ANALGESIC AND OTHER PHARMACOLOGICAL PROPERTIES OF 1-$\triangle^3$-PIPERIDEINO-3-*O*-TOLOXY PROPAN-2-OL HYDROCHLORIDE (TOLPRONINE)

BY A. DAVID, FIONA LEITH-ROSS AND D. K. VALLANCE

From the Biological Laboratories, The British Drug Houses, Ltd., Godalming, Surrey

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1-Δ^3-Piperideino-3-*o*-toloxypropan-2-ol hydrochloride, Tolpronine, possesses marked analgesic activity, being approximately 20 times as active as metamizolum subcutaneously and approximately 11 times as active orally. The induction time after subcutaneous injection is shorter than with metamizolum and the duration similar. Excessively large amounts constipate normal mice; a weak spasmolytic effect is seen in the isolated guinea pig ileum and rabbit duodenum. The jejunum of the anaesthetised cat shows a spasmogenic effect. Chronic administration to rats and rabbits caused a reduction in the growth rate of rats.

THE local anaesthetic activity of some 1-amino-3-aryloxy-propan-2-ols was described by Petrow, Stephenson and Thomas[1]. Certain of these compounds were also found to possess analgesic properties (*cf.* Fourneau[2]) and a comprehensive series of 1-amino-3-aryloxypropan-2-ols (I)

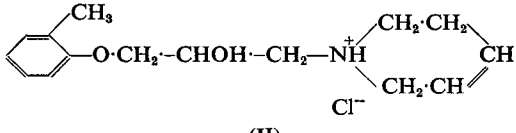
$$ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2NRR'$$

(I)

was prepared by Dr. V. Petrow and his colleagues[3], and evaluated for analgesic activity. The results proved encouraging and the work was extended to the formally related types indicated below.

$ArO{\cdot}(CH_2)_n{\cdot}NRR'$
$ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}OCH_2{\cdot}CH_2{\cdot}NRR'$
$ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}O{\cdot}CH_2CHOH{\cdot}CH_2{\cdot}NRR'$
$ArO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2NRR'$
$ArO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NRR'$
(o)–$HO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}O{\cdot}C_6H_4{\cdot}CONRR'$

1-Δ^3-Piperideino-3-*o*-toloxypropan-2-ol hydrochloride, Tolpronine (II) was the most promising of more than 50 compounds examined and was selected for further studies.



(II)

It is a white crystalline substance, molecular weight 283·8, with a melting point of 136 to 137·5°. It has a bitter taste and is readily soluble in water, a 10 per cent solution having a pH of approximately 5·8. Aqueous solutions are stable to heat but unstable to acids and alkalies.

METHODS

Acute Toxicity

This was estimated in male albino mice by the oral, intraperitoneal and subcutaneous routes. Mice weighing approximately 20 g., were fasted overnight before oral or intraperitoneal administration. The compounds were given in aqueous solution adjusted to 0·5 ml./20 g. body weight. Oral and subcutaneous toxicities were estimated by giving the compound at four, five or six dose levels, increasing in geometrical progression by a factor of 4/3. Ten animals were used at each dose. Intraperitoneal toxicities were estimated by giving the compounds at five dose levels increasing by a factor of 1·5, five animals being used at each dose level. The LD50s were calculated by Litchfield and Wilcoxon's[4] method or Kärber's[5] formula from the mortalities in seven days.

Acute oral and subcutaneous toxicities were also estimated in adult male albino rats. The animals were fasted overnight before oral administration and the compounds were given at four, five or six dose levels, with a dose ratio of 1·5. The volumes were adjusted to 25 ml./kg. for the oral route and to 10 ml./kg. for the subcutaneous route. Ten animals were used at each dose level. LD50s were calculated from the mortalities in seven days using Kärber's[5] formula.

Chronic Oral Toxicity

Immature male albino rats in two groups of ten animals were given an aqueous solution of the compound by stomach tube five days a week or water only. The daily dose of 100 mg./kg. was adjusted to the individual body weights at the beginning of each week and all volumes were adjusted to 25 ml./kg. The animals were kept under identical conditions and were allowed diet 41 and water *ad lib.* At the end of the six weeks experimental period five animals from each group were sacrificed and the spleen, liver, kidney, heart and lung were examined histologically. The remaining animals were kept under observation for a further period of seven weeks.

Five immature rabbits were given 50 mg./kg. in aqueous solution by stomach tube five days a week for eight weeks. Five controls were given water, 5 ml./kg. daily. The animals were kept under identical conditions and were allowed diet 18 and water *ad lib.* Red, white and differential cell counts, haemoglobin values, and weights were determined weekly.

Analgesic Activity

Analgesic acitivity after oral, intraperitoneal and subcutaneous administration was estimated in male albino mice, by the method of Bianchi and Franceschini[6]. The animals weighed 15 to 20 g. and were fasted overnight for the oral and intraperitoneal tests. A bulldog artery clip covered with catheter tubing was applied to the base of the tail; only those making continuous attempts to remove the clip within 15 seconds were regarded as sufficiently sensitive for use.

The compounds were given in aqueous solution, in volumes adjusted to 0·5 ml./20 g. body weight. They were given at four or five dose levels,

with dose ratios of 1·5 for the oral and 2·0 for the intraperitoneal and subcutaneous routes. Five animals were used initially at each dose level, the doses being injected in a random order. Thirty minutes after administration, the clip was applied to each mouse in turn. If no attempt was made to remove the clip within 30 seconds an analgesic state was assumed to be present.

In certain experiments the clip was also applied at 60 and 90 minutes after administration. The ED50 was calculated, using Litchfield and Wilcoxon's[4] method or Kärber's[5] formula.

Duration of Analgesic Action

The compounds were given subcutaneously to groups of 20 mice at the ED80 dose level. The clip was then applied at 30 minute intervals after injection. Onset of analgesia was taken to be 15 minutes before the first failure to respond and the time of recovery to be 15 minutes before the normal response reappeared. The difference between the two provided an estimate of the duration of analgesia to the nearest half-hour.

Local Anaesthetic Activity

The intracutaneous wheal test of Bülbring and Wajda[7] was employed. On the day before the test, the hair on the posterior half of each guinea pig's back was clipped and shaved. The animals were divided into three groups of six, the groups being allocated to high, medium or low concentrations. Three animals from each group were injected in the anterior half of the shaved area with the test compound and with the reference drug in the posterior half, the positions being reversed in the other three animals. The order of injection was unknown to the observer. Each dose was injected intracutaneously in the mid-line in 0·2 ml. of normal saline and the resultant wheal was outlined in ink. The response to six pin pricks applied at various points inside each wheal area was determined 5 minutes after injection and at 5 minute intervals for 30 minutes. The number of negative responses to the total of 36 stimuli was recorded. The mean of the six results for each concentration was calculated and plotted against the log of the concentration.

Anticonvulsant Activity

The leptazol seizure test described by Goodman and others[8] was used. Aqueous solutions or suspensions in 5 per cent acacia mucilage were given orally to groups of five fasted male albino mice at four dose levels, in volumes adjusted to 0·5 ml./20 g. body weight. The doses increased by a factor of 2·0. Two hours after administration the animals were given a rapid intravenous injection of a convulsant dose (50 mg./kg.) of leptazol. Those animals not developing the hindleg tonic extensor component of the convulsion were counted. The dose required to give protection to 50 per cent of the animals was calculated.

Action on the Gastrointestinal Tract

Isolated rabbit duodenum. Segments of rabbit duodenum were suspended in Ringer solution in a 70 ml. bath at 35°. A mixture of 95 per

cent oxygen and 5 per cent carbon dioxide was bubbled through the solution. When rhythmic contractions started, varying volumes of 0·1, 1·0 and 10 per cent aqueous solutions of the compound were added at suitable intervals and usually allowed to act for 2 minutes before washing out.

Isolated guinea pig ileum. Spasmolytic activity was estimated on the isolated guinea pig ileum. A 4 cm. segment was suspended in Tyrode's solution in a 25 ml. bath at 35°. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the solution. Submaximal doses (0·8 μg.) of acetylcholine were added at 3 minute intervals and allowed to act for 30 seconds. Varying amounts of the compounds were added 30 seconds before the addition of acetylcholine. The response to acetylcholine was allowed to return to normal between doses of the spasmolytics. The heights of the contractions immediately before and after the addition of the spasmolytic were measured. The inhibition per cent was plotted against log dose and the amount causing 50 per cent inhibition determined.

The effect on the peristaltic reflex was investigated on a 5 cm. segment suspended in oxygenated Tyrode's solution at 36° in a 70 ml. bath. Using the method of Trendelenberg, a record of peristaltic contractions was obtained by raising the pressure in the lumen by 3 cm. of Tyrode's solution for 2 minutes at 5 minute intervals. The compound was added to the bath 1 minute before raising the pressure and was washed out on reducing the pressure.

Defaecation. The method was based on that described by Lou[9] for the assay of vegetable purgatives. Male albino mice, weighing approximately 20 g., were divided into five groups of nine animals. Four groups were given varying amounts of the compound in aqueous solution by stomach tube, the volume being adjusted to 0·5 ml./20 g. body weight. The fifth group served as controls and were given a similar volume of water. Fifteen minutes after administration, the mice were placed in individual compartments over a wire grid and the faeces collected on blotting paper. The 15 minute interval was considered advisable as defaecation frequently occurred after handling. The mice were allowed free access to a paste made of Rat Diet 41 and water. The total number of faecal pellets from each animal was counted at 8 and 24 hours after administration.

Cat jejunum in situ. The cat was anaesthetised with ether followed by chloralose, 60 mg./kg. intravenously. A water filled balloon was inserted into the jejunum through an abdominal incision and contractions were recorded by means of a water/air transmission system connected to a small piston recorder. The pressure in the balloon was approximately 10 cm. of water. Aqueous solutions of the compound were injected intravenously when spontaneous rhythmic contractions had become established.

Actions on the Cardiovascular and Respiratory Systems

Cat blood pressure. The carotid blood pressure was recorded in cats anaesthetised with ether followed by chloralose, 60 to 80 mg./kg.,

ntravenously. Heparin, 500 to 1000 units, was injected intravenously to prevent clotting. All doses were given in aqueous solution through the cannulated femoral vein.

Isolated rabbit heart. A Langendorff preparation was perfused with Ringer-Locke's solution at 39° and aerated with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The amplitude of the cardiac

TABLE I

ACUTE TOXICITY IN MALE ALBINO MICE

Compound	Route	LD50 mg./kg.	Limits of error (P=0·95) mg./kg.
Tolpronine ..	Oral	330	290–380
Metamizolum ..		4030	3570–4550
*Tolpronine ..	Intraperitoneal	200	—
Metamizolum ..		3050	—
Tolpronine ..	Subcutaneous	500	430–590
Metamizolum ..		3440	2940–4020
*Tolpronine ..	Subcutaneous	390	—
Codeine phosphate		220	—
Tolpronine ..	Subcutaneous	530	460–620
Procaine hydrochloride		1210	1050–1390

*Calculated by Kärber's formula.

contractions was recorded by means of a thread attached to a heart lever. Varying volumes of an aqueous solution of the compound were administered by injection into the perfusion cannula.

Respiration of the anaesthetised cat. Respiration was recorded by Paton's[10] method.

RESULTS

Acute Toxicity

The acute toxicity of Tolpronine in mice was compared with metamizolum, codeine phosphate and procaine hydrochloride by various routes. Metamizolum was usually employed as the reference compound as it is not related chemically to the opium alkaloids and possesses moderate analgesic properties. Table I records the LD50s.

TABLE II

ACUTE TOXICITY IN MALE ALBINO RATS

Route	Compound	LD50 mg./kg.
Oral	Tolpronine	340
	Metamizolum	4250
Subcutaneous	Tolpronine	1030
	Metamizolum	2780

Tolpronine was rapidly fatal by the oral and intraperitoneal routes but death was frequently delayed for 24 hours after subcutaneous doses. Loss of the righting reflex was observed at near lethal subcutaneous doses.

Table II records LD50 values in rats. Death, as in mice, occurred somewhat more rapidly following oral than subcutaneous administration.

Loss of the righting reflex was observed with near lethal amounts by the latter route.

Chronic Oral Toxicity

The mean growth curves of control animals and rats given 100 mg./kg. of Tolpronine on five days a week are compared in Figure 1. The mean increase in weight of the treated group after six weeks was 62·9 g., standard

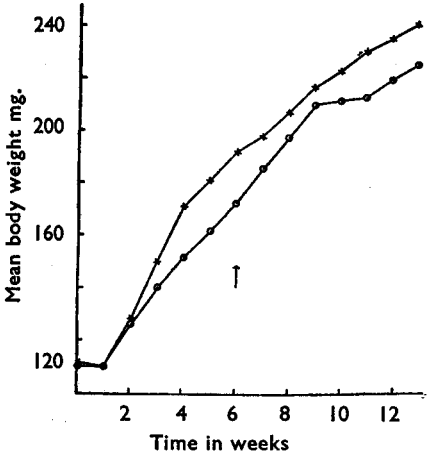


FIG. 1. Effect of Tolpronine (100 mg./kg./day) on the growth of immature male rats. O——O treated group, ×——× control group, ↑ indicates where treatment discontinued.

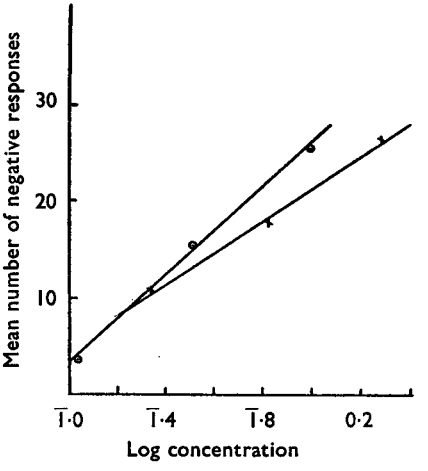


FIG. 2. Local anaesthetic activity of Tolpronine (O——O) and procaine hydrochloride (×——×). Guinea pig intracutaneous wheal test.

error 5·25 g., compared with 81·1 g., ± 5·28 g. for the controls. This difference was statistically significant ("Student's" test). No deaths occurred. Histological examination of spleen, liver, kidney, lung and heart from animals killed at the end of six weeks revealed no significant changes.

TABLE III

ANALGESIC ACTIVITIES IN MALE ALBINO MICE

Compound	Route	ED50 mg./kg.	Limits of error (P=0·95) mg./kg.
Tolpronine	Oral	130	90–180
Metamizolum		1470	1100–1976
*Tolpronine	Intraperitoneal	65	—
Metamizolum		710	—
Tolpronine	Subcutaneous	35	23–54
Metamizolum		730	495–1070
*Tolpronine	Subcutaneous	45	—
Codeine phosphate		30	—

*Calculated by Kärber's formula.

There was no significant difference between the weekly body weights and haematological values of rabbits given Tolpronine 50 mg./kg. daily by mouth for eight weeks and the control animals. Histological examination of liver, spleen, kidney and stomach also failed to demonstrate any abnormalities.

Analgesic Activity

Table III compares the analgesic activities of Tolpronine and metamizolum by various routes. For oral and subcutaneous routes, 15 animals were used at each dose level. Intraperitoneally, five animals at each dose level were tested at 30 and 60 minutes following administration. Table III gives the relative analgesic activities of Tolpronine and codeine phosphate by the subcutaneous route. Five animals at each dose level were tested at 30, 60 and 90 minutes after the injection.

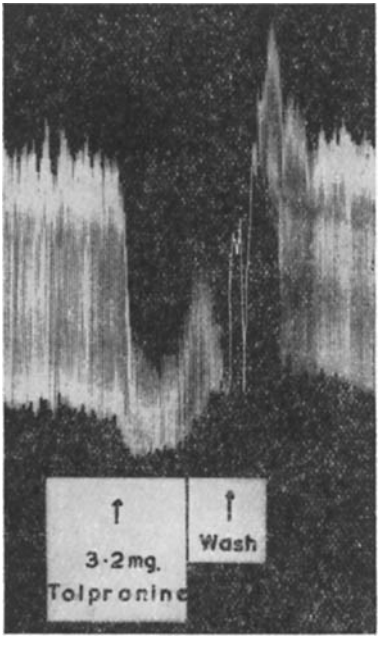


FIG. 3. Effect of Tolpronine (3·2 mg. in 70 ml. bath for two minutes) on pendular movements of isolated rabbit duodenum.

Duration of Analgesic Action

Tolpronine and metamizolum were compared for duration of analgesia at the ED80 level, i.e., 85 and 1600 mg./kg. respectively. After subcutaneous injection of Tolpronine, 17 of 20 animals showed analgesia. The mean induction time was 15 minutes and the mean duration 180 minutes (range 30 to 270 minutes). Metamizolum induced analgesia in 19 of 20 mice with a mean induction time of 30 minutes and a mean duration of 195 minutes (range 60 to 330 minutes).

Local Anaesthetic Activity

Tolpronine was injected as 0·11, 0·33 and 1·00 per cent solutions and procaine hydrochloride as 0·22, 0·67 and 2·00 per cent solutions. Figure 2 shows the mean number of negative responses plotted against log concentration. From this graph the concentration producing 18 out of a possible 36 negative responses, was determined. The value for Tolpronine was 0·45 per cent and for procaine hydrochloride 0·67 per cent. Tolpronine produced no local irritation or tissue damage.

Anticonvulsant Activity

Tolpronine had no anticonvulsant properties in mice at doses up to 200 mg./kg., i.e., approximately 60 per cent of its oral LD50. Troxidone,

TABLE IV

THE EFFECT OF TOLPRONINE AND METHANTHELINE BROMIDE ON THE ACETYLCHOLINE INDUCED CONTRACTION OF THE ISOLATED GUINEA PIG ILEUM

Compound	Dose μg.	Per cent reduction	Dose causing 50 per cent reduction μg.
Tolpronine	10	28	25
	20	40	
	40	61	
	80	77	
Methantheline bromide	0·01	22	0·03
	0·02	42	
	0·04	54	
	0·08	71	

suspended in 5 per cent acacia, had a PD50 of approximately 300 mg./kg., corresponding to approximately 10 per cent of its oral LD50.

Action on the Gastrointestinal Tract

Isolated rabbit duodenum. Tolpronine had no effect on normal rhythmic contractions in amounts below 0·4 mg. Larger amounts, up to 12·8 mg., had slight spasmolytic action. The higher concentrations cause a reduction in tone, but complete recovery occurred on washing out. Figure 3 records a typical response.

Isolated guinea pig ileum. The spasmolytic activity of Tolpronine was estimated using methantheline bromide as the reference compound. The results are recorded in Table IV.

At 0·1 mg. Tolpronine produced little or no inhibition of the peristaltic reflex, while 0·2 mg. caused a partial or complete inhibition. The addition of 0·4 mg. or of larger amounts caused complete inhibition. Figure 4 records typical responses.

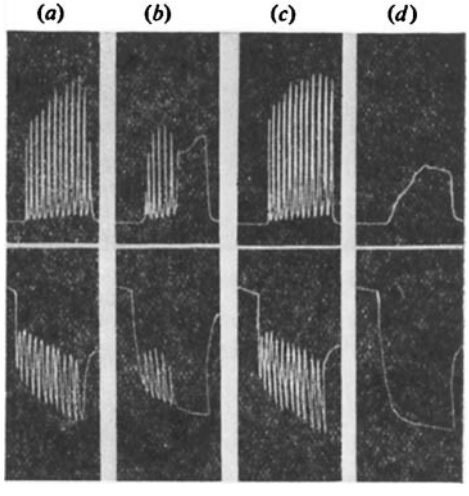


FIG. 4. Effect of Tolpronine on peristaltic reflex. Guinea pig ileum; 70 ml. bath; Trendelenberg preparation. Upper record-longitudinal contractions; lower record-volume changes. (*a*) Normal record, (*b*) one minute after 0·2 mg. Tolpronine, (*c*) normal record, (*d*) one minute after 0·4 mg. Tolpronine.

Defaecation. Tolpronine, 240 mg./kg., had no effect on the number of pellets passed in the first 8 hours. In the next 16 hours the number of faecal pellets passed was reduced to 35 per cent of the controls. There was little or no effect at lower doses.

Cat jejunum in situ. Tolpronine, 0·25 to 2 mg./kg., produced a considerable increase in the tone of the jejunum of an anaesthetised male cat weighing 2·7 kg. The increase occurred within a few seconds of injection and persisted for several minutes before a rapid fall to initial level. Amounts below 0·25 mg./kg. had no effect. Figure 5 illustrates a typical response.

Action on the Cardiovascular and Respiratory Systems

Cat blood pressure. Four cats weighing between 2·7 and 4·8 kg. were used. Amounts from 0·5 to 2·0 mg./kg. caused falls in blood pressure which did not exceed 50 mm. Hg., followed by a gradual return towards the initial level. The fall in blood pressure was not abolished by the intravenous injection of 2 mg. atropine sulphate nor by section of both vagi. Figure 6 shows a typical response following 1 mg./kg. of Tolpronine and codeine phosphate. No effects were observed following 20 mg./kg. of metamizolum.

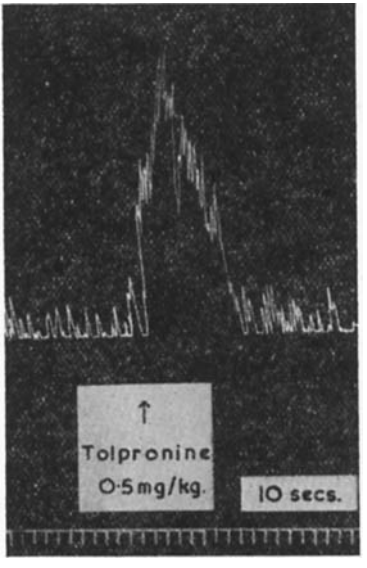


FIG. 5. Effect of intravenous injection of Tolpronine on intact jejunum of 2·7 kg. cat anaesthetised with chloralose, 60 mg./kg. intravenously.

Isolated rabbit heart. 2 mg. of Tolpronine caused a 50 per cent reduction in the amplitude of contraction of the isolated rabbit heart with recovery in approximately 90 seconds. Smaller amounts had little effect.

Respiration of the anaesthetised cat. Respiratory changes in three cats used in the blood pressure experiments were also recorded. Doses of Tolpronine from 0·55 to 1·1 mg./kg. usually produced a transient slight reduction in respiratory minute volume although the rate was increased. Figure 6 illustrates a typical response and also shows the slight respiratory depression produced by a similar amount of codeine phosphate.

DISCUSSION

Tolpronine possesses significant analgesic properties in mice by the subcutaneous, oral and intraperitoneal routes. The compound is most active subcutaneously, having approximately 20 times the activity of metamizolum on a weight for weight basis. The therapeutic indices of Tolpronine and metamizolum are 14·3 and 4·3 respectively, giving Tolpronine a wider margin of safety. Subcutaneously, the duration of action of the compounds is similar, but the new compound appears to have a rather more rapid onset of action. Compared with codeine phosphate, Tolpronine is approximately 0·7 times as active and has a

therapeutic index of 8·7 compared with 7·3 for codeine phosphate. Given orally, tolpronine is approximately 11 times as active as metamizolum; their therapeutic indices are similar, being 2·5 and 2·6 respectively. Tolpronine is also approximately 11 times as active as metamizolum when injected intraperitoneally.

It appears that there is a decrease in toxicity and an increase in activity of Tolpronine by the subcutaneous as compared with the oral and intraperitoneal routes, thus changes in activity and toxicity do not parallel

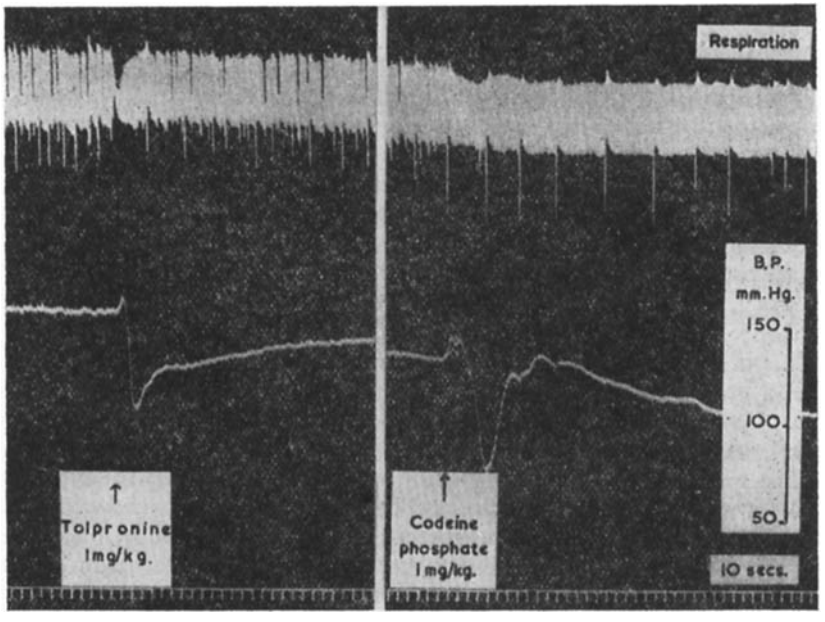
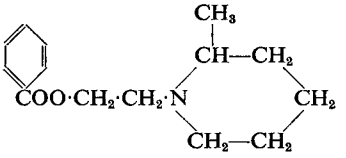


FIG. 6. Effects of intravenous injection of Tolpronine and codeine phosphate on blood pressure and respiration of chloralosed cat.

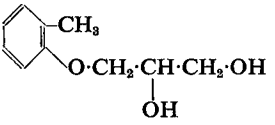
each other. This indicates that the analgesic action of Tolpronine in mice is not merely a manifestation of general toxicity. Bianchi and Franceschini[6] and Jackson[11], using mice and rats respectively, have reported similar findings with other analgesics.

The tail pinch technique, which is rapid and easy to carry out, appears to be particularly useful for screening new compounds with moderate analgesic activity.

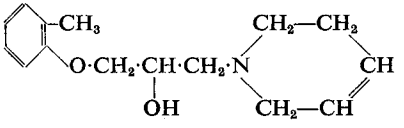
In addition to its analgesic activity, Tolpronine also possesses local anaesthetic properties, being approximately 1·5 times as active as procaine hydrochloride in the guinea pig intracutaneous wheal test. It is, however, at least twice as toxic by the subcutaneous route. The local anaesthetic action is perhaps not surprising as Tolpronine has structural similarities with piperocaine and mephenesin, the latter also having local anaesthetic activity of the same order as procaine.



Piperocaine



Mephenesin



Tolpronine

Its effect on the gastrointestinal tract is somewhat variable but a weak spasmolytic effect is usually observed. It has an anti-acetylcholine action on the isolated guinea pig ileum, although it has only about 0·1 per cent of the activity of methantheline bromide. In large amounts it causes inhibition of the pendular movements of the isolated rabbit duodenum and of the peristaltic reflex in the isolated guinea pig ileum. It has a constipating effect in normal mice only at very high dose levels. In contrast, the intravenous injection of Tolpronine has a spasmogenic effect on the jejunum of the anaesthetised cat.

Rapid intravenous injection of moderate amounts causes a fall in blood pressure and transient respiratory depression in the anaesthetised cat. The effects produced, however, are no greater than those observed after similar amounts of codeine phosphate.

The continued oral administration of 100 mg./kg. daily to immature rats for six weeks and of 50 mg./kg. daily to rabbits for eight weeks did not produce any untoward effects, apart from some reduction in the growth rate of rats. In particular, there was no deleterious action on the haemopoietic system of rabbits.

The authors wish to thank Professor T. Crawford for carrying out the histological investigations.

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