Brit. J. Pharmacol. (1953), **8**, 230.

THE EVALUATION OF "MYSOLINE"—A NEW ANTICONVULSANT DRUG

BY

J. YULE BOGUE AND H. C. CARRINGTON

From Imperial Chemical Industries Limited, Research and Biological Laboratories, Blackley, Manchester, 9

(RECEIVED JANUARY 27, 1953)

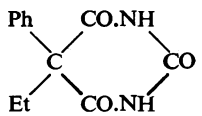
Mysoline* is a new anticonvulsant which was first prepared and evaluated by us and subsequently subjected to clinical trial (Handley and Stewart, 1952). This paper describes some of the methods by which the drug was evaluated.

The drugs commonly used for the treatment of epilepsy generally belong to the same chemical classes as the hypnotics and sedatives, but the hypnotic and anticonvulsant activities do not necessarily go together. In the barbiturate series, phenobarbitone is both a hypnotic and an anticonvulsant, but many other powerful hypnotic barbiturates are almost devoid of anticonvulsant action. The hydantoins, too, have both types of activity, but they are weaker hypnotics than the barbiturates, and in recent years several members of the series with little or no hypnotic action have been introduced as anticonvulsants. These include 5:5-diphenylhydantoin (phenytoin) and 5-ethyl-3-methyl-5-phenylhydantoin (mesantoin, methoin).

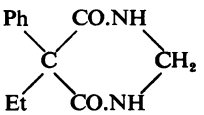
It is unfortunate that the hydantoins in general are rather toxic substances. 5-Ethyl-5-phenylhydantoin (nirvanol), which was introduced many years ago for the treatment of chorea, fell out of use after a short time because of its toxicity. Complaints of toxic side-effects have also been made of both phenytoin and methoin. With the latter this is not surprising in view of recent evidence that in the animal body it is metabolized to nirvanol (Butler, 1952).

The barbiturates are less liable to produce chronic toxic effects than the hydantoins. It is therefore rather surprising that more effort has not been made to obtain non-hypnotic anticonvulsants in this series. The only analogue of phenobarbitone which has found wide use in epilepsy is phemitone (5-ethyl-1-methyl-5-phenylbarbituric acid), which has somewhat less hypnotic and anticonvulsant activity.

Mysoline (5-ethyl-5-phenylhexahydropyrimidine-4:6-dione), formula II, may be considered as a derivative of phenobarbitone, formula I, in which the oxygen in the urea grouping of the barbituric acid is replaced by two atoms of hydrogen. Indeed, among the methods by which it may be prepared (B.P.666,027, W. R. Boon, H. C. Carrington, C. H. Vasey, and I.C.I., 6.2.52) are the electrolytic reduction of phenobarbitone itself, and the catalytic desulphurization of the corresponding 2-thiobarbituric acid.



(I)



(II)

Mysoline is a colourless, remarkably stable, crystalline substance which melts at 281–282° C. It is sparingly soluble in water (60 mg./100 ml. at 37° C.) and in most organic solvents, and, unlike the parent barbiturate, it has no acidic properties.

METHODS

Seizures Induced by Electrical Shock.—A standard convulsion was produced in rats by electrical stimulation, and the effects of drugs on the inhibition of these convulsions were measured.

The method chosen was a modification of that described by Kozelka, Hine, and Grieber (1942) and Alles, Ellis, Feigen, and Redman (1947) in that time rather than current was the variable. The electrical circuit was similar to that used by Tainter, Tainter, Lawrence, Neuru, Lackey, Luduena, Kirtland, and Gonzales (1943). Seizures were induced by passing a 7.5-mA. 50-cycle sinusoidal waveform current by means of padded ear clips. A timing switch was incorporated in the circuit and set so that the maximal duration of the shock did not exceed 10 seconds.

Within a period of 2–7 seconds the normal rat exhibited the characteristic tonic extension of the hind legs. When this was attained the current was switched off and the elapsed time recorded.

Albino rats of both sexes within a weight range of 85–105 g. were used. The initial threshold in terms of the energy required to produce the tonic extensor component of the electrically induced seizure was

* "Mysoline" is the registered trade-mark of Imperial Chemical (Pharmaceuticals) Ltd.

determined for each animal, which then served as its own control. Only those which exhibited the tonic extensor component after a shock intensity of 23–34 milliampere seconds or 600–800 mW. sec. (shock duration 3–4.5 seconds) were selected for anticonvulsant assay. Each assay of a drug at the chosen dose level was carried out on a group of 10 rats.

On the day after the initial threshold determination the drug was administered orally. The maximum dose never exceeded 500 mg./kg.; all doses were given in a volume of 0.5 ml./100 g. body weight. A control group of 10 rats was included in each experiment and was given the same volume of diluent or dispersing agent as was used for the drug under test. Unless otherwise stated, mysoline and other insoluble drugs were administered as an aqueous dispersion in Dispersol OG and LN. This dispersing agent was a mixture of polyglyceryl ricinoleate (OG) and the disodium salt of a disulphonate of dinaphthyl methane (LN).

Seizures were induced at intervals of 1, 3, 6, and 24 hours after dosing and thereafter at 24-hour intervals until the rats returned to within the normal threshold range. The maximum shock intensity delivered to a protected animal was 75 mA. sec. or 2,000 mW. sec. (a 10-second shock).

Anticonvulsant activity was assessed in terms of the dose of drug required to abolish the tonic extensor component of the seizure at this maximal intensity.

Toman, Swinyard, and Goodman (1946), Toman and Goodman (1948), and Goodman, Toman, and Swinyard (1949) have criticized the use of currents of relatively long duration. One of their assay methods is based on the ability of anticonvulsant agents to modify the different phases of the seizure pattern elicited by supramaximal shocks of very short duration (150 mA. for 0.2 sec.). They were able to study these phases independently of the effects of drugs on the energy threshold required to produce maximal seizures. They found that long periods of stimulation concealed the occurrence of drug-modified seizures. Nevertheless, the method as used by us has never failed to show the anticonvulsant activity of clinically recognized anti-epileptic agents or of other compounds reported in the literature. We were not, of course, able to follow the five components of the supramaximal induced seizure described by Toman, Swinyard, and Goodman (1946).

Leptazol (Metrazol) Seizures.—A standard dose of 90 mg./kg. made from a crystalline preparation of leptazol was given intraperitoneally to our own strain of albino rats at 160–180 g. At this dose all untreated rats exhibited maximal seizures followed by collapse, with not less than four deaths out of a group of six. Comparative anticonvulsant activity was assessed in terms of the dose required to prevent maximal seizures and death. Treatment varied from 1–6 hours before leptazol injection. Two groups of 6 treated and 6 control animals were used at each dose level.

TABLE I

THE ACTIVITY OF MYSOLINE AND OTHER ANTICONVULSANTS AGAINST ELECTRICALLY INDUCED SEIZURES IN RATS

Anticonvulsant	Oral Dose mg./kg.	No. of Rats in a Group of 10 in which the Tonic Extensor Component was Abolished						
		Hours After Dosing						Total*
		1	3	6	24	48	72	
Mysoline ..	500	6	10	10	10	10	1	10/10
	200	7	10	10	9	1	—	10/10
	100	5	10	10	5	0	—	10/10
	50	3	9	10	4	—	—	10/10
	20	1	9	10	1	0	—	10/10
	10	1	8	10	0	—	—	10/10
	10	2	7	8	1	—	—	9/10
	5	1	6	5	0	—	—	7/10
Phenobarbitone sodium	20	5	10	10	1	—	—	10/10
	10	3	5	8	1	—	—	8/10
	10	1	6	3	0	—	—	6/10
	5	0	0	2	0	—	—	2/10
5:5-Diphenyl-hydantoin	200	9	10	10	2	—	—	10/10
	100	9	10	10	0	—	—	10/10
	50	4	5	4	1	—	—	7/10
Methoin (Mesantoin; 5-ethyl-3-methyl-5-phenyl-hydantoin)	50	9	10	10	0	—	—	10/10
	20	3	9	8	0	—	—	9/10
	10	1	2	0	0	—	—	2/10
Troxidone (Tridione; 3:5:5-trimethyloxazolidine-2:4-dione	500	2	6	2	0	—	—	6/10
	100	0	0	0	0	—	—	0/10
Phenylacetylurea (Phenurone)	100	10	10	10	1	—	—	10/10
	50	8	8	5	0	—	—	9/10
	20	0	4	0	0	—	—	4/10
	10	0	1	0	0	—	—	1/10

* The last column includes all rats which did not exhibit the tonic extensor component at one or more of the test times.

RESULTS

Electrical Seizures.—Table I summarizes the results obtained with mysoline and compares them with those found with phenobarbitone, 5:5-diphenylhydantoin, methoin (mesantoin), troxidone (tridione), and phenylacetylurea (phenurone).

At 5 mg./kg. mysoline was found to be more active than phenobarbitone at the same dose level. The tonic extensor component was abolished in not less than 6 out of 10 animals as compared with 2 out of 10 with phenobarbitone. At 10 mg./kg. mysoline protected 9 or 10 out of 10 as compared with 6 to 8 with phenobarbitone. This activity was not approached by the other anticonvulsants until 50 or 100 mg./kg. was given. At doses from 50 mg./kg. upwards mysoline showed a prolongation of the duration of activity. Mysoline appeared to be slowly absorbed and reached its maximum activity at 3–6 hours after administration at low or moderate dosage. At high dosage (500 mg./kg.) the duration of protection exceeded 48 hours, but there was marked ataxia in rats. Some ataxia was apparent at 250 mg./kg.

A few additional tests were carried out at other time intervals with the object of determining the duration of highest activity. These results at 3 dose levels are summarized in Table II. At 50 and 100 mg./kg. 80–90% of the rats were protected at 6 hours, and this degree of protection persisted until at least the 12th hour after dosing. At 25 hours both groups returned to their normal threshold. At 10 mg./kg. 70% of the rats were protected at one or more periods of the test; here again the degree of protection afforded by this low dose was apparent at the 12th hour. At 25 hours the activity had gone and all the animals responded within the normal threshold range. Phenobarbitone at 10 mg./kg. is also included in the table for comparison; 80% of the animals were protected, and this degree of protection lasted for 12 hours. All this group exhibited the tonic extensor component within the normal threshold range at 25 hours.

The activity of the other anticonvulsants listed in Table I was virtually lost at 24 hours at subtoxic doses.

Fig. 1 summarizes the results obtained in measuring the energy threshold in 140 rats. Six groups of 10 served as controls. It will be seen

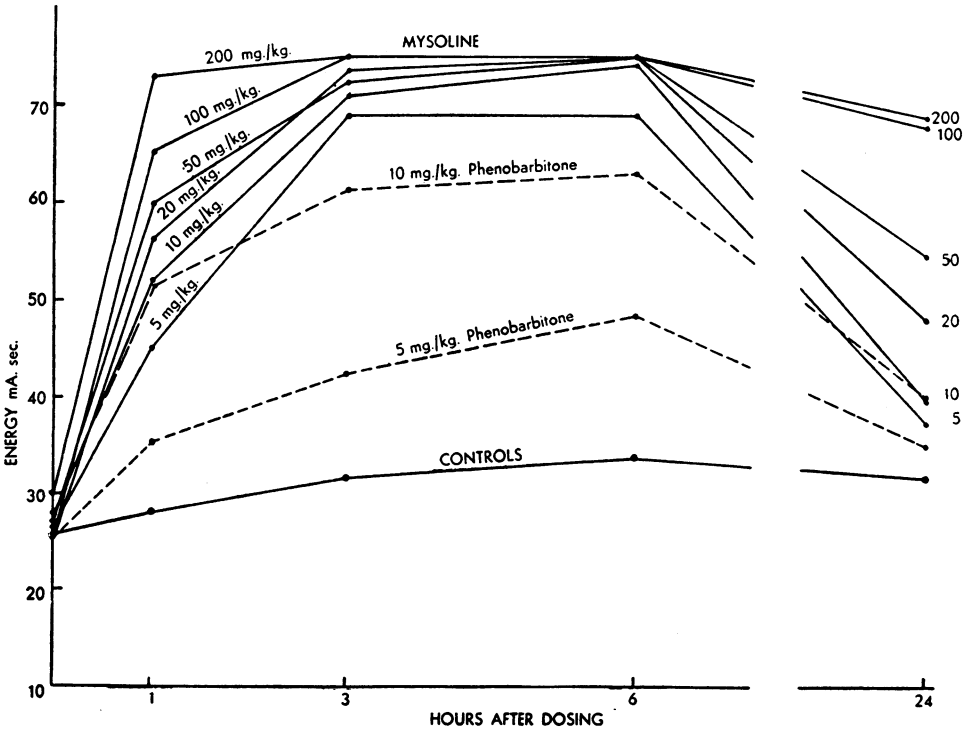


FIG. 1.—The effect of mysoline, continuous lines, and phenobarbitone, interrupted lines, on the energy threshold of electroshock seizures in rats.

TABLE II
DURATION OF PROTECTION AFFORDED BY MYSOLINE AGAINST ELECTRICALLY INDUCED SEIZURES

Anticonvulsant	Oral Dose mg./kg.	No. of Rats in which the Tonic Extensor Phase of the Seizure was Abolished					
		Hours After Dosing					Total*
		3	6	9	12	25	
Mysoline..	100	—	9	10	9	2	10/10
	50	—	8	9	8	1	10/10
	10	4	7	6	6	0	7/10
Phenobarbitone ..	10	6	7	5	6	0/9	8/10

* The last column includes all rats which did not exhibit the tonic extensor component at one or more of the test times.

that those animals selected for test when the initial threshold was determined remained remarkably constant in terms of the energy required to produce the tonic extensor component of the seizure. It will also be seen that there is a slight rise in threshold in the controls due to the shock regime which levels off at three hours. This increase in threshold, however, lies within the initial selected threshold range.

The elevation of the threshold in groups of not less than 10 rats by mysoline is shown at 6 dose levels, namely 5, 10, 20, 50, 100, and 200 mg./kg. At 5 mg./kg. 60% were protected, but, while the

maximum activity was apparent at 3 hours, a few rats show the beginning of a fall in threshold between the third and sixth hour. At 10, 20, and 50 mg./kg. the threshold rose rapidly during the first 3 hours and reached its maximum at 6 hours. At about 10 mg./kg. all animals were protected and received 2,000 mW. sec. or 75 mA. sec. without exhibiting the end-point. At 200 mg./kg. the threshold had virtually attained its maximum at 1 hour. At 50, 100, and 200 mg./kg. the thresholds were still significantly elevated at 24 hours. The elevation of the threshold by phenobarbitone at 5 and 10 mg./kg. is also shown for comparison.

Mysoline was also given intravenously and intramuscularly in order to determine any modification in the onset and duration of activity. When given intravenously the effects were apparent within the first hour, but after intramuscular injection the effects were somewhat delayed when compared both with intravenous and oral administration. At the third hour, however, there was little to choose between any of these routes. The prolongation of activity was only apparent when 50 mg./kg. was given intramuscularly, there being 100% protection at 24 hours as compared with 40% when 50 mg./kg. was given by the oral route.

The minimal single oral dose of mysoline required to abolish the tonic extensor component of the electrically induced seizure in more than 50% of rats was therefore 5 mg./kg.

Leptazol Seizures.—Fig. 2 shows the results obtained with mysoline and other anticonvulsants in rats given a standard dose of leptazol (90 mg./kg. i.p.). The anticonvulsant was given orally. The period of treatment before the administration of leptazol was governed by the results obtained from the electrically induced seizure experiments. With mysoline and those drugs which were insoluble it was found that a pretreatment period of 4–6 hours was desirable in order to obtain the maximum effect. The effect of a longer treatment period was more apparent at low dosage.

Complete control in terms of the prevention of maximal seizures in every animal in a group was obtained with mysoline at 200 mg./kg. The most active drug in this test was phenobarbitone, there being no maximal seizures at 20 mg./kg. and only 5 out of a group of 12 at 10 mg./kg. with one death. Methoin at 50 mg./kg. also prevented maximal seizures in a similar group.

While at 10 and 20 mg./kg. mysoline was found to be slightly more active than methoin, phenylacetyl urea, and troxidone (tridione), there was little to choose between the protective activity of mysoline, phenurone, and troxidone, all of which were completely effective at 200 mg./kg. At 100 mg./kg. there was 1 maximal seizure with phenurone, 2 with mysoline, and 11 out of 12 including 3 deaths with troxidone. With troxidone the females seemed to be afforded a higher degree of protection against death as compared with males in the same experiment. 5:5-Diphenylhydantoin showed little or no activity in this test at 200 mg./kg.

The minimal single oral dose of mysoline required to prevent maximal leptazol seizures in 50% of rats was found to be 20 mg./kg.

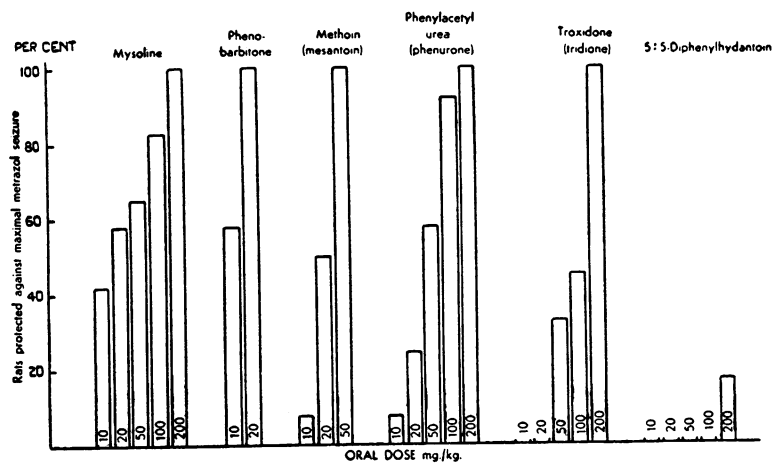


FIG. 2.—The degree of protection afforded by mysoline and selected anticonvulsants against maximal seizures in rats given a standard dose of metrazol, 90 mg./kg. i.p.

Toxicity of Mysoline

Acute Toxicity in Rodents.—The LD50 in mice was from 600 to 800 mg./kg. In rats the LD50 was 1.5 g./kg. for the 100 g. rat and greater than 2.0 g./kg. for the 200 g. rat. Some ataxia was apparent in rats following a single dose of 250 mg./kg. At 500 mg./kg. this was quite marked except in the 200 g. male, which did not show this effect. Fifty per cent of rats of 100 g. showed hypnosis after a single dose of 1.5 g./kg. Rats of 200 g. or over, particularly males, were more resistant to the onset of hypnosis.

Chronic Toxicity in Rats.—Three dose ranges, 125, 250, and 500 mg./kg., were used in groups of 10 male and 10 female rats of 120–150 g.

weight. Twenty doses were given in 4 groups of 5 daily doses over a period of 4 weeks. The rats were not subjected to any treatment over the week-end.

At 125 mg./kg. the first signs of ataxia were noted in the males on the fourth day 3 hours after dosing; this was also apparent in both sexes on the fifth day. On the eighth day, i.e., after the sixth dose, ataxia was noted in 5/10 males. All rats showed mild ataxia on the ninth day. At the beginning of the third week there was no ataxia after dosing, and subsequent dosing during this week produced no more than a trace of ataxia. During the fourth week the males appeared to be more resistant than before. No symptoms were observed in either sex from the 17th to the 20th dose, which ended the experiment. There were two deaths, one female on the fifth day and one male on the 17th day. The females showed an average gain in weight of 46 g. and males 90 g.

At 250 mg./kg. there was some ataxia after the first dose, but it was not until the fourth dose that any marked ataxia was apparent in both sexes. After the sixth dose ataxia was seen in the males, while the females showed milder symptoms. During the third week ataxia did not develop until the 14th dose. In the fourth week, only a trace of ataxia was observed. There was one death in the females during the third week. The females showed an average gain in weight of 41 g. and the males 85 g.

At 500 mg./kg. there was severe ataxia 5 hours after the first dose, and 24 hours later 6/10 females appeared ill. Subsequent doses produced torpor and hypnosis. Six deaths occurred between the fourth and sixth day and another four during the second week. Severe ataxia and torpor persisted throughout the period of the experiment. The surviving four females showed an average weight gain of 28 g. and the six males 69 g.

Growth of Rats.—The growth rate of 191 newly weaned 4-week-old rats from 19 litters was determined over a test period of one week. The rats were then divided into three groups so that each group showed a similar weight gain and contained the same number of rats from each litter. Each group was made up of 25 males and 25 females. Two groups were given 45 doses of mysoline at 100 and 250 mg./kg. respectively over a period of 9 weeks. The third group served as control, received no treatment, and were also weighed daily. The mysoline-treated groups showed a percentage weight gain of 314 at 100 mg./kg., 307 at 250 mg./kg., and the controls 341.

The appearance and subsequent reduction of ataxia observed in the chronic test despite continued dosing were also apparent in the growth trials.

Histological Examination.—On completion of both the chronic toxicity and growth experiments in rats a detailed histological examination was made at all dose levels.

In the chronic toxicity tests, at 25 to 100 times the dose required to protect 60% of rats against seizures induced by electrical shock, there was a suspicion of change in the convoluted tubules which could be attributed to the drug. This damage was more definite in the growth experiment in which rats received mysoline for more than double the time at 100 and 250 mg./kg. There was swelling of the cells of the convoluted tubules, vacuolation of these cells, and desquamation of damaged cells into the tubular lumina. The occasional presence of dividing cells in the tubules suggested regeneration after desquamation. One or more of the above changes were seen in the kidneys of 40% of the rats examined at both dose levels. Many of these changes were seen to a lesser degree in rats given dispersing agent only. However, they were more severe at the higher dose level and can therefore be attributed to the chronic administration of the drug.

The thyroid showed morphological change in the growth experiment in 44% of rats at 100 mg./kg. and 56% at 250 mg./kg. This consisted of a deepening of the usual cubical epithelium to low columnar or even to true columnar, which was sometimes associated with poorly staining colloid and mild peripheral vacuolation. The thyroids of the controls were all within normal limits.

All other organs failed to show any consistent change which could be attributed to the drug.

The above results indicate that a single dose of 100 times the therapeutic dose required to suppress electrically induced seizures in over 50% of rats was necessary to produce definite neurotoxic symptoms, and that with repeated administration over a period of 9 weeks at least 25 times this dose was required to produce any appreciable pathological change.

Toxicity of Mysoline in Monkeys.—Three monkeys were each given mysoline at 50, 100, and 250 mg./kg. respectively by stomach tube once daily in 8 groups of 5 doses on successive days for 8 weeks. The largest dose caused ataxia, which increased with daily dosing and disappeared over the week-end. There were no symptoms at the two lower doses. All three monkeys gained weight

during the course of the experiment. Weekly blood counts were normal.

A further five monkeys were given mysoline by stomach tube at 500 mg./kg. on four successive days. The first dose produced little or no effect. The first signs of ataxia were seen 3 hours after the second dose; torpor was apparent at 7 hours, and ataxia still evident 24 hours later. The third dose caused three of the monkeys to vomit, two showed hypnosis, and three marked ataxia. Torpor and ataxia were still marked before the fourth dose. Three were killed on the fourth day and two were kept. These latter were still torpid on the fifth day. Ataxia disappeared by the eighth day.

Histological Examination.—The changes seen in the kidneys of the monkey which received 250 mg./kg. for 8 weeks consisted of dilatation of the convoluted tubules with a rather low epithelium with ragged outlines. Many of the convoluted tubules contained granular or eosinophilic material together with an occasional desquamated epithelial cell. There were a few small areas of cellular infiltration around groups of tubules. There was probably some evidence of change in the monkey which received 100 mg./kg. for 8 weeks. At 500 mg./kg. there were some pyknotic nuclei in the medullary tubules, otherwise the kidneys did not differ materially from the control. The changes in the thyroid at 250 mg./kg. and 500 mg./kg. did not differ from those found in the control dosed with dispersing agent. All other organs were within normal limits at all dose levels. In the control monkey which received dispersing agent only, the majority of the convoluted tubules were dilated. There was also some eosinophilic amorphous substance in the lumen of the tubule with an occasional desquamate cell.

These findings were in agreement with those obtained in the rat toxicity trials.

Discussion

The anticonvulsant potency of mysoline compares favourably with phenobarbitone, phenylacetylurea, methoin, 5:5-diphenylhydantoin, and troxidone. This potency, however, is only of clinical significance when the effective dose is considered in relation to the toxic dose. Mysoline has been shown to possess a low toxicity in laboratory animals. It is much less toxic than phenobarbitone, a dose of the order of twenty times that of phenobarbitone being necessary to produce a similar degree of neurotoxicity. If the effective dose of mysoline required to protect 50% of rats in the electrical test, 5 mg./kg., is compared with the LD50, 1.5 to 2.0 g./kg., then the therapeutic ratio is between 300 and 400:1 for a single dose. If the therapeutic ratio is related to the single dose required to produce neurotoxicity, the ratio is then at least 50:1. Therapeutic ratios based on single-dose observations are of little value when considering drugs which have to be taken chronically.

In our opinion, a more realistic figure is that obtained by relating the effective dose to the chronic dose required to produce neurotoxicity, and possibly some pathological change, when administered over a period of at least one or two months. In our experiments the chronic neurotoxic dose is 250 mg./kg., which also gives a therapeutic ratio of 50:1.

A peculiar feature of the chronic trials, including the growth experiment, is the disappearance of ataxia with continued dosing at 250 mg./kg. from the beginning of the fourth week onwards. At double this dose the ataxia persists.

In the leptazol test the effective dose required to prevent maximal seizures in 50% of rats is 20 mg./kg., which gives a ratio of 12.5:1. If, however, the effective dose is taken as that required completely to prevent even submaximal leptazol seizures, then the ratio, based on the single neurotoxic dose, approaches unity.

The ultimate proof of the validity of our methods is only to be found in clinical trial on man. Such a trial has been reported by Handley and Stewart (1952). They confirmed the anticonvulsant activity of mysoline in grand mal epilepsy and its lack of serious toxicity.

At 1.6 g. per day they reported that 4 of their patients (10%) showed transient side-effects such as nausea, dizziness, and mild ataxia at the beginning of treatment. All these symptoms pass off in a few days and treatment was not interrupted. These observations are paralleled to some extent by disappearance of ataxia in rats and dogs even with continued dosage. Nausea was observed in monkeys at a very high dosage, 500 mg./kg. The patients observed by Handley and Stewart have now been under mysoline treatment for two years and have failed to show any blood dyscrasias or other abnormality which could be attributed to the drug. The clinical trials on grand mal epileptics suggest that the toxicity and therapeutic ratio agree more closely with the electric shock than the leptazol observations. The electrically evoked seizure is probably more comparable with human grand mal than the leptazol induced seizure.

The low toxicity of mysoline in experimental animals cannot be attributed solely to low water solubility and lack of absorption. A blood level

of over 20 mg. % has been observed in monkeys, while in rats a blood level of the order of 12 mg.% has been found six hours after a single dose of 250 mg./kg.

Summary

1. The anticonvulsant action of mysoline, 5-ethyl-5-phenylhexahydropyrimidine-4 : 6-dione, against electroshock and leptazol seizures in rats has been demonstrated and compared with phenobarbitone, 5:5-diphenylhydantoin, methoin (mesantoin), troxidone (tridione), and phenylacetylurea (phenurone).

2. A single oral dose of 5 mg./kg. of mysoline was sufficient to abolish the tonic extensor component of the electrically induced seizure in over 60% of rats, while a single dose of 20 mg./kg. of mysoline was required to prevent maximal leptazol seizures in over 50% of rats. This compares favourably with other anticonvulsant drugs.

3. Mysoline was shown to have a remarkably low toxicity in animals, both acute and chronic, whether expressed in terms of the LD50 or as the dose required to produce neurological symptoms or morphological change.

We are greatly indebted to Miss S. Bentley for her most valuable and skilful technical assistance.

We wish to thank Dr. E. Weston Hurst and Dr. D. C. Roberts for the histological examinations.

References

Alles, G. A., Ellis, C. H., Feigen, G. A., and Redman, M. A. (1947). *J. Pharmacol.*, **89**, 356.

Butler, T. C. (1952). Ibid., **104**, 299.

Goodman, L. S., Toman, J. E. P., and Swinyard, E. A. (1949). *Arch. int. Pharmacodyn.*, **78**, 144.

Handley, R., and Stewart, A. S. R. (1952). *Lancet*, **242**, 742.

Kozelka, F. L., Hine, C. H., and Grieber, M. F. (1942). *Fed. Proc.*, **1**, 256.

Tainter, M. L., Tainter, E. G., Lawrence, W. S., Neuru, E. N., Lackey, R. W., Luduena, F. P., Kirtland, H. B., and Gonzales, R. I. (1943). *J. Pharmacol.*, **79**, 42.

Toman, J. E. P., and Goodman, L. S. (1948). *Physiol. Rev.*, **28**, 409.

—— Swinyard, E. A., and Goodman, L. S. (1946). *J. Neurophysiol.*, **9**, 231.