PHOSPHATASES OF RAT LIVER

II. ADENOSINETRIPHOSPHATE DEPHOSPHORYLATION
IN REGENERATING LIVER*

BY ALEX B. NOVIKOFF, ESTELLE PODBER, AND JEAN RYAN

(From the Departments of Pathology and Oncology and of Biochemistry, College of Medicine, University of Vermont, Burlington, Vermont)

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In the dephosphorylation of ATP by adult rat liver homogenates, a major rôle is played by a magnesium-stimulated ATPase localized in the isolated mitochondrial fraction (1). However, in liver tumors, as in other tumors tested, ATP dephosphorylation is catalyzed mostly by an apyrase, stimulated equally by calcium and magnesium ions and localized in the isolated microsome fraction (2, 3). It seemed of interest, therefore, to study ATP dephosphorylation in “regenerating” liver in which cell division, although no less rapid than in tumors, lacks the relative autonomy from organismal influences which characterizes tumor growth.

The data to be presented show that rapidly growing regenerating liver resembles normal adult liver, rather than tumors, both in the general pattern of intracellular distribution of ATP-dephosphorylating activity and in the relative effectiveness of magnesium and calcium ions in stimulating this activity.

EXPERIMENTAL

Four male Sprague-Dawley rats, weighing 175 to 200 gm., were used in these experiments; they were given Purina dog chow and water ad libitum before and after surgery. Partial hepatectomies were performed according to the method of Higgins and Anderson (4); the animals were killed 70 to 72 hours later. The liver tissue removed at partial hepatectomy was used as the control for the regenerating liver of each animal (5). The extent of liver restoration was calculated, as in an earlier investigation (5), from the weights of the liver mass removed at the time of surgery and the total liver weights when the animals were killed; corrections were made for changes in animal weights. Random portions of the original and regenerating

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1 The following abbreviations are used: ATP = adenosinetriphosphate, ADP = adenosinediphosphate, A-5'-P = adenosine-5'-phosphate, PNA = pentose nucleic acid, DNA = desoxypentose nucleic acid.
livers were used for the preparation of microscopic slides and for the determination of water content. Homogenates in 0.88 M sucrose were prepared from the remaining liver. Six intracellular fractions were separated by the differential centrifugation procedure described elsewhere (6): nuclear fraction (NF), mitochondrial fraction (Mt), mixed fraction (Mx), two microsomal fractions (Mc1 and Mc2) and supernatant fluid (SF). Homogenates (H) and fractions were assayed for nitrogen content (7) and for PNA and DNA (8). ATP dephosphorylation was measured without addition of bivalent ions and in the presence of $10^{-3}$ M MgCl₂ or $10^{-3}$ M CaCl₂ (the "standard system" previously described (1)); these assays were performed after the homogenates and fractions had been frozen at $-25^\circ$ and thawed, since this often leads to a considerable increase in activity (6).

ATP-1 was used as substrate in the first two experiments, and ATP-2 in the last two. They were prepared in the following fashion: ATP-1, the disodium salt purchased from the Pabst Laboratories, Milwaukee, Wisconsin, was dissolved in water to a concentration of approximately 0.04 M (based on absorption of ultraviolet light at 2600 Å), adjusted to pH 6.8, and kept in frozen state until used. ATP-2, the dibarium salt, obtained from the Ernst Bischoff Company, Inc., Ivorytown, Connecticut,2 was dissolved in dilute acid and converted to the sodium salt by passage through a column of sodium-charged Amberlite IR-120. It was then diluted to 0.03 to 0.04 M, adjusted to pH 6.8, and frozen. When thawed and assayed by the method of Cohn and Carter (9), ATP-1 contained no A-5'-P, 4 per cent ADP, and 96 per cent ATP; ATP-2 had no A-5'-P, 7 per cent ADP, and 93 per cent ATP. Neither ATP preparation gave evidence of metallic or other impurities when tested by the method of LePage and Potter (10).

Results

The following facts indicate that the regenerating livers were actively growing at the time the animals were killed. (a) In all four experiments the PNA contents of the homogenates were higher in the regenerating than in the normal control livers.3 When expressed on a dry weight basis, the PNA increases were 20 to 44 per cent (average, 32 per cent), and when on a total nitrogen content, the increases were 19 to 37 per cent (average, 30 per cent); (b) 65 to 82 per cent (average, 72 per cent) of the original liver weights had been restored (see Novikoff and Potter (5)); and (c) the regenerating livers had numerous mitotic figures. Of 1000 cells counted in

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2 We are indebted to Dr. Edward Harvill for a generous supply of this ATP.

3 Among the isolated fractions, the "nuclear fraction" showed the greatest increase (46 per cent) in PNA/N, (cf. Price and Laird (11)). In the mitochondrial fraction the increase was 27 per cent; in the two microsome fractions it was 19 and 23 per cent. The "mixed fraction" showed essentially no increase, suggesting the presence of granules not present in either the mitochondrial or two microsomal fractions (see Novikoff et al. (6)).
each, nineteen to forty-six (average, twenty-nine) were in metaphase or anaphase in the regenerating livers, as compared with one to three (average, two) in the original control livers.

The average figures for the total ATP-dephosphorylating activities recovered in the six fractions were 100 and 104 per cent of those in the homogenates for normal and regenerating livers, respectively, in the presence of MgCl$_2$; 107 and 103 per cent in the presence of CaCl$_2$; and 69 and 74 per cent without added bivalent ions (see the legend to Fig. 1).

The activities of each of the isolated fractions are shown graphically in Fig. 1, and the values for each of the four separate experiments, as well as the averages, are given. The data demonstrate that (1) in regenerating liver, as in normal liver, magnesium ions are much more effective in stimulating ATP-dephosphorylating activity than are calcium ions, and (2) the general pattern of distribution of such activity among the isolated fractions is the same in regenerating as in normal liver.

Comparison of normal and regenerating liver may be made with either the activity or specific activity graphs, except for the "nuclear fraction." In that fraction the specific activity comparison is more valid, since the higher activity probably signifies only that a greater number of cells remained unbroken in the regenerating than in the normal liver. In the presence of calcium ions, the specific activities of regenerating liver were lower than those of its normal control liver in all fractions: 19, 10, and 10 per cent in NF, Mt, and Mx, respectively, and 27 and 36 per cent in Mc$_1$ and Mc$_2$. However, with magnesium ions, only the microsome fractions showed decreases: 36 per cent in Mc$_1$ and 47 per cent in Mc$_2$. In the case of the mitochondrial fraction (Mt), the regenerating liver had a higher level of specific activity in one of the four experiments, a lower level in one, and essentially the same levels in two; hence the average values were not significantly different. Similarly, the average specific activities of the "nuclear" and "mixed" fractions were not significantly different in regenerating and normal liver.

The results obtained with ATP-1 and ATP-2 were essentially the same, except for somewhat higher levels of activities with ATP-1, in all fractions (except the "supernatant fluid") with magnesium ions and in the mitochondrial and nuclear fractions with calcium ions. Earlier experiments (2), in which another ATP preparation was used, have not been reported here, since in the presence of calcium ions only 72 per cent of the homogenate activity was recovered in the isolated fractions. However, the data were similar to those shown in Fig. 1.

$^4$ The average specific activity (two experiments) in the regenerating liver (66 to 72 hours) was, for homogenate, 20 per cent less than that of the original liver with either magnesium or calcium ions; for the microsomes, with magnesium ions, it was 72 per cent less; and for mitochondria, with magnesium ions, it was 10 per cent less.
Fig. 1. ATP dephosphorylation by normal and regenerating liver of the rat. The graphs to the left show activity, expressed as micrograms of P liberated in 1 hour by 1 mg. of tissue (wet weight); the graphs to the right show specific activity, expressed as mg. of P liberated in 1 hour by tissue containing 1 mg. of nitrogen. Upper graphs, without added bivalent ions; middle graphs, in the presence of $1 \times 10^{-3}$ M CaCl$_2$; lower graphs, in presence of $1 \times 10^{-3}$ M MgCl$_2$. Normal liver, solid symbols; regenerating liver, open symbols. Lines are drawn through the averages, shown as larger circles; each of four experiments is shown by a different symbol. O and △, ATP-1; ▽ and □, ATP-2. Levels of homogenate activities (micrograms of P per mg. of tissue per hour), without added bivalent ions, O 12.2, △ 22.5, ▽ 7.0, □ 11.0 (average, 13.2); O 7.4, △ 20.0, ▽ 8.9, □ 11.0 (average, 11.9); with CaCl$_2$, 30.1, 37.8, 25.9, 26.1 (average, 30.0); 26.5, 29.5, 25.2, 21.8 (average, 25.8); with MgCl$_2$, 71.9, 88.3, 60.0, 64.2 (average, 71.1); 63.4, 74.9, 56.8, 48.4 (average, 60.9). Recoveries (in per cent of homogenate), without added bivalent ions, normal, 59, 73, 65, 78 (average, 69); regenerating, 57, 68, 92, 77 (average, 74); with CaCl$_2$, normal, 109, 105, 110, 103 (average, 107); regenerating, 118, 92, 103, 100 (average, 103); with MgCl$_2$, normal, 103, 90, 109, 98 (average, 100); regenerating, 107, 93, 108, 105 (average, 104).
DISCUSSION

The most significant feature of our data is the qualitative similarity of regenerating liver to normal adult liver. Whether growing rapidly or not, rat liver apparently has the same enzymatic machinery for dephosphorylating ATP.

It is difficult to evaluate the significance of the quantitative differences in ATP-dephosphorylating activities of the microsome fractions of regenerating and normal liver. The specific activities of MCI and Mcz, based on total nitrogen content, were approximately 30 per cent lower in the regenerating liver when calcium ions were present and 40 per cent lower when magnesium ions were present. From analyses of protein nitrogen on these fractions in two of the experiments (after the extraction of acid-soluble substances, lipides, and nucleic acids), the same appears to be true when the specific activities were based on protein nitrogen rather than total nitrogen. These decreases in ATP-dephosphorylating activity may not be related at all to the rapid growth of the regenerating liver, but rather to factors like reduced food intake following surgery. Rosenthal et al. (12) have observed that after 2 weeks of protein depletion the livers of rats lost 43 per cent of their original protein content and 50 per cent of their ATP-dephosphorylating activity.

The ATP-dephosphorylating activity of the mitochondrial fractions was no lower in regenerating than in normal liver when magnesium ions were present. From the slightly lower values (10 per cent) when calcium ions were used, it cannot be concluded that a decrease in enzyme quantity had occurred. It has been observed (1) that with some ATP preparations calcium ions were unable to yield full recovery of the homogenate activity in the isolated fractions, whereas magnesium did so. We have also found that after repeated freezing and thawing ATP-dephosphorylating activity of liver homogenates becomes very much reduced when calcium ions are used, at a time when magnesium ions still yield the original levels of activity.

Allard and Cantero (13) have recently reported experiments on the ATP-dephosphorylating activity of regenerating liver, using calcium ions as enzyme activator. They found the levels of activities in homogenates and isolated mitochondria to be much lower in regenerating liver than in normal liver. On the 3rd day following partial hepatectomy the activity of homogenates had decreased by 47 per cent and the mitochondrial fraction by 71 per cent. These differences are much larger than those which we found, even with calcium ions as activator. Our average values for re-

In fetal liver, too, ATP-dephosphorylating activity is stimulated much more by magnesium than by calcium ions, and the largest share of activity is found in the isolated mitochondrial fraction (2).
generating liver, in the presence of calcium, were only 14 per cent lower in the case of homogenate and 22 per cent in the case of the isolated mitochondria. The discrepancies between the two sets of data may be due to differences in the ATP preparations used. In our experience, commercial ATP preparations frequently contain considerable quantities of ADP and other phosphate esters which, under the conditions employed to test ATP dephosphorylation, are hydrolyzed at varying rates by different isolated intracellular fractions. On the other hand, it is possible that the discrepancies between the data of Allard and Canter0 and our own may reflect differences in factors such as the extent of "labile protein" depletion in the animals (12). In the absence of nitrogen values, it is not known whether in their experiments the regenerating liver showed decreases in protein content paralleling the decreases in ATP-dephosphorylating activity.

From what has been said earlier, it would appear that the quantitative levels of ATP-dephosphorylating activity of isolated fractions have considerably less significance when calcium rather than magnesium ions are used as activator (12, 14). It may be that the response of the enzymes to added bivalent ions is dependent upon delicately poised relationships which are altered during the isolation of fractions from the homogenate. These altered relations seem of importance when calcium ions are used for activation, but of little significance when magnesium ions are employed.

SUMMARY

1. Intracellular fractions isolated from 0.88 M sucrose homogenates of normal liver and rapidly growing liver after partial hepatectomy were analyzed for ATP-dephosphorylating activities without addition of bivalent ions and in the presence of magnesium and calcium ions.

2. There were no essential differences between regenerating and normal livers in either (a) the relative stimulation of ATP-dephosphorylating activity by magnesium and calcium ions, or (b) the qualitative pattern of distribution of this activity among the isolated fractions.

3. The quantitative levels of ATP-dephosphorylating activities in the microsome fractions of regenerating liver were lower than those of normal liver; in the mitochondrial fraction, when magnesium ions were present, the levels of activities were the same in regenerating and in normal liver.

4. Differences in the effects of calcium and magnesium ions are discussed.

BIBLIOGRAPHY

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ADENOSINE TRIPHOSPHATE
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