Histochemistry of Normal and Ethionine-Treated Rat Testis

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Summary. Testicles of mature rats were seen to be very sensitive to a low level of DL-ethionine (0.1% in the diet) for a period varying from seven to ten months, while the drug failed to interfere with the body growth. By means of histoenzymological techniques, the fate of some energetic and oxidative enzymes was tested both in control and treated animals: Alkaline phosphatase, which was evident in the connective tissue membrane of the tubuli, was seen to increase in the atrophic testicles, perhaps as a consequence of a decrease in tubular diameter. Acid phosphatase, weakly visible in control rat, showed a high activity in the hyperplastic interstitial tissue which accompany the inhibition of spermatogenesis produced by ethionine treatment. Adenosine triphosphatase showed a decreasing activity in the thick basement membrane of the treated rats whereas a strong deposit was still in the interstitial structures. Thiamine pyrophosphatase which was demonstrated in all stages of spermatogenesis in the mature testes showed a net reduction of enzymatic activity in the damaging germinal cells. α-Glycerophosphate dehydrogenase, Glucose-6-phosphate dehydrogenase, and 3β-hydroxysteroid dehydrogenase were absent in the ethionine treated rats, although important in the metabolic pathway of steroidogenesis. Lactic dehydrogenase was tested with two different substrates — Potassium Lactate and DL-α-hydroxyvalerate. In the ethionine fed rats, the enzyme activity disappears in the tubuli as well as in the interstitial cells.

So, the steroid synthesizing systems, dependent of oxidative enzymes, were found to be very sensitive to the drug, and the hyperplastic interstitial cells, found after ethionine treatment, stay physiologically inactive.

Introduction

The effect of ethionine on male rats was extensively studied, and characterized by inhibition of growth, intrahepatic bile duct proliferation (H. Ungar and M. G. Goldberg, 1957; 1959), hepatoma formation (E. Farber, 1963; 1967), and atrophy of the exocrine pancreas followed by atypical regeneration (M. Alvizouri, and S. Warren, 1954). Complete inhibition of spermiogenesis was described by Kaufman et al. (1956), Goldberg et al. (1959a, b, 1961). These authors observed after twenty-four days of ingestion of ethionine extensive destruction of the seminiferous epithelium, the persistence only of Sertoli cells in the tubules, and simultaneous hyperplasia of the Leydig cells. Subsequent treatment with testosterone, did not prevent the severe tubular lesions but this led to atrophy of the Leydig cells. Benson and Clare (1966) after daily intraperitoneal injections of ethionine (25 mg per 100 g body weight) for two 19 days periods separated by a rest period of 20 days, observed considerable variations in degenerative changes within the same testis. Pfau and Eyal (1963) reported on partial protection by methionine against the ethionine induced pathological changes of the germinative epithelium after twenty-four days, but complete protection was not obtained. In contrast to the observation by Farber et al. (1963) that adenosine triphosphate will
protect liver against the effect of ethionine, Eyal and Pfau (1968) found no similar effect on the testicles. On the contrary, injections of adenosine triphosphate accelerated the pathological changes in the testicles.

Most observations reported in the literature were made over short periods of time, following doses of ethionine which were high enough to produce the entire spectrum of lesions in different organs.

In our experiment, we tested the chronic action of ethionine on rats fed a diet containing ethionine 0.1 per cent over periods of seven to ten months.

It was found that this diet caused selective and severe lesions in the testicles but failed to interfere with growth and did not produce histological changes in the liver or the pancreas.

While the histological testicular changes caused by ethionine have been studied in detail (Kaufman et al., 1956; Goldberg et al., 1959, 1961; Ungar et al. in preparation), there is only sparse information about the fate of different enzymes associated with the cell organelles during the different stages of spermatogenesis. Niemi and Ikonen (1962) interpreted the functional significance of the oxidative enzymes in the Leydig cells of normal and hypophysectomized and gonadotrophin-treated animals. Ambadkar and George (1964) noted that both components of the testicular tissue, interstitial tissue as well as the seminiferous tubules, are sites of high metabolic activity.

The aim of the present investigation has been to study the localization of some phosphatases and dehydrogenases in the germinal elements and the interstitial cells, after ingestion of the ethionine during a seven to ten months period.

**Material and Methods**

Twelve male albino rats, random-bred, of the Hebrew University strain were used in this experiment. The animal weighed initially 130±20 g. They were maintained on a diet of ground lab-chow (Ambar No. 931), which contains 18% protein. To this diet 0.1% of DL-ethionine was added (purchased from Sigma Chemical Co. — Tel Aviv). Four animals of the same weight were used as controls. The animals were weighed at regular intervals.

Animals were killed by bleeding after seven to ten months. Liver and testis were weighed. Liver, pancreas and part of one testis were fixed in Zenker’s and Bouin’s fixatives. Slices of liver were also fixed in Carnoy’s solution. Paraffin sections were stained with hematoxylin-eosin and the periodic acid-Schiff-method, with and without prior diastase digestion. From each section of testis, stained with Hematoxylin-eosin, sixty tubules were measured by a calibrated ocular micrometer, to estimate the average diameter of seminiferous tubules in control and treated animals.

From the second testis Cryotome sections, 10 μ in thickness, were cut at −20° temperature and stored in a refrigerator overnight.

All the reactions for phosphatases were carried out on sections prefixed in 6% neutral formaldehyde for thirty minutes. For demonstration of the dehydrogenases, sections were post-fixed in formaldehyde.

The cryostat sections were investigated by the methods as listed below (Table 1): **Oil Red-O for Neutral Fat, Alkaline phosphatase (ALP), Adenosine-triphosphatase (ATP), two enzymes required in energy source protein synthesis and molecular transport, Thiamine pyrophosphatase (TPP), which catalyses the decarboxylation of α-ketoacids and is considered as a marker of the Golgi apparatus. Acid phosphatase (AcP), well known in the metabolic activity of phagocytosis and in the process of degradation. α-Glycerophosphate dehydrogenase (a-GPDH) which catalyses the triglyceride synthesis, Glucose-6-phosphate dehydrogenase (G-6-PDH) which regenerates the cofactor NADPH, involved in several steps of steroid biosynthesis, lipoge-