small amount of water-insoluble debris excluded in the present work may have possessed this activity. Mild means for solubilizing this fraction would be required to investigate this aspect further. Also, it is conceivable that agglutination of canine erythrocytes by appropriate antisera depends on the presence of a complex antigen composed of protein components, either alone or in combination with mucopolysaccharide(s). If protein components as well as mucopolysaccharide were necessary to complete the antigen, then gastric mucopolysaccharide alone would not be expected to inhibit the hemagglutination reaction. Further investigation will require liberation, fractionation and concentration of canine erythrocyte surface proteins and protein-mucopolysaccharide complexes.

Summary. Canine gastric secretions—both fluid acid and viscous non-acid—and hexosamine-rich fractions derived therefrom, were assayed for blood group substance activity in canine and in human test systems. None of the specimens tested was active in the canine test systems, but all exhibited A and H activity in the human test systems; no human B activity was detected. Hexosamine-rich fractions obtained by peptic digestion and phenol extraction of canine gastric secretion were largely of the neutral mucoid type, and were active in human A and H systems at the microgram level.

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Anticonvulsant Properties of 1-(1-phenylcyclohexyl) piperidine·HCl and Certain Other Drugs. (26425)

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1-(1-phenylcyclohexyl) piperidine · HCl (PCP) was found to be an extremely active agent in prevention of electrically induced tonic extensor seizures in mice(1). In man PCP has been shown to induce interceptive sensory deprivation at low doses and general anesthesia at high dose levels(2,3). This investigation dealt with a comparison of the anticonvulsant properties of PCP, Dilantin, phenobarbital and barbital in convulsions induced by sound, by pentylenetetrazol and by electroshock.

Materials and methods. The experiments on audiogenic seizures were conducted on DBA/2 male mice, 28 to 35 days old, obtained from the Roscoe B. Jackson Memorial Laboratory. The age range between 4 to 5 weeks was found to be the critical period for audiogenic seizure susceptibility in this strain of animals.

The apparatus consisted of a small insulated plywood chamber (40″ x 32″ x 30″) with a hinged door at the front. In it were wired a 4″ electric fan and 2 electric door

bells. A 12″ x 12″ glass window was inserted in the front door for full-view observation. A Petri dish (4¾″), covered with an inverted cylindrical wire basket (4½″ x 4½″) was used to house each mouse during the experiment.

The sound, approximately 90 decibels, was turned on for 90 seconds. During this period the seizure pattern was observed according to the criteria adopted by Frings and Frings(4) in the following sequence: the running phase, clonic spasms, clonic-tonic seizures with extension of limbs and death. Because the clonic spasms were not consistently seen in all animals between the running and the extensor seizure phase, this seizure pattern was not used in evaluation of the anticonvulsant effect of drugs. Under the experimental conditions employed, violent running in the cage occurred within 10 to 30 seconds during sound stimulation in every mouse; this was usually followed by tonic extension of limbs and death. In 50 untreated mice, 10 animals each from 5 groups received from the supply house at different times during the year, 90% of them at least in each group had extensor seizures and succumbed in respiratory failure. Because of the lethal effect of audiogenic stimulation, it was not possible to examine animals audio-susceptibility prior to drug testing. Consequently, 10 DBA/2 mice from each supply of 120 animals were examined for audiogenic seizures as controls; anticonvulsant activities of drugs as determined with the same group of mice were estimated on basis of the control response. And because of the brief age interval during which DBA/2 mice will respond in convulsions to sound, they were used only once in our experiment. Two mice were on observation simultaneously. Four groups of 10 animals each were employed to examine the drug effect at graded dosages.

Ordinary male Webster albino mice, weighing 18-24 g, were used to study the effect of drugs on pentylenetetrazol and electrically induced convulsions. A 0.9% pentylenetetrazol solution was given intramuscularly at 90 mg/kg, this being a submaximal convulsive dose for mice. In the present study, only suppression of the initial clonic seizures was taken as a measure of anti-pentylenetetrazol activity of drugs(5). The supramaximal shock procedure of Toman, Swinyard and Goodman(6) was followed to produce tonic extensor seizures. A current of 20 milliamperes was applied to animal's ears by clips for 0.2 seconds.

PCP, Dilantin (5,5 diphenylhydantoin sodium), phenobarbital (5-phenyl 5 ethyl barbiturate sodium), and barbital (5,5 diethyl barbiturate sodium) were dissolved in water and administered intraperitoneally. Their anticonvulsant activities were examined at time of pre-determined peak effect. Four graded doses of each drug, which would suppress seizures in 10 to 90% of the mice (10 animals per dose) were used to determine the 50% effective dose. ED_{50} was estimated graphically by the probit-log dose procedure of Miller and Tainter(7). Standard errors of a ratio of the means were computed from individual standard errors in percentages(8).

Results. In Table I are given the doses of drugs which would prevent seizures in 50% of the animals (ED_{50}) in respond to sound, pentylenetetrazol or electrical stimuli. Their relative anti-seizure activities, in reference to that of PCP, are expressed in ED_{50} ratios of molecular equivalents. There is also given for each drug a ratio of ED_{50}'s that were required for suppression of running and for prevention of extension of limbs in audiogenic seizures. This, as indicated by a/b in the table, signifies the relative effectiveness of each drug in combating the 2 types of seizure response. In the last column of the table is shown a comparison by ED_{50} ratios for phenobarbital and barbital of their anti-extensor seizure activity in convulsions produced by electroshock to their anti-clonic seizure activity in pentylenetetrazol-induced convulsions.

It may be seen first in audiogenic seizures that PCP and barbital were the most and the least effective drug respectively in suppressing both running and the extensor phase of the convulsion. Whereas Dilantin and phenobarbital were approximately equally capable of protecting animals from the extension of limbs, the former was less effective than the latter in prevention of running ac-

TABLE I. Anticonvulsant Effect of PCP and Other Drugs in Audiogenic, Pentylenetetrazol- and Electrically-Induced Seizures.

Compound	Time after inj., hr	Audiogenic (a) Running —ED_{50} ± S.E., mg/kg—	Audiogenic (b) T.E.S.* —ED_{50} ± S.E., mg/kg—	a/b§	Pentylenetetrazol (c) Int. Cl. S.* —ED_{50} ± S.E., mg/kg—	Electroshock (d) T.E.S. —ED_{50} ± S.E., mg/kg—	d/c§
PCP	¼	.73 ± .10 (1)†	.45 ± .07 (1)	1.62 ± .34	Ineffective at 5-40	5.20 ± .30 (1)	
Dilantin	1	8.50 ± 1.65 (.09 ± .02)	3.08 ± .36 (.15 ± .03)	2.76 ± .62	*Idem*	12.4 ± .45 (.43 ± .07)	
Phenobarbital	½	4.44 ± .39 (.18 ± .03)	3.80 ± .57 (.13 ± .03)	1.17 ± .21	31.5 ± 1.1	33.4 ± 1.42 (.18 ± .01)	1.06 ± .06
Barbital	1	16.5 ± 1.5 (.06 ± .01)	15.2 ± .2 (.04 ± .01)	1.08 ± .10	34.2 ± 1.5	218.0 ± 6.204 (.03 ± .01)	6.37 ± .34

* Int. Cl. S. = Initial clonic seizures; T.E.S. = Tonic extensor seizures.
† In parentheses, relative activity in reference to PCP on basis of mol equivalents.
‡ Loss of righting reflex.
§ a/b, d/c = ED_{50} ratios indicating relative effectiveness of each drug in combating 2 types of seizures.

tivity. As indicated by the ED_{50} ratios for each drug, a larger dose of PCP or Dilantin was required for suppression of running than that for extensor seizures. On the other hand, doses of phenobarbital or barbital taken for suppression of these 2 seizure responses were the same.

In regard to suppression of pentylenetetrazol-induced clonic seizures, phenobarbital and barbital were equally effective. PCP and Dilantin were ineffective at 5 to 40 mg/kg, the latter being a maximal tolerated dose of PCP in mice. In protecting animals from extensor seizures to electroshock, Dilantin is more effective than phenobarbital. Otherwise, the antiextensor seizure effects of these drugs were in the same order of activity as found in audiogenic seizures.

A striking difference in anticonvulsant properties of phenobarbital and barbital is shown by their ED_{50} ratios of electrically-induced extensor seizures to pentylenetetrazol-induced clonic convulsions. While the ED_{50}'s of phenobarbital for suppression of the 2 types of seizures were the same, the ED_{50} of barbital for extensor seizure was 6 times greater than that for clonic seizures.

Discussion. As shown by its anticonvulsant spectrum, PCP and Dilantin seem to possess certain neuropharmacological properties in common. The inability of both drugs to suppress pentylenetetrazol-induced clonic seizures correlates with lack of a hypnotic action in man. This appears to support the assumption that the central depressant action of phenobarbital and barbital responsible for suppression of pentylenetetrazol-induced clonic seizures is the same as that for induction of hypnosis(9). PCP, on the other hand, possesses an anesthetic action which is absent in Dilantin. The fact that PCP will produce sensory deprivation in man at low doses and general anesthesia at high dosages points to the possibility that this drug acts on both the afferent and the efferent nervous system in suppression of audiogenic seizures.

Summary. 1-(1-phenylcyclohexyl) piperidine · HCl was found to be a very effective agent in suppression of audiogenic seizures in mice. It was approximately 7 times more potent than Dilantin and phenobarbital and 25 times more potent than barbital. PCP was likewise the most active anticonvulsant in electrically induced tonic extensor seizures. Like Dilantin, on the other hand, PCP was devoid of any anti-clonic seizure activity in pentylenetetrazol-induced convulsions. A comparison was also made of the anticonvulsant effects of Dilantin, phenobarbital and barbital in audiogenic, pentylenetetrazol and electrically induced convulsions.

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Effect of Protein on Utilization of Vitamin A in the Chick.* (26426)

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Conflicting observations have been reported on the effect of dietary protein level upon vit. A requirement. Baumann *et al.* (1) observed that rat diets low in protein reduced the storage of vit. A in the liver and increased rate at which stored vitamin A was depleted. Arnich and Pederson(2) found the concentration of vit. A per gram of liver to be almost doubled in rats receiving diets low in protein as compared to faster-growing rats receiving adequate protein; the total vit. A per liver, however, being approximately equivalent. Dye *et al.*(3) observed little effect upon utilization of vit. A by rats, regardless of dietary protein level.

On the other hand, Mayer and Krehl(4) observed in rats fed diets deficient in vit. A, that as dietary protein increased, there was a concomitant increase in severity of vit. A deficiency symptoms. Bohman *et al.*(5) observed a reduced level of vit. A and carotene in the liver and blood plasma when extra protein supplement was fed to wintering beef calves. Olsen *et al.*(6), using Columbian Rock progeny of hens maintained on diets low in vit. A, observed that as dietary protein in a practical chick diet increased from 16.9% to 24.6%, liver storage of vit. A in the chicks decreased about 40%.

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In view of the lack of agreement on the effects of dietary protein levels upon vit. A requirements, experiments were undertaken in our laboratory to study this relationship in the chick, and to investigate blood uric acid levels of chicks fed various amounts of protein and vit. A, in an effort to extend the findings of Elvehjem and Neu(7) and Stoewsand and Scott(8) as to factors concerned in producing elevated blood uric acid levels, and to determine if these levels can be used as a criterion of assessment of vit. A deficiency in the chick.

Methods. Male White Plymouth Rock chicks were randomly distributed among the various lots at one-day of age, using duplicate lots of 10 chicks per treatment in Experiment 1 and duplicate lots of 14 chicks per treatment in Exp. 2 and 3. Chicks in Exp. 1 were fed *ad libitum*. At the end of one week, 12 chicks per lot in Exp. 2 and 10 chicks per lot in Exp. 3 were selected according to uniformity of weights within each lot, and continued on treatment for the subsequent 3 weeks. To achieve approximately equivalent growth, the feed consumption of the chicks in Exp. 2 and 3 was limited during this 3 week period by equalized paired feeding by groups to that of the chicks receiving the highest protein treatment within each level of vit. A supplementation.

The diets are presented in Table I. Protein levels were 71.5% (high); 47.5% (mod-