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A modification of a method for the evaluation of topical anaesthesia in the earthworm

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The method of Block, Potts & Finney (1964) for the evaluation of local anaesthetic activity using the response of the earthworm tail to hydrochloric acid has been found to give false negative results with control solutions. This was because the acid progressively damaged and thus desensitized the tail. This has been overcome by arranging the test so that no tail is immersed in the acid more than three times during an assay.

THE method of evaluating local anaesthetic activity using the response to acid of the tail of the earthworm, as described by Block, Potts & Finney (1964), although originally designed for qualitative screening purposes, was also recommended for quantitative comparison of the potencies of local anaesthetic agents.

Adhering *strictly* to this method, we evaluated several local anaesthetic agents and found that positive anaesthetic responses were yielded by control worms immersed in Ringer solution alone (Tables 1 and 2). Although sensation was not abolished during the initial three exposures to the acid, over 30% of the acid dippings produced no detectable reactions. In view of this a step-by-step review of the procedure was made.

Experimental

The conditions were re-examined and were found to conform to those of Block & others (1964) in genus and size of worm,* preparation and pH of the Ringer solution, normality of the hydrochloric acid solution and storage of the worms.

During all determinations, the initial responses were pronounced and unmistakable, but after a few dippings into the acid this sharpness diminished and was accompanied by an apparent change in the gross aspects of the skin of the tails which appeared dry and hard. The tails of worms were therefore examined microscopically after exposure to 0·0125 N hydrochloric acid. One group was dipped in the acid at 1 min intervals for 12 min; a second group was dipped twice at 1 and 3 min, while a third group was untreated and served as a control.

Photomicrographs showed that after two dippings in the acid the ectoderm had, to a large extent, been removed. That remaining was ragged and the cell morphology was disturbed. Some of the bundles in the circular muscle layer were swollen but the longitudinal layer had not been affected. After exposure of the tail to the acid at 1 min intervals for 12 min, the cuticle and ectoderm had been removed and the muscle bundles within the circular layer were swollen and oedematous. There was some degree of oedema in the longitudinal layer also.

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* They were identified specifically as *Lumbricus terrestris*.

MODIFIED PROCEDURE

As a result of these findings, we modified the original procedure.

Eathworms of the same genus and size were stored in a minimum of 250 ml of Ringer solution which was aerated while it contained the worms —a period of not less than 2 nor more than 3 hr (for periods in excess of this the condition of the animals progressively deteriorates).

Immediately upon removal from the Ringer solution, at least 18 worms were dipped in the acid solution to determine the acceptability of the responses. After testing, they were immersed in the experimental anaesthetic solution for 1 min and then divided into three equal groups. The tails of the first group were dipped into the acid solution at 1 and 3 min, after removal from the anaesthetic solution. Those of the second group were similarly dipped at 5 and 8 min, and those of the third group at 12 and 16 min. Thus, no tail was dipped into the acid on more than three occasions.

The data were then treated in a manner which differed from that recommended by Block & others (1964), purely on personal preference. The total number of "no responses", elicited by exposure to a given concentration of an anaesthetic solution, was divided by the total number of applied stimuli and the resultant product was multiplied by 1,000 to yield a value we called the Anaesthetic Index (see Table 3). These indices were plotted against concentration, %, and the ED50 for each anaesthetic agent was determined by the method of Litchfield & Wilcoxon (1949).

Results

The results of testing some anaesthetic agents using the modified method, have been summarized in Table 3. The differences between these results and those in Table 1 are obvious. Even though benzocaine still exhibited the greatest, and lignocaine the least potency, the ranges of gradient activity varied widely.

TABLE 1. RESULTS OF DETERMINING LOCAL ANAESTHETIC EFFICACY BY THE METHOD OF BLOCK & OTHERS (1964)

Drug	Conc. %	No. of worms	Total no. of stimuli	No. of "no responses"
Lignocaine	0·25	10	120	73
	0·50	10	120	103
	1·00	10	120	119
Diperodon	0·25	10	120	110
	0·50	10	120	111
	1·00	10	120	120
Benzocaine	0·0001	8	96	74
	0·001	10	120	109
	0·01	10	120	114
	0·10	10	120	120
	0·25	10	120	119
	0·50	10	120	118
	1·00	10	120	118
Ringer solution*		10	120	28

* NaCl, 3·67; Dextose, 1·33; $NaHCO_3$, 0·20; NaH_2PO_4, 0·67; 15% KCl, 0·62 ml; 16% $CaCl_2$, 0·20 ml; distilled water to 1 litre. Final pH, 6·6.

TABLE 2. DISTRIBUTION OF NEGATIVE REACTIONS OBSERVED WHILE ASSESSING POTENCY BY THE METHOD OF BLOCK & OTHERS (1964)

Drug	Conc. (%)	Number of "no responses" at (min)											
		1	2	3	4	5	6	7	8	9	10	11	12
Lignocaine	0·25	0	0	1	4	4	8	9	8	10	9	10	10
	0·50	6	8	7	7	9	8	9	9	10	10	10	10
	1·00	10	10	10	10	10	10	10	9	10	10	10	10
Diperodon	0·25	6	7	9	9	10	10	10	10	10	10	10	10
	0·50	9	8	7	9	9	10	9	10	10	10	10	10
	1·00	10	10	10	10	10	10	10	10	10	10	10	10
Benzocaine	0·0001	1	3	3	6	6	7	7	7	7	7	7	7
	0·001	5	5	8	10	10	10	10	10	10	10	10	10
	0·01	5	9	10	10	10	10	10	10	10	10	10	10
	0·10	10	10	10	10	10	10	10	10	10	10	10	10
	0·25	10	10	10	9	10	10	10	10	10	10	10	10
	0·50	10	9	9	10	10	10	10	10	10	10	10	10
	1·00	9	9	10	10	10	10	10	10	10	10	10	10
Ringer solution		0	0	0	1	2	3	3	3	3	5	4	4

TABLE 3. RESULTS OF DETERMINING LOCAL ANAESTHETIC EFFICACY AS DETERMINED BY THE MODIFIED PROCEDURE

Drug	Conc. (%)	No. of worms	Total no. of stimuli	No. of "no responses"	Anaesthetic* Index
Lignocaine	1·0	18	36	7	190
	2·0	18	36	19	520
	3·0	18	36	23	630
	4·0	36	72	49	680
	6·0	36	72	60	830
Diperodon	0·01	36	72	3	40
	0·05	36	72	13	180
	0·10	36	72	20	270
	0·50	36	72	44	610
	1·00	36	72	47	650
Benzocaine	0·01	36	72	6	80
	0·05	36	72	20	270
	0·10	36	72	58	800
Ringer solution ..		36	72	2	28

* See page 457.

It was determined that the ED50 of lignocaine equalled 2·30 (1·81 to 2·92)%; that of diperodon equalled 0·14 (0·11 to 0·18)%; and that of benzocaine equalled 0·07 (0·06 to 0·08)%. These were calculated with a 95% limit of probability.

Discussion

Our observations would appear to explain the progressive lack of response noted in worms which had received repeated exposure to acid, since this degree of tissue destruction would also involve receptor sites. According to Block & others (1964), concentrations of acid higher than 0·0125N caused damage but worms of the genus *Lumbricus* tolerated this degree of acidity. It would appear that this statement should be qualified and that some limitation should be placed on time and frequency of acid exposure.

TABLE 4. DISTRIBUTION OF NEGATIVE REACTIONS OBSERVED IN ASSESSING POTENCY BY THE MODIFIED PROCEDURE

Drug	Conc. (%)	Number of "no responses" at (min) 1	3	5	8	12	16
Lignocaine	1·0	1	1	1	2	1	1
	2·0	3	3	5	4	2	2
	3·0	3	3	4	5	3	5
	4·0	4	5	8	8	12	12
	6·0	6	6	12	12	12	12
Diperodon	0·01	1	2	0	0	0	1
	0·05	1	1	1	3	2	5
	0·10	1	5	2	4	1	7
	0·50	6	8	4	7	2	7
	1·00	5	7	6	12	8	8
Benzocaine	0·01	0	0	0	1	2	3
	0·05	0	4	2	3	7	4
	0·10	7	9	9	11	11	11
Ringer solution ..		0	1	0	1	0	0

The fact that the modification had yielded different results, however, does not of itself support a conclusion that these were more valid than those obtained by the original method.

Attention was first directed to the responses yielded by the control animals which had been exposed to the Ringer solution alone. Using the modified method, a 2·8% incidence of negative responses was elicited by this group (Table 3); compared with 23% after the original method (Table 1). Data illustrating the distribution of "no responses" in these instances have been presented in Tables 2 and 4.

References

Block, B. P., Potts, D. J. & Finney, R. S. H. (1964). *J. Pharm. Pharmac.*, **16**, *Suppl.* 85*T*–88*T*.

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