Diethylstilbestrol-Induced Intrahepatic Bile Duct Hyperplasia in the Rabbit.* (26158)

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While investigating the effect of carbon tetrachloride-induced liver insufficiency and iatrogenic hyperestrogenism upon the renal glomerulus, previously unreported changes in the intrahepatic bile ducts were produced. This report deals with severe hyperplasia of the intrahepatic bile ducts of rabbits receiving diethylstilbestrol. Similar changes have not been found in over 600 rabbits from the same source previously examined histologically in this laboratory. In addition, neither spontaneous hyperplasia nor development of cholangioma in the rabbit has been recorded (1). This study, therefore, indicates a previously unreported effect of diethylstilbestrol on the liver.

Materials and methods. Young adult, male, albino rabbits of a mixed strain, weighing initially 1700-4000 g, were used. All rabbits were fed a standard rabbit pellet from which the usual vitamin-antibiotic supplement was omitted.† The rabbits were divided into 4 groups. The first group of 10 rabbits each received 1 ml/kg initial body weight, of a 1:1 mixture of carbon tetrachloride and olive oil (CCl_4) twice weekly, subcutaneously. The rabbits were treated for 100-200 days until cirrhosis, as indicated by abnormal liver function tests, was obtained. Cirrhotic rabbits were then given both CCl_4 and one mg of diethylstilbestrol‡ in 0.2 ml of corn oil (DES) subcutaneously each day for an additional 50-100 days. The second group of 9 rabbits were each given CCl_4 and DES concurrently for 50-200 days. The third group of 10 rabbits received only DES for 50-200 days. The fourth group of 6 rabbits served as controls. All rabbits were subjected to complete postmortem examination.

Results. All rabbits of Groups 1, 2, and 3, which received DES for 50 days or more, regardless of other treatment, showed a distinctly abnormal bile duct proliferation. In most animals the proliferation was consistent with a severe reactive hyperplasia, while in one animal which received only DES the proliferation was suggestive of neoplasia. The animals in Group 1 were all severely cirrhotic at time of autopsy. Portal areas were enlarged by fibrous tissue and lobules were transected by thick fibrous bands connecting the portal areas. Within the portal areas and the fibrous bands there was a moderate to marked proliferation of small but well-circumscribed bile ducts which often almost completely filled the portal areas. Although polarity of cells was maintained the ducts appeared nonfunctional and the lumina were frequently nonexistent. Animals in Group 2, which received both CCl_4 and DES concurrently, developed only a mild cirrhosis but showed mild to moderate bile duct proliferation. Animals in Group 3 which received only DES were free of cirrhosis but showed the most severe degree of bile duct proliferation. In most animals there was a moderate to severe hyperplasia of the bile ducts which not only filled the portal areas but extended into the lobules and often completely across the lobule connecting adjacent portal areas. One animal from this group showed a severe proliferation of the bile ducts suggestive of neoplasia. In this rabbit which received DES for 200 days the liver had a "cauliflower-like appearance" with nodules varying from 1-4 mm in diameter. Microscopically the lesion consisted of massive proliferation of bile ducts with invasion of lobules, replacing approximately 80% of the liver cells. The proliferating cells were occasionally formed into recognizable biliary ducts with an intact

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† Red Rose Rabbit Pellets—O.S.U. Formula, purchased from John W. Eshelman and Sons., Circleville, O.

‡ Donated by Dr. William R. Kirtley, Eli Lilly and Co.

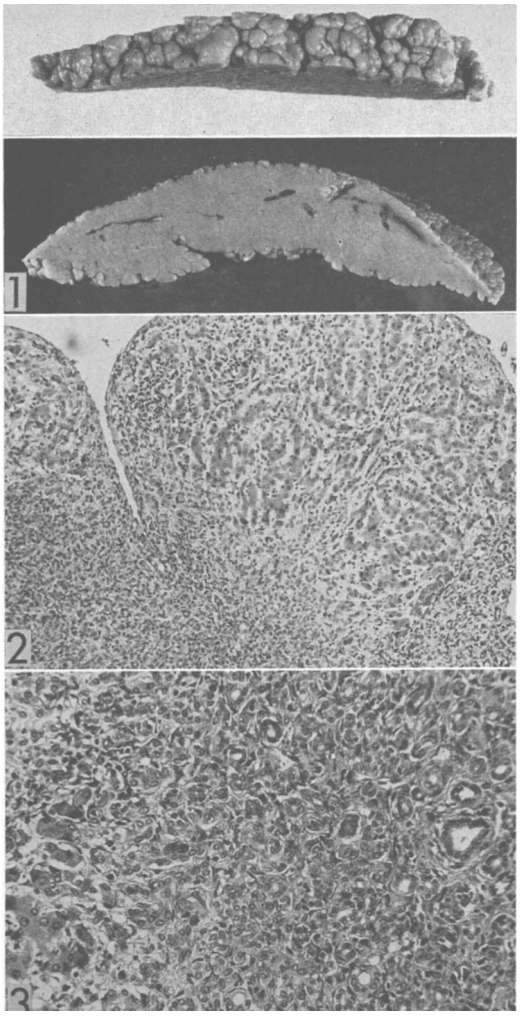

FIG. 1. Gross appearance of liver of rabbit from group 3.

FIG. 2. Microscopic appearance of liver of rabbit from group 3 which showed almost complete replacement of liver cells.

FIG. 3. Higher magnification of liver. A few remaining liver cells are present at left and a normal bile duct at right of photograph.

basement membrane, but more often consisted of irregular cords or sheets of cells in a connective tissue stroma. Polarity of cells was disrupted and in several areas blood vessel invasion was suspected but no distant mestatases were found. The control animals in Group 4 showed an occasional mild cholangitis but no evidence of cirrhosis or bile duct proliferation. All rabbits receiving CCl_4, Groups 1 and 2, lost weight while those rabbits receiving only DES, Group 3, and the control rabbits, Group 4, showed a normal growth curve. Subcutaneous abscesses developed at sites of injection of both CCl_4 and DES. Adrenal cortical hyperplasia was present in all rabbits receiving either CCl_4 or DES, Groups 1, 2, and 3. Testicular atrophy was present in all rabbits receiving DES, Groups 1, 2, and 3.

Discussion. Hyperestrogenemia is known to cause many growth abnormalities(2-10). However, the effect varies depending upon species of animal, estrogen level, dosage schedule, and type of estrogen administered. Hyperplasia, polyp formation and carcinoma of the endometrium, adenomyosis, uterine and extrauterine leiomyomas and fibromas, carcinoma of uterine cervix, adenoma and adenocarinoma of breast, hyperplasia of prostatic stroma, interstitial cell tumors of testis, chromophobe adenoma and adenoma of pars intermedia of the pituitary, lymphosarcoma of thymus, lymphomas, adrenal cortical neoplasms, and bone neoplasms have been reported after estrogen administration(2,8-10). Kirkman(11) reported that ethinylestradiol would produce hepatoma of the liver cells in hamsters. However, he was unable to produce similar changes with diethylstilbestrol and stilbestrol benzoate. Dow(4) found only fatty and hydropic degeneration of the livers of cats given estrogens for extended periods. No reports have been found of experimental estrogen-induced bile duct hyperplasia or neoplasia. The normal estrogens are inactivated and excreted in the bile(12). Synthetic estrogens such as diethylstilbestrol are more resistant to intrahepatic inactivation and are largely excreted intact, and reported, therefore, to be more tumorigenic(8). Some reports(7,13-16) state that inactivation of estrogens is decreased when the liver is partially excised or damaged by carbon tetrachloride while Cantarow *et al.*(17) disagree with this claim. Our data suggest that liver damage actually decreases the effect of estrogens on bile duct epithelium. Bile duct hyperplasia and an increased incidence of carcinoma of liver are often present in human patients with cirrhosis of the liver, most of whom also exhibit hyperestrogenemia. How-

ever, similar bile duct hyperplasia was not observed in cirrhotic rabbits receiving only CCl_4 in this laboratory. Estrogen has been reported to cause a decrease in growth, decrease in body weight of adult animals, increase in basal metabolic rate, hair loss, testicular atrophy, infertility, and hyperplasia of pituitary, thyroid and adrenal(3-7). In view of the well-established effect of estrogens on growth processes and excretion of estrogens in the bile, one might expect hyperestrogenemia to cause changes in bile duct epithelium. However, due to the body-wide endocrinopathy produced by estrogens one can only speculate as to whether the bile duct changes are due to a direct toxic effect of the estrogen, which seems most plausible, or to some other endocrine abnormality.

Summary. Administration of diethylstilbestrol to intact rabbits, or to rabbits made cirrhotic with carbon tetrachloride, produced in all animals a marked bile duct proliferation and in one animal changes suggestive of neoplasia of the bile ducts. The possibility that hyperestrogenism may be responsible for the increased incidence of carcinoma of the liver in cases of liver cirrhosis is suggested.

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An Improvement in Quantitative Separation of Phospholipids with Silicic Acid Impregnated Filter Paper. (26159)

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Recent developments in filter paper chromatography and the perfection of micro technics of analysis for specific substances have made it possible to quantitate compounds separated on paper. We have developed a simple method for quantitating minute amounts of the phospholipids, lecithin, lysolecithin, and sphingomyelin, following chromatographic separation on silicic acid impregnated filter paper, which uses the solvent systems and identification technics developed by Marinetti and coworkers(1,2). However we found that some of the lipid material from serum extracts, which was allowed to dry at point of application, became bound to the silicic acid at the origin and did not move with the chromatographic solvents employed. In addition to staining with Rhodamine G, it gave a positive test for choline. We avoided loss of this material, which was principally lecithin, by a technic we call "running start" chromatography. The technic is applicable to phospholipid analyses in tissue extracts as well as various biological fluids.