REDUCED RATE OF ATP CATABOLISM AND THE ENHANCED AMINO ACID INCORPORATING ACTIVITY OF REGENERATING RAT LIVER MICROSOMES

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It has been reported by several investigators (Hultin and Von der Decken, 1958; Hoagland et al., 1964; Cammarano et al., 1965) that the ability of cell-free liver preparations to carry out amino acid incorporation into protein increases markedly in the course of the vigorous regeneration following subtotal hepatectomy. The elevated activity was shown to be attributable both to the microsomal particles and to the soluble cytoplasmic fraction (Hoagland and Askonas, 1963; Cammarano et al., 1965). On the other hand, ribosomes derived from normal and regenerating liver proved to be equally efficient in mediating amino acid incorporation (Cammarano et al., 1965). It was inferred that the microsomal membranes of the normal liver contain, in excess over the amount present in the regenerating microsomes, a factor that inhibits protein synthesis (Hoagland et al., 1964; Cammarano et al., 1965). The results of the present study, though consistent with the above observations, suggest, however, that the enhanced amino acid incorporation capacity of the regenerating-liver microsomes is largely determined by their diminished ATP catabolism.

RESULTS AND COMMENTS

Comparative assays of amino acid incorporation into protein in cell-free extracts from normal and regenerating rat livers indicated that the outcome of the

experiment was critically dependent upon the amount of extract used in the test system. Thus, parallel experiments performed with increasing amounts of 15,000 xg

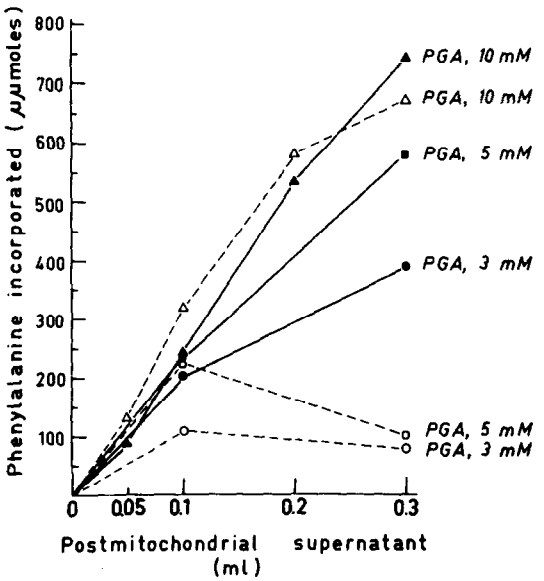

Fig. 1. Effect of varying the supply of ATP generator on the amount of amino acid incorporated by increasing concentrations of postmitochondrial fractions from normal and regenerating livers. In each group pooled livers from 5 male rats (starved overnight) were used. The minced livers were homogenized in 2 vol. of cold medium consisting of 0.25 M sucrose, 0.05 M Tris (pH 7.5), 0.025 M KCl and 0.001 M $MgSO_4$. The postmitochondrial 15,000 xg supernatants (S_{15}) of these homogenates (about 40 mg protein/ml) were added in the amounts indicated to the reaction mixture containing in a final vol. of 0.5 ml the following components (in μmoles): 25 Tris buffer (pH 7.5), 50 KCl, 5 $MgSO_4$, 0.2 (1-^{14}C) DL-phenylalanine (spec. act. 3 μC/μmole) 0.1 GTP and ATP-generating system. The latter system consisted of 0.5 μmoles ATP and the specified amounts of sodium 3-phosphoglycerate (PGA), and an excess of a 52-72% $(NH_4)_2SO_4$ - saturation enzyme fraction from rabbit muscle extract (Hershko et al., 1961). Following incubation for 30 min at 37°C, the reaction was stopped with 5% trichloroacetic acid (final conc.). Protein was isolated and the radioactivity was counted as previously described (Mager et al., 1965).

Dashed lines (open symbols): normal liver; solid lines (closed symbols): regenerating liver 22 hr after subtotal hepatectomy.

supernatants (S_{15}) from normal and regenerating liver homogenates, revealed that up to a certain level of S_{15} (equivalent to about 2 mg protein per ml reaction mixture) the two systems incorporated nearly equal quantities of (^{14}C)-labeled amino acid per mg protein. However, on raising the concentration of S_{15} two to three-fold above the critical level, the total counts incorporated by the normal-liver extract failed to increase or even declined beyond a certain maximal value, whereas the activity of the regenerating-liver S_{15} showed little or no departure from the expected linear course within the range of concentrations tested (Fig. 1). An essentially similar pattern of differential response was obtained, when graded amounts of microsomes from regenerating and resting liver were tested in the presence of a saturating quantity of the same postmicrosomal fraction (sap) derived from either normal or regenerating liver. Furthermore, by interchanging microsomes and saps from normal and regenerating livers, the overall behavior of the combined system was shown to be determined primarily by the origin of the microsomal particles and to a lesser extent by the source of the sap employed, both fractions acting in a cooperative fashion. In constrast, under the same experimental conditions, ribosomes from either source displayed closely similar incorporation yields, thus confirming the results of Cammarano et al., (1965).

It appeared likely that the divergent incorporation kinetics exhibited by the two systems reflected a difference in their respective rates of utilization of some essential reaction component, tending to become rate limiting in the course of incubation. The identity of the critical component with ATP was suggested by the finding that a suitable increase in the amount of phosphoglycerate, supplied as part of the ATP-generating system, induced in the normal-liver extract a substantial widening of the range of linear response to increasing enzyme concentrations. Moreover, in different experiments the increased supply of ATP generator greatly reduced or completely abolished the difference between the incorporation yields of the normal and regenerating-liver preparations. Similar results were obtained when the phospho-

glycerate system was replaced by equimolar amounts of phosphoenol pyruvate or creatine phosphate and an excess of the corresponding phosphotransferases. It appeared reasonable to assume that the lesser demand for ATP displayed by the regenerating microsomes was inherent in a diminished rate of ATP splitting relative to that of their normal counterpart. In fact, ATP-ase activity was found to be 30-50% lower in the regenerating than in the resting-liver microsomes (Table 1).

Additional support for the above interpretation was afforded by the correlation observed between the magnitude of amino acid incorporation and the efficacy of the ATP-generating system in maintaining a requisite level of ATP and preventing its exhaustion in the course of the incorporation process. Furthermore, microsomes from

TABLE 1. ATP-ase activity of microsomes from normal and regenerating liver

Amount of microsomal protein mg	ATP split μmoles		Pi released μmoles	
	Normal liver	Regenerating liver	Normal liver	Regenerating liver
2	2.8	1.7	3.2	1.7
4	5.5	3.6	6.2	4.2

The reaction mixture contained in a final volume of 1 ml : 90 μmoles Tris-chloride buffer (pH 7.5), 120 μmoles KCl, 12 μmoles $MgSO_4$, 10 μmoles ATP and the specified amounts of microsomes suspended in 0.05M Tris (pH 7.5)-0.15M KCl. At the end of 15 min incubation at 37°C the reaction was stopped by adding 1 ml of 1N perchloric acid. Following centrifugation, the supernatant fluid was neutralized with 0.5N $KHCO_3$ and used for determining ATP by the firefly luciferin-luciferase procedure of Strehler and McElroy (1957) and inorganic phosphate (Pi) by the method of Fiske and SubbaRow (1925). For other details see legend to Fig. 1.

regenerating liver proved to be much more efficient than their normal congeners in utilizing an excess of ATP (8-20 mM), in the absence of an ATP generator, as a source of energy for mediating amino acid incorporation into protein (Table 2). In this respect, therefore, the behavior of regenerating microsomes resembled that of ribosomal preparations, in which ATP-ase activity is of a negligibly low order (cf. Korner 1961). The significance of the ATP catabolism was further attested to by the observation that the adverse effect of ATP excess on amino acid incorporation

TABLE 2. Correlation between the extent of ATP splitting and the magnitude of its requirement for amino acid incorporation by normal and regenerating microsomes.

Additions per ml reaction mixture		Normal liver		Regenerating liver	
$ATP\text{-}Mg^{++}$ μmoles	Creatine phosphate μmoles	Phenylalanine incorporated into protein μμmoles per mg microsomal RNA	ATP found at the end of incubation μmoles per ml reaction mixture	Phenylalanine incorporated into protein μμmoles per mg microsomal RNA	ATP found at the end of incubation μmoles per ml reaction mixture
1	5	130	0.0	257	0.26
1	15	290	0.72	303	0.84
8	0	24	2.5	65	4.7

The reaction mixture was the same as in Fig. 1, except that the ATP - generating system consisted of an equimolar mixture of ATP and Mg^{++} and creatine phosphate in the amounts specified and 20 μg creatine kinase (EC 2.7.3.2). The cell-free extract was composed of microsomes (6 mg protein) and 100,000 xg supernatant (8 mg protein). The complete reaction mixture was incubated for 5 min at 37°C and the reaction was terminated by adding an equal volume of 1M perchloric acid. Following centrifugation, amino acid incorporation into protein was determined in the sediment and a sample of the supernatant fluid was used for ATP estimation by the procedure described in Table 1. Other details as in Fig. 1.

was largely eliminated in the presence of an ATP-generating system, presumably by obviating the accumulation of inhibitory products of ATP breakdown. It should be pointed out that in all these experiments ATP was added together with equivalent amounts of Mg^{++}, so as to counteract the deleterious effect of its metal chelating action.

The present data seem, therefore, to warrant the conclusion that the diminished ATP-splitting activity of the liver microsomes during the restorative phase following subtotal hepatectomy constitutes a major determinant of their augmented capacity for protein synthesis in vitro. Work is being pursued to elucidate the various aspects of this phenomenon, as well as the contributory role of some other factors in enhancing the amino acid incorporating activity of the regenerating microsomes.

Cammarano, P., Melli, M. and Novelli, G.D., Biochim. Biophys. Acta, 108, 329 (1965)
Fiske, C.H. and SubbaRow, J., J. Biol. Chem., 66, 375 (1925)
Hershko, A., Amoz, S. and Mager, J., Biochem. Biophys. Res. Comm., 5, 46 (1961)
Hoagland, M.B. and Askonas, B.A., Proceed. Nat. Acad. Sci., 49, 130 (1963)
Hoagland, M.B. Scornik, O.A. and Pfefferkorn, E.C., Proceed. Nat. Acad. Sci., 51, 1184 (1964)
Hultin, T. and Von der Decken, A., Exper. Cell Res., 15, 581 (1958)
Mager, J., Bornstein, S. and Halbreich, A., Biochim. Biophys. Acta, 95, 682 (1965)
Strehler, B.L. and McElroy, W.D., in Methods in Enzymology, vol. III, ed. Colowick, S.E. and Kaplan, N.O., Academic Press, Inc., New York, 1957, p. 871.