Tableau II. Activité de la glycogène phosphorylase en fonction de la température d'incubation (exprimée en pourcentage de l'activité observée à 25 °C)

Tissu	Nombre d'essais	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
Myocarde de Carpe	5	76,4 ± 6,6	84,6 ± 6,0	100	91,3 ± 4,1	78,2 ± 6,0	52,4 ± 7,2
Muscle rouge de Carpe	5	79,0 ± 7,8	88,7 ± 6,2	100	94,2 ± 3,2	73,1 ± 5,9	47,3 ± 8,8
Foie de Tanche	4	73,2 ± 8,0	86,9 ± 7,1	100	87,5 ± 6,1	71,6 ± 8,6	50,2 ± 10,1

Pourcentage moyen ± Sm. t 5%. Valeur arbitraire 100 à 25 °C.

même expérience, cette diminution était proportionnelle à la durée de l'incubation.

Le foie de Rat ou le foie de Tanche, poisson proche de la Carpe, incubés dans les mêmes conditions expérimentales, ont montré des activités normalement mesurables. Ce résultat très étonnant nous a amené à envisager plusieurs hypothèses que nous nous efforçons actuellement de vérifier.

Nous avons pensé qu'il était possible que dans le foie de Carpe, par suite d'une particularité structurale de l'enzyme, la réaction ne se fasse in vitro que dans le sens physiologique, la surcharge en glucose-1-phosphate étant soit insuffisante, soit inhibitrice pour l'enzyme.

Nous avons vérifié que des concentrations 10 fois supérieures en glucose-1-phosphate dans le milieu de réaction ne modifiait pas l'anomalie.

D'autre part, le foie de Carpe est en fait un hépato-pancréas (comme le foie de Tanche d'ailleurs). Aussi avons nous ajouté dans une série d'expériences un inhibiteur de protéase (extrait de l'œuf) pour prévenir une éventuelle dénaturation de l'enzyme par la trypsine pancréatique. Cela n'a donné aucun résultat.

Il se peut également que le phosphore libéré à partir du glucose-1-phosphate soit utilisé très rapidement par une autre réaction, malgré la présence de NaF et les conditions expérimentales très strictes utilisées classiquement pour ce dosage. Il resterait dans ce cas à expliquer pourquoi cette réaction parasite n'apparaît pas pour les autres tissus et en particulier le foie de Rat ou de Tanche.

On est peut être en droit de se demander si, dans tous les cas, la quantité de phosphore libéré, n'est pas la résultante de deux réactions enzymatiques, l'une libérant le phosphore, et en général dominante, et l'autre utilisant ce phosphore. Dans le foie de Carpe la deuxième réaction serait plus rapide que la première. Il conviendrait alors d'interpréter avec prudence les mesures d'activité de la phosphorylase.

Summary. In this paper we give some values of glycogen phosphorylase activity of heart, red muscle and white muscle from the Carp. We show that in vitro optimum temperature is 25 °C for the fish, and not 30 °C as in Mammalians. We also made measurements with rat and tench liver, but it has been impossible, under the same experimental conditions, to observe normal release of inorganic phosphorus from G-1-P with carp liver.

J. C. Murat, J. P. Parent et A. Serfaty

Université Paul Sabatier, Laboratoire d'Ecophysiologie animale, 38, rue des 36 ponts, F-31 Toulouse 04 (France), 25 Février 1972.

Distribution of 5 S RNA in the Liver of the South American Rattlesnake, *Crotalus durissus terrificus*

Since its discovery in *Escherichia coli*[1], 5 S RNA has been described in several classes of animals[2]. In all systems studied it was found attached to the large ribosomal subunit. However, there is no information about this type of ribosomal RNA in Reptilia. In the present study it was found that 5 S RNA of the liver of the South American rattlesnake is also attached to the large ribosomal subunit. The electrophoretic analysis on polyacrylamide gel showed that 5 S RNA of this snake has the same electrophoretic mobility as that extracted from honey bee (*Apis mellifera* L.) and *Escherichia coli*.

Material and methods. Snakes weighing 200–300 g were killed by decapitation and the livers were quickly removed and washed in cold physiological saline. The livers were homogenized in a Potter-Elvehjelm, teflon pestle, motor-driven homogenizer. The ribosomes were prepared from the tissue homogenate as described by Moldave and Skogerson[3]. The concentration of ribosomes was estimated on the basis of UV-absorption (1 unit of $A_{260\ nm}$ = 90 µg of ribosomes). Ribosomes were dissociated into subunits by EDTA treatment and by dialysis according to Martin et al.[4]. The subunits were separated by sucrose density gradient centrigufation. Gradient centrifugations were performed with 28 ml of a 10–30% (w/v) linear sucrose gradient containing 0.05 M KCl — 0.01 M *Tris*, pH 7.3. The tubes were centrifuged at 17,500 rpm for 14 h at 4 °C in the SW 25.1 rotor with Spinco model L ultracentrifuge. After centrifugation, the bottom of the tubes was punctured with a hypodermic needle and the absorbance at 254 nm was continuously recorded with Instrumentation Specialities Co. (ISCO), model UA-2 UV-analyser. In some experiments the fractions of the gradient were directly collected in 1 ml of distilled water and the absorbance at 260 nm

[1] R. Rosset and R. Monier, Biochim. biophys. Acta *68*, 653 (1963).
[2] M. Aubert, R. Monier, M. Reynier and J. F. Scott, Proc. 4th. FEBS Meeting, Oslo 1967 (Eds. L. O. Fröholm and S. G. Laland; Academic Press, New York 1968), p. 151.
[3] K. Moldave and L. Skogerson, in *Methods in Enzymology* (Eds. L. Grossman and K. Moldave; Academic Press, New York 1967), vol. 12, part A, p. 478.
[4] T. E. Martin, F. S. Rolleston, R. B. Low and I. G. Wool, J. molec. Biol. *43*, 135 (1969).

was read in a Zeiss spectrophotometer model PMQII. The subunits were precipitated with 3 volumes of absolute ethanol at −15 °C for at least 12 h and collected by centrifugation at 2,000 *g* for 30 min. The resulting pellets were suspended in 0.04 *M* *Tris*-HCl, pH 7.3, containing 0.02 *M* sodium acetate, 0.001 *M* EDTA and 0.1% sodium dodecyl sulphate. Aliquots of each ribosomal subunit were analyzed by electrophoresis on polyacrylamide gel (7.5%) according to LOENING[5]. The 5 S RNA of *Escherichia coli* used in these experiments was kindly supplied by Dr. T. YAMANE and the RNA of pupae of honey bee (*Apis mellifera* L.) was extracted as described by GREENBERG[6].

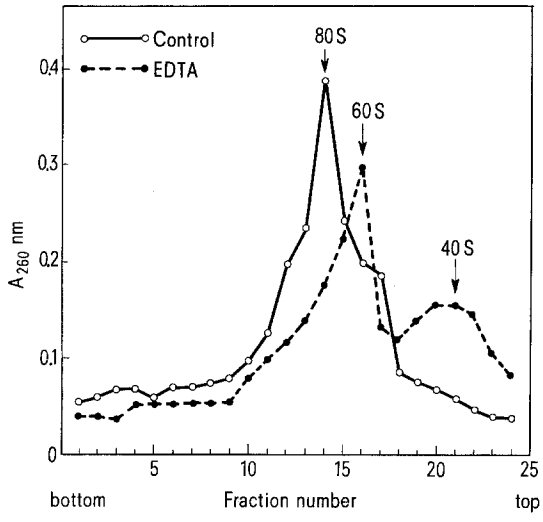

Fig. 1. Dissociation of ribosomes by EDTA treatment. Ribosomes were analyzed in a 10–30% sucrose gradient containing 0.05 *M* KCl – 0.01 *M* *Tris*, pH 7.3. Centrifugation was carried out at 4 °C with a SW 25.1 rotor at 17,500 rpm for 14 h.

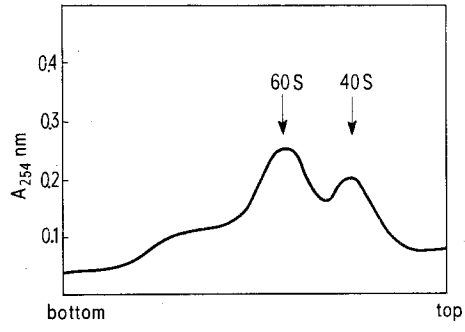

Fig. 2. Dissociation of ribosomes by dialysis. Analysis on sucrose gradient was performed as described in Figure 1.

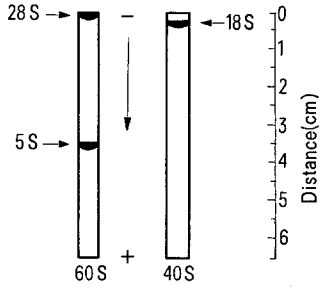

Fig. 3. Polyacrylamide gel electrophoresis of the RNA from the ribosomal subunits treated with sodium dodecyl sulphate.

Results and discussion. This report is the first investigation to demonstrate the ribosomal localization of 5 S RNA in Reptilia. Figure 1 shows the sedimentation profile of ribosomes isolated from the liver of rattlesnake and their dissociation by EDTA treatment. As can be seen, the ribosomes obtained using the same method described for the rat liver[3], sediment as a unique peak with a sedimentation coefficient of approximately 80 S. This finding indicates that ribosomes of this snake are obtained as monomers. Further studies are required to elucidate if the ribosomes are found as monomers in the cytoplasm of the living cells of snake liver, or if the polysomes are degradaded during their isolation. It was also observed that such monomers are unstable even when stored at −20 °C. The sedimentation profile of the ribosomes stored at −20 °C for 12 h shows a large amount of heterogenous material throughout the gradient. This suggests that our ribosomal preparations are contaminated with nucleases. It is well established that the addition of chelating agents (such as EDTA) to mammalian ribosomes promotes dissociation in their subunits[4,7,8]. This effect of EDTA was also observed with ribosomes of rattlesnake liver (Figure 1) and the resulting subunits were assumed to be 60 S and 40 S as described for other eukaryotes. However, electrophoretic analysis on polyacrylamide gel of RNA extracted from these subunits shows that EDTA treatment releases 5 S RNA of the large ribosomal subunit. A similar observation was made by COMB and SARKAR[9] with ribosomes of the aquatic fungus *Blastocladiella emersonii*. For this reason, we tried the dissociation of the monomers by dialysis in the conditions used by MARTIN et al.[4]. Figure 2 shows the sedimentation profile of ribosomes after dialysis. This result indicates that dissociation of ribosomes was not complete and a small shoulder can be seen in the region of the gradient corresponding to 80 S monomers. The samples obtained after alcohol precipitation of the two subunits and after treatment of the pellets with detergent (sodium dodecyl sulphate) in order to dissociate protein from ribosomal RNA, were submitted to electrophoresis on polyacrylamide gel. The result of a typical experiment is presented in the Figure 3. This finding shows clearly that 5 S RNA of the liver of the South American rattlesnake is attached to the large ribosomal subunit. On the other hand, 5 S RNA was never found in the soluble fraction of cell extracts.

The electrophoretic analysis on polyacrylamide gel showed that 5 S RNA of the liver of this snake and those extracted from pupae of honey bee (*Apis mellifera* L.) and *Escherichia coli* have the same electrophoretic mobility, at least in the conditions of ionic strength and pH used in these experiments. This result indicates that the molecular weight of 5 S RNA of the South American rattlesnake is of the same magnitude as those described in other organisms.

The findings presented here support the hypothesis that 5 S RNA is a universal component of living cells and is exclusively ribosomal.

[5] U. E. LOENING, Biochem. J. *102*, 251 (1967).
[6] J. R. GREENBERG, J. molec. Biol. *46*, 85 (1969).
[7] H. LAMFROM and E. GLOWACKI, J. molec. Biol. *5*, 97 (1962).
[8] Y. TASHIRO and P. SIEKEVITZ, J. molec. Biol. *11*, 149 (1965).
[9] D. G. COMB and N. SARKAR, J. molec. Biol. *25*, 317 (1967).

Résumé. Nous avons étudié la distribution du RNA 5 S dans le foie du serpent sud-américain *Crotalus durissus terrificus*. Nos résultats ont montré que le RNA 5 S de ce reptile est associé à la sous-unité ribosomique 60 S. D'autre part, la mobilité electrophorétique de ce RNA de faible poids moléculaire est la même que celle d'*Escherichia coli* et de pupe d'*Apis mellifera* L.

E. M. B. Rodrigues[10] and F. L. De Lucca[11]

Faculdade de Medicina, Departamento de Bioquímica, 14.100 – Ribeirão Preto (S. P.) (Brazil), 15 June 1972.

[10] Fellow of the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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De novo RNA Biosynthesis in Isolated Bone Marrow Nuclei

Bone marrow cells are in constant process of proliferation and differentiation, in which the nucleic acids occupy a central role[1]. Furthermore, there is convincing evidence that most of the RNA synthesis takes place in the nucleus of the animal cells[2]. Some of these RNAs are restricted to the nucleus while others are transported to the cytoplasm where they will form part of the biochemical machinery for the synthesis of the proteins[3, 4]. When the synthesis of different types of RNA is high, the requirements for RNA simple precursors of RNA must be very extensive.

In a previous paper we have shown[5] that isolated bone marrow nuclei are capable of synthesizing RNA from ribonucleotide triphosphate precursors, which are likely to be furnished by the cytoplasmic pool. It has also been found that isolated nuclei from both rat liver and Hela cells can incorporate labelled aminoacid into nuclear proteins[6]. These findings suggest that the nuclei of the animal cells have a large degree of autonomy, expressed in their capacity to synthesize complex macromolecules from simple precursors.

This paper describes experiments in which isolated bone marrow nuclei incorporated (^{14}C) formate and (^{14}C) glycine into the RNA purine bases. Using this criterion we show that the isolated rat bone marrow nuclei can synthesize RNA de novo from simple precursors. A preliminary report has already been given in abstract form[7].

Normal male rats of the Wistar strain weighing 230–270 g were used. The bone marrow nuclei were obtained as indicated by Perretta and Bosco[5]. The resulting nuclear pellet was washed[8] with 0.25 *M* sucrose solution containing 10^{-3} *M* MgCl2a and 0.1% Triton X-100 and twice rewashed with 0.25 *M* sucrose solution 10^{-3} *M* MgCl2. All the steps were performed between 0°–4°. The purity of the nuclei fraction was tested by examining the pellet by light and phase microscopy and for the presence of the cytoplasmic marker enzymes glucose 6 phosphate, succinic dehydrogenase, cytochrome oxidase and urate oxidase. In all cases, the nuclei were found to be free of cytoplasmic contaminants.

The purified nuclear fraction was suspended in 0.25 *M* sucrose containing 10^{-3} *M* MgCl2; 5 μCi of (^{14}C) formate or (U-^{14}C) glycine to a final volume of 1.25 ml at pH 6.5 was added and kept at 37 °C for variable periods of time. The reaction was stopped by the addition of cold 2.1 *M* perchloric acid and then the RNA and DNA were isolated following the method described by Smellie, Thomson and Davidson[9]. The DNA and RNA bases were separated by bidimensional chromatography and their concentrations were estimated by measuring the UV-absorption. The radioactivity was counted in a Nuclear Chicago glas-flow counter and expressed as specific activity in count per minute μmole of base.

The pattern of in vitro incorporation of (^{14}C) formate into the nucleic acid bases of isolated marrow nuclei is shown in Table I. Notice that the labelled precursor is extensively incorporated into the RNA purine bases while the DNA bases are not labelled. When the experiment was performed with previously boiled bone marrow nuclei, the RNA and DNA bases did not incorporate radioactivity. It is worth adding since that the thymine in DNA was not labelled, the incubation system was free from bacterial contamination.

In order to confirm de novo RNA synthesis, the uptake of a precursor that is incorporated in the first stages of the metabolic pathway was studied. The incorporation of (U-^{14}C) glycine into the RNA adenine is shown in Table II. As a control another nuclear sample incubated with

Table I. In vitro incorporation of (^{14}C)-formate into the purine bases of the nucleic acids of isolated rat bone marrow nuclei

Base analyzed	Specific activity Counts/min/μmole of bases obtained from RNA	DNA
Adenine	2,065	0
Guanine	1,302	0
Thymine		0

The results are of a typical experiment from a series of 10. Each value represents the average of 2 samples. The nuclei were incubated with 5 μCi of ^{14}C-formate for 2 h. The specific activity of the (^{14}C)-formate was 19 mCi/m*M* (New England Corporation). Other experimental conditions are indicated in the text.

[1] R. Y. Thomson, G. Ricceri and M. Perretta, Biochim. biophys. acta *45*, 87 (1960).
[2] J. E. Darnell, Bact. Rev. *32*, 262 (1968).
[3] K. Scherrer and Lise Marcaud, J. Cell. Physiol. *72*, 181, Sup. 1 (1968).
[4] W. K. Roberts and V. D. Quinlivan, Biochemistry *8*, 288 (1969).
[5] M. Perretta and Cleo Bosco, Experientia *26*, 594 (1970).
[6] E. Zirmmerman, J. Hackey, P. Nelson and I. M. Arias, Biochemistry *8*, 2636 (1969).
[7] A. Romero and M. Perretta, Archs biol. Med. Exptl. *7*, R84 (1970).
[8] W. Sierralta and M. Minguell, Bioch. biophys. Res. Comm. *41*, 50 (1970).
[9] R. M. S. Smellie, R. Y. Thomson and J. N. Davidson, Biochim. biophys. acta *29*, 59 (1958).