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A simple method for the evaluation of local anaesthetic activity using earthworms

B. P. BLOCK,* D. J. POTTS AND R. S. H. FINNEY

Earthworms are immersed in local anaesthetic solution and tested for anaesthesia at 1 min intervals by dipping their tails into weak acid. The time of onset of anaesthesia is used as a measure of anaesthetic activity. The method is simple, inexpensive and has an end-point which is unmistakable.

SEVERAL tests have been described for assessing topical anaesthesia using frogs (Munch, Pratt & de Ponce, 1933), guinea-pig cornea (Chance & Lobstein, 1944, and others), the guinea-pig sneeze reflex (Nieshultz, Hoffman & Popendiker, 1958), rabbit cornea (McIntyre & Sievers, 1937, and others) and the human larynx (Clarke, Orkin & Rovenstine, 1954).

We now describe a method for estimating topical anaesthesia using earthworms which was designed primarily for screening purposes but is equally suitable for comparing local anaesthetic potencies.

Experimental

MATERIAL

Earthworms from several genera were collected but those from the genus *Lumbricus* were used in all of the later experiments. They can be identified by the following features (Cernosvitov and Evans, 1947): (*a*) They are tanylobic; (*b*) they are thicker than other worms of similar length; (*c*) they are less opaque than worms of other genera; (*d*) they are reddish rather than brown in colour; (*e*) they have a spatulate tail.

The worms varied in weight from 0·5 to 5·0 g. They were kept in groups of about 50 at room temperature in 6½ inch flower pots filled with garden soil containing 25–30% moisture. Powdered dried cow manure was sprinkled daily on the surface of the soil to provide extra organic matter (Guild, 1957).

METHOD

This is based on a sharp withdrawal response produced when the tail is dipped into 0·0125N hydrochloric acid to a depth of about 0·25 in. The response can be abolished by total immersion in local anaesthetic solution.

Before testing, the worms are maintained in aerated worm "Ringer,"† which is also used for preparing the local anaesthetic solutions.

From the Department of Biology, College of Technology, The Newarke, Leicester.

* Present address: British Industries Biological Research Association, Carshalton, Surrey.

† NaCl, 3·67; dextrose, 1·33; $NaHCO_3$, 0·20; NaH_2PO_4, 0·67 g; KCl, 15%, 0·62 ml; $CaCl_2$, 16%, 0·50 ml; distilled water to 1 litre. Final pH 6·6. (Modified after B. I. Roots, personal communication to J. A. Edson.)

Worms, in groups of five, were first tested for a positive response to acid, washed with water and dried with absorbent paper. Five worms were now immersed in anaesthetic solution and after 1 min were removed in turn for the acid test. No individual identification of the animals was made. Thereafter, the group was tested at minute intervals. The time of response was noted and the means calculated. Separate groups were used for each concentration of anaesthetic.

FACTORS INFLUENCING THE METHOD

Concentrations of acid higher than 0·0125N caused damage to the tail, lower concentrations gave an unreliable response.

Differences in weight affected the mean time of onset of anaesthesia, large worms taking a longer time to show inhibition of the response. Consequently the animals were distributed evenly by weight amongst the groups.

Worms stored in small volumes of aerated Ringer solution showed a decreased sensitivity to the acid with length of storage time. This effect was not seen when they were stored in 250 ml of Ringer in which they retained their responsiveness for over 8 hr.

Worms from the genus *Lumbricus* showed (1), a characteristically sharper response to acid; (2), greater sensitivity to acid; (3), no acid damage which had been seen in other worms. They were, however, less sensitive to the anaesthetics than other genera.

Worms used two or three times each day showed a less sharp response to acid on the second and third occasions, thus affecting the end-point. If less than 2 hr were allowed for recovery, damage occurred and the results were unreliable. Although groups tested on consecutive days gave reproducible results, deterioration occurred if the worms were used more often than once in about 4 days.

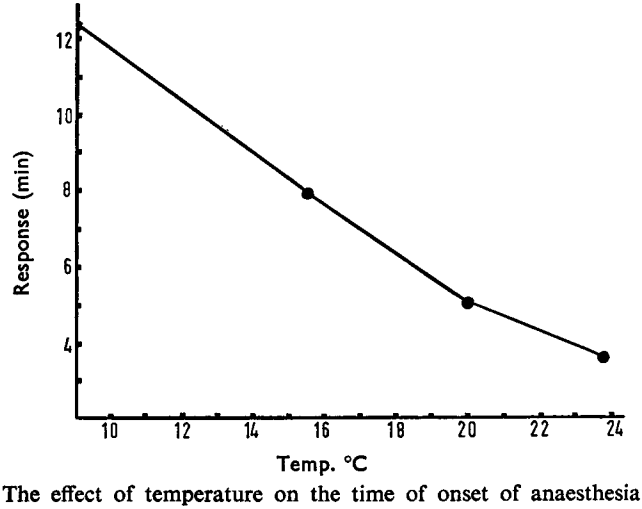

FIG. 1. The effect of temperature on the time of onset of anaesthesia.

Groups of 10 worms were tested at different temperatures with the same concentration of anaesthetic. An increase in temperature reduced the time of onset of anaesthesia at a rate of 0·6 min/°C (Fig. 1). Variation in temperature should therefore be avoided.

Results

A linear relationship between log concentration of cinchocaine hydrochloride and response was obtained (Fig. 2). This was an early experiment using worms of mixed genera. The slope of the curve ($b = -15{\cdot}5$) is significantly different from zero ($s/b = 2{\cdot}26$). The straight line portion of the graph lies between responses of about 4 and 16 min.

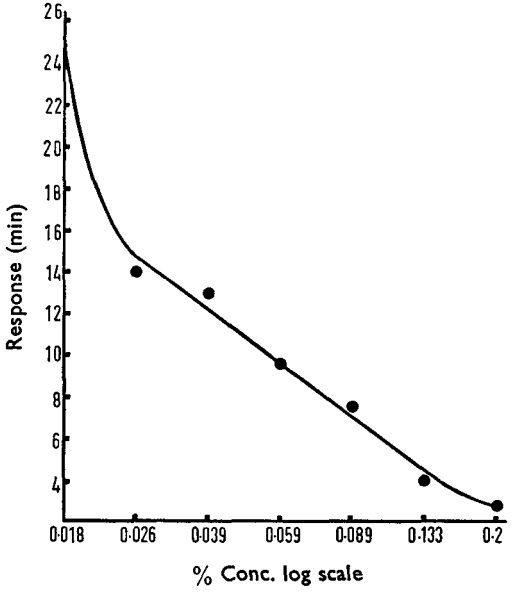

FIG. 2. Dose response relationships for cinchocaine hydrochloride. Each point is the mean of the responses of 10 worms from several genera.

Log concentration/response curves for cinchocaine, lignocaine and cocaine using *Lumbricus* are given in Fig. 3. The limits of linearity for cocaine and lignocaine are approximately 5 and 15 min.

The relative potencies were expressed as the antilog of the difference between the log concentrations producing the mean response (10 min) for each drug. Lignocaine was 7% and cocaine 13% as potent as cinchocaine. These figures agree with those recorded by Adriani (1956).

Discussion

The method of evaluating local anaesthetic activity using *Lumbricus* is simple, requires little skill and uses materials which are cheap and easily available. It eliminates the difficulty, inherent in the cornea

method, of obtaining reproducible stimulations. The end-point is unmistakable.

The sensitivity of the method is comparable with that of other methods and the ratio of potencies of the three drugs tested agreed well with those recorded in the literature.

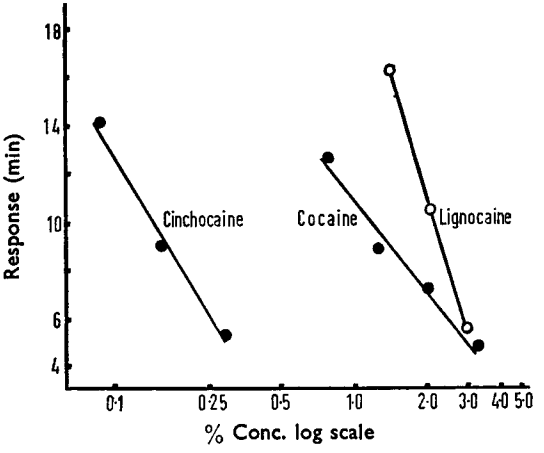

FIG. 3. Dose response curves for cinchocaine, lignocaine and cocaine hydrochlorides. Worms of the genus *Lumbricus* only were used.

Although the method measures the time of onset of anaesthesia, the duration of anaesthesia can be determined easily by removing the animals from the anaesthetic solution and replacing them in aerated Ringer.

The slopes of the dose response curves are steep. Although the use of worms of varying weights within groups is not conducive to low standard errors, the reproducibility of group means is good (± 1 min for those results in which anaesthesia occurred after 10 min immersion in anaesthetic solution).

The method is quick and suitable for rapid screening as well as for comparison work.

References

Cernosvitov, L. & Evans, A. C. (1947). *Synopsis of the British Fauna* No. 6 Lumbricidae. Linnean Society of London.

Chance, M. R. & Lobstein, H. (1944). *J. Pharmacol.*, **82**, 203–210.

Clarke, R. E., Orkin, L. R. & Rovenstine, F. A. (1954). *Anaesthesiology*, **15**, 161–173.

Guild, W. J. M. (1957). The U.F.A.W. Handbook on *The Care and Management of Laboratory Animals*, 2nd ed., pp. 875–888, London: U.F.A.W.

McIntyre, A. R. & Sievers, R. F. (1937). *J. Pharmacol.*, **61**, 107–120.

Munch, J. C., Pratt, M. J. & de Ponce, A. M. (1933). *J. Amer. pharm. Ass.*, **22**, 1078–1080.

Nieshultz, O., Hoffman, I. & Popendiker, K. (1958). *Arzneimitt. Forsch.*, **8**, 539–544.

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