of retrolental fibroplasia.

In this connection, it is of interest that two water-miscible preparations of vit. A (the alcohol and acetate) have proven, in our laboratory, to be less stable than solutions of the same two isomers in cottonseed oil. Both the oil and water-miscible preparations were secured from the same laboratory at the same time and stored together, unopened, at 0°C. After 18 months, vitamin A bioassays demonstrated that the vit. A potency of the oil solutions was unimpaired, whereas the water solutions had lost so much vit. A activity that even when fed at a level of 3.0 units per day (estimated from original potency), the depleted animals with few exceptions either died or lost weight during the 4-week assay period. These data suggest that vitamin A is protected from oxidation to a lesser degree in an aqueous medium than in a cottonseed oil medium, and for this reason may render a correspondingly larger amount of vit. E in the digestive tract unavailable for metabolic processes in the body.

These observations are presented with the thought that studies to prevent the occurrence of retrolental fibroplasia in premature infants might be facilitated by further experimentation with vitamin E-deficient rats to develop a standardized technic for the production of a similar abnormality in young rats.

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Effect of Dilantin and Mesantoin on the Giant Axon of the Squid.* (18469)

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The effectiveness of dilantin (sodium diphenyl hydantoinate) and mesantoin (sodium phenyl ethyl methyl hydantoinate) in raising cortical thresholds to electric convulsive shocks(1) and their successful applications in the therapy of convulsive disorders are yet unexplained in terms of mechanism. The present experiments employing the isolated giant axon of squid are an attempt to study the actions of these compounds under simplified conditions and to approach an understanding of their mode of action.

Procedure. The giant axon of squid *(Loligo pealii)* was dissected in connection with its ganglion. The nerve was kept for ½ hour in a solution of artificial sea water to attain ionic equilibrium(2), then it was exposed to various solutions and the electrical signs were examined periodically. In other experiments the ganglion was removed and the nerve was threaded through a small bore polystyrene tube into which the electrodes had been sealed as described previously(3). The nerve was then perfused continuously and its electrical activity was recorded on a dual beam cathode ray oscilloscope. Stimulation was delivered by a dual square wave monophasic stimulator. The results of the two procedures were similar. A reduction in the content of both calcium and magnesium of the sea water produced spontaneous firing of the giant axon for long periods without showing electrical signs of injury. Omitting either calcium or magnesium did not produce an adequate condition, since the fibers lost their excitability after a brief period of hyperirritability. The concentration of dilantin or mesantoin in the

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artificial sea water was about 0.6 mg per ml. The insolubility of these compounds makes it difficult to estimate accurately how much remained in solution during the course of the entire experiment. The solutions were adjusted to the usual pH of sea water, *i.e.* pH 8.0-8.3. The penetration of dilantin into the interior of the axon was studied by exposing the fiber to dilantin labelled with N^{15}.† After one hour of exposure the axoplasm of the nerve was extruded and the N^{15} determined in a manner similar to that described previously(4).‡

Results and discussion. a. *Effect on the fiber.* Exposure of the giant axon for ½ to 2 hours to solutions of dilantin and mesantoin produced no appreciable effect on its electrical activity. Although a moderate increase in threshold was noted, it did not differ significantly from the controls. However, in another way an effect of dilantin and mesantoin has been obtained. Reducing the concentrations of calcium in the artificial sea water from .012 M to .006 M and of magnesium from .024 M to .012 M results after 10-15 minutes of exposure in spontaneous firing which persists during the period of perfusion with the sea water deficient in ions. This increased irritability is reversed within 10 to 15 minutes by re-exposing the axon to sea water containing calcium and magnesium in normal concentrations. A similar decrease in irritability has been observed within 2 to 3 minutes by the addition of dilantin or mesantoin to the sea water deficient in ions. After the spontaneous activity ceases, further soaking in the solutions containing dilantin or mesantoin and a reduced concentration of calcium and magnesium causes a reduction in the size of the action potential induced by stimulation and in 5 to 10 minutes its disappearance. However, excitability may be restored by washing the axon in sea water of normal composition. The entire cycle can be reproduced several times with the same axon. The action potential decreases and finally disappears in a way which has no special features.

The results are of interest in view of the application of dilantin and mesantoin to convulsive disorders. Apparently they do not demonstrably affect the normal fiber. Nevertheless, when the physiological equilibrium has been altered and a state of hyperirritability produced, as in the experiments described, these compounds are effective.

b. *Penetration into the axon.* By labelling dilantin with N^{15} the penetration of the compound into the interior of the axon may be determined. The presence or absence of dilantin within the fiber is an important consideration for the problem of its site of action. The nerves were exposed to 0.5 mg dilantin per ml for one hour. The atom per cent excess N^{15} of the dilantin was 28.5%. In the 80 mg of axoplasm collected the atom per cent excess was 1.2%. On the basis of these data the internal axonal concentration of dilantin was 85 per cent of that in the external medium. Since the concentration of dilantin of the outside solution does not remain constant because of a tendency to precipitate during the course of the experiment, the value of 85% is an approximation which may be regarded as conservatively low. It is more likely that equilibrium between the interior of the axon and the outside medium actually was attained during the period of exposure. The rate of penetration of dilantin is relatively rapid. Its entry, for example, is many times faster than that of glycine, alanine and aspartic acid (5). The observation classifies dilantin among those other compounds which have been shown to enter the interior of the nerve fiber (6). Thus far it would appear that only compounds which penetrate affect the conductive process, although mechanisms of their action may be different.

Summary. An action of dilantin and mesantoin on the nerve fiber is described. The

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addition of these compounds to a perfusion of artificial sea water does not affect the electrical activity of isolated giant axons. In the presence of reduced calcium and magnesium spontaneous firing of the fiber occurs. This may be reversed by returning the nerve to sea water with normal concentrations of calcium and magnesium. A decrease in hyperirritability may also be obtained by adding dilantin or mesantoin to water deficient in calcium and magnesium. Continued exposure to the latter solution reversibly abolishes signs of electrical activity. Dilantin labelled with N^{15} was found to penetrate into the interior of the giant axon of squid. The concentration in the axoplasm of the fiber was close to equilibrium within one hour.

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Effect of Histamine and Antihistaminics on Coagulation of Normal and Heparinized Rabbit Plasma. (18470)

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Haley and Harris(1) recently reported that several antihistaminic compounds significantly decreased the coagulation time in both normal and roentgen ray irradiated guinea pigs. This indicated that the antihistaminic compounds were able to inactivate the circulating anticoagulant (heparin?) found in the blood of animals subjected to whole body ionizing radiation(2). However, when one considers the chemical structure of the antihistaminics in relation to other compounds shown to be heparin inactivators both chemically and in a coagulation system(3,4), it becomes difficult to explain the antiheparin activity of the antihistaminics. The structure of these drugs, with the exception of the phenothiazine derivatives, bears no resemblance to the nuclear structure (phenazine, thiazine or oxazine) previously shown to be essential for heparin inactivation(3). Furthermore, the essential primary amine group is absent and the antihistaminics have a tertiary or cyclized amine group in its place. It was these considerations which prompted our *in vitro* investigation of the effect of both histamine and the antihistaminics upon the coagulation of normal and heparinized rabbit plasma.

Experimental. Blood was obtained from 5 rabbits by cardiac puncture every 2 weeks. The pooled blood was mixed with 3.8% citrate at a ratio of 1:4, then centrifuged and the supernatant plasma removed and stored at 4°C until used. All the plasma was filtered through glass wool before use and no plasma was used which was more than 4 days old. The method and chemicals used for studying the effects of histamine and the antihistamines on coagulation were the same as previously described for the dyes(4). Histamine and the antihistamines were dissolved in physiological saline to give final concentrations of 10, 25, 50, 100, 250, 500, 750, and 1000 μg per 0.1 cc. Six separate determinations were made with each concentration of drug, with and without heparin 0.1 μg (0.12 Toronto Units) in the system. The following antihistaminic drugs were investigated: diphenhydramine, tripelennamine, phenazoline, pyranisamine, thonzylamine, phenindamine, Tagathen, metaphenilene,

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