(1.33×10^{-6} M methyl D-galactopyranuranosyl residues), and the value of the V_{max} of the inhibited one was 1.8×10^{-6} mol min^{-1} and of the non-inhibited one 2.33×10^{-6} mol min^{-1}, the action of iodine on the isolated form of pectinesterase can be characterized as non-competitive.

In order to determine the number of molecules of iodine binding to the enzyme, a Hill plot was used. When log $[v_i/(v_o-v_i)]$ was plotted against the log $[I_2]$, where v_i is the enzyme activity in the presence of inhibitor and the v_o the activity without the inhibitor, a straight line was obtained (figure 3). The slope of this line (0.71) indicates that 1 molecule of iodine is involved in the interaction with the catalytic site of the pectinesterase.

The reaction of iodine with proteins can cause SH oxidation, or iodination of tyrosyl and histidyl residues; tyrosyl residues are iodinated more readily than histidyl residues[7]. To ascertain if the inhibition of pectinesterase with iodine could be due the oxidation of free SH-groups, the action of Ellman's reagent on the isolated form of pectinesterase was examined. Nevertheless Ellman's reagent in concentrations of 10^{-4}–10^{-3} M in phosphate buffer pH 8.0 was not found to be an inhibitor of 1.1×10^{-7} M pectinesterase. Based on the results obtained with the iodination of tomato pectinesterase, the presence of tyrosine in the enzyme's active site can be assumed.

7 W. L. Hughes, Ann. N. Y. Acad. Sci. *70*, 3 (1957).

Different salt concentration requirement of nuclear and cytoplasmic Mn^{++}-stimulated poly(A) synthetase activities from rat liver

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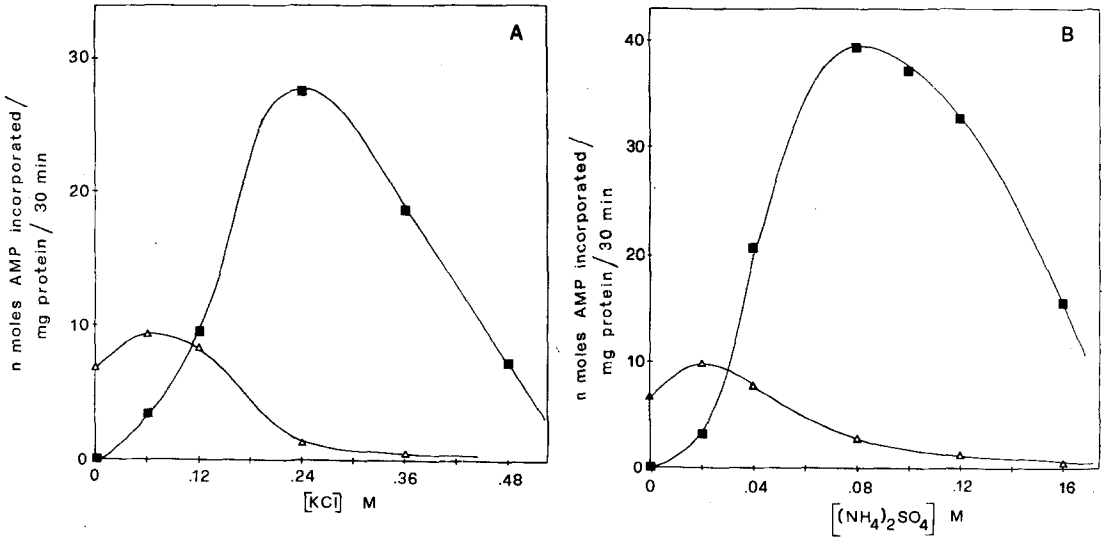
Summary. Mn^{++}-stimulated poly(A) synthetase activity of dialyzed ammonium sulfate fraction (0–50% saturation) of nuclear extracts from rat liver is dependent on salt addition to the incubation mixture. The corresponding soluble cytoplasmic activity is inhibited by the salt concentration required for nuclear activity.

Poly(A) synthetase activities (poly(A) polymerase, terminal riboadenylate transferase) have been found in prokariotes as well as in the nucleus[1-3] and the cytoplasm[3-6] of eukaryotic cells. Enzymes from both subcellular compartments of the latter organisms have been extensively purified[2-3]; the question, however, still remains, whether they are separate entities or the same enzyme undergoing redistribution upon subcellular fractionation. We have found that Mn^{++}-stimulated poly(A) synthetase activities of dialyzed ammonium sulfate fractionated extracts from nucleus and cytoplasm of rat liver cells, behave differently with regard to salt concentration in the test system, thus providing a means for distinguishing the 2 activities.

Methods. Male rats of the Wistar strain, 3 months old and weighing about 300 g were used. 3 animals, fasted for 24 h, were killed for each experiment, their livers were washed and pooled. All the subsequent operations were conducted at 0–2 °C. The organs were minced and homo-

1 M. A. Winters and M. Edmonds, J. biol. Chem. *248*, 4756 (1973).
2 J. Niessing, Eur. J. Biochem. *59*, 127 (1975).
3 A. Hadidi and V. S. Sethi, Biochim. biophys. Acta *425*, 95 (1976).
4 C. W. Chung, H. R. Mahler and M. Enrione, J. biol. Chem. *235*, 1448 (1960).
5 C. M. Tsiapalis, J. W. Dorson and F. J. Bollum, J. biol. Chem. *250*, 4486 (1975).
6 A. Corti, J. Wilson and H. G. Williams-Ashman, 9th FEBS Meeting, Budapest (1974).



Effect of increasing concentrations of KCl *A* or $(NH_4)SO_4$ *B* on dialyzed Mn^{++}-stimulated poly (A) synthetase activity of ammonium sulfate fractions (0–50% saturation) of nuclear and cytoplasmic extracts from rat liver. The experimental conditions are described in the text. Each point represents the average of 4 determinations on 4 different pools of livers. The SEM was less than 10%. △—△, cytoplasmic activity; ■—■, nuclear activity.

genized with 4 vol. of 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, 2.5 mM KCl, 1 mM $MgCl_2$, passed through 4 layers of cheese-cloth and centrifuged at 600 × g for 10 min. The supernatant was further centrifuged at 10,000 × g for 10 min to sediment the mitochondria, and then the 'crude soluble cytoplasmic fraction' was obtained by centrifuging 90 min at 105,000 × g. The 600 × g nuclear pellet was washed with the homogenization medium containing 0.5% Triton-X-100 and twice with the same medium without Triton-X-100. The washed nuclear fraction was suspended in 0.2 M KP_i, pH 7.5, and extracted for 90 min with continuous stirring[5]. The nuclear suspension was then centrifuged for 90 min at 105,000 × g. This was the 'crude nuclear extract'.

The 2 crude extracts were fractionated with solid $(NH_4)_2SO_4$ (0–50% saturation), the precipitates were resuspended in a small volume of 10 mM Tris-HCl, pH 8.2, and dialyzed exhaustively against the same buffer. For the assay of poly(A) synthetase activity, the test system contained[7] 100 mM Tris-HCl, pH 8.2 (37 °C), 2 mM dithiothreitol, 1 mg/ml poly(A) (from Miles), 1 mM [8-^{14}C]ATP (1,200–1,500 cpm/nmole), 1 mM $MnCl_2$, approximately 0.200 mg of protein, KCl $(NH_4)_2SO_4$ as indicated in the legend to the figure, and water to a final volume of 0.25 ml. That a polyadenylation reaction is always measured is demonstrated by the complete dependence of AMP incorporation on the addition of synthetic polyadenylate to the reaction mixture (data not shown).

Results and discussion. The figures A and B show the effect of increasing concentrations of KCl and $(NH_4)_2SO_4$ on the polyadenylation activity of dialyzed nuclear and cytoplasmic ammonium sulfate fractions. The 2 preparations exhibit a different response to increasing ion concentration both with KCl and $(NH_4)_2SO_4$. With both salts maximum effect is obtained when the same values of ionic strength are reached.

The dialyzed ammonium sulfate fractions from nuclei have no detectable poly(A) synthetase activity with no addition of salt to the incubation mixture. This activity rises steeply by increasing salt concentrations and reaches a peak much higher than that of the cytoplasmic activity. The latter is slightly enhanced by the lowest concentration of both salts used, but it is strongly inhibited by the concentrations causing highest activities with the nuclear extract. Thus, under our experimental conditions, it appears possible to distinguish the Mn^{++}-dependent poly(A) primed poly(A) synthetase activity of nuclear extracts from that of cytoplasmic ones on the basis of a different salt requirement.

Since we have been working with a crude enzyme preparation, besides the existence of at least 2 separate enzymes for polyadenylation activity within the cell of eukaryotes, other possibilities cannot be ruled out, such as dissociation and reassociation of subunits of the same oligomeric protein, as a consequence of subcellular fractionation and salt addition to the test system.

7 A. Corti, A. Casti, N. Reali and C. M. Caldarera, Biochem. biophys. Res. Comm. *71*, 1125 (1976).

Enzymatic activities of muscle fibres differentiated, in vitro, from pectoralis major (white) and adductor magnus (red) muscles of chick embryos[1]

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Summary. Specific activities of NADP isocitrate dehydrogenase and acetylcholinesterase were significantly higher in muscle fibres differentiated, in vitro, from myoblasts of adductor magnus (red) than pectoralis major (white) muscles 10-day-old chick embryos. This is evidence, as far as enzyme activities are concerned, that myoblasts from different types of skeletal muscles are able to give, in tissue culture, muscle fibres of different properties, even in the absence of nerve supply.

It is now firmly established that muscle fibres arise by the fusion of mononucleated myogenic cells[2,3], and that in vivo differentiated muscle fibres manifest differences in structural, contractile and metabolic characteristics. This permits them to be classified into 3 main types: white fibres αW (fast twitch and glycolytic), red fibres αR (fast twitch and oxidative) and βR (slow twitch and oxidative). Moreover it is known from denervation and cross-innervation[4,5], tenotomy[6–8], or local applications of anaesthetics[9,10], that the maintenance and to some extent the acquisition of the mature characteristics of the muscle fibres are determined by their motor innervation and functional activity.

However, it is not yet well-known, whether, during the ontogenesis of muscle fibres, the various characteristics of fibres, as they appear in vivo, result only from nervous and functionnal influences or whether some of them are myogenic in origin. That is to say, are some of the properties of the different types of fibres related to the existence of populations of myogenic cells with different potentialities, the cells of a same population fusing to give a specific type of fibre?

We investigated this possibility by determining the capacity of embryonic myoblasts obtained from muscles which are known to be composed in the adult by only one type of fibre (α or β), to give separately, in vitro, fibres with similar or distinctive characteristics. This present work has been carried out on myoblasts of 10-day-old

1 Supported by a grant No. 75 7 1641 from the French Délégation à la Recherche scientifique et technique.

2 K. Okazaki and H. Holtzer, Proc nat. Acad. Sci. USA *56*, 1484 (1966).

3 R. Bischoff and H. Holtzer, J. cell. Biol. *41*, 188 (1969).

4 A. J. Buller, J. C. Eccles and R. M. Eccles, J. Physiol., Lond. *150*, 417 (1960).

5 F. A. Sréter, A. R. Luff and J. Gergely, J. gen. Physiol. *66*, 811 (1975).

6 G. Vrbová, J. Physiol., Lond. *166*, 241 (1963).

7 A. J. Buller and D. M. Lewis, J. Physiol., Lond. *178*, 326 (1965).

8 M. Nicolet, P. Thibert and P. Bechtel, C. r. Séanc. Soc. Biol. *168*, 1251 (1974).

9 R. Jones and G. Vrbová, J. Physiol., Lond. *236*, 517 (1974).

10 A. Pestronk, D. B. Drachman and J. W. Griffin, Nature *260*, 352 (1976).