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# A Histochemical Study on the Activity of Several Enzymes in Rat Liver Tissues with Special Reference to the Age-factor<sup>1), 2)</sup>

By

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(With 8 Text-figures)

It has been known to certain extent that aging of the animal, one of the interesting biological phenomena, is associated with morphological and physiological changes in various organs. However, very little has been known about the relationship between aging process and enzymatic activity histochemically. It is then evident that the histochemical approach is needed for elucidation of this problem.

The author (Matsuzawa 1964a) has reported that the activity of glucose-6-phosphatase (G-6-Pase) and of phosphorylase varies with glycogen content in rat livers in connection with the increase in age of rats, and that intra-lobular distribution of these enzyme activities became heterogeneous with age: namely in old rats the G-6-Pase activity is higher in periportal areas of hepatic lobules than in central areas, while it is evenly distributed throughout the lobule in young rats.

The present paper describes additional data pertaining to some histochemical changes occurring in rat livers in relation to age, with special attention to activities of adenosine triphosphatase (ATPase), succinic dehydrogenase (SDH) and nonspecific esterase.

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Materials and Methods: Liver tissues from fetuses (just before birth), newborn (5-7 day old), young (30-40 day old) and old rats (more than 10 month old) of Fischer strain

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were used for the present study.

Frozen-substituted sections were made at 10 micra by the method of Chang and Hori (1961, 1962a, b).

For the demonstration of cellular substances, the following staining methods were employed: the method of Wachstein and Meisel (1956) for ATPase; the technique of Chang and Hori (1962a) for SDH; Burstone's method (1956) for non-specific esterase; the toluidine blue method for ribonucleic acid (RNA) (Lison 1953); and Hematoxylin-eosin staining for general histological observations.

### Results

*SDH and ATPase:* In fetal rats, the activities of both SDH and ATPase were either absent or demonstrated slightly in hepatic cells, as well as hematopoietic cells.

After birth, the SDH and cytoplasmic ATPase activities increased gradually with the increase in age. The activity of ATPase in bile canaliculi was absent in newborn and some young rats, while it was higher in the rest of the young and all the old rats.

Histologically, in newborn and some young rats, the activity of SDH and cytoplasmic ATPase were almost evenly distributed throughout the lobule, whereas in old rats these enzyme activities were higher in periportal areas of hepatic lobules than in central areas (Figs. 1, 2, 3, and 4). The bile-canal ATPase was demonstrated only in periportal areas in young rats and in both the areas in adult rats, being stronger in activity in periportal areas.

*Non-specific esterase:* No activity of esterase was found in fetal rat livers, while in livers of postnatal rats the activity increased with age.

On the histological basis, a few layers of hepatic cells surrounding central veins were stained weakly and diffusely in newborn rats. In young rats, however, a moderate activity of the enzyme was observed in hepatic cells in central areas (Fig. 5). Old rats showed a strong activity in the whole areas of the lobule, though precise examination revealed that the activity was higher in central than in periportal areas (Fig. 6). The activity of the enzyme was distributed evenly in the cytoplasm of hepatic cells in both young and old rats.

*RNA:* Staining reaction with toluidine blue was more intense in the liver of fetal and newborn rats than in those of young and old rats.

Histologically, hepatic cells of both fetal and newborn rats showed no lobular pattern after staining with toluidine blue. Basophilic materials stained with toluidine blue were demonstrated as fine granules which were distributed evenly in the cytoplasm (Fig. 7). In young rats, hepatic cells in periportal areas exhibited stronger reaction to toluidine blue than those in central areas. Basophilic materials were observed as clumps and distributed rather unevenly in the cytoplasm of hepatic cells (Fig. 8). No significant difference was observed between livers of young and old rats.

### Discussion

It has been shown by means of both histochemical and biochemical studies that the SDH is exclusively localized in mitochondria (Hogeboom *et al.* 1948, Barnett and Palade 1957), while on the other hand the ATPase activity detectable by means of Wachstein and Meisel's method has been localized in mitochondria and endoplasmic reticulum (Lazarus and Barden 1962, Wachstein and Fernandez 1964). Furthermore, Novikoff *et al.* (1952, 1958) have presented quantitative biochemical data showing that about 60 per cent of ATPase activity is present in mitochondrial fraction. On the basis of the possibility that the ATPase activity demonstrated by means of Wachstein and Meisel's method could be due largely to the enzyme in mitochondria, it would be appropriate to state that the results, obtained in the present study, showing the increase of both SDH and ATPase activities with the increase of age, are suggestive of progressive change in physiological function of mitochondria with advance in age.

On the other hand, a biochemical study by Wienbach and Garbus (1956) revealed that the ATPase activity of mitochondria was lower in livers of 37 month old rats than in 3 month old ones. Difference between the present results and those of Wienbach and Garbus (1956) might be attributable to the difference of the materials and methods used.

It is well documented and accepted fact that G-6-Pase and cytoplasmic RNA are associated with endoplasmic reticulum (Swanson 1950, Palade and Siekevitz 1956, Hultin 1957, Tice and Barnett 1963). From the previous and present studies, the author has found that in hepatic cells of young rats both RNA and G-6-Pase were generally localized in basophilic clumps, while in newborn rats basophilic substance and G-6-Pase were evenly distributed in the cytoplasm as fine submicroscopic granules. It has also been reported that cytoplasmic basophilic clumps became disintegrated into fine granules in livers of both fasted and tumor-bearing rats (Chang *et al.* 1961, Matsuzawa and Hori 1963). It is interesting to note there is a similarity in the morphology and activities of endoplasmic reticulum between newborn and fasted or tumor-bearing rats. The above feature may probably be regarded as homeostatic response of the liver to the energy requirement of growth of an animal itself or of developing of tumors.

A considerable amount of biochemical data have presented the fact that the activities of various hepatic enzymes are affected by hormones, such as epinephrine, glucagon, and cortisone (Rall *et al.* 1957, Weber *et al.* 1955).

It has been observed histochemically that hepatic enzyme activities in central areas of the lobules responded to both adrenalectomy and hypophysectomy in different manners from enzyme activities in periportal areas (Matsuzawa 1964b). Evidence presented above seems to suggest that the heterogeneous distribution of cellular substances within a hepatic lobule occurring in the course of growth of rats may be controlled by the action of certain hormonal glands.

### Summary

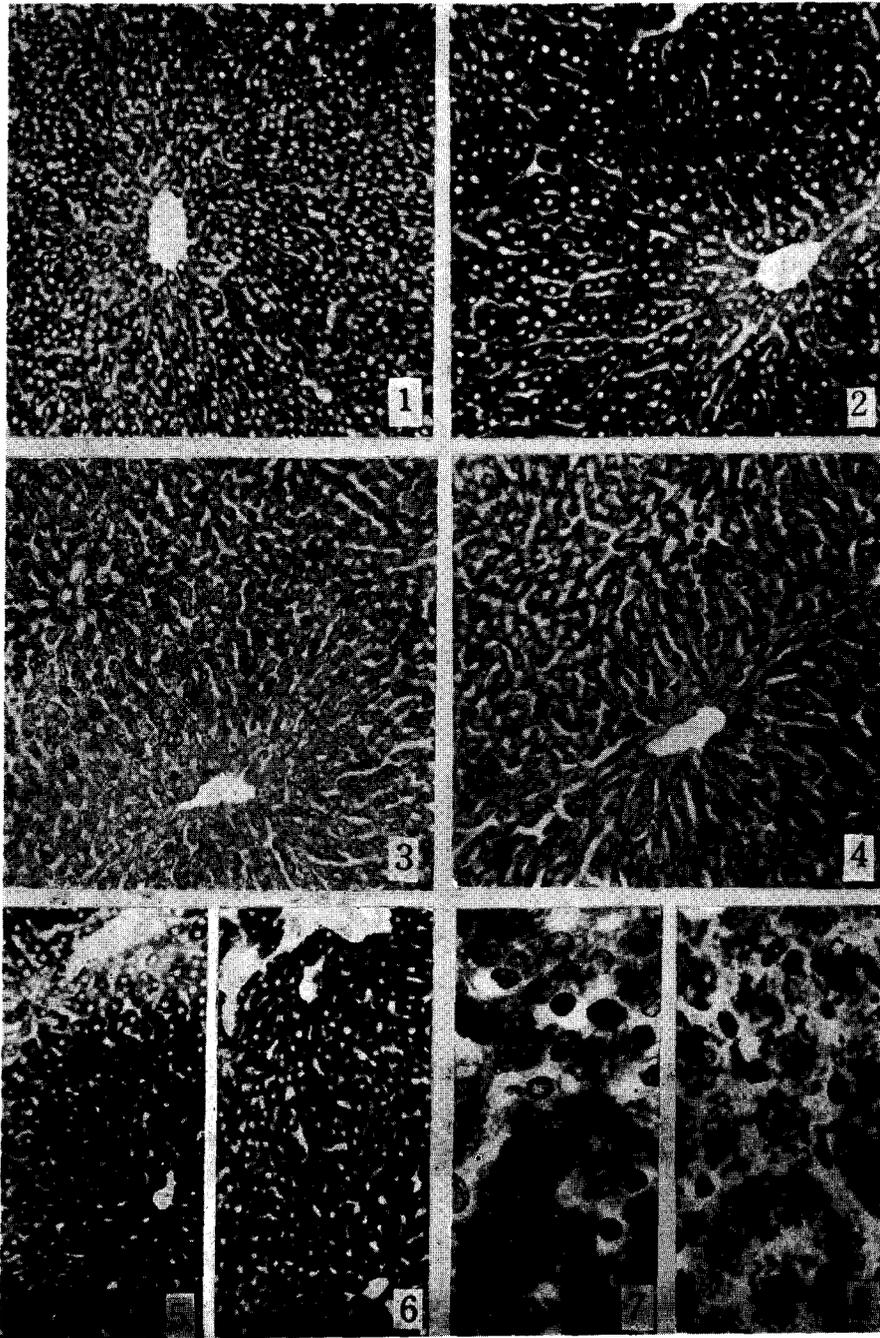
In the liver of rats, the activities of succinic dehydrogenase (SDH), ATPase and non-specific esterase and RNA content were investigated histochemically with special regard to the age factor.

The activities of SDH, ATPase and non-specific esterase increased gradually after birth. In striking contrast, no such enzyme activities were demonstrated in the liver of fetuses. RNA was more demonstrated in hepatic cells of newborn rats than in those of both young and old ones.

Histologically, intra-lobular distribution of the enzyme activities investigated appeared to have become heterogeneous with age.

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#### Explanation of Figures 1-8.

- Fig. 1. Succinic dehydrogenase. Young rat liver.  $\times 100$
- Fig. 2. Succinic dehydrogenase. Old rat liver.  $\times 100$ .
- Fig. 3. ATPase. Young rat liver.  $\times 100$ .
- Fig. 4. ATPase. Old rat liver.  $\times 100$ .
- Fig. 5. Esterase. Liver of a young rat.  $\times 100$ .
- Fig. 6. Esterase. Liver of an old rat.  $\times 100$ .
- Fig. 7. Toluidine blue stain. Newborn rat liver.  $\times 400$ .
- Fig. 8. Toluidine blue stain. Young rat liver.  $\times 400$ .