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STUDIES ON RAT LIVER RIBONUCLEASES

IV. LIVER RIBONUCLEASES IN DEVELOPING, 2-ACETYLAMINOFLUORENE FED AND PARTIALLY HEPATECTOMIZED RATS

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SUMMARY

A relationship was found between the activity of three ribonucleases and liver growth.

All three liver ribonucleases showed high activity in fetuses 3 days before birth, in new-borns and in suckling rats; these enzyme activities decreased gradually and attained the adult level between 30 to 40 days of age. Acid phosphatase was relatively constant during the developing stage of the rat.

Acid ribonuclease (*i.e.*, ribonuclease I) increased soon after feeding 2-acetylaminofluorene while acid phosphatase decreased. Ribonuclease II and ribonuclease III increased during the first 5–6 weeks and decreased subsequently for approx. 10 weeks before returning to the control level.

In regenerating liver, all three ribonucleases were increased soon after partial hepatectomy with a maximal increase at about 4 h after the operation. Ribonuclease II activity was significantly decreased from 8 h to 24 h. Ribonuclease I and ribonuclease III remained increased up to 72 h after partial hepatectomy.

The possible implications of the relationship between ribonuclease activities and phenomena of liver growth is discussed.

INTRODUCTION

A number of ribonucleases are known to exist in rat liver[1], and a few of them have been partially purified[2,3] and their enzymic specificity determined. The biological functions of these ribonucleases, however, are largely unknown.

One purpose of this study is to investigate a possible relationship between the activity of ribonucleases, and the phenomenon of growth.

This report is on studies of three known cytoplasmic ribonucleases[4–7] in rat liver during normal growth as well as during some types of abnormal growth. As

models of abnormal growth, we studied the livers from rats fed the hepatocarcinogen 2-acetylaminofluorene, which causes hepatocyte hyperplasia prior to the appearance of hepatomas[8], and the regenerating liver of partially hepatectomized rats.

Our data show a clear relationship between the phenomenon of growth, both normal and abnormal, and the activities of these three ribonucleases.

MATERIALS AND METHODS

Rats of SD/Anl (Anl 66) strain were fed *ad libitum* on a standard laboratory chow diet. For experiments on fetuses and new-born rats, pregnant females of known gestational age were obtained from the animal facility of the Biology Division at the Argonne National Laboratory. Since liver ribonuclease activities are not found different between male and female rats, both sexes were used in these experiments.

2-Acetylaminofluorene was mixed at a level of 0.01–0.02% into a nutritionally adequate diet containing 30% protein, and the diet was pelleted. (General Biochemicals, Chagrin Falls, Idaho). Groups of 3 animals were used for each time point, and 3 control rats of the same age were also killed during each experiment. The rats were 30 days old at the beginning of the experiment, and the duration of 2-acetylaminofluorene feeding was 140 days.

Partial hepatectomy, resulting in the removal of about 70% of the entire liver, was performed by the method of HIGGINS AND ANDERSON[9]. All hepatectomies were performed between 9:30 and 10:30 a.m. The two lobes of liver removed were used as the control for the corresponding hepatectomized animal.

Liver homogenate was prepared in the same manner as was described previously[7].

Acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) was assayed as described by WATTIAUX AND DE DUVE[10], acid ribonuclease or ribonuclease I (ribonucleate pyrimidine-nucleotido-2'-transferase, EC 2.7.7.16) and the ribonuclease III (ref. 7) (alkaline ribonuclease with pH optimum at 9.5) were assayed in the presence of liver supernatant, which inhibits the ribonuclease II (alkaline ribonuclease with a pH optimum at 8.0 (ref. 5)). The ribonuclease II was assayed as follows: the liver homogenate was prepared in 0.25 M sucrose in the presence of 0.02 M acetate buffer at pH 5.0. The acidified homogenate was then heated at 60° for 30 min to inactivate the ribonuclease I and ribonuclease III (ref. 7). The incubation medium for ribonuclease II contained $4 \cdot 10^{-4}$ M *p*-chloromercuribenzoate, which inactivates the natural inhibitor for the ribonuclease II.

For all three ribonucleases, a volume of 0.2 ml liver homogenate (equivalent to 20 mg liver wet weight) was used, and the time of incubation was 60 min. Under these conditions, we found that the values of our enzyme determinations varied not more than 15% between the animals used for each experimental point.

Protein nitrogen was determined by nesslerization of H_2SO_4–H_2O_2 digests.

RESULTS AND DISCUSSION

Fig. 1 shows the activities of all the three ribonucleases as well as acid phosphatase in fetal, new-born, and developing rats. Liver ribonucleases in fetuses 3 days before birth were found to have as much as 200–300% specific activity of the adult

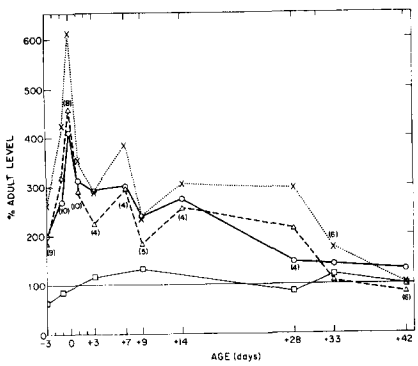
level; these ribonucleases reached a peak at birth and then decreased and attained the adult level at about 2 weeks after weaning (28 days after birth). Ribonuclease activities at birth differ significantly ($P > 0.001$) from those of all other ages, whereas the activities at days 3, 7, 9 and 14 do not differ significantly from each other. By contrast, acid phosphatase activities of fetal, new-born and developing rats were not markedly different from the activity level of the adult liver. From the results shown in Fig. 1, the following points are made.

(1) High enzyme activities of all three ribonucleases are found in livers of fast growing rats. The relation of ribonuclease activity to growth has been reported in bacteria[11,12] and in plants[13–15], as well as in mammalian tissues[16,17]. In all these cases, the ribonuclease activity (in refs. 11–17, only one of the ribonucleases was reported) was found to be increased during the period of increased growth. BRESNICK, SAGE AND LANDOS[16] reported that in the nuclei of fetal rat liver, one of the ribonucleases (*i.e.*, ribonuclease II) was found to be 10 times higher than that of the adult rat liver. In our present study, the liver homogenate was used for enzyme assays and no attempt was made to separate the nuclei from the cytoplasm of the liver cells. Because all three ribonucleases reported in the present paper are essentially distributed in all major groups of cytoplasmic particles (*i.e.*, in mitochondria and microsomes in the case of ribonuclease II and III, and mainly in lysosomes in the case of ribonuclease I (refs. 18, 19), we tentatively conclude that the increases of these three ribonucleases are not exclusively in a single group of subcellular particulates; hence most (or all) subcellular particles are involved in the process of growth.

(2) Ribonuclease I activity is markedly higher in fetal, new-born and young rats than in adult rats, while acid phosphatase activity is relatively constant at all age levels. Assuming that ribonuclease I and acid phosphatase are indeed localized within the same group of subcellular particles, *i.e.*, lysosomes, as has been suggested by DE DUVE[20], we have to presume that there is a difference in the ratio of ribonuclease I to acid phosphatase within the liver lysosomes of developing rats, on the one hand, and the liver lysosomes of adult rats, on the other. Another alternative explanation is that ribonuclease I has indeed a bimodal distribution, with one mode being in the lysosomes (within which this enzyme is more or less constant throughout the period of development), whereas the increased ribonuclease I activity belonged to yet another group of subcellular particles, different from the lysosomes, and it is the ribonuclease activity of this latter group which changes with the age of the rat. Further work is needed to clarify these possibilities.

(3) Since liver is a blood-forming organ during the embryonic stage of the rat, one can argue that part of the increased ribonuclease activities are indeed derived from the erythropoietic cells. While this possibility is not entirely ruled out by the present experimental results, it seems unlikely because the enzyme activities in the fetuses increase as birth approaches (Fig. 1), whereas the hematopoietic activity of the liver is known to decrease during this period[21].

(4) From the available data on changes of various enzymes in liver of fetal, new-born, and young rats[22–27], most enzymes in embryonic and early postnatal periods showed neglibible activity, but this increased soon after birth, with the exception of the phospholipid-synthesizing enzymes[25], and the activities of hexokinase and phosphofructokinase[27] which were shown to be much higher in the liver of the fetuses and the new-borns than in adult liver. The pattern of ribonuclease



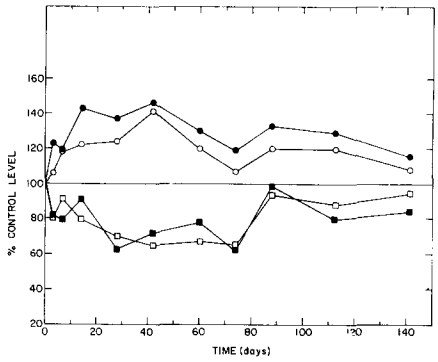


Fig. 1. Liver ribonucleases and acid phosphatase activities in fetal, new-born and developing rats. The enzymes are expressed in percent of specific activity of the adult level. The specific activity of the ribonucleases are expressed as: ΔA/mg nitrogen per min, and the acid phosphatase as μg phosphorus/mg nitrogen per min. The numbers in parentheses represent the number of individual animals used for all the enzyme determinations. The adult level of all the enzymes is based on the average value obtained from 30 adult rats. ○—○, ribonuclease I; △— — —△, ribonuclease II; × · · · ×, ribonuclease III; □—□, acid phosphatase.

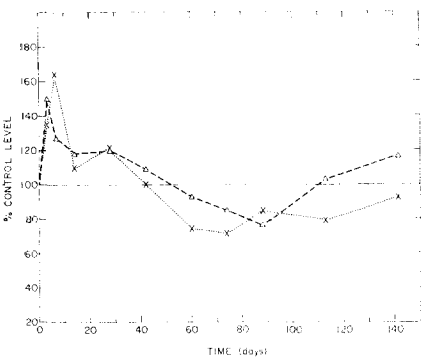
Fig. 2. Liver ribonuclease I and acid phosphatase activities of rats fed 2-acetylaminofluorene. The enzymes are expressed in percent of specific activity of the control rats. ○—○, ribonuclease I of rats fed 0.01% 2-acetylaminofluorene; ●—●, ribonuclease I of the rats fed 0.02% 2-acetylaminofluorene; □—□, acid phosphatase of rats fed 0.01% 2-acetylaminofluorene; ■—■, acid phosphatase of rats fed 0.02% 2-acetylaminofluorene.

activities reported in the present paper is another example with a similar result, whereas the pattern of acid phosphatase activity is relatively constant throughout the developing period of the animals. Further work is required to understand the biological functions of these RNA-degrading enzymes with respect to both RNA and protein synthesis during the early developmental stages of the mammalian liver.

Fig. 2 shows the specific activity of ribonuclease I and acid phosphatase in liver of rats fed 0.01% and 0.02% 2-acetylaminofluorene. The activity of ribonuclease I is significantly increased as soon as 3 days after the carcinogen feeding when, at the same time, the activity of acid phosphatase is clearly decreased. This difference in response between the two enzymes can be seen throughout the experiment, with a maximal difference at 40 days of 2-acetylaminofluorene feeding, after which time both enzymes tend to return to the control level at day 140. Expression of the activity based either per g of liver or on per mg of nitrogen gave similar results.

Fig. 3 shows the changes of ribonucleases II and III during 2-acetylaminofluorene feeding. Both of these ribonucleases show increased activity during the first 3 weeks, with a peak around the first week, but decreased subsequently during the entire period preceeding the appearance of tumors in the livers which occurred at about 100 days after 2-acetylaminofluorene feeding.

Based on results shown in Fig. 2 and Fig. 3, the following summary can be made: (1) acid phosphatase in liver of rats fed 2-acetylaminofluorene is clearly and specifically depressed while ribonuclease I is conversely activated. The present result is in general agreement with that of SHIBKO AND FRIEDMAN[28]. Ribonucelase I is generally found to be elevated during carcinogen feeding[1], the significance of this increase being unknown. (2) Ribonucleases II and III are found to have a similar



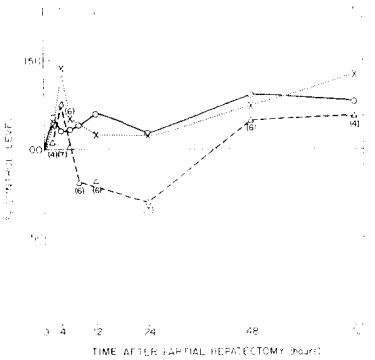


Fig. 3. Liver ribonuclease II and ribonuclease III activities of rats fed 2-acetylaminofluorene. The enzymes are expressed in percent of specific activity of the control rats. △ · · · · · △, ribonuclease II of rats fed 0.02% 2-acetylaminofluorene; × · · · ×, ribonuclease III of rats fed 0.02% 2-acetylaminofluorene.

Fig. 4. Activity of ribonucleases of regenerating rat liver. The enzymes are expressed in percent of specific activity of the control liver. (The two lobes of liver removed were used as each corresponding control.) The number marked at each time point represents the number of individual animals used for that particular time point. ○—○, ribonuclease I; △— — —△, ribonuclease II; × · · · ×, ribonuclease III.

response during 2-acetylaminofluorene feeding. (3) All three ribonucleases are increased soon after the beginning of 2-acetylaminofluorene feeding. Ribonucleases II and III, however, are subsequently decreased when ribonuclease I is found elevated during the entire course of the experiment.

Fig. 4 shows the activity of all three ribonucleases in regenerating rat liver. There is an increase of activity in all these three enzymes as early as 2 h after partial hepatectomy, and these increases reached a peak at 4 h. At subsequent times, ribonuclease I and ribonuclease III remained moderately increased, whereas ribonuclease II showed a significant decrease at 8,12 and 24 h after hepatectomy.

Our data are in good agreement with those of other investigators[29–31], who used fewer time intervals and studied only one or two of the ribonucleases.

RNA synthesis was reported to be stimulated soon after partial hepatectomy, and reached the maximum at 6 h (ref. 32). The increase of all three ribonucleases during the first few hours after partial hepatectomy as reported in the present paper may be related to their biological functions in RNA metabolism during liver regeneration. The decrease of ribonuclease II activity between 8 and 24 h after partial hepatectomy (Fig. 4) is yet another interesting phenomenon, which has now been reported in three independent studies (refs. 30, 31 and the present paper). SHORTMAN[31] also found that the inhibitor to ribonuclease II was increased beginning at 12 h after partial hepatectomy with a maximum at 48 h. Assuming that ribonuclease II inhibitor is equally active *in vivo*, this means that in regenerating liver (at least up to 48 h after operation), ribonuclease II activity is in reality much lower than found by the enzyme assay method used, since in all three studies (refs. 30, 31 and the present paper), ribonuclease II activity was assayed *after* the removal of its inhibitor.

There are indirect indications that ribonuclease II is responsible for the degradation of the mRNA in rat liver *in vivo*[33–35]. The fact that this enzyme was decreased

between 8 and 24 h after partial hepatectomy (Fig. 4), at the time when protein synthesis is at its maximum[36], as well as the fact that the polyribosomes in regenerating liver 14 h after hepatectomy were found more stable than the polyribosomes from control liver[37] seem to support this suggested biological function of the ribonuclease II. By the use of specific antibodies, we hope to be able to understand further the biological functions of this ribonuclease II in relation to the phenomena of liver growth.

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