A PARTICULATE FRACTION OF LIVER CONTAINING A RIBONUCLEASE-RESISTANT SYSTEM FOR AMINO ACID INCORPORATION INTO PROTEIN

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During a study of the influence of diet on liver ribonucleic acid (RNA) metabolism (Prosser, Mallinson & Munro, 1960), cell sap was prepared by centrifuging rat liver homogenates for 1 hr. at 105,000 g and was then fractionated by more prolonged centrifugation to yield a post-microsomal pellet (Goldthwaite, 1959). When this pellet was incubated for 10 min. with ATP and ^{14}C-leucine but without cell sap, incorporation of the labelled amino acid occurred to an appreciable extent, the greatest activity being observed with pellets prepared from protein-depleted animals. Although the post-microsomal pellet is rich in RNA, preliminary experiments indicated the absence of soluble RNA (sRNA). The nature of the incorporation was therefore studied further.

Methods

Liver cell fractions were prepared from protein-depleted rats, since our earlier experiments had shown more active uptake of leucine into the RNA of post-microsomal pellets obtained from such animals. Male rats were fed a protein-deficient diet for 5 days before homogenising their livers in 2.5 vol. ice-cold phosphate-buffered medium (Rendi & Campbell, 1959). After spinning at 18,000 g for 1 hr. to remove nuclei, mitochondria and heavier microsomes, the homogenate was centrifuged at 105,000 g for 1 hr. to provide a light-microsome pellet. The supernatant fluid from this spin was then centrifuged for a further 3 hr. at 105,000 g to yield a second pellet (post-microsomes) and a final cell sap. The microsomal and post-microsomal pellets were resuspended in medium. These suspensions and the cell sap were each adjusted to pH 5.2, the resulting precipitates separated and redissolved in medium, and assayed for protein by the method of Lowry, Rosebrough, Fair &

Randall(1951) and for RNA content by ultraviolet measurements after perchloric acid (PCA) extraction.

Amounts of the three cell-fraction preparations containing approximately the same quantity of RNA were incubated individually with ATP and ^{14}C-leucine in the presence or absence of ribonuclease (RNAase). The reaction was terminated with ice-cold 0.4 N PCA, the precipitate washed three times with 0.2 N PCA at 0° and then with lipid solvents. The dry powder remaining was either (a) dissolved in N NaOH, plated directly on planchets using lens-paper (Garrow & Piper, 1955), and counted with a windowless flowcounter (total activity), or (b) extracted twice with 0.4 N PCA at 70° for 20 min., the residue being then plated and counted as above (hot PCA residue activity). The difference between the total and PCA residue activity provides the hot PCA soluble activity (Tables 1 & 2).

Results

Table 1 shows the uptake of radioactivity by each cell fraction when incubated with ATP and ^{14}C-leucine for 1 hr. The greatest activity extractable with hot PCA was obtained with the cell-sap preparations; this activity, presumably associated with RNA, was wholly suppressed by adding RNAase at the start of incubation. A smaller but still significant amount of radioactivity was obtained in the hot PCA extracts of the post-microsomal preparations and was also RNAase-sensitive. The microsome fractions showed little uptake of label which could be extracted with hot PCA.

Incorporation of leucine by the protein (hot PCA extracted residues) of the cell sap and the microsomes was slight. However, incorporation into the protein of the post-microsomal pellet was considerable and addition of RNAase at the start of incubation slightly stimulated this uptake. Because the incubation mixtures for each fraction had been adjusted to provide the same initial RNA content, the post-microsomal pellet preparations contained less protein per tube than the other two fractions (Table 1); consequently, the difference in uptake between the post-microsomes and the other two fractions is accentuated when related to the amount of protein incubated.

Using detergents to disrupt liver microsomes, Zalta (1960) obtained evidence of a RNAase-resistant system for amino acid

TABLE 1

Effect of ribonuclease on ^{14}C-leucine uptake by different liver-cell fractions

Cell fraction	RNAase added	RNA at end of incubation	Hot PCA soluble activity	Hot PCA residue activity
		per cent	cts/tube	cts/tube
Cell sap	0	71	978	21
	+	4	9	19
Post-microsomes	0	48	53	119
	+	10	10	147
Microsomes	0	59	13	9
	+	10	4	13

Amounts of each cell-fraction preparation were taken to provide approximately 225 µg RNA per tube initially; the initial protein content per tube averaged 4.56 mg for cell sap, 0.87 mg for post-microsomes and 1.01 mg for microsomes. The samples were incubated for 1 hr. at 37° in 1 ml of phosphate-buffered medium to which were added 10 µmoles ATP and 1 µC 1-^{14}C-DL-leucine (Rendi & Campbell, 1959). RNAase, when added, provided 300 µg per tube. Data are the average of 2 identical experiments.

incorporation. In some of his preparations, the combined addition of all four nucleoside triphosphates, an amino acid mixture and RNA resulted in a considerable increase in incorporation. In our system, inclusion of GTP, CTP and UTP increased total uptake of ^{14}C-leucine to about the same extent in the presence and absence of added amino acids (Table 2). However, the presence of the amino acids in the reaction mixture resulted in a redistribution of radioactivity when the triphosphates were added : the hot PCA-soluble activity fell by 234 counts and activity in the protein rose by 561 counts.

Discussion

Ribonuclease-resistant systems for amino acid incorporation have been described in bacteria (Beljanski & Ochoa, 1958; Wachsmann, Fukuhara & Nisman, 1960) and in fragments obtained from liver microsomes following the action of detergents (Zalta, 1960). Our studies suggest that RNAase-resistant incorporation into protein may be restricted to one fraction of the liver

TABLE 2

Effect of nucleoside triphosphates and an amino acid mixture on ^{14}C-leucine uptake by the post-microsome fraction

Additions	RNA at end of incubation	Total activity	Hot PCA soluble activity	Hot PCA residue activity
	percent	cts/tube	cts/tube	cts/tube
None	80	1283	322	961
GTP,CTP,UTP	59	1479	402 (+80)	1077 (+116)
Amino acids	61	1113	379	734
Amino acids + GTP,CTP,UTP	58	1440	145 (-234)	1295 (+561)

Conditions of incubation as in Table 1, the samples being incubated for 2 hr. at 37°. Nucleoside triphosphates added to give a final concentration of 60 mμmoles per tube; the amino acid mixture provided 5 mμmoles per tube of each of 17 L-amino acids (leucine being omitted). Data are the average of two identical experiments. Addition of the nucleoside triphosphates increased uptake into the PCA residue to a significantly greater extent in the presence of the amino acids (P less than 1%).

cell, the post-microsomes. Unlike Zalta's microsomal fragments, incorporation by the post-microsomes depends on the provision of ATP. There is reason to think that sRNA is not involved in the labelling process, since (i) the amounts of RNAase used were sufficient to remove any sRNA within the first 10 min. of incubation, yet labelling of protein has been obtained almost linearly for at least 2 hr. in the presence of RNAase; (ii) no sRNA fraction was observed when RNA extracted with phenol from the post-microsomes was separated on Ecteola columns; (iii) when phenol-extracted RNA from the post-microsomes was added to pH 5 enzyme preparations made from cell sap, it failed to stimulate uptake of labelled amino acids, although this system is sensitive to addition of phenol-extracted sRNA.

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