested, larger doses of all three hemlock alkaloids (coniine and *N*-methylconiine, 100–120 μg./10 g.; γ-coniceine, 5–10 μg./10 g.) and of nicotine (5–10 μg./10 g.) caused a spastic paralysis resembling that produced by decamethonium. However, this response to the hemlock alkaloids, and to nicotine, differed from that to decamethonium as follows. With lethal doses of decamethonium, the chicks died in spastic paralysis which persisted for about half an hour after death. With sublethal doses, recovery was abrupt and as the spasticity wore off, the chick stood up and was apparently normal (Buttle and Zaimis, 1949). The spasticity produced by the hemlock alkaloids and by nicotine, on the other hand, was always followed by a further period of flaccid paralysis during which death occurred or from which recovery took place.

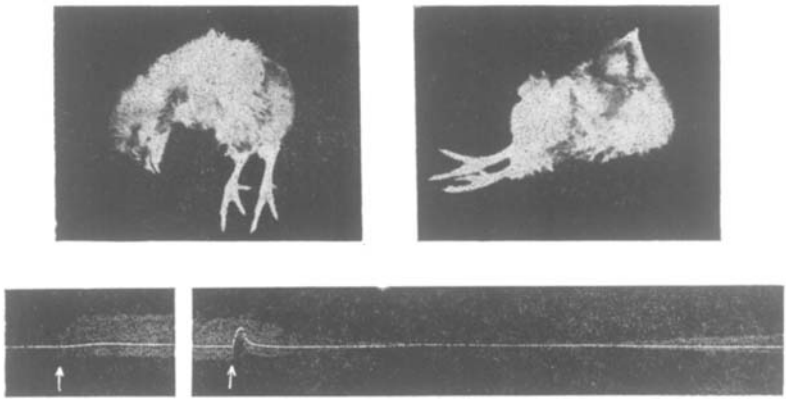

FIG. 10. The whole figure illustrates experiments on the same chick. Upper panels: before anaesthesia. Lower panels: maximal twitches of gastrocnemius muscle elicited indirectly once every 10 sec. during anaesthesia with pentobarbitone sodium (12 mg. intraperitoneally). Left-hand panels: 75 μg./10 g. coniine intravenously caused flaccid paralysis of the conscious chick but did not block the maximal twitches of its gastrocnemius muscle. Right-hand panels: 110 μg./10 g. coniine caused spastic paralysis (illustrated) followed by flaccid paralysis in the conscious chick and contracture followed by block of the gastrocnemius muscle.

It appeared unlikely that the flaccid paralysis often produced by the smallest effective doses of coniine and of nicotine could be due to depressed excitability of the muscle, since larger doses, given immediately afterwards, caused contracture. The initial flaccid paralysis could, however, be explained if the most powerful action of these substances were a depressant one in the central nervous system. This possibility was tested by anaesthetising one of the chicks, which had responded by flaccid paralysis to small doses of coniine, and recording maximal twitches of its gastrocnemius muscle elicited by stimulation of the sciatic nerve. The results obtained from this chick, both before and after it was anaesthetised, are shown in Fig. 10. They show that a small dose of coniine (75 μg./10 g.) which produced flaccid paralysis in the conscious chick, was completely without effect on the indirectly excited maximal twitches of the gastrocnemius muscle. This result indicated that the initial flaccid paralysis in the conscious chick had been a consequence of an action in

the central nervous system and this led to the experiments on spinal reflexes to be described later. A larger dose (120 μg./10 g.) which had caused spastic paralysis followed by flaccid paralysis in the conscious chick, produced contracture followed by a block of the maximal twitches of the gastrocnemius muscle.

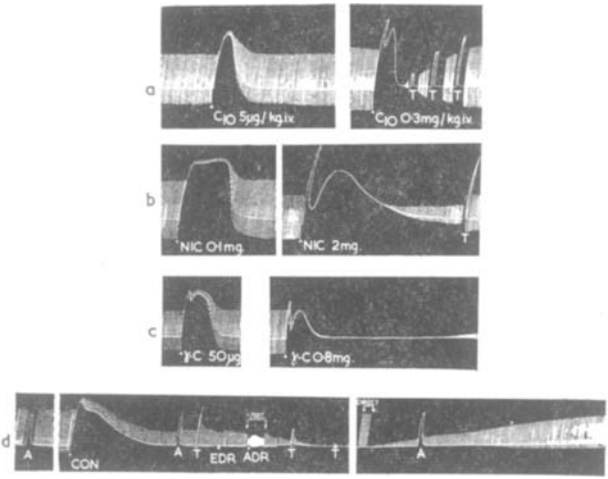

FIG. 11. Hens, chloralose anaesthesia. Maximal twitches of gastrocnemius muscles elicited indirectly once every 10 sec. *a*. At C_{10}, decamethonium intravenously and at T, tetanus (50/sec. for 10 sec. on faster kymograph). Each panel depicts a different experiment. *b*. At NIC, nicotine close-arterially. Each panel depicts a different experiment. *c*. At γ-C, γ-coniceine close-arterially. Both panels are from the same experiment. *d*. At A, 5 μg. acetylcholine; at CON, 4 mg. coniine and at EDR, 10 μg. edrophonium injected close-arterially. At ADR, 10 μg./kg. adrenaline intravenously. At T, tetanus (50/sec. for 10 sec.). At DIRECT, direct stimulation of the muscle.

In the anaesthetised adult hen, all three hemlock alkaloids and nicotine caused a sustained contracture of the gastrocnemius muscle on intravenous or close arterial injection. With small doses (γ-coniceine and nicotine, 50–100 μg.; coniine and *N*-methylconiine, 1–2 mg. close arterially) the maximal twitches following the contractures were not depressed below the pre-injection level but with larger doses the subsequent twitches remained depressed after the contracture (Fig. 11*b* and *c*). With the hemlock alkaloids, the doses necessary to produce a biphasic response were about three times greater than those which caused only a contracture, while with nicotine the ratio was about 10:1. Decamethonium was also capable of producing a biphasic response (Fig. 12*a*) but with this drug the ratio of the two doses was of the order of 50:1. Tridecamethonium in which the two quaternary nitrogens are separated by a chain of 13 methylene groups, produces a biphasic response in all effective doses and the secondary depression of the maximal twitches shows the characteristics of block produced by tubocurarine (Zaimis, 1953). Zaimis used the term "dual block" to describe this type of paralysis in which both depolarising and curare-like phases are present. With large doses of decamethonium, the secondary depression of the twitches did not resemble a tubocurarine

block. Neostigmine was without antagonistic action, tetanic tension was well sustained and the course of the block was not altered by the tetanus (Fig. 11*a*). This secondary effect of large doses of decamethonium was probably a consequence of membrane inexcitability outlasting the depolarisation. Burns and Paton (1951) showed in the tenuissimus muscle of the cat, that the block produced by depolarising substances is a direct consequence of an electrical inexcitability of the muscle membrane to which prolonged depolarisation gives rise in the region surrounding the motor end-plates.

The secondary block of the maximal twitches produced by the hemlock alkaloids differed from that produced either by tubocurarine or by large doses of decamethonium. In most respects, it resembled that produced by the alkaloids in the skeletal muscles of the cat. During the paralysis, direct stimulation of the muscle produced normal contractions; tetanic tension was poorly sustained and the post-tetanic twitches were slightly increased in tension; neostigmine or edrophonium produced only very small antagonistic effects; tubocurarine or decamethonium administered during the block caused a further increase in the depth of paralysis; adrenaline exerted some antagonistic action. The effect differed from that in the cat in that during the paralysis the response of the muscle to close-arterially injected acetylcholine was only slightly reduced. Fig. 11*d* illustrates some of these responses during block produced by coniine.

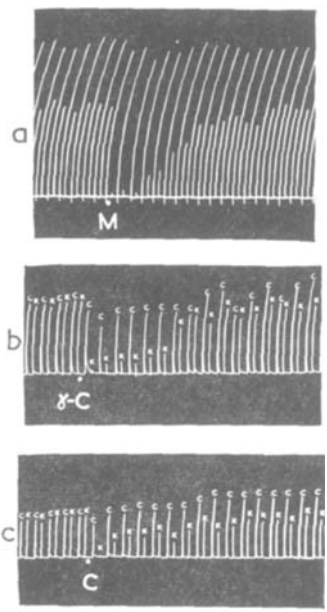

FIG. 12. Cats, light chloralose anaesthesia. Alternate crossed extensor and patellar reflex contractions of the quadriceps femoris muscle every 30 sec. (i.e. 1 min. between reflex contractions of the same type). *a*. Patellar reflex contractions are the larger deflections. At M, 10 mg./kg. mephenesin. *b* and *c*. C = crossed extensor reflex and K = patellar reflex (knee jerk). At γ-C, 300 μg./kg. γ-coniceine and at C, 4 mg./kg. coniine. All injections intravenously.

Spinal reflexes. In these experiments similar results were obtained in anaesthetised cats and in cats previously decerebrated or spinalised. Therefore no distinction is made in the following description. Small intravenous doses of the hemlock alkaloids and of nicotine blocked the response of the quadriceps muscle to tapping the patellar tendon (patellar reflex) or stimulating the central end of the contra-lateral sciatic nerve (crossed-extensor reflex). With the first injection of each hemlock alkaloid the patellar reflex was often more affected than the crossed extensor reflex. Figure 12*b* and *c* illustrates experiments in which γ-coniceine and coniine caused complete block of the patellar reflex while leaving the crossed extensor reflex almost unaffected. The contrast between this effect and that of the spinal relaxant, mephenesin, which selectively abolishes the crossed extensor reflex, is also illustrated (Fig. 12*a*). With subsequent injections of the hemlock alkaloids, both reflexes were roughly equally depressed. Nicotine always blocked both reflexes to a similar extent. Doses of the hemlock alkaloids and of nicotine sufficient to cause complete paralysis of the patellar and crossed extensor reflexes were without effect on the response of the tibialis anterior muscle to stimulation of the central end of the musculocutaneous branch of the ipsilateral peroneal

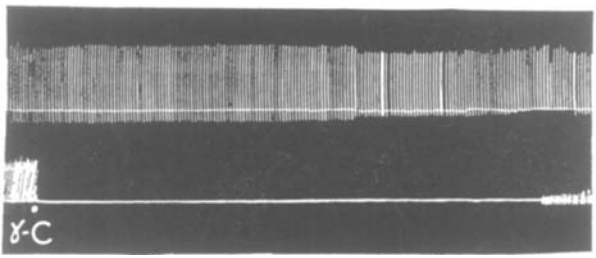

FIG. 13. Cat decerebrate. Upper record: flexor reflex contractions of right tibialis anterior muscle once every 10 sec. Lower record: patellar reflex contractions of left quadriceps femoris muscle, once every 5 sec. At γ-C, 300 μg./kg. γ-coniceine intravenously.

nerve (flexor reflex). Fig. 13 illustrates this effect of γ-coniceine. When the limb, from which the patellar and crossed extensor reflexes were recorded, was deprived of its circulation by clamping the ipsilateral iliac artery just below the aorta, the hemlock alkaloids and nicotine still abolished these reflexes when administered intravenously. Recovery occurred after the expected time showing that the lack of circulation had not contributed to the block. These results rule out the neuromuscular junction and the muscle spindles as the site of this action and show that the effects were a consequence of an action in the spinal cord.

Larger doses of hemlock alkaloids and of nicotine blocked the flexor reflex. The block was usually preceded by potentiation of the contractions and powerful fasciculations of the muscle (Fig. 14). The latter were more pronounced after nicotine than after the hemlock alkaloids. After large doses, fasciculations of the quadriceps muscle sometimes occurred even though the elicited reflex contractions of this muscle were completely abolished. This stimulant activity was reduced but not abolished on

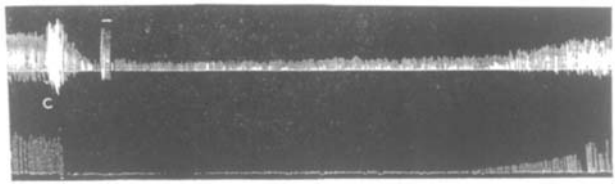

FIG. 14. Spinal cat. Upper record: Flexor reflex contractions of right tibialis anterior muscle once every 10 sec. During period marked by horizontal bar, maximal twitches of tibialis anterior muscle were elicited by stimulation of the motor nerve. Lower record: Patellar reflex contractions of left quadriceps femoris muscle once every 10 sec. At C, 7·5 mg./kg. coniine intravenously.

sectioning the sciatic-nerve. An action of the drugs on the intrafusal fibres of the muscle spindles was unlikely to have contributed to the stimulant action. The muscle spindles are the sensory receptors involved in the patellar reflex and the doses required to produce fasciculations were greatly in excess of those which completely abolished this stretch reflex.

TABLE II

APPROXIMATE INITIAL INTRAVENOUS DOSES (MG./KG.) NECESSARY TO CAUSE COMPLETE BLOCK OF REFLEX CONTRACTIONS AND THE DURATIONS OF THE EFFECTS

Alkaloid	Patellar	Crossed extensor	Flexor
Coniine	4 mg. 25–35 min.	4–5 mg. 25–35 min.	10 mg. 15–30 min.
N-Methylconiine ..	4 mg. 5–15 min.	4–5 mg. 5–15 min.	10 mg. 10–20 min.
γ-Coniceine	0·12 mg. 25–35 min.	0·15 mg. 25–35 min.	0·8 mg. 15–30 min.
Nicotine	0·1 mg. 25–35 min.	0·1 mg. 25–35 min.	0·6 mg. 30–50 min.

The fasciculations were therefore probably partly due to motor end-plate stimulation and partly due to a central stimulant action. Table II shows the doses of the alkaloids necessary to cause block of the three types of reflex contraction and the approximate durations of the effects. The duration of the effect of *N*-methylconiine was always shorter than that of the other alkaloids. Fig. 15 illustrates the short duration of action of *N*-methylconiine compared with that of coniine. The doses of the

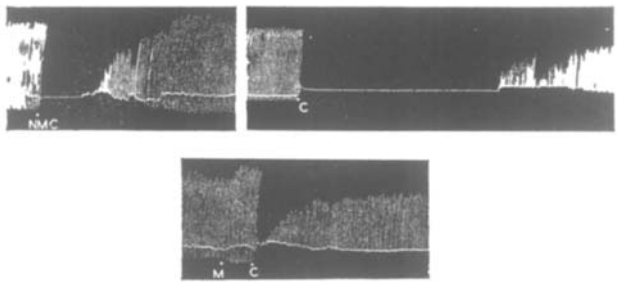

FIG. 15. Cat decerebrate. Patellar reflex contractions of quadriceps femoris once every 10 sec. At NMC, 4 mg./kg. *N*-methylconiine and at C, 4 mg./kg. coniine. 5 min. before the second injection of coniine, 20 mg./kg. mephenesin (M) was injected. All injections intravenously.

alkaloids necessary to block the patellar and crossed-extensor reflexes were much smaller than those necessary to cause peripheral neuromuscular block but the doses necessary to block flexor reflex contractions were only slightly smaller. However, at the time of maximal block of the flexor reflex, it was always possible to elicit twitches by motor nerve stimulation and such twitches were frequently not reduced below the control level (Fig. 14).

The previous administration of mephenesin reduced the ability of small doses of the hemlock alkaloids to block the patellar reflex. Fig. 15 illustrates the reduction in the action of coniine when injected after mephenesin. Furthermore, when administered during a partial block of the patellar reflex produced by the hemlock alkaloids, mephenesin caused an immediate increase in the size of the contractions (Fig. 16). However, this antagonistic effect of mephenesin was limited and could be demonstrated only when the extent of the block was small.

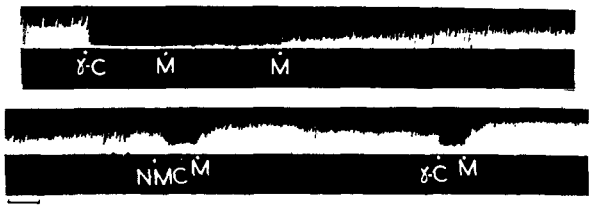

FIG. 16. Cat decerebrate. Patellar reflex contractions of quadriceps femoris elicited once every 5 sec. At γ-C, 200 μg./kg. γ-coniceine; at M, 20 mg./kg. mephenesin and at NMC, 2 mg./kg. *N*-methylconiine intravenously. Time calibration, 5 min.

Strychnine, administered intravenously in doses of 20–50 μg./kg. caused a powerful increase in the tension of the reflex contractions, particularly in the flexor and crossed-extensor reflexes. All three hemlock alkaloids in doses necessary to block the reflexes, temporarily reduced the potentiation produced by strychnine. When administered during block of all three types of reflex contraction produced by the hemlock alkaloids, strychnine caused a powerful increase in the tension of the partially blocked contractions (Fig. 17).

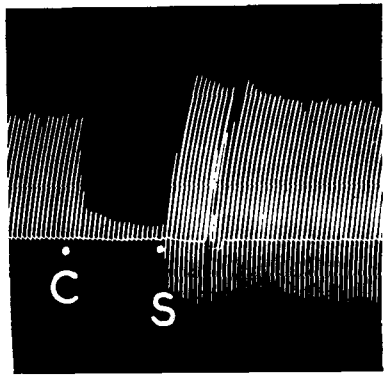

FIG. 17. Cat, decerebrate. Patellar reflex contraction of quadriceps femoris elicited once every 10 sec. At C, 6 mg./kg. coniine and at S, 60 μg./kg. strychnine intravenously.

Discussion

The most pronounced action of the three hemlock alkaloids and of nicotine was shown to be their ability, in relatively small doses, to block the crossed extensor reflex and the knee-jerk by an action exerted in the spinal cord. This action of nicotine on the knee-jerk was first demonstrated by Schweitzer and Wright (1938). With small doses, these effects occurred without any evidence of a stimulant action. The results do not, therefore, support the conclusion of de Boer (1950) that the action of coniine on the spinal cord resembles that of strychnine. In fact, the actions of the hemlock alkaloids and of strychnine were shown to be mutually antagonistic. As with most drugs possessing a central depressant action on spinal reflexes, the flexor reflex was much more resistant to the hemlock alkaloids and to nicotine than the other reflexes studied. The large doses necessary to depress this reflex initially potentiated the contractions and caused fasciculations of the muscle. This stimulant action appeared to be partly central and partly peripheral in origin.

Small doses of the hemlock alkaloids, particularly the first injection, often blocked contractions of the quadriceps muscle elicited by tapping the patellar tendon while leaving contractions of the same muscle, elicited by stimulating the central end of the contra-lateral sciatic nerve, almost unaffected. This apparently selective action is difficult to account for since the patellar reflex has been shown to be monosynaptic (Lloyd, 1952) and the final path, that is the large α-neurons to the extra-fusal muscle fibres, is common to both reflexes. Such a selective action might be explained if there is a difference in the synaptic gaps in the two reflex arcs. It may be that the boutons of the afferent fibres from the muscle spindles make closer contact with the anterior horn cells than those of the interneurons involved in the crossed extensor reflex. Alternatively, the two sets of boutons might release different transmitters and the alkaloids may preferentially block the actions of one of them. However, perhaps the most likely explanation of the selective action is that it was an artifact arising from the different methods of initiating the reflexes. The afferent sensory discharge from the muscle spindles elicited by tapping the patellar tendon may well be weaker than that induced by electrical stimulation and so the knee jerk may appear to be selectively blocked. Further work is therefore necessary to determine whether the selective action is a true one.

The action of the hemlock alkaloids in the spinal cord is complicated by the fact that both inhibitory and excitatory neurons may be affected. Both mephenesin and strychnine antagonised the depressant action of the alkaloids on the patellar reflex. This similar effect of two substances which themselves are mutually antagonistic might be explained if the alkaloids depress the patellar reflex, initially at least, by stimulating inhibitory neurons rather than by blocking excitatory ones. Although the patellar reflex arc is monosynaptic there is evidence that its central synapse is controlled by polysynaptic inhibitory pathways. Mephenesin acts preferentially on spinal interneurons (Tavener, 1952) and might,

therefore, antagonise the effect of the alkaloids by blocking the interneurons of the inhibitory pathways. There is also evidence that strychnine exerts its effects at least partly by antagonising the actions of inhibitory transmitters in the spinal cord (Bradley, Easton and Eccles, 1953; Eccles, Fatt and Koketsu, 1954).

The peripheral effects of the hemlock alkaloids on involuntary structures appeared to be mainly a consequence of a stimulant, and with larger doses, a blocking action in both parasympathetic and sympathetic ganglia. With *N*-methylconiine the blocking action predominated, the stimulant phase of the action being transient, or, in some tissues, non-existent. With γ-coniceine, on the other hand, the stimulant phase was always pronounced while the blocking action was relatively difficult to demonstrate. The action of coniine fell between those of γ-coniceine and *N*-methylconiine; in most experiments it resembled γ-coniceine and produced powerful stimulant effects but in others, the stimulant phase was absent and only the blocking action could be demonstrated. The pressor effects of coniine and γ-coniceine were shown to be a consequence both of stimulation of sympathetic ganglia and of the release of catecholamines from the adrenal medullae. The results obtained in experiments on local blood flow were in accordance with these findings. When injected intra-arterially into muscle or skin, only a weak vasodilatation was produced. With this route of injection, the drugs do not reach the sympathetic ganglia and therefore do not cause vasoconstriction. When injected intravenously, vasoconstriction occurred in the skin but an increase in flow still occurred in the muscles. The muscle vessels of the cat differ from those of the skin in that they possess a dual innervation-sympathetic cholinergic vasodilator fibres (Folkow and Uvnäs, 1948*b*, 1950) and sympathetic vasoconstrictor fibres which liberate noradrenaline (Folkow and Uvnäs, 1948*a*). The effects of stimulation of both types of fibre must counteract each other and the resulting change in calibre will therefore be small. Both adrenaline and noradrenaline released from the adrenal medullae may cause vasodilatation in skeletal muscles (Bowman, 1959) and the rise in blood pressure caused by vasoconstriction in other areas probably forces more blood through the muscle vessels whatever the slight change in their calibre, so that only an increase in flow is recorded.

The ability of the alkaloids to cause a quick contraction on close-arterial injection into the tibialis anterior muscle of the cat and a slow contracture of the frog rectus abdominis and gastrocnemius muscles of the hen may be taken as evidence of a depolarising action. It was of interest that in the mammal, *N*-methylconiine was more powerful than coniine in stimulating skeletal muscle but considerably less powerful in stimulating autonomic ganglia.

Although the alkaloids appeared to possess a depolarising action, the neuromuscular block which they produced differed in many respects from that produced by the depolarising blocking drugs, decamethonium and suxamethonium. With the first close-arterial injection of coniine and γ-coniceine there was a considerable latent period between the immediate contraction they produced and the development of neuromuscular block.

With decamethonium and suxamethonium, both the contraction and the block are known to be a consequence of the same mechanism, namely depolarisation of the motor end-plates. With these drugs therefore neuromuscular block is always present immediately after the initial contraction. For persistent depolarisation to occur, the affinity of the drug for the acetylcholine receptors must presumably be high so that the drug remains in dynamic equilibrium with the receptors and keeps the end-plates depolarised. It seems likely that the hemlock alkaloids have a low affinity for the receptors. When injected close-arterially in high concentration they are able to react with the receptors to cause a sudden but fleeting depolarisation and consequent contraction, after which they probably dissociate rapidly and are quickly diluted to subthreshold strength. It appears therefore that their blocking action may be unrelated to their ability to cause end-plate depolarisation. The block produced by the hemlock alkaloids also differed from block produced by tubocurarine and from the "dual block" which is sometimes produced by depolarising drugs and which was first described by Zaimis (1953).

In the experiments on the cat, the alkaloids blocked the response of the muscle to close-arterially injected acetylcholine showing that at least part of their blocking action is post-junctional. The alkaloids are secondary and tertiary amines and they are well absorbed after oral or subcutaneous administration, penetrating the blood brain barrier to exert central actions. These facts imply that the alkaloids can penetrate cell membranes readily and suggest that their blocking action at the motor end-plate might be a consequence of an intracellular action which renders the end-plate inexcitable by acetylcholine. del Castillo and Katz (1955) have shown that the acetylcholine receptors are located only on the external surface of the motor end-plate and reaction with these receptors would account for the initial contraction produced by large amounts of the alkaloids. The slow onset of the subsequent block might then be explained if the alkaloids require time to penetrate the cell membrane. An intracellular mechanism of action would also account for the finding that in the rectus abdominis muscle of the frog, concentrations of the alkaloids, smaller than those necessary to cause contracture, blocked the response of the muscle to acetylcholine providing they were left in contact with the tissue for a sufficient time. Bennett, Tyler and Zaimis (1957) postulated a similar intracellular mechanism of action for the secondary amine mecamylamine.

Although, in the cat, the response to close-arterially injected acetylcholine was blocked by the hemlock alkaloids, it returned sooner than it did following a similar degree of block produced by tubocurarine. In the hen, the response of the muscle to acetylcholine was only slightly reduced by the alkaloids even at the time of maximum depression of the indirectly excited twitches. These results suggest that the failure in neuromuscular transmission produced by the alkaloids is partly due to an action on the nerve endings through which the amount of acetylcholine released by a nerve impulse is reduced. The local anaesthetic, procaine, is known to cause neuromuscular block through both pre- and

post-junctional mechanisms (Harvey, 1939; Straughan, 1961) and this action is usually attributed to its local anaesthetic action stabilising the cell membranes and preventing the abrupt changes in permeability which are necessary for depolarisation of both nerve terminals and motor end-plates to occur. The local anaesthetic activity of the hemlock alkaloids was tested in the present experiments but did not appear powerful enough to account for their neuromuscular blocking actions although it may have contributed to the effect.

The blocking action of *N*-methylconiine was quickest in onset after close-arterial injection. This alkaloid was the least readily absorbed after oral administration and these findings may indicate that its blocking action has a large extra-cellular component in it. This might be expected since at body pH, the tertiary amine will carry the strongest positive charge. The finding that, unlike the other two alkaloids, *N*-methylconiine was without effect on respiration when injected into the carotid artery may also reflect an extracellular action.

The mechanism of action of the alkaloids on neuromuscular transmission is obviously complex and may well involve more than one site of action. This is not unlikely with substances of this type which combine with acetylcholine receptors but whose action, unlike that of quaternary ammonium compounds, is not confined to extra-cellular sites.

In general, Sollman's (1957) statement that the effects of coniine resemble those of nicotine both centrally and peripherally, was confirmed. With few exceptions, *N*-methylconiine and γ-coniceine had qualitatively similar actions. With the possible exception of the effects of small doses of the alkaloids on the spinal cord their actions are clearly too widespread to be of real therapeutic use. Indeed it seems unlikely that hemlock could ever have been administered in doses sufficient to produce the peripheral actions for which it was recommended in the older clinical literature. The central depressant action of the alkaloids is of interest, that on the knee-jerk and crossed extensor reflex occurring with doses which produced only minor additional actions. This action of the alkaloids was obviously the basis of the use of hemlock in spastic states in man, and not, as was believed by many, the peripheral neuromuscular blocking action which requires much larger doses. The central depressant action was much longer lasting and occurred with smaller doses than that produced by mephenesin, and the alkaloids might therefore serve as a starting point for the synthesis of more specific and less toxic spinal relaxants.

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