Brit. J. Pharmacol. (1962), **19**, 111–119.

AN ESERINE-LIKE ACTION OF CHLORAL HYDRATE

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(*Received March* 6, 1962)

The intra-arterial injection of chloral hydrate potentiated the transmission of nerve impulses through the cat superior cervical ganglion, antagonized the ganglion-blocking action of hexamethonium, and greatly enhanced the ganglion-stimulant action of acetylcholine. Effects on the ganglion-stimulant actions of carbachol, nicotine, tetramethylammonium and potassium chloride were slight or absent. Chloral hydrate itself usually had no direct stimulant action. The neuromuscular-blocking action of tubocurarine on the isolated rat diaphragm preparation was completely and rapidly reversed by chloral hydrate. This reversal was prevented by previous treatment of the muscle with neostigmine. Chloral hydrate potentiated the actions of acetylcholine and nicotine on the isolated rabbit duodenum, and, in concentrations exceeding 1 mg/ml., produced a spasm which was abolished by hyoscine but not by mepyramine. It was concluded that these eserine-like effects were manifestations of an anticholinesterase action of chloral hydrate. Neither chloralose nor trichlorethanol showed evidence of this property.

During the course of an investigation of the effects of central depressant drugs on synaptic transmission, it was observed that chloral hydrate could facilitate transmission through the cat superior cervical ganglion. In view of the *in vitro* anticholinesterase activity of chloral hydrate reported by Dybing & Dybing (1955), an attempt has been made to determine how far the effects of chloral hydrate on synaptic transmission could be ascribed to the inhibition of cholinesterase.

METHODS

Cat superior cervical ganglion preparation. Cats were anaesthetized with 60 mg/kg of chloralose, injected intravenously after induction with ethyl chloride and ether, or with an intraperitoneal injection of 35 to 45 mg/kg of sodium pentobarbitone. Transmission through the superior cervical ganglion was studied by recording the contraction of the nictitating membrane on a smoked paper, using a lever giving a magnification $\times 10$. The preganglionic cervical sympathetic trunk was isolated, sectioned approximately 2 cm from the superior cervical ganglion, and maintained at body temperature under liquid paraffin. The peripheral end of the nerve was stimulated electrically through bipolar platinum electrodes with rectangular pulses of 0.2 msec duration, delivered through an isolating transformer from an electronic stimulator of the type used by Bell (1957). Bursts of impulses of 15 sec duration each minute at a frequency of 50/sec, or more prolonged trains of impulses at 10/sec, were used. The stimulus strength employed was sufficient to evoke a contraction of the membrane which was maximal at the selected frequency. In many experiments the cervical sympathetic trunk was partially resected between the point of stimulation and the ganglion, so that supramaximal nerve stimulation produced a submaximal membrane contraction (Kamijo & Koelle, 1952).

Drugs were administered either intravenously *via* the femoral vein, or by retrograde intra-arterial injection through a cannula inserted into the lingual artery, the external carotid artery being occluded during the injection (Morrison & Paton, 1953). The drugs were dissolved in 0.9% sodium chloride solution, and an injection volume of 0.5 ml. was always used.

Contractions of the nictitating membrane were also elicited by the intra-arterial injection of the ganglion-stimulant drugs acetylcholine, nicotine, tetramethylammonium, carbachol and potassium chloride. In 8 cats the preganglionic nerve on one side was sectioned three weeks prior to the experiment. Degeneration of the nerve distal to the point of section was confirmed by visual inspection of the cut nerve, by the relaxation of the nictitating membrane, and by the increased sensitivity of the ganglion to acetylcholine. In these animals ganglion-stimulant drugs were injected intra-arterially to the ganglia on both sides, enabling the effects of chloral hydrate on normal and on chronically denervated ganglia to be compared in the same cat.

In every experiment 1 mg/kg of hyoscine was injected intravenously approximately 1 hr before recording commenced.

Isolated rat phrenic nerve-diaphragm preparation. Adult rats were killed with a blow on the head and bled out. A segment of the diaphragm with the phrenic nerve attached was rapidly obtained, mounted on an electrode assembly, and immersed in a bath containing 50 ml. of Krebs solution. The bath fluid was maintained at 38° C and was bubbled with a 5% carbon dioxide/95% oxygen mixture. The phrenic nerve was stimulated supramaximally by single electrical pulses of 0.1 msec duration delivered at a frequency of 5/min. The twitches of the diaphragm muscle were recorded on a smoked paper by means of a spring-loaded lever (magnification, ×10).

Isolated rabbit duodenum preparation. A segment of rabbit duodenum, approximately 2 cm long, was mounted in a bath containing 30 ml. of Tyrode solution, maintained at 38° C and bubbled with a 5% carbon dioxide/95% oxygen mixture. The contractions of the longitudinal muscle were recorded on a smoked paper using a frontal writing lever (magnification, ×5).

Isolated frog rectus abdominis muscle preparation. The muscle was suspended in a 10 ml. bath containing frog Ringer solution at room temperature and bubbled with air. The contracture responses to acetylcholine and potassium chloride were recorded on a smoked paper with a Gimbal lever (magnification, ×10).

Drugs. Eserine salicylate, acetylcholine chloride, carbachol chloride, nicotine hydrogen tartrate, tetramethylammonium bromide, tetraethylammonium bromide, hexamethonium chloride, tubocurarine chloride, neostigmine sulphate and hyoscine hydrobromide were administered as the salt, and doses refer to the weight of salt. Doses of chloral hydrate, trichlorethanol and chloralose refer to the pure compound.

RESULTS

Cat superior cervical ganglion preparation

Action of chloral hydrate and other drugs on the effect of preganglionic nerve stimulation. In each of 5 experiments, the intra-arterial injection of 0.5 mg (or more) of chloral hydrate in 0.5 ml. augmented the contractions of the nictitating membrane elicited by stimulating the cervical sympathetic nerve intermittently for 15 sec/min at 50/sec (Fig. 1, at CH). The action of chloral hydrate was transient, and was readily repeatable in the same cat. It closely resembled the effect of an intra-arterial injection of 10 μg/ml. or more of eserine (Fig. 1, at Es). In no instance was the effect of postganglionic stimulation enhanced.

The intra-arterial injection of chloral hydrate also increased the height of the membrane contraction when the preganglionic nerve was stimulated at 10/sec, provided that transmission through the superior cervical ganglion had been rendered

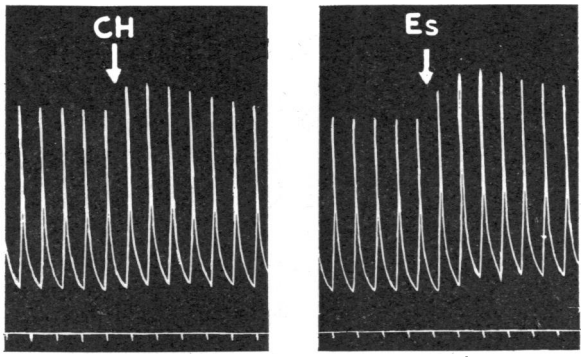

Fig. 1. Cat, 3.3 kg. Pentoba bitone anaesthesia. Records of the contractions (upwards) of the nictitating membrane evoked by maximal intermittent stimulation of the preganglionic cervical sympathetic nerve at 50/sec for 15 sec/min. Hyoscine, 1 mg/kg, was injected intravenously about 1 hr before recording. The intra-arterial injection of 2.5 mg chloral hydrate (at CH) or of 20 μg eserine (at Es) to the superior cervical ganglion through the lingual artery while the external carotid artery was occluded augmented the height of the membrane contractions. Time, 1 min.

submaximal by partial section of the preganglionic nerve distal to the region where the stimulus was applied (16 experiments). Otherwise, little effect was seen with this stimulus frequency. In contrast, transmission was never increased by intra-arterial injections of chloralose (0.2 to 10 mg), trichlorethanol (20 μg to 5 mg), ethanol (1 to 100 mg), paraldehyde (0.5 to 2 mg), methylpentynol (0.5 to 2 mg), methylpentynol carbamate (0.1 to 1 mg), or the central stimulant drugs leptazol (2 μg to 1 mg) or bemegride (5 μg to 500 μg).

Effect of chloral hydrate on the action of ganglion-stimulant drugs. Chloral hydrate greatly augmented the stimulant action of acetylcholine on the cat superior cervical ganglion in each of 8 experiments. In 3 cats, the preganglionic cervical sympathetic nerve on one side was sectioned three weeks before the experiment. In these animals, chloral hydrate potentiated the effect of acetylcholine on both normal and chronically denervated ganglia (Fig. 2).

A single intra-arterial injection of chloral hydrate enhanced the effect only of the immediately subsequent injection of acetylcholine, whereas the effect of a single injection of eserine usually persisted for 30 min or more.

The lowest dose of chloral hydrate observed to potentiate the action of acetylcholine was 50 μg (in 0.5 ml.). Intra-arterial injections of from 10 μg to 1 mg of chloralose, or from 100 μg to 1 mg of trichlorethanol, were without effect.

Carbachol was injected intra-arterially to both normal and chronically denervated superior cervical ganglia in 2 cats. In both experiments, the denervated ganglion was slightly more sensitive to carbachol. Chloral hydrate slightly increased the effect of carbachol, but by an amount considerably less than the potentiation of the action of acetylcholine. The augmented response to carbachol was greater on the normal than on the preganglionically denervated side of the preparation. Eserine slightly increased the effect of carbachol on the normal ganglion of one cat, but did not change the sensitivity of the denervated ganglion to carbachol in either experiment.

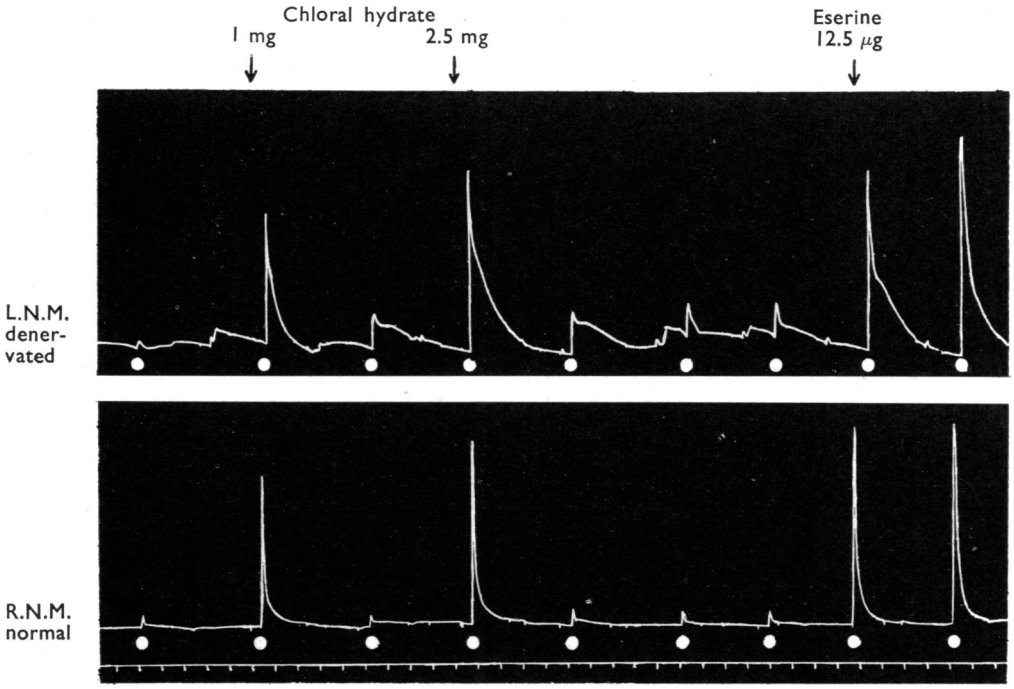

Fig. 2. Cat, 6.3 kg, anaesthetized with chloralose. Responses of the left and right nictitating membranes (upper and lower traces respectively) following stimulation of the corresponding superior cervical ganglia by acetylcholine (injected intra-arterially at the white dots). The left cervical sympathetic nerve had been sectioned preganglionically three weeks previously. The intra-arterial dose of acetylcholine was 20 μg on the left (denervated) side and 50 μg on the right (normal) side. Chloral hydrate, 1 mg and 2.5 mg, and eserine 12.5 μg, were injected intra-arterially to both ganglia at the arrows, 30 sec before the injection of acetylcholine. Hyoscine, 1 mg/kg, was administered intravenously 1 hr before recording started. Time, 1 min.

Eserine did not potentiate the actions of either nicotine or tetramethylammonium on normal or denervated ganglia in each of 2 experiments. The effect of chloral hydrate on the action of these stimulant drugs was variable, but was neither as great nor as consistent as its effect on acetylcholine activity, and in over half of the cats tested (5 out of 9) produced no change in sensitivity.

The action of potassium chloride on normal and denervated ganglia in 2 cats was potentiated neither by chloral hydrate nor by eserine.

The intra-arterial injection of chloral hydrate in doses up to 50 mg evoked no contraction of the nictitating membrane in the absence of other forms of stimulation in 38 out of 40 cats. In the other 2 animals, both of which had received repeated injections of nicotine and tetramethylammonium, the intra-arterial injection of 5 mg of chloral hydrate produced an immediate contraction of the membrane which was abolished by tetraethylammonium in doses of 2 mg/kg intravenously or of 200 μg intra-arterially.

Antagonism to hexamethonium. In 3 cats in which an almost complete block of ganglionic transmission was produced by the intravenous injection of hexamethonium, the subsequent intra-arterial injection of chloral hydrate or of eserine

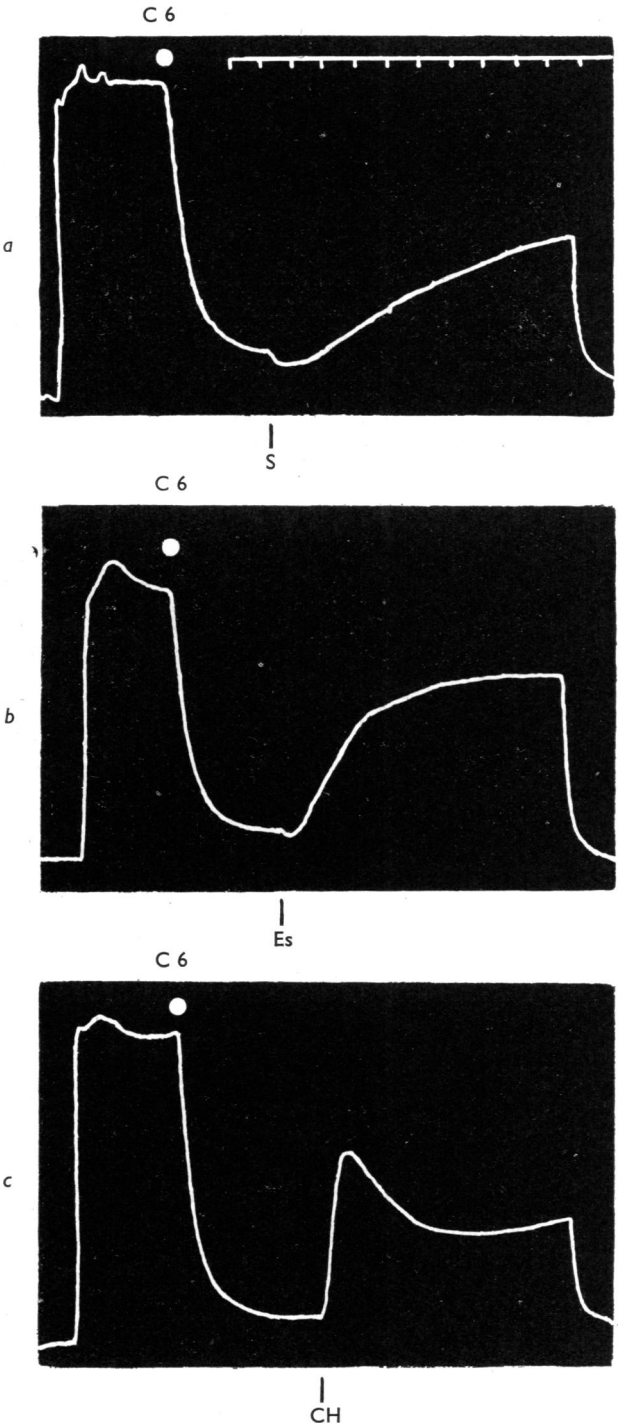

Fig. 3. Cat, 4.4 kg, anaesthetized with pentobarbitone. Records of the contracture of the nictitating membrane elicited by continuous maximal stimulation of the preganglionic cervical sympathetic nerve at 10/sec. Hyoscine, 1 mg/kg, was injected intravenously 1 hr before recording. At the white dots, 0.2 mg/kg of hexamethonium (C6) was injected intravenously. During the ensuing ganglion-block, 0.5 ml. of saline solution (at S in record *a*), 5 μg of eserine (at Es in record *b*), and 5 mg of chloral hydrate (at CH in record *c*) were injected intra-arterially to the superior cervical ganglion. Time, 1 min.

reversed the block. Fig. 3 illustrates this reversal, and shows that the action of chloral hydrate was more rapid in onset and more transient than that of eserine.

Isolated rat phrenic nerve-diaphragm preparation

The neuromuscular-blocking action of tubocurarine on this preparation was rapidly reversed by chloral hydrate in a concentration of 100 μg/ml. or greater (Fig. 4*a*). The antagonism to tubocurarine was abolished by soaking the preparation in Krebs solution containing 10 μg/ml. of neostigmine (Fig. 4*b*). Concentrations of

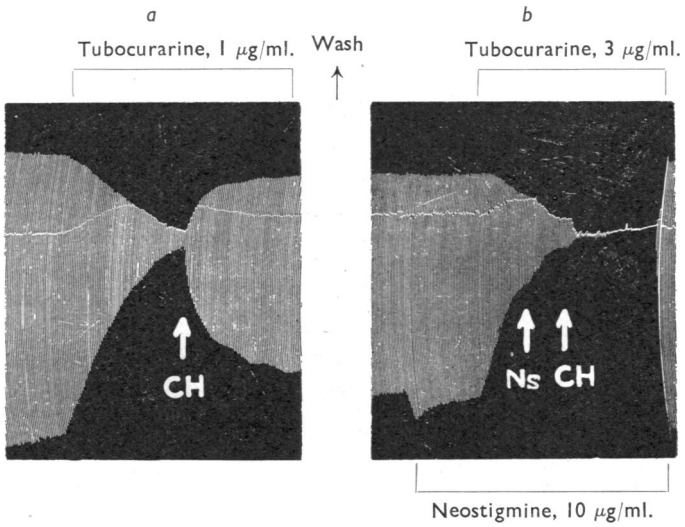

Fig. 4. Contractions of the isolated rat diaphragm elicited by single maximal stimuli applied to the phrenic nerve at a frequency of 5/min. *a*, The neuromuscular block produced by 1 μg/ml. of tubocurarine was reversed on adding chloral hydrate (at CH) to give a final bath concentration of 500 μg/ml. *b*, In the presence of 10 μg/ml. of neostigmine, 3 μg/ml. of tubocurarine was required to produce a similar degree of neuromuscular block. Now, the administration of a further 5 μg/ml. of neostigmine (at Ns), or of 500 μg/ml. of chloral hydrate (at CH), only increased the block of transmission. A time interval of approximately 30 min elapsed between the two records, during which the preparation was washed several times.

chloralose or trichlorethanol up to 1 mg/ml. did not reverse the neuromuscular block produced by tubocurarine.

Isolated rabbit duodenum preparation

Concentrations of chloral hydrate between 100 μg/ml. and 1 mg/ml. often increased the amplitude of the spontaneous movements of the duodenum (Fig. 5*b*), and enhanced the responses of the tissue to acetylcholine (Fig. 5*a*) and to nicotine (Fig. 5*b*). This action resembled the effect of 1 to 10 μg/ml. of eserine, with the exception that the potentiating effect of chloral hydrate was more readily reversed by washing.

Concentrations of chloral hydrate exceeding 1 mg/ml., or concentrations of eserine exceeding 10 μg/ml., produced a slowly increasing spasm of the duodenal

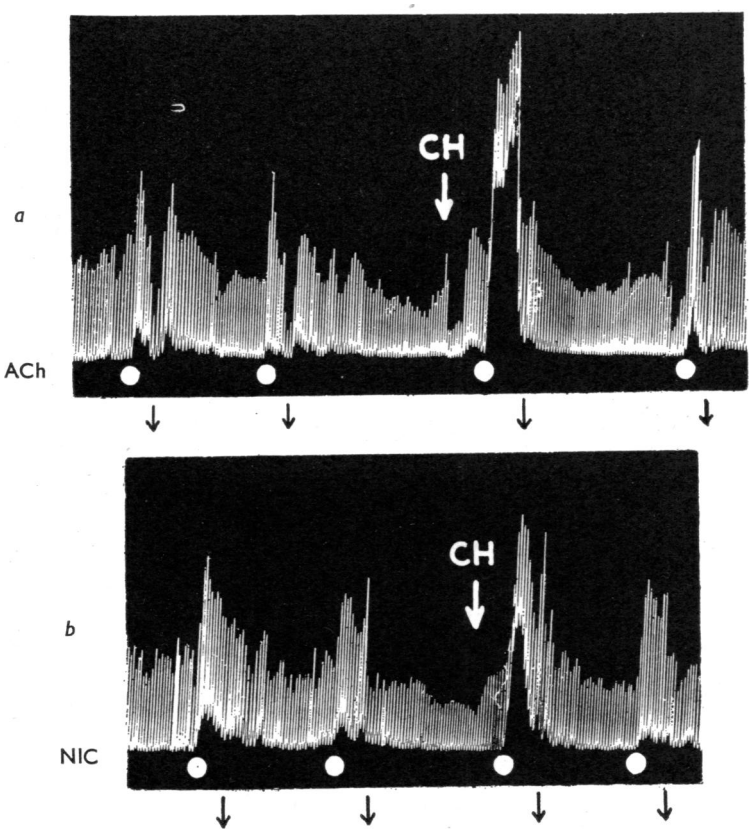

Fig. 5. Records of the effects of (*a*) 0.4 μg of acetylcholine (ACh), and (*b*) 20 μg of nicotine (NIC), on isolated segments of duodenum from the same rabbit suspended in 30 ml. baths. The stimulant drugs were added to the bath at the white dots, and the bath fluid was changed at the arrows. Chloral hydrate, 15 mg, was added at CH ↓.

muscle. The spasm evoked by either agent was abolished by 0.1 μg/ml. of hyoscine but not by a similar concentration of mepyramine.

Neither chloralose nor trichlorethanol increased the activity of acetylcholine on the duodenum; 1 mg/ml. of chloralose slightly reduced spontaneous motility and depressed the effect of added acetylcholine. Trichlorethanol acted in a similar manner at about one-third of this concentration. After washing out the trichlorethanol, a contraction occurred which declined without further washing within 5 min.

Isolated frog rectus abdominis muscle preparation

Chloral hydrate (0.2 to 1 mg/ml.) potentiated the stimulant action of acetylcholine on the rectus muscle. At a concentration of 1 mg/ml. of chloral hydrate, the effect of potassium chloride was also augmented. A reversible contracture of the muscle was produced by a concentration of chloral hydrate above 1 mg/ml. Eserine (5 to 10 μg/ml.) enhanced the activity of acetylcholine but not that of

potassium chloride. After immersing the rectus for 30 min in Ringer solution containing 10 μg/ml. of eserine, a concentration of 1 mg/ml. of chloral hydrate still increased the height of the contracture evoked by added acetylcholine, but the potentiating action of concentrations of chloral hydrate below 1 mg/ml. was abolished.

Trichlorethanol (0.1 to 0.3 mg/ml.) increased the height of the contracture elicited by both acetylcholine and potassium chloride. Like the potentiation produced by chloral hydrate, this effect was quickly reversed on washing; 1 mg/ml. of trichlorethanol itself evoked a contracture of the muscle which was immediately reversed on removing the trichlorethanol from the bath fluid.

DISCUSSION

Chloral hydrate potentiated the transmission of nerve impulses through the superior cervical ganglion, reversed the ganglion-blocking action of hexamethonium, and greatly augmented the ganglion-stimulant action of acetylcholine. In contrast, it was without effect on the ganglion-stimulant action of potassium chloride, and produced at most only a slight and variable potentiation of the actions of nicotine and tetramethylammonium. In all but 2 out of 40 experiments chloral hydrate failed to evoke a retraction of the nictitating membrane when injected intra-arterially to the ganglion.

These actions of chloral hydrate closely resemble those of eserine, which suggests that they arise from inhibition of cholinesterases. Reports that concentrations of chloral hydrate comparable to those employed in the present study (100 μg/ml. or more) inhibit the *in vitro* cholinesterase activity of homogenates of rat brain (Bernheim & Bernheim, 1936; Dybing & Dybing, 1955) and of plasma (Heim & Fahr, 1940) support this suggestion.

Further pharmacological actions of chloral hydrate compatible with an anticholinesterase action were recorded on the isolated rabbit duodenum and at the neuromuscular junction. Chloral hydrate evoked a spasm of the duodenum which was reversed by a low concentration of hyoscine. Chloral hydrate also antagonized the neuromuscular-blocking action of tubocurarine on the isolated rat diaphragm, but was ineffective after inactivation of the cholinesterases by prior treatment of the muscle with neostigmine. The latter observation is in agreement with that of Dybing & Dybing (1955), who reported that eserine likewise abolished the anticurare action of chloral hydrate on the isolated rat diaphragm.

On the frog rectus abdominis muscle, chloral hydrate appears to have other effects in addition to an anticholinesterase action. In concentrations greater than those required to potentiate the action of acetylcholine, chloral hydrate also increased the effect of potassium chloride. Still larger concentrations evoked a contracture. These effects differed from those of eserine and suggest that chloral hydrate has also a direct stimulant action. Butler (1949) has shown that many tissues, including striated muscle, rapidly convert chloral hydrate into trichlorethanol, a substance which has a much lower anticholinesterase activity than its parent compound (Dybing & Dybing, 1955). Since trichlorethanol was a more potent stimulant of the frog rectus muscle than was chloral hydrate itself, it may be that trichlorethanol was

formed from chloral hydrate in the presence of the muscle, and that the direct stimulant action of trichlorethanol became superimposed on the anticholinesterase action of the parent compound when large doses of the latter were used. On two occasions, chloral hydrate also stimulated the superior cervical ganglion. This could not have been due to the formation of trichlorethanol, for trichlorethanol had a depressant action at this synapse. Since the stimulation was abolished by tetraethylammonium, one explanation may be that chloral hydrate provoked a massive discharge of transmitter from the preganglionic fibres, perhaps rendered unduly excitable by the previous administration of many injections of nicotine and tetraethylammonium.

The eserine-like actions of chloral hydrate reported in this paper serve to distinguish it pharmacologically from related central depressant drugs. However, the high concentrations necessary to produce these effects are only transiently attained *in vivo* even after the intravenous or intraperitoneal injection of sufficient chloral hydrate to induce anaesthesia (Butler, 1948; MacKay & Cooper, 1962). Hence, unless the official dosage be greatly exceeded or administered concurrently with other drugs having marked activity of a similar nature, it is highly unlikely that anticholinesterase effects would be produced by the dose of chloral hydrate normally employed for hypnotic purposes.

This work was undertaken in partial fulfilment of the requirements for the Ph.D. degree of the University of London and was supported by a grant from the U.S.A.F., No. 61(052)-25. The author is grateful to Professor J. P. Quilliam for his advice and encouragement; to Dr D. F. J. Mason for help in the denervation of the superior cervical ganglion; to Mr P. M. G. Bell for the electronic stimulating apparatus; and to Miss J. Allen for technical assistance.

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