Summary. High blood phenylalanine levels and low phenylalanine hydroxylase activity were found in pregnant female rats fed excess phenylalanine. Fetal plasma phenylalanine levels averaged twice as high as the maternal levels. Tyrosine levels were elevated in pregnant females and in their fetuses. The ratio of fetal tyrosine to maternal tyrosine was lower in phenylalanine fed pregnant rats than in normally fed pregnant rats.

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Antimalarials and Other Anticonvulsant Drugs: Predictive Validity of Laboratory Tests. (28927)

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A convincing number of reports have attested to the efficacy of quinacrine in juvenile petit mal seizures(1-11). Quinacrine has been effective in cases resistant to other treatment (2,9,11) where it has controlled seizures for up to 4½ years(9,11). Pathological EEG's were often normalized(8,11). While petit mal episodes were controlled, major attacks were sometimes precipitated in patients showing no previous major convulsive seizures (2,6). Twenty-four to 72 hours were needed for the drug to exert its effect(1,9,11) and seizure control was sometimes preceded by a period of excitation(1).

The clinical profile of quinacrine then, as an anticonvulsant has been fairly well established and it was considered pertinent to assay quinacrine for its effects on several of the standard laboratory anticonvulsant procedures. Of specific interest was the performance of this compound on the maximal electroshock seizure test (MES), maximal Metrazol (Pentylenetetrazol) seizure test (MMS), and the minimal Metrazol seizure test (mMS). Jenney and Pfeiffer(12) on the basis of their modifications of these tests suggest that pentylenetetrazol provides qualitatively different information from electroshock. According to their hypothesis, compounds exhibiting high anti-pentylenetetrazol activity correlate with clinical effectiveness in juvenile petit mal. High anti-electroshock activity is correlated with grand mal efficacy, and broad spectrum laboratory effectiveness suggests clinical trial for psychomotor and adult petit mal as well as other seizure syndromes. Mitchell and Keasling(13) similarly concluded that there are qualitative differences between electroshock and pentylenetetrazol as convulsant agents. To the contrary, Chen(14) suggests that petit mal drugs be assayed against minimal clonic seizures induced either by pentylenetetrazol or electroshock while grand mal drugs should be tested

against the tonic extensor convulsions initiated by supramaximal electroshock or large doses of pentylenetetrazol. He suggests that pentylenetetrazol induced extensor seizures are not different from electroshock tonic extensor convulsions in testing grand mal drugs.

In this study 3 antimalarial agents, quinacrine, chloroquine and hydroxychloroquine, were assayed as anticonvulsants in the laboratory to discern qualitative and quantitative differences on the MES and MMS tests. In addition, these agents were tested against electrically induced psychomotor seizures (Psym) and minimal Metrazol seizures (mMs). The test drugs were compared with phenobarbital, diphenylhydantoin Na, trimethadione, and phenacemide.

Methods. Maximal electroshock seizures (MES). The technique used was that of Toman *et al* for mice(15). Fifty milliamps of alternating current were applied through corneal electrodes for 0.3 second. Tonic hindleg extensor seizures were obtained in 98% of the control male mice used in these electroshock studies. The criterion for drug efficacy was the abolition of the tonic extensor component of the seizure.

Maximum metrazol seizure (MMS). Goodman *et al*(16) employed intravenous injections of 38 mg/kg of pentylenetetrazol to elicit seizures. In this study, 50 mg/kg of pentlyenetetrazol (0.5% solution) given by rapid intravenous injection was necessary to elicit the tonic extensor convulsion in 96% of a large series of control mice. Again abolition of this extensor seizure was the criterion for drug action.

Minimal metrazol seizures (mMS). Rapid intravenous injections of 32.5 mg/kg of pentylenetetrazol are followed within one minute by a loss of the righting reflex and asymetrical, bilateral, coordinated clonic seizures without a tonic extensor phase. Complete protection from these convulsions was the criterion for a positive drug effect.

Psychomotor seizures (Psym). Modifications of the methods of Toman(17) and Brown *et al*(18) were employed to elicit these seizures. A Grass model S4 stimulator was programmed to deliver 40 volt DC pulses at 6/sec, 0.15 msec pulse duration. The current was applied for 3 seconds through corneal electrodes. Immediately following the shock mice become catatonic for a few seconds. They then assume an erect posture with the forepaws crossed. Often the forepaws undergo pumping movements. The head is retroflexed and a Straub tail position is always seen. Gradually the paws and tail are lowered and suddenly, as the forepaws and tail touch the table the animals "break" taking several running steps. They may then huddle quietly or resume normal activity. The seizure was timed from the termination of the shock stimulus to the "break." Seizure durations were remarkably constant, control group means varying between 22.5-25.5 seconds from day to day.

All drugs were prepared as suspensions in 1% Gum Tragacanth and tested 30 or 90 minutes after intraperitoneal (i.p.) or oral (p.o.) administration, except diphenylhydantoin which was tested at 60 and 90 minutes for the MES test. In addition, quinacrine, chloroquine and hydroxychloroquine were administered orally once daily for 4 days and tested one hour after the final medication. All solutions were prepared so that the desired dose was contained in 0.1 ml/10 g of body weight.

Where applicable, ED_{50}'s were estimated graphically from a log-dose probit plot and standard errors were calculated by the Miller-Tainter approximation(19).

Results and discussion. Table I contains the ED_{50}'s $\pm$ s.e. of the compounds on the MES, MMS and mMS tests.

Since Trimethadione's potency is in the order mMS $>$ MMS $>$ MES, these procedures appear to predict the clinical profile of this drug in accordance with both the Jenney and Pfeiffer(12) and Chen(14) postulations. Diphenylhydantoin's profile stands in opposition to the Jenney and Pfeiffer hypothesis since its greatest potency is against MMS.

Of the antimalarials, only hydroxychloroquine was active after a single dose against MMS and to a lesser extent against mMS.

The slopes of the hydroxychloroquine regressions, as evidenced in part by the large

TABLE I. Activity of Antimalarials and Reference Compounds on Maximal Electroshock Seizure, Maximal Metrazol Seizure and Minimal Metrazol Seizure in Mice, After Single Doses.

Compound	Min	Maximal electroshock seizure $ED_{50} \pm$ s.e. (mg/kg) I.P.	Maximal electroshock seizure $ED_{50} \pm$ s.e. (mg/kg) P.O.	Maximal metrazol seizure $ED_{50} \pm$ s.e. (mg/kg) I.P.	Maximal metrazol seizure $ED_{50} \pm$ s.e. (mg/kg) P.O.	Minimal metrazol seizure $ED_{50} \pm$ s.e. P.O.
Phenobarbital	30	19.8 ± 3.6	27 ± 7.4	6.0 ± .6	8.4 ± .8	—
	90	17.7 ± 3.5	23 ± 3.8	3.6 ± .6	5.6 ± 1.0	5.4 ± 1.0
Diphenylhydantoin	30	—	—	3.5 ± .7	5.7 ± 1.4	—
	60	8.2 ± .6	16.5 ± .9	—	—	—
	90	7.3 ± 1.1	13.6 ± 2.3	2.7 ± .7	3.2 ± .5	10.6 ± 3.2
Trimethadione	30	Inactive up to 600 mg/kg		160 ± 16.7	218 ± 18.4	—
	90			205 ± 14.2	225 ± 20.0	140 ± 32
Phenacemide	30	84 ± 16.5	68 ± 13.4	32 ± 5.1	42 ± 8.6	—
	90	88 ± 23.9	61 ± 8.7	54 ± 12	50 ± 11.7	—
Quinacrine	30	Inactive up to 100 mg/kg potentiates death*		Inactive up to 100 mg/kg potentiates death*		—
	90					Inactive
Chloroquine	30	Inactive up to 100 mg/kg potentiates death*		Inactive		—
	90					—
Hydroxychloroquine	30	Inactive up to 100 mg/kg potentiates death*		38 ± 11	73 ± 34	—
	90			47 ± 12	83 ± 21	60% at 300†

* Incidence of death after electroshock or pentylenetetrazol injection was 20%-30% greater than controls.

† Maximum protection occurred at 300 mg/kg. Higher doses were not more effective.

standard errors, were shallower than those of the other MMS active compounds and no meaningful potency ratios could be calculated.

Table II contains the data from the MMS and mMS tests run one hour after the last of 4 daily oral medications. Quinacrine and chloroquine, ineffective after a single administration, do afford some protection from MMS after 4 oral doses although the dose-response regression is not linear for the 3 doses employed. Hydroxychloroquine, active after a single oral dosage (Table I) is also active after repeated medications. The appearance of activity in the MMS test, after 4 daily doses of quinacrine and chloroquine may be correlated with the clinical observations that several days are necessary to secure an effect(1,9,11) and that excitation may precede control(1). The mMS activity after 4 doses is poor (Table II). In a high percentage of cases the pentylenetetrazol injection elicited major tonic seizures rather than the minimal clonic convulsions observed in control mice. While these data confirm clinical reports of the conversion of petit mal seizures to major episodes(2,6) they do not conform to the hypothesis that in the laboratory mMS as opposed to MMS studies may differentiate drugs effective in petit mal from those clinically active in grand mal(14). Since these compounds are completely inactive in MES after repeated administration but do protect to some degree against MMS on the same dosage regimen, it can be concluded that, for quinacrine at least, the MES-MMS activity profile was predictive of clinical efficacy(12,13).

TABLE II. Protection from MMS and mMS Seizures in Mice After 4 Daily Oral Medications at Indicated Dosages. (N=40 animals per dose for MMS, 20 for mMS.)

	Protection %					
	Quinacrine		Chloroquine		Hydroxy-chloroquine	
	MMS	mMS	MMS	mMS	MMS	mMS
100 mg/kg	33	5 (75)*	40	45 (10)	52	15 (15)
30 "	48	5 (30)	60	0 (55)	40	20 (0)
10 "	35	0 (0)	20	10 (20)	32	20 (0)
Controls	0	0	0	0	0	0

* Numbers in parentheses are % of animals showing tonic hind leg extensor seizures.

The appearance of activity after 4 days of chloroquine treatment is probably not the result of a build-up of metabolites. The primary metabolite of chloroquine, the N-monoethyl derivative is not active on the metrazol tests either after single administration or after 4 daily doses. Similarly, the metabolite of hydroxychloroquine, the N-hy-

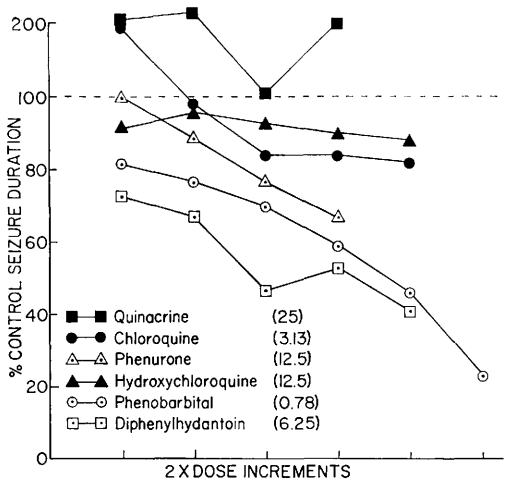

FIG. 1. Effect of anticonvulsant agents on psychomotor seizure duration. Figures in parentheses are lowest oral doses tested. Each dose increment doubles previous dose.

droxyethyl derivative is also inactive.

In general it would appear that the inconsistencies among the drug profiles preclude the use of either of the hypotheses under discussion to predict accurately the clinical efficacy of experimental anticonvulsant drugs. Predictions of clinical activity should be made only after examination of experimental compounds in as many laboratory situations as possible. Probably no single test or pair of laboratory tests serve to identify the clinical usefulness of an anticonvulsant compound. Such specificity requires detailed knowledge of seizure etiology and drug mechanisms.

Electrically induced psychomotor seizures are sometimes used in the laboratory in addition to the precedures discussed above. Fig. 1 graphically presents the effects of the compounds under study on psychomotor seizures. The plotted points are not comparable among the different agents since each dose increment on the abscissa represents a dose double that of the preceding point.

It is apparent that diphenylhydantoin and phenacemide, clinically effective psychomotor agents, are also effective in depressing the duration of electrogenic psychomotor seizures. Chloroquine and hydroxychloroquine at higher doses depress seizure duration ($P = 0.05$) though the absolute magnitude of this depression is small. At the lower doses used, both quinacrine and chloroquine significantly increase seizure duration, an observation again confirming the clinical impression that quinacrine can "stimulate" seizures before control becomes evident.

Four daily medications with quinacrine fail to reverse the potentiating effects of this drug on Psym duration. In fact at the lower doses (12.5 and 25 mg/kg) the psychomotor seizure was often converted into a maximal tonic hind leg extensor seizure. Daily doses of hydroxychloroquine do not change this compound's activity on this test.

The data obtained with diphenylhydantoin in experimental psychomotor seizures are at variance with those reported by Brown *et al* (18). It is suggested that these authors, in utilizing a quantal seizure—no seizure assay with a cut-off point at 7 seconds could not observe the decrease in seizure duration.

It is not suggested that the laboratory psychomotor seizure is necessarily a model of the clinical entity. It does extend the body of data available on any one compound, however, and in the present instance seems to correlate with already established clinical information.

Of the 3 antimalarials studied hydroxychloroquine possesses the most favorable activity profile. In contrast to quinacrine and chloroquine it is effective on MMS after a single oral administration; it does not enhance psychomotor seizure duration nor does it convert psychomotor seizures to tonic convulsions; it is not so potent as the other 2 in converting minimal metrazol seizures to major tonic episodes. Within the limitations of the predictability of laboratory profiles for clinical efficacy these data suggest that hydroxychloroquine might be as effective clinically as quinacrine in petit mal disorders without a preliminary period of excitation and without a propensity to elicit major seizures.

Summary. The antimalarials quinacrine, chloroquine and hydroxychloroquine were investigated for their anticonvulsant activities against maximal electroshock seizures, maximal and minimal metrazol seizures and electrogenic psychomotor seizures. Phenobarbital, trimethadione diphenylhydantoin, and

phenacemide were employed as reference standards. 1. All 3 antimalarials were ineffective in protecting mice from maximal electroshock tonic extensor seizures, both after single oral doses and after 4 daily doses. 2. Hydroxychloroquine was effective against maximal metrazol seizures after a single oral dose. Quinacrine and chloroquine exhibited activity after 4 daily medications. 3. Quinacrine and chloroquine (low doses) enhanced psychomotor seizure duration while hydroxychloroquine and chloroquine (high doses) slightly reduced seizure duration. 4. Difficulties in predicting clinical efficacy from laboratory data are discussed.

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Antibodies to Mouse Hepatitis Viruses in Human Sera. (28928)

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Epidemiologic studies of viruses of the mouse hepatitis group have long been hampered by the lack of sensitive and specific serologic techniques. By using the continuous C3H mouse liver tissue culture cell line, clone NCTC 1469, which was originally cultivated by Hobbs *et al*(1) and applied to mouse hepatitis virus studies by Manaker *et al*(2), we have recently developed a plaque assay for many strains of this group(3). In addition, the fluids from infected cultures provide complement fixing (CF) antigens which are not only somewhat higher in titer than antigens prepared from infected suckling mouse liver and brain, but are often more sensitive for detection of antibody in mouse antisera (unpublished data); for example, the titer of MHV-S hyperimmune mouse antiserum was 1:128 when tested against 8 antigen units of tissue culture-grown MHV-S, but was only 1:32 with 8 units of MHV-S suckling mouse brain antigen.

This report describes the successful use of the plaque neutralization test and the tissue culture-produced CF antigens for demonstration of antibodies to mouse hepatitis viruses in human sera. The chief purpose is to present evidence for the specificity of the reac-

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