Table 2. TBA-reacting substances in serum, abdominal aorta, brain and liver after feeding under conditions of reduced oxygen supply

	0 (control) (n=8)	2 weeks (n=7)	4 weeks (n=7)
Serum	4.3±0.4	5.6± 0.9*	4.6± 0.5 nmoles/ml
Abdominal aorta	12.1±2.3	23.3± 7.7**	45.1± 6.0 nmoles/100 mg wet wt***
Brain	82.5±8.7	122.2±13.3***	84.2±10.0 nmoles/100 mg wet wt
Liver	43.3±4.7	46.2± 6.3	42.9± 5.5 nmoles/100 mg wet wt

Results are expressed as mean value±SD. * $p < 0.01$; ** $p < 0.005$; *** $p < 0.001$ for difference from controls by Student's t-test.

levels of TBA-reacting products were slightly increased, but the change was insignificant at 2 weeks. At 4 weeks of feeding under conditions of reduced oxygen supply, the serum, brain tissue and liver tissue levels of TBA-reacting substances declined to a level near the values obtained before the beginning of the experiment. But TBA-reactants in the abdominal arterial wall showed a still higher increase (table 2).

Discussion. The arterial tissue levels of TBA-reactants increased with advancing hypoxia. These observations indicated the possibility that hypoxia might be one of the factors predisposing to the accumulation on lipid peroxide in the arterial wall. At 2 weeks of feeding under conditions of reduced oxygen supply the serum and brain tissue levels of TBA-reactants were remarkably elevated, but at 4 weeks were restored to the values obtained before onset of the experiment. It appears that scavenging mechanisms exist in the serum and in brain tissue. It deserves attention that this condition differs from the change occurring in the arterial wall. In the liver, on the other hand, the increase of TBA-reacting products due to hypoxia was not as remarkable as in other tissues. At 2 weeks there was only a slight increase in the liver tissue level of TBA-reactants. This may be attributed to the fact that the liver contains more antioxidative mechanisms or more efficient mechanisms than other organs. We cannot explain the increase in TBA-reactants in conditions of hypoxia exactly, but the fact that TBA-reactants increase in conditions of hypoxia suggests that peroxidation may occur not only in conditions of excessive supply of oxygen but also in conditions of short supply of oxygen. This phenomenon may provide an important clue to the elucidation of the causes of various clinical disorders arising from hypoxia.

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Urinary acidic glycosaminoglycans in Werner's syndrome[1]

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Summary. The composition of urinary acidic glycosaminoglycans (AGAG) in 4 patients with Werner's syndrome was determined by an enzymatic assay system using chondroitinases and hyaluronidase. In Werner's syndrome, the amount of hyaluronic acid and heparan sulfates in the total AGAG increases. A compositional change in the chondroitin sulfate isomers occurs. The change of urinary AGAG in Werner's syndrome appears to reflect age-related changes.

Werner's syndrome has unique clinical manifestations resembling those found in the aging process of normal subjects[2-4]. It is a genetic disease affecting mesodermal connective tissues[5] of which acidic glycosaminoglycans (AGAG) are 1 component. The degraded components of these AGAG might be excreted, in part, in urine as catabolic products. Urinary AGAG in normal subjects consist, in decreasing order of concentration, of chondroitin 4-sulfate (C-4S), chondroitin 6-sulfate (C-6S), and heparan sulfates (HS) with other minor components of chondroitin sulfate (CS) isomers[6-9]. Hyaluronic acid (HA) was reported to be absent or, if present, in a very small amount[6].

The present study was on the components of urinary AGAG in Werner's syndrome, investigated by using an enzymatic assay method with chondroitinases and hyaluronidase, with which they can be analyzed at the constitutional level[10,11], with other chemical assays.

Materials and methods. Approximately 10 l of pooled urine from 4 patients with Werner's syndrome was used for this experiment. Urinary AGAG were prepared by a modification of the method reported previously[7]. The urinary AGAG were precipitated by adding 5% cetyl pyridinium chloride at the ratio of 15 ml/l of urine. After the precipitated specimens were digested with pronase, trichloroacetic acid was added to the solutions at a final concentration of 10%. The supernatants were dialyzed against water in dialysis tubing (Visking Co.) which had been pretreated with pyridine to reduce the pore size. The nondialyzable AGAG were concentrated and 4 vol. of ethanol was added to precipitate the AGAG. The crude AGAG were applied to Dowex 1-X2 columns (Cl^- form, 200-400 mesh, 1×25 cm). Stepwise elution was performed with 0.25 M and 3.0 M NaCl[7], and the latter eluate was desalted before use.

Composition of urinary glycosaminoglycans in Werner's syndrome and normal subjects

Age in years and sex with number of samples	Werner's syndrome Case 1 51M (N = 4)	Case 2 28F (N = 1)	Case 3 38F (N = 3)	Case 4 43F (N = 3)	Normal 5 cases 27–51M & F (N = 5)
Glycosaminoglycans	0.112 ± 0.018**	0.073	0.081 ± 0.014	0.085 ± 0.015	0.055 ± 0.07
Hyaluronic acid* (%)	11.9 ± 0.8	9.9	11.0 ± 1.9	13.4 ± 2.8	1.1 ± 1.2
Chondroitin (%)	15.7 ± 2.8	18.6	13.1 ± 1.7	13.1 ± 2.2	19.4 ± 2.1
Chondroitin 4-sulfate (%)	15.5 ± 0.9	16.0	16.5 ± 1.1	15.3 ± 4.3	28.5 ± 1.5
Dermatan sulfate (%)	3.2 ± 1.9	1.8	2.6 ± 0.8	2.3 ± 0.5	0.4 ± 0.3
Chondroitin 6-sulfate (%)	15.6 ± 1.2	17.2	15.7 ± 0.8	15.1 ± 1.9	17.4 ± 4.2
Oversulfated chondroitin sulfate (%)	5.2 ± 1.5	3.5	5.6 ± 0.9	3.4 ± 0.3	4.4 ± 1.0
Heparan sulfates (%)	33.0 ± 1.2	33.0	35.6 ± 3.4	37.5 ± 2.8	28.8 ± 1.8

* All values were determined by the borate carbazole reaction and expressed as percent. Numbers indicate the mean ± SE. ** Yield of glycosaminoglycans was determined by the borate carbazole reaction and expressed as mg/kg body weight. Hyaluronic acid, chondroitin and chondroitin 6-sulfate were calculated from the mean values of the unsaturated non-sulfated and 6-sulfated disaccharides derived from hyaluronic acid, chondroitin and chondroitin 6-sulfate by digestion with chondroitinase-ABC and -AC. The yield of dermatan sulfate was estimated by subtraction of the value for the unsaturated 4-sulfated disaccharide from digestion with chondroitinase-AC from that with chondroitinase-ABC. Oversulfated chondroitin sulfate was estimated from the value for the unsaturated disulfated disaccharide after digesting with chondroitinase-ABC. Heparan sulfates were calculated from the value of glycosaminoglycans undigested with chondroitinase-ABC[11].

The AGAG were then subjected to an enzymatic assay using chondroitinase-ABC and -AC to identify the constitutional disaccharide units of CS isomers by separating them from the other macromolecular AGAG[10,11], as mentioned below. The AGAG (approximately 400 µg as uronic acid) were digested with chondroitinase-ABC (1.0 unit) in 0.1 M tris buffer, pH 8.0, and with chondroitinase-AC (1.2 units) in 0.1 M acetate buffer, pH 6.0, at 37 °C for 120 min. The macromolecular AGAG undigested by these enzymes were characterized by electrophoresis[7,11].

The unsaturated disaccharides were applied to Whatman No. 1 filter paper and then separated in 1-butyric acid – 0.5 M ammonia (5:3, v/v) for 60 h[11]. The separated disaccharides as well as the origin were cut out in pieces and eluted for measurement by the borate carbazole reaction[12].

Results and discussion. The daily yield of urinary AGAG in the patients with Werner's syndrome (1–4 samples of urine pooled from each individual) was greater than that of the healthy subjects, when the yield was expressed on the basis of kg body weight. The constitution of urinary AGAG in Werner's syndrome at the disaccharide unit level with digestion by chondroitinase-ABC and -AC is summarized in the table. Detectable HA amounted to 10–13% of the total AGAG in the urine of Werner's syndrome. In contrast, none or very little may be found in normal subjects. In Werner's syndrome, the proportion of C-4S to total AGAG was found to be less than the normal, though that of C-6S was similar to the normal. Therefore, the ratios of C-4S to C-6S in Werner's syndrome were significantly less than those in normal subjects. HS accounted for approximately one third of the total AGAG. The relative proportion of HS in Werner's syndrome was greater than normal. Thus, the increased amount of urinary AGAG in Werner's syndrome may be ascribed to the increases of HA and HS. In electrophoretic analysis, the band corresponding to standard HA was detected in the urinary AGAG of all cases of Werner's syndrome. The HA band was not detected after digestion with Streptomyces hyaluronidase, a specific HA-lyase.

The urinary AGAG components may reflect the metabolism of connective tissue with aging. The increased proportion of HS to total AGAG with the advance of age has been reported[8]. Previous papers indicated that the proportion of C-4S to total AGAG decreased with aging, whereas that of C-6S tended to increase[13,14]. Actually, the ratios of C-4S to C-6S decreased from 1.53 at premature to nearly or below 1.0 with aging and to 0.82 at the 8th decade[13,14]. On the other hand, the presence of HA in urinary AGAG in Werner's syndrome has been indicated[15,16]. However, the constitution of the other individual urinary AGAG in this syndrome remains to be defined. The present data clearly show that the most characteristic feature of urinary AGAG in Werner's syndrome was the increased proportion of HA followed by that of HS. The increased urinary HA in Werner's syndrome may be used for diagnosis. In addition, the decreased ratios of C-4S to C-6S with aging corresponded to those found in these patients. Thus, the present study indicated that the composition of urinary AGAG in Werner's syndrome reflects aging changes of connective tissue.

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