Pyridoxal phosphate was essential for the enzyme activity. At all the stages of purification, it was necessary to add pyridoxal phosphate. As in the case of OAT from other sources[12-14], the brain enzyme was completely inhibited by 1 mM hydroxylamine confirming the requirement of pyridoxal phosphate.

L-Valine, L-leucine and L-isoleucine inhibited the brain enzyme (table 2). As with OAT from other sources[14-16], the inhibition by L-valine was competitive with ornithine. Cysteine and N-acetylornithine also inhibited the brain enzyme. Similar to the rat liver[15] and chick liver[14] enzymes, the brain enzyme was not inhibited by L-arginine. Significant inhibition of the enzyme activity was seen when either histamine, spermidine or spermine was included in the assay system (table 2). Putrescine did not have a significant effect on the enzyme activity.

Heavy metal ions like Hg^{++}, Co^{++}, Ni^{+++}, and Al^{+++} inhibited the enzyme activity (table 2). Iodoacetate and p-chloromercuribenzoic acid (PCMB) also inhibited the enzyme activity demonstrating the requirement of $-SH$ groups for the enzyme activity. Similar results have been reported for rat liver and chick liver enzymes[7,14].

The metabolic function of OAT in the brain is not known. Brain tissue contains appreciable amounts of arginase[18]. Ornithine thus formed from arginine cannot be converted to citrulline because ornithine carbamyl transferase (E.C. 2.1.3.3) is absent from the brain[19]. Ornithine formed by the action of arginase may either be decarboxylated to form putrescine (a precursor of the polyamines spermidine and spermine) or transaminated to glutamic γ semialdehyde (a precursor of GABA). Both spermine (table 2) and GABA[20] inhibited OAT from rat brain.

In conclusion, the properties of the brain enzyme were comparable to those of OAT from rat, fish and chicken liver and rat kidney. Brain OAT showed a pH optimum, substrate specificty and K_m similar to those of liver, kidney and small-intestinal enzymes. In other studies such as inhibition by branched chain amino acids or the effect of p-chloromercuribenzoic acid and heavy metals, the brain enzyme did not differ from kidney and liver enzyme. Since brain has a very low level of activity of carbamyl phosphate synthetase[17], and lacks ornithine carbamyl phosphate transferase[19] activities, it is possible that brain OAT, like that of rat kidney and Chang's liver cells, is mainly used for ornithine degradation.

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A rat mutant unable to synthesize vitamin C[1]

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Summary. A colony of Wistar rats with a hereditary defect in L-ascorbic acid-synthesizing ability was established. This rat, like primates and guinea pigs, lacks L-gulonolactone oxidase (EC 1.1.3.8) which catalyzes the last step of L-ascorbic acid biosynthesis. When L-ascorbic acid was added to their drinking water, the rats grew almost normally and were fertile. These mutant rats should be useful not only for nutritional and parmacological studies on vitamin C, but also for genetic studies on the lack of this enzyme.

Most animals can synthesize L-ascorbic acid from D-glucose via the D-glucuronic acid pathway[3-5], in which the last step is the conversion of L-gulono-γ-lactone into L-ascorbic acid, catalyzed by L-gulonolactone oxidase (EC 1.1.3.8)[6]. This step is missing in primates, guinea-pigs and a few other animals which require a dietary supply of this vitamin. In our laboratories, a rat strain with a hereditary osteogenic disorder controlled by a single autosomal recessive gene with the gene symbol *od* was established from Wistar rats[7]. This paper reports that the homozygotes of this strain, like primates and guinea-pigs, do not

Table 1. L-Ascorbic acid content of rat tissues

Age	Phenotype	Genotype	No. of rats	L-Ascorbic acid content Liver (μg/g)	Kidney (μg/g)	Adrenal (mg/g)
3 weeks	Normal	+/?	8	202 ± 5	176 ± 5	1.92 ± 0.06
	Osteogenic disorder	*od/od*	8	24 ± 1[b]	27 ± 1[b]	0.48 ± 0.01[b]
4 weeks	Normal[a]		5	216 ± 11	157 ± 3	1.78 ± 0.02
	Osteogenic disorder	*od/od*	4	11 ± 3[b]	8 ± 1[b]	0.15 ± 0.06[b]

[a] Rats from another subline of Wistar rats. Data represent mean ± SE. [b] Significantly different from normal rats in phenotype at $p < 0.01$.

Table 2. Biosynthesis of L-ascorbic acid from L-gulono-γ-lactone by rat liver microsomes

Phenotype	Genotype	No. of rats	L-Ascorbic acid formed (μmol/g protein/min)
Normal	+/?	8	1.88 ± 0.04
Osteogenic disorder	*od/od*	8	0.00 ± 0.00

4-week-old rats (male 4, female 4) were used. Data represent mean ± SE.

Table 3. Reproductive performance of osteogenic-disorder rat

Mating	Delivery rate (%)	Litter size	Weaning rate (%)
Between heterozygotes	83.3 ± 10.8 (12)	8.8 ± 2.7 (10)	91.9 ± 2.9 (86)
Between homozygotes	75.0 ± 15.4 (8)	9.3 ± 3.9 (6)	96.0 ± 3.9 (25)

The rats were bred in our barrier facilities. The heterozygotes were given tap water and the homozygotes were given tap water containing L-ascorbic acid (40 mg/100 ml) to drink from weaning. The period of mating was 2 weeks. Data represent mean ± SD. The number of rats is given in parentheses.

have L-ascorbic acid-synthesizing ability as they lack L-gulonolactone oxidase.

Materials. History of osteogenic-disorder rat (OD rat). In 1973, a rat with an osteogenic disorder in the limbs was found in a subline of Wistar rats maintained in our laboratory. Makino and Katagiri[7] established a rat strain in which a similar disorder spontaneously occurs in 15-16-day-old animals, by backcross mating and full sib-mating between normal individuals for the sake of appearance. The strain was named the osteogenic-disorder rat (OD rat) and this characteristic is controlled by a single autosomal recessive gene with the gene symbol *od*. The pedigree is shown in figure 1.

Animals. The rats were bred under conventional conditions. Normal breeding pairs were obtained from parents both of which were known heterozygotes (+ /*od*), because rats with the osteogenic disorder (*od/od*) were sterile. Therefore, one quarter of the offspring from the pairs were expected to have the caracteristic. The parent rats and weaned offspring were fed commercial pellets which had been disinfected in an oven at 100 °C for 30 min before use.

Assay methods. The L-ascorbic acid conent in rat tissues was determined by the method of Ogawa and Kishigami[8] based on oxidation by 2,6-dichlorophenolindophenol and coloration by 2,4-dinitrophenylhydrazine[9]. L-Ascorbic acid-synthesizing activity in the liver microsomes was assayed by the method of Nishikimi et al.[10] using as the substrate L-gulono-γ-lactone,

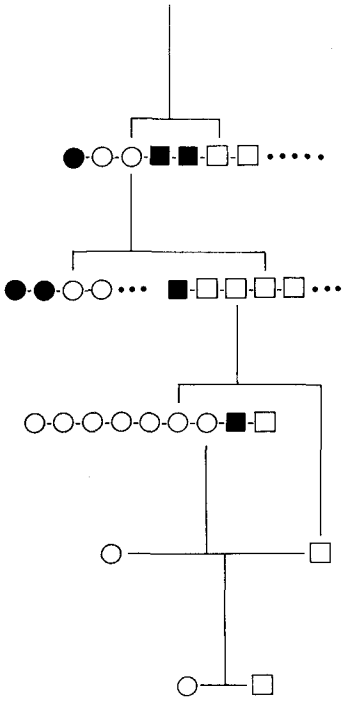

Figure 1. Pedigree of osteogenic disorder rat found in Wistar/shi strain. ○, Normal female; □, Normal male; ●, Osteogenic disorder female; ■, Osteogenic disorder male.

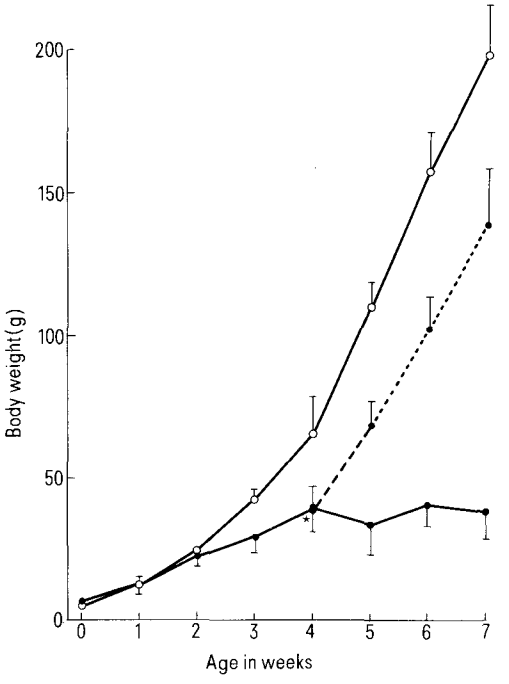

Figure 2. Effect of daily administration of L-ascorbic acid on the body weight of homozygotes of osteogenic-disorder rat. Experiments were performed under conventional conditions. Infants with the osteogenic disorder were divided into 2 groups at weaning and 1 group (●—●, n = 4) was given tap water as were the osteogenic normal rats (○—○, n = 7) and the other group (●—●, n = 4) was given tap water containing L-ascorbic acid (40 mg/100 ml) to drink. Each point represents the mean and the vertical bar represents SD; *, Start of L-ascorbic acid administration.

which was synthesized by the method of Wolfrom and Anno[11]. The reaction mixture (2 ml) contained 50 mM potassium phosphate buffer (pH 7.5), 2.5 mM L-gulono-γ-lactone, 1 mM EDTA, and liver microsomes of about 3 mg of protein equivalents. The mixture was incubated for 30 min at 37 °C, and the reaction was stopped by the addition of 2 ml of 10% trichloroacetic acid. After the protein had been precipitated, L-ascorbic acid in the supernatant was determined by the method of Roe and Kuether[9]. Protein was determined by the method of Lowry et al.[12] with a slight modification.

Results and discussion. Table 1 shows the L-ascorbic acid concentrations in the rat organ tissues. Only trace amounts of L-ascorbic acid were found in the liver, kidney and adrenal glands of the 3-week-old rats with osteogenic disorder (homozygotes, *od*/*od*) compared with those of the littermates, of which the external appearance was normal (heterozygotes, +/*od* and normal +/+). The levels in the OD rats decreased still further at 4 weeks of age. Table 2 shows the activities of L-gulonolactone oxidase in the liver microsomal fractions of OD rats and the littermates of normal phenotype. The littermates had the enzyme activity, but rats with the disorder did not. Figure 2 shows the growth curves of rats with the disorder given tap water or water containing L-ascorbic acid (40 mg/100 ml) to drink from weaning. In the rats given tap water, hind limb disorders were observed after 3 weeks, body weight gain stopped after 4 weeks and death due to incomplete development occurred after 7 to 8 weeks. However, the rats given L-ascorbic acid grew up almost normally and were fertile (table 3).

In the homozygous OD rat, disorder of the hind limbs becomes clear from 15–16 days of age and mere traces of the long bones of the 4 limbs and deformed epiphyseal cartilages are observed from 4 weeks of age at autopsy. Hematomas around the femurs, humerus and scapulae are also observed[7]. These observations resemble the symptoms of infantile scurvy. Collagen is one of the main structural materials of bone, and L-ascorbic acid is essential for the biosynthesis of collagen[13]. Therefore, we determined the tissue levels of this vitamin. Only traces of L-ascorbic acid were found in rats with the osteogenic disorder compared with the levels in the littermates of normal phenotype and normal rats of other substrains. Usually rats can synthesize L-ascorbic acid[3-5] and a dietary supply is not necessary. Therefore, the commercial diet for rats contains lower amounts of vitamin C than that for guinea pigs. Our analysis showed that the vitamin C content in the diet, disinfected at 100 °C for 30 min, was about 20 mg/kg. Addition of L-ascorbic acid to the drinking water prevented development of the osteogenic disorder in the homozygotes and the rats grew up normally and were fertile. These observations clearly proved that the homozygous of OD rat, like primates and guinea pigs, can not synthesize L-ascorbic acid. The enzyme system catalyzing the conversion of L-gulono-γ-lactone into L-ascorbic acid is located entirely in the liver microsomal fraction in rats[14]. The littermates of normal phenotype had the enzyme activity but rats with the osteogenic disorder did not. This shows that the homozygotes of the OD rat, like primates and guinea pigs, lack L-gulonolactone oxidase (EC 1.1.3.8).

We conclude that homozygous OD rats do not have L-ascorbic acid-synthesizing ability because of a lack of L-gulonolactone oxidase (EC 1.1.3.8). These rats with a hereditary defect in L-ascorbic acid-synthesizing ability should be useful not only for nutritional and pharmacological studies on vitamin C, but also for genetic studies on the lack of L-gulonolactone oxidase.

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Specific inhibition of human leukocyte elastase by substituted alpha-pyrones[1]

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Summary. Several α-pyrones have been synthesized and investigated for their in vitro inhibitory activity using α-chymotrypsin (α-CT), porcine pancreatic elastase (PPE) and human leukocyte elastase (HLE). 4-Hydroxy-6-undecyl-2H-pyran-2-one **4**, 4-Hydroxy-6-[(1-butyl)heptyl]-2H-pyran-2-one **5** and 4-Methoxy-6-[(1-butyl)heptyl]-2H-pyran-2-one **6** were found to be specific inhibitors of HLE. These compounds constitute a promising new class of HLE inhibitors.

The chronic destruction of the elastic component of lung connective tissue by human leukocyte elastase (HLE) and cathepsin G is currently believed to result in the onset of chronic obstructive lung disease[2-6]. These proteases are primarily inhibited by the major serum proteases inhibitor α_1-proteinase inhibitor (α_1-PI), which is also a normal constituent of bronchioalveolar lavage fluid (BAL). However, α_1-PI is readily inactivated by oxidants such as those present in cigarette smoke or oxidative enzymes (i.e. myeloperoxidase) that are normally functioning in phagocytic cells during inflammatory states. In addition, some individuals are genetically deficient in α_1-PI with levels of the inhibitor which are 25% of normal.