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THE PHARMACOLOGICAL ACTIONS OF MUREXINE (UROCANYLCHOLINE)

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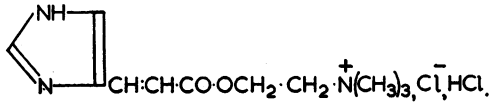
Murexine or urocanylcholine is a naturally occurring choline ester of urocanic acid which was found in very large amounts in the hypobranchial body of *Murex trunculus* and other prosobranchiate molluscs. In vertebrates and invertebrates, it was found to possess marked neuromuscular blocking and nicotinic actions, but was almost devoid of muscarinic effects. The blocking action of murexine was considered, on the basis of experimental and clinical evidence, to be of the " depolarizing " type. It was weaker than, but qualitatively very similar to, that produced by suxamethonium. The nicotinic action of murexine was stronger than that of suxamethonium in both experimental animals and human beings.

Murexine or urocanylcholine (β-[imidazole-4(5)]-acryl-choline) is a naturally occurring choline ester found in the hypobranchial body of some prosobranchiate molluscs, which might use it for defence or, more likely, for procuring food.



Murexine was first isolated by Erspamer (1948); its chemical constitution was determined by Erspamer and Benati (1953a, 1953b); and the proposed formula was confirmed by synthesis (Pasini, Vercellone and Erspamer, 1952). Sufficient quantities of the substance were made which permitted extensive pharmacological studies and a preliminary clinical trial. The present paper gives an account of these studies.

METHODS AND MATERIALS

Murexine Samples.—Both natural and synthetic murexine chloride hydrochloride (mol. w., 296.2; 1 mg.=0.76 mg. base) were used throughout the experiments. The natural product was prepared as described by Erspamer and Benati (1953b). The synthetic product was obtained according to the procedure of Pasini, Vercellone and Erspamer (1952) which has recently been improved by Pasini (unpublished observation).

During the course of experiments carried out to improve the synthetic procedure, it was observed that different murexine samples, identical according to analysis for elements and paper-chromatographic characteristics, showed slightly different biological activity. This may perhaps be attributed to the presence of isomers or rearrangement derivatives of murexine. Here we wish only to point out that the activity of the synthetic murexine samples used in the present experiments differed from that of the natural product by less than 5%; thus the synthetic product was at least 95% pure.

Preparation of Hypobranchial Extracts.—The hypobranchial bodies (median zone), removed from the living animal (*Murex trunculus*, *Murex brandaris* and *Tritonalia erinacea*) after cautious rupture of the shell, were placed in 3 to 4 volumes of acetone. After standing for 24 hr., the solvent was poured off and filtered, and the tissue re-extracted with a further 3 volumes of 90% acetone. The combined filtrates were stored in the refrigerator until used. The solvent was then evaporated under reduced pressure at 45° C., and the liquid brought to the desired volume with distilled water.

The bioassay was carried out on the isolated frog rectus abdominis muscle suspended in a 10 ml. bath of frog Tyrode solution bubbled with air.

Pharmacological Methods.—The doses required to cause head-drop in 50% of rabbits and to paralyse 50% of dogs were determined after rapid intravenous injection.

The anaesthetics used were chloralose (70 mg./kg., i.v.), pentobarbitone (30 mg./kg., i.v.), Pernocton (70 mg./kg., i.m.) and urethane (1 to 1.3 g./kg., i.v.). Cats and dogs received intravenous injections or infusions into the femoral vein, rabbits into the ear marginal vein, rats and mice into the tail vein. Blood pressure was recorded from the femoral or carotid artery. Respiration was recorded by means of a tracheal cannula connected to a tambour. Adequate oxygenation of the blood during apnoea was maintained by a respiratory pump or by passing oxygen through a tracheal catheter.

Muscarine-like action was tested on the guinea-pig and rabbit intestine, on the rabbit atrium and on the isolated frog heart. The isolated frog rectus abdominis

muscle and the spinal cat prepared as described by Burn (1950) were used to test for nicotinic effects.

Other preparations employed in customary fashion were the leech dorsal muscle, the enucleated frog and octopod eye (the latter being kept in sea water) and the heart of *Helix pomatia*, suspended in the liquid recommended by Zettler and Schlosser (1954). The cat, dog and rabbit sciatic nerve gastrocnemius preparations were prepared as described by Burn (1950). Maximal stimuli (rectangular current pulses of 7 msec. duration) were applied to the sciatic nerve through a unipolar electrode at the rate of 5 or 10/min., and the contractions were recorded semi-isometrically.

The rat phrenic nerve-diaphragm preparation as described by Bülbring (1946) was used in a few experiments. The drug was left in contact with the preparation for 4 min.

Results

Occurrence and Distribution of Murexine

Table I shows the murexine content (expressed in terms of free base) in different areas of the hypobranchial body of *M. trunculus*, *M. brandaris* and *T. erinacea*. Only traces of murexine were found in other tissues.

TABLE I

THE MUREXINE CONTENT OF THE HYPOBRANCHIAL BODY

M indicates the median zone (MA, pars anterior; MP, pars posterior); B, the branchial zone; and R, the rectal zone of the hypobranchial body (see Erspamer and Dordoni, 1947). HB indicates the entire hypobranchial body. The numerals in parentheses give the number of specimens examined. The weights are expressed in mg./g. of fresh tissue.

Tissue		Dry Residue of Acetone Extract (mg./g.)	Murexine Content (mg./g.)
M. trunculus	MP (151)	114	46·0
,, ,,	MA (124)	60	16·5
,, ,,	B (132)	24	1·2
,, ,,	R (242)	31	1·0
,, ,,	MP (50)	—	33·0
,, ,,	MA (50)	—	11·0
,, ,,	M (3,350)	82	23·0
M. brandaris	M (22)	50	1·3
,, ,,	MP (19)	—	0·1
T. erinacea	HB (23)	—	7·6

The results in Table I show: (a) that virtually all murexine in the hypobranchial body was contained in the median zone, and that it was most concentrated in the posterior part (75%) in *M. trunculus*, and in the anterior part (90%) in *M. brandaris*; and (b) that there were very conspicuous differences in the murexine content of the hypobranchial body of different *Murex* species. For *M. trunculus*, the dry residue of the acetone extract of the median zone may contain 35% of murexine, for *M. brandaris* only 2.6%. Since the weight of the median zone of a hypobranchial body of *M. trunculus* is approximately 0.1 to 0.15 g. (average of 3,350 specimens), it is easy to calculate that each *Murex* contains 2 to 4 mg. of murexine base.

Whittaker (1955) and Keyl and Whittaker (1955) have added to the list of murexine-yielding molluscs two North Atlantic species related to *Murex*, *Urosalpinx cinerea* and *Thais lapillus*. The concentration of the choline ester in the whole organism was 360 and 230 μg./g. fresh tissue, respectively. Welsh (1955) has tentatively identified as murexine one of the quaternary ammonium bases responsible for the paralysing action displayed by acetone extracts of the tentacles of certain jellyfishes and siphonophores.

Pharmacological Actions

General Effects.—The basic action of murexine in both vertebrates and invertebrates is to provoke a paralysis of the skeletal musculature. Death is caused by anoxia secondary to peripheral respiratory arrest. The heart continues to beat vigorously for several minutes after respiration has ceased.

It is not worth while describing in detail the pattern of the acute intoxication provoked by murexine in mammals. It corresponds to that described for other neuromuscular blocking agents, particularly decamethonium (Paton and Zaimis, 1949) and suxamethonium (Bovet and Bovet-Nitti, 1955). In the dog, for example, relaxation occurred within 1 min. of a single intravenous dose of 0.5 mg./kg. of murexine chloride hydrochloride, became maximal within 2 min. and disappeared within 4 to 7 min. Transient apnoea sometimes occurred at the time of maximal effect. Muscular relaxation of longer duration was easily obtained by repeated injections at appropriate intervals, or by intravenous infusion.

Particularly if murexine was administered too rapidly, neuromuscular depression was regularly preceded by transient stimulation, as shown by an increase in muscular tension and the appearance of muscular fasciculations. Respiratory movements, too, before being depressed by the drug, were powerfully stimulated for some seconds. At the time of maximal effect there was a conspicuous increase in salivary secretion, signs of increased lachrymal and bronchial secretion and, frequently, evacuation of urine and faeces.

When injected by the subcutaneous route in rabbits, rats and mice, about 5 to 8 times the intravenous dose was required for equal maximal effects and the paralysis did not appear for 10 to 20 min. and lasted for about 1 to 2 hr. Muscular paralysis and respiratory depression were preceded or accompanied by polypnoea, muscular twitches and fasciculations all over the body, salivary hypersecretion, intestinal borborygmi (in the rabbit) and defaecation.

TABLE II
COMPARISON OF THE DOSES OF MUREXINE WITH THOSE OF OTHER NEUROMUSCULAR BLOCKING AGENTS WHICH HAVE THE SAME PHARMACOLOGICAL ACTIVITY AND TOXICITY
Doses are given in mg./kg.

	Murexine (Chloride Hydrochloride)	Suxamethonium (Iodide)	Decamethonium (Iodide)	Gallamine (Iodide)	Tubocurarine (Chloride)
Mouse: LD50 by i.v. route	8·1–8·7	0·55–0·60	0·75–1·33	3·14	0·15–0·16
Rabbit: head drop, ED50	0·65	0·20–0·25	0·10–0·14	0·45	0·15–0·20
Dog: paralysing dose ED50	0·35	0·20	0·10	0·40	0·20
Man: mean clinical dose by single injection ..	1·0–1·2	0·50–1·0	0·04–0·1	1·2–2·0	0·10–0·25

When death occurred, terminal asphyxial convulsions sometimes appeared, but these were mild owing to the almost complete muscular paralysis. Muscular twitchings and fasciculations persisted for 10 to 15 min. after death.

The activity and toxicity of murexine is compared with that of other neuromuscular blocking agents (see Bovet and Bovet-Nitti, 1955) in Table II.

When given subcutaneously, the LD50 of murexine in mice is approximately 50 mg./kg.; when administered by mouth the drug is ineffective in doses up to 1 g./kg. Under artificial respiration dogs can resist more than 200 times the paralysing dose of murexine.

In birds murexine, like suxamethonium and decamethonium, provokes contracture (extension cramp of the legs, opisthotonos) instead of muscular paralysis. Myosis and evacuation of the bowels are sometimes observed at the same time. In pigeons the minimum active dose by the intravenous route is approximately 0.05 mg./kg., the lethal dose 0.2 to 0.3 mg./kg. Frogs and fishes are paralysed, like mammals. In fishes, the last muscles to be paralysed and the first to recover are the branchial ones.

The injection of 30 to 40 mg./kg. of murexine into the branchial heart of *Eledone moschata*, an octopod, is followed by a short period (1 to 2 min.) of stimulation and motor agitation with deep, forced respiratory movements. Soon afterwards, muscular weakness supervenes and the animal lies motionless on the bottom of the aquarium. At this time pupil becomes constricted and iris discoloured. Within 7 to 10 min., the pupils are maximally constricted and the eyeballs have almost entirely disappeared, as they have apparently been retracted and covered by the adjacent tissues. From the eye the depigmentation extends progressively all over the body, affecting first the tentacles and then the mantle. The musculature of the animal is flaccid and the tentacles hang inertly; respiratory movements cease completely within 10 min. Respiratory paralysis is followed by terminal asphyxial convulsions, reappearance of intense pigmentation and then, finally, by slow, paralytic emission of the ink and arrest of the branchial hearts (15 min.). A tenth of the above dose produces similar phenomena, which, however, are less severe and disappear completely within 1 or 2 hours.

Although not directly observed, it is highly probable that murexine is capable of paralysing other molluscs, such as small bivalve molluscs, which can in this way be opened and devoured by the *Murex*.

Neuromuscular Block

Cat Gastrocnemius.—A comparison of the paralysing action of different doses of murexine chloride hydrochloride on the cat, dog, and rabbit gastrocnemius is given in Table III.

The majority of our experiments were carried out in cats anaesthetized with chloralose. In some experiments in which pentobarbitone was used, murexine appeared to be somewhat less effective. Indeed, after administration of 200 μg./kg. of the drug, the twitch depression was 40%, as compared with 74% in cats anaesthetized with chloralose; after 300 μg./kg. the twitch depression was 70% and 96% respectively.

As with all other blocking agents, the repeated administration of murexine at brief intervals resulted in drug cumulation. Whereas, for example, a first injection of 100 μg./kg. of murexine produced in one experiment a maximal twitch reduction of 25%

TABLE III
THE PARALYSING ACTION OF MUREXINE CHLORIDE HYDROCHLORIDE ON THE SCIATIC NERVE-GASTROCNEMIUS MUSCLE PREPARATION OF DIFFERENT SPECIES
The results are the mean of 3 to 10 experiments. M.t.r. = maximum twitch reduction (% of normal twitch); t = time required for 50% recovery.

Murexine (μg./kg.)	Cat Gastrocnemius		Dog Gastrocnemius		Rabbit Gastrocnemius	
	M.t.r.	t (min. and sec.)	M.t.r.	t (min.)	M.t.r.	t (min.)
100	31	3	0	—	0	—
200	74	3 45	53	6	0	—
250	92	8	—	—	0	—
300	96	8 50	78	7	0	—
500	100	15	—	—	65	6
1,000	100	32 48	92	15	80	11
2,000	100	—	100	30	—	—

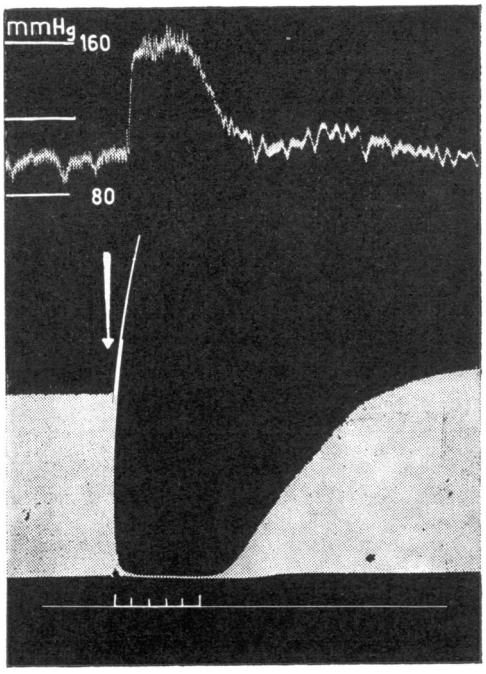


FIG. 1.—Cat sciatic nerve-gastrocnemius muscle preparation. Upper trace: blood pressure. Lower trace: muscle twitches in response to single maximal stimuli applied to the sciatic nerve. At the arrow murexine (300 μg./kg., i.v.). Neuromuscular block was preceded by an increased twitch height and a rise in blood pressure. Time, 1 min.

and required 3 min. for 50% recovery, a second injection, given 15 min. later, caused a maximum depression of 75%, and 50% recovery took 5 min.

Following the injection of small doses of murexine (250 to 300 μg./kg.), the twitch reduction was often

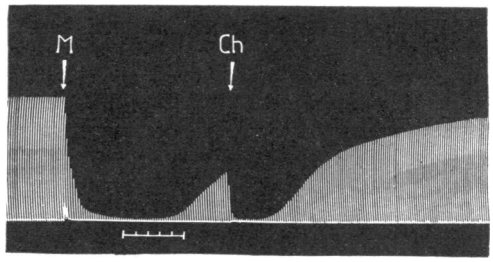


FIG. 2.—Cat sciatic nerve-gastrocnemius preparation. M, 300 μg./kg. murexine chloride hydrochloride. Ch, 5 mg./kg. choline chloride. Time, 1 min. Both injections were given intravenously. Choline, administered during the recovery phase following murexine paralysis, promptly caused further almost complete block.

preceded by a short-lived increase in the tension of the muscles as shown by the potentiation of some twitches (Fig. 1) and, between the contractions, fasciculations of the muscle could be seen. Another potentiation of the twitches was occasionally observed at the end of murexine paralysis.

5 mg./kg. of choline chloride, given intravenously during the recovery phase subsequent to 300 μg./kg. of murexine, promptly caused a renewed block (Fig. 2). A similar phenomenon was noted by Osterloch (1954) for suxamethonium. Eserine salicylate, intravenously in a dose of 50 μg./kg., did not affect appreciably the intensity of neuromuscular paralysis, but apparently prolonged it from 4 to 7 min. following 100 μg./kg. of murexine.

The intravenous infusion of 16 to 18 μg./kg./min. of murexine produced a gradually increasing twitch depression which began in 8 to 11 min., was 75% after 15 to 28 min., and was 95% to 100% after 30 to 60 min. A complete paralysis was then main-

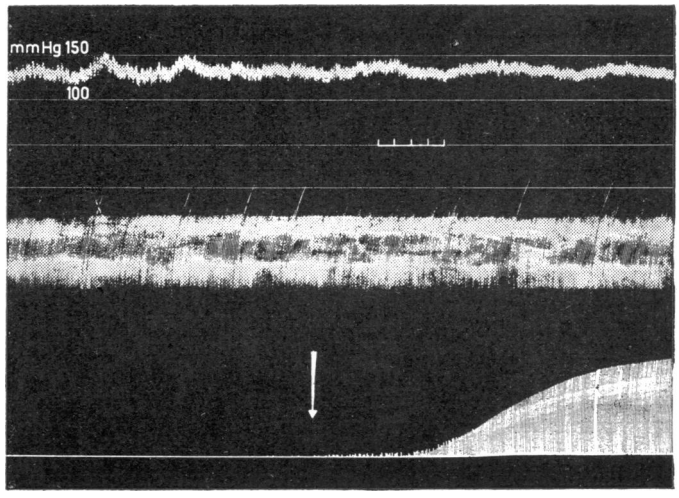


FIG. 3.—Cat anaesthetized with chloralose. Upper trace, blood pressure. Middle trace, respiration. Lower trace, twitches of the gastrocnemius muscle in response to single maximal stimuli to the sciatic nerve. Before the tracing commences, an intravenous infusion of murexine (17 μg./kg./min.) was started. At the arrow it was stopped. Time, 2 min. In spite of complete neuromuscular block, there was no change in blood pressure and no impairment of respiration.

tained as long as the infusion lasted. After the administration of the drug had been discontinued, a 25% recovery occurred within 8 to 20 min. (Fig. 3). With the same dose of suxamethonium both 100% twitch depression and complete recovery were attained more rapidly.

The intravenous injection of 5 mg./kg. of choline chloride, given during a murexine infusion when the twitch reduction was only 35%, immediately increased it to 90%. Tubocurarine proved to be a powerful antagonist to murexine, as it is for decamethonium and suxamethonium. Intravenous administration of 70 μg./kg. of tubocurarine completely abolished the paralysing action of a murexine infusion (Fig. 4). According to Philippot and Dallemagne (1956), 5-hydroxytryptamine partially

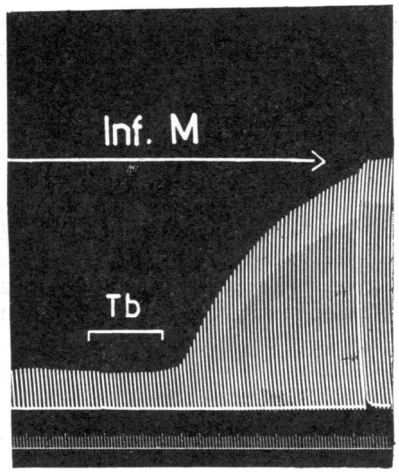


FIG. 4.—Muscle twitch as in Fig. 2. For the duration of the uppermost horizontal white line, an intravenous infusion of murexine (15 μg./kg./min.) was given (Inf. M.). The intravenous injection, during the period indicated by Tb, of tubocurarine (70 μg./kg.) antagonized the neuromuscular block due to murexine.

antagonizes the neuromuscular block caused by tubocurarine, whereas it has no effect on decamethonium paralysis. In our experiments, 25 to 50 μg./kg. of 5-hydroxytryptamine given intravenously did not affect the course of paralysis induced by murexine infusion.

In view of the close chemical and pharmacological similarity existing between murexine and suxamethonium, a comparison of the neuromuscular blocking action of the two drugs was carried out on the cat's gastrocnemius muscle preparation.

It is evident from the results shown in Table IV that suxamethonium is approximately 5 times as potent as murexine. The duration of the neuromuscular block is, however, longer for murexine.

TABLE IV

COMPARISON OF THE PARALYSING DOSES OF MUREXINE CHLORIDE HYDROCHLORIDE AND SUXAMETHONIUM CHLORIDE ADMINISTERED INTRAVENOUSLY IN THE CAT

No. of Expts.	Murexine (mg./kg.)	Suxamethonium (mg./kg.)	Maximum Twitch Reduction (% of Normal)	Time Required for 50% Recovery (min.)
8	0·1	—	31	3
3	—	0·025	48	2·33
10	0·2	—	74	3·75
5	—	0·05	91	4
3	0·25	—	92	8
3	—	0·1	100	7
8	0·3	—	96	9
2	—	0·2	100	8·5
2	0·5	—	100	15
2	—	0·5	100	12
5	1·0	—	100	33
2	—	1·0	100	14

It can be seen for example that, whereas after 1 mg./kg. of suxamethonium a 50% recovery can already be observed after 14 min., the same recovery after 1 mg./kg. of murexine takes place only after 33 min.

Dog and Rabbit Gastrocnemius.—Neuromuscular transmission in the dog was less sensitive to murexine than that in the cat, while that in the rabbit was still less sensitive. This example of variation of potency in different animal species can be seen in Table III.

Rat Diaphragm.—The preparation was not particularly sensitive to murexine. The minimum active dose is 15 to 20 μg./ml. Tubocurarine 0.5 μg./ml. almost completely antagonizes the twitch reduction produced by 25 μg./ml. of murexine. On the rat diaphragm, murexine is 2 to 3 times less potent than suxamethonium.

Blood Pressure

Cat.—In the intact animal, anaesthetized with chloralose or with pentobarbitone, the blood pressure response to rapid intravenous injections of murexine was rather variable. Small doses (20 to 50 μg./kg.) usually provoked a slight but transient hypotension; moderate doses caused transient hypotension (50 to 70 mm. Hg), transient hypertension (20 to 80 mm. Hg) (Fig. 1) or biphasic reactions (a rise followed by a fall of blood pressure). The injection of very high doses of murexine (5 to 10 mg./kg.) was generally followed by a sharp rise in blood pressure with subsequent fall, lasting for 10 to 15 min. The response often decreased progressively as doses were repeated. During intravenous infusion of murexine, at 15 to 30 μg./kg./min., which caused a complete muscular block, blood pressure changes were negligible (Fig. 3).

In contrast to the intact animal, the spinal cat always responded to intravenous murexine (0.2 to 3 mg./kg.) with a pure rise of blood pressure. In some cats this was proportional to the dose administered, while in others subsequent doses elicited decreasing reactions. Hexamethonium (2 to 5 mg./kg., i.v.) and Prosympal (diethylaminomethylbenzodioxan, 5 mg./kg., i.v.) reduce or abolish the murexine hypertension (Fig. 5). From this antagonism, from the characteristic form of the pressure curve and from the initial tachycardia it may be inferred that murexine hypertension is due to liberation of catecholamines in consequence of sympathetic ganglionic stimulation and/or suprarenal discharge; in fact it is a typical nicotinic effect of the substance.

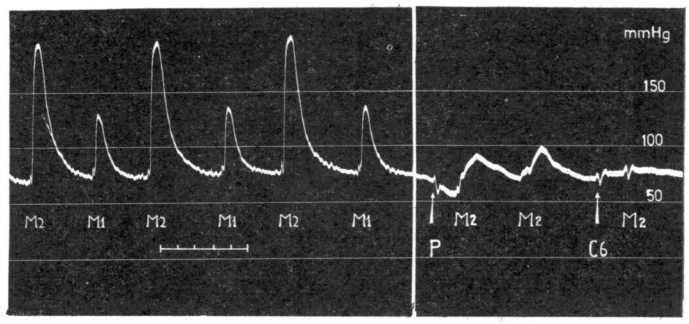


FIG. 5.—Blood pressure of a spinal cat. The pressure rise caused by murexine in this experiment was proportional to the dose. M1 and M2. 1 and 2 mg./kg. murexine chloride hydrochloride respectively. The blood pressure response to murexine was reduced by 5 mg./kg. of Prosympal (P), and was abolished by 5 mg./kg. of hexamethonium (C6). Time, 1 min.

Rabbit.—Doses of murexine which neither affected respiration (25 to 100 μg./kg.) nor caused hyperpnoea (100 to 500 μg./kg.) regularly provoked in the unanaesthetized rabbit transient and moderate hypotension (20 to 50 mm. Hg) which was barely proportional to the dose. With larger amounts of the drug, which produced respiratory arrest, the pressure response was often polyphasic (hypotension, hypertension, hypotension). If apnoea was completely avoided by the introduction of artificial respiration, there was no hypertension, and the fall of blood pressure, too, was conspicuously reduced. Hexamethonium (2 to 5 mg./kg.) had no effect on the murexine hypotension.

The behaviour of rabbits anaesthetized with urethane, and with urethane and pentobarbitone, was very similar to that observed in the unanaesthetized animal.

Dog.—Intravenous doses of 100 to 200 μg./kg. of murexine or more caused, in the animal anaesthetized with pentobarbitone or chloralose, an abrupt increase in the blood pressure. In some dogs the hypertension was roughly proportional both in intensity and duration to the doses of murexine, but in others there was tachyphylaxis. If hypertension was particularly intense and prolonged, it might be followed by a secondary fall of blood pressure. In one experiment, the intravenous injection of 25 mg./kg. of murexine caused a great increase in blood pressure, which rose from 140 to 280 mm. Hg and was followed by a fall to 105 mm. Hg lasting 40 min. As in the cat, murexine hypertension was opposed by hexamethonium (5 mg./kg.).

Heart

The isolated rabbit atrium was not affected by murexine chloride hydrochloride in concentrations up to 150 μg./ml. in nutrient liquid. The isolated frog heart responded to murexine concentrations of 1/1,000 to 1/3,000 with a moderate but transient decrease in the amplitude of beat. The effect was resistant to atropine, but promptly disappeared upon washing with fresh Tyrode solution.

On the isolated heart of *Helix pomatia*, murexine produced a negative chronotropic effect in a concentration of 10 μg./ml. in the nutrient liquid. Higher concentrations (30 to 40 μg./ml.) also reduced the amplitude of the beat or provoked (100 μg./ml.) a transient diastolic arrest followed by complete recovery.

In the intact anaesthetized dog, murexine in doses of up to 1 to 2 mg./kg. did not produce specific electrocardiographic changes other than a compensatory bradycardia with occasional manifestations of ventricular escape.

Respiration

Cat.—In the cat anaesthetized with chloralose or pentobarbitone, the minimum dose of murexine which affected respiration was 50 to 100 μg./kg. The usual response was a transient depression, followed by a period of respiratory stimulation; more rarely a short-lived stimulation was the only effect. It was not possible to correlate the respiratory changes induced by the drug with its effects on the blood pressure.

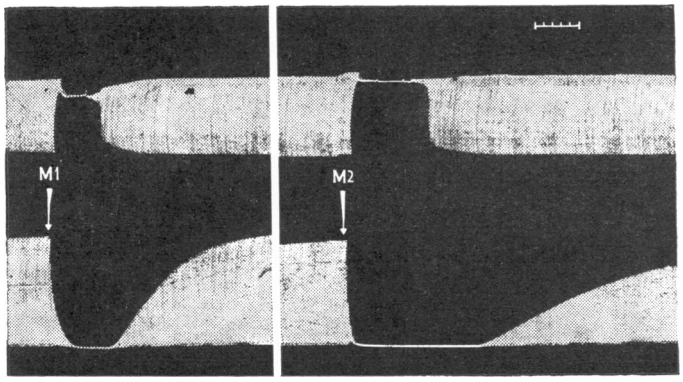


FIG. 6.—Cat anaesthetized with chloralose. Upper trace, respiration. Lower trace, contractions of the gastrocnemius muscle. After murexine injections (M1, 500 μg./kg.; M2, 1,000 μg./kg.) oxygen was administered through a tracheal catheter. Time, 1 min. The recovery of respiratory musculature was more prompt than that of the gastrocnemius muscle.

Higher doses of murexine (500 to 1,000 μg./kg.) regularly provoked respiratory arrest due to paralysis of the skeletal musculature. If oxygen was administered through a catheter introduced into the trachea, it was readily observed that recovery of respiratory muscles preceded that of other skeletal muscles (Fig. 6).

Rabbit.—In the unanaesthetized animal, doses of 25 to 50 μg./kg. did not affect respiration. Higher doses (100 to 500 μg./kg.) regularly increased the respiratory rate and volume. Doses of 1 mg./kg. and more caused respiratory arrest. Paralysis was sometimes followed by a period of hyperpnoea.

The behaviour of rabbits anaesthetized with urethane or pentobarbitone was similar, but animals anaesthetized with urethane and morphine always responded to murexine with a respiratory depression which lasted for several minutes. When murexine stimulated respiration, there was no correlation between intensity and duration of the moderate hypotension produced by the drug and the intensity and duration of hyperpnoea. It seems therefore probable that this was a nicotinic effect of murexine.

Dog.—The minimum dose of murexine active on respiration was 150 to 200 μg./kg. There was a very short-lived intense stimulation of respiratory movements, followed by depression or, according to the dose, by respiratory arrest. The changes were very similar in normal dogs and in animals anaesthetized with chloralose or pentobarbitone.

Smooth Muscle

Intestine.—The minimum dose of murexine which caused stimulation of the guinea-pig small intestine was approximately 5 to 10 μg./ml. There was no constant dose-response relationship. Both atropine 10^{-7} and hexamethonium 10^{-4} reduced the spasmogenic effect of murexine.

The rabbit small intestine was rather insensitive to murexine, and the weak spasm provoked was very irregular and not proportional to the dose. Acetylcholine was at least 5,000 times as potent as murexine.

On the rabbit large intestine, doses of 10 to 15 μg./ml. of murexine, or above, regularly produced a decrease in tonus which was sometimes proportional to the drug concentration. Hexamethonium 10^{-4} strongly reduced this adrenaline-like effect.

The results obtained on the intestinal smooth muscle demonstrated, in full accordance with those obtained on the isolated heart, that murexine completely lacked muscarinic effects.

Iris Muscle.—If murexine chloride hydrochloride was added to sea water in which the enucleated eye of an octopod (*Eledone moschata, Octopus vulgaris*) was immersed, there was a mydriasis and an expansion of the iris chromatophores (Fig. 7). The effect began to appear with a drug concentration of 1/20,000 to 1/50,000 and was very intense with 1/1,000 murexine. A maximal mydriasis was attained within 20 to 30 sec., and expansion of the chromatophores, preceded by alternate movements of expansion and retraction, was very conspicuous.

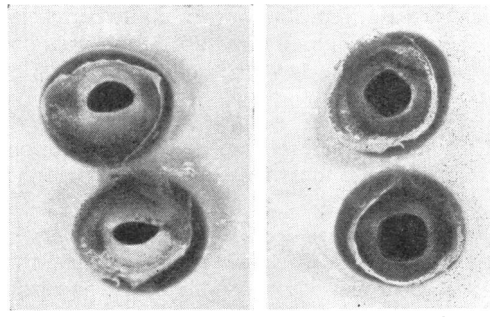


FIG. 7.—Enucleated eyes of *Octopus vulgaris*. On the left, norma eyes in sea water; on the right, the same eyes after adding murexine chloride hydrochloride (0.2 mg./ml. sea water). Murexine provoked an intense mydriasis and expansion of the iris chromatophores.

The toad and the frog enucleated eyes responded to murexine, 1/5,000 being required to produce a mydriasis.

D'Ermo (1947) has shown that crude extracts of the hypobranchial body of *M. trunculus* exerted a potent spasmogenic action on the isolated sphincter muscle of the horse iris. There was little doubt that this action was due to murexine which was active in a concentration of about 1/300,000.

Other Tissues

Frog Rectus Abdominis.—In our first study (Erspamer and Dordoni, 1947) murexine was found to induce a contracture in the frog rectus. This response was very similar to that produced by the methonium compounds and by suxamethonium, but differed from that provoked by acetylcholine, which had a slower onset and did not reach a plateau so readily. The relative potencies in stimulating the frog rectus were murexine chloride hydrochloride, 100; decamethonium iodide, 50; suxamethonium chloride, 180 to 200; acetylcholine bromide, 1,000.

Eserine salicylate 10^{-5} potentiated the response to murexine only by about 40%. Tubocurarine showed a strong antagonistic action; after 0.3 μg./ml. and 1.5 μg./ml. the contracture caused by 0.3 μg./ml. of murexine was reduced by 80 and 90% of the control respectively.

Leech Muscle.—Murexine causes a contracture in this preparation which closely resembled that produced by acetylcholine. Eserine salicylate 5×10^{-6} potentiated the response to murexine by about 2 to 4 times and that to acetylcholine by 1 to 2,000 times.

Isolated Cervical Ganglion of the Cat.—Kewitz (1955) found that the isolated superior cervical ganglion of the cat is stimulated by murexine, when the drug is added to the perfusion liquid. The action, which was as intense as that caused by acetylcholine, was reduced by adrenaline but not by scopolamine.

Isolated Brain Preparation of the Cat.—The intravenous injection of murexine, in doses of up to 10 mg./kg., did not produce any appreciable change in the electroencephalographic record.

Clinical Trial of Murexine

A preliminary clinical trial of murexine was carried out by Deblasi and Leone (1955) on 47 patients and by Ciocatto, Cattaneo and Fava (1956) on 123 patients. Murexine proved to be an interesting short-lasting muscle relaxant, worthy of a more extensive clinical investigation. The mean paralysing dose in adult patients was approximately 1 to 1.2 mg./kg., intravenously. Maximum effects were attained in 45 to 60 sec., and, following a single intravenous dose, the paralysis lasted 3 to 6 min. A long-lasting, satisfactory muscular relaxation could be obtained by slow intravenous infusion of a 1/1,000 solution of murexine in physiological saline.

Murexine caused several side-effects, which were mainly attributable to the nicotinic actions of the drug. Muscular paralysis was always preceded by and followed by fasciculations of the musculature; copious salivation was very common but was easily blocked by atropine; a moderate rise in blood pressure (10 to 15 mm. Hg) was frequent, but unimportant. Persistence of pharyngeal and laryngeal reflexes, lachrymation and sweating were rarely observed.

Table V summarizes the relative potency, rapidity of onset and duration of action of murexine and other curarizing agents in man (Ciocatto, Cattaneo and Fava, 1956). The potency of tubocurarine is arbitrarily taken as 100.

TABLE V

ACTIVITY OF MUREXINE IN MAN AS COMPARED WITH THAT OF OTHER MUSCLE RELAXANTS

The potencies are related to that of tubocurarine arbitrarily taken as 100.

	Blocking Potency	Time for Appearance of Maximum Effect (min.)	Duration of Action (min.)
Tubocurarine ..	100	1–2	25–30
Gallamine ..	21	1–2	20–25
Decamethonium ..	578	1–2	20–25
Suxamethonium ..	34	½–¾	3–5
Murexine ..	20	¾–1	3–6

DISCUSSION

Murexine is one of the first neuromuscular blocking agents of animal origin to be chemically defined. It is highly probable that systematic studies in the field of invertebrates will lead to the discovery of the existence of murexine or closely related substances in other invertebrates, for defence or for securing food.

The materials available to *Murex* for the biosynthesis of murexine are common in the living organism, being choline and urocanic acid or some precursors of it, probably derived from the metabolism of histidine. It would certainly be worth while investigating in detail the processes involved in the synthesis of this new choline ester, particularly in view of the idea that similar processes may take place in the central nervous system of vertebrates (Gruner and Kewitz, 1955).

Murexine possesses two of the three types of pharmacological action seen in choline and its

derivatives, namely the nicotinic and the neuromuscular blocking actions. The muscarinic action, if any exists, is very weak. The relation between the chemical structure and the pharmacological actions of murexine derivatives will be discussed in greater detail elsewhere, but in connexion with the present work it is interesting to note the following: (*a*) Hydrogenation of the acrylic lateral chain into propionic caused a powerful increase in both nicotinic and neuromuscular blocking actions. The blocking effect of dihydromurexine on the cat gastrocnemius preparation was 4 to 5 times as great as that of murexine, and the nicotinic action on the frog rectus was 7 times as great. (*b*) The length of the lateral chain of the imidazole acid was of critical importance for the pharmacological effects. A three carbon atom lateral chain seemed to be optimal both for maximal blocking and nicotinic potency. Imidazoleacetyl- and imidazolecarboxylcholine on the one hand, and imidazolebutyrylcholine on the other, are less potent than imidazolepropionyl-choline. (*c*) Substitution of the ester linkage by an ether linkage reduced the blocking potency of dihydromurexine by about 6 times on the cat gastrocnemius, but considerably prolonged this action, and the nicotinic effect on the frog rectus was reduced by about 250 times. (*d*) Glässer and Pasini (1957) found that murexine chloride hydrochloride was twice as potent, on the cat gastrocnemius preparation and the frog rectus, as murexine bromide. The explanation of this surprising fact remains obscure.

In the absence of direct evidence, all the available experimental and clinical evidence suggests that murexine should be listed among the blocking agents acting by depolarization. Table VI shows the differences and similarities between tubocurarine (an example of the " competitive " blocking agents or " pachycurares "), suxamethonium choline (an example of the " depolarizing " blocking agents or " leptocurares ") and murexine.

TABLE VI

COMPARISON OF DIFFERENCES AND SIMILARITIES BETWEEN TUBOCURARINE, SUXAMETHONIUM AND MUREXINE

NMB = Neuromuscular block.

	Tubocurarine	Suxamethonium	Murexine
Action in mammals	NMB	NMB preceded by muscular fasciculation	NMB preceded by muscular fasciculation
,, ,, birds ..	,,	Nicotinic contraction	Nicotinic contraction
Effect of anticholinesterases on NMB	Antagonism	No antagonism	No antagonism
Effect of tubocurarine on NMB	Potentiation	Antagonism	Antagonism
Action on frog rectus	No contracture	Contracture	Contracture

Among the known " depolarizing " agents, the substance most closely related to murexine is suxamethonium. The blocking potency of murexine in intact dogs and human beings was approximately 50% to 60% and in the cat was about 20% of that of suxamethonium. As with gallamine, the acute toxicity of murexine was surprisingly low in mice, being about 13 to 15 times lower than that of suxamethonium. Murexine was considerably more potent than suxamethonium in its nicotinic actions on the blood pressure of mammals, and in its power to produce muscular fasciculations which precede paralysis. Apart, however, from the above quantitative differences, murexine behaves very similarly to suxamethonium and, like the latter, it is characterized by the rapid onset and short duration of its pharmacological effects.

In preliminary clinical trial as an adjuvant in anaesthesia murexine gave a good neuromuscular blocking effect, accompanied by marked nicotinic side-effects, some of which were easily counteracted by atropine, while others apparently cannot be avoided. More extensive clinical investigation is necessary to establish whether murexine can be employed as a safe and valuable muscle-relaxant drug.

REFERENCES

Bovet, D., and Bovet-Nitti, F. (1955). *Scientia med. italica*, **3**, 484.

Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.

Burn, J. H. (1950). *Biological Standardization*, pp. 347 and 351. Oxford: Oxford University Press.

Ciocatto, E., Cattaneo, A., and Fava, E. (1956). *Mi nerva anestes.*, **22**, 197.

Deblasi, S., and Leone, U. (1955). Ibid., **21**, 137.

D'Ermo, F. (1947). *Boll. Oculistica*, **26**, 763.

Erspamer, V. (1948). *Experientia*, **4**, 226.

—— and Benati, O. (1953a). *Science*, **117**, 161.

—— —— (1953b). *Biochem. Z.*, **324**, 66.

—— and Dordoni, F. (1947). *Arch. int. Pharmacodyn.*, **74**, 263.

Glässer, A., and Pasini, C. (1957). *Experientia*, in the press.

Gruner, G., and Kewitz, H. (1955). *Naturwissenschaften*, **42**, 628.

Kewitz, H. (1955). *Arch. exp. Path. Pharmak.*, **225**, 111.

Keyl, J., and Whittaker, V. P. (1955). *Amer. J. Physiol.*, **183**, 634.

Osterloch, G. (1954). *Arch. exp. Path. Pharmak.*, **222**, 360.

Pasini, C., Vercellone, A., and Erspamer, V. (1952). *Ann. Chem.*, **578**, 6.

Paton, W. D. M., and Zaimis, E. J. (1949). *Brit. J. Pharmacol.*, **4**, 381.

Philippot, E., and Dallemagne, M. J. (1956). *Arch. int. Pharmacodyn.*, **105**, 426.

Welsh, J. H. (1955). *Deep Sea Research*, **3**, Suppl., 287.

Whittaker, V. P. (1955). *3ème Congrès internat. Biochimie, Bruxelles.* Rés. des Communicat., p. 31.

Zettler, G., and Schlosser, L. (1954). *Arch. exp. Path. Pharmak.*, **222**, 345.