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.

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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

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Enzyme activities of muscle fibres differentiated, in vitro, from myogenic cells of pectoralis and adductor muscles

Protein mg/g wet weight		Specific activities: milli-unit (mU)/mg protein							
		ICDH		Aldolase		AChE		CPK	
Pectoralis culture	Adductor culture	Pectoralis culture	Adductor culture	Pectoralis culture	Adductor culture	Pectoralis culture	Adductor culture	Pectoralis culture	Adductor culture
42.98 ± 4.12 (10), N.S.	40.21 ± 4.10	96.52 ± 10.34 (10), *	137.76 ± 16.48	193.52 ± 19.75 (10), N.S.	200.60 ± 30.42	79.06 ± 17.14 (10), *	130.12 ± 17.20	618.03 ± 95.74 (10), N.S.	805.27 ± 103.53

Mean ± S.E. ( ) Number of each type of cultures. * Significant ($p < 0.01$). N.S. Non-significant. ICDH: NADP isocitrate dehydrogenase (E.C.: 1-1-1-42). Aldolase: Fructose 1–6 diphosphate aldolase (E.C.: 4-1-2-13). AChE: Acetylcholinesterase (E.C.: 3-1-1-7). CPK: Creatine kinase (E.C.: 2-7-3-2).

chick embryos. In some of the previous work on this subject, sufficient attention has not been paid to the choice of muscle from which myogenic cells are isolated: In some cases[11,12] all the muscles of the thigh have been used to obtain myoblasts of 'red muscle'. However the great majority of the muscles of the thigh are mixed with varying proportions of fibre types, leading to an heterogeneous starting population of myoblasts.

In this study, cells were obtained from 2 different muscles: the pectoralis major, which differentiates, in vivo, into one type of fibre[13] (αW), and the adductor magnus, which differentiates into 80% βR fibres and 20% αW fibres[14]. These αW fibres are grouped in a thin strip located on the posterior side of the muscle and were easily discarded under binocular lens by dissecting beyond this strip, to obtain pure βR myoblasts.

Separate cultures of myoblasts from these two muscles were established on the same day from the same embryos according to a procedure previously described[15]. In order to detect any differences between the 2 cultures and to relate them to the type of fibres found in pectoralis and adductor muscles, in vivo, the activities of 4 enzymes were assayed: these were NADP isocitrate dehydrogenase (oxidative metabolism), which is higher in βR fibres, and the activity of fructose 1–6 diphosphate aldolase (glycolytic metabolism), which is higher in αW fibres. In addition, the activities of creatine kinase[16,17] and acetylcholinesterase[18,19], which are related to the differentiation and function of muscular tissue were also measured.

Material and methods. Trypsin-dissociated myogenic cells were plated on 60 mm Falcon plastic tissue culture dishes, coated with rat-tail collagen, at a nominal density of 1.10^6 cells/dish. Culture medium consists of: Eagle's Minimum Essential Medium (with Earle's balanced salt solution), sheep serum, chick embryo extract in the ratio 8:1:1. Cultures were grown in a water-saturated atmosphere of 5% CO_2 in air at 37 °C. The medium was changed every day after plating. The pattern of development of the 2 cultures was the same. Cells began to fuse at the end of the second day, forming multinucleated myotubes, which towards the sixth day showed cross-striations and spontaneous contractions. When the cultures were first prepared, nearly 30% of the cells were fibroblasts and in order to prevent overcrowding of the cultures by fibroblasts, they were treated with Ara-C (10 μM) for 24 h at the end of the period of myoblast fusion. Almost pure cultures of muscle fibres were thus obtained.

For each type of culture, the muscle fibres from 5 plates were pooled, suspended (1:5 dilution) in phosphate buffer 0.1 M, pH 7.25, and homogenized at 4 °C in a Potter homogenizer. Enzyme activities were performed at 37 °C on centrifugal supernatants using a Gilford Model 240 recording spectrophotometer. Aldolase, ICDH and CPK activities were detected on the 35,000 g supernatant by Bergmeyer's methods[20], using the absorption band at 340 nm of coenzymes NAD and NADP; AChE activity was determined on the 500 g supernatant by the method of Ellman et al.[21] using BW 284 C 51 as an inhibitor of AChE. Protein determinations were carried out on a part of the whole homogenate by Lowry's method[22] with bovine serum albumin as a standard. Specific activities of these enzymes were assayed on 7-day-old cultures.

Results and discussion. The results (table) show that the activities of AChE and ICDH were significantly different between the fibres which originated from myogenic cells of the pectoralis and adductor muscles. There was no significant difference between the other 2 enzymes in any of the cultures.

In the case of AChE, Wilson et al.[23] showed that the enzyme was released by the muscle fibres into the medium. Assays of AChE activity were carried out, on the 6th, 7th and 8th day, on the culture medium which was changed every day. These showed no significant differences between pectoralis and adductor cultures (48.8 ± 3.6 and 46.7 ± 2.5 mU/ml, respectively). Thus the enzyme activity observed between the 2 types of cultures cannot be explained by a differential loss of enzyme into the medium. It is known that AChE is present in the muscle cell membrane (in both the plasma membrane and synaptic folds) and it has been suggested[24] that this enzyme is a part of the basic excitation unit which also includes the acetylcholine receptor protein; the numbers of these 2 molecules are believed to be the

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same[25]. Meanwhile, it is known that the distribution and the number of receptors vary not only with the degree of differentiation of myotubes[26], but also with its state of innervation[27], the same holds true for cholinesterase[28]. Recently is has been shown[10] that the number of acetylcholine receptors of neuromuscular junction-free muscle strips of rat soleus (red) was almost 2 times greater than that of the extensor digitorum longus (white). The difference between the AChE activity in the 2 cultures suggest that this is related to the different structural properties of excitable membranes of pectoralis and adductor muscle differentiated in vitro. These differences in the AChE activity, as in ICDH, show that myoblasts obtained from 2 different homogeneous muscles differentiate, in vitro, under the same conditions of development, into muscle fibres with different functional and metabolic properties. This result between the 2 cultures may be related to the existence of myogenic cells with different potentialities.

In a histochemical study of in vitro differentiated muscle fibres from breast and leg muscles myoblasts of 10-day-old chick embryos, Askanas et al.[11] found no difference in phosphorylase (glycolytic metabolism) staining between the 2 cultures, this result being in agreement with our observation for aldolase, another enzyme of the glycolytic pathway. As we observed significant differences in ICDH activities (citric acid cycle), these authors also observed a difference in succinate dehydrogenase (oxidative metabolism): staining for this enzyme was more pronounced in cultured leg muscles than cultured breast muscle, and this difference could be related to a greater amount of mitochondria as observed in electron micrographs of cultured leg muscles. However, these leg muscle cultures were established from ill defined populations of myogenic cells coming from heterogeneous muscles samples, the majority of them being of mixed fibre populations: In these circumstances it is difficult to relate the observations to the type of myogenic cell. We feel that with our results, particularly in the case of adductor muscle cultures, we can with certainty relate the differences to the type of myoblast from which they originated.

This is evidence, therefore, that myoblasts from potentially different skeletal muscle differentiate in different ways in tissue culture, even in the absence of the nerve supply. This certainly seems to be true as far as enzyme activities are concerned. From a general point of view, this seems to be the expression of those characteristics which are myogenically determined, other characteristics may well be determined by exogenous factors including the nerve supply.

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Reduction of gallstone formation by ascorbic acid in hamsters

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Summary. The addition of 0.5% of ascorbic acid to the lithogenic diet of golden hamsters whose body pool was labelled with 26-^{14}C-cholesterol, lowered the formation of gallstones, the cholesterol concentration and half-life in blood plasma and in the liver, and accelerated cholesterol transformation to bile acids.

Bile acids and lecithin are the major solubilizing agents for biliary cholesterol, the predominant component of at least 90% of gallstones. An initial phase in gallstone formation is a disorder of liver cell metabolism, the production of bile that is supersaturated with cholesterol[1]. Persons with cholesterol gallstones have a decrease in bile acid pool size[2]. The increase in biliary cholesterol secretion and the reduction of bile acid pool size could lead to a decrease in the proportion of bile salts to cholesterol which could result in the precipitation of cholesterol and aggregation of cholesterol crystals into gallstones. A similar metabolic situation occurs in guinea-pigs during a chronic latent vitamin C deficiency: the rate of cholesterol transformation to bile acids in the liver is decreased[3,4] and the size of the bile acid pool is also reduced[5]. Marginal vitamin C deficiency interferes with the biosynthesis of bile acids at the stage of microsomal 7 α-hydroxylation of the cholesterol nucleus[6]. Björkhem and Kallner[7] have suggested that ascorbate affects the synthesis or breakdown of the cholesterol 7 α-hydroxylating system, in particular the cytochrome P-450 component. In scorbutic guinea-pigs, frequent occurence of gallstones was observed[8–10]. These facts have prompted us to consider the potential role of a chronic latent vitamin C deficiency in the pathogenesis of cholelithiasis and the possibility of preventing the formation of gallstones by a permanent supply of vitamin C[11]. The aim of the experiment reported here has been to verify this hypothesis.

Materials and methods. 200 male golden hamsters aged approximately 6 weeks (body weight about 70 g) were put on a semi-purified fat-free, high-glucose diet[12] known to produce cholesterol gallstones. 100 animals were fed this diet without any vitamin C addition, the others had the same diet plus 5 g of ascorbic acid per kg of diet. The

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