CHARACTERISTICS OF METAMYOSIN

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Metamyosins isolated from the muscles of rabbits of different ages differ in their cholinesterase activity, substrate specificity of cholinesterase, and content and relative percentages of electrophoretic protein fractions. The cholinesterase activity is linked with three fractions of fetal and neonatal metamyosin and two fractions of metamyosin from 12-day and adult animals.

The discovery of the myofibrillary protein metamyosin was reported in 1955 [8]. The discoverers described some of the characteristic properties of metamyosin in subsequent papers [5-10].

The physiological role of this fraction of the myofibrillary proteins is not known. It has been postulated that metamyosin is the precursor of other myofibrillary proteins [7]. So far this hypothesis has not received factual confirmation.

According to Yur'ev et al. [3], metamyosin preparations possess high cholinesterase activity. The heterogeneity of this protein has been demonstrated by the salting out method. These workers consider that metamyosin is either a cholinesterase with unusual action or contains a mixture of different types of cholinesterases, and also that only certain of its components apparently possess enzymic activity. To clarify the situation, the enzymic activity of metamyosin has been studied in animals of different ages.

EXPERIMENTAL METHOD

The skeletal muscle of 28-day rabbit fetuses, and newborn, 12-day, and adult rabbits was used. All stages of treatment of the tissue and extraction of the protein were carried out in the cold, using cooled apparatus and reagents. The muscles were minced or cut up with scissors and then ground in a mortar with quartz sand. Metamyosin was obtained by the method of Marcaud-Raeber et al. [7]. To determine the cholinesterase activity, the protein was incubated in phosphate buffer (pH 7.8; $\mu = 0.4$) with various substrates (acetylcholine, butyryl choline, acetyl-β-methylcholine). The concentration of cholinesters in the experimental and control samples was determined by Hestrin's method [4]. Protein nitrogen was determined by the micro-Kjeldahl method. The metamyosin was fractionated by electrophoresis in agar gel in the cuvette designed by Kadykov [2], using phosphate buffer (pH 7.8; $\mu = 0.4$). The conditions of electrophoresis were: voltage gradient 1.3-1.5 V/cm, current 19-22 $\mu A/cm^2$, duration 22-24 h. For preparative electrophoresis, a cuvette taking 8 samples was used. In this case 2.3-2.4 ml of the protein solution was fractionated at the same time. After the end of electrophoresis, two strips of gel were cut along the length of the cuvette, to correspond to the outside wells, and these were stained with amido black. Strips of gel with fractions developed by the dye were returned to the cuvette in their former position, and using these as "witnesses," areas of the agar corresponding to the separate protein components and containing native protein were cut out. The agar was chopped up, frozen, turned out into a closely woven piece of cloth, and placed in a mold. Solutions of the metamyosin fractions were obtained during thawing under pressure.

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TABLE 1. Cholinesterase Activity of Rabbit Metamyosin in Ontogenesis (in μg substrate/mg protein/h)

Rabbits	Substrate		
	acetylcholine	butyrylcholine	acetyl-β-methylcholine
Fetuses	1 250—1 471	1 408—1 399	225—220
Newborn	1 094—1 428	950—1 527	200—251
12-day	496—517	230—255	152—170
Adult	72—91	18—25	27—45

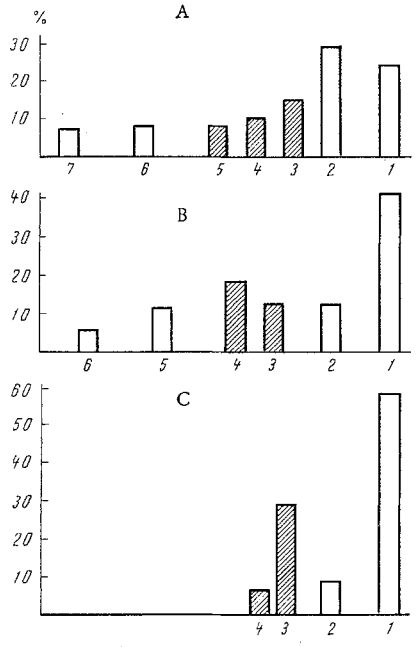

Fig. 1. Composition, relative content, and cholinesterase activity of metamyosin fractions from rabbit fetuses and newborn rabbits (A), rabbits aged 12 days (B), and adult rabbits (C). Shaded columns correspond to fractions possessing enzymic activity. Abscissa, electrophoretic fractions of metamyosin; ordinate, content of metamyosin fractions, in %.

EXPERIMENTAL RESULTS

It is clear from the results in Table 1 that metamyosin isolated from the muscles of animals of different ages possessed cholinesterase activity. Metamyosin of the fetal and newborn rabbits possessed the highest enzyme activity and it decreased with age. The metamyosin hydrolyzed all choline esters tested. The results of these experiments agree with those obtained previously in the writer's laboratory, [3]. The rates of hydrolysis of acetyl- and butyrylcholine by fetal and neonatal metamyosin were about equal. With age, the intensity of hydrolysis of acetylcholine increased compared with the intensity of hydrolysis of butyrylcholine. At the same time, the rate of hydrolysis of butyrylcholine decreased compared with that of acetyl-β-methylcholine.

Metamyosin is not a homogeneous protein. By free electrophoresis, Marcaud-Raeber demonstrated the presence of two components [5, 8]. Fetal metamyosin was separated by salting out into seven protein fractions [3]. The method of electrophoresis in agar gel was chosen this time to fractionate the metamyosin. Investigation of the protein by this method also demonstrated its marked heterogeneity. The fractions obtained were designated by numbers starting from the anode end of the agar. The results showing the number of electrophoretic components, their relative content, and the presence or absence of cholinesterase activity in metamyosin of animals of different ages are given in Fig. 1. A tendency for the fractional composition of the metamyosin to change during ontogenesis will be seen. For instance, in specimens of fetal and neonatal metamyosin 7 principal electrophoretic components were detected. Metamyosin from rabbits aged 12 days separated into 6, and metamyosin from adult rabbits into 4 fractions. A considerable increase in the content of the most mobile component of metamyosin, fraction 1, also was observed. At the same time, a definite inconstancy both of the fractional composition and of the content of individual electrophoretic components of this protein must be pointed out. This fact is evidently attributable to the marked lability of some of the protein fractions of metamyosin.

To determine which of the metamyosin components possessed cholinesterase properties, all the above-mentioned fractions were separated and their cholinesterase activity determined. Activity was found in solutions of fractions 3, 4, and 5 of metamyosin from the fetuses and newborn animals and in fractions 3 and 4 of metamyosin from 12-day and adult rabbits. The total content of active components remained virtually unchanged in ontogenesis, with a mean value of 32% protein nitrogen of metamyosin (Fig. 1). The cholinesterase activity of the fractions, calculated on the basis of their percentage content, was about three times higher than the activity of the original metamyosin of animals of the corresponding age. Enzymically active components of metamyosin, despite their high activity, were not pure enzymes and, besides cholinesterase, they also contained large quantities of proteins. This is clear from the following findings. The activity of rabbit fetal and neonatal metamyosin as regards hydrolysis of acetylcholine was 15-17 times stronger than the activity of metamyosin from the adult animal. At the same time, as has already been mentioned, the content of enzymically active fractions remains unchanged during ontogenesis. These

experiments confirmed the results of earlier investigations showing that metamyosin is not an individual protein. They also showed that metamyosins from animals of different ages are not identical but differ from each other in the number of their electrophoretic components and in their quantitative content. Both their cholinesterase activity and the ratio between the rates of hydrolysis of different substrates by the cholinesterase of metamyosin vary with age.

Another important fact is that only certain components of metamyosin possess enzymic activity: three fractions from fetal and neonatal rabbits and two fractions of metamyosin from 12-day and adult animals. In other words, a substantial change in the fractional composition and the properties of metamyosin and its components takes place in ontogenesis. The formation of a tetanic reaction of skeletal muscle is thus accompanied by a change in the content and properties not only of the chief contractile proteins of muscles, myosin and actomyosin [1], but also of metamyosin.

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