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Effect of Cortisone and Growth Hormone upon Ductular Cell Proliferation in Liver Ethionine Intoxication.* (24233)

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Recently interest was focused upon accumulation of interstitial cells between liver cell plates, as apparent in biliary obstruction and dietary disorders(1), and in intoxication with carcinogenic drugs(2). These interstitial cells have been considered to be epithelium of bile ductules(2). Injection of bile ducts demonstrated that interstitial cells are to a large extent bile ductular cells even though morphologically they resemble mesenchymal elements (3). The interstitial cell reaction has been considered a response of the liver to injury, and this raised the question whether the reaction can be suppressed by anti-inflammatory agents, such as cortisone. Since this reaction can be elicited with reproduceable regularity in ethionine intoxicated rats(4), the influence of simultaneous cortisone administration was studied in view of known effect of cortisone upon inflammatory reactions in the liver(5,6). For comparison the effects of administration of growth hormone upon ethionine intoxication was also studied because of its influence on liver cells(7) and its antiphlogistic effect possibly antagonistic to cortisone(8).

Methods. Female Sprague-Dawley rats weighing approximately 175 g were placed in individual cages for 25 to 35 days and kept on synthetic diet containing vitamin free case-

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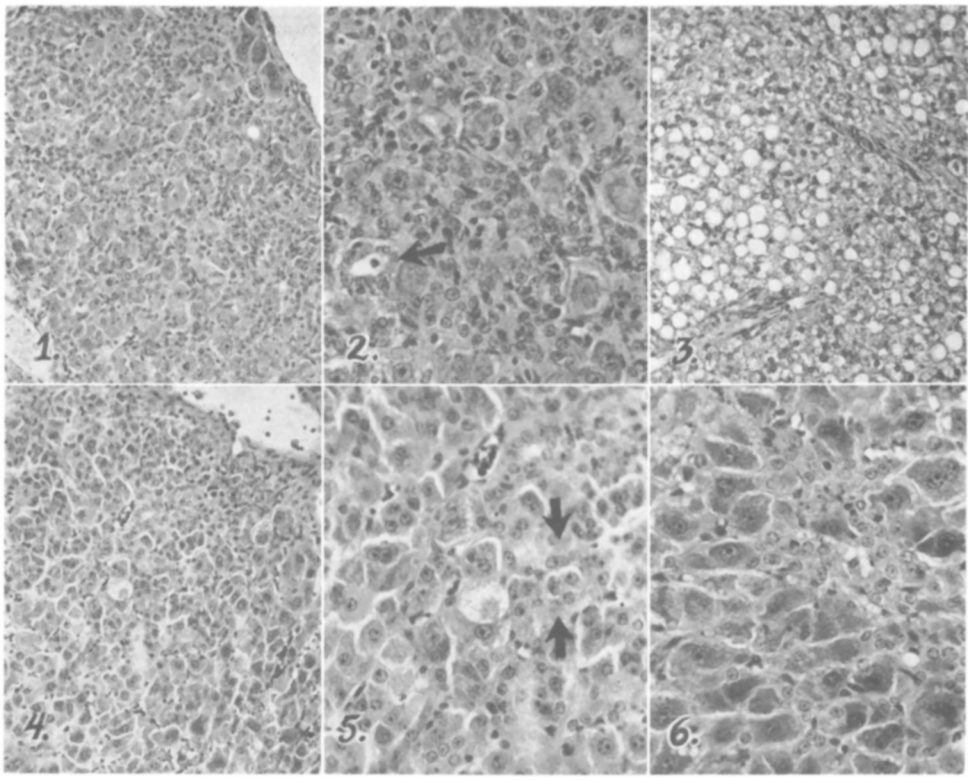

FIG. 1. Liver of rat on ethionine diet. Diffuse single cell degeneration and regeneration and interstitial cell reaction. H & E. 80×.

FIG. 2. Liver of rat on ethionine diet. Interstitial cells consist of ductular cells occasionally arranged around lumina (arrow) intermixed with inflammatory cells including segmented leucocytes. H & E. 160×.

FIG. 3. Liver of rat which had received cortisone. Severe diffuse fatty metamorphosis. H & E. 80×.

FIG. 4. Liver of rat on ethionine diet which had received cortisone. Liver damage is the same as in rats not receiving cortisone. However, the interstitial cell reaction is almost entirely absent. H & E. 80×.

FIG. 5. Liver of rat on ethionine diet which had received cortisone. In places (arrow) transitional stages between liver cells and ductular cells are noted. H & E. 160×.

FIG. 6. Liver of rat on ethionine diet which had received growth hormone. The interstitial cell reaction is almost as severe as in rats not receiving growth hormone and the liver cells show damage but no fatty metamorphosis which is seen in rats on growth hormone in the absence of ethionine administration. H & E. 160×.

in, 160 g; corn oil, 50 g; sucrose, 750 g; salt mixture U.S.P., for depletion diet, 40 g/kilo; and adequate vitamin supplements, except for riboflavin, 4 mg; and choline hydrochloride, 30 mg. One group of 10 rats, serving as control, were on the basal diet. A second group of 20 rats had a dietary supplement of 0.5% ethionine. A third group of 10 rats on basal diet received 5 mg of cortisone intramuscularly 3 times a week. A fourth group of 25 rats on ethionine supplemented diet had the same amount of cortisone as Group 3. A fifth group of 10 rats on basal diet received 0.5 mg of growth hormone‡ (Armour and Company's "SOMAR," lot no M208) intramuscularly 6 days/week. A sixth group of 20 animals were on ethionine diet while receiving same amount of growth hormone. At completion of the experiment, the animals were

‡ The authors are indebted to Dr. Irby Bunding of the Armour Laboratories, Chicago, for supplying the growth hormone. It was crystallized in a lyophilized form. Prior to use it was reconstituted with triple distilled water. Portions of rehydrated hormone not used immediately were frozen at -20°C, then thawed at room temperature prior to experimental use.

TABLE I. Effect of Cortisone and Growth Hormone on the Rat Liver in Ethionine Intoxication.

Procedure	No. of rats	Duration (days)	Parenchymal degeneration	Cell regeneration	Fatty metamorphosis	Interstitial cells		Collagen	Reticulum
						Ductular	Inflamm.		
Basal diet, 7 d/wk	10	30	0	0	2.0	0	0	0	0
.5% ethionine in basal diet, 7 d/wk	20	30	2.0	4.0	0	4.0	1.0	0	4.0
Cortisone, 5 mg IM 3× /wk + basal diet, 7 d/wk	10	30	0	0	1.0	0	0	0	
Cortisone, 5 mg IM 3× /wk + .5% ethionine in basal diet, 7 d/wk	25	30	1.0	2.0	.25	.25	Nil	0	1.0
Growth hormone, .5 mg IM 6 d/wk + basal diet, 7 d/wk	10	30	0	0	4.0	0	0	0	0
Growth hormone, .5 mg IM 6 d/wk + .5% ethionine in basal diet, 7 d/wk	20	30	1.0	2.0	.25	2.0	1.0	0	2.0

sacrificed and their livers were subjected to routine histologic analysis. The histologic study was done without knowledge of experimental history and individual morphologic findings were graded from 0 to 4+ to be subsequently averaged for the entire group.

Results. Rats on basal diet exhibited a varying but slight degree of fatty metamorphosis and showed occasionally focal necrosis associated with accumulation of mononuclear cells. Animals on ethionine in the diet showed severe single cell degeneration and regeneration characterized by focal ballooning and coagulation of cytoplasm on the one hand and presence of very large vesicular nuclei with prominent nucleoli on the other (Fig. 1 and 2). The liver cell plates were widely separated, throughout the lobule, by accumulation of cells with vesicular, round or oval nuclei and relatively little cytoplasm. In previous studies these cells were shown to be ductular cells(3) since occasionally they surrounded thin lumina and connected with bile canaliculi or portal bile ducts. The ductular cells were intermixed with unquestionable inflammatory cells including segmented leucocytes. The sinusoidal lumen appeared obstructed. Argentaffine fibers were conspicuously increased but collagenous fibers were virtually unaltered (Table I).

Rats receiving cortisone with the basal diet exhibited moderate fatty metamorphosis (Fig. 3) far in excess of that seen on the basal diet alone. In rats on ethionine and cortisone, fatty changes were minimal but liver cell alterations were of almost equal degree as in ethionine fed rats. The ductular cell reaction in contrast was almost entirely absent (Fig. 4) and the enlarged and damaged liver cells were closer to each other. The capillaries were clearly recognizable and the Kupffer cells were enlarged. The ductular cell reaction was noted only in portal spaces and about foci of eosinophilic necrosis. The cytoplasm of numerous cells in these areas appeared slightly basophilic and more abundant than in ductular cells, creating a resemblance to liver cells and suggesting a transition between ductular cells and hepatic epithelial cells. Mononuclear inflammatory cells and segmented leukocytes were entirely absent (Fig. 5). Silver impregnations exhibited considerably fewer argentaffine fibers than were seen in non-cortisone treated rats on ethionine diets.

Rats on basal diet receiving growth hormone exhibited a severe fatty metamorphosis particularly on the lobular periphery. In contrast, in rats receiving ethionine and growth hormone, fatty metamorphosis was not conspicuous and the picture was similar to that of rats receiving ethionine alone (Fig. 6) except that epithelial cell damage and ductular cell reaction were less intense. Distribution of the latter was less diffuse and the cells revealed a

tendency to arrange themselves around lumina. In principle, the alterations in this group resembled far more those produced by ethionine alone than by ethionine with cortisone.

Discussion. The repeatedly described alterations of liver produced by ethionine(4,2) appeared in this series particularly early and regularly, probably because use of single cages equalized food intake. The alterations are characterized by hepatocellular and interstitial cell changes. Cortisone distinctly suppresses both components of the interstitial cell reaction by practically eliminating the inflammatory as well as bile ductular cells. The anti-inflammatory effect of cortisone is well established(9). Suppression of the diffuse ductular cell reaction in the liver deserves emphasis and has to be compared with suppression of other epithelial cell reactions(10-13). In circumscribed areas such as portal tracts or foci of necrosis, cells are seen which suggest a transition betwen liver cells and ductular cells. In contrast to previously held belief, it appears now established(14) that in the embryo liver cells develop into ductular cell reaction(2). However, under cortisone the direction of the transition under cortisone also goes from liver cells into ductular cells. One could speculate that under the influence of cortisone such a transition is more conspicuous because of retardation of the process, and may represent a response to injury.

The previously demonstrated(3) relation between ductular cells and reticulum fiber proliferation is confirmed by the smaller number of reticulum fibers in cortisone-treated ethionine rats. The liver cell damage has been considered an effect of interstitial cell reaction(2). However, under cortisone treatment they are clearly dissociated. The simultaneous disappearance of ductular and inflammatory cell reaction suggests that they are produced by the same stimulus or that the ductular reaction may induce an inflammatory cell response.

The fatty metamorphosis present to a moderate degree in rats on basal diet or on cortisone, and to a very conspicuous degree in rats on basal diet supplemented by growth hormone, is absent in ethionine intoxicated rats even with the supplements above. This lipotropic effect may be the result of marked metabolic disturbance created by ethionine comparable to the lipotropic effect of reduced metabolic needs in starvation(15). The effect of growth hormone itself is not conspicuous. It does not exaggerate the interstitial cell reaction but, if anything, mitigates it to produce a low-grade cortisone effect, perhaps explained by its corticotropic effect or cortisone-growth hormone antagonism(16).

Summary. Administration of cortisone inhibits accumulation of interstitial cells between liver cell plates, otherwise produced by oral administration of ethionine to rats. The inflammatory component of interstitial cells consisting of mononuclear cells and segmented leucocytes is entirely abolished whereas the otherwise prevalent ductular cell reaction, also considered a response of liver to injury, is almost completely prevented. Where ductular cells form, transitions between these and liver cells are observed. Liver cell degeneration characteristic of ethionine intoxication is not influenced by cortisone. Administration of growth hormone has a greatly reduced cortisone effect. Ethionine intoxication prevents the fatty metamorphosis produced by administration of cortisone and growth hormone.

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Fractionation of Cell Types in Ehrlich Ascites Fluid by Sucrose Gradient Centrifugation.* (24234)

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For studies in our laboratories concerning the influence of X-irradiation on cell metabolism, it was necessary to obtain a supply of Ehrlich Mouse Ascites Tumor Cells free from erythrocytes, leucocytes and other cell types usually present in ascites fluid.

Erythrocytes may be removed by lysis with hypotonic saline which appears to leave ascites cells intact(1,2). Centrifugation of the mixed cells in Krebs-Ringer bicarbonate or isotonic saline affords a rough separation between erythrocytes and tumor cells whereby either layer may be scooped out with a glass spatula (3). The counter-streaming centrifugal procedure of Lindahl and Klein(4) yields considerably purified tumor cell preparations, but none in which contaminants are completely absent.

We have shown that centrifugation of the mixed cells through a sucrose gradient affords a separation into 4 distinct bands of different cell types. One of these appears to consist of pure ascites tumor cells which are viable.

Methods. Fractionation of cell types. A suitable sucrose gradient was prepared in a centrifuge tube (17 x 3 cm diameter). After first filling with 0.07 M phosphate buffer of pH 7.4, a layer of 50% w/v sucrose in phosphate buffer was introduced into the bottom 2 cm of the tube using a long Pasteur pipette with a small upturned tip. Successive 1 cm layers of sucrose solutions in phosphate buffer, each solution 5% w/v less in concentration than the previous one, were then introduced by upward displacement through the Pasteur pipette until a concentration gradient of 10-50% w/v of sucrose was obtained. After standing 15-30 minutes a glass rod was moved gently up and down through the solution to ensure an even gradient. Mouse ascites fluid (10 ml) was mixed with sodium citrate anticoagulant solution on withdrawal, then centrifuged. The cell mass (2-3 g wet wt) was twice washed in isotonic saline at 5°C and finally resuspended in 10 ml saline. A portion of the cell suspension (1.5-2.0 ml) was carefully layered on top of the sucrose gradient, and the tube centrifuged at 250-300 rpm for 30 minutes at 10-15°C.

Viability tests. Separate experiments were carried out to test the effect of strongly hypertonic (1.17 M) 40% sucrose solution on the viability of ascites tumor cells.

1. *Oxygen uptake of ascites cells treated with 40% sucrose solution in phosphate buffer.* A suspension of saline-washed mixed ascites cells was prepared in phosphate buffer containing 40% w/v sucrose. The suspension was divided into 2 portions, one of which was allowed to stand 40 minutes at 23°C,

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