TABLE III. Composition of Anolis Ash.

Sex	Wet wt	Dry wt	Ash wt	Na	K	Ca	Mg	Cl
	g			mg				
♂	3.50	1.32	.175	3.35	5.87	58.3	1.34	3.85
♂	3.26	.99	.135	2.86	5.23	43.2	1.10	2.73
♂	3.02	.90	.132	2.23	5.03	45.6	1.19	3.30
♂	3.71	1.26	.173	2.39	6.23	57.9	1.30	4.20
♀	3.22	1.00	.153	2.80	5.65	51.6	1.39	3.12

and urine urea, ammonia, uric acid, Cl, Na, K, pH, CO_2; blood and plasma proteins; urine creatinine, and Na, K, Ca, Mg and Cl of the ashed animal.

1. van Allen, C. M., *J. Am. Med. Assn.*, 1925, v84, 202.

2. Schales, O., and Schales, S., *J. Biol. Chem.*, 1941, v140, 879.

3. Gilmont, R., *Anal. Chem.*, 1948, v20, 1109.

4. Kirk, P. L., *Quantitative Ultramicroanalysis*, 1950, Wiley and Sons, New York.

5. Scholander, P. F., and Roughton, F. J. W., *J. Biol. Chem.*, 1943, v140, 573.

6. Roughton, F. J. W., and Scholander, P. F., *J. Biol. Chem.*, 1943, v148, 541.

7. Sobel, A. E., and Sobel, B. A., *J. Biol. Chem.*, 1939, v129, 721.

8. Kuttner, T., and Cohen, H. R., *J. Biol. Chem.*, 1927, v75, 517.

9. Kolthoff, I. M., and Sandell, E. B., *Textbook of Quantitative Inorganic Analysis*, 1936, Macmillan, New York.

10. Denis, W., *J. Biol. Chem.*, 1922, v52, 411.

11. Conway, E. J., *Micro-diffusion Analysis and Volumetric Error*, 1940, van Nostrand, New York.

12. Brown, H., *J. Biol. Chem.*, 1945, v158, 601.

13. Folin, O., *J. Biol. Chem.*, 1934, v106, 311.

14. Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, v38, 81.

15. ———, *J. Biol. Chem.*, 1920, v41, 367.

16. Coulson, R. A., Hernandez, T., and Brazda, F. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 203.

17. Smith, H. W., *J. Biol. Chem.*, 1929, v82, 651.

18. O'Malley, E., Conway, E. J., and Fitzgerald, O., *Biochem. J.*, 1943, v37, 278.

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Effects of Liver Injury and Nephrectomy on the Anticonvulsant Activity of Phenacemide.*† (19749)

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Although phenacemide (phenacetylurea; Phenurone) is one of the most useful agents for the treatment of psychomotor epilepsy, comparatively little is known concerning the fate and the excretion of this agent in animals or man. In a brief report on the pharmacology of Phenurone, Everett(1) mentioned experiments in which the duration of anticonvulsant action remained unchanged in bilaterally nephrectomized mice, but was significantly prolonged by carbon tetrachloride-induced liver damage and hepatectomy. We are aware of no published information concerning the fate and excretion of Phenurone in man.

We have reported(2,3) the results of experiments in which biological methods were used to measure the effect of liver injury and nephrectomy on the potency and the duration of anticonvulsant action of clinically employed hydantoins and oxazolidine-2,4-diones. We have now applied the same biological methods in the study of Phenurone. It was anticipated that the results would contribute information

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on the relative importance of the liver and the kidney for the fate and the excretion of this compound, as interpreted from the alterations in potency and duration of action induced by the experimental conditions employed.

Methods. Adult male albino rats obtained from the Sprague-Dawley or Holtzman-Rolfsmeyer farms were used as experimental animals. They were maintained on an adequate diet and allowed free access to food and water except during experimental testing. Because of its insolubility, Phenurone was given orally as a 0.5 or 1.0% suspension in a 10% acacia solution. Anticonvulsant potency, as measured by the maximal electroshock seizure test, was determined in normal, nephrectomized and liver-injured rats at 3, 6 and 12 hours after administration of the drug. The details of the maximal electroshock seizure test have been described elsewhere(4). Briefly, it consists in determining the dose of a drug which abolishes the hindleg tonic extensor component of the maximal electroshock seizure pattern in 50% of animals. The ED_{50}s were calculated by the method of Litchfield and Wilcoxon(5). From 14 to 72 animals were used for the determination of each ED_{50}. Liver injury was induced by a single subcutaneous injection of 2 ml/kg of a 50% (w/v) solution of carbon tetrachloride in peanut oil, and the drug was administered 36 to 48 hours later. Histological studies of liver sections taken from rats subjected to such carbon tetrachloride treatment showed that moderate to severe liver damage was consistently produced. Nephrectomy was performed under ether anesthesia at such a time interval before drug administration that 12 hours always elapsed between time of operation and time of electroshock test; in this manner, the effect of accumulated metabolic products was kept relatively constant. All animals (normal, nephrectomized and liver-injured) were previously examined for their ability to exhibit a maximal electroshock seizure pattern. In all instances they were found able to respond with a normal pattern; thus nephrectomy and liver injury *per se* did not alter the response to electroshock. Therefore, any changes observed could reasonably be attributed to Phenurone.

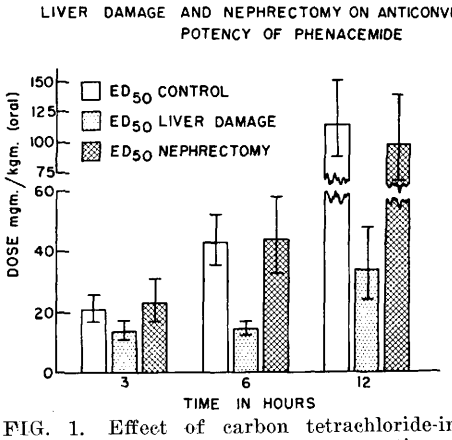

FIG. 1. Effect of carbon tetrachloride-induced liver injury and of nephrectomy on anticonvulsant potency of phenacemide. Anticonvulsant doses are expressed in mg/kg along the ordinate and the time intervals at which tests were made after drug administration are shown on the abscissa. The significance of the various vertical bars is indicated by the legend in the figure. The 95% confidence limits are shown by the bracketed vertical lines.

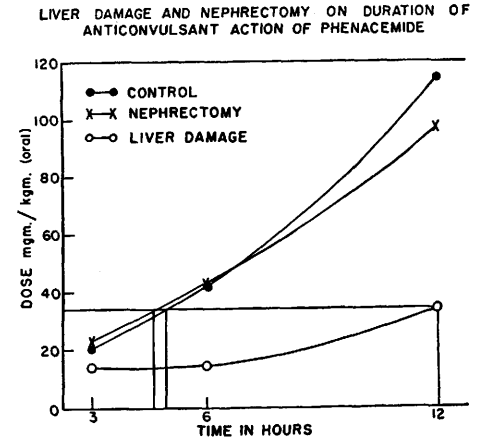

FIG. 2. Effect of liver injury and nephrectomy on duration of anticonvulsant action of phenacemide. See text for explanation.

Results. The effects of nephrectomy and of liver injury on the anticonvulsant *potency* of Phenurone are shown in Fig. 1. It may be seen from Fig. 1 that liver injury tends to reduce significantly the dose of Phenurone required to modify the maximal electroshock seizure pattern in 50% of rats at all 3 time intervals studied. For example, a dose of 115 mg/kg of Phenurone was required to protect 50% of normal rats at the 12-hour interval, as compared with a dose of only 34 mg/kg in rats with damaged livers [potency ratio with

95% confidence limits = 3.4 (2.2 to 5.2)]. In contrast, bilateral nephrectomy has no statistically significant effect on the anticonvulsant potency of Phenurone. With regard to duration of action, pertinent information was obtained by plotting the ED_{50}s against time, as shown in Fig. 2. In a recent publication(2) duration of anticonvulsant action was calculated indirectly rather than by the direct graphic method employed herein. The 2 methods give essentially the same results but the graphic technic is easier and more readily comprehended. In Fig. 2 the smooth curves which join the observed values were fitted by eye. A horizontal line was drawn to intercept the ordinate at the 34 mg/kg dose level. Where this line crosses the dose-time curves, perpendicular lines were drawn to the abscissa. From an inspection of this plot of the data, it can be seen that a dose of 34 mg/kg of Phenurone protects at the 50% level for less than 5 hours in normal rats and for slightly over 4½ hours in nephrectomized animals. In sharp contrast, the same dose of Phenurone protects liver-injured rats for 12 hours. It is thus evident that liver injury, but not nephrectomy, significantly increases the duration of anticonvulsant action of Phenurone.

Discussion. The data presented substantiate the observation of Everett(1) that carbon tetrachloride-induced liver injury, but not nephrectomy, increases the duration of anticonvulsant action of Phenurone. In addition, carbon tetrachloride-induced liver injury significantly increases the anticonvulsant potency of Phenurone at all time intervals studied; the average increase in potency was approximately 250%. In contrast, bilateral nephrectomy had no significant effect on the anticonvulsant potency or the duration of action of Phenurone.

The fact that hepatic damage increases both potency and duration of action of Phenurone can be interpreted to mean that the liver is important for the degradation of Phenurone into substances devoid of anticonvulsant activity as measured by the maximal electroshock seizure test. The observation that bilateral nephrectomy neither increases anticonvulsant potency nor prolongs the duration of action of Phenurone suggests that the kidney is not the principal organ for the elimination of this drug or any active product into which it may be converted.

Summary. Phenurone was tested at various time intervals after administration in normal, liver-injured and nephrectomized rats for its ability to prevent the tonic extensor component of maximal electroshock seizures. The results were analyzed for the effect of liver injury and nephrectomy on the potency and the duration of anticonvulsant action of this compound. Liver injury significantly increased the potency and the duration of anticonvulsant action; on the other hand, bilateral nephrectomy had no significant effect on the anticonvulsant properties of this agent. The results suggest that, in the rat, the liver is the principal organ for the degradation of Phenurone into products devoid of anticonvulsant action, and that the kidney plays no important role in the metabolic alteration or excretion of this compound.

1. Everett, G. M., *Federation Proc.*, 1949, v8, 289.
2. Swinyard, E. A., Weaver, L. C., and Goodman, L. S., *J. Pharmacol. and Exp. Therap.*, 1952, v104, 309.
3. Swinyard, E. A., Schiffman, D. O., and Goodman, L. S., *J. Pharmacol. and Exp. Therap.*, In press.
4. Swinyard, E. A., *J. Am. Pharm. A.* (Scient. Ed.), 1949, v38, 201.
5. Litchfield, J. T., and Wilcoxon, F., *J. Pharmacol. and Exper. Therap.*, 1949, v96, 99.

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