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A METHOD OF ASSAYING THE ANTI-TETANUS POTENCY OF DRUGS ON EXPERIMENTAL LOCAL TETANUS IN THE RABBIT

BY

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Local tetanus was induced in rabbits by the intramuscular injection of tetanus toxin. The relative potency of drugs which abolished the muscle spasm was determined using quantitative electromyography and the accuracy of the method tested with mephenesin and thiopentone. It is suggested that the method is suitable for screening new drugs for clinical use.

There is no general agreement on the best method of controlling the convulsions of clinical tetanus, which still carries a high mortality rate.

One of the reasons for this lack of agreement is the absence of information as to which drugs possess desirable anticonvulsant properties in tetanus. These properties are an ability to suppress the convulsions without interfering with respiration and without causing loss of consciousness.

Clinical tetanus is now usually treated with general anaesthetics and hypnotics or with neuromuscular-blocking agents, but control can only be achieved at the price of respiratory depression or unconsciousness with the former and respiratory paralysis with the latter. In both cases the treatment promotes complications which may be fatal.

Mephenesin has been widely used in clinical tetanus because it has the required properties to some degree, but it is impotent in the most severe cases and has other disadvantages. However, the existence of mephenesin suggests that a search for more effective drugs would be worth while. The first requirement for such a search is a method for quantitative comparison of drug activity and this forms the subject of the following report.

METHOD

Rabbits in which local tetanus had been induced in one hind limb by injection of tetanus toxin into the gastrocnemius muscle were suspended in a canvas sling so that their hind limbs just touched the bench.

The electrical activity of the developed tetanus was recorded with intramuscular electrodes placed 10 to 15 mm. apart with an indifferent electrode attached to the skin of the back. The recorded potentials were amplified by a condenser-coupled pre-amplifier and a main amplifier and displayed on an oscillograph (Wright, Morgan, and Payling Wright, 1952). These voltages were then summed by an integrating circuit to an arbitrary level at which a pulse was emitted to a "Dekatron" counter. Every 100th pulse so obtained was led off through a relay circuit and signal marker to a smoked drum. Thus a certain degree of muscular activity was converted into a quantitative unit and the number of units was directly proportional to the degree of electrical activity.

It was found, however, that spontaneous local tetanus was too variable to give a consistent activity from which small deviations could easily be measured, and so an electrically operated hammer was arranged to strike the frame supporting the animal in its sling at intervals of 1 min. With this

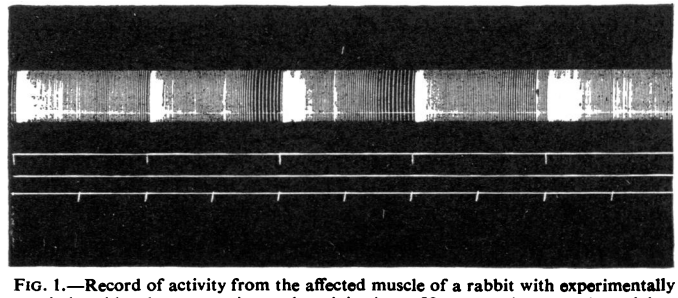

FIG. 1.—Record of activity from the affected muscle of a rabbit with experimentally induced local tetanus prior to drug injection. Upper tracing, muscle activity; middle tracing, applied stimulus; lower tracing, injection signal line; time, 30 sec. Note the burst of activity coincident with each applied stimulus and then the gradual decline in activity towards the end of the minute.

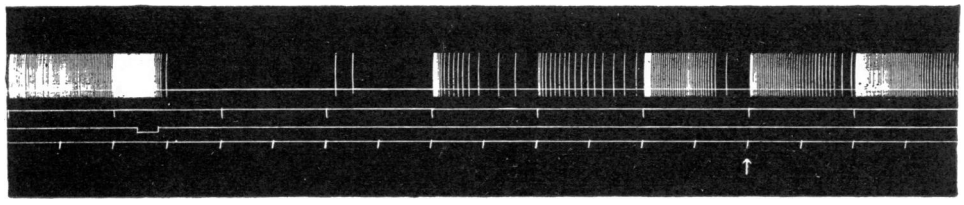

FIG. 2.—Record of the effect of 15 mg./kg. mephenesin (at signal) on the activity of the affected muscle of a rabbit with experimentally induced local tetanus. Upper tracing, muscle activity; middle tracing, applied stimulus; lower tracing, injection signal line; time, 30 sec. In this instance the end of the drug effect, based on the arbitrary criteria chosen, is indicated by the arrow. The duration of abolition of tetanus is 5½ min.

standard afferent stimulus it was possible to achieve a more steady state (see Fig. 1).

In order to minimize the effect of the physical disturbance of making the injection in the ear, the base of the ear was infiltrated with 2.0 ml. of 2% procaine before each experiment. This amount of procaine did not affect the tetanus.

Before the administration of any drug, tetanus activity was recorded until a base-line of 5 min. constant activity was achieved. The experimental drug was then injected intravenously and a quantitative measure of the potency obtained as follows. The beginning of a drug effect was taken from the time all activity ceased. The drug effect was considered to have finished when a minute was reached in which activity, starting coincidentally with the stimulus, was maintained so that at least 10 units of activity were recorded in the second half of that minute. Thus in Fig. 2 the end of the drug effect is indicated by the arrow and the response time is 5½ min.

If further doses were to be given the animal was left undisturbed for 10 min. before reapplying the stimulus preparatory to the next injection.

Mephenesin and thiopentone, both drugs known to be active against clinical tetanus, were given in a Latin square arrangement of four doses. The rows in each Latin square represented different days of the experiments, the columns different times of day when the doses were given. Each square was then carried out on one rabbit.

TABLE I

DOSE/RESPONSE RELATIONSHIP FOR ABOLITION OF TETANUS ACTIVITY BY MEPHENESIN

The values above the broken line are the results of a Latin square of four doses performed on one rabbit on four consecutive days. Those below the broken line represent a similar experiment performed on another rabbit.

Dose (mg./kg.) ..	10	15	20	30
Response (min.) ..	4·5	5·0	6·75	7·5
	2·0	5·0	6·5	10·5
	3·5	4·75	5·5	15·0
	3·25	4·25	5·75	7·0
	3·0	5·25	6·0	11·5
	3·5	5·5	6·5	9·5
	3·0	3·75	5·5	10·0
	3·0	3·75	5·0	9·5
Mean ..	3·22	4·66	5·94	10·06

RESULTS

Dose/Response Relationship

Mephenesin.—The results of two complete Latin squares for mephenesin are shown in Table I. The mean responses, when plotted against dose, were found to give a linear regression that extends through zero (Fig. 3).

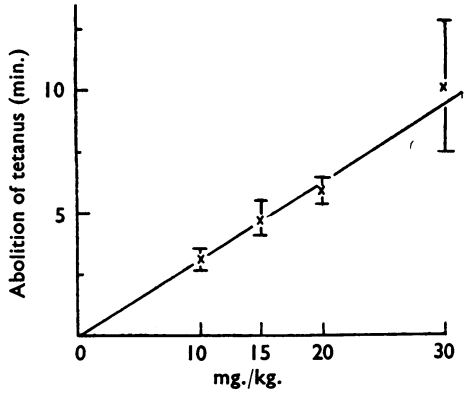

FIG. 3.—Dose/response relationship for the anti-tetanus activity of mephenesin. Each point is the mean of eight observations and the standard deviations are also shown.

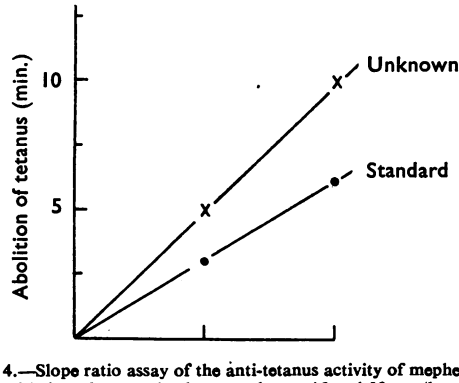

FIG. 4.—Slope ratio assay of the anti-tetanus activity of mephenesin. With the unknown, the doses used were 15 and 30 mg./kg., while with the standard, 10 and 20 mg./kg.

If two of the doses (10 and 20 mg./kg.) are treated as standard and two (15 and 30 mg./kg.) as unknown, then their two regression lines are not parallel but intersect at a point for which the dose is zero (Fig. 4). The relative potency of the two solutions is then the ratio of the slopes of the two regression lines (Wood, 1951). The regression coefficients $b_u . b_s$ can be calculated and the relative potency of the two solutions $= b_u/b_s = R = 1.625$. The theoretical value $= 1.5$, the "unknown" doses being 50% greater than the "standard" doses. The 5% limits of error, calculated according to Burn (1950), were 1.31 and 1.94.

Thiopentone.—The results of two similar Latin squares for thiopentone, shown in Table II, were treated as for mephenesin (Figs. 5 and 6). The relative potency of the two solutions $= b_u/b_s = R = 1.32$, with limits of error ($S = 0.05$) of 1.10 and 1.54. The theoretical value of the ratio is, in this case, 1.33.

TABLE II

DOSE/RESPONSE RELATIONSHIP FOR THE ABOLITION OF TETANUS ACTIVITY BY THIOPENTONE

See Table I for an explanation of the arrangement of results.

Dose (mg./kg.) ..	6	8	12	16
Response (min.) ..	3·5	5·0	5·25	9·0
	2·5	6·5	10·5	13·5
	3·5	6·5	12·5	8·5
	3·5	4·5	7·5	10·5
	3·0	6·25	8·0	14·0
	4·0	4·25	8·5	18·0
	9·0	7·0	14·0	14·5
	8·0	7·0	11·0	18·0
Mean ..	4·625	5·875	9·660	13·25

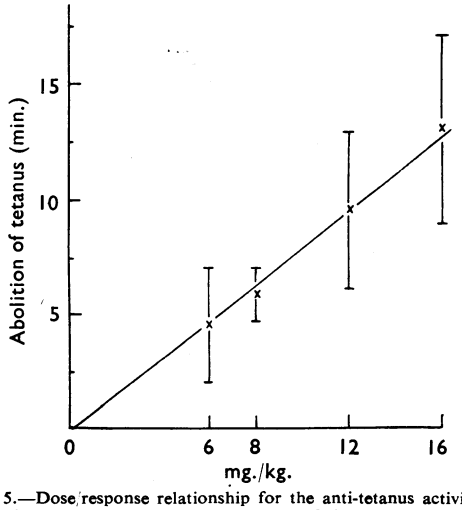

FIG. 5.—Dose/response relationship for the anti-tetanus activity of thiopentone. Each point is the mean of eight observations and the standard deviations are also shown.

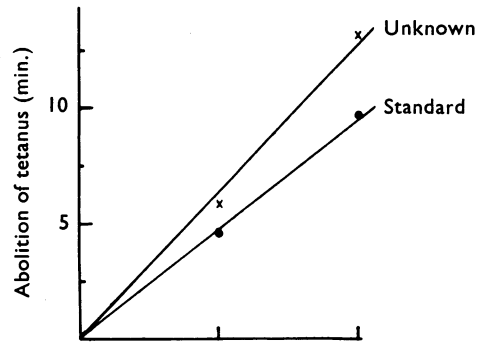

FIG. 6.—Slope ratio assay of the anti-tetanus activity of thiopentone. With the unknown, the doses used were 8 and 16 mg./kg., while with the standard, 6 and 12 mg./kg.

Analysis of Variance

The significance of a number of the possible sources of error in this assay can be established statistically. They are: (1) A systematic variation in the response of the animals with the order in which the doses are given. (2) A daily variation in the overall response of each rabbit depending on the stage of tetanus reached. (3) A variation in the sensitivity of different animals to the same drug.

The four doses were given to each rabbit in a Latin square arrangement covering four days so that all doses were given each day, and during the period of the experiment the four possible dose orders were covered. Thus an analysis of variance performed on the combined results for the two squares can be made to determine the significance of the three sources of error. From the results of the analysis shown in Tables III and IV it will be seen that: (1) The order in which the doses are given is not important. (2) There is no significant daily fluctuation in the response of the experimental animals. (3) There is a significant difference in the overall response of the different animals to the same drug in the case of thiopentone.

TABLE III

SLOPE/RATIO ASSAY FOR MEPHENESIN

Analysis of variance of the results in Table I.

Nature of Variation	Sum of Squares	d.f.	Mean Square	F.
Between doses	208·36	3		
Regression	207·11	2	103·44	57·340
Non-convergence at zero dose	1·25	1	1·25	
Between experiments	12 812	7		
Between animals	0·195	1	0·195	
Between days within animals	12 617	6	2 103	1·166
Dose order (between columns)	7·047	3	2·349	1·234
Error	32 48	18	1·804	
Total ..	260·7	31	..	

TABLE IV

SLOPE/RATIO ASSAY FOR THIOPENTONE

Analysis of variance of the results in Table II.

Nature of Variation	Sum of Squares	d f.	Mean Square	F.
Between doses	365·09	3		
Regression	364·077	2	182 04	32 741
Non-convergence at zero dose	1·013	1	1·013	
Between experiments	103·885	7		
Between animals	53·854	1	53·854	9·515
Between days within animals	50·031	6	8·3385	1·499
Dose order (between columns)	11·454	3	3·818	0·687
Error	100·071	18	5·560	
Total ..	580·50	31		

DISCUSSION

The results reported show that this assay is sufficiently sensitive to differentiate between small changes in dose of mephenesin and thiopentone and therefore may reasonably be used to screen drugs for their anti-tetanus activity.

However, with thiopentone the assay is obviously at its limit of discrimination because a reversal of results with closely spaced doses occurred on one occasion (Table II). Differences in the rate of return of tetanus after a drug are liable to cause this occasional reversal where the lower dose appears to have a greater effect than the higher. This is due mainly to the relative insensitivity introduced by the arbitrarily selected end-point. Steps are being taken to remove this anomaly, which has only occurred with thiopentone, although it is rare enough not seriously to affect the assays in which it arises.

It is possible that the greater scatter of responses with thiopentone than with mephenesin is due to differences in their site of action.

The established difference in the response of different animals to the same drug means that whenever possible the comparison of different drugs should be performed on the same rabbits.

We should like to thank the Wellcome Research Laboratories for the preparation of tetanus toxin, British Drug Houses for the supply of mephenesin, and May & Baker Ltd. for the thiopentone. We are indebted to Dr. H. O. Schild for statistical advice, Dr. O. C. J. Lippold for advice on the apparatus, and D. Sayers for technical assistance. The salary of one of us (R. A. W.) and part of the cost of the apparatus was generously donated by May & Baker Ltd.

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