Enzyme Activity in Aging Articular Cartilage

Both ultrastructure and enzymatic activity of articular cartilage change with advancing age[1,2]. During growth and midlife, the activities of several glycolytic enzymes, of myokinase, of creatine phosphokinase and of isocitric dehydrogenase varied inversely of DNA content; the latter had risen slightly during the first three months, dropped steeply at the end of the first year of life and remained about stationary thereafter. However in old age, total enzyme activity of the cartilage as well as that of individual chondrocytes rose again. This functional stimulation was associated with rapid turnover and organellar development of the cells.

The present investigation was carried out in order to determine whether or not similar aging changes might occur in regard to other enzyme systems involved in cartilage homeostasis or degradation.

Material and methods. The samples of articular cartilage were obtained from the same untreated animals used in the previous investigation, except for a few instances in which the tissue had to be supplemented from additional animals. Articular cartilage was removed from the femoral heads and knee joints of untreated female guinea-pigs, 2 weeks, 12 weeks, 1 year, $2^1/_2$ years and $5^3/_4$ years of age. 4 animals were used for each age group. The methods used for collection, and preparation of the cartilage were the same as those reported earlier, except that 0.5% Triton X-100 was added during the general dilution of the samples.

The following enzymes were presently assayed: uridine diphosphate glucose dehydrogenase (UDPDH)[3], 6-phosphogluconic dehydrogenase (6GPDH)[4], malic enzyme[5], glutamic oxaloacetic transaminase (GOT)[6], malic dehydrogenase (MDH)[7], glutamic dehydrogenase (GLDH)[8], β-galactosidase[9], α-mannosidase[10], α-glucosidase[11], β-acetyl glucosaminidase[7], sulfatase[12,13], and cathepsin D[9] (Table I). Sulfatase was stopped with alkaline quinol. β-galactosidase and α-mannosidase were stopped with glycine (0.2 *M*)-NaCl (0.1 *M*)-Na_2CO_3 (0.125 *M*) buffer pH 10.7. Cathepsin D and α-glucosidase were stopped with 5% Trichloroacetic Acid.

Results. The activity of UDPGDH changed little during the first $2^1/_2$ years, but had risen to a life time peak at the end of the sixth year of life, the increase being about twofold on the basis of dry weight and fourfold as calculated per unit DNA (Figure 1).

The activity of 6PGDH dropped continuously to the age of $2^1/_2$ years, increasing thereafter to more than twice the previous level as calculated per dry weight and-

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Table I. Reagents and incubation periods for enzyme determinations

Enzyme	Buffer	Substrate (s)	Other additions	Standard	Incubation time
UDP-glucose dehydrogenase	0.1 *M Tris* 8.6	0.5 m*M* DPN 2 m*M* UDPG		DPNH	15 min
6-P-gluconic dehydrogenase	0.04 *N* glycyl glycine 7.6	0.5 m*M* TPN 2 m*M* 6-P-gluconate	5 m*M* $MgCl_2$	TPNH	30 min
Malic enzyme	0.1 *M Tris* 7.5	0.2 m*M* TPN 3 m*M* malate	0.02% BSA 0.1 m*M* $MnCl_2$	TPNH	30 min
Glutamic oxaloacetic transaminase	0.1 *M Tris* 8.2	16 m*M* aspartic acid 10 m*M* αKG 0.5 m*M* DPNH	1 µg/ml MDH 30 m*M* K_2HPO_4	DPN	15 min
Malic dehydrogenase	0.04 *M* Imidazole 7.3	0.5 m*M* DPNH 1 m*M* oxaloacetate	0.02% BSA 15 m*M* nicotinamide	DPN	30 min
Glutamic dehydrogenase	0.1 *M Tris* 7.6	5 m*M* αKG 0.5 m*M*	30 m*M* K_2HPO_4 40 m*M* $(NH_4)_2SO_4$ 1 m*M* ADP 5 m*M* sodium amytal	DPN	30 min
β-galactosidase	0.02 *M* lactate 4.0	1 m*M* methylum belliferyl-β-D-galactopyranoside		4-methylum belliferone	2 h
α-mannosidase	0.02 *M* citrate 5.5	10 m*M* 4-methylum belliferyl-α-D-mannopyranoside		4-methylum belliferone	2 h
α-glucosidase	0.02 *M* lactate 4.0	10 m*M* maltose		Glucose	2 h
β-acetyl glucosaminidase	0.05 *M* acetate 5.0	1 mg/ml, 2-nitrophenyl-2-acetamido-2-deoxy-B-D-glucopyranoside		*p*-nitrophenol	2 h
Cathepsin	0.1 *M* borate-acetate-cocodylate 4.0	5 mg/ml bovine serum albumin		Bovine serum albumin	24 h

Table II. Age-linked anges i enzyme activity[a] in articular cartilage of the lower extremity of female guinea-pigs

Enzyme	2 weeks	12 weeks	1 year	$2^1/_2$ years	$5^3/_4$ years
UDP glucose dehydrogenase (mM)	37.7 ± 5.5	37.8 ± 1.4	28.8 ± 2.5	32.1 ± 4.7	64.5 ± 8.6
6-phosphogluconic dehydrogenase (mM)	145 ± 14	43 ± 5	32 ± 3	30 ± 3	84 ± 8
β-galactosidase (mM)	10.16 ± 1.67	3.10 ± 0.77	1.46 ± 0.17	1.78 ± 0.27	0.56 ± 0.17
α-mannosidase (μM)	398 ± 57	164 ± 15	115 ± 7	132 ± 8	49 ± 13
Sulfatase (μM)	2.38 ± 0.24	2.74 ± 0.18	1.98 ± 0.09	2.70 ± 0.34	4.65 ± 0.59
α-glucosidase (mM)	241 ± 20	259 ± 29	175 ± 11	207 ± 37	379 ± 56
Glutamic oxaloacetic transaminase (mM)	634 ± 81	489 ± 94	374 ± 54	297 ± 58	247 ± 51
Glutamic dehydrogenase (mM)	50.0 ± 3.7	58.3 ± 6.7	35.6 ± 9.1	28.4 ± 3.7	< 3.5
Malic dehydrogenase (mM)	2137 ± 346	1541 ± 197	1506 ± 147	1492 ± 141	1918 ± 142
β-acetyl glucosaminidase (mM)	19.5 ± 3.1	12.8 ± 1.8	12.7 ± 1.2	6.7 ± 1.3	1.1 ± 0.3
Cathepsin (BSA) (g)	1.006 ± 0.124	0.723 ± 0.058	0.691 ± 0.089	0.587 ± 0.115	0.749 ± 0.174
Malic enzyme	Activity < 20 mM/Kd/h for all samples				

[a] Calculated as moles (M), millimoles (mM), micromoles (μM), or grams (g)/kilogram dry weight/h. ±, indicates standard error.

to 6 times the previous level as calculated on the basis of DNA.

Sulfatase, after showing only minor variations through growth and midlife reached a peak of activity at the end of the sixth year. The increase amounted to almost twice or 4 times the previous level as calculated per dry weight, or on the basis of DNA respectively (Figure 2).

The activity of α-glucosidase as calculated per dry weight dropped after cessation of growth, but rose again during midlife and in old age. As calculated on the basis of DNA the enzyme changed little during growth and midlife, but rose slightly late in life.

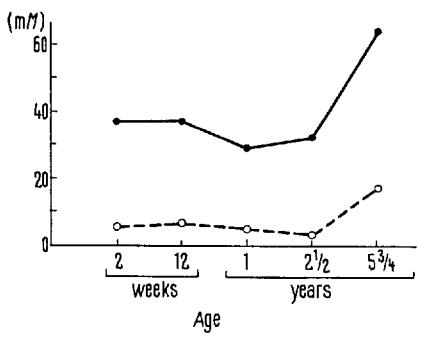

Fig. 1. UDP glucose dehydrogenase. —, mM/kg dry weight/h; ---, mM/g DNA/h.

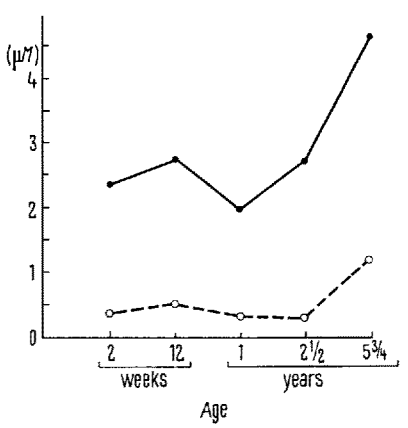

Fig. 2. Sulfatase. —, μM/kg dry weight/h; ---, μM/g DNA/h.

Changes in MDH activity calculated per dry weight were characterized by a sharp initial drop followed by a more or less stationary phase during the first half of the life span, and rising again in old age. As calculated per DNA, the enzyme changed inversely with the former during the first year but increased in activity during the latter part of life (Figure 3).

Cathepsin activity decreased during growth and midlife, rising slightly during the last period of observation, this rise being about 25% if based on dry weight and about 65% if calculated on the basis of DNA (Figure 4).

Malic enzyme was too low in activity to be measured by the method used. The activities of the other enzymes assayed declined more or less continuously with advancing age, either in relation to both dry weight and DNA, or in relation to dry weight only and at the same time changing inversely to DNA.

Discussion. Quantitative histochemical analysis of articular cartilage disclosed age-linked changes in enzyme activity in addition to those observed previously. There was a general trend for all enzymes to decrease in activity after cessation of active growth and during midlife. In old age, a number of enzymes continued to decline in activity: glutamic oxalacetic transaminase, α-mannosidase, β-galactosidase, glutamic dehydrogenase and β-acetyl glucosaminidase. For UDPGDH, 6-PGDH, sulfatase, α-glucosidase and MDH, however, the downward trend was reversed: the increase from the preceding level being highly significant or, for MDH and cathepsin, significant at the 5% level.

All enzymes increasing in activity in relation to dry weight, also increased if calculated per unit DNA. The curve for MDH in particular closely resembled that of isocitric dehydrogenase[2], another enzyme of the citric acid cycle, which thus seems to participate in the stimulation of metabolic activity of aging cartilage.

The increase in sulfatase activity is of particular interest in view of the increased uptake of S^{35} by aging human cartilage[5] on the one hand and the presumed loss of chondroitin sulfate from aging cartilage on the other[13]. The rather narrow range of the activity of cathepsin throughout the period of observation suggests 2 possibilities: either this enzyme so far as it is derived from chondrocytes does not play a major role in the age-linked degradation of articular cartilage, or the particular degradative processes in which it participates proceed at comparatively uniform rate throughout life. The decline in the activities of other enzymes potentially active in degradative processes – such as β-acetyl glucosaminidase,

Table III. Age-linked changes in enzyme activity[a] in articular cartilage of the lower extremity of female guinea-pigs

Enzyme	2 weeks	12 weeks	1 year	$2^1/_2$ years	P^b	$5^3/_4$ years
UDPGDH (mM)	5.98 ± 0.65	6.67 ± 0.99	4.84 ± 0.83	3.75 ± 0.62	[d]	16.65 ± 2.17
6PGDH (mM)	22.69 ± 0.48	7.44 ± 0.48	4.55 ± 1.05	3.44 ± 0.34	[d]	22.14 ± 3.06
β-galactosidase (mM)	1.691± 0.430	0.572± 0.093	0.247± 0.033	0.172± 0.023	NS	0.137± 0.022
α-mannosidase (μM)	63.0 ± 8.5	23.1 ± 0.6	18.8 ± 3.2	17.5 ± 2.9	NS	14.8 ± 1.3
Sulfatase (μM)	0.384± 0.058	0.508± 0.090	0.337± 0.040	0.312± 0.044	[d]	1.209± 0.129
α-glucosidase (mM)	37.7 ± 1.2	24.7 ± 3.2	29.3 ± 3.9	28.4 ± 4.3	[d]	97.8 ± 10.7
Glutamic oxaloacetic transaminase (mM)	422 ± 46	264 ± 32	282 ± 35	173 ± 22	NS	191 ± 31
Glutamic dehydrogenase (mM)	34.2 ± 5.5	32.9 ± 2.6	25.4 ± 1.5	15.9 ± 1.2	[d]	<2
Malic dehydrogenase (mM)	1405 ±167	849 ± 68	1167 ±187	887 ± 47	[c]	1516 ±153
β-acetyl glucosaminidase (mM)	12.51 ± 0.84	7.11 ± 0.35	11.98 ± 0.67	3.84 ± 0.38	[d]	0.81 ± 0.26
Cathepsin (BSA) (mg)	680 ± 90	410 ± 29	641 ± 13	333 ± 27	[c]	568 ± 51

[a] Calculated as micromoles (μM), millimoles (mM), moles (M), or milligrams (mg)/g DNA/h. [b] Statistical significance of difference between $2^1/_2$ and $5^3/_4$ year values. [c] $P<0.05$. [d] $P<0.001$. NS, P not significant. ±, indicates standard error.

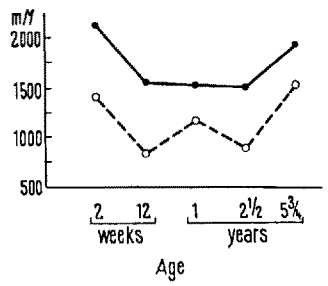

Fig. 3. Malic dehydrogenase. —, mM/kg dry weight/h; ---, mM/g DNA/h.

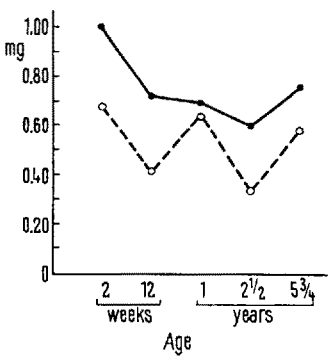

Fig. 4. Cathepsin (BSA). —, mg/g dry weight/h; ---, mg/g DNA/h.

α-mannosidase and β-galactosidase – seems remarkable in old age, when deterioration of the ground substance is supposed to be prominent.

No clear cut correlation between organellar morphology and function is recognizable beyond the fact that in a non-specific manner the development of the cellular components is consistent with increased function. Of the enzymes assayed, 3 are presumed to be present in mitochondria and/or microsomes. In aging articular chondrocytes mitochondria may be numerous[1]; yet no corresponding change was noted in microsomes. Of the enzymes associated with these organelles, GOT, GLDH and alkaline P'ase decreased, whereas MDH considered tobe mitochondrial was enhanced in activity. Of lysosomal enzymes, the activity of sulfatase increased markedly and that of α-glucosidase and cathepsin D slightly, but still significantly at the 5% level. Conversely, the activity of other lysosomal enzymes, such as β-galactosidase, α-mannosidase, β-acetylglucosaminidase and acid phosphatase declined or changed insignificantly, inspite of a slight increase in the number of lysosomes. The activities of various enzymes thus vary independently of each other an effect observed also following mechanical injury to the articular cartilage[15].

A decreasing ratio of MDH/LDH has, on the basis of observations in the rotifer, been considered as an indicator of aging[16]. If the values obtained presently for MDH are compared with those recorded earlier for LDH[2], MDH/LDH ratios of 0.46, 0.32, 0.12, 0.28 and 0.11 are obtained for the 5 age groups respectively. With the exception of the two and one half year stage, the MDH/LDH ratio in articular cartilage thus also declined into old age.

The present findings differ from earlier reports[17] in that 6-phosphogluconic dehydrogenase could be demonstrated in all age groups examined[18].

Zusammenfassung. Ergebnisse quantitativ histochemischer Untersuchungen am Gelenkknorpel von Meerschweinchen verschiedener Altersstufen werden diskutiert. Unmittelbar nach Abschluss des Längenwachstums sinkt die Enzymtätigkeit ab; im vorgeschrittenen Alter senkt sich die Tätigkeit einiger Enzyme weiter, während für andere wie UDPGLDH, 6-PGDH, MDH, α-Glucosidase, Sulfatase und Kathepsin D eine Erhöhung der Aktivität beobachtet wurde.

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