can be seen from Table I, active enzyme preparations were found in both soluble and insoluble protein fractions. The activity per mg of protein and the stability of the insoluble fraction was usually slightly greater than that of the soluble one. With beef lungs, about 1/3 of the total activity was found in the soluble fraction; with dog lungs, a smaller fraction of the total activity was soluble.

Summary. Saline extraction of ground lung tissue (beef or dog) followed by ammonium sulfate fractionation, dialysis, and isoelectric precipitation at pH 5.1 yields a protein extract which catalyzes the inactivation of a purified preparation of serum vasoconstrictor.

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Anticonvulsant Properties of 5,5-Phenyl Thienyl Hydantoin in Comparison with Dilantin and Mesantoin.*†‡

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Dilantin (Diphenylhydantoin, U.S.P.) has been successfully employed for a decade in the treatment of grand mal and psychomotor epilepsy. In the past 2 years, Mesantoin (3-methyl, 5,5-phenyl ethyl hydantoin) has been used for the same purposes.[1-4] Still another congener, 5,5-phenyl thienyl hydantoin (Lilly 00079), has recently been used clinically with encouraging results.[5] A number of previous communications have dealt with the ability of Dilantin, Mesantoin and other congeneric substituted hydantoins, some of which have had initial therapeutic trial, to protect against or modify experimental seizures.[6-16] Comparable data on 5,5-phenyl

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TABLE I.
Protective Indices of 3 Antiepileptic Hydantoins by 4 Different Assay Methods in Rats.

Drug	TD_{50} mg/kg	Protective indices*			
		Max. electroshock seizure pattern	Normal electroshock threshold	Hydration electroshock threshold	Metrazol seizure protection
Mesantoin	50 ± 3 (24)	11.1 ± 0.71 (43)	0.69 ± 0.07 (25)	1.42 ± 0.25 (16)	0.91 ± 0.08 (26)
5,5-phenyl thienyl hydantoin	75 ± 6.5 (25)	4.47 ± 0.42 (49)	0 (20)	0.53 ± 0.05 (28)	0 (29)
Dilantin	104 ± 8 (20)	2.36 ± 0.24 (22)	0 (22)	1.1 ± 0.09 (26)	0 (30)

* For end-points employed and calculation of protective indices, see text.
± S.E. Figures in parentheses, No. of rats.

thienyl hydantoin have not as yet been published. This report is therefore concerned with the assay of the anticonvulsant properties of 5,5-phenyl thienyl hydantoin in comparison with Dilantin and Mesantoin.

Methods. (A). *Animal Assay.* Adult albino rats of the Sprague-Dawley strain were employed. For assays based on electrically-induced convulsions, Spiegel corneal electrodes were used and electroshock was delivered by means of a 60-cycle A.C. apparatus designed by Dr. L. A. Woodbury; the current delivered is independent of the external resistance. Stimulus duration was 0.2 second. Maximal electroshock seizures were obtained with a current of 150 m.A., which is 5- or 6-fold that required for minimal (threshold) seizures. For the assay based on experimentally lowered electroshock threshold ("hydration" threshold),[17] rats were injected intraperitoneally with isosmolar glucose solution, 10 cc/100 g, and determinations of drug-modified thresholds made 4 hours later. Chemoshock seizures were induced by 70 mg/kg. Metrazol injected subcutaneously, a dose which elicits convulsions in 97% of rats (CD_{97}).

Dilantin and the thienyl homolog were administered subcutaneously in aqueous solutions of their sodium salts; Mesantoin was injected intraperitoneally in solution in propylene glycol. Single doses were injected and the tests conducted after an interval (60 to 300 minutes) previously established as the time for peak effect for the particular drug and test. The occurrence of minimal neurological deficit (such as loss of placing responses, ataxia, etc.) was considered as evidence of toxicity, and the toxic dose for 50% of rats (TD_{50}) was calculated for each drug by probit analysis. The effective dose of each drug for 50% of rats (ED_{50}) was similarly calculated for each test. Protective indices could then be obtain (TD_{50}/ED_{50}) for purposes of comparison of drugs.

The details of the assay methods employed have been reported elsewhere,[10,11,17-19] but the end-points employed may be briefly summarized, as follows:

(1). *Maximal Electroshock Test.* A characteristic tonic-clonic seizure which is constant in duration and pattern follows the application of supramaximal current in non-medicated animals. The ED_{50} is based on the abolition of the extensor component of the tonic phase of the convulsion.

(2). *Normal Electroshock Threshold Test.* The least current necessary for a minimal seizure was determined for each rat, which then served as its own control. The ED_{50} is based on a 20% increase in threshold.

(3). *"Hydration" Electroshock Threshold.* The ED_{50} is based on a 50% increase in the threshold previously lowered by reduction in extracellular cation.

(4). *Metrazol Seizure.* The ED_{50} is

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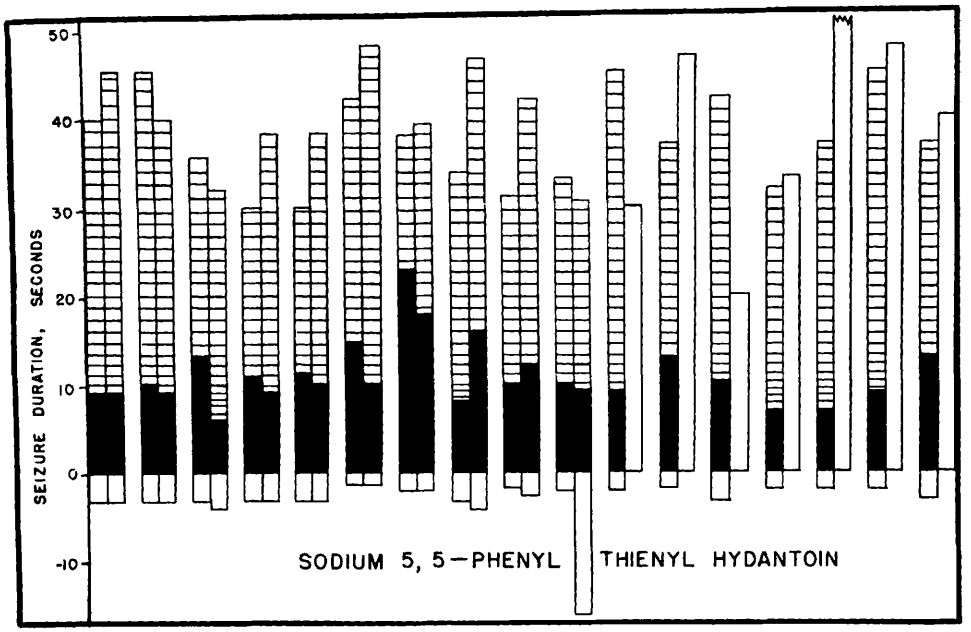

FIG. 1.

The Effect of 5,5-Phenyl Thienyl Hydantoin on Electroshock Seizure Pattern in Man.

Results of 17 separate electroshock tests on 10 non-epileptic patients. Each column represents a separate test, the left-hand column indicating the control seizure pattern and the right-hand column the seizure pattern after 3 days of oral medication with 5,5-phenyl thienyl hydantoin.

Open segment below the zero base-line indicates latency between current application and beginning of seizure; solid black segment, tonic component; horizontal-barred segment, clonic component; height of column, total duration; open columns, "missed shocks" (psychomotor seizures); serrated upper edge, indeterminate duration.

First 3 tests (starting at left of figure, first 3 pairs of columns), 0.13 g, twice daily; no change in seizure pattern. Next 2 tests, 0.13 g, 3 times daily; no change. Next 5 tests, 0.13 g, 6 times daily; no change. Next 3 tests, 0.13 g, 6 times daily; complete abolition of tonic-clonic seizures. Final 4 tests, 0.13 g, 9 times daily; complete abolition of tonic-clonic seizures.

based on complete protection against the CD_{97} of Metrazol.

(B). *Human Assay.* In addition to assays in rats, the ability of the 3 hydantoins to modify seizure patterns in non-epileptic patients undergoing electroshock therapy for psychiatric disorders was measured. The method was essentially a modification of the maximal electroshock test used in animals and has been fully described elsewhere.[13] Control seizure patterns were compared with those obtained after 3 days of treatment with various dose levels of the hydantoins and several days after withdrawal of medication. Electroshock was delivered by a Rahm clinical apparatus through temporal electrodes, 120 to 150 volts, 0.2 to 0.4 sec. duration. Current intensity and stimulus duration were selected on the basis of previous experience with each patient.

Results. The results of the animal experiments are shown in Table I. Mesantoin has the highest protective index by the maximal electroshock test, Dilantin the lowest and the thienyl compound is intermediate. In contrast to Mesantoin, neither Dilantin nor its thienyl homolog elevates the threshold for minimal electroshock seizures or affords protection against Metrazol-induced convulsions. All 3 compounds are capable of elevating the experimentally lowered (hydration) electroshock threshold; Mesantoin is the most effective by this test, Dilantin next and the thienyl derivative least.

All 3 hydantoins modify the pattern of electroshock seizures in man. As also occurs in animals, the tonic component of seizures is characteristically abolished. In-

deed, the tonic-clonic seizure may be entirely replaced by a "missed shock," especially when Mesantoin or the thienyl compound has been administered. Such "missed shocks" more nearly resemble psychomotor seizures.[13] The doses of the 3 hydantoins necessary to modify electrically-induced seizures in non-epileptic subjects are in the upper range employed for the control of grand mal epilepsy. No toxic effects were noted from these doses. The results obtained with 5,5-phenyl thienyl hydantoin (Fig. 1) differ from those with Dilantin in that the convulsions were entirely replaced by psychomotor seizures and purely clonic convulsions were not observed. Mesantoin was intermediate in this respect.

Discussion. In general, 5,5-phenyl thienyl hydantoin resembles Dilantin in its anticonvulsant spectrum of activity in animals. Both compounds differ from Mesantoin in their inability to elevate threshold for minimal electroshock convulsions or to prevent Metrazol seizures. A striking antagonism exists between Metrazol and certain derivatives of oxazolidine 2,4-dione, such as Tridione and Paradione, which are effective in the petit mal triad; the relationship between this antagonism and drug efficacy in petit mal has been discussed.[11,14,19] Inasmuch as the hydantoins at present employed clinically are generally ineffective against petit mal, the modest degree of Metrazol antagonism manifested by Mesantoin cannot as yet be related with its specificity of action in epilepsies. The methylated nitrogen and the ethyl radical on the carbon in position 5 independently bestow on Mesantoin the ability to antagonize Metrazol because both Nirvanol (5,5-phenyl ethyl hydantoin) and N-methyl diphenylhydantoin possess this action in slight measure.

On the basis of the experimental findings reported above, the prediction is advanced that 5,5-phenyl thienyl hydantoin will exhibit the same order of clinical usefulness as does Dilantin, that is, efficacy in grand mal and psychomotor epilepsy. The preliminary report of Peterman[5] who employed the drug in 44 patients with epilepsy appears to confirm such a prediction. However, this observer also recorded improvement in some cases of petit mal but made no mention of EEG diagnostic criteria. Although it is known that an occasional patient with pyknoepilepsy responds to hydantoin therapy, the results are usually not impressive and Tridione is the agent of choice. It will be interesting to observe whether other workers verify Peterman's results in petit mal.

Attention has already been directed to the advantages of multiple anti-convulsant assay tests, as employed here.[10,11,19] No one test is sufficient to measure the potential antiepileptic potencies of a new drug, and a battery of indices permits a better comparative estimate. Both electrical and chemical methods should be included. Simple elevation of electrical threshold for minimal seizures is not adequate alone, as indicated by the inefficacy of Dilantin and 5,5-phenyl thienyl hydantoin by this test. However, both drugs elevate the experimentally reduced electroshock threshold, and markedly alter the pattern of maximal seizures in animals and man. It is believed at present that the maximal electroshock and Metrazol tests are most useful for preliminary screening of new drugs and that the other tests are best reserved for compounds exhibiting adequate indices by these two methods. The electroshock test in man also provides a means of preliminary screening for drug efficacy and toxicity. The many non-epileptic psychiatric patients receiving electroshock therapy provide adequate material for this assay, and the beneficial effect of the electroshock treatment is not compromised by a short period of antiepileptic drug administration.

Summary. A new anticonvulsant, 5,5-phenyl thienyl hydantoin, proposed for the treatment of epilepsy, has been compared with Dilantin and Mesantoin by 4 different assay methods in rats and examined for its ability to modify the electroshock seizure pattern in man. The thienyl congener more closely resembles Dilantin than Mesantoin in its spectrum of antiepileptic actions.