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STUDIES ON THE HETEROGENEITY OF CATALASE IN RAT LIVER

I. Changes of Catalase pI-Isozyme Pattern during Hepatic Cell Differentiation and Growth

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INTRODUCTION

Studies on the problem of normal cell differentiation and growth are concerned with elucidation of the mechanisms involved in abnormal tumor cell proliferation. In recent years, our knowledges on intracellular control mechanisms, especially those related to biosyntheses and activities of various enzymes, have considerably increased.

Among these enzymes, catalase (E. C. 1.11.1.6.), one of enzymes located in peroxisomes, has been widely discussed to have a heterogeneous nature (2, 4, 6, 7, 9, 10, 14-17). Although the precise function of this enzyme in cell metabolism has not been fully clarified as yet, several studies have been made on the isozymal variations which occur during cell differentiation and growth (12, 17).

The present report, as one of series of the investigations on the changes in the heterogeneous nautre of peroxisomal enzymes

during cell differentiation and proliferation, deals with an alteration of the multiple forms of catalase in the growing and differentiating liver of rats during pre- and postnatal growth, and also in regenerating liver of the animals after partial hepatectomy. Furthermore, multiple forms of the enzyme in the kidney of rats were also examined to ascertain the characteristic of the enzyme each in the kidney and liver.

MATERIALS AND METHODS

Animals

Male Wister/Mk strain rats, ranging in age between the 20th fetal and 60 post-natal day, were used. The fetal ages were estimated from the time of conception and were accurate to within 24 hours. The animals were kept at 22 $\pm$ 1°C and were fed on a compressed diet (Oriental, No. NMF), and received drinking water *ad libitum*. Adult rats were lightly anesthesized with ether and their livers and kidneys were perfused *in vivo* with PBS. Fetal and newborn rats were killed by decapitation and were exsanguinated. The liver and kidney tissues thus obtained were immediately placed on a glass plate cooled on ice. For examining the effect of 3-amino-1,2,4-triazole (A. T.) on the enzyme, adult rats were administered intraperitoneally with the drug dissolved in PBS, using a dosage of 1 gm/kg body weight. In partial hepatectomy, median and left lateral

lobes of the liver were surgically removed under light anesthesia by the method of Higgins and Anderson (8).

Preparation of Enzyme Solution for Isoelectric Fractionation

Liver and kidney tissues were homogenized in 4 volumes (v/w) of cold distilled water using a Potter-Elvehjem homogenizer. The homogenates were sonicated for 7 minutes at 200W with a Kubota KMS-250 ultrasonic generator, and were centrifuged at 105,000 x g for 60 minutes at 0°C in a Beckman Model L2-65B ultracentrifuge. The resulting supernatants were concentrated and dialysed for about 2 hours at 0°C against cold distilled water using collodion bags. The enzyme solution thus obtained, corresponding to 15 mg of protein, was applied to an isoelectric focusing column. Protein were determined by the method of Lowry, _et al._ (13).

Isoelectric Fractionation by Ampholine-carrier Ampholites

Electrofocusing was carried out by the method of Vesterberg and Svensson (20). The details of the procedure in the present examinations were the same as the described previously (12). The measurement of catalase activity was made according to Adams (1).

RESULTS

pI-Isozyme Pattern of Catalase in Adult Rat Liver

A typical pattern of the multiple forms of the activity of catalase extracted from adult rat liver is shown in Fig. 1. It was clearly demonstrated that the enzyme activity was found to be separated into five major components, pIs of which were pH 6.95, 6.75, 6.10 and 5.90, respectively.

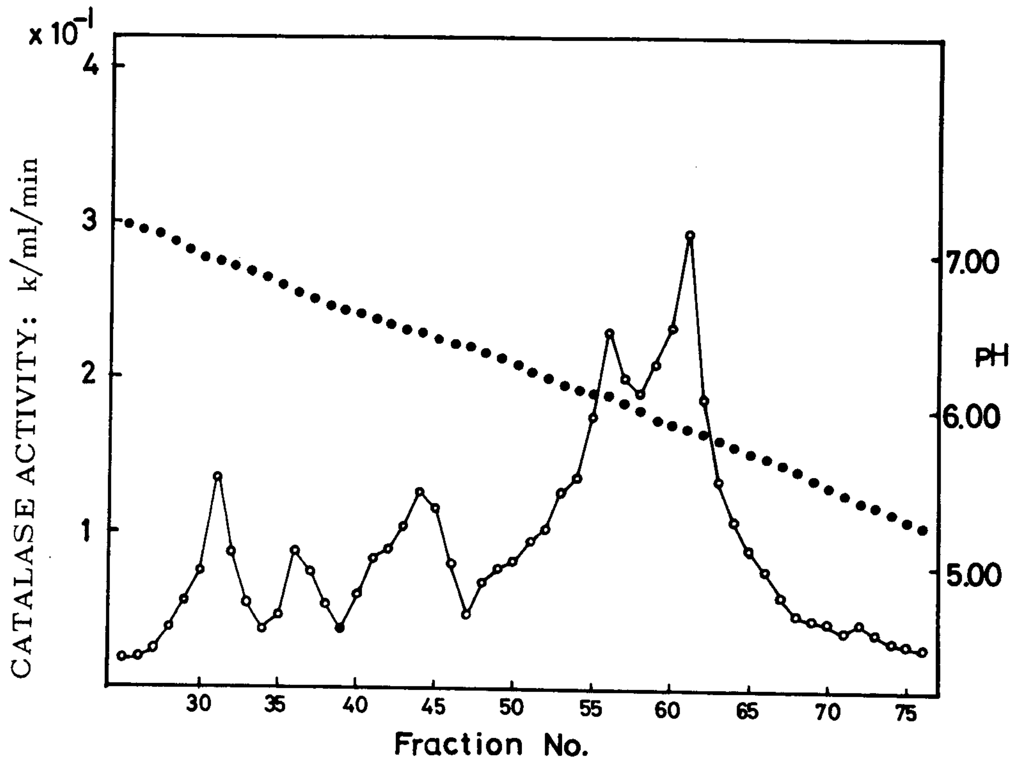

Fig. 1. A typical pattern of catalase pI-isozyme of adult rat liver. Open circles and solid line illustrate catalase activity, which is expressed in terms of catalase coefficient (k)/ml/min. Solid circles indicate pH value of each fraction.

Effect of Aminotriazole on pI-Isozyme Pattern of Catalase

It is known that 3-amino-1,2,4-triazole (A. T.) produces a rapid fall in liver catalase activity, followed by a return over a period of several days to the steady-state level, which was resulted from a new synthesis of the enzyme (5, 18). An experiment was made to determine whether the pI-isozyme pattern of catalase in adult rat liver alters during the course of biosynthesis of this

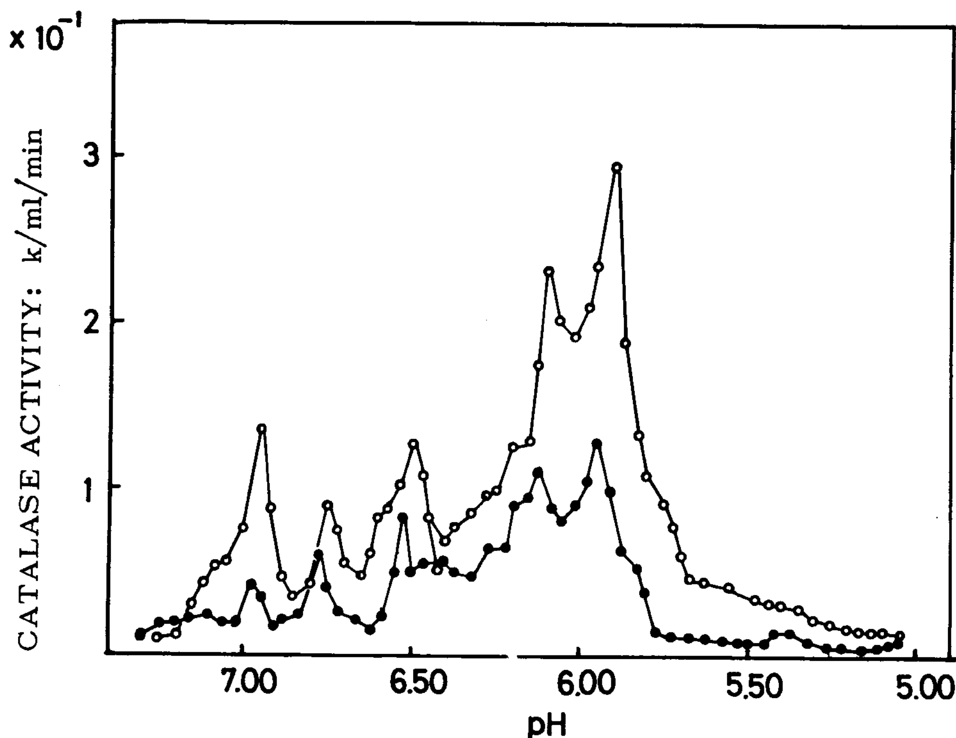

Fig. 2. Catalase pI-isozyme pattern in liver of rat after intraperitoneal administration of A. T. (1 g per kg of body weight) (—●—●—).

Livers were removed from animals at the 24th hour after injection of A. T. Catalase pI-isozyme pattern of untreated adult liver as control is also plotted (—○—○—). Catalase activity is expressed in terms of catalase coefficient (k)/ml/min.

enzyme. 24 hours after the injection of A. T., the enzyme activity showed one-third of that in normal liver. As seen in Fig. 2, all of the five catalase pI-isozymes were found, and also the general profile was observed to be similar to that in normal adult liver except for a low activity of each component.

pI-Isozyme Pattern of Catalase at Various Stages of Development

Catalase activity in the livers from fetal, newborn and adult rats was measured with supernatants of the homogenates centrifuged at 105,000 x g. As shown in Fig. 3, the enzyme activity

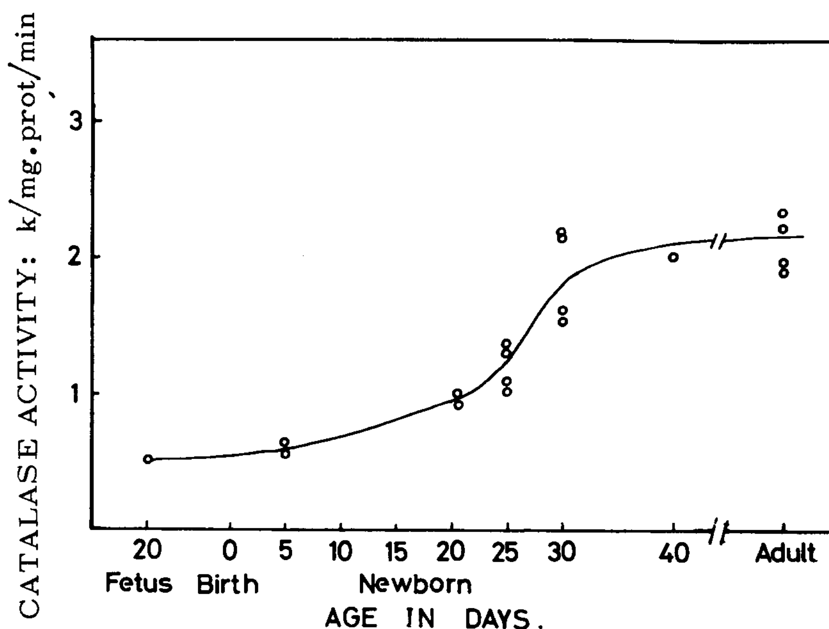

Fig. 3. Catalase activity in the liver during the course of growth of rats. Livers were removed from animals at fetal and various newborn stages, as shown. Catalase activity was determined in 105,000 x g supernatants of tissues, and the specific enzyme activity is expressed in terms of catalase coefficient (k)/mg protein/min.

in the 20 day-old fetal liver showed approximately one-third of that in the adult liver. After birth, the activity was found to be increased gradually until the third week. Then, a rapid rise in the activity was observed during the following about 10 days, and two-thirds of the normal catalase level were measured in 30 day-old animals.

Fig. 4-a shows the catalse pI-isozyme pattern of 20 day-old fetal liver. It demonstrates the existence of five different fractions each showing isoelectric points at pH 7.23, 7.13, 6.95, 6.75 and 6.50, respectively. The patterns of pI-isozyme appeared in 5 day-old and 3 week-old newborn rat liver (Fig. 4-b and c) were essentially the same as that seen in fetal liver. On or around the 25th day after birth, the isozyme pattern was observed in two forms, i.e., either the adult or fetal form (Fig. 4-d). Lastly, 30 day-old newborn animals showed the isozyme pattern analogous to that in adult liver (Fig. 4-e).

pI-Isozyme Pattern of Catalase in Regenerating Liver

In Fig. 5 are plotted mitotic indices determined at certain intervals during the first 2 days after partial hepatectomy. Mitotic figures appeared approximately at the 30th hour, increased rapidly showing a peak around the 36th hour after the hepatectomy. At this stage, catalase activity in the liver displayed an approximately 50% decrease. Fig. 6 illustrates the catalase pI-isozyme pattern of the regenerating liver at the 36th post-operative hour. Five components of the isozymes were found to have pI-values the same as those in control

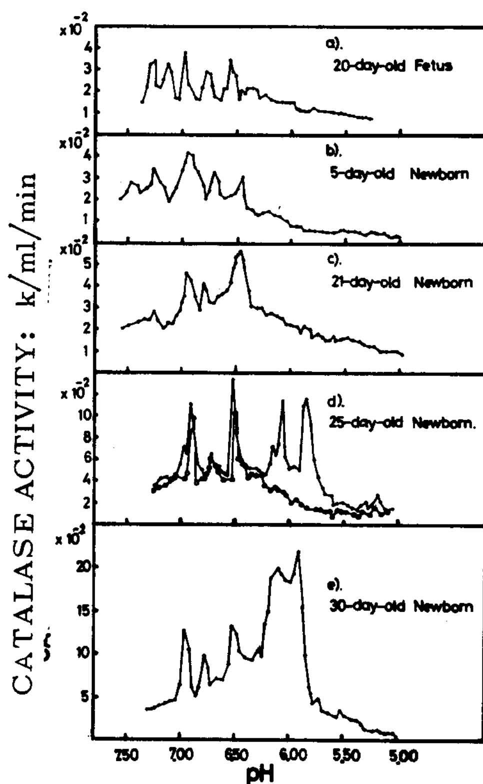

Fig. 4. Changes in pI-isozyme pattern of catalase in rat liver during the course of growth. Enzyme activity is expressed in terms of catalase coefficient (k)/ml/min.
Note a disappearance of basic catalase activity and an appearance of the enzyme activity of acidic catalase during the course of growth of rats.

liver. The activities of the pI-5.90 and 6.10 components were strikingly lower than those in control, though the activities of pI-6.95, 6.75 and 6.50 of fractions were the same as those in control.

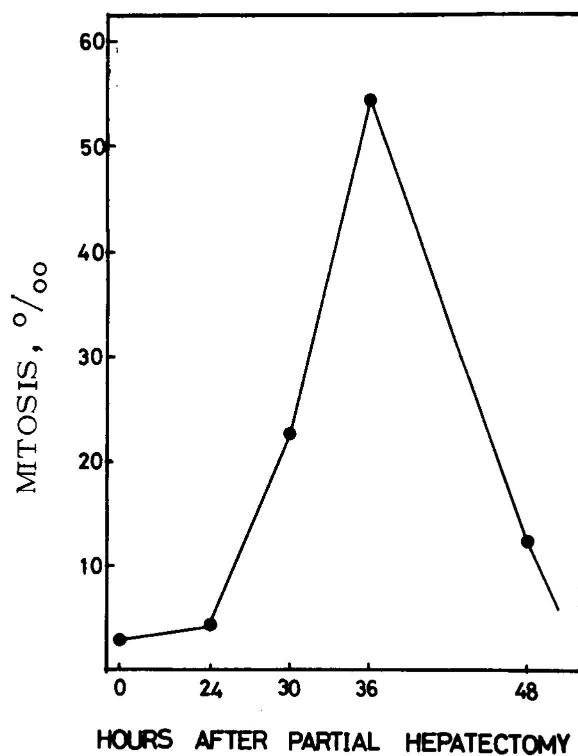

Fig. 5. Mitotic indices of hepatocytes at various time intervals after partial hepatectomy.

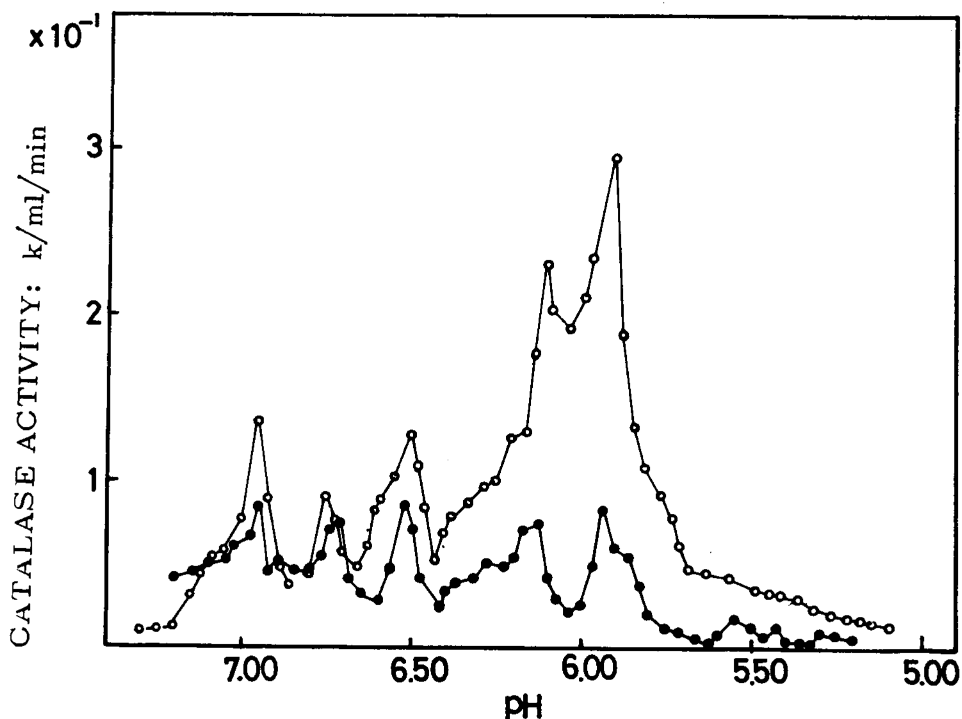

Fig. 6. Effects of partial hepatectomy at the 36th hour after operation on catalase pI-isozyme pattern (—●—●—).
The pI-isozyme pattern of adult rat liver as control is also plotted (—○—○—).
Catalase activity is expressed in terms of catalase coefficient (k)/ml/min.

pI-Isozyme Pattern of Catalase in Adult Rat Kidney

Catalase was reported to be found in peroxisomes in hepatocytes and epithelial cells of proximal tubules in the kidney (11). pI-isozyme pattern of catalase in the rat kidney was studied to determine whether it was different depending on the species of organs and/or tissues. In our experiments, an absence of the

pI-6.95 and 6.75 catalase activities was shown to be characteristic of the kidney tissues (Fig. 7).

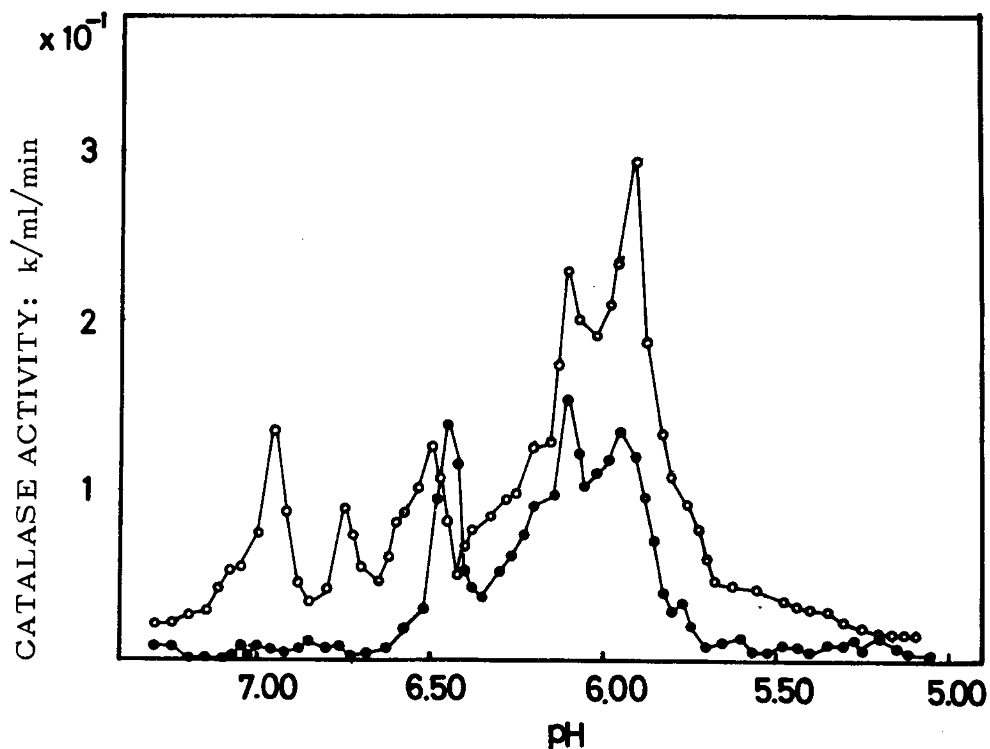

Fig. 7. A typical pattern of catalase pI-isozyme of adult rat kidney (—●—●—).
Catalase pI-isozyme pattern of rat liver as control is also plotted (—○—○—).
Catalase activity is expressed in terms of catalase coefficient (k)/ml/min. Note the absence of pI-6.95 and 6.75 catalase activities.

DISCUSSION

Nakamura, et al. (14) reported that catalase in the liver of normal rats could be divided into six or more different types; the major ones had pI values at pH 5.90, 6.08, 6.75 and 6.90 $\pm$ 0.04 by isoelectric fractionation using Ampholine-carrier Ampholite. These multiple forms of catalase were called catalase pI-isozymes. In our experiments, however, catalase in adult rat liver extracted with cold distilled water with the aid of sonication was separated into mainly five active components, pIs of which were pH 6.95, 6.75, 6.50 6.10 and 5.90, respectively. Repeated experiments showed that the activity-pH curves for each fraction were essentially the same and were reproducible. These data were also in agreement with catalase pI-isozyme pattern of Tetrahymena pyriformis (GL), as described by Koyama and Tsukada (12).

After the administration of A. T. into rats, the return of catalase activity in the liver was reported to occur in parallel with a corresponding uptake of Fe^{59} into the catalase, indicating that the return might result from the formation of new enzyme (18). During this de novo synthesis of catalase in rat liver, catalase pI-isozyme pattern appeared in five forms. This result suggests that all of the five catalase pI-isozymes in adult rat liver might be synthesized at a similar rate.

In fetal and early postnatal stages of rats, both cell differentiation and proliferation of hepatocytes are seemingly in progress. During these developmental stages, it was clearly verified that the time of appearance of each isozymal component of catalase in the liver was various. Namely, basic catalase (pI-7.23, 7.13) and neutral catalase (pI-6.95, 6.75 and 6.50) appear to develop early, and then are accompanied by an appearance of acidic catalase (pI-6.10 and 5.90) as well as by a disappearance of basic catalase around the 3rd to 4th week after birth. On the other hand, in an immunoelectrophoretic study on hepatic catalase of rats by Patton and Nishimura (17), it was reported that the anodic subcomponents of the enzymes appeared in fetal and early in newborn periods, while on the 14th postnatal day, the enzyme of the liver contained cathodic subcomponent, in addition to the above-mentioned anodic subcomponents. Futhermore, on the 25th day after birth the electrophoretic pattern was observed to be similar to that in the adult liver. Our data seems to be in good agreement with the results of Patton and Nishimura (17), with respect to the changes in catalase heterogeneity during postnatal development. Thus, the alteration in catalase pI-isozyme pattern during liver organogenesis may imply that a difference in the rate of synthesis of the individual catalase subcomponents exists at least between neutral and acidic catalase. These facts might be correlated with a specific functional need of liver tissues during the cell differentiation.

The absence of acidic catalase activity during prenatal and early postnatal stages might correspond to be immature state of the cell development, and may reflect a repression of the ability to synthesize acidic subcomponents of catalase. In the studies on Morris hepatomas in our laboratory (19), it was shown that acidic components of catalase appeared to be missing in the hepatomas, as in the case of fetal and early newborn liver. These changes in heterogeneous nature of catalase in hepatomas may indicate a reversion of catalase subcomponents to a more primitive or fetal nature. These alterations during the cell differentiation and proliferation of catalase pI-isozyme pattern have recently been observed by Koyama and Tsukada (12) on _Tetrahymena pyriformis_.

The concept of differentiation in protozoan is considered to differ in some respects from that in multicellular organizms. When _T. pyriformis_ of a maturated form was cultivated in exponential growth, only catalase of pI-5.85 was found to retain a stability and the other components disappeared. On the other hand, hepatocytes, in an proliferating stages after partial hepatectomy, exhibited all of the five major components of the isozymes, though acidic enzyme was significantly decreased. Hepatocytes, even in a proliferative regenerative phase, may essentially retain their specificity. In this aspect, a type of differentiation, which assures stable cell diversities in metazoan cells, is apparently lacking in _Tetrahymena_ cells. According to Fujita (3), metazoan cells had at least two

different types of differentiation, "major differentiation", which is virtually irreversible and determines the cell specificity in certain tissues, and "minor differentiation", which is usually reversible and related rather to functional states of the cells. These features of major and minor differentiation are the outstanding characteristics that distinguish the differentiation of metazoan from that of protozoan cells.

As to the heterogeneity of catalase in the kidney, Nishimura, et al. (16) reported that the heterogeneous pattern characteristic of the kidney was identical with that observed on the liver by immuno-electrophoresis. On the other hand, Holmes and Masters (10) described that the enzyme in kidney exhibited different mobility characteristics from that in liver by means of gel electrophoresis. In our results, catalase of rat kidney was found to lack in some of the neutral catalase components, i.e., pI-6.95 and 6.75. The pI-isozyme pattern is thus considered to be characteristic of tissue species. It might be important that the morphological and physiological changes during the organogenesis are probably associated with the changes in enzyme constitution which are not only characteristic of different organ species, but also developmental stages of the tissues.

SUMMARY

Catalase extracted from the liver of adult rats was distinguished into 5 main catalatically active components, with pI values at pH 6.95, 6.75, 6.50, 6.10 and 5.90, respectively, at 0°C by isoelectric fractionation with Ampholine-carrier ampholite. A. T. injected rat liver, which showed approximately one-third catalase activity of the control, also showed five multiple forms of the enzyme. Fetal and early newborn rat liver showed neutral components (pI-6.95 - 6.50) and basic components (pI-7.50 - 7.10) of catalase. As the animals grew older, the acidic components of catalase (pI-6.10 and 5.95) appeared and basic components disappeared around the 3rd to 4th week after birth. On the other hand, in regenerating liver after partial hepatectomy, catalase activity showed approximately half of that in the control, but all five multiple forms of the enzyme, including both neutral and acidic catalase, were observed, although a remarkable decrease in acidic components was found. Catalase extracted from the kidney of adult rats was resolved into three fractions, which indicated isoelectric points at pH 6.50, 6.10 and 5.90. Kidney catalase was observed to be lacking in pI-6.95 and 6.75 enzyme activities.

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