to *Bartonella muris* infection. 4. The lesser effect on mortality observed in Experiment 300 was probably due to antibacterial activity of the chlortetracycline on minor incident infections antagonized by the X-irradiation.

1. Miller, C. P., Hammond, C. W., Tompkins, M., *Science*, 1950, v111, 540.

2. Miller, C. P., Hammond, C. W., Tompkins, M., *J. Lab. & Clin. Med.*, 1951, v38, 331.

3. Miller, C. P., Hammond, C. W., *Trans. Assn. Phys.*, 1950, v63, 155.

4. Miller, C. P., Hammond, C. W., Tompkins, M., Shorter, G., *J. Lab. & Clin. Med.*, 1952, v39, 462.

5. Howland, J. W., Furth, F. W., Coulter, M., 1949. The Effect of Aureomycin and Antibiotics on Whole Body Irradiation. Unclassified. Rep. 94, Univ. Rochester, Atomic Energy Project 10-14-49.

6. Furth, F. W., Coulter, M., 1950. The Effect of Aureomycin on the Radiation Syndrome on Dogs. Unclassified Rep. 116, Univ. Rochester, Atomic Energy Project 4-27-50.

7. Warren, S., *Arch. Path.*, 1942, v34, 749 (Subject reviewed by Dunlap).

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Anticonvulsant Effect of Nialamide and Diphenylhydantoin.*‡ (27033)

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Evidence was accumulated during the past decade indicating that 5-hydroxytryptamine (5-HT), a normal constituent of the central nervous system, is of importance in the functioning of the system. Among its multiple functions, several investigators have suggested a possible interrelation between the levels of brain 5-HT and the excitability of the central nervous system. Prockop *et al.*(1) reported that the monoamine oxidase inhibitors have a pronounced anticonvulsant effect and that this effect is closely associated with an elevation of brain 5-HT and norepinephine (NE). Reserpine which releases brain amines was reported to facilitate convulsions(2). Bonnycastle and co-workers(3,4) and Anderson *et al.*(5) have shown that a number of anticonvulsant compounds including diphenylhydantoin, mesantoin, trimethadione and paramethadione caused a significant elevation of brain 5-HT levels in rats and suggested a relationship between an increase in 5-HT in the brain and the anticonvulsant action of these drugs. Prockop *et al.* (1), on the other hand, observed that diphenylhydantoin does not increase amines in the brain of rats.

The purpose of this paper is to report the influence of pharmacological agents such as nialamide or reserpine which increase(6,7,8) or decrease, respectively, the levels of brain amines on the anticonvulsant action of diphenylhydantoin. A study of the interaction of diphenylhydantoin with these agents was of particular interest because nialamide has been reported to have anticonvulsive action(7,9) and reserpine to facilitate convulsions. Simultaneous measurement of brain amine levels under the various experimental conditions was also made.

Experimental. A total of 595 Swiss-Webster male white mice weighing 17 to 25 g and 125 Wistar male white rats weighing 100-120 g were used in these studies.

Two methods were used in testing the anticonvulsant action: 1. Protection against maximal electroshock seizures (MES) as described by Swinyard, Brown and Goodman(10) in which alternating current of 50 mA intensity in mice and 150 mA in rats with 0.2 second duration was delivered through corneal electrodes from a Hans Technical Associates Stimulator. The dose of anticonvulsant drug necessary to protect 50% of animals (ED_{50}) from the hindleg tonic extensor component of the maximal

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seizure pattern was determined. 2. Determination of the maximal electroshock seizure threshold, i.e., amount of current necessary to produce maximal tonic extensor seizures in 50% of animals (CD_{50}). All ED_{50}'s or CD_{50}'s and standard deviations were calculated by the use of Thompson & Weil's(11) tables for moving average interpolation. Unless otherwise stated, electroshocks were applied to the animals at 1, 4 and 5 hours after administration of diphenylhydantoin, nialamide and reserpine, respectively. No animal was shocked more than once.

To determine the influence of reserpine or nialamide on the anticonvulsant effect of diphenylhydantoin, groups of 20 to 30 mice were given a fixed dose of reserpine, 5 mg/kg I.P., or nialamide, 100 mg/kg I.P. Four hours later the animals were divided into groups of 5 each and a series of 4 to 6 geometrically spaced doses of diphenylhydantoin sodium was given to groups of the nialamide or reserpine pretreated animals. One hour after diphenylhydantoin, maximal electroshock (50 mA, 0.2 sec.) was applied, and the dose of diphenylhydantoin required to protect 50% of the nialamide or reserpine pretreated animals was determined.

For determination of brain amines, the animals were sacrificed after application of electroshock and the concentrations of 5-HT and NE were determined spectrophotofluorometrically by the single extraction method of Mead and Finger(12).

Results. Effect of Nialamide on Maximal Electroshock Seizures and on Brain Amines. In mice, nialamide in doses up to 400 mg/kg had no protective action against maximal electroshock seizures using 50 mA current but it did increase the maximal electroshock seizure threshold. The convulsive threshold was unchanged after intraperitoneal doses of 20, 35 or 50 mg/kg of nialamide(Fig. 1). The levels of 5-HT in the brain, however, were twice that of controls after an intraperitoneal dose of 20 mg/kg and more than double the normal values after doses of 35 or 50 mg/kg of nialamide. A moderate increase in electroshock seizure threshold occurred only after the dose of nialamide had reached 100 mg/kg. It is interesting to note that in spite of the fact that brain levels of 5-HT had reached a plateau after 100 mg/kg of nialamide, there were further increases in electroshock seizure thresholds with increasing doses of nialamide. There was no significant change in brain NE content at all dose levels at the time the determinations were made.

Further evidence that the anticonvulsant effect of nialamide is independent of changes in brain amines may be found in the time course studies (Fig. 2). After intraperitoneal administration of nialamide, 200 mg/kg, there was a definite increase in brain 5-HT during the first hour and a marked increase during the second hour whereas an increase in electroshock seizure threshold was not observed until 4 hours after injection of nialamide. Sixteen hours after injection of nialamide when the brain 5-HT was still elevated, the antielectroshock action had disappeared and the electroshock seizure threshold had returned to normal. No consistent change in NE level was observed.

In rats, nialamide also increased the maximal electroshock seizure threshold and this effect again appeared unrelated to changes in brain amine levels. After intraperitoneal injection of nialamide, 35 mg/kg, the brain 5-HT reached its peak at about 4 hours and remained elevated for at least 24 hours (Fig. 3). The maximum elevation of seizure threshold was obtained 8 hours after nialamide. Twenty-four hours after injection of nialamide, when the brain amine levels were still as high as at 4 hours after the drug, the seizure threshold had almost returned to predrug levels. NE levels were practically unchanged.

Effect of Nialamide or Reserpine on Brain Amine Levels and on Anticonvulsant Activity of Diphenylhydantoin. The influence of nialamide and reserpine on the antielectroshock seizure effect (MES) of diphenylhydantoin in mice is shown in Fig. 4. When given alone, diphenylhydantoin (6.6 mg/kg) protected 50% of the mice (ED_{50}) from maximal electroshock seizures due to 50 mA intensity. The potent anticonvulsant action of diphenylhydantoin in mice was not associated with any measurable change in brain amines. Diphenylhydantoin in dose of 10 mg/kg protected 100% of the mice from MES, but even doses as high as 55 mg/kg did not cause any change in level of brain amines. When mice were pretreated

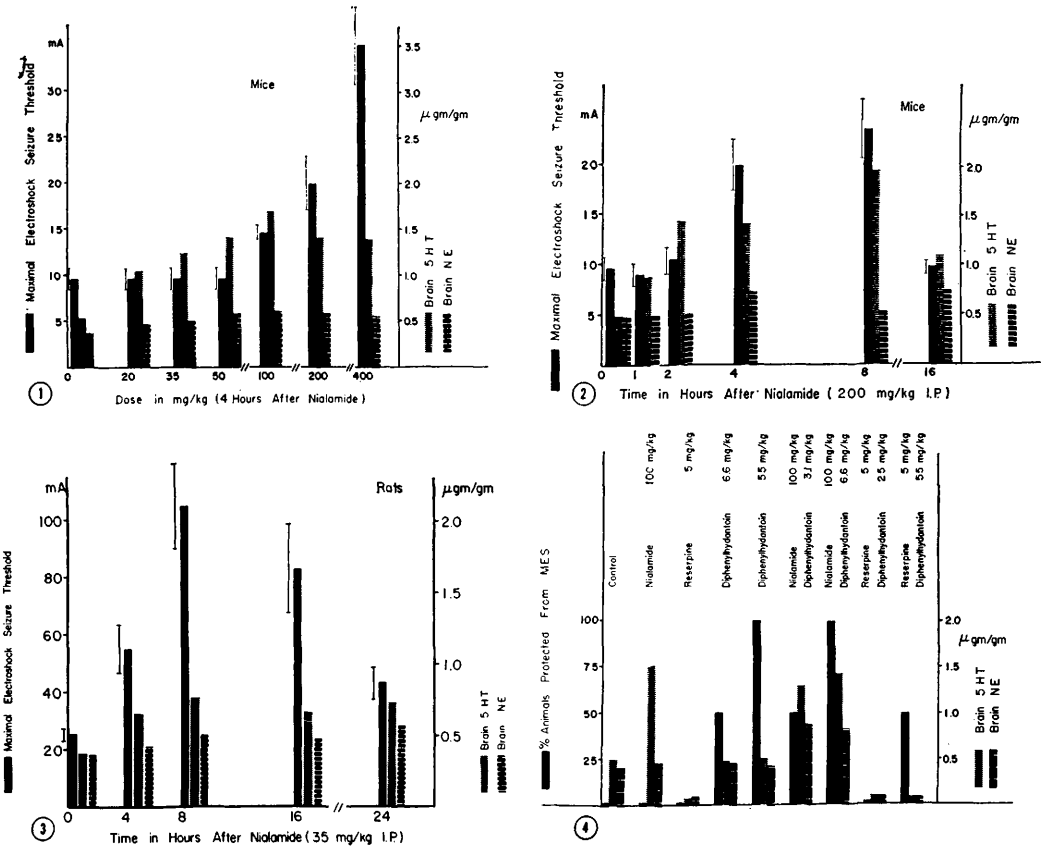

FIG. 1. Relationship between increase in maximal electroshock seizure threshold and brain 5-HT and NE after intraperitoneal injection of increasing doses of nialamide in mice. Vertical brackets represent ±1 standard deviation.

FIG. 2. Relationship between increase in maximal electroshock seizure threshold and brain 5-HT and NE at different times after a single dose of nialamide in mice. Vertical brackets represent ±1 standard deviation.

FIG. 3. Relationship between increase in maximal electroshock seizure threshold and brain 5-HT and NE at different times after a single dose of nialamide in rats. Vertical brackets represent ±1 standard deviation.

FIG. 4. Interaction of nialamide or reserpine with diphenylhydantoin on maximal electroshock seizures and brain amine levels in mice. Nialamide or reserpine was given 4 hours before diphenylhydantoin. All drugs were injected intraperitoneally.

4 hours previously with 100 mg/kg of nialamide, the anticonvulsant effect of diphenylhydantoin was greatly potentiated, the ED_{50} was reduced to 3.1 mg/kg, i.e., one half of the dose required when administered alone. Administration of diphenylhydantoin to nialamide pretreated animals did not further increase the levels of 5-HT in the brain from levels of those that received nialamide alone. Levels of NE were, however, increased. Pretreatment of mice with reserpine 4 hours prior to administration of diphenylhydantoin markedly decreased the anticonvulsive effects of diphenylhydantoin; the ED_{50} for diphenylhydantoin in these reserpine pretreated animals was 55 mg/kg, i.e., almost 9 times the dose required when given alone. Administration of high doses of diphenylhydantoin to the reser-

pine pretreated mice did not reverse the depletion of brain amine levels elicited by reserpine.

Discussion. The elevation of the convulsive threshold produced by administration of nialamide to mice and rats does not appear to be directly related to changes in brain serotonin or norepinephrine for the following reasons: 1. Doses of nialamide which more than doubled the brain serotonin level did not increase the convulsive threshold in mice. 2. The brain serotonin concentration was not further elevated by doses of nialamide in excess of 100 mg/kg while the convulsive threshold continued to rise as the dose was increased. 3. A 2- to 3-fold elevation of brain serotonin was achieved at a time (2 and 16 hours) when no significant changes in convulsive threshold were observed. 4. Changes in convulsive threshold occurred without corresponding changes in brain norepinephrine. These results indicate that the changes in convulsive threshold obtained after nialamide are due to a mechanism of action other than elevation of brain serotonin and norepinephrine.

Diphenylhydantoin in doses from 6.6 to 55 mg/kg, which was almost 9 times the anticonvulsive dose, did not change 5-HT or NE levels in the brain of mice. Prior administration of nialamide potentiated the anticonvulsant activity of diphenylhydantoin but the levels of brain 5-HT were not higher than when nialamide was given alone. The concentration of brain NE was elevated by the combination of the two drugs. However, since it was shown that the anticonvulsant effect of nialamide or diphenylhydantoin was independent of changes in brain NE concentrations it does not seem likely that nialamide potentiated the effect of diphenylhydantoin by increasing the level of NE. Administration of reserpine facilitated tonic extensor seizures and while diphenylhydantoin was capable of counteracting this effect, very large doses of the anticonvulsant drug were required. This blockade of reserpine-induced facilitation, however, was not accomplished by reversal of the effects of reserpine on the brain amines. The failure of diphenylhydantoin to reverse the amine depleting effects of reserpine appears to corroborate the report of Chen and Bohner(13) who found that diphenylhydantoin did not reverse reserpine-induced ptosis, a response attributed by Costa and Pscheidt(14) to depletion of brain serotonin.

The results of this study demonstrated that while the elevation of the seizure threshold elicited by nialamide was not related to changes in brain amines, the possibility remains that a modifying action of nialamide or reserpine on the anticonvulsant action of diphenylhydantoin could be due to changes in brain serotonin and norepinephrine.

Summary. 1. Nialamide increased the maximal electroshock seizure threshold in mice and rats. This effect was independent of the elevation of brain 5-HT and NE elicited by nialamide. 2. Diphenylhydantoin in doses from 6 to 55 mg/kg, which was almost 9 times the anticonvulsive dose, did not significantly alter the content of 5-HT or NE in the brain of mice. 3. Nialamide potentiated and reserpine decreased the anticonvulsant effect of diphenylhydantoin. No correlation could be found between these effects and brain amine levels.

1. Prockop, D. J., Shore, P. A., Brodie, B. B., *Ann. N. Y. Acad. Sci.*, 1959, v80, 643.

2. Chen, G., Ensor, C. R., Bohner, B., PROC. SOC. EXP. BIOL. & MED., 1954, v86, 507.

3. Bonnycastle, D. D., Bradley, A., *Fed. Proc.*, 1956, v15, 403.

4. ————, Giarman, N. J., Paasonen, M. K., *Brit. J. Pharmacol.*, 1957, v12, 228.

5. Anderson, E. G., Bonnycastle, D. D., *Pharmacologist*, 1960, v2, 77.

6. Rowe, R. P., Bloom., B. M., P'an, S. Y., Finger, K. F., PROC. SOC. EXP. BIOL. & MED., 1959, v101, 832.

7. Schneider, J. A., Bloom, B. M., Delahunt, C. S., Finger, K. F., Finkelstein, M., Rowe, R. P., Stebbins, R., Weissman, A., *J. Soc. Cienc. Med. Lisboa*, 1959, v123, 43.

8. Finger, K. F., *Fed. Proc.*, 1960, v19, 278.

9. Pisanty, J., Pineyro, A., Moreira, R., Todd, L. E., *J. Soc. Cienc. Med. Lisboa*, 1959, v123, 58.

10. Swinyard, E. A., Brown, W. C., Goodman, L. S., *J. Pharmacol. & Exp. Therap.*, 1952, v106, 319.

11. Thompson, W. R., Weil, C. S., *Biometrics*, 1952, v8, 51.

12. Mead, J. A. R., Finger, K. F., *Biochem. Pharmacol.*, 1961, v6, 52.

13. Chen, G., Bohner, B., *J. Pharmacol. & Exp. Therap.*, 1961, v131, 179.

14. Costa, E., Pscheidt, G. R., PROC. SOC. EXP. BIOL. & MED., 1961, v106, 693.

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