animals after a period of starvation has induced a central necrosis of the liver lobules which has varied in extent. The initial concentration and percentage retention of phenoltetrachlorphthalein has been extremely variable, apparently depending upon the extent of the liver injury.

The 8 dogs which were demonstrated to have acquired a morphologically altered type of liver epithelium and later intoxicated by alcohol, and the 7 animals with a similar type of liver cell anesthetized with chloroform, showed both anatomically and functionally a resistance to these poisons. All of the animals which received alcohol as an intoxicant failed to show the characteristic injury commencing at the periphery of the lobules and extending toward the central vein. Five of the dogs anesthetized with chloroform failed to develop a central necrosis of the liver lobules. The 2 remaining animals showed an early injury to this part of the lobules.

The functional expression of this lack of injury to the morphologically altered liver epithelium by ethyl alcohol and chloroform has consisted in a lower initial concentration of phenoltetrachlorphthalein in the plasma and a more rapid disappearance of the dye from the plasma than was the case with the control animals in which the liver epithelium was susceptible to injury from ethyl alcohol and chloroform.

The number of senile dogs with a naturally acquired, morphologically altered type of liver epithelium is insufficient to permit final deductions concerning the resistance of such epithelium to intoxication by ethyl alcohol and chloroform.

6439

Acquired Resistance of Altered Type of Liver Epithelium to Uranium Nitrate and to Chloroform.*

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During the past nine years, studies in this laboratory were concerned with the type of liver injury induced in dogs by uranium nitrate and other chemical substances, with the mechanism of repair to the liver lobules and the influence of secondary intoxications by

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these substances and by chloroform on the regenerated liver cells. A total of 161 dogs of ages varying from 4 months to 14 years have been used. In 73 dogs phenoltetrachlorphthalein has been employed according to the technique of Rosenthal[1] as an index of liver function. The dye was used in normal dogs before the intoxications, during the initial acute liver injury and at the end of a 4-week period, allowed for liver repair and during the secondary intoxication by uranium or when chloroform was used as the hepatoxic agent following a liver repair from uranium. Before such periods of functional study the animals were lightly anesthetized by ether, an incision made below the right costal margin, and liver tissue removed from one or more areas of the organ by means of a nasal cutting forceps or punch. Such tissue was fixed in corrosive-acetic, and in 10% formaline, and sections stained with Scharlach R. for lipoid material.

The uranium intoxications with the resulting liver injury have been induced by the subcutaneous injection of 2 to 4 mg. of uranium nitrate per kilo. The liver injury from uranium is diffuse, involving the cells of the liver lobules as a whole. In a number of animals this injury was not as marked immediately around the central vein of the lobules as it was in their central and peripheral portions. This variation from the diffuse character of the lobular injury may have some influence on the type of epithelial regeneration in the lobules. The injured cells have shown cloudy swelling, edema, vacuolation and a variable amount of necrosis. The severity of injury is associated with the dosage of uranium and the age of the animal. The injured liver cells, regardless of the extent of the necrosis, have shown a marked accumulation of stainable lipoid material, usually in the form of large droplets. The functional expression of this diffuse liver injury from uranium consisted in an increase in concentration of phenoltetrachlorphthalein in the plasma as compared with the same animal before the use of uranium. There is also a retention of the dye in the plasma for a longer period than with normal animals.

At the end of 4 weeks, allowed for liver repair, the animals which recovered from the initial intoxication by uranium were again studied functionally by phenoltetrachlorphthalein, and were then subjected to a second uranium intoxication. On the basis of the data obtained the animals could be divided into 2 general groups. The first showed a normal percentage retention of dye in the plas-

[1] Rosenthal, S. M., *J. Pharm. and Exp. Therap.*, 1922, **19**, 384.

ma followed by its rapid disappearance. Liver tissue from such animals showed repair which in general restored the liver to its normal histological structure.

In the second group the percentage retention of the dye in the plasma was higher, and the dye was retained for a longer time (23 minutes to over 1 hour), repair in the liver had not resulted in the formation of a normal type of liver cell. The type of cell developing as a process of repair in this second group of animals was of a very flattened type, with large deeply staining nuclei. Such cells were found in narrow cord-like structures which radiated in an irregular course from the central vein to the periphery of the lobule. Not infrequently such liver cords failed to show cell differentiation but existed in the form of syncytium. A similar type of epithelial regeneration has been previously described[2, 3] for the kidney following a uranium nephritis.

When dogs in the first group recovered from the initial uranium intoxication, with the regeneration of liver cells of a normal type and with a normal functional response as shown by phenoltetrachlorphthalein, are then given a second injection of uranium nitrate, there is no evidence of such animals developing any resistance for this poison. The liver cells rapidly degenerate and, associated with this change and dependent upon its extent, there occurs a high percentage concentration of phenoltetrachlorphthalein in the plasma and a delayed disappearance of the dye from the plasma. In the second group of dogs, which failed at the end of 4 weeks to show a normal functional response when the dye was used as a liver function test, and which showed the repair of the liver injury from uranium to have consisted in the formation of atypical, flattened cords of liver cells, have also shown a marked resistance to a second intoxication by uranium. This resistance of the liver cell of altered morphology not only occurs when the second injection of uranium is similar in amount to the first injected, but when the amount injected is increased twofold. Such regenerated cells fail to become edematous and vacuolated, the nuclei stain in a normal manner and only a small amount of stainable lipoid material in the form of granules and small droplets can be microchemically demonstrated within the cells.

Chloroform has been given to animals in the 2 groups after 2 days of starvation according to the method of Whipple and Sperry.[4] A

[2] MacNider, Wm. deB., *J. Exp. Med.*, 1929, **49**, 411.

[3] MacNider, Wm. deB., *Science*, 1931, **73**, 103.

[4] Whipple, G. H., and Sperry, J. A., *Johns Hopkins Hosp. Bull.*, 1909, **20**, 278.

similar though less marked resistance has been shown by the altered type of liver cell to an anesthesia from chloroform lasting for 2 hours.

In such animals that have developed a cytological resistance to uranium and chloroform, the percentage concentration of phenoltetrachlorphthalein in the plasma is not as high as it is in those animals with the formation of a normal type of liver cell which has less resistance to a secondary intoxication by uranium or to the use of chloroform for its hepatoxic effect. The removal of the dye from the plasma is more readily effected by those animals with a resistant type of regenerated epithelium than it is by the animals with a normal type of nonresistant epithelium.

6440

Synthetic Diets for Herbivora.

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Synthetic diets for herbivora present two special problems, first, because these species are accustomed to diets containing large amounts of cellulose, and second, they are accustomed to high levels of inorganic elements that tend to keep the urine alkaline. The chief problem in selecting a suitable source of pure cellulose is one of physical composition. We attempted to maintain goats upon synthetic diets in which the cellulose portion was furnished by paper, but never succeeded in maintaining the animals upon such a ration. They usually refused the feed entirely after a period of 2 weeks. On the other hand, we have found both goats and rabbits eat regenerated cellulose as readily as the rats, discussed in our earlier report.[1] In devising suitable inorganic mixtures for herbivora we have adopted the plan of imitating the ash constituents of a diet of natural feedstuffs known to be satisfactory for goats and sheep. Such a mixture, however, is rich in the element, silicon. Although it has long been recognized that skin, hair and wool are relatively rich in silicon, we know little of the nutritional requirements for this element. Furthermore, we know little about the form in which it occurs in plants and almost nothing concerning its metabolism in the animal body.

[1] McCay, C. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 209.