Brit. J. Pharmacol. (1956), **11**, 141.

ANTICONVULSANT ACTION OF SOME ANTI-EPILEPTIC DRUGS IN MICE PRE-TREATED WITH *RAUWOLFIA* ALKALOIDS

BY

CAMILLO BIANCHI

From the Dr. Recordati Laboratorio Farmacologico S.p.A., Milan, Italy

(RECEIVED OCTOBER 7, 1955)

It has been demonstrated that reserpine, in spite of its depressant action on the CNS, does not protect rats and mice against convulsions induced by electrical or chemical stimuli (Chen, Ensor, and Bohner, 1954; Tripod, Bein, and Meier, 1954). However, it protects normal rats against audiogenic seizures (Tripod *et al.*, 1954; Schneider and Earl, 1954), and protects chronically epileptic monkeys against convulsions provoked by prodding with a stick (Chusid, Kopeloff, and Kopeloff, 1955).

Chen, Ensor, and Bohner (1954), and Chen and Ensor (1954), further proved that reserpine, administered to mice, lowers the threshold of the CNS to electrical stimuli and suppresses the anti-electroshock effect of phenytoin and phenacemide, while enhancing their general depressant action.

The foregoing results suggest that reserpine has different points of action in the CNS: these are not yet clearly understood. Since the authors limited their investigations to reserpine and, generally, to electrically induced convulsions, we thought it worth while to extend the experiments by using some other convulsant drugs and other alkaloids of *Rauwolfia*. Moreover, since the foregoing authors generally used reserpine in doses that only provoke a slight sedative effect, we thought it opportune to employ *Rauwolfia* alkaloids and reserpine at the maximal doses permitted by their acute toxicities, in order to explore possible new properties not hitherto detected. The purpose of the researches has been to show whether *Rauwolfia* alkaloids, administered in high doses, (1) prevent or potentiate the convulsions elicited by three convulsant drugs having different types of action; (2) modify the spectrum of action of some anti-epileptic drugs having different types of action; (3) nullify, depress, or potentiate the anticonvulsant actions of these anti-epileptic drugs.

METHODS

The experiments were done on adult albino mice of both sexes.

The acute toxicities of the following drugs were determined: phenytoin sodium, troxidone, phenacemide, selected *Rauwolfia* alkaloids, called RA throughout this paper ("Rauserfia," Penick Brand, batch LMX 30–X 41659), and reserpine. The LD95, LD50, and LD5 (and the corresponding CD values) were calculated by the method of Litchfield and Wilcoxon (1949).

The CD95, CD50, and CD5 of the following drugs were determined: natural camphor, leptazol, and strychnine.

The anticonvulsant activities of the following drugs were determined: phenytoin sodium, troxidone, phenacemide, selected *Rauwo fia* alkaloids (RA) and reserpine. The ED95, ED50, and ED5 (anticonvulsant dose) were calculated for leptazol-induced seizures.

Camphor, leptazol, and strychnine were given intraperitoneally; phenytoin, troxidone, RA, and reserpine subcutaneously; and phenacemide by stomach tube.

Leptazol, strychnine, phenytoin, and troxidone were used in aqueous solution. Phenacemide was suspended in 10% w/v gum acacia solution. The solution of camphor was: natural camphor 0.5% w/v, acetone 2% v/v, polyoxyethylene sorbitan mono-oleate (Tween 80) 5% v/v. RA and reserpine were dissolved in: glacial acetic acid 2% v/v, Tween 80 6% v/v, N-NaOH 16%. A 1% or 0.5% solution of RA or reserpine was used in toxicity studies and a 0.1% solution in activity studies.

All activity studies were performed as follows: (1) Phenytoin, troxidone, phenacemide, RA, and reserpine, at 1/10 LD50, were administered 3 hr. before intraperitoneal injection of the convulsant drug at its CD95. Whenever the percentage of animals showing convulsions was sensibly below the expected 95%, the compound under examination was presumed to exert a protective action. With phenytoin, troxidone, and phenacemide, the same investigations were performed after administration of half the LD50. (2) Phenytoin, troxidone, phenacemide, RA, and reserpine at 1/10 LD50, were administered 3 hr. before intraperitoneal injection of the convulsant drug at its CD5. Whenever

the percentage of animals showing convulsions was sensibly above the expected 5%, the compound under examination was presumed to exert a facilitating action. With phenytoin, troxidone, and phenacemide, the same investigations were performed after administration of half the LD50. (3) Phenytoin, troxidone, and phenacemide at one half or one tenth their LD50, plus RA or reserpine at 1/10 LD50, were administered 3 hr. before the intraperitoneal injection of convulsant drugs, which were administered at the CD95 or CD5. The variations in the expected percentages of animals showing convulsions were evaluated as above.

Results

The acute toxicities of phenytoin, troxidone, phenacemide, RA, and reserpine are shown in Table I.

Table I

ACUTE TOXICITY IN MICE OF PHENYTOIN, TROXIDONE, PHENACEMIDE, RA, AND RESERPINE

(Phenacemide was given orally, the others subcutaneously. Confidence limits are for a probability (*P*) of 0·05)

Compound	LD50 (mg./kg.)	No. of Animals	Experimental Doses	
			1/2 LD50	1/10 LD50
Phenytoin sodium	160·0 (180·8–141·5)	60	80	16
Troxidone ..	>1,000	30	750	150
Phenacemide ..	About 3,000	50	1,000*	450*
RA	147·0 (155·8–138·7)	90	—	14·7
Reserpine ..	>200	25	—	30·0

* Phenacemide doses explained in text.

The mice treated with RA and reserpine were observed for only 24 hr., because in these animals, which showed a high degree of sedation, later deaths might not be directly correlated with the toxicity of the drugs. With other drugs the mice were observed for 48 hr. It was not possible to determine the exact LD50 for phenacemide, because its low acute toxicity and low solubility would have required the administration of very high doses. Moreover, no dose of reserpine higher than 200 mg./kg. and no dose of troxidone higher than 1,000 mg./kg. were given, since these doses were well tolerated by nearly all animals injected, and were considered sufficiently high to give a clear idea of the low acute toxicity of the drugs. The LD50's were arbitrarily presumed to be 50% greater than these highest doses administered. Throughout the experiments all the drugs, except phenacemide, were administered at 1/2 or 1/10 of the LD50, or of the presumed LD50. Phenacemide, on the contrary, was used in doses not directly correlated with its presumed LD50. The dose corresponding to 1/2 LD50 (1 g./kg.) was chosen as the highest dose causing mild sedation only, and the dose corresponding to 1/10 LD50 (450 mg./kg.) as the highest dose causing mild ataxia only. The doses used (reported in Table I) were never lethal.

The results obtained on testing phenytoin, troxidone, phenacemide, RA and reserpine as anticonvulsant drugs against camphor-, leptazol- or strychnine-induced seizures are reported in Tables II, III, and IV and are summarized below.

Table II

CONVULSANT ACTIVITY OF CAMPHOR (INTRAPERITONEALLY) IN MICE PRE-TREATED WITH ANTICONVULSANT DRUGS, RA, OR RESERPINE, AND WITH THEIR COMBINATIONS

(All pre-treatment drugs were given subcutaneously, except phenacemide, which was given orally 3 hr. before camphor)

Pre-treatment	% Mice (*) Convulsing at:	
	CD95	CD5
Control (0·5 ml. of vehicle for RA or reserpine) ..	90	0
Reserpine (1/10 LD50)	100	63
RA (1/10 LD50)	100	45
Phenytoin (1/10 LD50)	90	0
,, (1/2 LD50)	100	60
,, (1/10 LD50)+RA (1/10 LD50) ..	100	80
,, (1/10 LD50)+reserpine (1/10 LD50)	100	70
,, (1/2 LD50) +RA (1/10 LD50) ..	100	80
,, (1/2 LD50)+reserpine (1/10 LD50)	100	60
Troxidone (1/10 LD50)	90	0
,, (1/2 LD50)	100	0
,, (1/2 LD50)+RA (1/10 LD50) ..	50	0
,, (1/2 LD50)+reserpine (1/10 LD50)	80	0
Phenacemide (450 mg./kg.)	100	35
,, (1 g./kg.)	0	0
,, (450 mg./kg.)+RA (1/10 LD50) ..	30	0
,, (450 mg./kg.) + reserpine (1/10 LD50)	70	0
Phenacemide (1 g./kg.)+RA (1/10 LD50) ..	0	0
,, (1 g./kg.)+reserpine (1/10 LD50)..	0	0

(*) Each percentage is calculated on 10 or more animals.

Table III

CONVULSANT ACTIVITY OF LEPTAZOL (INTRAPERITONEALLY) IN MICE PRE-TREATED WITH ANTICONVULSANT DRUGS, RA, OR RESERPINE, AND WITH THEIR COMBINATIONS

(All pre-treatment drugs given subcutaneously, except phenacemide, which was given orally 3 hr. before leptazol)

Pre-treatment	% Mice (*) Convulsing at:	
	CD95	CD5
Control (0·5 ml. vehicle for RA or reserpine) ..	80	0
Reserpine (1/10 LD50)	100	100
RA (1/10 LD50)	100	84
Phenytoin (ED95)†	5	0
,, (ED95)+RA (1/10 LD50)	100	80
,, (ED95)+reserpine (1/10 LD50) ..	100	100
Troxidone (ED95)..	5	0
,, (ED95)+RA (1/10 LD50)	100	0
,, (ED95)+reserpine (1/10 LD50) ..	100	30
Phenacemide (ED95)	5	0
,, (ED95)+RA (1/10 LD50).. ..	20	10
,, (ED95)+reserpine (1/10 LD50) ..	20	50

(*) Each percentage is calculated on 10 or more animals.
† Anticonvulsant doses given in Table VI.

TABLE IV

LETHAL ACTIVITY OF STRYCHNINE (INTRAPERITONEALLY) IN MICE PRE-TREATED WITH ANTICONVULSANT DRUGS, RA, OR RESERPINE, AND WITH THEIR COMBINATIONS

(All pre-treatment drugs given subcutaneously, except phenacemide, which was given orally 3 hr. before strychnine)

Pre-treatment	% of Mice Killed* by Strychnine at: LD95	LD5
Control (0·5 ml. vehicle for RA or reserpine) ..	100	0
Reserpine (1/10 LD50)	90	50
RA (1/10 LD50)	100	70
Phenytoin (1/10 LD50)	90	0
,, (1/2 LD50)	80	0
,, (1/10 LD50)+RA (1/10 LD50) ..	100	40
,, (1/10 LD50)+reserpine (1/10 LD50)	100	70
,, (1/2 LD50)+RA (1/10 LD50) ..	70	10
,, (1/2 LD50)+reserpine (1/10 LD50)	90	0
Troxidone (1/10 LD50)	100	0
,, (1/2 LD50)	80	0
,, (1/2 LD50)+RA (1/10 LD50) ..	100	0
,, (1/2 LD50)+reserpine (1/10 LD50)	80	0
Phenacemide (450 mg./kg.)	85	11
,, (1 g./kg.)	0	0
,, (450 mg./kg.)+RA (1/10 LD50) ..	90	0
,, (450 mg./kg.) + reserpine (1/10 LD50)	100	0
Phenacemide (1 g./kg.)+RA (1/10 LD50) ..	0	0
,, (1 g./kg.)+reserpine (1/10 LD50)	0	0

* Each percentage is calculated on 10 or more animals.

Camphor.—Camphor provoked only clonic convulsions. These were observed a few minutes after intraperitoneal injection and consisted, generally, in only one fit of very short duration. The mice, immediately after the injection, were placed on vertically suspended wire netting and, until the fit occurred, moved freely without falling. During the fit, preceded by a squeak, they were unable to cling to the net, and fell down. A linear relation was observed between the logarithms of the doses injected and the probits of animals which fell down (Fig. 1). The convulsant doses, CD95, CD50, and CD5, are given in Table V. The rapid occurrence of the fit, and the accuracy of the results obtained, depended on the vehicle used in preparing the camphor for injection. If an oily instead of an aqueous solution was used, the results were quite different. The vehicle used to dissolve the camphor had no effect on the behaviour of normal mice. The constancy of the CD95 and CD5 was frequently controlled. The values showed no significant differences in different batches of mice.

The convulsant activity of camphor was not modified by phenytoin at 1/10 LD50 and by troxidone at 1/2 or 1/10 LD50. The convulsant activity, however, was facilitated by phenytoin at 1/2 LD50 and, perhaps, by phenacemide at 450 mg./kg. Although this action of phenytoin might be due to the excitation which the compound elicited at the dose used, the action of phenacemide is not readily explicable since its effect at this dose was depressant. It appeared to possess a protective action at 1 g./kg.

Although normal mice generally had only one fit of very short duration after the administration of camphor, animals pre-treated with ineffective doses of anticonvulsant drugs had repeated fits.

RA and reserpine not only did not have any protective action, but facilitated the convulsions. The amount of solvent used had no effect alone. After RA or reserpine administration, convulsions were no longer only clonic: clonic-tonic convulsions similar to those induced by leptazol occurred, with maximal extensor spasm of the hind legs.

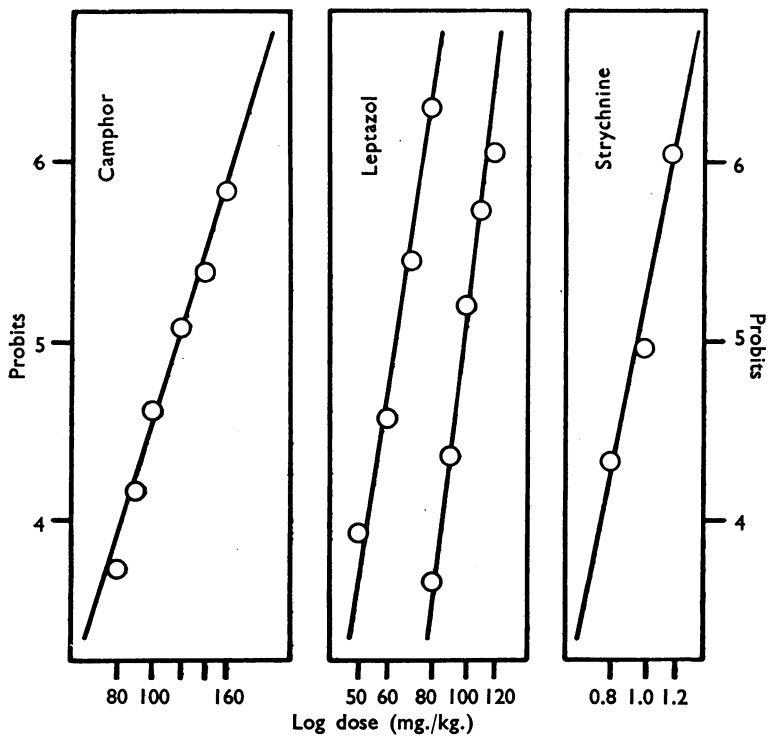

FIG. 1.—Showing the convulsant activity of camphor, leptazol and strychnine in mice. Abscissa, log dose in mg./kg. Ordinate, probits of animals convulsing.

TABLE V

CONVULSANT ACTIVITY IN MICE OF CAMPHOR, LEPTAZOL, AND STRYCHNINE ADMINISTERED INTRAPERITONEALLY

(Confidence limits $P=0.05$)

Compound	CD95 (mg./kg.)	CD50 (mg./kg.)	CD5 (mg./kg.)	No. and Batch of Mice Used
Camphor ..	213 (292–155)	118 (131–106)	66 (90·4–48·1)	100 B
Leptazol ..	89 (102–77·3)	66 (69·9–62·2)	49 (56·3–42·6)	110 A
,, ..	130 (143–118)	102 (105–99)	81 (89·1–73·6)	85 B
Strychnine ..	1·45 (1·74–1·2)	0·98 (1·03–0·92)	0·66 (0·79–0·55)	75 B

Phenytoin at 1/10 LD50 as well as at 1/2 LD50 did not nullify the facilitating effect, on clonic convulsions, of the RA and reserpine. This effect, however, was completely nullified by troxidone and phenacemide. All three compounds completely nullified the tonic convulsions elicited by camphor in mice pre-treated with RA or reserpine. After the administration of phenytoin (1/2 LD50), troxidone and phenacemide, the mice showed ataxia and succeeded only with great difficulty in clinging to the net. Clinging to the net became impossible after treatment with RA or reserpine plus one of the three anti-epileptic drugs. The animals were therefore considered to be convulsing when they manifested clonic movements of the legs.

The depressant and sedative actions of RA and reserpine and of the three anti-epileptic drugs were mutually enhanced. The enhancement was very clear after phenacemide, and was small after troxidone. Only very slight enhancement was observed after phenytoin.

Leptazol.—The convulsions elicited by leptazol are of the clonic-tonic type. Only animals showing maximal, tonic extension of the hind legs were considered to be convulsing. The animals which died without showing maximal tonic extension were considered to be protected. There was a linear relation (Fig. 1) between the logarithms of the doses injected and the probits of animals showing maximal tonic extension. The convulsant doses (CD95, CD50, and CD5) are reported in Table V. During the investigations two different batches (batch A and batch B) of mice were used. The sensitivity of each batch to the convulsant drugs was evaluated separately. Their sensitivity to the convulsant activity of leptazol was different, corresponding to the two different probit/log dose lines in Fig. 1; whereas sensitivity to camphor and strychnine was not different. Sensitivity to the toxic action of phenytoin was unchanged.

The values of CD95 and CD5 for leptazol were frequently controlled. They did not vary within a single batch of mice.

Since phenytoin, troxidone, and phenacemide possess marked protective action against the tonic convulsions elicited by leptazol, it was thought opportune to calculate for each of the three compounds the doses which nullified completely the maximal extension of the hind legs provoked by the CD95 of leptazol. There was a linear relation (Fig. 2) between the logarithm of the dose employed and the probit of animals not showing maximal extension of the legs. The anticonvulsant doses, ED95, ED50, and ED5, are given in Table VI. The CD95 of leptazol was administered by the intraperitoneal route 3 hr. after the subcutaneous injection of the anticonvulsant drug. The difference in sensitivity to leptazol noticed between the two batches of mice found expression also in a difference in sensitivity to the anticonvulsant action of phenytoin. The values, ED95, ED50, and ED5, did not vary within a single batch of mice.

RA and reserpine did not protect against leptazol-induced seizures; they had, on the contrary, a marked facilitating action which was not counteracted by phenytoin but was partially counteracted by troxidone and phenacemide. The vehicle used for dissolving RA and reserpine for injection had no effect upon leptazol-induced convulsions.

In this group of experiments enhancement of the depressant and sedative effect of RA or reserpine and anti-epileptic drugs was again observed. Here, too, the greatest effect was obtained with phenacemide, the least with phenytoin.

Strychnine.—As the tonic convulsions provoked by strychnine are always followed by death, the lethal (LD95, LD50, and LD5) instead of the convulsant doses were calculated. The results are given in Fig. 1 and in Table V.

TABLE VI

ANTICONVULSANT ACTIVITY AGAINST LEPTAZOL-INDUCED SEIZURES OF SUBCUTANEOUS PHENYTOIN AND TROXIDONE AND OF ORAL PHENACEMIDE

(The anticonvulsant drugs were administered 3 hr. before the leptazol. The dose of leptazol was 130 mg./kg. i.p. except in the first experiment with phenytoin on mice of batch A, where it was 89 mg./kg.)

Compound	ED95 (mg./kg.)	ED50 (mg./kg.)	ED5 (mg./kg.)	No. and Batch of Mice Used
Phenytoin sodium	12·5 (19·0–0·82)	6·6 (7·5–5·7)	3·5 (5·3–2·3)	59 A
,, ,,	6·6 (12·8–3·3)	1·9 (2·3–1·5)	0·54 (1·05–0·27)	80 B
Troxidone ..	490 (671–358)	270 (300–243)	150 (205–109)	85 B
Phenacemide ..	100 (171–58·4)	28 (34·4–22·7)	8 (13·7–4·6)	109 B

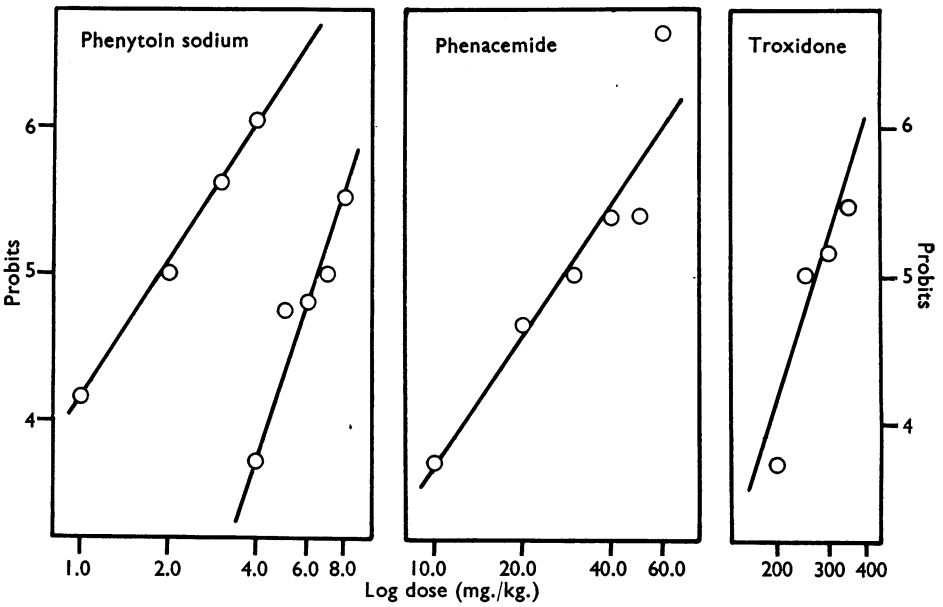

FIG. 2.—Showing the anticonvulsant activity of phenytoin, phenacemide and troxidone against leptazol - induced seizures. Abscissa, log dose in mg./kg. Ordinate, probits of animals not convulsing.

Among the anti-epileptic drugs tested, only phenacemide at a dose of 1 g./kg. exerted a protective action. RA and reserpine had a marked facilitating action, which could be inhibited by phenytoin (1/2 LD50), by troxidone, and by phenacemide. Enhancement in activity of the anti-epileptic drugs was never observed.

Deaths caused by strychnine, however, were delayed by anti-epileptic drugs as well as by RA and reserpine. While normal mice died generally from 30 to 60 min. after the injection of strychnine, pre-treated mice died many hours later. All the results reported were recorded approximately 20 hr. after the injection of strychnine.

DISCUSSION

The results obtained show that alkaloids of *Rauwolfia* do not protect against but, on the contrary, facilitate the convulsions elicited by camphor, leptazol, and strychnine.

It seems possible that they do this not only by lowering the threshold to convulsant drugs in the CNS (demonstrated by the fact that a dose almost inactive in normal mice became highly active in mice pre-treated with RA or reserpine), but also by facilitating the spread of stimuli from one region of the CNS to another—possibly from cortical to sub-cortical regions. Thus camphor provoked in normal mice only convulsions of the clonic type, whereas in pre-treated animals it also elicited tonic convulsions.

We supposed at first that a low blood sodium might account for the increased sensitivity of the CNS, for it is known that mice with a low blood-sodium level have an increased susceptibility to leptazol- or electrically-induced seizures (Swinyard, Brown, and Goodman, 1952; Swinyard, Schiffman and Goodman, 1955). This hypothesis, however, had to be discarded, because mice with a low blood sodium are no more susceptible to camphor-induced convulsions than are normal mice (the CD5 of camphor, given to 20 mice 2½ hr. after intraperitoneal administration of 1 ml./10 g. of a solution of 5.5% glucose, provoked convulsions, which did not have a tonic phase, in only two animals).

Since it is known that changes in the pituitary-adrenocortical axis can alter the excitability of the CNS, we then postulated that the adrenals were implicated in the facilitating action of *Rauwolfia* alkaloids. Previous studies demonstrated that adrenalectomy as well as hydrocortisone enhanced the susceptibility of mice and rats to convulsant drugs (Timiras, Woodbury, Despain, and Baker, 1955; Timiras, Woodbury, and Goodman, 1954), whereas DOCA decreased it (Timiras, Woodbury, and Goodman, 1954). ACTH has been shown to counteract the DOCA effect (Woodbury and Sayers, 1950). Furthermore, reserpine has been reported by Schneider (1954) to nullify the analgesic action of morphine in a similar way to ACTH and cortisone (Winter and Flataker, 1951), and has been shown to possess a stimulant action on the adrenals: in rats

the weight of the adrenals increases after reserpine treatment, whilst the thymus weight decreases (Gaunt, Renzi, Antonchak, Miller, and Gilman, 1954). Cronheim and Koster (1955) found that the adrenal ascorbic acid was depleted after reserpine treatment of rats. Mukherjee and Werner (1954) found a similar depletion in rats after treatment with " rauwolscine."

We accordingly injected 20 adrenalectomized mice (60 hr. after operation, and kept alive with NaCl 0.9%) with reserpine (30 mg./kg. s.c.). Six mice died within 3 hr. Thus adrenalectomized mice were more susceptible to the toxic effect of reserpine than were normal ones. To the 14 survivors, the CD5 of camphor was given intraperitoneally 3 hr. after the reserpine. Ten mice convulsed, and 6 of these died in tonic convulsions. On the whole, therefore, it can be said that adrenalectomized mice did not show any significant change in their susceptibility to the convulsant effect of camphor.

Summary

1. The anticonvulsant activity of soluble phenytoin, troxidone, phenacemide, reserpine, and a preparation containing mixed alkaloids of *Rauwolfia*, was evaluated in mice against camphor-, leptazol- and strychnine-induced convulsions. The anticonvulsant activity of combinations of the antiepileptic drugs with reserpine or mixed *Rauwolfia* alkaloids was also tested.

2. Reserpine and *Rauwolfia* alkaloids facilitate camphor-, leptazol-, and strychnine-induced seizures.

3. Reserpine and *Rauwolfia* alkaloids nullify the anticonvulsant activity of phenytoin, troxidone, and phenacemide against leptazol-induced convulsions, but do not nullify the activity of phenacemide against camphor- or strychnine-induced convulsions.

4. The facilitating action of reserpine and *Rauwolfia* alkaloids toward camphor- or leptazol-induced convulsions is partially counteracted by troxidone and by phenacemide. The facilitating action toward strychnine-induced convulsions is counteracted by troxidone, by phenacemide, and, partially, by phenytoin.

5. Camphor-induced convulsions in normal mice are exclusively clonic, but in mice pre-treated with reserpine or *Rauwolfia* alkaloids they become tonic. The anticonvulsant drugs studied suppress this action of *Rauwolfia*.

6. The facilitating action of *Rauwolfia* does not seem to be mediated by a lowering of the blood sodium level or by effects on the adrenals.

References

Chen, G., and Ensor, C. R. (1954). *Proc. Soc. exp. Biol., N.Y.*, **87**, 602.

—— —— and Bohner, B. (1954). Ibid., **86**, 507.

Chusid, J., Kopeloff, L. M., and Kopeloff, N. (1955). Ibid., **88**, 276.

Cronheim, G., and Koster, S. (1955). *J. Pharmacol.*, **113**, 12.

Gaunt, R., Renzi, A., Antonchak, N., Miller, G., and Gilman, M. (1954). *Ann. N.Y. Acad. Sci.*, **59**, 22.

Litchfield, J. T., and Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99.

Mukherjee, K., and Werner, G. (1954). *Sci. and Cult.*, **20**, 144.

Schneider, J. A. (1954). *Proc. Soc. exp. Biol., N.Y.*, **87**, 614.

—— and Earl, A. (1954). *Neurology*, **4**, 657.

Swinyard, E. A., Brown, W. C., and Goodman, L. (1952). *J. Pharmacol.*, **106**, 319.

—— Schiffman, D. O., and Goodman, L. S. (1955). *Fed. Proc.*, **14**, 389.

Timiras, P. S., Woodbury, D. M., Despain, L. G., and Baker, D. H. (1955). Ibid., **14**, 391.

—— —— and Goodman, L. (1954). *J. Pharmacol.*, **112**, 80.

Tripod, J., Bein, H. J., and Meier, R. (1954). *Arch. int. Pharmacodyn.*, **96**, 406.

Winter, C. A., and Flataker, L. (1951). *J. Pharmacol.*, **103**, 93.

Woodbury, D. M., and Sayers, G. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 398.