ation injury may have disrupted the blood-CSF barrier in some unknown fashion. There are no data which bear out this point. It is conceivable that leukopenia influenced the growth characteristics of bacteria in some unknown fashion to prevent utilization of glucose. This idea gains support in the observation that, *in vitro,* rapidly-growing pneumococci consume more glucose than organisms growing at a slow rate(2). Since only the absolute numbers of bacteria in the CSF were measured in our studies no information is available concerning their rate of multiplication.

These experiments do not provide the answer to the problem of hypoglycorrhachia in tuberculous meningitis which is usually characterized by relatively few cells and bacteria or in the occasional patient with pneumococcal meningitis with a large number of bacteria in the absence of significant pleocytosis. Nor do they shed light on the mechanism of transfer of glucose from the blood to the CSF. They do emphasize, however, that the mechanism governing the decrease in CSF glucose in bacterial meningitis is a complex one, and provide support for the argument that both cells and bacteria must be present for hypoglycorrhachia to occur.

Summary. Pneumococcal meningitis was produced in normal and irradiated dogs. The latter became leukopenic and the pleocytosis occurring in the CSF in response to the infection was suppressed. The number of bacteria in the CSF was the same in both groups, but CSF sugars remained consistently normal in the irradiated animals while hypoglycorrhachia occurred in 50% of controls. These findings suggest that both cells and bacteria are necessary to lower the CSF sugar in bacterial meningitis.

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Liver Regeneration in Experimental Carbon Tetrachloride Intoxication.* (25357)

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Hepatic regeneration which follows carbon tetrachloride induced centrilobular necrosis has been assessed previously by histological (1), cytological(2-3), and chemical(4) means. These methods often yielded conflicting results and provided only limited insight into the process of liver cell proliferation. Availability of tritiated thymidine for detection of cells that are actively synthesizing DNA (5-6) made it possible to investigate carbon tetrachloride induced liver regeneration in more detail. Our purpose was to determine the time at which maximum regeneration occurs following a single dose of carbon tetrachloride; to localize the anatomical site and time sequence of parenchymal cell proliferation; and to attempt demonstration of a humoral factor responsible for hepatic regeneration after toxic liver injury.

Material and methods. Forty-five male Sprague-Dawley rats weighing 190-340 g were housed in individual cages and fed a standard diet of Purina Lab. Chow and water *ad lib.* Thirty-six rats were given 0.2 ml of CCl_4/100 g weight intragastrically *via* a polyethylene catheter; saline was given in the same manner

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to 9 controls. At intervals of 6, 12, 24, 36, 40, 48, 72, 96, and 120 hours after administration of carbon tetrachloride, 4 rats were injected intraperitoneally with 1 microcurie/g weight of tritiated thymidine‡ (specific activity 0.360 curie/millimol). Control rats were given labeled thymidine 8 hours after administration of saline. Experiments were scheduled so that tritiated thymidine was always given between 7 and 8 a.m. Four hours after administration of tritiated thymidine the animals were killed by decapitation and the liver removed, sliced and fixed in cold 10% neutral buffered formalin. After fixation tissue slices were washed in running tap water for 24 hours and then dehydrated, cleared and imbedded in paraffin. Sections of 7 to 8 μ thickness were prepared and stained with hematoxylin and eosin. Fine grain stripping film was applied over similar sections and exposed in a light tight box at 10°C for 30 days. The autoradiographs were developed in Kodak D-19 Developer for 7 minutes, fixed in Kodak D-19 Developer for 10 minutes, washed in cool running tap water for 1 hour and mounted in polyvinyl pyrrolidone. Estimation of mitotic activity and number of hepatic cell nuclei bearing the label was carried out as follows: for each liver, total number of hepatic cells present in an average of 10 (400 X) fields was determined in sections stained with hematoxylin and eosin. Fifty consecutive (400 X) fields were then searched for mitotic figures and results expressed/100,000 hepatic cells. In autoradiographs the number of labeled liver cell nuclei present in 100 consecutive (400 X) fields was counted and results expressed/100,000 hepatic nuclei. In 16 rats given orally 0.2 ml/100 g of carbon tetrachloride 42 hours earlier, whole blood was obtained at laparotomy under light ether anesthesia by cannulating the renal vein. Blood was heparinized and pooled and the plasma separated by centrifugation in the cold. Five ml of this pooled plasma was injected into a saphenous vein of each of 5 normal male rats. Five ml of pooled plasma obtained from 16 untreated animals was injected into each of 7 control recipients. Sixteen hours later recipients of plasma were given 1 microcurie/g of tritiated thymidine by intraperitoneal route and 4 hours later the animals were sacrificed. Histological sections and autoradiographs were prepared as described above.

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Results. Histological changes in liver, consisting of a slight increase in intracellular fat and infiltration with inflammatory cells of the centrilobular area was detected 6 hours after carbon tetrachloride administration. At 12 hours these changes were more pronounced and at 24 hours marked centrilobular necrosis and inflammation involving ¼ to ½ of each lobule was present. Pyknotic nuclei, karyorexis and polymorphonuclear and monocytic cellular infiltration was noted. Intact liver cells were slightly larger than normal. At 6, 12 and 24 hours the number of hepatic mitoses was comparable to controls. Increased mitotic activity was first noted 36 hours after carbon tetrachloride and the mitotic index reached a maximum at 40 hours. Between 48 and 96 hours the degree of both necrotic and regenerative changes decreased and at 120 hours there was complete restoration of the parenchymal pattern without evidence of active necrosis or increased mitosis, mononuclear inflammatory cells being the only indication of prior liver injury.

The number of labeled hepatic nuclei in autoradiographs was similar in saline treated controls (Fig. 1) and in animals given tritiated thymidine 6 and 12 hours after receiving carbon tetrachloride. An increase in number of labeled hepatic nuclei was first noted at 24 hours and a maximum in labeling was reached at 40 hours (Fig. 2). The labeling of parenchymal cell nuclei was not limited to the centrilobular or periportal area but was diffuse and also involved the acinar region(7) and intermediate areas. In necrotic areas labeling was chiefly confined to nuclei of inflammatory and supporting tissue cells. At 48 and 72 hours the number of labeled nuclei was still markedly increased but a significant reduction in number occurred at 96 hours. At 120 hours labeling returned to control values. Comparative counts of mitoses and labeled nuclei showed a good correlation (Fig. 3) at each time interval after administration of carbon tetrachloride except at 24 hours when the

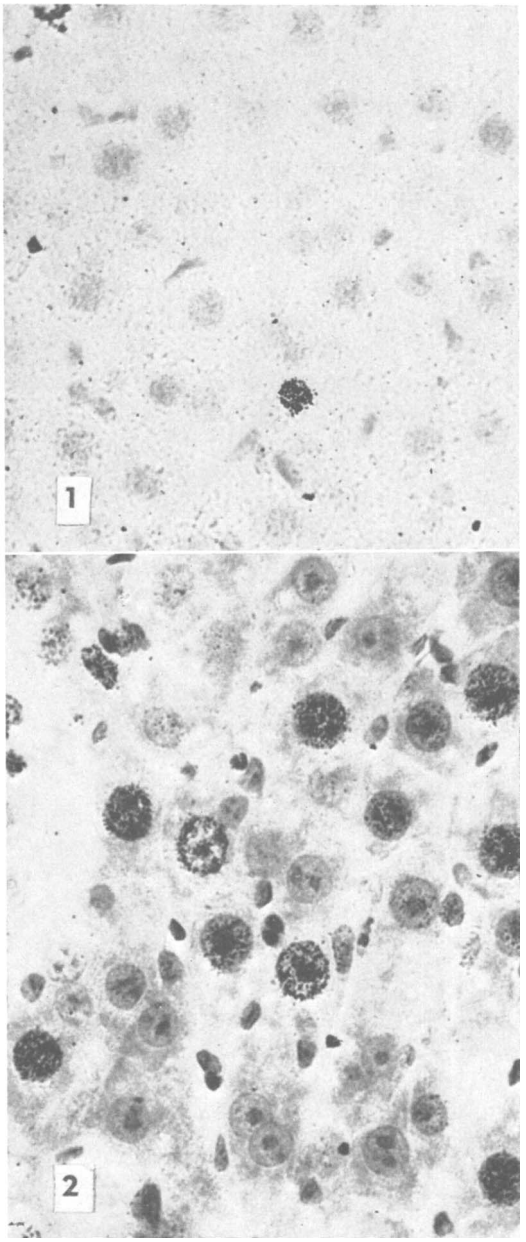

FIG. 1. Control rat liver, autoradiograph × 300. A single hepatic nucleus is labeled.

FIG. 2. Autoradiograph of liver 40 hr after a single oral dose of CCl_4 (400 ×).

average number of labeled nuclei increased to 282/100,000 cells while the mitotic index remained at control levels.

Pooled plasma from carbon tetrachloride treated rats had an albumin concentration of 2.65 g% and total protein 6.3 g% by chemical analyses(8,9); total serum bilirubin was 0.52 mg% by Malloy-Evelyn technic(10). Plasma from untreated rats had an albumin concentration of 2.15 g%, total protein 6.06 g%, and total serum bilirubin 0.12 mg%. There was no significant difference in number of mitoses and of tritium labeled hepatic nuclei between animals that had received plasma from rats treated with carbon tetrachloride and those given normal plasma. The carbon tetrachloride group had a mean average of 3.2 (S.D. ± 1.3) mitoses, and 44.6 (S.D. ± 21.8) labeled nuclei/100,000 liver cells; the controls had a mean average of 2.4 (S.D. ± 1.09) mitoses, and 28.4 (S.D. ± 12.2) labeled nuclei/100,000 liver cells. Neither group exhibited histological abnormalities in the liver.

Discussion. Carbon tetrachloride produces an increase in permeability of liver mitochondria(11), loss of diphosphopyridine nucleotides(12), and uncoupling of oxidative phosphorylation(13). This is associated with loss of hepatic glycogen(14) and co-enzyme A(15), and increase in neutral fat(14). A fall in level of nucleic acids during the period of necrosis(4) has not been confirmed by others(16). Our study shows that in early phases after carbon tetrachloride administration the number of labeled nuclei and mitoses is not less than that found in saline treated controls.

The importance of simultaneous histological, spectrophotometric, cytochemical, and chemical studies in evaluating the dynamics of

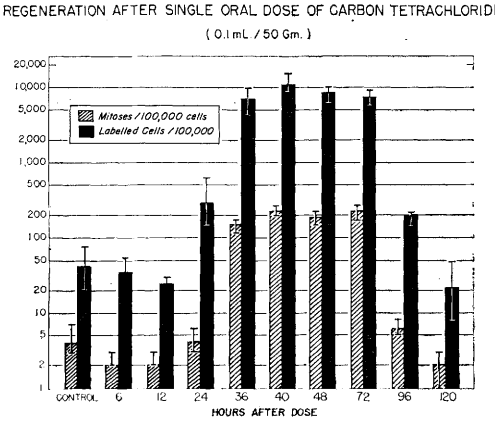

FIG. 3. Hepatic mitoses and labeled nuclei after single oral dose of 0.2 ml of CCl_4/100 g rat wt.

the regenerative process has been emphasized. This is illustrated by dissociation between mitotic counts and number of labeled nuclei at 24 hours after administration of carbon tetrachloride which probably reflects the increase in DNA synthesis occurring prior to actual cell division. Between 36 and 72 hours a concomitant increase in number of labeled hepatic nuclei and mitotic activity was noted (Fig. 3).

The magnitude and diffuse nature of the regenerative process in liver following carbon tetrachloride administration suggests that it may be due to other than local necrotic and reparative factors. Studies with partial hepatectomy in rats have demonstrated presence in plasma of a humoral principle for liver regeneration(17-21). In our study, plasma obtained from carbon tetrachloride poisoned rats at time of maximal liver regeneration failed to induce an increase in number of labeled cells or mitosis in livers of recipient animals. However, these preliminary results do not necessarily disprove the presence of a plasma factor for liver regeneration; changes in technic, use of plasma concentrates or variations in time between administration of CCl_4 and procurement of plasma or between injections of plasma and of tritiated thymidine might reveal the presence of a humoral principle which governs the repair phase following acute liver injury.

Summary. An autoradiographic technic employing tritiated thymidine was used to study liver regeneration in rats treated with a single oral dose of carbon tetrachloride. Results demonstrate that following toxic liver injury synthesis of desoxyribonucleic acid, indicating new cell formation, occurs chiefly in nonnecrotic areas of the liver; the regenerative process is diffuse and involves the acinar, intermediate, centrilobular and periportal regions. The highest rate of regeneration is reached 36 to 72 hours following administration of hepatotoxin, when the number of labeled liver cell nuclei is increased more than 250 fold over control values; after 120 hours, the regenerative process is virtually completed and the histological and autoradiographic pattern returns to normal. Under conditions of this experiment, the presence of a plasma factor for liver regeneration could not be demonstrated.

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