Polyribosomes in Rat Liver Slices During Incubation in vitro

Most of the studies on protein synthesis are now carried out with cell-free preparations, but tissue slices are still useful research tools, particularly in the field of cellular pathology. It is known that tissue slices can sustain a good protein synthesis for a long time and respond to changes in the environment with direct or mediated alterations in their metabolic activity, but little attention has been paid to the state of aggregation of the ribosomes in tissue slices engaged in protein synthesis. Therefore we have attempted to isolate and characterise the ribosomal population of rat liver slices incubated at 37 °C and tried to understand how polysomes break down during incubation.

The rats were starved overnight and killed by decapitation. The liver was quickly removed and sliced by hand with a razor. Slicing was done at room temperature; the slices were collected in Krebs-Henseleit saline under constant bubbling of $O_2 + CO_2$ (95:5)[1]. The same medium was used for the incubation at 37 °C. At the specified times, the medium was removed by suction and the slices were quickly blotted and transferred to a Potter tube containing ice-cold 0.25*M* sucrose in 50 m*M* *Tris*-HCl (pH 7.8), 25 m*M* KCl and 5 m*M* $MgSO_4$ (TKM), 50 slices (approx. 600–700 mg wet tissue) were homogenized in 3 ml of medium, with a Teflon pestle and 10 strokes at 500 rpm. The Post-mitochondrial supernatant (PMS) was obtained by centrifuging the homogenate at 12,000 *g* for 20 min; PMS was then treated with deoxycholate (1.3% final concentration) and 1 ml samples were layered over 14-ml linear gradients 15–50% (w/v) sucrose-TKM. Centrifugation was performed at 40,000 *g* for 3 h in the SB-283 rotor of the International B-60 centrifuge. The pattern of the gradients was recorded at 260 nm in a Gilford 2400 spectrophotometer, with the flow cell attachment, In the experiments with amino acids, the medium contained the mixture described by CLEMENS and KORNER[2], at 10 times the concentration found in rat serum. Cycloheximide, dissolved in isotonic NaCl, was injected into the femoral vein at the dose of 5 mg/100 g body wt., 5 min before killing the animals; cycloheximide (1 mg/ml) was also present in the medium where the slices were collected and incubated. Poly-U directed polyphenylalanine synthesis was studied with monosomes recovered from the gradients; incubations and determination of the radioactivity were carried out as previously described[3,4].

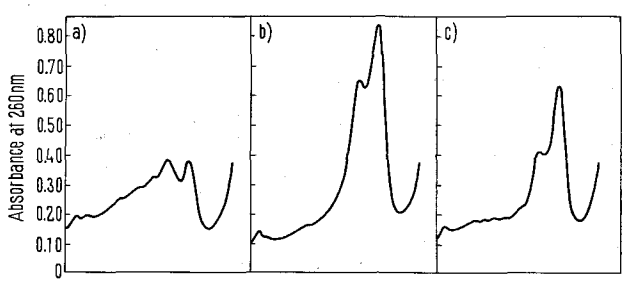

Fig. 1. Size-distribution patterns of rat liver ribosomes; post-mitochondrial supernatants from liver slices: a) at the start of the incubation, b) after 30 min at 37°C, c) after 30 min at 37°C in the presence of amino acids.

The ribosomal size-distribution patterns from liver slices before the start of incubation at 37°C have lost most of the heavier forms found in normal rat liver, but still retain a fairly good amount of polysomes (Figure 1a). After 30 min of incubation at 37°C, the polysomes disappear and the monomer and dimer peaks become very prominent (Figure 1b). We consider both these peaks as monosomes, since it is known that monomeric ribosomes from rat liver tend to dimerize during isolation[5]. In the presence of a full complement of amino acids, liver slices increase their rate of protein synthesis[2]; concurrently, after 30 min of incubation in the presence of amino acids, the ribosomal size-distribution pattern still contains a certain amount of polysomes, while the monosomes are much less than in the corresponding slices incubated in the absence of amino acids (Figure 1c). Thus, the presence of a high consentration of amino acids prevents the shift towards the monosomes, possibly by stimulating the ribosomes to become and (or) to remain attached to messenger RNA, as has already been shown to occur – more markedly – in vivo[6], in the perfused liver[7] and in a cell-free system[8]. In further experiments we have seen that the process of polysomal disaggregation occurs fairly promptly in incubated liver slices; patterns identical with Figure 1b are obtained as shortly as after 10 min at 37°C. Of the possible mechanisms of polysome breakdown occurring in liver slices during incubation, 2 in particular should be considered: 1. Metabolic release of the ribosomes from the messenger, with concurrent slowing down of the recycling process. 2. Enzymic degradation of the messenger RNA, linking the ribosomes together. The metabolic release of ribosomes, which run off from the messenger strand, should be prevented by agencies that inhibit the readout process and should yield monomers responsive to poly-U stimulation. On the contrary, monosome formation caused by enzymic (i.e. ribonuclease) action will not be affected by inhibition of the flow of ribosomes along the messenger; the resulting monomers, carrying a piece of attached m-RNA should not respond to poly-U. The patterns of Figure 2 show that the treatment with cycloheximide protects the polysomes of the incubated liver slices from degradation. Moreover, monosomes isolated from incubated slices respond to poly-U with an incorporation of labelled phenylalanine very similar to that displayed by monosomes isolated from

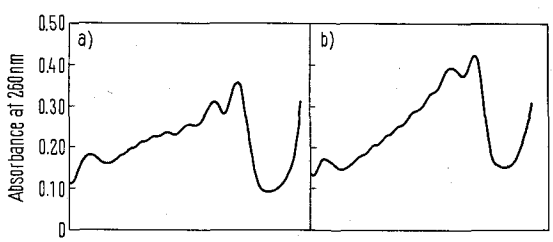

Fig. 2. Size-distribution patterns of rat liver ribosomes in the presence of cycloheximide. Post-mitochondrial supernatants from liver slices: a) at the start of the incubation, b) after 30 min at 37°C.

1 A. BERNELLI-ZAZZERA, G. GAJA and G. RAGNOTTI, Biochem. J. *100*, 114 (1966).
2 M. J. CLEMENS and A. KORNER, Biochem. J. *119*, 629 (1970).
3 G. RAGNOTTI, F. CAJONE and A. BERNELLI-ZAZZERA, Expl. molec. Path. *13*, 295 (1970).
4 F. CAJONE, G. RAGNOTTI, A. BERNELLI-ZAZZERA and M. BASSI, Expl. molec. Path. 14, 392 (1971).
5 T. E. WEBB and H. P. MORRIS, Biochem. J. *115*, 575 (1969).
6 W. H. WUNNER, J. BELL and H. N. MUNRO, Biochem. J. *101*, 417 (1966).
7 L. S. JEFFERSON and A. KORNER, Biochem. J. *111*, 703 (1969).
8 B. S. BALIGA, A. W. PRONCZUCK and H. N. MUNRO, J. molec. Biol. *34*, 199 (1968).

the corresponding liver (Table). These results suggest that the prompt degradation of polysomes in incubated liver slices is the outcome of a metabolic release from the messenger, rather than the consequence of enzymatic breakdown. The action of amino acids in the preservation of part of the polysomes can be satisfactorily explained in this context. The reason why liver slices, with a ribosomal population almost entirely reduced to monosomes, can still sustain a good and linear rate of protein synthesis for a fairly long time is not clear and will need further investigation.

Polyphenylalanine synthesis by monosomes isolated from a) liver or b) liver slices after 30 min incubation in vitro

	Time (min) 5	10	15
a) Liver	429,700	666,570	790,360
b) Liver slices	491,150	695,200	814,680

Results of a typical experiment: cpm/mg RNA.

Riassunto. Durante l'incubazione a 37°C i poliribosomi delle sezioni di fegato di ratto subiscono un processo di monomerizzazione. Il processo viene rallentato dalla presenza di forti concentrazioni di aminoacidi ed è inibito dal trattamento con cicloheximide. I monomeri che si formano durante l'incubazione in vitro rispondono al poly-U come i monosomi isolati direttamente dal fegato. Si ritiene quindi che la degradazione dei polisomi sia il risultato di un distacco metabolico dal messaggero con un rallentamento del riciclo dei monomeri piuttosto che la conseguenza di una azione enzimatica.

A. BERNELLI-ZAZZERA, F. CAJONE and LUISA SCHIAFFONATI[9]

Istituto di Patologia Generale dell'Università di Milano, Via Mangiagalli, 31, I-20133 Milano (Italy),
18 August 1971.

[9] Acknowledgments. The work was supported by a grant from the Consiglio Nazionale delle Ricerche. The IEC-B 60 centrifuge was a gift from Mrs. GIULIA DEVOTO FALCK.

The Active Form of Local Anesthetic Drugs

Most local anesthetics are secondary or tertiary amines and can therefore exist either as uncharged molecules or as ammonium ions, depending on the pK_a of the molecule and on the pH of the solution. The question whether the active form of the drug is the cationic or the undissociated one received considerable attention, but the experimental results are conflicting[1-14], because of the variety of methods employed and of the large number of parameters which are involved. Therefore the problem remains to be clarified; we therefore looked for a different experimental approach and carried out experiments on the action of tetracaine on the transfer of ions across a model of membrane, at different pH values.

Methods. The three-phases partition model system described by ROSANO and SCHULMAN[15,16] has been used because it behaves like a physiological membrane as to the different rate of transfer of various inorganic cations[17-19] and because the 2 interfacies are equivalent to monomolecular phospholipidic layers[15,16,20]. The model system allowed us to study the facilitated diffusion of ions from one water compartment to the other one, across a phospholipidic layer[21]. For this purpose we adopted a 3.5 mg/ml solution of brain cephaline (Koch and Light, Bucks.) in a diffusion blocking solvent, namely 1-pentanol+petrol ether 4:1. Salt solutions of KCl and $MgCl_2$ (both $\times 0{,}17\,M$) were allowed to exchange against NaCl and $CaCl_2$ ($0{,}17\,M$), which were placed in the opposite water compartment. The effect of local anesthetics has been studied by using 3 mM tetracaine in both water compartments. The pH value has been set at 5.0–7.3–9.3 with $0.01\,M$ piperazine buffer; in these conditions, tetracaine is dissociated >99%–90%–10%, respectively.

The ion concentration has been measured at different time intervals by atomic absorption (Atomspec, Hilger and Watts). The Figures are the average of 4 to 6 determinations.

Results. In the above-mentioned experimental conditions, a negligible passage of ions occurs by simple diffu-

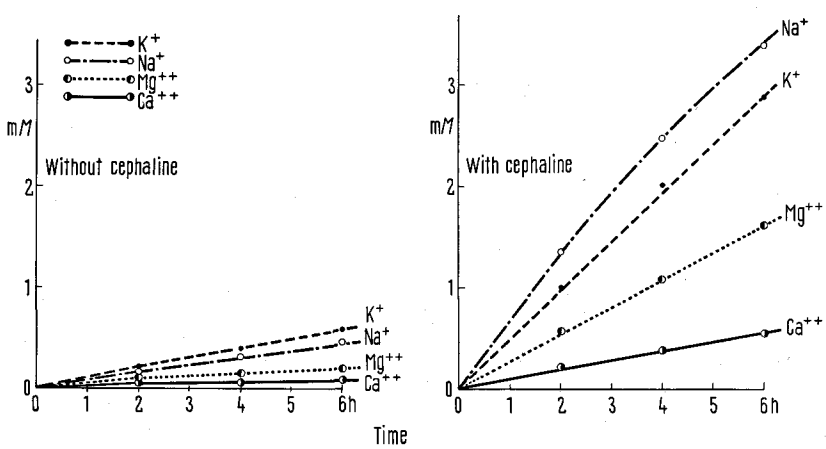

Fig. 1. Effect of cephaline (3,5 mg/ml) on the passage of Na^+, K^+, Ca^{++}, Mg^{++} accross pentanol-petrol ether 4:1. Water solutions: pH 5,0.