RIBONUCLEASE ACTIVITY OF THE "MYOFIBRILLARY" FRACTION OF NORMAL AND AUTOLYZED MUSCLE TISSUE

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The separation in a sucrose gradient of the "myofibrillary" fraction of normal and autolyzed muscle tissue gave 4 components. During post-mortem destruction of the tissue there was observed a slight decrease of the "myofibrillary" fraction yield and also certain changes in the distribution of protein between different components. Under the selected conditions RNase activity was found in all 4 components. During the course of autolysis enzymatic activity increased in the whole "myofibrillary" fraction, as well as in the lysosomal-mitochondrial components of "myofibrils."

KEY WORDS: *Ribonuclease; muscles; autolysis.*

The study of the action of ribonucleases (RNases) is an essential condition for the elucidation of the pathways and mechanisms of RNA breakdown in the tissues. The writers showed previously [3] that the fraction of a muscle homogenate, precipitated by centrifugation at 30,000g for 30 min ("myofibrillary"), possesses RNase activity.

Since this fraction is heterogeneous in composition, in the investigation described below an attempt was made to fractionate it and to investigate the RNase activity directly in the components of normal and autolyzed tissue obtained in this way.

EXPERIMENTAL METHODS

Hind limb muscles of noninbred rats, killed before or 24, 48, and 72 h after the experiment, were used. The "myofibrillary" fraction of the muscles was obtained as described previously [3]. The residue of "myofibrils" was suspended in buffer A (0.03 M Tris-HCl, pH 7.6, 0.25 M KCl, 0.01 M $MgCl_2$, and 0.11 M sucrose) and sedimented by centrifugation at 30,000g for 30 min. After repetition of this procedure the residue was again suspended in a minimal volume of buffer A and filtered through Kapron gauze. Next, 7 ml of the suspension was applied to a stepwise sucrose gradient made up as follows: 3 ml of 2.3 M sucrose solution, 12 ml of 1.75 M sucrose, 15 ml of 1.3 M, and 15 ml of 1.0 M sucrose solution. The sucrose solutions were made up in buffer B of the following composition: 0.03 M Tris-HCl, pH 7.6, 0.25 M KCl, 0.01 M $MgCl_2$. Centrifugation was carried out at 10,000 rpm in the SW 4 × 60 rotor of a "high speed-24" centrifuge for 1 h. The contents of the tubes were passed through a Uvicord II-8300 densitometer at the rate of 90 ml/h, with simultaneous recording of the optical density of the solution at 255 mm. Fractions corresponding to the peaks of optical density were collected and dialyzed overnight against buffer B (1:100). The protein concentration by Lowry's method [4] and the RNase activity of the samples obtained after dialysis were determined. Labeled ribosomal RNA (rRNA) was obtained from the liver of animals into which [^{32}P]orthophosphoric acid (10 μCi/g) was injected intraperitoneally 36 h before sacrifice. To determine RNase activity samples (2.5 ml) containing 0.1 mg protein of the test fraction and 10,000-15,000 cpm of ^{32}P-labeled rRNA (3-5 E_{260} optical units) were incubated in buffer B for 20 min at 70°C. The reaction was stopped by the addition of 3 M $HClO_4$ to a final concentration of 0.5 M. The residues were separated by centrifugation and the radioactivity of the supernatant determined in Bray's reagent by means of an ABAC-SL-40 counter. Samples not containing the enzyme fraction served as the control. The activity of each fraction was calculated in conventional units (E.U.), 1% rRNA hydrolyzed by 1 mg protein of the test preparation at 70°C during 20 min being taken as 1 E.U. The preparations obtained by centrifugation in a sucrose density gradient were fixed for 1 h in 1% OsO_4 solution [5], dehydrated in alcohols, and embedded in the epoxide resin Araldite. Ultrathin sections were cut on the Om U2 ultramicro-

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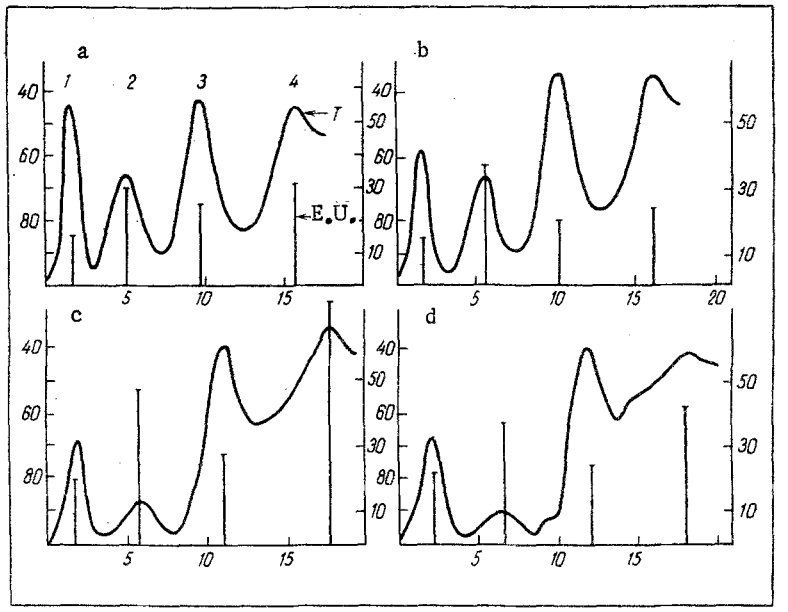

Fig. 1. Distribution of material and RNase activity (in E.U.) during fractionation of "myofibrils" from normal and autolyzed tissue in a stepwise sucrose gradient: a) normal tissue; b) autolysis for 24 h, c) for 48 h, d) for 72 h. Sucrose concentrations: 1) 2.3 M, 2) 1.75 M, 3) 1.3 M, 4) 1 M. Abscissa: No. of fraction; ordinate: left) optical density (in % absorption; curves); right) RNase activity (in E.U.; columns).

tome (Reichert), stained with uranyl acetate and lead salt [6], and examined in the JEM-7A electron microscope (Japan).

EXPERIMENTAL RESULTS

The "myofibrillary" fraction of the muscles, after fractionation in a sucrose gradient, was separated into four components (Fig. 1). Electron-microscopic investigation of these components showed that they are heterogeneous in composition and contain chiefly cell organelles which preserve their ultrastructural organization sufficiently well. Fraction No. 1, isolated in 2.3 M sucrose, consisted of isolating nuclei and a small number of mitochondria (Fig. 2a). Fraction No. 2, isolated in 1.75 M sucrose, consisted mainly of secondary lysosomes in the form of residual bodies (Fig. 2b). Fraction No. 3, isolated in 1.3 M sucrose, consisted of a highly concentrated residue of mitochondria with some contamination with small vesicles and glycogen granules (Fig. 2c). Fraction No. 4, isolated in 1.0 M sucrose, contained many mitochondria together with a few vesicles (Fig. 2d). The fractions obtained from "myofibrils" of autolyzed tissue were almost identical in their morphological composition but contained numerous vesicles, bounded by a single membrane, and a very small number of mitochondria with moderately swollen cristae.

During postmortem destruction of the tissue a small decrease in the yield of the "myofibrillary" fraction was observed (Table 1). With an increase in the duration of the postmortem period changes also took place in the distribution of the material among the components obtained after centrifugation of the "myofibrils" in the sucrose gradient. After autolysis for 3 days a considerable shift of material into fraction No. 4 was observed from the other fractions and, in particular, from fraction No. 2. Since the greater part of the material of fraction No. 4 consisted of destructively changed material, this suggests that the number of these structures increases in the course of autolysis.

Determination of RNase in the various fractions of the "myofibrils" showed that all the fractions possessed nuclease activity under the chosen conditions of incubation (Fig. 1). Since the "myofibrils" contained a certain quantity of adsorbed ribosomal material, possessing intrinsic RNase activity [1], the possibility of contamination of each fraction with ribosomes

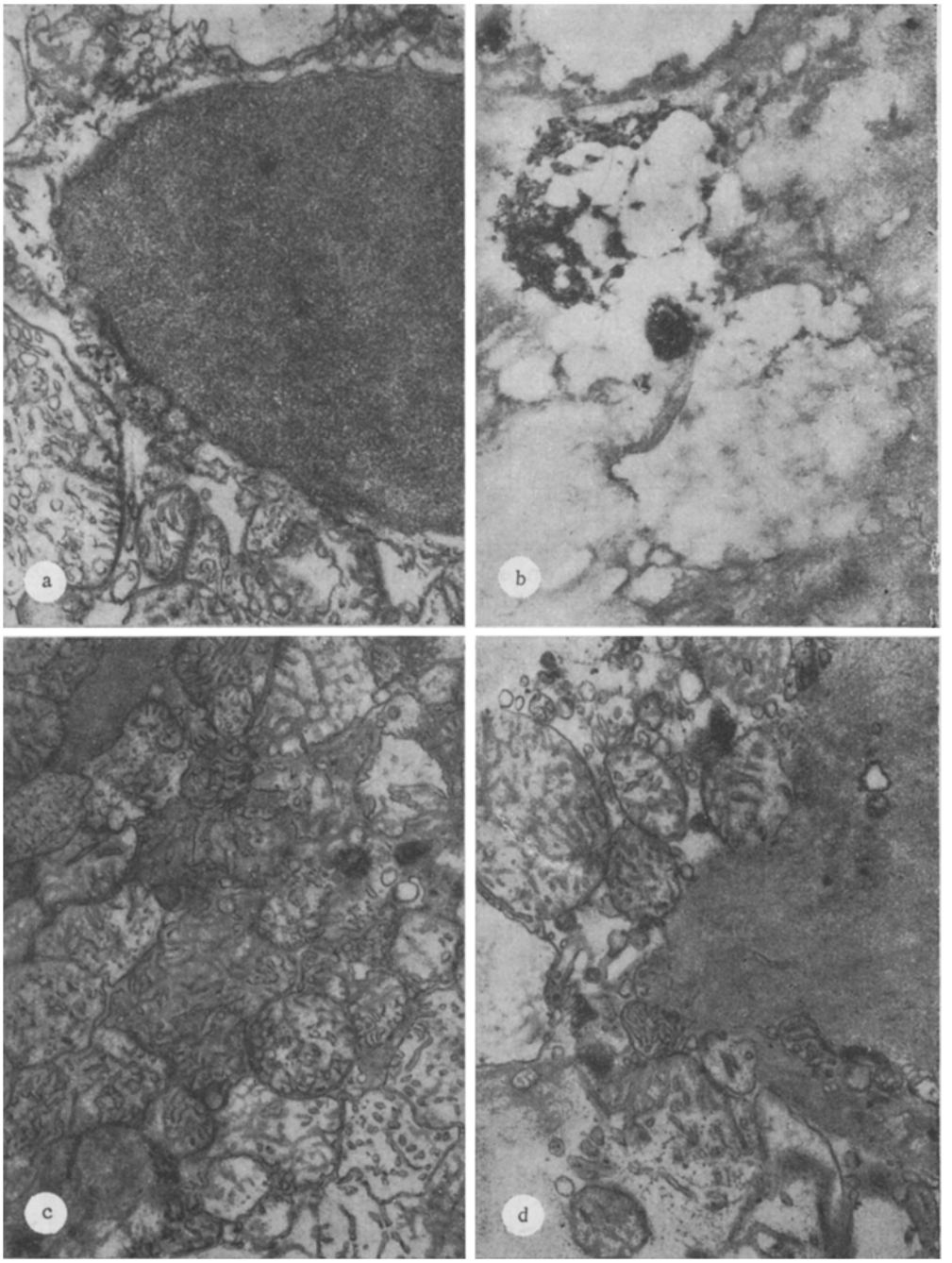

Fig. 2. Electron micrographs of fractions obtained by fractionation of "myofibrils" of normal tissue in stepwise sucrose gradient: a) 2.3 M sucrose (40,000×); b) 1.75 M sucrose (46,250×); c) 1.3 M sucrose (27,500×); d) 1.0 M sucrose (33,750×).

was investigated. For this purpose, 1 ml of a suspension of ribosomes (15,000 rpm) obtained from the liver of rats into which [^{32}P]orthophosphoric acid has been injected, was added to the washed fraction of "myofibrils" before gradient centrifugation. The results demonstrated negligible contamination of fractions Nos. 1-3 with ribosomal material and showed that the free ribosomes were located mainly in fraction No. 4 (Fig. 3). These results, together with the electron-microscopic investigation of the fractions, suggested that fractions Nos. 1-3 of the "myofibrils" were virtually free from additional RNase activity on account of the presence of ribosomes.

In the process of postmortem destruction of the tissue an increase in RNase activity was observed in fractions Nos. 2 and 4, together with an increase in the activity of the total

TABLE 1. Changes in Yield and Distribution of Protein among Fractions of "Myofibrils" during Autolysis

Duration of autolysis, h	Yield, mg/g tissue	Distribution of protein, %			
		fraction No. 1 (2.3 M)	fraction No. 2 (1.75 M)	fraction No. 3 (1.3 M)	fraction No. 4 (1.0 M)
normal tissue	21,2±1,7	15,1	20,7	18,1	46,1
24	20,1±1,6	11,4	11,8	23,9	52,9
48	20,4±1,8	13,1	7,2	14,6	65,1
72	16,0±0,1	7,2	4,8	18,6	69,4

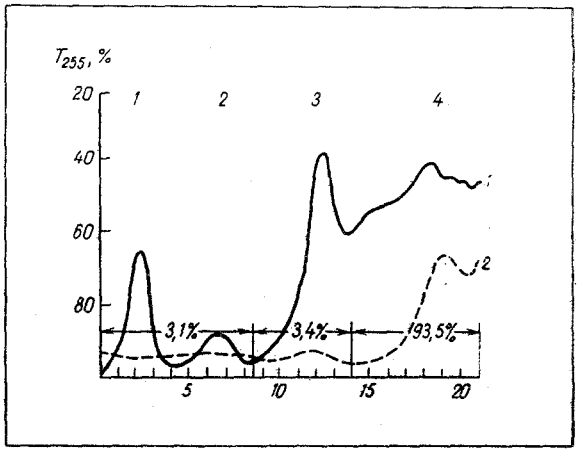

Fig. 3. Distribution of mixture of "myofibrils" of muscle tissue autolyzed for 72 h and of ribosomes (1) and of free ribosomes (2) in stepwise sucrose gradient. Percentage of ribosomes adsorbed by the various fractions of "myofibrils" indicated in this figure. For remainder of legend, see Fig. 1.

"myofibrillary" fraction (Fig. 1). Presumably after death of the animal the enzyme activity of the mitochondrial-lysosomal fraction of the muscles increased. The ribonuclease activity of fraction No. 4 may be associated with the presence of ribosomes in it, and also with the lysosomes found in this fraction. The increase in RNase activity in fraction No. 4 was accompanied by the transfer of material into it from the lower fraction than, in particular, from fraction No. 2, a possible cause of the increase in enzyme activity.

The RNase activity thus increases in the course of autolysis to reach a maximum by 48 h after death. By this time rigor mortis is resolving and this is accompanied by activation of autolytic processes in the muscle tissue [2].

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