i.p. or in the cerebral ventricle of the mouse[8]. Numerous other animal species, including rat, rabbit, sheep, goat and ox, exhibited qualitatively similar hypothermia (reviews by Preston and Schönbaum[11] and by Jacob and Girault[12]). A resolution of this dichotomy is difficult at present, but it must be realized that in some cases the reported alteration of body temperature was actually part of a multiphasic temperature response[8]. Also, even with microinjection techniques, it is difficult to avoid rapid diffusion of the test compound in the brain. For example, depending upon whether serotonin was injected into the posterior or anterior region of the hypothalamus, the pigeon exhibited hyperthermia or hypothermia, respectively[13]. Perhaps a partial explanation for the conflicting data may be found in the observation that in chick pineal gland in organ culture, endogenously formed serotonin and exogenously supplied serotonin entered into distinct metabolic pools, i.e., different metabolic products arose[14]. If this were generally true in other animal species, one would expect to see varying results in the literature, depending on the source of the serotonin. Also, this suggests that temperature regulation studies which are based on serotonin depletion may be more meaningful, physiologically, than experiments in which serotonin is administered. The present results would tend to favor the possibility that serotonin produces hyperthermia in mice. The observation that serotonin depletion was not accompanied by hypothermia if the mice were exposed to an ambient temperature of 30 °C is not without precedent. Francesconi and Mager[15] observed that injection of tryptophan (the precursor of serotonin) into rats maintained at cool ambient temperatures resulted in body temperature alteration, whereas no temperature change occurred if the ambient temperature was raised to 31 °C. A similar effect of ambient temperature was noted in the cat[16].

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Multiple esterase forms in isolated hepatocytes and Kupffer cells of partially hepatectomized rats

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Summary. The esterase patterns of isolated parenchymal liver cells of rats consisted of 6 bands of enzymatic activity, whereas the patterns of iron-loaded Kupffer cells showed 5 bands. Both patterns become simpler in the early prereplicative period of liver regeneration. During simultaneous replication of DNA, i.e. 24 h after partial liver removal, an additional band of esterase activity appears in patterns of hepatocytes and Kupffer cells. At the moment of maximum hepatocyte mitotic rate, i.e. 36 h after partial hepatectomy, both esterase patterns lose the single band of activity again. 2 or 3 days after surgery the initial esterase patterns in hepatocytes return whereas the patterns of Kupffer cells remain incomplete.

The patterns of multiple esterase forms may serve as markers of the state of maturity of isolated cells and cellular cultures[1]. By means of starch-gel electrophoresis of normal liver extracts 10 bands of esterase activity may be revealed. At the same time the data on the esterase patterns in isolated hepatocytes and Kupffer cells are scanty[2]; nothing is known about esterase patterns in regenerating liver and liver parenchymal and nonparenchymal cells. Here some results are presented showing the transient changes of multiple esterase patterns in hepatocytes and Kupffer cells from intact and regenerating liver.

Materials and methods. In all experiments, Wistar male rats, weighing 180–230 g were used. $^2/_3$ of their liver tissue was removed under urethan anesthesia[3]. Each operation was performed between 0900 and 1100 h. At each sampling time, 2,5, 24, 36, 48 and 72 h after surgery, 5–6 animals were sacrificed and the livers perfused in situ via the v.portae with 0.25 M cold sucrose solution containing 0.01 M EDTA. Kupffer cells were separated from hepatocytes in a magnetic field[4]. For the overloading of Kupffer cells, the rats received 1 ml of a 10% starch stabilized suspension of colloid carbonic iron (R-100F, Scientific Institute of Electroorganic Compounds, Moscow) 2 h before sacrifice, via the v.femoralis. To test the viability of

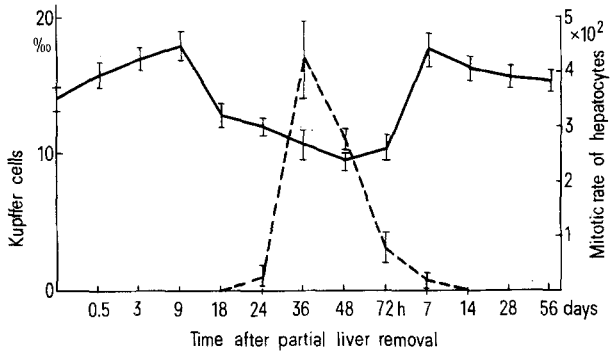

Fig. 1. The relative number of Kupffer cells per 1000 hepatocytes (——) and mitotic rate of hepatocytes in ‰ (– – –) in partially hepatectomized rats.

isolated hepatocytes the trypan blue dye-exclusion reaction was applied. For enzyme analysis 10% homogenates of hepatocytes and Kupffer cells in 0.25 M sucrose with 0.01 M EDTA, pH 7.4, were prepared. Enyzmes were separated on 2 mm thick plates of starch gel mixed with 0.5 M Tris-EDTA-borate buffer, pH 8.6. Conditions for electrophoresis: 100 V, 50 mA, 4 °C, 15 h. The substrate mixture contained equal volumes of α- and β-naphthylacetate. The mitotic rate for the hepatocytes and the relative number of Kupffer cells were counted in liver slices stained with hematoxylin-eosin.

Results. As can be seen from figure 1, in the early prereplicative period of liver regeneration the relative number of Kupffer cells per 1000 hepatocytes increases. It is due perhaps to their migration into the liver from extrahepatic sources[5]. The esterase pattern of whole liver consists of 9 bands of activity. All bands may be grouped into 2 zones. The 1st consists of slow fractions located at the anodic end (bands 1–4), the 2nd, of rapidly-moving types of enzyme (bands 5–9). The esterase patterns of isolated hepatocytes comprise 6 bands of activity. They are in the positions of bands 1, 2, 3, 5, 6, 7 of whole liver extracts (figures 2, 3). In isolated Kupffer cells from normal livers we find 5 bands of activity which are in the positions of bands 2, 3, 4, 6, 7 from total liver homogenates (figures 2, 3). In regenerating liver the esterase patterns of both hepatocytes and Kupffer cells are changing. 2.5 h after partial liver removal bands 1 and 5, in hepatocytes, and band 4 in Kupffer cells disappear. At 9 h the esterase patterns tend to be restored. Moreover, an extra band 4 in hepatocytes and band 8 in Kupffer cells may be seen. At 24 h the patterns are just the same, but at 36 h bands 1 and 4 in hepatocytes and bands 4 and 8 in Kupffer cells disappear again. At 48 and 72 h the initial esterase pattern in hepatocytes is restored but in Kupffer cells band 4 is absent.

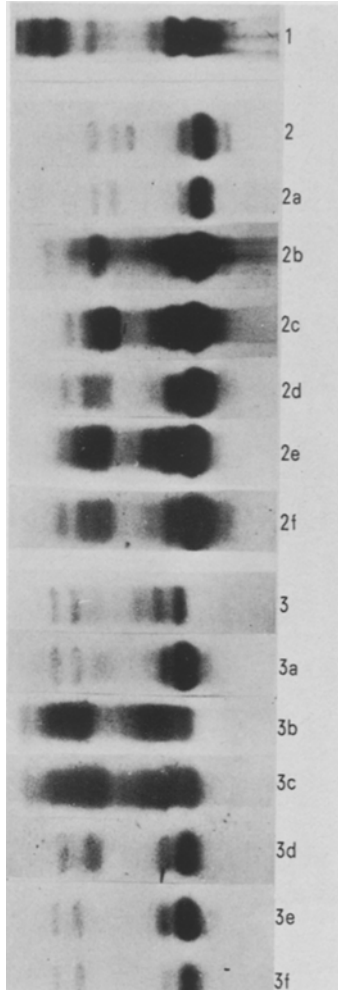

Fig. 2. Esterase patterns of regenerating rat liver. 1, whole normal liver; 2, isolated hepatocytes from normal liver; 2, a–f hepatocytes obtained 2.5, 9, 24, 36, 48 and 72 h respectively after partial liver removal; 3 isolated Kupffer cells from normal liver; 3, a–f, Kupffer cells obtained 2.5, 9, 24, 36, 48 and 72 h respectively after partial liver removal.

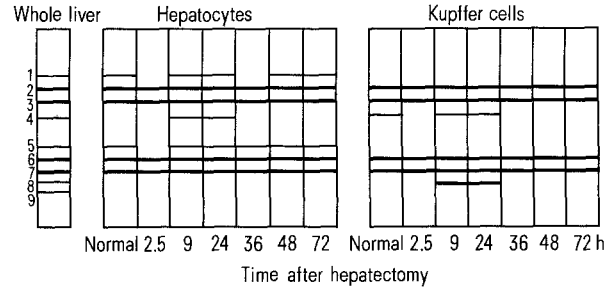

Fig. 3. The distribution of multiple esterase forms in hepatocytes and Kupffer cells of regenerating rat liver in starch-gel electrophoresis.

Thus in the early period of regeneration before simultaneous replication of DNA in hepatocytes, and at the moment of their maximum mitotic rate, the esterase patterns of parenchymal liver cells become less complex than in normal hepatocytes. On the contrary, in hepatocytes from the replicative phase of regeneration an extra band of activity may be revealed. The main finding in Kupffer cells is biphasic simplification of their esterase patterns. The 1st phase coincides with the prereplicative period of liver regeneration, the 2nd with the mitotic peak and early postmitotic period. The 1st phase is connected with an influx of less differentiated precursors of liver macrophages from bone marrow[5,6]. The 2nd is perhaps linked with the division of unmatured Kupffer cell precursors fixed in regenerating liver tissue, which usually lasts from 48 to 96 h after partial hepatectomy[7].

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