# ON THE PHARMACOLOGY OF PETALINE CHLORIDE, A CONVULSANT ALKALOID FROM *LEONTICE LEONTOPETALUM* LINN

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The alkaloid petaline chloride, obtained from extracts of *Leontice leontopetalum* Linn. is a more potent convulsant poison than leptazol. At lower dose levels it appears to reduce the convulsant activity of leptazol and apparently gives some protection from electrically induced seizures. Petaline chloride depresses both the patellar tendon reflex and the crossed extension reflex; it also has muscle relaxant activity and increases the rate, force and amplitude of the beat of the isolated auricle depressed in a low calcium medium. These properties are discussed in relation to its use in folk medicine.

EXTRACTS made from the fresh tuberous roots of *Leontice leontopetalum*, a plant which grows wild in the Lebanon, are used there as a folk remedy for *grand mal* epilepsy. An account of their preparation, actions, dosage and use is given by McShefferty and others[1] who, quoting a personal communication by Dr. W. M. Ford Robertson and Dr. A. S. Manugian of the Lebanon Hospital for Mental and Nervous Disorders, Asfuriyeh, Beirut, state that initially the juice from the fresh tubers is given in a dose of one teaspoonful three times daily for 3 days. This induces *status epilepticus*. After the initial treatment, large quantities of an aqueous extract of the marc left after preparation of the juice are given each day for several months. McShefferty and others[1] have investigated the chemistry of *L. leontopetalum* and Nelson and Fish[2,3] have described its history, sources, macroscopical and microscopical characters. A preliminary pharmacological investigation has also been made of the alkaloids petaline chloride and leonticine[1].

Petaline chloride, 0·05 mg. caused sedation and slowed respiration in 25 g. mice. Larger doses (0·075 mg.) induced respiratory distress and varying degrees of central nervous system stimulation. 0·08 or 0·1 mg. caused death from acute respiratory failure. It was concluded that the MLD of petaline chloride in the mouse was approximately 3·1 mg./kg. In the rabbit a total dose of 30 to 40 mg. of petaline chloride caused respiratory depression, mild clonic convulsions and death and the approximate MLD was 15·6 mg./kg. Petaline chloride was also shown to antagonise the stimulant actions of acetylcholine on the isolated frog rectus abdominis muscle. On this preparation it had about 65 per cent of the potency of gallamine triethiodide and its effects were antagonised by eserine.

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Extracts of the tubers of *L. leontopetalum* are undoubtedly complex mixtures and probably contain several pharmacologically potent compounds each of which may possess different types of activity. Results obtained from a study of one compound can naturally not be taken as expressing the whole activity of the plant, but the bizarre manner in which extracts are made use of by local inhabitants, the reports of confirmed cures of epilepsy[1] and the convulsant activity of the extracts seemed of sufficient interest for us to investigate more fully the properties of petaline chloride before considering the properties of extracts and other active principles. We are much indebted to Dr. J. B. Stenlake of the School of Pharmacy, Royal College of Science and Technology, Glasgow, for supplies of petaline chloride.

## Methods and Results

*Convulsant: Anticonvulsant Activity*

*Comparison with leptazol.* The method used was based on that of Goodman and others[4]. Male albino mice weighing 20 ± 1 g., or 25 ± 1 g., were used. Each group contained not less than 10 mice, the weight of the individuals remaining constant. Drugs in aqueous solution were injected into the tail vein. The volume of solution injected at each dose level was constant and the duration of injection did not exceed 2 to 3 seconds. The range of doses used is shown in Table I and the approximate CD50 and LD50 were obtained graphically for petaline chloride and leptazol. (Figs. 1 and 2). The CD50 of leptazol was found to be 40 mg./kg. and that for petaline chloride 6·6 mg./kg. The LD50 of leptazol was 56 mg./kg. and the LD50 of petaline chloride was 9·2 mg./kg. Petaline chloride is clearly a more potent convulsant and is more toxic than leptazol.

*Effect of pre-treatment with petaline chloride on the response to leptazol.* Three groups, each containing not less than 50 mice, were used. The weights of individuals in each group were constant at 20 ± 1 g. or 25 ± 1 g.

TABLE I

CONVULSANT ACTIVITY AND MORTALITY IN MICE AFTER PETALINE CHLORIDE OR LEPTAZOL (FIGS. 1 AND 2)

| Petaline chloride dose mg./kg. | Per cent convulsing | Per cent mortality |
|---|---|---|
| 4 | 10 | — |
| 6 | 40 | — |
| 8 | 70 | 20 |
| 10 | 100 | 70 |
| 12 | 100 | 100 |
| **Leptazol dose mg./kg.** | **Per cent convulsing** | **Per cent mortality** |
| 30 | 13 | — |
| 40 | 51·4 | 11·4 |
| 50 | 83·3 | 22·2 |
| 60 | 92·8 | 57·1 |
| 80 | 100 | 100 |

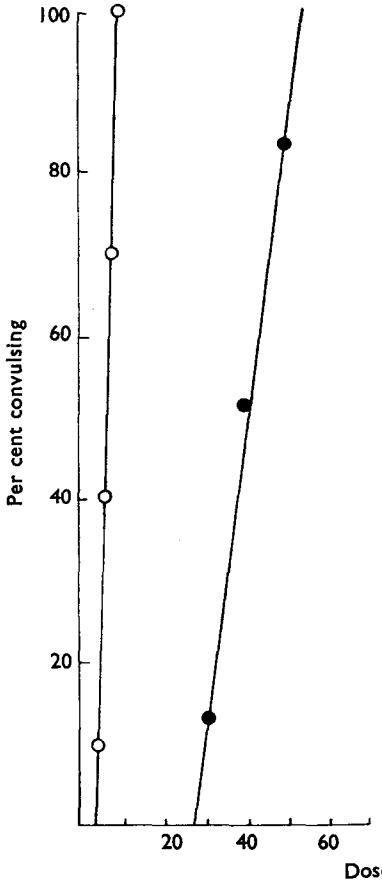


FIG. 1. Convulsant activity of petaline chloride (O—O) and leptazol (●—●) on the mouse.

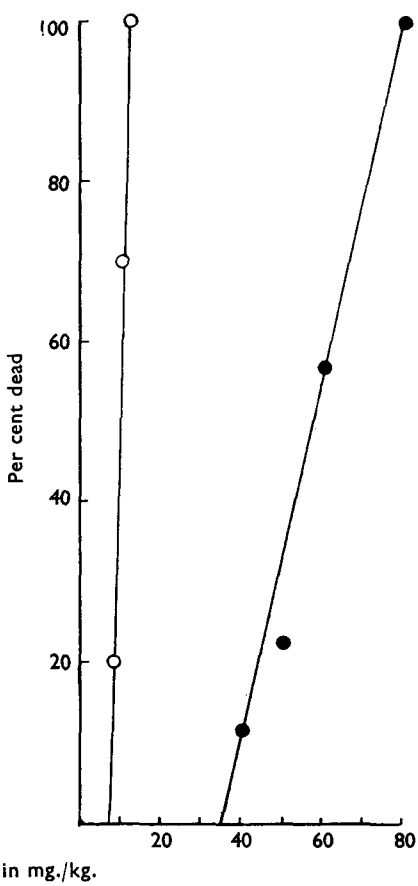


FIG. 2. Mortality in mice from petaline chloride (O—O) and leptazol (●—●).

The groups were given respectively 2·5, 5·0, or 8·0 mg./kg. of petaline chloride by intravenous injection. After an interval of 45 to 60 minutes the survivors of each large group were divided into four groups of about 10 and each group was given leptazol by intravenous injection. The doses of leptazol used were from 20 to 75 mg./kg. (Table II). The CD50 and LD50 of leptazol were calculated for the pre-treated animals and found to be respectively 42·8, 43·5 and 35 mg./kg. and 64, 70·7 and 48 mg./kg. (Figs. 3 and 4). Lower doses of petaline chloride may, therefore, give some protection but higher doses increase both convulsant activity and toxicity.

*Effect of petaline chloride on electro-shock seizures.* The method used was based on that of Swinyard[5] but ear clip electrodes[6] were employed. A supra-threshold current intensity of 20 mA applied for 5 seconds caused tonic extension of the hind limbs, which was taken as the end point.

TABLE II

CONVULSANT ACTIVITY AND MORTALITY OF LEPTAZOL GIVEN 45 TO 60 MINUTES AFTER PETALINE CHLORIDE. (FIGS. 3 AND 4)

| Leptazol dose mg./kg. | Petaline chloride dose mg./kg. | | | Petaline chloride dose mg./kg. | | |
|---|---|---|---|---|---|---|
| | 2·5 | 5·0 | 8·0 | 2·5 | 5·0 | 8·0 |
| | Per cent animals convulsing | | | Per cent mortality | | |
| 20 | — | — | 0 | — | — | — |
| 25 | 0 | — | 10 | — | — | 0 |
| 30 | — | 0 | — | — | — | — |
| 37·5 | 20 | 40 | — | 0 | — | — |
| 40 | 62·5 | — | 68·75 | — | — | 12·5 |
| 50 | 90 | 60 | 100 | 10 | 0 | 58·3 |
| 60 | — | — | — | 42·8 | — | 70·5 |
| 62·5 | — | 100 | — | 71·4 | 30 | — |
| 75 | 100 | — | — | 80 | 60 | — |

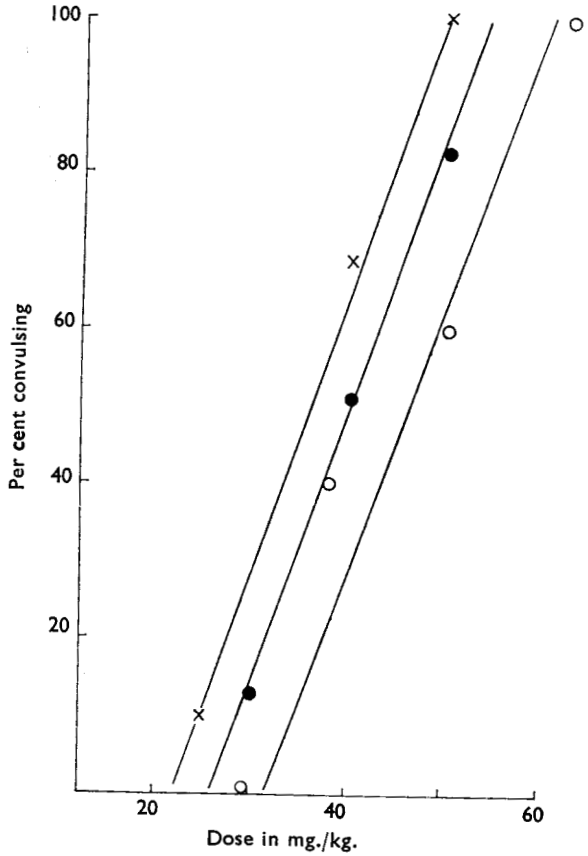


FIG. 3. Effects of pretreatment with petaline chloride, 5 (O—O) or 8 (×—×) mg./kg. on convulsant activity of leptazol (●—●).

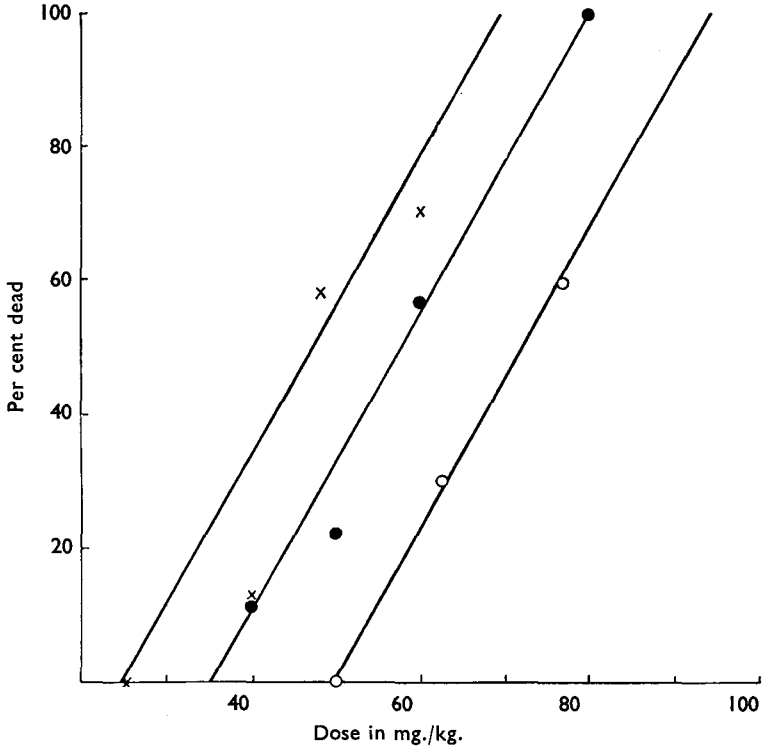


FIG. 4. Effects of pre-treatment with petaline chloride 5 (O—O) or 8 (×—×) mg./kg. on mortality due to leptazol (●—●).

Seven groups of 10 male albino mice were employed, the weights of the members of each group were constant at 10 ± 1, 15 ± 1 or 20 ± 1 g. All animals to be used for the tests were treated with a supra-threshold current of 20 mA for 5 seconds, 24 hours before the experiment and those which did not give the end point were discarded. Three groups of 10 mice were pre-treated with 4·0, 5·0 or 6·6 mg./kg. of phenobarbitone by intraperitoneal injection. The remaining four groups were given by the same route 1·66, 2·5, 3·3, or 5·0 mg./kg. of petaline chloride. After an interval of 1 hour electroshock treatment was repeated. The number of mice in each group failing to show the end point was counted and the percentage protection calculated (Table III, Fig. 5). Petaline chloride is apparently capable of conferring some protection but it is very much less potent than phenobarbitone. At higher doses petaline chloride protection was reduced.

*Muscle Relaxant Activity*

*Neuromuscular transmission in the cat.* The method adopted was that described by Lewis and Muir[7]. Cats weighing from 2·0 to 3·0 kg. were anaesthetised by intraperitoneal injection of 60 mg./kg. of sodium pentobarbitone. The gastrocnemius muscle was partially freed from the

TABLE III

PROTECTION OF MICE FROM ELECTROSHOCK BY PRE-TREATMENT WITH PETALINE CHLORIDE OR PHENOBARBITONE (FIG. 5)

| Petaline chloride dosage mg./kg. | Per cent protection |
|---|---|
| 1·6 | 10 |
| 2·5 | 25 |
| 3·3 | 37·5 |
| 5·0 | 20 |
| **Phenobarbitone dosage mg./kg.** | **Per cent protection** |
| 4 | 20 |
| 5 | 50 |
| 6·6 | 80 |

surrounding tissues and the achilles tendon severed near to its insertion into the calcaneus. The tendon was attached by means of a strong linen thread and pulley system to a myograph lever. The sciatic nerve was exposed on the lateral aspect of the thigh and stimulated by means of platinum electrodes using square impulses at a frequency of 8 to 10 per minute, 15 to 20 volts, pulse width 3 to 4 msec. In any given experiment frequency, voltage and pulse width were constant. In some experiments,

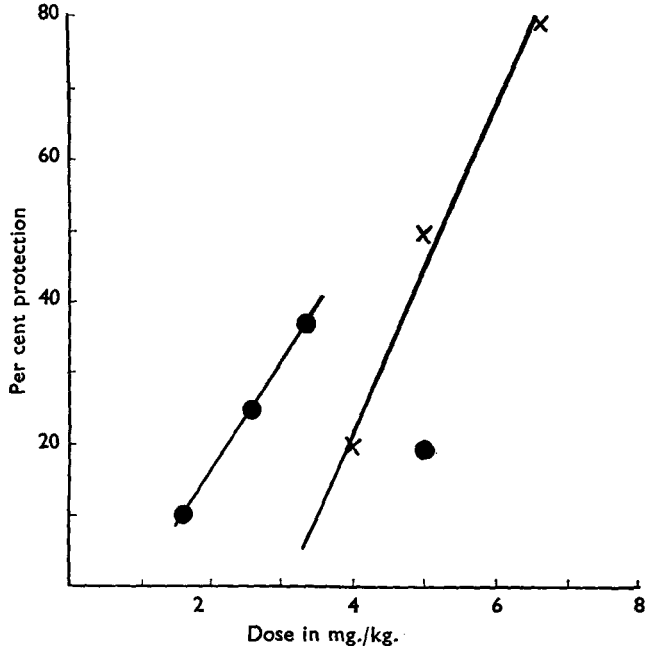


FIG. 5. Protection of mice from electroshock seizures by treatment with petaline chloride (●—●) or phenobarbitone (×—×).

however, a tetanizing current of 1,500 square impulses per minute was applied, voltage and pulse width remaining constant at the values stated. Drugs in aqueous solution were administered by way of the cannulated external jugular vein. Petaline chloride (3·0 to 10·0 mg./kg.) reduced twitch height; recovery occurred in about 15 to 20 minutes and the effects of petaline chloride were reversed by edrophonium (1·0 mg./kg.) or neostigmine methylsulphate (0·10 mg./kg.). When a tetanizing current was applied to the nerve of a petaline chloride treated muscle, the tetanus was not maintained—an effect similar to that seen when tubocurarine is used.

*Neuromuscular transmission in the rat diaphragm.* A method similar to that described by Bülbring[8] was used. The preparation was set up in a 100 ml. bath containing "double glucose" Tyrode's solution (NaCl, 0·8; KCl, 0·02; $CaCl_2.6H_2O$, 0·02; $MgCl_2$, 0·01; $NaHCO_3$, 0·1, $NaH_2PO_4$,$2H_2O$, 0·005 and glucose 0·2 per cent) at 29°. The solution was gassed with oxygen or a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The electrode described by Bell[9] was used so that the muscle could be stimulated both directly and indirectly. Stimulation of the nerve was by square impulses at a frequency of 6 to 8 per minute, 10 to 12 volts, pulse width 0·5 to 1·0 msec. When the muscle was stimulated directly the frequency was 6 to 8 per minute at 25 to 50 volts, pulse width 1·5 to 2·0 msec. In any given experiment, frequency, voltage and pulse width were constant. Drugs in aqueous solution were added directly to the bath. Petaline chloride (0·2 to 0·3 mg./ml.) abolished the response of the diaphragm to indirect stimulation but when the muscle was stimulated directly at a higher voltage and with a wider pulse, regular contractions were produced (Fig. 6).

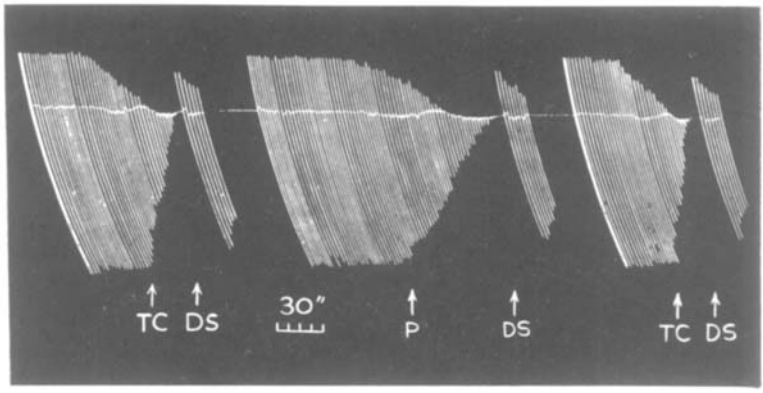


FIG. 6. Rat phrenic nerve-diaphragm preparation. Contractions induced by indirect stimulation of the phrenic nerve or by direct stimulation of the muscle.
Bath 100 ml., temperature 29°, bath fluid, oxygenated "double glucose" Tyrode's solution.
At TC, 3·0 μg./ml. tubocurarine added to bath.
At P, 0·20 mg./ml. petaline chloride added to bath.
At DS, direct stimulation of the muscle.

*Frog rectus abdominis muscle.* The method used was essentially that of de Jalon[10]. Frogs of either sex were decapitated and pithed. The rectus abdominis muscle was dissected and set up in a 10 ml. bath containing oxygenated frog Ringer's solution (NaCl, 0·65; KCl, 0·014; $CaCl_26H_2O$, 0·012; $NaHCO_3$, 0·02; $NaH_2PO_42H_2O$, 0·001 and glucose 0·2 per cent) at room temperature. Acetylcholine chloride (0·10 to 1·0 $\mu$g./ml.) was added to the bath and left in contact with the tissue for 90 seconds, the contraction being recorded. Drugs were added to the bath 30 seconds before a subsequent addition of acetylcholine and the contraction again recorded for 90 seconds. The bath was then washed out. Between successive additions of acetylcholine, there was an interval of 7 minutes. 5 to 20 $\mu$g./ml. of petaline chloride antagonised contractions caused by acetylcholine chloride (0·1 to 1·0 $\mu$g./ml.); a graded effect was seen. Petaline chloride was found to have about 50 per cent of the potency of gallamine triethiodide on this preparation. (Fig. 7).

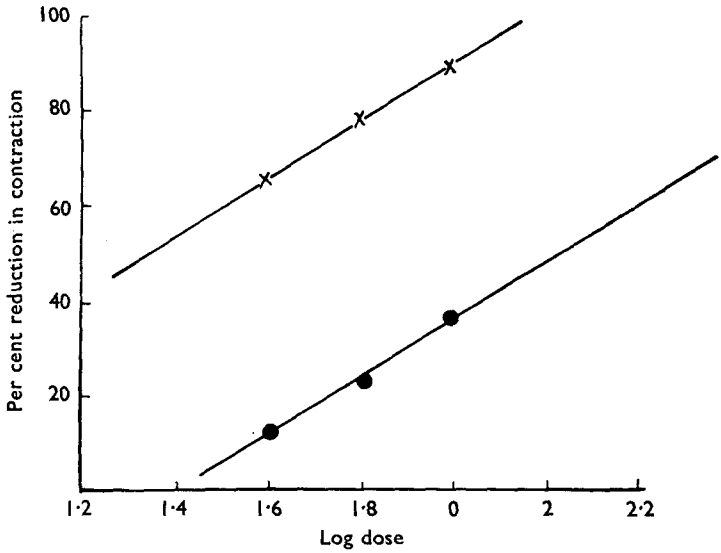


FIG. 7. A comparison of the effects of gallamine triethiodide (×—×) and petaline chloride (●—●) on acetylcholine-induced contractions of the frog rectus abdominis muscle.

*Other Effects*

*Cardiovascular Actions*

*Blood pressure of anaesthetised cat.* Cats weighing from 2·0 to 3·0 kg. were anaesthetised by intraperitoneal injection of 60 mg./kg. of sodium pentobarbitone. Blood pressure was recorded from the common carotid artery using a mercury manometer and drugs in aqueous solution were injected by way of the cannulated external jugular vein. Petaline chloride (2·0 to 4·0 mg./kg.) caused an immediate fall of blood pressure. The

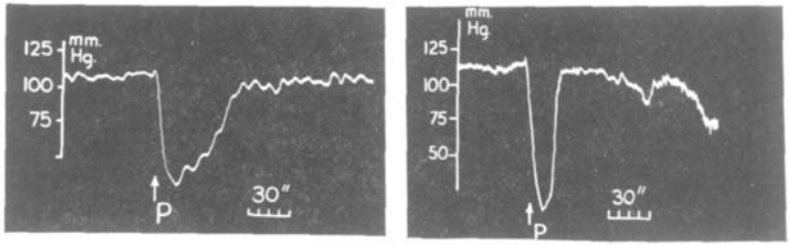


FIG. 8. Effect of petaline chloride on arterial blood pressure. Left hand record, cat carotid artery blood pressure. At P, 2 mg./kg. petaline chloride. Right hand record, rat carotid artery blood pressure. At P, 58 $\mu$g./100 g. petaline chloride.

level returned to normal in from 5 to 10 minutes (Fig. 8). In some cases there was a slow secondary fall. This was only seen with higher doses. There was no effect on the blood pressure level of spinal cats even at doses of up to 8·0 mg./kg.

*Blood pressure of anaesthetised rat.* The technique employed was similar to that described by Dekanski[11]. Rats weighing 300 to 350 g. were used. Anaesthesia was induced by subcutaneous injection of 175 mg./100 g. of urethane. Blood pressure was recorded from the cannulated common carotid artery by means of Condon's manometer[12] and drugs in aqueous solution were administered by way of the cannulated external jugular vein. Petaline chloride (20 to 60 $\mu$g./100 g.) caused an immediate but short lived fall in blood pressure (Fig. 8). In some experiments when higher doses of petaline chloride were used there was a gradual secondary fall. There was slight antagonism by petaline chloride (30 to 40 $\mu$g./100 g.) to the pressor effects of 0·05 to 0·10 $\mu$g./100 g. of adrenaline bitartrate or noradrenaline bitartrate.

*Rat hind-quarters preparation.* The isolated hind-quarters of rats were perfused with oxygenated Locke's solution (NaCl, 0·9; KCl, 0·042; $CaCl_26H_2O$, 0·024; $NaHCO_3$, 0·05 and glucose 0·1 per cent) at room temperature, using the method described by Burn[13]. Outflow was recorded, using Stephenson's[14] recorder and drugs in aqueous solution were administered by way of the perfusion cannula. Petaline chloride (2·0 to 3·0 mg.) caused slight vasodilatation and slight antagonism to the vasoconstrictor actions of adrenaline bitartrate (1·0 $\mu$g.) or noradrenaline bitartrate (1·0 $\mu$g.) (Fig. 9).

*Isolated perfused rabbit or kitten heart.* Hearts were perfused using Langendorff's method[15]. The perfusion fluid was well oxygenated "double glucose" Locke's solution at 37°. Outflow was recorded by Stephenson's[14] recorder and drugs in aqueous solution were injected into the perfusion cannula. Petaline chloride 2 to 4 mg. increased slightly the rate and amplitude of the ventricular contractions and caused a slight increase in outflow. A similar effect was seen when the heart was perfused with 200 $\mu$g./ml. of petaline chloride in Locke's solution.

*Isolated guinea pig auricles.* The auricles were set up in a 10 ml. bath containing well oxygenated Locke's solution at 29°. Drugs in aqueous

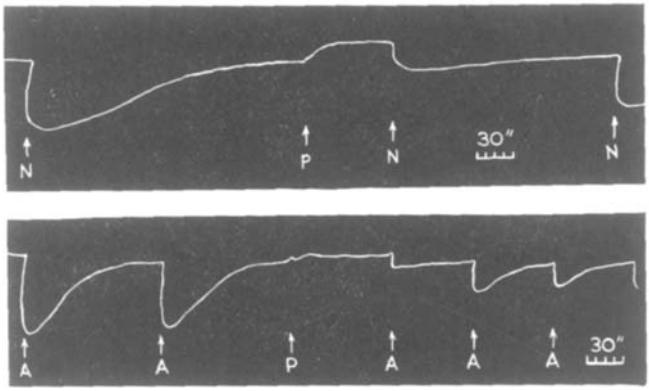


FIG. 9. Perfused rat hind quarters. Perfusion with Locke's solution at room temperature. Outflow recorded by Stephenson's outflow recorder. Drugs in aqueous solution injected into the perfusion cannula.

At N, 1·0 μg. noradrenaline bitartrate.
At A, 1·0 μg. adrenaline bitartrate.
At P, 2·0 mg. petaline chloride.

solution were added directly to the bath and kept in contact with the auricles for 60 seconds. Petaline chloride (200 to 400 μg./ml.) increased the rate and amplitude of the contractions but after washing they returned to normal. There was no antagonism or very slight antagonism to the effects of adrenaline bitartrate (0·10 μg./ml.), noradrenaline bitartrate (0·10 μg./ml.) or histamine acid phosphate (0·10 μg./ml.), but the effects of acetylcholine chloride (0·02 μg./ml.) were antagonised. When the auricles were perfused with Locke's solution containing half the usual amount of calcium chloride the amplitude and rate were reduced and the auricles gradually came to rest. Petaline chloride (200 μg./ml.) added to the bath restored the rate to normal and the amplitude to supranormal levels (Fig. 10).

*Effects on Smooth Muscle*

*Guinea-pig ileum.* The method used was a modification of that described by Guggenheim and Loffler[16]. A 5 ml. bath containing oxygenated Tyrode's solution at 37° was used. Drugs in aqueous solution were added to the bath and kept in contact with the tissue for 15 seconds. Large doses of petaline chloride (1 mg./ml.) caused a slight increase in spontaneous activity. The responses to acetylcholine chloride (1·0 μg./ml.), histamine acid phosphate (0·3 μg./ml.), 5-hydroxytryptamine creatinine sulphate (1·0 μg./ml.), barium chloride (0·6 mg./ml.) and potassium chloride (5·0 mg./ml.) were antagonised by petaline chloride (100 μg. to 1·0 mg./ml.), added 30 seconds beforehand. The effect was reversible on washing and there was a graded inhibition according to the dose.

*Rat uterus.* The method adopted was a modification of that described by Amin, Crawford and Gaddum[17]. Virgin female rats weighing from 150 to 180 g. were employed and brought into oestrus by subcutaneous

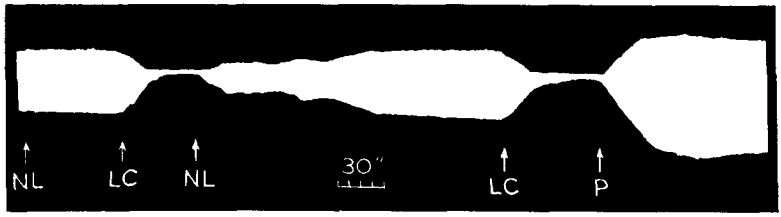


FIG. 10. Isolated guinea pig auricles. Record of spontaneous contractions. Bath 10 ml., temperature 29°. Bath fluid oxygenated Locke's solution. At NL, Locke's solution.
At LC, the bath fluid changed to "half calcium" Locke's solution.
At P, 200 μg./ml. petaline chloride added to bath.

injection 24 hours before use of 0·10 mg./100 g. of stilboestrol in arachis oil. One horn of the uterus was set up in a 5 ml. bath containing de Jalon's solution (NaCl, 0·9; KCl, 0·042; $CaCl_26H_2O$, 0·006; $NaHCO_3$, 0·05 and glucose 0·1 per cent) at 29°. The solution was gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. Drugs in aqueous solution were added to the bath and allowed to remain in contact with the tissue for 30 seconds. In doses of 20 to 60 μg./ml. petaline chloride reduced the height of contractions produced by 0·10 to 0·50 μg./ml. of acetylcholine chloride. There was a graded inhibition according to dose and recovery to control levels was usually complete. No direct effects were seen.

*Toxic Effects in the Rabbit*

Only two rabbits were used. One (2·75 kg.) was given 30 mg. petaline chloride by intravenous injection. It immediately developed convulsive jerky movements, respiration stopped and about 3 minutes later the heart ceased to beat. After death the animal was flaccid. Similar effects were seen in a second rabbit (2·25 kg.) given 35 mg. of petaline chloride. These results are similar to those of McShefferty and others[1].

## DISCUSSION

Petaline chloride is a potent convulsant and is apparently 5 to 7 times as powerful as leptazol. Lower doses (2·5 and 5 mg./kg.) may reduce the convulsant activity of a subsequent dose of leptazol and apparently give some protection from electrically induced seizures, but higher doses (8 mg.) are additive with leptazol both with respect to convulsant activity and acute toxicity. Petaline chloride has muscle relaxant activity, it also depresses the patellar tendon reflex and the crossed extension reflex and is more effective than mephenesin with respect to the latter. Similar effects to these are seen when tubocurarine is used and the effect is probably due to the muscle relaxant activity. When these actions are taken, in conjunction with the stimulant effects upon isolated cardiac muscle, in particular the effect on the isolated auricles depressed in a low calcium medium, then there may be some basis for its use in folk medicine. It

must not, however, be forgotten that the extracts used undoubtedly contain other substances[1] and that the disease reported to have been treated may not have been correctly diagnosed by the native physicians or the sufferers' relatives. We hope to have the opportunity of testing other extracts and pure substances.

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