and elevation of plasma potassium with a consequent imbalance of these ions. If this were the cause, it would be expected that abnormalities of the electrocardiogram would be reversed toward normal only by restoration or maintenance of adequate concentrations of sodium and potassium; such was found to be the case in our experiments.

It is currently taught that the difference in potential which exists across the cell membrane is dependent, in part, upon the difference in concentration of potassium between the intra- and extracellular compartments. Alterations in this concentration, as are seen in conditions of hyperkalemia, result in a variety of disturbances in neuro-muscular activity and, more particularly, myocardial function (9,11,12), which result in such clinically overt findings as described above.

In adrenal insufficiency it has been repeatedly observed(4,5,7), that sodium chloride administration in adequate amounts will lower plasma potassium concentration and restore the electrocardiographic abnormalities toward normal; from this it would appear that these disturbances are directly related to abnormalities in sodium and potassium metabolism and are reversible only if adequate concentrations of these ions are restored.

Since cortisone has been found to correct the renal functional defect on sodium excretion and to maintain normal plasma concentrations of sodium and potassium, it is conceivable that the disturbances in the electrocardiogram seen in adrenal insufficiency are effected by this steroid only in so far as it corrects the abnormalities in electrolyte metabolism.

That this is true under the conditions of these experiments is evident from the finding that cortisone is without effect when marked abnormalities in the plasma concentration of these ions has already occurred; but adequate maintenance with or administration of this steroid early in adrenal insufficiency, without added sodium, is effective in maintaining a normal electrocardiogram.

The author is grateful to Dr. Robert F. Pitts for his kind suggestions and advice concerning this work.

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Received November 20, 1951. P.S.E.B.M., 1952, v79.

## Hepatic Fibrosis Produced by Chronic Ethionine Feeding.* (19263)

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Ethionine ($\alpha$ - amino- $\gamma$ -ethylthiobutyric acid), an analogue of methionine, probably inhibits protein synthesis, since it has been shown to inhibit the incorporation of radioactive methionine into glycine(1). It produces within 24 to 48 hours a fatty infiltration of the liver in female but not in male rats(2,3).

* Supported by a grant from the Dr. Jerome D. Solomon Memorial Foundation.

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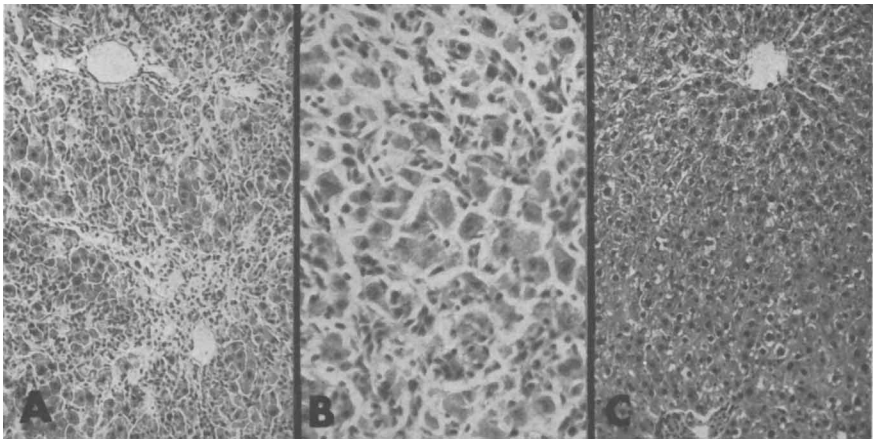


FIG. 1. Photomicrographs of livers of rats after prolonged feeding of ethionine. A. (165 ×). The lobular architecture is distorted and connective tissue septa start to connect central fields. The liver cells in the center of the lobule have disappeared and the liver cell plates are irregularly arranged. The interlobular connective tissue is increased. B. (330 ×). Small groups and individual liver cells revealing differences in size and staining qualities are surrounded by connective tissue membranes which contain fibroblasts, inflammatory cells and proliferated cholangioles. C. (165 ×). With methionine. The lobular architecture is intact. The liver cells show moderate variations in staining qualities of nuclei and cytoplasm.

This is prevented not only by glucose, but also by an amount of methionine more than equivalent to that of ethionine(4). The question arose as to what liver changes are produced by chronic ethionine administration.

*Material and methods.* Wistar strain white female rats were divided into 3 groups and one group of male animals was added. All animals were on a basal diet, containing 16% casein. All but one group received 0.5% ethionine in their synthetic diet. The daily intake and the weight of the animals were recorded. To exclude any changes possibly caused by the relatively low food intake, when ethionine is added, one female group was pair-fed without ethionine. One group received in addition to 0.5% ethionine, 1.0% methionine in the diet. All animals were killed after 28 days, the histological picture of the liver evaluated, and the total lipids of the liver determined gravimetrically after extraction with chloroform and re-extraction with petrolether.

*Results.* The livers of the pair-fed animals without ethionine showed slight fatty metamorphosis without fibrosis or other liver damage. The livers of the rats, males and females alike, which received ethionine showed a pale granular outer and cut surface and were of increased consistency. Under the microscope, the lobular architecture appeared somewhat distorted in at least part of the liver. In addition, the arrangement of the liver cell plates was irregular (Fig. 1A). The liver cells varied from very small, atrophic or degenerated to large, regenerating ones. Especially in the latter, the nuclei were rich in chromatin and often contained several large, strongly acidophilic nucleoli. The cytoplasm was usually dark, irregular stained and appeared homogenous in some necrobiotic cells. In the center of the lobule the liver cells had often disappeared. In the rest of the lobule single liver cells or small groups of them appeared separated from each other by connective tissue membranes of variable thickness. These revealed uniform fine reticulum fibers as well as many mesenchymal cells, apparently fibroblasts, and also proliferating cholangioles. Only rarely were the membranes condensed to thicker septa dissecting the lobular architecture. In these septa some of the remnants of sinusoids appeared as wide vessels. Brown, granular pigment was found in epithelial and mesenchymal cells, especially near the center. It was not fluorescent and failed to give iron reaction. Few fat droplets were found in isolated liver and Kupffer cells (Fig. 1B). The

TABLE I. Results of Acute and Chronic Ethionine Administration, With and Without Methionine Supplements, upon Histologic Changes and Total Liver Lipids in Rats.

| No. of rats | Sex | Ethionine | Duration, days | Methionine, % of diet | Wt loss in % | Liver: Degree of liver damage | Liver: Histologic fat | Total lipids, g/100 g wet wt |
|---|---|---|---|---|---|---|---|---|
| 7 | ♀ | — | 2§ | — | 5.6 ± .6 | 0 | 0 | 5.3 ± .2 |
| 2 | ♂ | — | 2§ | — | | | | |
| 12 | ♀ | +† | 2§ | — | 8.8 ± 1.1 | 0 | +++ | 19.4 ± 1.7 |
| 6 | ♂ | +† | 2§ | — | 6 ± .8 | 0 | 0 | 6.2 ± .2 |
| 11 | ♀ | +‡ | 28 | — | 24.5 ± 4.8 | +++ | 0 | 3.1 ± .7 |
| 7* | ♀ | — | 28 | — | 10.4 ± 1.6 | 0 | + | 7.8 ± 1.8 |
| 8 | ♀ | +‡ | 28 | 1 | 9.6 ± 1.9 | + | 0 | 6 ± .8 |
| 7 | ♂ | +‡ | 28 | — | 17.8 ± 3.3 | +++ | 0 | 3.3 ± .6 |

* Pair-fed to previous group. † 100 mg/100 g body wt intraper. ‡ .5% of diet.
§ Fasting.

pancreas in these animals showed atrophy of the acini and fibrosis of the interstitial tissue with infiltration by lymphocytes and histiocytes.

The livers of the rats which had received supplementary methionine were grossly normal. Microscopically, the arrangement of the liver cell plates was slightly irregular. The liver cells in the central zone were vacuolated and, in the peripheral zone, they were larger and darker, probably due to regeneration. Around the widened portal triads some proliferation of the cholangioles, fibrosis and pigment deposition was noticed. Without specific location in the lobule, isolated cells or groups of cells revealed many small fat droplets (Fig. 1C).

After chronic ethionine administration, less than the normal amount of liver lipids was extracted chemically and at the same time, the normal body fat depots had disappeared in both sexes while in the pair-fed controls and methionine-supplemented rats the normal amount of lipids in the liver and the fat depots was preserved. For comparison results obtained 48 hours after the intraperitoneal administration of ethionine(2) were included (Table I).

*Comments.* The extensive fibrosis which is observed after chronic administration of ethionine is of predominantly intralobular location, distorting only slightly the lobular pattern. It represents an unusual type of cirrhosis. The proliferation of the cholangioles reveals some similarity with the lesion produced by butter yellow. So far, however, the marked distortion of the lobular pattern, massive necrosis and excessive regeneration which precede carcinoma formation in the latter, have not been observed. Whether the severe liver cell damage with the associated regeneration is the primary cause of the fibrosis is as yet unproven. It should be stressed that the chronic administration produces not only no increase in liver fat, but oddly enough, a decrease. This decrease parallels the depletion of the entire fat depots of the body and suggest a basic, so far not understood effect of ethionine administration upon the lipid metabolism. The analogy to the gradual transformation of the human fatty cirrhotic liver into a fat-free scarred organ deserves emphasis despite the rapidity of the experimental process. Sex difference in the response to ethionine found in the acute fatty liver(2) and in kidney changes(5) is not present in the chronic lesion here presented and in the pancreatic necrosis and inflammation recently reported and confirmed(6-8). In common with these acute lesions, the chronic hepatic fibrosis can be prevented by simultaneous administration of methionine. These findings suggest that, like the acute damage produced by ethionine, the hepatic fibrosis is an expression of a chronic interference with the utilization of methionine. Nevertheless, to date, a specific toxic effect of ethionine, ameliorated by methionine, cannot be entirely excluded.

*Summary.* Oral administration of ethionine produces severe liver cell damage and an intralobular type of hepatic fibrosis with reduction of the fat depots of the entire body and of the

normally present fat in the liver. The lesion is prevented by simultaneous administration of methionine.

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Received November 20, 1951. P.S.E.B.M., 1952, v79.

## Potentiation by Adrenaline of Protective Effect of Cortisone* on Histamine Toxicity in Adrenalectomized Mice. (19264)

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It is well known that mice are relatively insensitive to histamine. According to Halpern and Wood(1) the lethal dose of histamine dihydrochloride, injected intraperitoneally, is for normal mice 50 mg per 20 g‡ of body weight. Adrenalectomy renders the mice considerably more sensitive to histamine so that it requires only 0.5 mg per 20 g of histamine to kill the adrenalectomized mice (2). It has been shown by the same authors that pretreatment with Promethazine increases the resistance of the adrenalectomized mice to histamine and brings it up to that of the normal mice. In the same conditions adrenaline increases also the resistance of the adrenalectomized mice to histamine, but in this case the toxicity of histamine is not reduced to normal; the lethal dose of histamine is brought up from 0.5 mg to 5 mg per 20 g. Adrenaline therefore does not seem to be the only factor responsible for the high resistance of normal mice to histamine.

It is the purpose of this study to investigate whether cortisone increases the resistance of adrenalectomized mice to histamine. It has been reported by various authors that cortisone can protect this species of animals against anaphylaxis(3,4). It should be noted, however, that cortisone does not protect other species of animals either against histamine or anaphylactic shock(5-7).

*Experimental. Technic.* 112 adult mice (female) weighing about 20 ± 5 g were used. Bilateral adrenalectomy was performed under light ether anesthesia and the animals allowed access to normal diet with plenty of fluid. Right after the operation, the animals received subcutaneously 250 γ of desoxycorticosterone, and 0.5 ml of isotonic saline. The animals treated with cortisone were injected with 0.5 mg of the drug twice a day for 3 doses, the last dose was given 4 to 5 hours before the injection of histamine. The doses of histamine dihydrochloride were administered intraperitoneally in 0.5 ml of normal saline per 20 g of body weight 48 hours after the operation. In some animals, hemoconcentration was measured by the change of the hemoglobin concentration of the blood. Blood samples were obtained by puncture of the ophthalmic venous plexus through the inner anode of the eye with a capillary glass pipette (8), before injection and again 20 minutes after injection of histamine. A group of adrenalectomized animals treated with cortisone was also treated with adrenaline. To these

* We wish to acknowledge the help of Merck who supplied us graciously through l'Institut National d'Hygiene with Cortisone used in this experiment.

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‡ It is considered that the average weight of an adult mouse is 20 g.