The kinetics of the change in MDA concentration thus reflects superposition of three processes: activation of LPO of the erythrocyte membranes under the influence of complement, spontaneous oxidation of erythrocyte ghosts, and the reaction of MDA with amino groups of proteins and phospholipids.

These results are evidence that LPO of erythrocyte membranes participates in the mechanism of complement-dependent hemolysis.

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GLUCOCORTICOIDS IN INTRACELLULAR CATABOLISM OF MYOSIN AND ACTIN

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Glucocorticoids are known to increase the proteolytic activity of skeletal muscles [8] and to stimulate, in the first place, degradation of myofibrillary proteins [10, 12]. The atrophy developing under these circumstances, as well as the increase in alkaline proteinase activity in them, depends on the type of skeletal muscle [1, 11]. Considering that nonlysosomal proteinases also participate in the intracellular catabolism of muscle proteins [3], and also that the number of lysosomes in skeletal muscles is very small and that inhibition of lysosomal proteinases does not inhibit myosin degredation [7], it can be concluded that there are good grounds for the opinions of those workers who consider that the initial step in muscle protein degredation does not involve the participation of lysosomal proteinases [7]. It has been shown, for instance, that thiol proteinases, which are activated at neutral pH values by Ca^{++} ions, degrade troponin I and T [7], whereas serine proteinases, at the same pH values, destroy both structural and regulatory proteins of the contractile system of muscles [9, 13]. This suggests that glucocorticoids, by increasing proteolytic activity of alkaline proteinases in the muscle cell, may initiate degradation of myofibrillary proteins.

The aim of this investigation was to study connections between proteolytic activity of alkaline proteinases and the intensity of their synthesis, and also the degradation of actin and myosin in muscle cells when the blood glucocorticoid level is raised.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats aged 16-17 weeks, kept under the conditions described previously [11]. Alkaline poteinases were isolated from m. gastroc-

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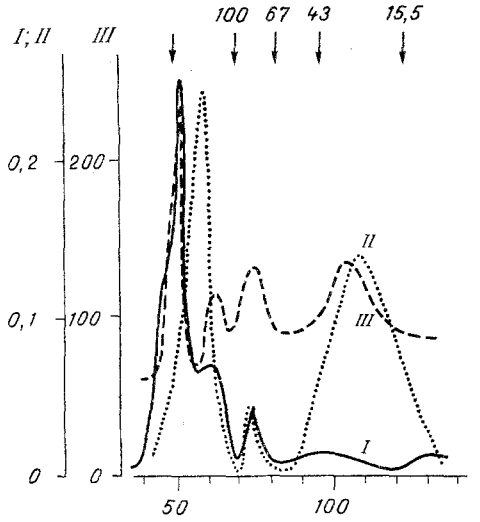

Fig. 1

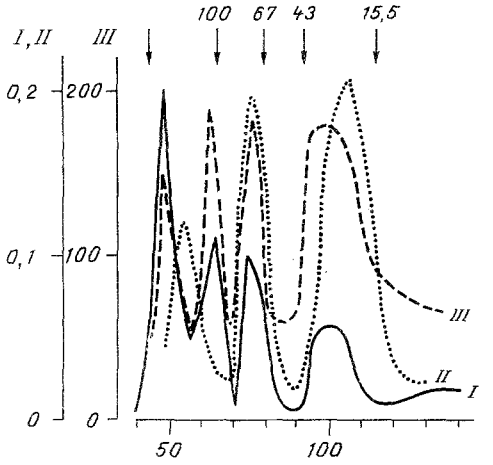

Fig. 2

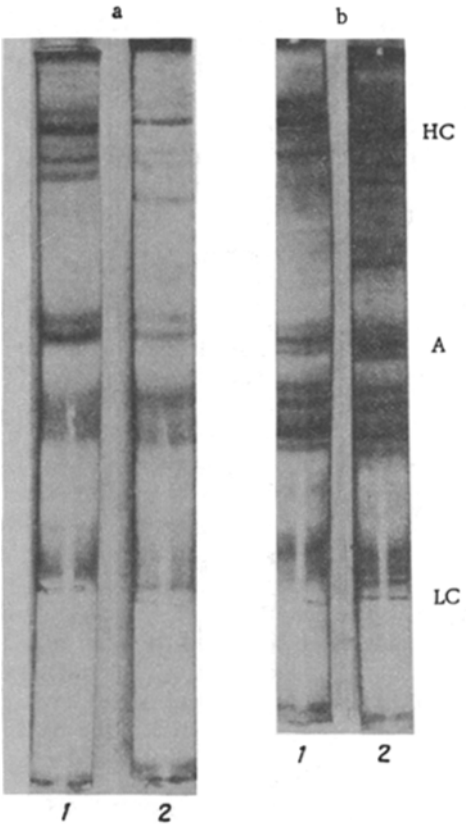

Fig. 3

Fig. 1. Gel-filtration of alkaline proteinases of skeletal muscles of control animals on Ultrogel AcA-44. Abscissa, Nos. of fractions of eluate; ordinate: I) optical density (o.d.) at wavelength of 280 nm (in o.d. units); II) proteolytic activity (in U/ml); III) incorporation of ^{14}C-leucine (in counts/ml). Arrows indicate positions of elution of standard (in kilodaltons): from left to right — blue dextran, α-actin, serum albumin, ovalbumin, and hemoglobin.

Fig. 2. Gel-filtration of alkaline proteinase of sleketal muscles from animals injected with dexamethasone, on Ultrogel AcA-44. Legend as to Fig. 1.

Fig. 3. Electrophoretic profile on myofibrils incubated with alkaline proteinase (a) and trypsin (b). 1) Control myofibrils; 2) myofibrils after incubation; A) actin.

nemius by the method in [5] and purified by gel filtration on Ultrogel AcA-44. The column measured 2 × 105 cm, the rate of flow was 45 ml/h, and the volume of the eluate fractions was 2.3 ml. Their proteolytic activity was determined by hydrolysis of azocasein, as described previously [6], except that 1% azocasein in 1 M Tris-HCl, pH 9.0, was used. The intensity of synthesis of the above-mentioned proteinases was determined by measuring incorporation of ^{14}C-leucine (30 mCi/mmole), which was injected into the animals in a dose of 150 μCi/100 g body weight [10]. Myofibrils, actomyosin, actin, and the heavy and light chains (HC and LC respectively) of myosin were isolated and purified as described previously [10]. To study the heterogeneity of the turnover of actomyosin proteins, the double label method was used [6]. The relative turnover of actin and myosin was determined as the ratio ^{3}H/^{14}C. L-(U-

^{14}C)-lysine (336 mCi/mmole) and L-(4,5-^{3}H)-lysine (40 Ci/mmole) were used. If the turnover of the proteins studied was identical, the $^{3}H/^{14}C$ ratio was higher. Radioactivity was measured by means of a Minibeta 12 liquid scintillation counter. Myofibrils (5 mg/ml) were incubated for 60 min at 37°C in buffer containing 1 mM KCl and 50 mM Tris-HCl, pH 8.5, and alkaline proteinases (0.7 U/ml). They were then sedimented in 10 volumes of dithiothreitol (0.1 mM) and Tris-HCl (1.75 mM), pH 7.0, at 0°C and eluted 3 times in the same buffer.

The myofibrils were incubated with trypsin (4000:1) at 25°C for 30 min in the presence of KCl (1 mM), NaN_3 (1 mM), $MgCl_2$ (2 mM), EGTA (2 mM), and Tris-maleate (10 mM), pH 7.0. The reaction was stopped by addition of trypsin inhibitor in the ratio of 4:1 with trypsin (at 0°C).

Electrophoresis was carried out in 10% polyacrylamide gel in the presence of sodium dodecylsulfate. Protein was determined by Lowry's method or by the biuret method. Dexamethasone was injected intraperitoneally into the animals in a dose of 1 μg/g body weight and excretion of 3-methylhistidine was determined [10].

EXPERIMENTAL RESULTS

The intensity of degradation of actin and myosin in response to injection of dexamethasone was judged from the increased excretion of 3-methylhistidine — from 0.34 ± 0.04 to 0.55 ± 0.07 μmole/mg creatine/day. As was shown previously [11], the degree of atrophy and the proteolytic activity of the muscles were maximal when the oxidation potential was low. However, the increase in proteolytic activity of the alkaline proteinases under these circumstances was not accompanied by intensification of the rate of their synthesis [2]. When the blood glucocorticoid level was raised, the spectrum of alkaline proteinase subunits of the skeletal muscles was changed, the intensity of synthesis and the proteolytic activity of the light subunits increased, but activity of the heavy subunits, on the other hand, decreased (Figs. 1 and 2). On incubation of the myofibrils with alkaline proteinase, myosin HC separated into fractions (Fig. 3a). Similar changes also took place in myosin HC on incubation of the myofibrils with trypsin (Fig. 3b). Fibrillary actin also had a tendency toward degradation on incubation with alkaline proteinase (Fig. 3a) but was insensitive to the action of trypsin (Fig. 3b). The rate of turnover of myosin HC under these circumstances showed a tendency to decrease (12%), and the content of myosin LC increased by 50% ($P < 0.01$) and of actin by 31% ($P < 0.05$).

The increased proteolytic activity of muscle cells, initiated by glucocorticoids, is thus realized mainly on account of degradation of actin and myosin HC, i.e., on account of proteins with a slow turnover. Myosin LC, with a relatively rapid turnover, were insensitive to the action of alkaline proteinases, at least in experiments *in vitro*. The fact will also be noted that, on administration of glucocorticoids to the experimental animals, the intensity of synthesis of the light subunits of alkaline proteinase was increased, but this was not reflected in the level of the enzyme as a whole, as the writers demonstrated previously [2].

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