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AGE RELATED CHANGES IN MUSCLE PROTEIN DEGRADATION

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SUMMARY

In vitro autolytic degradation of sarcoplasmic proteins in red, white and cardiac muscles increases with advance in age and with increase in temperature, the rate varying with age. Higher activity is seen in the alkaline range in all age groups. Ca^{2+} activated proteolytic activity also increases with advance in age.

INTRODUCTION

Gutmann *et al.* [1–4] have shown significant similarities between denervated and senile muscles in the metabolic changes. Increased proteolytic activity seen in denervated and senile muscles is also characteristic of muscular atrophy. Increased *in vitro* autolytic [5, 6] and Ca^{2+} activated proteolytic activity of sarcoplasmic proteins following denervation have been reported. The present study is to see whether the muscles of old animals behave in a manner similar to denervated muscles with respect to changes in *in vitro* autolytic activity and Ca^{2+} activated proteolytic activity of sarcoplasmic proteins and also to find the functional relationship, if any, of the latter enzymes with ageing.

MATERIALS AND METHODS

Effect of temperature on in vitro *autolytic activity*

Red (*soleus*), white (*extensor digitorum longus*) and cardiac muscles of albino rats, Wistar strain aged 5, 10, 15, 20 and 25 months were used for the study. For *in vitro* autolysis, the animals were decapitated, the muscles were excised, chilled, weighed and homogenized immediately. The muscle homogenate in 0.25 *M* sucrose (pH 7) was centrifuged at 600 × *g* for 20 min to separate the sarcoplasmic proteins (supernatant) from contractile and collagenous proteins (sediments rapidly at 600 × *g*). 1 ml of the supernatant was incubated in closed tubes at the desired temperature (0°, 10°, 20°, 30° and 37 °C) for 2 h as described earlier [8]. Controls were run simultaneously at 0 min of incubation. The autolytic activity was stopped with 1 ml of 10% TCA and centrifuged.

The supernatant was neutralized with 0.5 *N* NaOH and the total free amino acid content was estimated [9]. The protein content was estimated by the method of Layne [10]. Q_{10} was calculated for different ranges of temperature according to van't Hoff's equation [11].

pH and in vitro autolytic activity

The muscle homogenates were mixed with an equal volume of buffer of 0.2 *M* potassium acid phthalate, potassium phosphate and Tris buffer (pH 2–9) [12] and incubated in a water bath at 37 °C for 2 h; at the end of the incubation period, 1 ml of 10% TCA was added to stop the activity and the supernatant was analyzed for total free amino acid content [9]. An unincubated, TCA denatured supernatant served as control.

Ca^{2+} activated proteolytic activity

The sucrose homogenate of sarcoplasmic proteins was centrifuged at 1000 × *g* for 20 min at 4 °C to remove contaminating contractile, fibrous and *Z*-line proteins and then dialyzed for 2 h against 0.25 *M* sucrose. 1 ml dialyzate, 1 ml of 1% casein in 5 m*M* $NaHCO_3$ and 1 ml of 0.01 *M* $CaCl_2$ were mixed and incubated at 37 °C for 3 h. At the end of the incubation period, the protein was precipitated with the addition of 2 ml of 10% TCA. The clear supernatant obtained after centrifugation at 600 × *g* for 10 min was

TABLE I

EFFECT OF TEMPERATURE ON *IN VITRO* AUTOLYTIC DEGRADATION OF SARCOPLASMIC PROTEINS IN RED, WHITE AND CARDIAC MUSCLES OF RATS DURING AGEING

Values are represented as mean ± SD of 7 observations. Autolytic activity is expressed as μg of amino acid released/mg protein/h.

Temp. (°C)	*Nature of muscle*	*Age (months)*				
		5	*10*	*15*	*20*	*25*
0	Red	8.5 ± 1.6	13.0 ± 1.4	12.3 ± 1.2	14.4 ± 0.9	14.9 ± 1.1
	White	6.0 ± 0.6	11.4 ± 0.9	9.6 ± 2.2	15.1 ± 0.7	15.6 ± 1.6
	Cardiac	7.3 ± 1.4	12.2 ± 1.8	11.8 ± 2.2	14.6 ± 1.6	15.8 ± 1.3
10	Red	11.7 ± 0.8	14.6 ± 1.0	16.8 ± 1.8	20.5 ± 1.0	20.1 ± 1.2
	White	9.3 ± 1.2	15.3 ± 2.2	14.8 ± 1.8	18.5 ± 1.9	19.9 ± 2.4
	Cardiac	10.5 ± 1.0	14.9 ± 1.3	16.5 ± 1.9	17.1 ± 2.1	19.1 ± 1.6
20	Red	14.8 ± 0.8	18.6 ± 1.0	20.5 ± 1.1	24.5 ± 1.4	25.6 ± 1.7
	White	12.8 ± 1.0	19.8 ± 0.6	19.7 ± 2.5	20.3 ± 1.7	22.0 ± 1.1
	Cardiac	12.1 ± 0.6	15.8 ± 1.6	19.5 ± 1.6	21.7 ± 1.9	22.8 ± 2.3
30	Red	18.5 ± 1.1	20.9 ± 1.0	23.1 ± 2.1	26.1 ± 2.8	28.2 ± 2.3
	White	16.8 ± 0.9	22.5 ± 1.2	24.8 ± 2.5	23.3 ± 1.2	24.4 ± 2.5
	Cardiac	16.1 ± 1.7	18.9 ± 2.3	22.3 ± 1.3	23.4 ± 1.3	24.8 ± 2.6
37	Red	19.2 ± 1.8	22.4 ± 0.8	25.5 ± 1.5	28.4 ± 2.1	32.4 ± 3.1
	White	17.7 ± 1.3	24.0 ± 1.3	25.3 ± 2.4	24.1 ± 3.1	27.2 ± 2.1
	Cardiac	18.2 ± 1.2	21.2 ± 2.1	24.3 ± 2.0	26.2 ± 2.6	30.7 ± 3.0

neutralized with 0.5 *N* NaOH and assayed for total free amino acid content [9]. The protein content of the homogenate prior to incubation was estimated according to the method of Layne [10]. The proteolytic activity in the assay medium was corrected to the *in vitro* autolytic degradation and expressed as μg of amino acid released/mg initial homogenate protein/h.

RESULTS

Table I shows the *in vitro* autolytic activity in red, white and cardiac muscles of rat as a function of temperature during ageing. The *in vitro* autolytic degradation of sarcoplasmic proteins increases with increase in temperature but the rate of increase varies with age. Q_{10} for *in vitro* autolytic activity increases upto 15 months in white muscle and upto 20 months in red and cardiac muscles. There is a sharp decrease at 25 months age in all the three muscles (Fig. 1). With advance in age, there is an increase in *in vitro* autolytic degradation of sarcoplasmic proteins in white, red and cardiac muscles (Fig. 2). The activity increases by 70% in red and cardiac and 54% in white muscle as the age advances from 5 months to 25 months (Fig. 3). Table II shows the influence of pH on proteolysis of the endogenous substrate. This indirectly suggests the effect of pH on cathepsin activity as the *in vitro* autolysis of the buffered homogenate is considered to be mostly due to intracellular cathepsins. Higher *in vitro* autolytic activity is seen in the alkaline range in all the three muscles of all five age groups. The reason for the peak seen at pH 4 in 5 month old animals needs further investigation with a different batch of rats. The Ca^{2+} activated proteolytic activity increases by two fold during ageing, the red muscle showing higher activity than the white (Fig. 4).

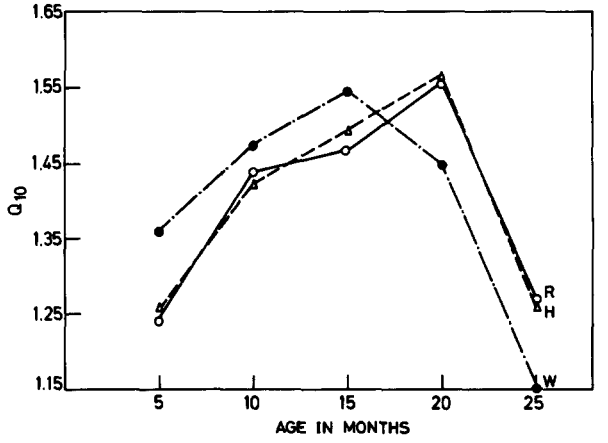

Fig. 1. Q_{10} for *in vitro* autolytic activity. R = red muscle (*soleus*); W = white muscle (*extensor digitorum longus*), H = cardiac muscle. Values are represented as mean ± of 7 observations.

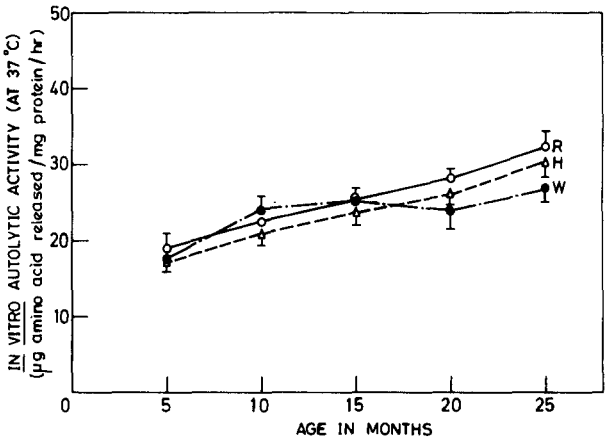

Fig. 2. *In vitro* autolytic degradation of sarcoplasmic proteins (at 37 °C). R = red muscle (*soleus*); W = white muscle (*extensor digitorum longus*), H = cardiac muscle. Values are represented as mean ± of 7 observations.

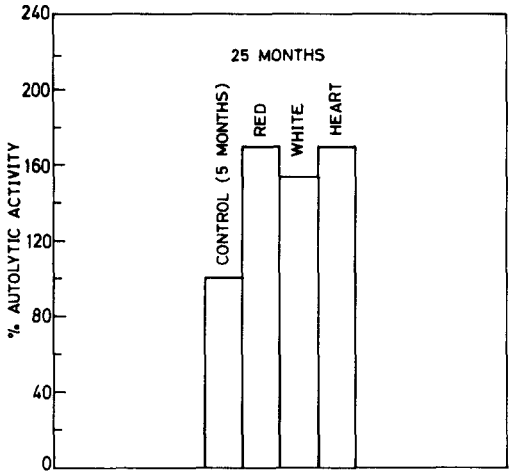

Fig. 3. Percentage increase in autolytic activity as the age advances from 5 months to 25 months. R = red muscle (*soleus*); W = white muscle (*extensor digitorum longus*), H = cardiac muscle. Values are represented as mean ± of 7 observations.

DISCUSSION

The increase in *in vitro* autolytic and Ca^{2+} activated proteolytic activity in red, white and cardiac muscles during ageing is concomitant with the results obtained for denervated muscle [5, 6]. There is no significant difference in *in vitro* autolytic degradation of the sarcoplasmic proteins between the three muscles in case of 5, 10 and 15 month old whereas at 20 and 25 months the activity is more in red than in white muscle. The increased *in vitro* autolytic activity of sarcoplasmic proteins in red, white and cardiac

TABLE II

EFFECT OF pH ON *IN VITRO* AUTOLYTIC DEGRADATION OF SARCOPLASMIC PROTEINS IN RED, WHITE AND CARDIAC MUSCLES OF RATS DURING AGEING

Values are represented as mean ± SD of 7 observations. Autolytic activity is expressed as μg of amino acid released/mg protein/h.

pH	*Nature of muscle*	*Age (months)*				
		5	*10*	*15*	*20*	*25*
2	Red	1.6 ± 0.3	2.3 ± 0.3	3.3 ± 0.3	3.8 ± 0.4	3.8 ± 0.3
	White	2.1 ± 0.4	2.9 ± 0.3	2.2 ± 0.2	2.5 ± 0.1	3.3 ± 0.7
	Cardiac	2.3 ± 0.5	2.9 ± 0.6	2.0 ± 0.1	3.4 ± 0.4	3.5 ± 0.4
3	Red	3.3 ± 0.3	3.1 ± 1.1	6.0 ± 0.8	5.4 ± 0.4	6.4 ± 0.5
	White	3.9 ± 0.6	4.0 ± 0.4	3.9 ± 0.2	5.0 ± 0.6	4.2 ± 0.7
	Cardiac	4.0 ± 0.2	2.9 ± 0.3	3.2 ± 0.1	4.2 ± 0.5	6.8 ± 0.6
4	Red	8.8 ± 1.7	4.7 ± 0.7	6.6 ± 1.1	10.3 ± 1.4	9.7 ± 0.8
	White	7.2 ± 0.8	3.4 ± 0.7	5.8 ± 0.5	4.8 ± 0.7	7.2 ± 0.9
	Cardiac	6.7 ± 0.7	5.0 ± 0.5	5.1 ± 0.6	7.0 ± 0.8	10.0 ± 0.7
5	Red	6.3 ± 1.1	6.8 ± 1.1	9.7 ± 1.3	11.3 ± 1.6	12.7 ± 0.9
	White	5.9 ± 1.4	4.4 ± 0.8	7.2 ± 0.6	7.4 ± 0.8	9.0 ± 0.8
	Cardiac	5.6 ± 0.3	6.6 ± 0.6	6.5 ± 0.8	11.0 ± 1.1	13.2 ± 0.8
6	Red	7.9 ± 0.9	9.5 ± 0.8	12.4 ± 1.6	14.3 ± 1.7	16.1 ± 1.3
	White	6.0 ± 1.5	6.9 ± 1.0	9.0 ± 0.9	10.6 ± 0.7	13.2 ± 1.4
	Cardiac	7.1 ± 0.6	7.3 ± 1.4	8.6 ± 0.7	12.6 ± 1.5	15.2 ± 1.1
7	Red	10.8 ± 1.0	12.4 ± 1.6	14.3 ± 0.7	17.4 ± 1.2	20.4 ± 1.9
	White	8.2 ± 1.0	8.3 ± 1.0	11.5 ± 0.7	13.6 ± 1.5	17.6 ± 0.8
	Cardiac	8.2 ± 0.8	9.4 ± 0.9	10.1 ± 0.7	14.5 ± 1.5	17.5 ± 1.2
8	Red	11.2 ± 1.4	12.1 ± 1.5	15.6 ± 1.0	20.0 ± 1.6	24.8 ± 1.1
	White	9.0 ± 0.4	9.8 ± 0.8	12.0 ± 0.9	16.7 ± 1.2	21.5 ± 1.7
	Cardiac	10.7 ± 1.2	10.8 ± 0.4	12.3 ± 0.5	18.7 ± 1.6	22.3 ± 1.3
9	Red	11.6 ± 0.8	12.8 ± 1.2	14.5 ± 0.9	20.4 ± 1.3	23.6 ± 0.9
	White	8.5 ± 0.5	8.7 ± 1.1	9.2 ± 0.5	16.7 ± 0.7	20.0 ± 0.7
	Cardiac	10.5 ± 0.7	11.0 ± 1.2	12.0 ± 0.4	18.7 ± 1.8	22.5 ± 0.5

muscles during ageing could be attributed to increased cathepsin activity as the autolysis of the homogenate is considered to be mostly due to intracellular cathepsins. Two types of cathepsins are reported to occur in the skeletal muscle, one with an optimum activity in the acid range [13], and the other in the alkaline range [14]; however, these studies were not with reference to ageing.

Increase in *in vitro* autolytic and Ca^{2+} activated proteolytic activity of sarcoplasmic proteins shown by the present results can be related to the increase in degradation of proteins during ageing. Senile and denervated muscles have similar pattern of change with reference to autolytic and proteolytic activity. The analogy of metabolic changes in muscles of old animals and those of denervated muscles is further strengthened by the present study.

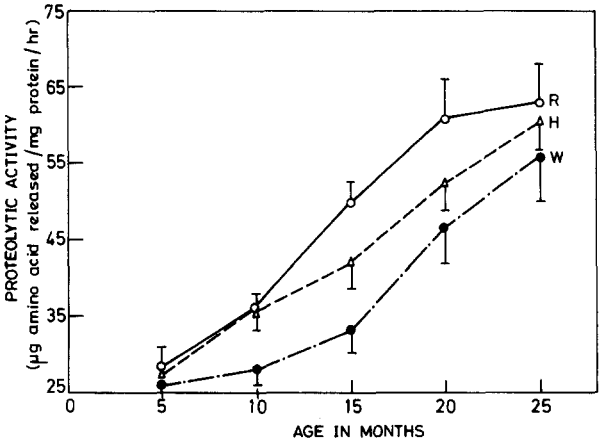

Fig. 4. Ca^{2+} activated proteolytic activity of sarcoplasmic proteins. R = red muscle (*soleus*); W = white muscle (*extensor digitorum longus*), H = cardiac muscle. Values are represented as mean ± SD of 7 observations.

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