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PHARMACOLOGICAL STUDIES ON HEMLOCK WATER DROPWORT

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The Umbellifer, *Oenanthe crocata*, is found in damp situations in many parts of the British Isles and Western Europe, and is held to be one of our most poisonous indigenous plants. Confusion in nomenclature renders many early reports unreliable, though Vaughan (1722) appears to have recognized the effects produced in man by ingestion of the tubers. Bloc (1873), Demoor (1896, 1897) and Witthaus (1911) report 124, 218, and 159 cases of poisoning respectively, with death rates of between 26 and 44%. In Demoor's series, the mortality among those who suffered convulsions was approximately 70%. Fatal instances of poisoning still appear in the contemporary literature (*Lancet*, 1947).

Clarke, Kidder, and Robertson (1949) were the first to obtain crystalline oenanthotoxin, and their paper should be consulted for references to earlier work. The extensive studies of Anet, Lythgoe, Silk, and Trippett (1953) have revealed much of its chemical nature, and a structural similarity to some antibiotics such as mycomycin and to related substances obtained from certain Basidiomycetes.

Boehm (1876) suggested that the convulsant properties of oenanthotoxin were similar to those of picrotoxin, a view supported by Grollman and Slaughter (1947). We are unaware of any detailed studies of the pharmacological actions of this plant and present our own in the following paper.

METHODS

Preparation of Material

Tubers of *Oenanthe crocata* were gathered from the Botanic Garden, Cambridge, in September, October, and March of the same and different years. They were stripped of stems and rootlets, cut into thin discs, dried to constant weight at 40° C., and then ground to a fine powder. The powders were extracted with 70% alcohol either by cold percolation or by hot continuous extraction (*British Pharmacopoeia*, 1953). The tinctures obtained by the first method were used as such; but, after hot percolation, water soluble crystals were deposited on cooling; these were discarded when it was found that they had no observable effect on intravenous injection into unanaesthetized rabbits. The final volumes of tinctures were such that 1 ml. corresponded to 0.5–1 g. of dried powder, but much variation in potency was expected because of obvious seasonal differences in the appearance of the tubers. The tinctures were stored at 4° C. and some samples of powder at room temperature.

In later work 4.3 kg. of tubers were obtained from South Devon in January and 8.7 kg. from Kent in February. The tubers were minced, placed in small muslin bags and extracted with 5–6 l. of ether in a 20 l. bolt-head flask standing in a large electrically heated water-tank. The ether distilled into a receiver outside the tank until about 1 l. remained in the bolt-head flask. This residual liquor was driven into a 5 l. flask also standing in the tank, whence it distilled into the same receiver as did the contents of the 20 l. flask. The distilled ether was next drawn back into the extraction flask and the procedure repeated as often as required. The marcs were expressed and re-extracted, the process ending when the ether in the extraction flask remained colourless. Further processes were carried out on the concentrate in the 5 l. flask and were essentially those of Anet *et al.* (1953) except that 40% instead of 20% NaOH was used as a precipitant. After the initial crops of crystals had been recovered from the various solvents, further crops were obtained from many of them by the addition of light petroleum. The total yields were 270 and 234 mg./kg. from the Devon and Kent samples respectively. The melting point of the crystals was 86° C.; Anet *et al.* (1953) found m.p. 87° C. The crystals (oenanthotoxin) were sealed in ampoules under nitrogen and kept at −20° C. They were soluble in propylene glycol and some specimens were stored under similar conditions in this solvent.

When freshly prepared crystals of oenanthotoxin were exposed to air they rapidly became brown; but stored at −20° C., in nitrogen, no change was observed after nine months. Under such conditions solutions of oenanthotoxin in propylene glycol showed no appreciable loss of convulsant properties; dried powders still furnished convulsant tinctures after four months at room temperatures. Tinctures were potent after a similar period at 4° C. The subsequent experiments were carried out using test solutions not more than three weeks old.

Animal Experiments

Most of the observations were made upon unanaesthetized rabbits after the intravenous injection of either 0.5–1 ml. oenanthe tincture or 1 mg./kg. oenanthotoxin in propylene glycol. Cats received similar injections. Oenanthotoxin was also administered intraperitoneally to mice and by stomach tube to rabbits and mice. The behaviour of unanaesthetized animals after such treatment was observed and compared with the effects of picrotoxin given intravenously in rabbits and intraperitoneally in mice.

Central Nervous System.—In cats, similar studies were made on hypothalamic, mid-brain and spinal preparations, and in some animals after decapitation and destruction of the spinal cord. Injections were also given after subaxial section of the cord. In rabbits, only mid-brain, spinal, and pithed preparations were used. Blood pressure was recorded in all these experiments. Respiratory movements were recorded in those animals in which artificial respiration was not required.

Respiration and Blood Pressure.—Changes in blood pressure and respiration produced by oenanthotoxin or oenanthe tincture were studied in intact rabbits anaesthetized with paraldehyde, pentobarbitone, or hexobarbitone. In later experiments, in addition to the blood pressure, the heart action in the open chest was recorded by means of a thread passed through the apex and operating a small Starling heart lever. The heart was protected from interfering respiratory movements by a plastic ring inserted within the pericardium and fixed in position near the base of the heart by an encircling ligature. This ring was attached to a slender steel rod which was clamped outside the chest. The effects of bilateral vagotomy and of atropine upon the response of the cardiovascular system to oenanthotoxin were observed in such preparations. In many of these experiments the complicating convulsions were prevented or arrested by the use of tubocurarine. Similar studies were made on decapitate rabbits after destruction of the spinal cord. These animals received 10 ml. of "Dextraven" (6% w/v Dextran in 0.9% saline) after decapitation, and their blood pressures were maintained subsequently by the continuous intravenous injection of 0.12 ml. of saline/min. containing 100 μg. noradrenaline bitartrate (Light and Co.), delivered by a motor-operated syringe.

Heart.—Oenanthotoxin is insoluble in water and satisfactory suspensions could not be obtained using lecithin as suggested by Clarke *et al.* (1949). When oenanthotoxin in a minimal volume of propylene glycol was added to plasma with gentle shaking, a clear solution was produced after an instant's opalescence. This mixture diluted with Dale-Ringer solution did not lose its convulsant properties after passing through fine filter paper. Perfused isolated rabbit hearts (Brodie and Cullis, 1908) ran well on a mixture of 1% of the rabbit's own plasma and 0.01% propylene glycol in Dale-Ringer solution. When constant values for heart rate, excursions and coronary flow had been obtained from such a preparation, the perfusion fluid was changed to a similar one containing the desired concentration of oenanthotoxin.

In the heart-lung preparation in the dog, recordings were made of arterial and venous pressures, and a cardiometer used to indicate the behaviour of the heart. Various amounts of oenanthotoxin in propylene glycol were added to the blood in the perfusion reservoir to give doses corresponding to 0.15–1 mg./kg. in the intact dog. A similar experiment was performed with a tincture which had been evaporated to dryness and re-dissolved in propylene glycol.

Oenanthotoxin-Barbiturate Antagonism

To investigate the antagonism between oenanthotoxin and pentobarbitone each member of two groups of six rabbits received oenanthotoxin or oenanthe tincture by an ear vein. As soon as convulsions were established in the test group they were arrested by the injection of a sufficient volume of a 1% solution of pentobarbitone sodium. The control group received no pentobarbitone. The test group of animals remained under observation for seven days. The relative effectiveness of picrotoxin and oenanthotoxin as stimulants of depressed respiratory activity was compared in rabbits by a method similar to that of Das (1939). Femoral arterial blood pressure records were made in addition to those of respiration. Hexobarbitone sodium was infused into a femoral vein, the correct dose—between 0.7 and 1.4 mg./kg./min.—to maintain a steady depression of respiration being determined by trial. The test substances in propylene glycol or propylene glycol-plasma were introduced into the inferior vena cava through a 1 mm. bore polythene tube passed by way of the other femoral vein. The rate and volume of the injections were the same for both substances. Comparisons started after 1 hour's anaesthesia, the test solutions being injected alternately. After each test the respiratory activity was allowed to return to the initial level before a further injection was given.

Additional comparisons were made in mice. The LD50 for oenanthotoxin was determined in 72 male albino mice maintained on identical diets, and used when between 15–25 g. in groups of similar mean weight. The same intraperitoneal injection technique was always employed, and the external conditions were maintained constant. Oenanthotoxin was dissolved in propylene glycol, suspended in rabbit plasma, and appropriate dilutions made in 0.9% saline. The volume received by a 20 g. mouse was 0.5 ml. containing 0.002 ml. propylene glycol and 0.1 ml. plasma. Other animals received proportionate doses. The LD50 for picrotoxin in saline was similarly determined. After injection, the times of onset of convulsions and death were noted and final mortalities assessed after 24 hr. All dead mice were subjected to autopsy.

Picrotoxin and oenanthotoxin were compared in their capacity to prevent death in mice depressed by large doses of pentobarbitone sodium. A dose-mortality curve for this was determined by intraperitoneal injection into a total of 64 mice. A convenient dose—the LD82—which would kill a high percentage of mice was selected and given to further groups of mice. The first group of

14 mice received no further injections and served as the control, but of the remaining four groups of 15 mice each, two received 1 × LD50 and 2 × LD50 of picrotoxin respectively and two 1 × LD50 and 2 × LD50 of oenanthotoxin. These injections were made within 3 min. of the pentobarbitone injections. The mortality after 24 hr. was noted.

In all experiments close attention was paid to the results of control studies with the solvents, since propylene glycol itself, in doses of about 0.5 ml., induces temporary changes in both blood pressure and respiratory activity. The use of plasma allowed the glycol to be diluted, in critical experiments, beyond its active range.

Results

Unanaesthetized Animals.—Convulsions were induced in forty rabbits by the intravenous injection of oenanthotoxin or oenanthe tincture. There was an initial, brief, quiescent period, often absent with doses of 1 mg./kg. of oenanthotoxin, during which the animals showed twitching of the facial muscles and whiskers with increased respiratory rate. There were occasional twitches of the limbs and often marked head retraction with characteristic backward shuffling. Widespread spasms of all four limbs heralded the onset of violent running movements, at the start of which the animal fell upon its side. Usually the " running " occurred in bouts. At the height of these attacks, respiration was impeded and the buccal mucosa was cyanosed. There was some salivation, but micturition and defaecation were not markedly increased. Chewing, and grinding of the teeth were constantly observed. Towards the end, the spasms became less frequent, and after the arrest of respiration the heart was beating feebly. During the action of the drug the pupils were inconstant. Death always occurred within 30 min. after 1 mg./kg. oenanthotoxin, and within 60 min. after 0.5–1 ml. oenanthe tincture.

Six cats, after intravenous injections of oenanthe tincture, behaved similarly except that the limb movements were those associated with scrabbling or scratching rather than running. Bristling of the fur and unsheathing of the claws were usually prominent. Micturition and defaecation were often noted. In two cats death followed within one minute of the injection and there were no convulsions. In three cats given oenanthotoxin the limb movements showed some tonic features and in these animals the increase in respiration was particularly striking.

In 72 mice intraperitoneal injection of oenanthotoxin gave effects comparable to those after intravenous injection to rabbits, though the onset of convulsions was delayed (Table I), and usually preceded by a marked erection of the tail.

The convulsions induced in rabbits and mice were in pattern indistinguishable from those produced by picrotoxin. The oral LD50 for oenanthotoxin in mice was about 7–8 mg./kg., the usual convulsive phenomena being followed by death, but in 6 rabbits doses up to 90 mg./kg. by mouth induced no observable change in behaviour.

Central Nervous System.—In the cat, the usual limb movements were still obtained when all cerebral tissue anterior to the superior colliculus had been removed, but tonic strychnine-like spasms of the limbs with rigid flexion of the trunk were observed in spinal animals, though larger doses were often required to elicit this response. After subaxial section of the spinal cord, clonic jaw and face movements and tonic spasms of the limbs

TABLE I

DOSE-MORTALITY DATA FOR OENANTHOTOXIN AND FOR PICROTOXIN GIVEN INTRAPERITONEALLY TO MICE
The control injection was a 20% solution of plasma in 0.9% saline containing 0.4% propylene glycol, and caused no change in the behaviour of the mice.

Drug	Dose (mg./kg.)	No. of Mice	Av. Wt. (g.)	Animals Convulsing (%)	Av. Time of Onset of Convulsions (min.)	Mortality (%)	Av. Time from Injection to Death (min.)
Oenanthotoxin	2·21	5	17	100	20	100	48
	1·80	10	17	100	24	90	41
	1·47	20	17	75	18	60	50
	1·20	20	16	80	32	50	59
	0·98	10	17	50	36	40	70
	0·80	5	17	40	41	0	—
	0·59	2	17	0	—	0	—
Control	0·5 ml./20 g. mouse	8	18	0	—	0	—
Picrotoxin	12·60	5	16	100	5	100	11
	10·00	10	18	100	7	90	13
	8·00	16	17	94	9	50	17
	6·40	10	17	80	15	40	33
	5·00	10	17	40	18	0	—
	4·00	5	16	20	30	0	—
	3·18	5	17	0	—	0	—

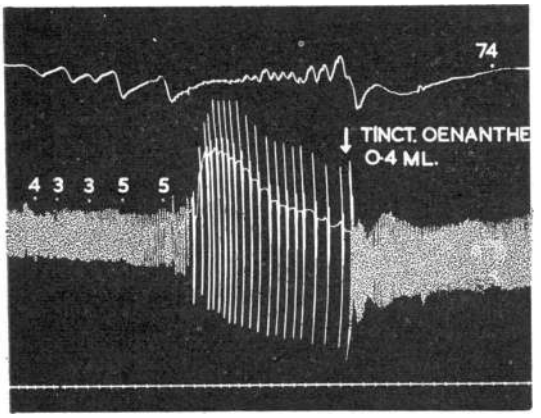

FIG. 1.—Rabbit, 1.2 kg., anaesthetized with pentobarbitone sodium 30 mg./kg. Upper record, arterial blood pressure. Lower record, respiratory movements. Time in 10 sec. Further doses of pentobarbitone (4, 3, 3, 5, and 5 mg.) were given until respiration was greatly slowed. Oenanthe tincture promptly restored the respiratory rate.

followed the intravenous injection of oenanthotoxin. After destruction of the central nervous system no motor activity was seen.

In the rabbit, the mid-brain preparation gave the typical running pattern following administration of the drug, and the other preparations gave results similar to those in the cat. Mid-brain animals showed an increase in respiratory rate and blood pressure. The latter change was absent in the pithed preparation though a slight rise was occasionally seen in the spinal rabbit.

Respiration and Blood Pressure.—In 30 intact rabbits anaesthetized with paraldehyde, urethane or barbiturate there were increases in respiratory rate, or amplitude, or both. These changes were most striking in deeply anaesthetized animals (Fig. 1). The blood pressure after an initial fall—hypotensive phase—usually rose steadily—hypertensive phase—attaining in some cases 160 mm. Hg. Oenanthe tincture and oenanthotoxin gave similar results. The hypotensive phase showed marked variation both in degree and duration in successive similar experiments and was frequently accompanied by a dilatation and slowing of the heart (Fig. 2). Bilateral vagotomy prevented the bradycardia, but the other changes were still seen. During the subsequent hypertensive phase the cardiac dilatation persisted, sometimes accompanied by slowing. These effects were especially marked at the peaks of fluctuations in the blood pressure. This dilatation was increased by bilateral vagotomy which further raised the blood pressure and restored the heart rate. In curarized preparations similar cardiovascular changes were seen. After destruction of the central nervous system in the rabbit, oenanthotoxin gave the usual dilatation of the heart in the hypotensive phase though without cardiac slowing (Fig. 3). The hypertensive phase was absent.

Heart.—Perfusion of the isolated rabbit heart with solutions containing oenanthe tincture or oenanthotoxin caused a reduction of the excursions of the recording lever. The rate was slightly increased with a small decrease in coronary flow. With concentrations in the blood corresponding to 1 mg./kg. body weight in the intact dog, the heart-lung preparation revealed, at constant arterial pressure, an increase in the volume of the heart with a slight rise in venous pressure. When an evaporated tincture was used, these changes were accentuated.

Oenanthotoxin-Barbiturate Antagonism

Sodium pentobarbitone prevented death in six

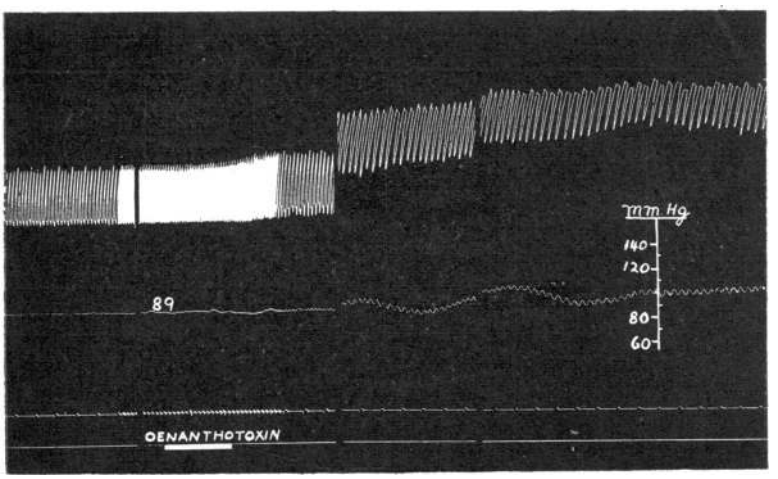

FIG. 2.—Rabbit, 1.8 kg., paraldehyde 1.7 ml./kg. by stomach tube. Heart movements (upper record) recorded in open chest by means of a thread attached to the apex and to a Starling heart lever. Downstroke, systole. Oenanthotoxin i.v. produced progressive dilatation and slowing of the heart. This preparation showed no marked fall in blood pressure (lower record). The hypertensive phase starts towards the end of the tracing. The intervals between the strips are from left to right 39, 78, and 29 sec. Time in sec.

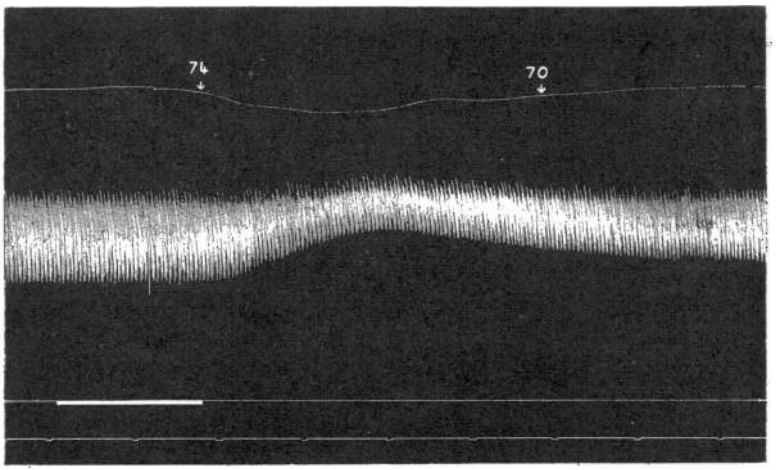

FIG. 3.—Rabbit, 1.9 kg., anaesthetized with pentobarbitone sodium 30 mg./kg., decapitated, and spinal cord destroyed. Blood pressure (upper record) maintained with an infusion of noradrenaline. Oenanthotoxin i.v. at signal produced a fall of blood pressure, impairment of ventricular contraction (lower record), but no cardiac slowing. Time, 0.25 min.

rabbits receiving a lethal dose of oenanthotoxin (Table II), and one of these delivered a living litter on the day after the experiment. In a large series of rabbits, none has survived 0.5–1.0 ml. of oenanthe tincture or 1 mg./kg. of oenanthotoxin without an antidote, but in addition to those shown in Table II many more rabbits and cats thus poisoned have recovered following adequate treatment with pentobarbitone. Fig. 4 shows that when oenanthotoxin was administered to a rabbit under continuous hexobarbitone narcosis a greater and more prolonged increase in respiratory amplitude and rate was observed than that given with picrotoxin in three times the dose. The intraperitoneal LD50 of oenanthotoxin for mice determined by the method of Bliss (1938) was 1.22 ± 0.11 mg./kg., and that of picrotoxin 7.56 ± 1.10 mg./kg. (Table I). The intraperitoneal LD50 of pentobarbitone for mice was 139 ± 1 mg./kg., which agrees closely with the value recorded by Chakravarti (1939). The recovery rates of mice given the LD82 of pentobarbitone were 80% with 1×LD50 of either oenanthotoxin or picrotoxin. With 2×LD50, the recovery rates were 80% and 93% respectively. Deaths in the oenanthotoxin series mainly occurred in the first hour, whereas those after picrotoxin were seen much later in the experiment. Mice receiving oenanthotoxin tended to recover more rapidly from the coma induced by pentobarbitone than those of the picrotoxin series.

TABLE II

OENANTHOTOXIN-PENTOBARBITONE ANTAGONISM IN RABBITS

The dose of oenanthotoxin was 1 mg./kg. i.v., except in experiments 5 and 6 in which 1 and 2 ml. of oenanthe tincture respectively were given i.v. As soon as convulsions were established they were arrested in the test series by the injection of a 1% solution of pentobarbitone. None of the animals given pentobarbitone died.

Expt. No.	Oenanthotoxin Alone (Control)			Oenanthotoxin + Pentobarbitone (Test)		
	Wt. (kg.)	Onset Convulsions after Injection (sec.)	Time after Injection to Death (min.)	Wt. (kg.)	Onset Convulsions (sec.)	Pentobarbitone to control Convulsions (mg.)
1	2·3	65	18·5	2·3	35	40
2	2·2	79	19·7	2·2	50	120
3	1·7	50	19·5	2·4	48	130
4	1·9	77	29·7	1·9	80	110
5	1·2	300	50	1·2	120	60
6	1·4	180	40	1·2	240	90

DISCUSSION

The crystalline product obtained appears similar to the oenanthotoxin of Anet *et al.* (1953) in general chemical and physical properties. The convulsions induced in mice were similar to those described by Clarke *et al.* (1949), but their stated LD50 is lower than ours. Their experimental results, however, are insufficient for an accurate determination of this value. Oenanthe tincture and oenanthotoxin gave qualitatively similar results in all the pharmacological actions examined except that in some cats the oenanthotoxin convulsions tended to be tonic. The similarity between convulsions induced in the mid-brain preparation and the intact rabbit suggests an origin in the brain stem—a view supported by the strychnine-like spasms observed in the spinal animals.

The inconstancy of the cardiovascular response of the rabbit to oenanthotoxin rendered difficult a precise analysis of the mechanisms involved. Since the fall in blood pressure with its associated cardiac dilatation was not prevented by bilateral vagotomy and appeared in rabbits in which the central nervous system had been destroyed, it seemed probable that this was due to a direct action on the heart or an obstruction of pulmonary blood flow. The

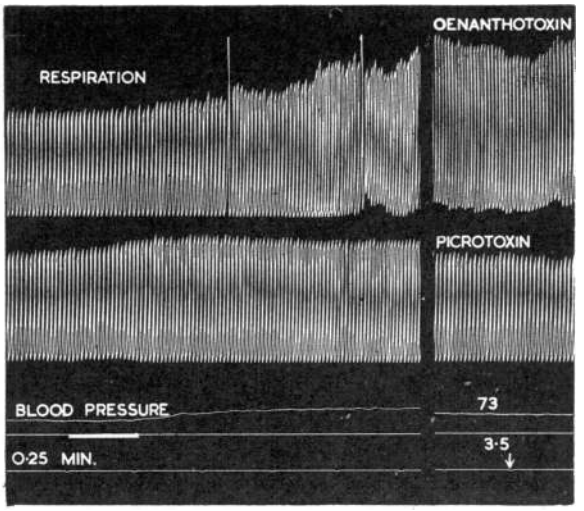

FIG. 4.—Rabbit, 1.8 kg., continuous narcosis with hexobarbitone sodium 1.3 mg./kg./min. Records of respiratory movements. Top line, expt. with oenanthotoxin; 2nd line, expt. with picrotoxin; 3rd line, b.p. in picrotoxin expt. Either 0.2 mg./kg. of oenanthotoxin or 0.6 mg./kg. of picrotoxin i.v. at signal. Arrow in second part of tracing marks 3.5 min. after beginning of each injection. The blood pressure record for oenanthotoxin was similar in form to that for picrotoxin except that the maximum rise was higher by 15 mm. Hg. Oenanthotoxin gave a greater and more prolonged increase in respiratory amplitude and rate than picrotoxin when administered at comparable levels of respiratory depression. Time, 0.25 min.

results obtained with the heart-lung preparation in the dog support the former possibility. The effects of small doses of oenanthotoxin upon the isolated rabbit heart suggest that the major cause of the hypotension is a direct depression of myocardial activity. The slowing of the heart in this phase is probably due to vagal discharge in that it was not seen in pithed rabbits, or after bilateral vagotomy in the intact animal.

Though the hypertensive phase was absent in the pithed animals the blood pressure rise may not be entirely central in origin since these preparations showed a reduced sensitivity to certain peripherally-acting hypertensive agents. Bilateral vagotomy obliterated the bradycardia of the hypertensive phase, increasing the blood pressure and cardiac dilatation. The slowing thus appears to be a reflex response to the raised blood pressure, though a contribution by central stimulation of the vagus is possible. Since the heart dilates as the blood pressure rises and *vice versa*, even in vagotomized animals, it is concluded that the cardiac dilatation in this phase is mainly a mechanical response to the hypertension. Similar responses can also be obtained by the injection of noradrenaline into intact or pithed animals.

Oenanthotoxin has been shown to be more potent than picrotoxin in all the actions examined, but a precise figure cannot be given for their relative effectiveness against pentobarbitone poisoning because of the small number of mice used. The higher early death rate of the mice treated with pentobarbitone and oenanthotoxin may be due to the slow absorption of the latter from the peritoneum.

SUMMARY

1. Crystalline oenanthotoxin and tinctures of *Oenanthe crocata* tubers have similar pharmacological properties.

2. Oenanthotoxin convulsions in rabbits and mice resemble those of picrotoxin; in rabbits and cats they have their origin in the brain stem.

3. Intravenous oenanthotoxin causes a transient fall followed by a sustained rise in blood pressure. The former is due to a direct action on the myocardium.

4. In mice, oenanthotoxin is six times as toxic as picrotoxin, and is more potent as an antidote for pentobarbitone poisoning. In the rabbit, it is a more powerful stimulant of respiration than picrotoxin.

5. Rabbits and cats given a lethal dose of oenanthotoxin can be saved by the early administration of pentobarbitones.

We wish to thank the director of the Botanic Garden, Cambridge, Dr. S. M. Walters, and Mr. P. C. Hall for supplies of *Oenanthe* tubers, and are indebted to Professor E. B. Verney for setting up the heart-lung preparations.

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