Lack of Influence of the Membranes of the Endoplasmic Reticulum on the Capacity for Protein Synthesis of the Rat Liver Polyribosomes

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The influence of the membranes of the endoplasmic reticulum on the functional properties of liver polyribosomes was studied in rats. The evidence seems to exclude the idea that the activity of the polyribosomes is under the control of the membranes.

When the liver is induced to synthesize protein at a rate higher than normal, the increase in protein-synthesizing activity is manifested by microsomal rather than by the detergent-treated polyribosomal fractions. This observation was made in regenerating liver (Campbell *et al.*, 1964, 1965, 1967; Campbell & Lowe, 1967) and in nephrotic (Marsh *et al.*, 1966) and phenobarbitone-treated animals (Kato *et al.*, 1966). From these results it was concluded that the rate of protein synthesis in rat liver is controlled by factors present in the membranes of the endoplasmic reticulum (Kato *et al.*, 1966). Since results obtained in this laboratory (Ragnotti, 1971) have cast some doubt on this interpretation, the occurrence of the control mechanism was reinvestigated by considering (i) if phenobarbitone had a significantly different effect on the amount of [^{14}C]leucine incorporated by the microsomal as compared with the detergent-treated polyribosomal fractions and (ii) if detergent treatment increased the protein-synthetic activity of polyribosomes from controls more than that of polyribosomes from phenobarbitone-treated animals.

Experimental

Male albino rats (Wistar strain) were starved for 48 or 96 h before being killed. A group was injected once daily intraperitoneally with phenobarbitone (Merck A.G., Darmstadt, West Germany) at a dose of 80 mg/kg body wt.

The reagents and media used and the experimental procedure adopted for the preparation of cell sap, microsomal and detergent-treated polyribosomal fractions have been described in detail by Ragnotti (1971). The microsomal fractions were prepared by sedimenting the postmitochondrial supernatant on a cushion of 2M-sucrose (B procedure; Ragnotti, 1971). [^{14}C]Leucine incorporation by subcellular fractions, radioactivity incorporated, density-gradient analysis of polyribosome profiles, determination of RNA and protein and the statistical treatment of the results were also as described previously (Ragnotti, 1971).

Results and Discussion

The results (Table 1) show that phenobarbitone administered to 48 h-starved animals significantly increased the activity of microsomal and detergent-treated polyribosomal fractions, provided the cell sap had not been treated with Sephadex. This clearly indicates that the capacity for protein synthesis of the microsomal fraction from 48 h-starved animals is not limited because of the membranes but because of an alteration in the composition of the cell sap. The incorporating activity of the microsomal and detergent-treated polyribosomal fractions was decreased by prolonging the period of starvation from 48 to 96 h. Also, in these latter experiments phenobarbitone significantly increased the rate of incorporation over that of the corresponding controls; this was also true when Sephadex-treated cell sap was used. However, in all situations tested, the phenobarbitone-induced increase of protein-synthesizing activity was very similar for microsomal and detergent-treated polyribosomal fractions.

Provided the cell sap had not been subjected to Sephadex chromatography, there was a significant increase in the incorporation observed in the detergent-treated polyribosomes compared with the microsomal preparations. This effect was seen in the livers from 48 h-starved animals, in both controls and phenobarbitone-treated animals. Smaller increases, none of which were significant, were observed for each of the other similar experimental comparisons shown in the Table. The effect of detergent treatment on the activity of polyribosomes was, however, very similar for control and experimental fractions.

The size distributions of the microsomal and detergent-treated polyribosomal fractions investigated in these experiments are shown in Fig. 1. In the controls, starvation decreases the amount of aggregation of the microsomal polyribosomes, the loss being proportional to the length of starvation. Very little, if any, degradation was observed in the profile of microsomal polyribosomes from phenobarbitone-treated

Table 1. *Protein-synthesizing activity of the microsomal and detergent-treated polyribosomal fractions from control and phenobarbitone-treated animals*

The microsomal and detergent-treated polyribosomal fractions, obtained from the same pooled livers, were prepared and incubated as specified in the Experimental section. Radioactivity incorporated is the mean $\pm$ s.e.m. of five (48 h) and three (96 h) experiments. The mean of RNA incubated (mg/ml of incubation mixture) was 0.096. The mean amount of cell-sap protein (mg/ml) incubated was 6.02 before and 5.28 after Sephadex treatment.

	Phenobarbitone ...	Microsomal fractions				Detergent-treated polyribosomes			
		-		+		-		+	
		Before Sephadex	After Sephadex	Before Sephadex	After Sephadex	Before Sephadex	After Sephadex	Before Sephadex	After Sephadex
(a) Animals starved for 48 h									
Radioactivity incorporated	...	14400	200750	22650*	228800†	20600‡	214600§	29900*†	238850†§
(c.p.m./mg of RNA)		± 550	± 3950	± 1800	± 13200	± 1350	± 6700	± 1750	± 11250
(b) Animals starved for 96 h									
Radioactivity incorporated	...	9150	105250	15850*	170400*	11650§	123350§	18200*§	186750*§
(c.p.m./mg of RNA)		± 900	± 7000	± 1350	± 10450	± 450	± 10200	± 350	± 4100

* Significance of difference between values for controls and phenobarbitone-treated rats, $P < 0.05$.

† Significance of difference between values for controls and phenobarbitone-treated rats, $P > 0.05$.

‡ Significance of difference between values for detergent-treated polyribosomes and microsomal fractions, $P < 0.05$.

§ Significance of difference between values for detergent-treated polyribosomes and microsomal fractions, $P > 0.05$.

Fig. 1. Sedimentation patterns of the microsomal and detergent-treated total polyribosomal fractions assayed for protein synthesis (Table 1)

The suspensions (0.5 ml), prepared as previously described (Ragnotti, 1971), were layered over a 12 ml linear gradient of sucrose (15–50%) in TKM1 buffer [50 mM-Tris-HCl (pH 7.8 at 20°C)–25 mM-KCl–5 mM-MgSO₄·7H₂O] and centrifuged for 150 min at 40000 rev./min in a Spinco SW40 (r_{av} , 11.1 cm) or in a International SB283 rotor (r_{av} , 10.3 cm). Arrows indicate the position of monomers. (a) Microsomal fractions from 48 h-starved (—) and 48 h-starved phenobarbitone-treated (----) animals (0.58 and 0.71 mg of RNA respectively); (b) microsomal fractions from 96 h-starved (—) and 96 h-starved phenobarbitone-treated (----) animals (0.57 and 0.57 mg of RNA respectively); (c) detergent-treated total polyribosomes from 48 h-starved (—) and 48 h-starved phenobarbitone-treated (----) animals (0.93 and 1.05 mg of RNA respectively); (d) detergent-treated total polyribosomes from 96 h-starved (—) and 96 h-starved phenobarbitone-treated (----) animals (0.49 and 0.50 mg of RNA respectively).

rats. Confirming previous results (Webb *et al.*, 1964; Munro, 1968), centrifugation of the polyribosomal suspensions through the 2 M-sucrose layer resulted in a loss of the lighter ribosomal species. Since the loss was not proportional to the amount present in the original microsomal fractions, a greater number of monomeric ribosomes, inactive in protein synthesis (Wettstein *et al.*, 1963; Norman *et al.*, 1973) was lost from the control than from the experimental fractions. This effect explains the somewhat higher rate of increment, over that of the microsomal fractions, of the activity of control than that of the polyribosomes isolated from phenobarbitone-treated rats.

Detergent-treated polyribosomes from 48 h- or 96 h-starved and starved phenobarbitone-treated animals show the same differences in incorporation capacity as the microsomal preparations from which they were derived, and this in spite of their identical sedimentation profiles.

This finding, together with the results previously discussed, clearly indicates that the membranes do not interfere in the regulation of the rate of protein synthesis, the activity of the polyribosomes from starved animals being decreased partly because of an intrinsic defect of the polyribosomes themselves in protein synthesis, partly because of a decreased degree of

aggregation and partly because of an alteration of the composition of the cell sap.

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