AN APPARATUS FOR TESTING ANTICONVULSANT DRUGS BY ELECTROSHOCK SEIZURES IN MICE

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An apparatus is described for assessing anticonvulsant drugs by the maximal electroshock seizure test in mice. It uses simple equipment which is normally available in pharmacological laboratories. The evaluation of three known and established anticonvulsant drugs show its application to routine testing procedures.

For the evaluation of anticonvulsant drugs the maximal electroshock seizure test described by Swinyard, Brown and Goodman (1952) has become well established. The apparatus described by Woodbury and Davenport (1952) is commonly used, a shock being administered to rats or mice through Spiegel (1937) electrodes applied to the eyes. The technique is time consuming and the stimulator output, which can reach 2,000 V, presents a potential hazard to the operator.

We describe here a simple application of the Palmer Electronic Square Wave Stimulator which has a maximum output of 100 V. Shocks were applied through ear electrodes.

Apparatus

The apparatus consists of an electronic square wave stimulator, providing an output easily adjustable for voltage, pulse rate and pulse width. The output is connected through a relay (2,000 ohms coil resistance) to silver electrodes. These were constructed from 0·04 in. diameter silver wire, sealed into glass tubes with the projecting ends fused to produce spherical tips. The relay controls the duration of the stimulus and is actuated photoelectrically through a rotating segment breaking a light beam focussed onto a phototransistor (Mullard OCP71). A segment of cardboard fixed to a kymograph provides a convenient rotating unit whereby the duration of the stimulus can be controlled by varying the speed of rotation of the kymograph and altering the size of the segment. The wiring diagram is shown in Fig. 1. For our experimental work a stimulus duration of 0·3 sec. was found to be satisfactory and this was obtained from a 90° segment rotating at 50 r.p.m.

Experimental

Methods

For the estimation of anticonvulsant activity, white mice (Schofield), weighing between 19 and 23 g., were used. Each mouse was only used once. The drugs compared were phenytoin, primidone and trimethadione, which were tested at the time of peak activity after dosing. They were administered orally, in aqueous solution, or suspended in mucilage

of tragacanth, to groups of mice at ascending dose levels in a constant volume of 0·5 ml. For administration of the shock the ears were filled with physiological saline and the mouse held with the electrodes placed in the ears. Whilst observing the rotating segment the impulse was switched on at the stimulator so that the shock was administered only once and for 0·3 sec.

From the numbers in each group showing tonic extensor convulsions, the ED50 values and confidence limits (P = 0·95) were calculated for each drug by the method of Litchfield and Wilcoxon (1949).

In order that protective indices could be calculated, the drugs were also tested for inco-ordination by the rotating rod method of Gross, Tripod and Meier (1955). The drugs were administered orally to groups of ten mice as before, and at hourly intervals after dosing they were placed individually on a $\frac{3}{4}$ in. metal rod rotating at 3 r.p.m. From the maximum number in each group failing to remain on the rod for 30 sec., the ID50 values and their confidence limits were calculated.

The ratio of the ID50 to the ED50 gives the protective index. Drugs effective clinically in grand mal epilepsy generally have a PI considerably greater than 1·0 in this test.

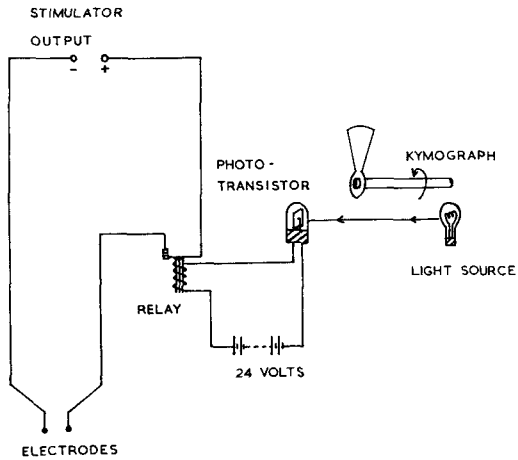

FIG. 1. Diagram showing arrangement of apparatus used in the maximal electroshock seizure test in mice.

RESULTS AND DISCUSSION

Stimulus

The stimulator output required for inducing the hind limb tonic extensor component of the maximal electroshock seizure pattern was first determined. Groups of ten mice were subjected to ascending stimuli by increasing the voltage and/or pulse width at the stimulator. Some indication of the current flowing was obtained by connecting an Avometer across the electrodes with the relay closed. The results (Table I) indicate that a supra-maximal effect was obtained at 80 V using a pulse rate of

100/sec. with a pulse width of 3 msec. for 0·3 sec. The recorded current output was 117 mA which was 2·5 times the current required to induce tonic extensor convulsions in 50 per cent of normal mice.

TABLE I

THE EFFECT OF INCREASING STIMULI ON TONIC EXTENSOR CONVULSIONS IN MICE

Stimulus		Recorded current, mA	Percentage of mice showing tonic extensor convulsions
Volts	Pulse width, msec.		
40	1	15	0
60	1	25·2	0
60	2	35	30
60	3	58	70
70	2	73	70
70	3	84	80
70	4	92	100
80	1·5	100	90
80	2	112	90
80	3	117	100
80	4	120	100
90	3	122	100

The pulse rate was 100/sec. and the duration of stimulation 0·3 sec. in all experiments.

Relative Activity of Drugs

The time of peak activity after dosing of each drug was determined in preliminary experiments. Groups of ten mice were dosed orally with each drug, the dose being the approximate ED50 as determined in a pilot test. At 1, 1½, 2, 2½, 3 and 4 hr. after dosing, groups of mice were subjected to the standard shock procedure and the numbers failing to exhibit tonic extensor convulsions were recorded. The times of peak activity were for phenytoin 2½ hr., primidone 3 hr. and trimethadione 1½ hr. respectively.

TABLE II

ANTICONVULSANT AND INCO-ORDINATION ACTIVITIES IN MICE

Drug	PAT* (hr.)	MES Test† ED50 mg./kg. orally	Inco-ordination Test ID50 mg./kg. orally	PI‡ (ID50/ED50)
Diphenylhydantoin ..	2½	5·7 (4·56 to 7·11)	36·3 (27·5 to 53·8)	6·37
Primidone	3	10·4 (3·9 to 19·5)	73·0 (33·1 to 161·1)	7·02
Trimethadione	1½	801·5 (622·7 to 1031)	420(267·6 to 659·1)	0·524

Figures in parenthesis indicate fiducial limits of error (P = 0·95).

* PAT = Peak activity time.
† MES = Maximum electroseizure test.
‡ PI = Protective index.

A comparison of the relative anticonvulsant activities (ED50) of the three drugs at their peak activity times in the maximum electroshock seizure test is shown in Table II, together with the results of the inco-ordination test (ID50) and the calculated protective indices.

The results show phenytoin to be the most potent drug both in the maximum electroshock seizure and the inco-ordination tests. The protective index was 6·37. Primidone was less active in both tests but

the protective index of 7·02 was very similar to that of phenytoin. Trimethadione showed a low activity in both tests and produced incoordination at a dose well below its anticonvulsant dose level, giving an index of 0·52. This drug is not used clinically in grand mal epilepsy.

These results are largely in accord with those reported in the literature for similar tests, with the exception of the ID50 for primidone, where Goodman and others (1953) found an ID50 in mice of 1,120 mg./kg. as opposed to the 73 mg./kg. we obtained. This discrepancy may be due to the difference in the method for determining neurological deficit or possibly the use by Goodman and his colleagues of a lower dose volume of this sparingly soluble compound. Their figure is not supported by the much higher toxicity they reported in cats, rabbits and humans.

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