Pferdeheilkunde 12 (1996) 4 (Juli August) 488–492

Concentration and composition of serum acid glycosaminoglycans in horses

*A. Calatroni, Rosalia Vinci, Alida M. Ferlazzo** and *Adriana Ferlazzo**

Istituto pluridisciplinare di Fisiologia Umana, and
*Istituto di Fisiologia Generale e Speciale degli Animali domestici e Chimica Biologica, University of Messina, Italy

Summery

Serum glycosaminoglycan (GAG) composition was studied in horses. Comparison was made between GAG mixtures obtained from four trained horses and four untrained horses, and with plasma GAGs from two trained horses. Moreover, the concentrations and characteristics of serum GAGs were evaluated in four trained horses at different times after a show jumping competition.
GAGs were isolated, fractionated and analysed by anion exchange chromatography.
GAG concentrations, expressed in terms of aminosugars, were significantly ($p<0.025$) higher in serum from trained horses than in serum from untrained horses.
Analytical data suggest similar serum GAG composition in trained and untrained horses. Chondroitin sulphate, measured in terms of galactosamine, and keratan sulphate, measured in terms of galactose, were the main components, and observed in the ratio 4 to 1. About 50% of the GAG chains were undersulphated. Following competition, a decrease of serum GAG concentrations was constantly observed.

Keywords: Glycosaminoglycan, horse serum, physical exercise, physical training, keratan sulphate

---

Konzentration und Zusammensetzung der Glykosaminoglykane im Serum von Pferden

An 10 gesunden Pferden im Alter von 5–12 Jahren wurde die Zusammensetzung des Glykosaminoglykans (GAG) im Serum untersucht. Die Serum-GAG-Gemische von trainierten Pferden (n=4) wurden mit denen von untrainierten (n=4), sowie mit den Plasma-GAG-Gemischen zweier trainierter Pferde verglichen. Außerdem wurden Konzentration und Charakteristika der Serum-GAGs bei 4 trainierten Pferden zu verschiedenen Zeitpunkten nach einem Springwettbewerb untersucht. Die GAGs wurden isoliert, fraktioniert und mittels Anionenaustausch-chromatographie analysiert. Die Konzentration an GAGs, angegeben als Aminozucker-Einheiten, war im Serum der trainierten Pferde signifikant höher ($p<0,025$) , als in dem der untrainierten. Die analytische Auswertung deutet an, daß die Zusammensetzung der Serum-GAGs bei trainierten und untrainierten Pferden gleich ist. Chondroitin-Sulfat, bestimmt als Galaktosamin-Einheiten, und Keratan-Sulfat, gemessen als Glukose-Einheiten, stellten die Hauptkomponenten dar; für sie wurde ein Verhältnis von 4 : 1 ermittelt. Ungefähr 50% der GAG-Ketten waren nicht ausreichend mit Sulfat gesättigt. Nach einem Wettkampf konnte ein kontinuierlicher Abfall der GAG-Konzentration beobachtet werden.

Schlüsselwörter: Glykosaminoglykane, Pferd, Serum, Belastung, Training, Keratan-Sulfat

Introduction

Acid Glycosaminoglycans (GAGs), the highly charged linear polysaccharides widely distributed in animal tissues that are composed of repeated disaccharide units containing hexosamine and hexuronic acid (or galactose, in keratan sulphate), have been measured (*Ferlazzo* et al., 1991) and characterized (*Calatroni* et al., 1995) in horse plasma and serum. The concentrations were similar to those measured in man and in other domestic animal species. Also the degree of sulphation, the glucosamine to galactosamine ratio, and the masking of anionic charges in native complexes with proteins were similar.
The origin of serum GAGs is not yet well understood. GAGs are supposed to exist mainly in proteoglycan form. In human plasma, covalent, electrostatic and specific interactions have been demonstrated to exist between plasma GAGs and plasma proteins (*Calatroni* et al., 1992). An undersulphated chondroitin-4-sulphate chain, covalently linked to proteins, has been detected in the inter-a-trypsin inhibitor of plasma (*Jessen* et al., 1988). The C1q inhibitor in serum is a chondroitin-4-sulphate proteoglycan, very similar to the proteoglycan isolated from platelets (*Kolset* and *Gallagher,* 1990). However, the presence of GAGs in circulation may be correlated with the metabolism of peripheral connective tissues, and some circulating GAG structures are probably degradation products originating from articular cartilage (*Thonar* et al., 1985) and from intramuscular connective tissue (*Muraca* et al., 1992). Vascular endothelial cells (*Bourin* and *Lindahl,* 1993) and blood cells (*Kolset* and *Gallagher,* 1990) may also release GAGs.
Studies on the composition of GAG mixtures isolated from horse plasma and serum showed that the main component is galactosamine-containing chondroitin sulphate, largely in undersulphated form, as in man; and that glucosamine-containing heparan sulphate and keratan sulphate are also present. Fucose was detected in GAG fractions (*Calatroni* et al., 1995).
No significant variations in plasma GAG levels were observed according to age or sex (*Ferlazzo* et al., 1991). Physical training appears to be the major factor affecting plasma (and serum) GAG concentration in humans (*Muraca* et al., 1992) and in horses (*Ferlazzo* et al., 1991).
In the present study, the composition of GAG mixtures isolated from serum in trained and untrained horses, and from plasma in

trained horses was investigated. The effect of physical exercise (show jumping competition) on the characteristics of serum GAG mixtures was evaluated in trained horses at different times after the end of a race. The aims of this study were a) to compare GAG concentrations in trained and untrained horses and b) to monitor the post exercise changes. Data obtained may prove useful for directing further studies to evaluate a possible role for serum GAGs, especially keratan sulphate, in monitoring the effects of physical exercise and to study the kinetics of recovery related to pre-exercise conditions.

Materials and methods

Animals and blood samples

Ten healthy horses were utilized, of both sexes and of different breeds, aged from 5 to 12 years. Four animals were untrained, the remaining six horses were trained. Four of the six were utilized in show jumping competition: two horses were 5 and 6 years old and two horses were 11 and 12 years old. A single blood sample was obtained in the untrained animals for serum separation. From the four subjects utilized in show jumping competitions, four blood samples were collected from each animal: at rest, at 30 minutes, 90 minutes and 24 hours after a competition, for serum separation.

A single blood sample was obtained from the two remaining trained horses, using tubes containing EDTA solution for plasma separation. The two horses were trained, but were not used for show jumping competition.

Isolation, purification and fractionation of serum or plasma acid GAG mixtures

Acid GAGs were isolated from plasma and serum (1–4 ml) as described (*Calatroni* et al., 1992). Each sample (serum or plasma) was held in alkaline conditions (0.05 M NaOH) at 40°C for 16 hours to cleave protein-carbohydrate covalent linkages and to release GAG chains from proteoglycans. The sample solution was filtered through weak anion exchanger Ecteola-cellulose (Fluka, 0.7×3 cm column, chloride form). The acid GAG molecules isolated on the resin were eluted with 2M NaCl and quantified in terms of hexuronic acid. The crude GAG solution from Ecteola-cellulose was applied to a Bio-Gel P-2 column (0.7×40 cm column) . Desalted and purified polysaccharide GAG fraction was isolated.

The purified GAG fraction was then subfractionated on a strong anion exchanger Dowex 1×2 (Fluka, 0.7×4 cm column, chloride form) into low sulphate (LS) and normally sulphated (NS) fractions, by eluting with 1 M and 2 M NaCl solutions, respectively. LS and NS fractions were desalted on Bio-Gel P-2 as described, and reduced in volume under vacuum. Glucosamine and galactosamine, galactose and fucose were measured in the resulting 1 ml volume salt-free solutions.

Analytical methods

Hexuronic acid was determined by the colorimetric method of *Bitter* and *Muir* (1962), using glucuronic acid (Sigma) as a standard. Glucosamine and galactosamine were determined following acid hydrolysis in 4M HCl, four hours at 100°C. Following HCl elimination under vacuum, the two aminosugars were separated in HPLC by two different methods:

- as dansyl derivatives, on reverse phase C18 column, with fluorescent detector, eluting with 18% acetonitrile;
- directly on anion exchange resin CarboPac PA1, with pulsed amperometric detection, eluting with 0.016 M NaOH.

Galactose and fucose were determined using HPLC and on anion exchanger CarboPac PA1, eluting with 0.016 M NaOH, with pulsed amperometric detection following acid hydrolysis in less severe conditions: 0.3 M HCl was utilized for fucose, and 1 M HCl for galactose determination, again at 100°C for 4 hours.

Accuracy and precision

Accuracy of the method was tested by measuring recovery of commercial (Sigma) GAG added (20 micrograms per ml of serum) to human serum. Accuracy was 3,8%. Precision was tested utilizing the same serum sample in different amounts (from 0.5 to 5 ml). Coefficient of variation (P=95%) was less than 5%.

Statistical analysis

The differences in the means were evaluated by Student's t-test.

Results

Concentrations of acid GAGs in terms of hexuronic acid in crude GAG fractions from Ecteola-cellulose

The acid GAGs concentrations in terms of hexuronic acid (component of every type of GAG, except for keratan sulphate) in the crude GAG fraction from Ecteola-cellulose were consistent with concentrations of 11.3±2.7 mg/l in serum and 9.5±0.8 mg/l in plasma from trained horses. These values were significantly higher ($p<0,0025$) than those measured in serum from untrained horses (5.2±0.7 mg/l). After the competition a mean decrease of about 40% at 30 minutes and 60% at 90 minutes was observed, with total recovery after 24 hours. By comparing these figures obtained in the crude fraction from Ecteola-cellulose and expressed in terms of hexuronic acid with those measured in the purified GAG fraction from Bio-Gel P-2 and expressed in terms of aminosugars (as shown in Tab.1), the purified polysaccharide GAG fraction from Bio-Gel P-2 appears to represent about 75–80% of the material isolated on Ecteola-cellulose resin.

Concentration and composition of purified fractions of acid GAGs in serum from trained and untrained horses (Tab. 1)

Tab. 1 lists the concentrations of purified GAGs isolated from serum of trained and untrained horses. The concentrations are given in terms of mg/l of serum of the main neutral and amino-monosaccharide components of GAGs. Every GAG chain contains aminosugars, glucosamine or galactosamine, and keratan sulphate contains galactose instead of hexuronic acid. Each type of monosaccharide represents about 30% by weight of the GAG chain. Fucose is present in GAGs only in some types of keratan sulphate.

In Tab. 1 GAG concentrations are relative to the low sulphate (LS) and normally sulphated (NS) fractions obtained from Dowex 1. The LS fraction accounted for 58% and 56% of total GAGs in terms of total aminosugars (glucosamine + galactosamine) in serum from untrained horses and trained horses, respectively.

In Tab. 1 the galactosamine concentrations are an estimate of chondroitin sulphate molecules, and galactose concentrations are an estimation of keratan sulphate chains. Glucosamine is an estimation of the three glucosamine-containing GAGs, keratan sulphate, heparan sulphate and hyaluronan. As the concentration of hyaluronan in horse serum is very low *(Engström-Laurent* et al., 1985) the difference between galactose and glucosamine concentrations becomes a measure of heparan sulphate.

In trained horses, compared to untrained horses, the GAG concentrations were higher in terms of galactosamine, glucosamine and galactose in the LS fraction and NS fraction.

Tab. 1: Acid glycosaminoglycan (GAG) concentrations in serum from four untrained and for trained horses.

	concentrations expressed in terms of: (mg/l of serum, mean value ± S.D.)				weight ratios between mean values of concentration		
	GalN (CS)	GlcN (KS + HS)	Gal (KS)	Fuc	GlcN / GalN	Gal / GlcN	Fuc / Gal
Untrained horses (n=4)							
LS fraction, mg/l	1.87±0.60	0.60±0.20	0.50±0.01	0.15±0.06	0.32	0.83	0.30
NS fraction, mg/l	1.52±0.40	0.26±0.08	0.25±0.04	0.06±0.04	0.17	0.96	0.24
Trained horses (n=4)							
LS fraction, mg/l	3.79±1.70*	1.16±0.4**	0.82±0.32*	0.25±0.11	0.30	0.71	0.30
NS fraction, mg/l	3.12±1.2**	0.78±0.50*	0.54±0.28*	0.11±0.05	0.25	0.69	0.20

* $p<0.05$, ** $p<0.025$ vs corresponding fractions in untrained horses. GalN=galactosamine; GlcN=glucosamine; Gal=galactose; Fuc=fucose; CS=chondroitin sulphate; KS=keratan sulphate; HS=heparan sulphate; LS=low sulphate; NS=normally sulphated.

In Tab. 1 values of the weight ratios between the mean values of monosaccharides concentrations are listed. Very similar values were obtained in serum GAG fractions from trained and untrained animals.

Concentration and composition of purified fractions of acid GAGs in plasma from two trained horses

Monosaccharides listed in Tab. 1 were also measured in purified fractions obtained from the two plasma samples from trained horses. The GAG concentrations in terms of galactosamine (2.6 ± 0.6 mg/l, LS fraction; 3.3 ± 0.1 mg/l, NS fraction), of glucosamine (0.87 ± 0.25 mg/l, LS fraction; 0.75 ± 0.01 mg/l, NS fraction), of galactose (0.65 ± 0.15 mg/l, LS fraction; 0.50 ± 0.18 mg/l, NS fraction) and of fucose (0.18 ± 0.11 mg/l, LS fraction; 0.12 ± 0.09 mg/l, NS fraction) were similar in NS fractions to those measured in NS fractions from serum of trained horses (Tab. 1). The GAG concentrations for the to LS fractions in plasma were consistently lower than in serum, although differences were not significant.

Concentration and composition of purified fractions of acid GAGs in serum from trained horses before and after show jumping competition (Tab. 2)

Changes of serum GAG concentrations and composition in trained horses following show jumping competition are shown in

Tab. 2: Changes in serum glycosaminoglycan concentrations in four trained horses following show jumping competition.

		concentration measured at rest	concentrations measured after the end of the competition, at: 30 minutes	90 minutes	24 hours
		mean value ±S.D.	mean value ±S.D.	mean value ±S.D.	mean value ±S.D.
Galactosamine (CS)					
LS fraction	mg/l	3.79±1.70	3.23±0.40	2.41±0.83	3.45±0.94
	(%)	(100)	(85±11)	(64±22)	(91±25)
NS fraction	mg/l	3.12±1.20	1.87±0.74	2.03±1.06	2.95±0.37
	(%)	(100)	(60±24)	(65±34)	(95±12)
Glucosamine (HS+KS)					
LS fraction	mg/l	1.16±0.40	0.80±0.05	0.50±0.16*	0.76±0.23
	(%)	(100)	(70±4)	(43±14)	(66±20)
NS fraction	mg/l	0.78±0.50	0.34±0.05	0.41±0.28	0.50±0.14
	(%)	(100)	(43±6)	(53±36)	(64±18)
Galactose (KS)					
LS fraction	mg/l	0.82±0.32	0.53±0.13	0.46±0.19	0.53±0.21
	(%)	(100)	(65±16)	(56±24)	(65±26)
NS fraction	mg/l	0.54±0.28	0.29±0.21	0.25±0.08	0.38±0.12
	(%)	(100)	(54±39)	(46±16)	(70±22)

* $p<0.0125$ vs value at rest. CS=chondroitin sulphate; HS=heparan sulphate; KS=keratan sulphate; LS=low sulphate; NS=normally sulphated

Tab. 2. Concentrations are given in terms of galactosamine, glucosamine and galactose, mg/l of serum, in both LS and NS fractions. Changes are expressed in Tab. 2 also in terms of percent of the value measured at rest.
The GAG concentration in serum from trained horses measured at rest, in terms of galactosamine, appeared to be lower at 30 and 90 minutes after the end of the competition. Values measured after 24 hours were similar to the values at rest. The decrease appeared to be greater for glucosamine and galactose, in both the LS and NS fractions, but the decrease was statistically significant only at 90 minutes in terms of glucosamine.
Changes at 90 minutes post-exercise listed in Tab. 2 appear different when the four horses are grouped according the age. Younger horses showed values at 90 minutes higher than those in Tab. 2, and in terms of galactosamine (recovery was 85% of the value at rest, LS fraction, and 95%, NS fraction) and in terms of glucosamine (56%, LS fraction, 88%, NS fraction) and of galactose (73%, LS fraction, 50%, NS fraction). Recoveries obtained in older horses at 90 minutes were lower than 40%, with the only exception of the recovery of galactosamine in LS fraction (50%).

Discussion

A major role for the acid glycosaminoglycans (GAGs) chain of proteoglycans outside the extracellular matrix appears to be the interaction with proteins, especially enzymes, or enzyme inhibitors, or growth factors, acting to regulate the physiological functions of these proteins, or simply to prevent their proteolytic degradation (*Kolset* and *Gallagher,* 1990). The role may also be explainable by the GAG chain of proteoglycans present in plasma or serum: indeed, plasma inter-a-trypsin inhibitor contains covalently linked low sulphated chondroitin sulphate (*Jessen* et al., 1988). This inhibitor should represent the main component of plasma proteoglycans.
The origin of the other GAG molecules in plasma or serum is still under study. Platelets contain chondroitin-4-sulphate that is released when platelets are stimulated (*Kolset* and *Gallagher,* 1990). Chondroitin sulphate concentration in serum is significantly increased in patients with thrombocythaemia (*Calabrò* et al., 1995), and C1q inhibitor in serum is supposed to originate from platelets (*Kolset* and *Gallagher,* 1990). The higher concentration of GAGs observed in serum compared with plasma in humans (*Calabrò* et al., 1995) and in horses (*Ferlazzo* et al., 1991; this work) may be explained by the release of chondroitin sulphate chains from platelets during coagulation.
Changes with training may suggest new hypotheses about the origin of serum GAGs. As shown in Tab. 1, GAG concentration in serum from trained horses is significantly higher than GAG concentration in serum from untrained horses in terms of galactosamine (chondroitin sulphate), in terms of galactose (keratan sulphate) and in terms of glucosamine (keratan sulphate and heparan sulphate). The relative increase is approximately the same: the composition of serum GAG mixtures from trained and untrained horses appears very similar. However an examination of larger groups appears necessary to support the finding that sites of origin are unchanged, and contribution to circulating GAG pool increases in trained subjects, because sites of origin are affected by training.
The increase in number of capillaries in muscle in trained individuals may suggest that vascular endothelial cells are releasing their surface GAGs, heparan sulphate and chondroitin sulphate (*Bourin* and *Lindhal,* 1993), in correspondingly increased amounts. The improvement with training of biochemical and biomechanical properties of cartilage (*Säämänen* et al., 1988) may suggest that also articular cartilage is increasing its contribution to serum GAG pool in trained subjects. The most abundant type of proteoglycan in the extracellular matrix of cartilage, aggregan, contains chondroitin sulphate and keratan sulphate side chains. Aggregan fragments generated by the degradation of cartilage appear in blood, as shown by *Thonar* et al. (1985) by measuring serum keratan sulphate by means of a sensitive immunoassay which quantifies highly sulphated sequences in keratan sulphate chains. Data shown in Tab. 1 add more information to characterize serum keratan sulphate and to support the hypothesis that GAGs are released from articular cartilage in blood, in higher amounts in trained horses. About 60% of the galactose-containing molecules, keratan sulphate, are undersulphated (LS fraction). These molecules are not detected by the method of *Thonar* et al. (1985). Furthermore, both LS and NS fractions of serum GAGs contain fucose, increasing with galactose in trained horses, although differences were not significant. The skeletal type of keratan sulphate is classified into articular and non-articular on the basis that the former contains fucose, which is absent in the latter (*Brown* et al., 1994).
Presence of fucose suggests that serum keratan sulphate was generated in articular cartilage, and that degradation of articular cartilage is affected by training.
Changes were observed in serum GAG concentrations in trained horses following show jumping competition, as shown in Tab. 2. Concentration of every type of GAG appears reduced at 30 and 90 minutes in the four animals, although the only statistically significant difference was found at 90 minutes in terms of glucosamine (kinetics of changes appear different in younger and older subjects, and values of standard deviations are high). Animal differences within a group of only four subjects, by preventing statistical support to the differences between group values, greatly limit the interpretation of the data. Further studies are needed to statistically support the constant decrease observed in this work. A suggested trend indicated by data listed in Tab. 1 (NS fractions appear more affected than LS fractions, and glucosamine-containing GAGs show slow recovery to pre-exercise levels) is that concentrations are decreased because degradation of circulating molecules was promoted: molecules of LS fraction, especially chondroitin sulphate, are tightly bound to proteins that prevent their interactions (*Calatroni* et al., 1992).
The hypothesis of degradation in circulation following exercise appears noteworthy. Exercise in horses results in a two- to threefold increase in plasma cortisol concentrations, with values returning to the pre-exercise level within a few hours (*Rose* and *Hodgson,* 1994). The effect of cortisol on protease activity and on liver gluconeogenesis could explain degradation of circulating GAGs, occurring in liver. On the other hand, diurnal variations were found in man (*Muraca* et al., 1992) and in horses (*Calatroni* et al., 1991), with minimum values in the morning, when cortisol reaches its highest levels in plasma.

Conclusions

The limited number of horses utilized in this study would suggest working hypotheses rather than conclusions. However, the significantly higher values of GAG concentrations in trained horses compared with untrained horses, confirming previous data (*Ferlazzo* et al., 1991), appear to be a valid result. Furthermore, the serum GAG composition, in terms of low sulphate (LS) and nor-

mally sulphated (NS) fractions, and in terms of aminosugars, galactose and fucose appears very similar in trained and untrained horses, although further studies are needed to reach final conclusions. At present, GAGs of the LS fractions represent 56–58% of total GAGs. Main components are chondroitin sulphate (75% of total GAGs in LS fraction, 80–85% in NS fraction) and keratan sulphate (16–20% in LS fraction, 14% in NS fraction).

Fucose was detected in serum GAG fractions. This strongly supports the hypothesis that serum keratan sulphate originated in articular cartilage by degradation of cartilage proteoglycans.

Data relative to changes of GAG concentrations in serum of trained horses at 30 minutes, 90 minutes and 24 hours after a show jumping competition provide suggestions for further studies. A decrease of GAG concentration after the competition was always observed. However, results of the present work suggest that the kinetics of the process depends upon the type of GAG and the age of the animal, and indicate that examination of larger groups of conveniently selected animals is needed.

References

Bitter, T. and *Muir, H. M.* (1962): A modified uronic acid carbazole reaction. Anal. Biochem. 4, 330–334.

Bourin, M. C. and *Lindahl, U.* (1993): Glycosaminoglycans and the regulation of blood coagulation. Biochem. J. 289, 313–330.

Brown, G. M., Huckerby, T. N., Morris, H. G., Abram, B. L. and *Nieduszynski, I. A.* (1994): Oligosaccharides derived from bovine articular cartilage keratan sulfates after keratanase II digestion: implications for keratan sulfate structural fingerprinting. Biochemistry 33, 4836–4846.

Calabrò, L., Vinci, R., Musolino, C. and *Calatroni, A.* (1995): Acid glycosaminoglycans in normal and pathological human serum. Eur. J. Histochem. 39 suppl., 20.

Calatroni, A., Vinci, R. and *Ferlazzo, A. M.* (1991): Variazioni diurne dei livelli sierici di glicosaminoglicani acidi nel cavallo (Diurnal variations of acid glycosaminoglycan levels in horse serum). Atti. Soc. Ital. Sci. Veter. 44, 425–429.

Calatroni, A., Vinci, R. and *Ferlazzo, A. M.* (1992): Characteristics of the interactions between acid glycosaminoglycans and proteins in normal human plasma as revealed by the behaviour of the protein-polysaccharide complexes in ultrafiltration and chromatographic procedures. Clin. Chim. Acta. 206, 167–180.

Calatroni, A., Campo, S., Vinci, R. and *Ferlazzo, A. M.* (1995): The measurement of aminosugars and neutral sugars in plasma and serum glycosaminoglycan fractions by anion exchange chromatography and pulsed amperometric detection. Ital. Biochem. Soc. Trans. 6, P46.

Engström-Laurent, A., Laurent, U. B. G., Lilja, K. and *Laurent, T. C.* (1985): Concentration of sodium hyaluronate in serum. Scand. J. Clin. Lab. Invest 45, 497–504.

Ferlazzo, A. M., Vinci, R., Panzera, M., Ferlazzo, A and *Calatroni, A.* (1991): Glycosaminoglycan concentrations in horse plasma and serum. Differences with other animal species and identification of affecting factors. Comp. Biochem. Physiol. 100B, 745–751.

Jessen, T. E., Faarvang, K. L. and *Ploug, M.* (1988): Carbohydrate as covalent crosslink in human inter-a-trypsin inhibitor: a novel plasma protein structure. FEBS Lett. 230, 195–200.

Kolset, S. O. and *Gallagher, J. T.* (1990): Proteoglycans in haemopoietic cells. Biochim. Biophys. Acta. 1032, 191–211.

Muraca, U., Vinci, R., Ferlazzo, A. M., Muraca, G. and *Calatroni, A.* (1992): Factors affecting glycosaminoglycan concentration in normal human plasma. Ital. J. Biochem. 41, 159–169.

Rose, R. J. and *Hodgson, D. R.* (1994): Hematology and biochemistry, in: The athletic horse (*Hodgson, D. R.* and *Rose, R. J.*, Eds), *W. B. Saunders Co.*, Philadelphia, USA, pp.63–78.

Säämänen, A.-M., Tammi, M., Kiviranta, I. and *Helminen, H. J.* (1988): Running exercise as a modulator of proteoglycan matrix in the articular cartilage of young rabbits. Int. J. Sports Med. 9, 127–133.

Thonar, E. J.-M. A., Lenz, M. E., Klintworth, G. K., Caterson, B., Pachman, L. M., Glickman, P., Katz, R., Huff, J. and *Kuettner, K. E.* (1985): Quantification of keratan sulfate in blood as a marker of cartilage catabolism. Arthritis. Rheum. 28, 1367–1376.

Acknowledgement

This study was supported by grants 40% and 60% of the Murst of Italy.

A. Calatroni
Rosalia Vinci

Istituto pluridisciplinare di Fisiologia Umana
Universitá di Messina
Facoltá di medicina e chirurgia
Via T. Cannizzaro, 278
98122 Messina, Italia

Tel. (0039 90) 71 34 48–77 45 97

Alida M. Ferlazzo
Adriana Ferlazzo

Istituto di Fisiologia generale e Speciale degli
Animali domestici e Chimica Biologica
Universitá di Messina
Facoltá di medicina e chirurgia
Via T. Cannizzaro, 278
98122 Messina, Italia

Tel. (0039 90) 71 34 48–77 45 97