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Mg^{2+} Dependent Adenosine Triphosphatase in Rat Liver during Acclimation to Hyperthermic Environment–Histochemical Studies

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This study was undertaken to demonstrate histochemically variation in ATPase activity that occurs in the liver parenchyma of rats exposed various time (until 60 days) to moderate hyperthermic environment. The obtained results indicated that the acute exposition (1 and 4 days) to high environmental temperature (35±1°C) cause diminution of ATPase activity, especially in the pericentral areas of the liver. Prolonged exposure of animals to hyperthermic environment (after 14 days) showed restoration of the enzyme activity probably as result of the acclimation of the rats to the changed conditions of the environment.

Kew words: ATPase, liver, high environmental temperature, rat.

Introduction

Adenosine triphosphatases (3.6.1.3) in animal organs are responsible for the physiological degradation of ATP. They utilise energy stored in ATP for metabolite or ion transport processes [2]. Several investigators have used histochemistry and cytochemistry techniques to study the location of Mg^{2+} dependent ATPase in the liver. They reported that ATPase was distributed at the canalicular borders of hepatocytes, bile canalicules, bile ductules [1, 13, 19, 23, 24] and Kupffer cells [16]. This location in the liver tissue is in the correlation with enzyme function in secretion of bile constituent from hepatocytes into bile canaliculi [4, 15].

Previous studies, in general, indicate that diminution of bile canalicular activity is associated with damage of the liver cells or changes of material transport across the cell membrane [6, 14]. For example: after bile destruction [17], bile duct ligation [24], administration of hepatotoxic drugs [6], adriamycin [1] and alcohol administration [13] disappearance of the ATPase activity has been noted. In addition some authors suggested seasonal variation in the activity of ATPase in the liver tissue [18].

It is well known that temperature fluctuations can have effects upon the organism, as well as the liver. An experimental study on the effect of short term exposition to the high temperature showed decrease of basal metabolism, body mass [10, 11], and relative mass of the liver as heat-producing organs [21, 25]. Furthermore, degenerative reactions in the

liver were described as a response to exposition of the animals to high environmental temperatures [2, 22]. In addition, exposure of rats to high temperature showed evidence for reversible effects of liver histopathology due to prolonged exposure to high temperature [9]. In this study our objective is to provide an answer to the question of whether reversible temperature-related liver changes correlating with Mg^{2+} dependent ATPase activity.

Material and Methods

Adult, male Wistar rats weighing 190-230 g maintained in controlled environmental temperature conditions (35±1°C), with constant humidity (30-40%) and a regular light-night cycle (light from 6:00 a.m. to 6:00 p.m.) were used. The experimental animals were exposed to a moderate hyperthermic environment 1, 4, 7, 14, 21, 30 and 60 days. Rats kept at room temperature (20±°C) served as a control.

Under ether anaesthesia of the rats (between 9:00 — 10:00 a.m.) the liver were removed. Tissue preparation for reveal of ATPase activity by lead method of W a c h s t e i n and M e i s e l [23] was carried out as follows. Immediately after removal, the liver was sliced into small pieces (0,5 cm^3) and frozen in liquid nitrogen. Cryostat sections 10 micrometers thin were made. Glass slides with attached sections were incubated at 37°C for 30 minutes at pH 7.2 using adenosine 5'-triphosphate disodium salt as substrate. The sections were embedded in glycerol-jelly. Depositions of lead sulphide (brown in colour) marked sites of enzyme activity. Controls of reaction included incubation of the sections in the absence of substrate and heat-inactivated sections equable processed.

Results and Discussion

The Mg^{2+} dependent ATPase activity in the liver sections was observed in control animals as fine granular reaction product localized in the bile canaliculi, ductules and bile duct (Figs. 1 and 2). Control sections did not show any lead sulphide precipitations. These observations are in agreement with previously histochemical findings (1, 13, 16, 23, 24). The enzyme activity appeared to be strongly positive in periportal zones compared with pericentral zones (Fig. 1). A higher ATPase activity in periportal zones of hepatic lobules, which reflect metabolically more active hepatocytes in this zone [7], has been reported for the rats [5] and rabbits [20].

After acute exposition (1 and 4 days) to high environmental temperature significant changes in Mg^{2+} dependent ATPase were noted. The enzyme reaction product was absent in bile canaliculi and ductules in pericentral areas, and week ATPase activity was seen in periportal zones (Fig. 3). According Munn (14), a general loss of phosphatases (including ATPase) was due to hepatocytes injury and decrease ATPase activity in liver parenchyma is observed under many conditions in which damage to liver cells occurs [1, 6, 13, 24]. So diminution of ATPase activity in pericentral zones may be explained by the fact that in centrilobular hepatocytes thermal exposure induced degeneration of the pericentral hepatocytes [2, 9, 22]. Moreover, the canalicular ATPase and bile acid excretion are preferentially located in periportal zones compared with pericentral ones [5, 8, 20]. Pericentral zones constitute only a reserve capacity for transportation of the bile constituents into the bile [8]. The absence of ATPase in pericentral zones suggested that during acute exposition to high temperature animals don't need to use reserve capacity of the pericentral located cells probably due decreased extrahepatic transport.

Compared with ATPase activity noted in rats exposed 1 or 4 days to high temperature, after 7 and 14 days of exposition to high environmental temperature enzyme activity

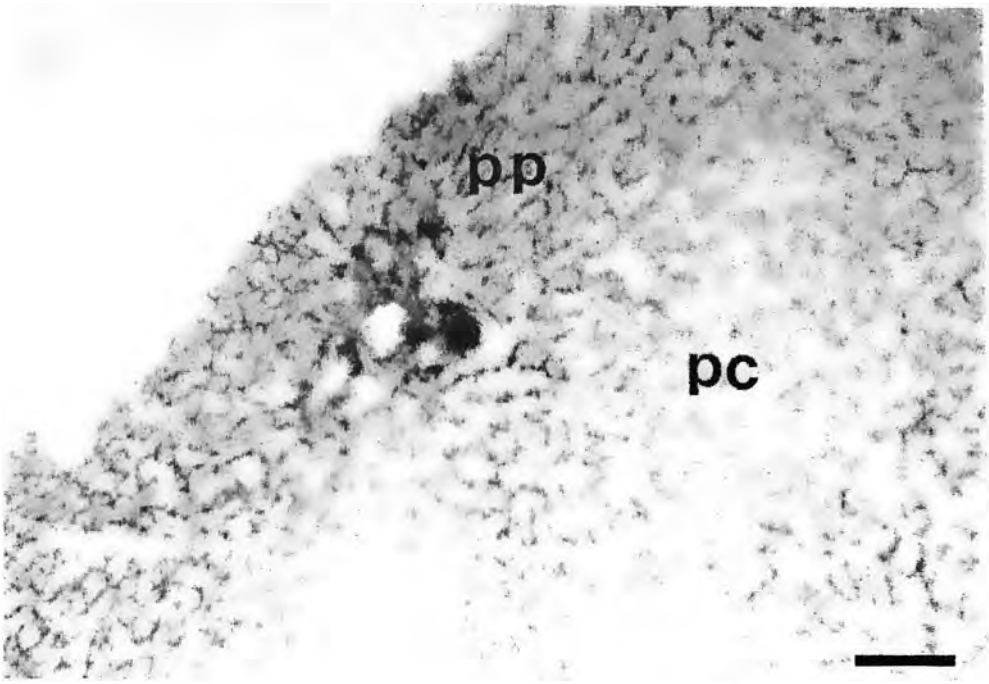

Fig. 1. Light micrograph of frozen liver sections treated for magnesium dependent ATPase in the control rats. pp — periportal; pc — pericentral; Bar = 100 μm

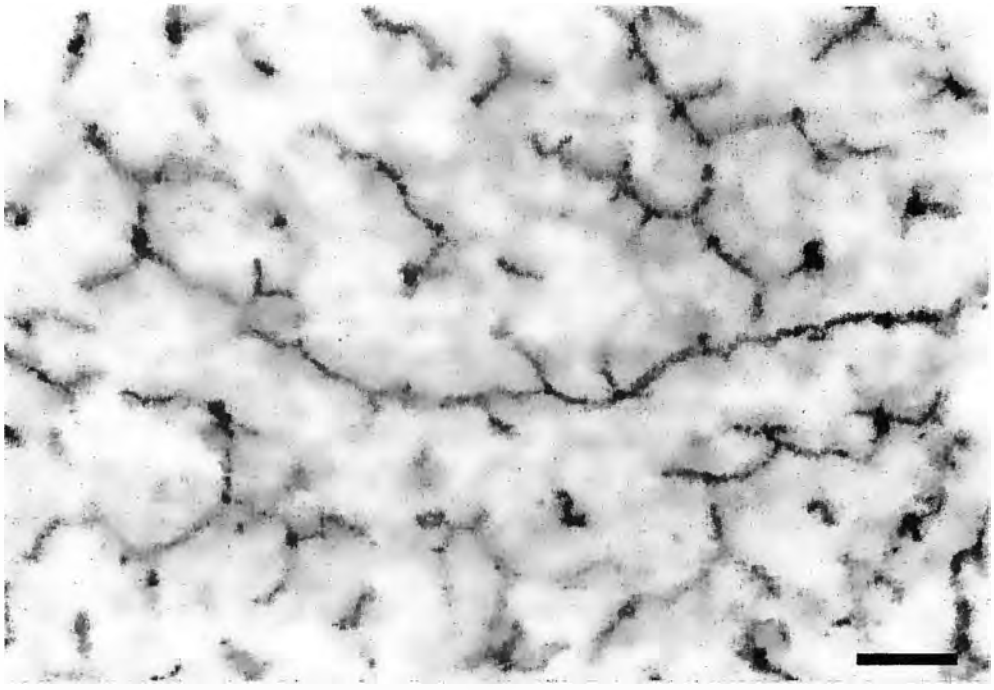

Fig. 2. Detail of frozen liver sections in the control rats. Note the reaction product within the biliary tree, apparently up to its smallest and thinnest portions. Bar = 20 μm

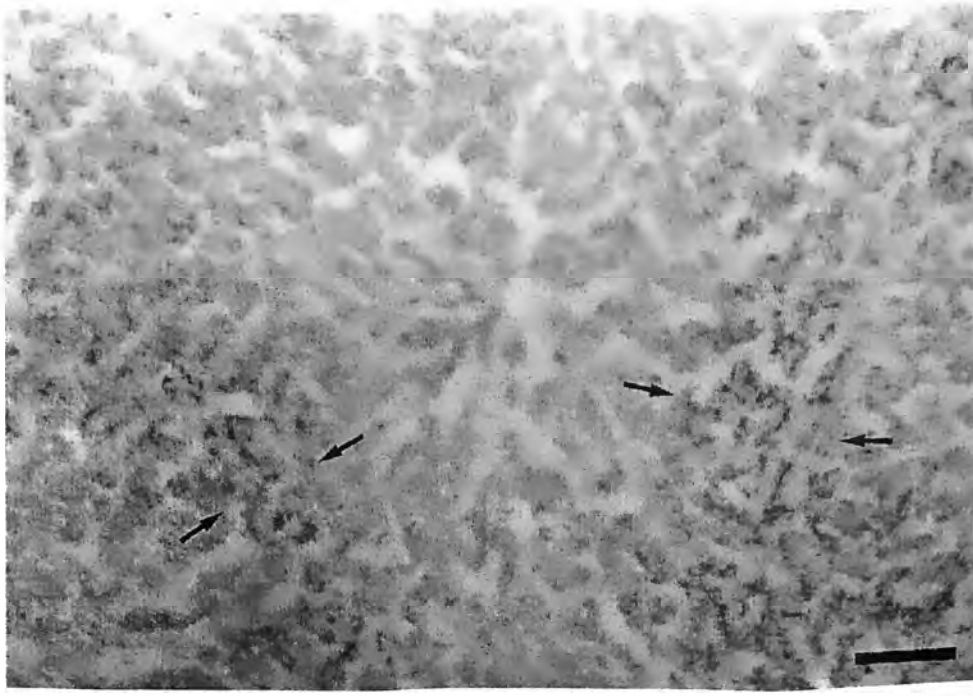

Fig. 3. Light micrograph of frozen liver sections in rats exposed 4 days to 35±1°C. A week reaction for ATPse in periportal zones (arrows). No reaction product in pericentral zone. Bar = 100 μm

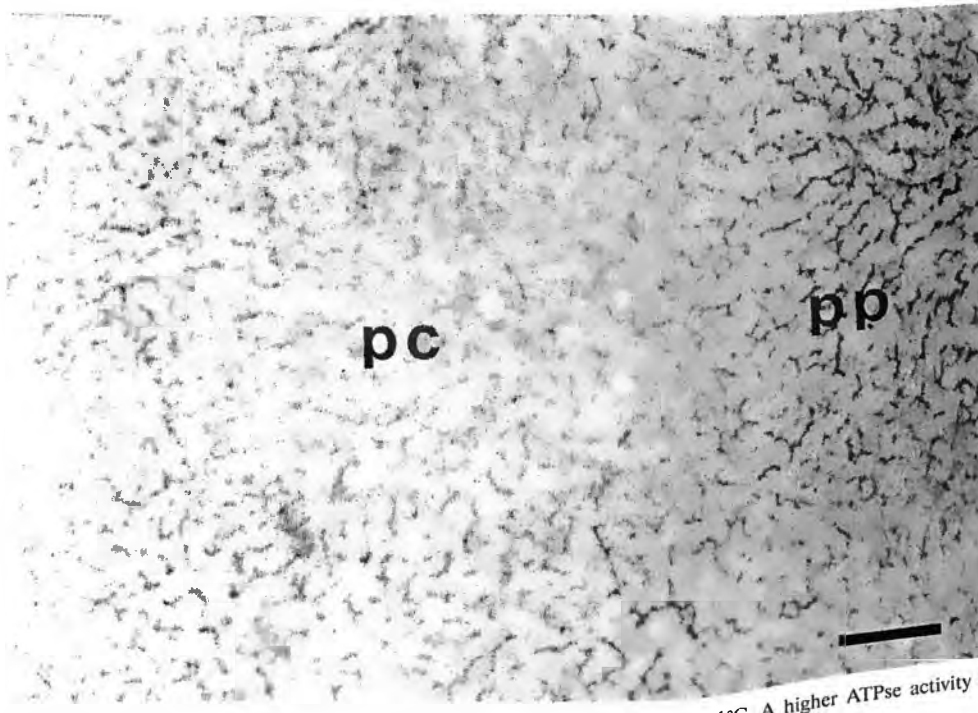

Fig. 4. Light micrograph of frozen liver sections in rats exposed 14 days to 35±1°C. A higher ATPse activity in periportal (pp) compared with pericentral zone (pc). Bar = 100 μm

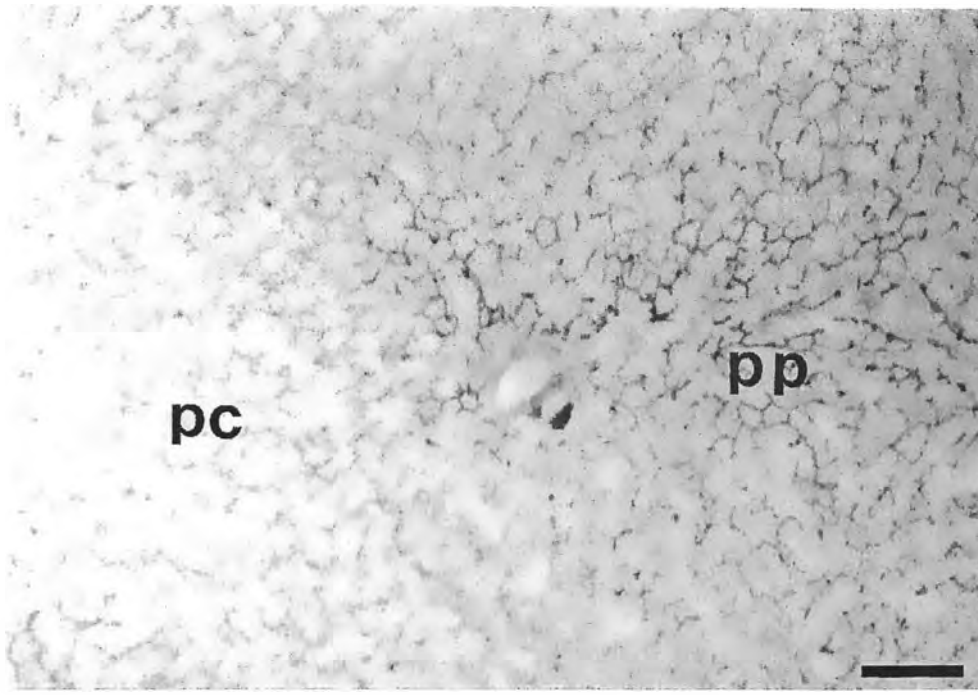

Fig. 5. Light micrograph of frozen liver sections in rats exposed 30 days to 35±1°C. A ATPse reaction product mainly homogenously distributed in periportal (pp) and pericentral zones (pc). Bar = 100 μm

increased in pericentral areas (Fig. 4). Increased enzyme activity could be associated with increasing in the cell membrane permeability due to hydropic degeneration present in pericentral hepatocytes after exposition of the rats one and two weeks to high temperature [9]. Increased permeability required increased active transport which probably acted as a compensatory mechanism for loosing of many molecules across the plasma membrane [3]. Also, cell membranes which are active in transporting molecules in liver, as well as in other tissue characterize the high levels of ATPase activity [16].

Reaction products in animals exposed 21 days to moderate hyperthermic environment showed same intensity and location as well as control rats acclimated to room temperature. Prolonged heat acclimation up to 60 days did not alter the amount of enzyme activity (Fig. 5). Degenerative changes were not recognisable in liver tissue of the rats' long-term exposed to high temperature [9].

Changes in Mg^{2+} dependent ATPase activity observed in rats liver in responses to heat during short-term exposition disappear over a period of two weeks, after a cessation of high environmental temperature. The normalisation or restoration in the enzyme activity obtained during long-term acclimation and maintained stable for a long period could be result of the acclimation of the organism to changeable environmental conditions.

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