low inorganic phosphate concentration[23]. Similarly an acid phosphatase[24], 2 extracellular nucleases[25] and ribonucleases[26] have been reported to be derepressed under orthophosphate limiting conditions. c) The high phosphate grown culture may produce an inhibitor of the enzyme activity. In our studies, high phosphate grown cultures were found to produce an inhibitor (unpublished results) which was found to be responsible for inhibition of the activities of glucose-6-P dehydrogenase and isocitrate lyase, but not of isocitrate dehydrogenase, since the addition of crude cell-free extract of a high phosphate grown culture to a low phosphate grown cell-free extract was found to inhibit the activities of these enzymes. The rate of inhibition was also proportional to the concentration of inhibitor (data not presented here).

These results indicate that phosphate plays an important role in the control of the activity of a variety of enzymes. This may help us in understanding the regulation of the production of secondary metabolites by inorganic phosphate, and hence these studies might help in increasing the production of secondary metabolites, through control of enzyme activities by control of inorganic phosphate levels.

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The increase of thiobarbituric acid reacting substances in rats with experimental chronic hypoxia

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Summary. Feeding under conditions of reduced oxygen supply prompted an increase in serum, arterial and brain tissue levels of thiobarbituric acid (TBA)-reacting substances. These observations indicated the possibility that hypoxia might be one of the factors predisposing to the accumulation of lipid peroxide.

It has been demonstrated in animal experiments and other studies that damage is caused to arterial tissue under conditions of oxygen insufficiency, such as hypoxia due to diminished lung function on diseases or smoking[1-3]. On the other hand, Glavind[4] has reported that the content of lipid peroxide in the arterial wall is elevated with increasing severity of arteriosclerosis. We focused our attention on lipid peroxidation as one of the important factors in hypoxia-induced damage, and an investigation was carried out in rats with experimental chronic hypoxia; the levels of TBA-reacting substances in the serum, artery, brain and liver were determined.

Materials and methods. Adult female Wistar strain rats weighing between 250 and 300 g were used in all experiments. In order to induce hypoxia, the rats were kept in airtight 60-l cages which were ventilated with a mixed gas containing 85% nitrogen and 15% oxygen at a rate of 0.5 l/min. After 2 or 4 weeks of feeding under these conditions, blood was drawn and liver, brain and abdominal aorta were removed. The tissues were perfused with cold saline solution to remove blood. The levels of TBA-reacting substances in the serum and tissue were determined by the method of Yagi et al.[5,6].

Results. At 2 weeks of feeding under conditions of reduced oxygen supply Po_2 decreased by about 16%, as compared to the level determined before the beginning of the experiment, and at 4 weeks Po_2 showed a tendency to decrease further. This observation indicates that hypoxia was induced in all the rats kept under this condition (table 1). When the animals were kept under conditions of reduced oxygen supply, TBA-reactants in the serum, in the abdominal arterial wall and in the brain tissue showed a significant increase at 2 weeks, whereas in the liver tissue

Table 1. Change of Po_2, PCo_2 and pH levels under hypoxic conditions

	0 (control) (n=8)	2 weeks (n=7)	4 weeks (n=7)
pH	7.43±0.03	7.47±0.04	7.41±0.03
PCo_2	35.9 ±3.5	32.1 ±4.9	34.8 ±4.6 mmHg
Po_2	90.2 ±4.5	76.4 ±3.2*	72.3 ±9.7 mmHg*

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

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.