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Variation of Serum α_2-Macroglobulin Concentration in Healthy Rats and Rats Inoculated with *Staphylococcus aureus* or Subjected to Surgery

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Background and Purpose: **The aim of the study reported here was to investigate variations in the serum concentration of α_2-macroglobulin (α2M) from healthy rats and rats inoculated with *Staphylococcus aureus* or subjected to surgery.**

Methods: **Concentration of α2M was measured by use of an enzyme-linked immunosorbent assay.**

Results: **Serum α2M in healthy rats at intervals of 3 h, 1 day, and 1 week ranged from 17.5 to 38.0 (mean ± SD, 27.2 ± 6.6) μg/ml, 15.8 to 48.2 (27.9 ± 8.7) μg/ml, and 17.2 to 39.6 (23.9 ± 5.9) μg/ml, respectively. Concentrations of α2M did not undergo significant variations within individuals or between rats. Serum α2M concentration increased at one day and peaked two days after inoculation with *S. aureus* or being subjected to surgery. Peak concentration was eight to 33 times pre-inoculation values after inoculation with *S. aureus,* four to 25 times pre-surgical values when rats were subjected to castration, and seven to 28 times pre-surgical values when rats were subjected to oophorohysterectomy, respectively.**

Conclusions: **Physiologic variation in the concentration of α2M in rats was not found. Induction of α2M in rats inoculated with *S. aureus* or subjected to surgery was documented.**

α_2-Macroglobulin (α2M) is a prototypic acute-phase reactant protein in rats. It exists in small amounts in clinically normal rats and increases after tissue damage (1, 2). Mean ± SD concentration of α2M in serum from healthy rats has been determined to be 32 ± 4 μg/ml (2) and 14 to 68 μg/ml (3) by electroimmunoassay and 16 ± 1 μg/ml (1) by single radial immunodiffusion, and in injured rats, values were 110 to 140 times the normal concentration. Recently, an enzyme-linked immunosorbent assay (ELISA) for rat α2M has been reported (4, 5). However, to the authors' knowledge, the physiologic circadian and day-to-day variation of α2M concentration in serum sequentially obtained from rats has not been reported.

Staphylococcus aureus has been isolated from a wide range of animals, including trapped wild rats in buildings (6). C-Reactive protein, which is an acute-phase reactant protein in humans and dogs, is markedly increased following bacterial infection or surgical trauma (7-9). Changes in α2M concentration in serum from rats experiencing these pathologic conditions been not reported to our knowledge. We describe the physiologic variation in the serum concentration of α2M from healthy rats as well as rats inoculated with *S. aureus* or subjected to castration or oophorohysterectomy.

Materials and Methods

Rats. Sixty-four (32 males and 32 females) specific-pathogen-free/virus antibody-free (SPF/VAF) CD (IGS) rats (Charles River Japan Inc., Yokohama, Japan) were studied. The SPF/VAF rats were examined and found to be free of *Corynebacterium kutscheri*, *Salmonella* spp., *Psudomonas aeruginosa*, *Pasteurella pneumotropica*, *Streptococcus* pneumoniae, *Bordetella bronchiseptica*, *Mycoplasma pulmonis*, and parasites, and antibody to Tyzzer's organism, Sendai virus, mouse hepatitis virus, pneumonia virus of mice, mouse polio virus, reovirus type 3, lymphocytic choriomeningitis virus, mouse adenovirus, rat coronvirus/sialodacryoadenitis virus, Toolan's H-1 virus, Kilham rat virus, and Hantaan virus. Rats were reared under sterile room conditions and received a diet of sterile CRF-1 and water until being sent to the Inoue Experimental Animals Center Co., Ltd. (Kumamoto, Japan). After arrival, these rats were reared at the institute, and individually housed in cages (280 × 440 × 180 mm) in an animal room adjusted to a temperature of 22 ± 2°C, relative humidity of 60 ± 10%, and illumination for 12 h (7 a.m. to 7 p.m.), with air being changed 15 times/h. They were fed a solid stock food (MF; Oriental Yeast Co., Ltd., Tokyo, Japan), and allowed ad libitum access to water. All experiments conformed to the Japanese law concerning animal care and use, following the *Guideline for Animal Experimentation* recommendation (10), and were approved by the institutional animal care and use committee.

Acquisition of sera from healthy rats. Rats were allotted to three groups containing 10 (five males and five females) rats/group for evaluation of physiologic variation of α2M concentration. Rats of group 1 were used to monitor circadian changes in α2M concentration. At nine weeks of age, blood was sequentially collected at 3-h intervals (9 a.m., 12 noon, 3, 6, and 9 p.m., midnight, and 3, 6, and 9 a.m.). Rats of group 2 were used to monitor day-to-day variations of α2M concentration. At 9 weeks of age, blood was sequentially collected 8 times at one-day intervals. Rats of group 3 were used to monitor week-to-week variations of α2M values. Blood was sequentially collected 9 times at one-week intervals. The blood was initially collected at 6 weeks of age from the subclavian vein of rats under ether anesthesia.

Culture of S. aureus. *Staphylococcus aureus* was cultured at 37°C for 24 h in blood agar (Difco Laboratories, Detroit,

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Mich.). The organism was supplied by Dr. M. Fukuyama of the Laboratory of Microbiology, Azabu University.

Inoculation of rats with S. aureus. Twelve (6 males and 6 female) rats were inoculated intradermally with 10^8 live *S. aureus* in 0.1 ml of sterile physiological saline. The suspension was sequentially diluted by a factor of 10. The presence of a number of *S. aureus* was confirmed by culturing *S. aureus* in blood agar plates and counting the colonies. The other ten (5 males and 5 female) rats used as a control were inoculated intradermally with 0.1 ml of sterile physiologic saline.

Surgery. Surgery was carried out on rats under inhalation anesthesia with Sevoflurane (Maruisi Pharmaceutical Co., Osaka, Japan). Sterile surgical appliances were used, and incised parts were disinfected with Povidone-iodine. The 6 male rats were subjected to castration; testes were excised, and the scrotum was sutured. The 6 female rats were subjected to oophorohysterectomy. The abdominal incision was about 1 cm long; after the ovaries and uterus were excised, the abdomen was closed by suturing. After these surgeries, the antibiotic Orbifloxacin (Dainihon Pharmaceutical Co., Osaka, Japan) was orally administered to the rats.

Quantification of rat α2M. Concentration of α2M in serum was determined by use of a capture ELISA and an anti-rat α2M monoclonal antibody (11). This monoclonal antibody, adjusted, using 0.05*M* sodium bicarbonate buffer (pH 9.6), to a concentration of 1.0 μg/ml, was incubated at a concentration of 100 μl/well at 37°C for 1 h. After blocking with 1% bovine serum albumin in sodium bicarbonate buffer (pH 9.6), serially diluted rat serum (100 μl/well), with a known α2M concentration (2,520 μg of α2M/ml), and the test serum was added. The plates were incubated at 37°C for 2 h. Peroxidase (Sigma Chemical Co., St. Louis, Mo.)-conjugated anti-rat α2M monoclonal antibody was prepared by use of the method of Nakane and Kawaoi (12), and was used at a concentration of 20.0 μg/ml. The plates were incubated at 37°C for 2 h with the substrate 0.05% 2,2-azino-di-(3-ethylbenzthiazoline sulphonic acid-6; ABTS) (Zymed Laboratories, South San Francisco, Calif.). After incubation for 50 min at room temperature, A_{415} values were measured. The results were compared with a standard curve obtained using serially diluted specimens from rats with known α2M concentration. Rat serum containing 2,520 μg of α2M/ml was used as the standard antigen for the ELISA.

Statistical analysis. Concentration of α2M was expressed as mean, SD, and coefficient of variation. To assess circadian, day-to-day, and week-to-week variations in and between individual rats, two-way analysis of variance was carried out, *F* values being considered significant at $P < 0.05$.

Results

Variation in rat serum concentration of α2M in at 24 h, eight days, and nine weeks. Evaluation of inter- and intra-individual variations and analytical variation is shown in Table 1. Concentration of α2M in serum obtained nine times within a 24-h period ranged from 17.5 to 38.0 (mean, 27.2 ± 6.6) μg/ml (Fig. 1). The concentration of α2M in sera obtained eight times within eight days ranged from 15.8 to 48.2 (mean, 27.9 ± 8.7) μg/ml (Fig. 2). The concentration of α2M in serum obtained eight times within nine weeks ranged from 17.2 to 39.6 (mean, 23.9 ± 5.9) μg/ml (Fig. 3). Intra-individual variations in the concentration of α2M at 24 h, eight days, and nine weeks were higher than intra-individual variations. Significant variation was not observed. Concentrations of α2M were not significantly different between rats.

Changes in α2M concentration in rats inoculated with *S. aureus*. Concentration of α2M in the serum of 10 rats increased at one day and peaked two days after inoculation with *S. aureus* (Fig. 4). Peak α2M concentration in male and female rats ranged from 328.9 to 846.3 (mean, 538.4 ± 156.9) μg/ml; eight to 33 times pre-inoculation values of 16.0 to 68.4 μg/ml. There was no significant difference in α2M concentration between males and females ($P > 0.05$). Concentration of α2M in serum of control rats did not change.

Changes in serum α2M concentration in rats subjected to surgery. Serum concentration of α2M in 6 rats subjected to castration increased one day and peaked two days after castration (Fig. 5). Peak α2M concentration ranged from 225.1 to 660.5 (377.5 ± 152.0) μg/ml), four to 25 times the pre-inoculation values of 15.5 to 61.7 μg/ml.

Serum α2M concentration increased one day and peaked two days after oophorohysterectomy (Fig. 6). Peak α2M concentration ranged from 314.4 to 672.5 (448.8 ± 136.0) μg/ml), seven to 28 times the pre-inoculation values of 18.4 to 67.0 μg/ml.

Discussion

The circadian or day-to-day variation of C-reactive protein, an acute-phase reactant protein in humans, has been reported (13, 14). Furthermore, day-to-day variations in C-reactive protein under conditions of cold or fatigue also have been observed (15). Reported circadian and day-to-day variations in canine C-reactive protein in healthy dogs were not significant (16). Some factors cause changes in the concentrations of some substances in the serum. Meals cause changes in the concentrations of serum glucose and lipid concentrations. Serum lipid concentration increases and in serum iron concentration decreases during pregnancy. Increases in serum α2M concentration of pregnant rats also have been reported (17). Knowing the physiologic variation of α2M concentration in rats is important for monitoring changes in such concentration associated with pathologic changes. However, to the authors' knowledge, other physiologic variations of α2M concentration have not been reported.

Results of this study suggest that the concentration of α2M does not change during the circadian cycle (e.g., influence of a meal), nor was there day-to-day variation (e.g., influence of the

Table 1. Average interindividual, intra-individual, and analytical variations

			Inter-individual variation		Intra-individual variation		Analytical variation	
Variation	n	Mean[a]	A[b]	CV(%)	B(A)[c]	CV(%)	E[d]	CV(%)
In 24h	10	27.4	22.0	80.1	3.2	11.4	2.0	7.3
In 8 days	10	28.0	25.2	80.1	4.2	15.0	1.7	5.9
In 9 weeks	10	23.9	16.2	68.0	4.6	19.3	1.9	8.0

[a]Expressed in micrograms per milliliter.
[b]Estimated $\sigma A = (S_A^2 - S_B^2/C)^{1/2}$, where S_A^2 is the between-rows variance, S_B^2 is the between-columns variance, and C is the number of columns.
[c]Estimated $\sigma B(A) = S_B^2 - S_E^2)^{1/2}$, where $S_E^2$is the error variance.
[d]Estimated $\sigma E = (S_E^2)^{1/2}$.

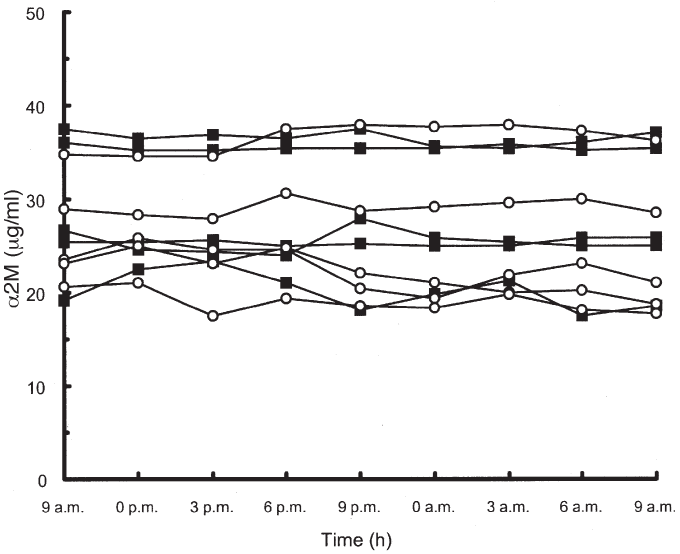

Figure 1. Changes in concentration of α_2-macroglobulin (α2M) in serum from healthy rats over 24 h (■ = male; ❍ = female).

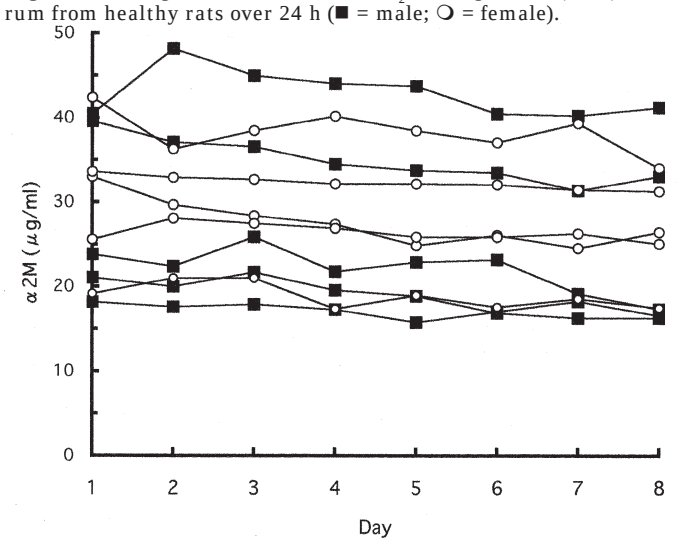

Figure 2. Changes in concentration of α2M in serum from healthy nine-week-old rats over seven days. *See* Figure 1 for key.

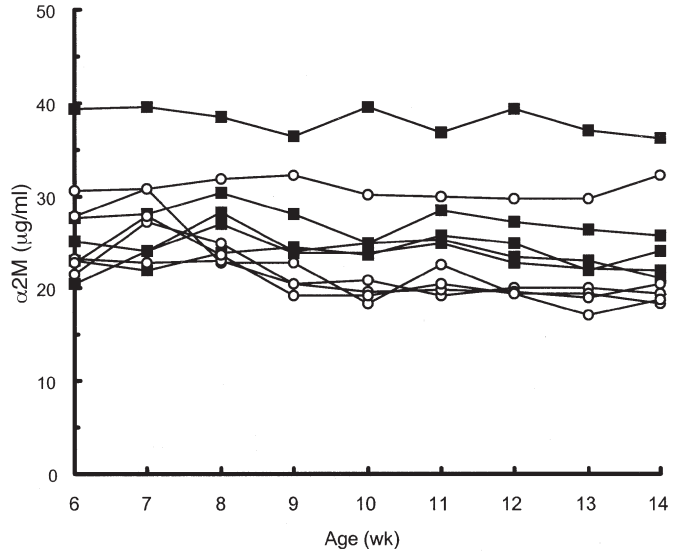

Figure 3. Changes in concentration of α2M in serum from healthy rats obtained at one-week intervals. *See* Figure 1 for key.

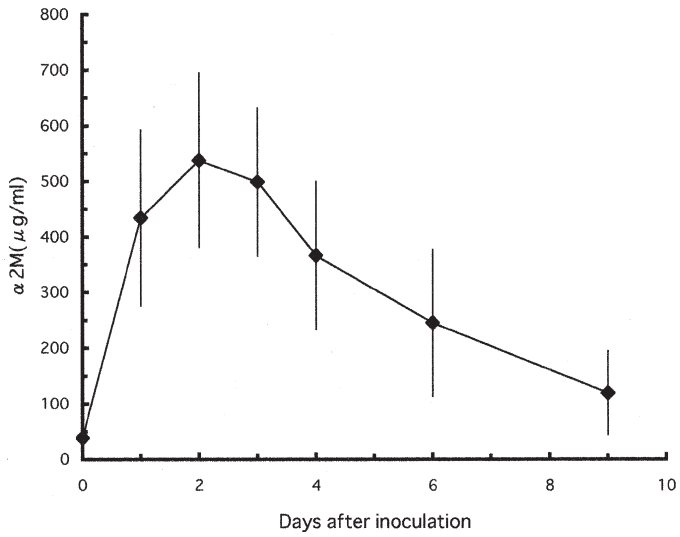

Figure 4. Changes in concentration of α2M in serum from10 rats inoculated with *Staphylococcus aureus*.

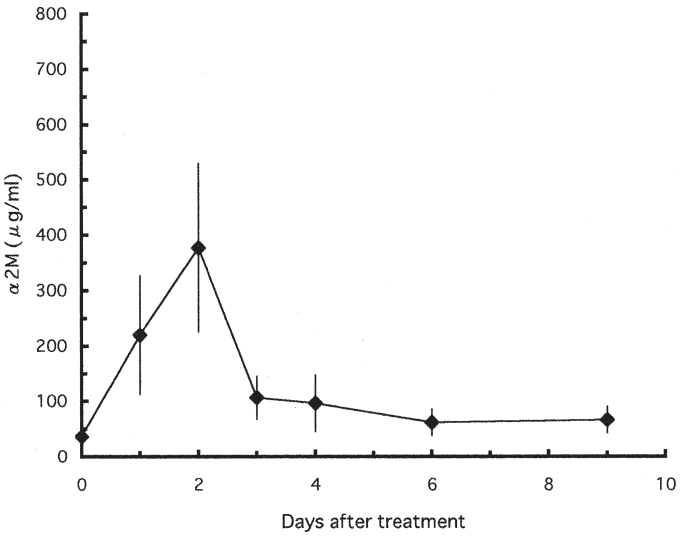

Figure 5. Changes in concentration of α2M in serum from six male rats subjected to castration.

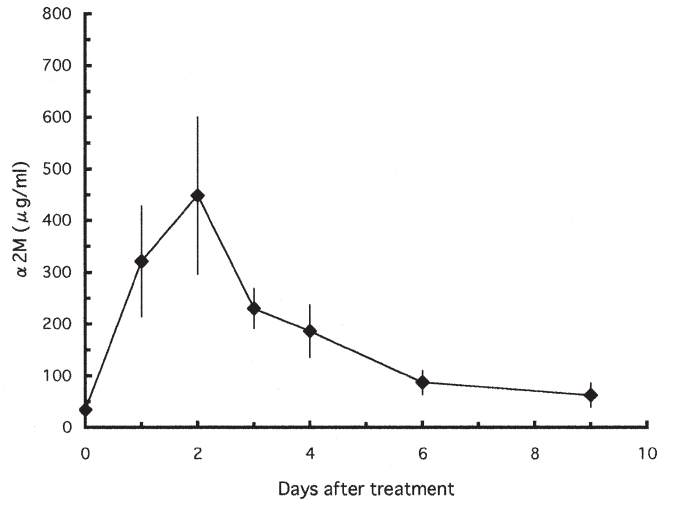

Figure 6. Changes in concentration of α2M in serum from six female rats subjected to oophorohysterectomy.

reproductive cycle), or variation by age. Changes in serum C-reactive protein values are not significant in healthy dogs (16). Similarly, a significant difference in serum α2M values was not found.

Induction of α2M in rats inoculated with *S. aureus* or subjected to surgery was documented. Increases in serum concentration of α2M in rats inoculated with turpentine oil have been reported (1, 2). Mean peak values were > 1 mg/ml. In contrast, peak values of α2M in rats inoculated with *S. aureus* or subjected to surgery were < 1 mg/ml. The inflammatory stimulation of the inoculation of *S. aureus* or the surgery might be weaker than that of the inoculation of turpentine oil.

About three million rats per year are used as experimental animals in Japan. However, quantification of α2M as an inflammatory marker has hardly been reported. Determination of serum α2M concentration may be useful for health assessment of rats, preclinical or toxicologic examination. This study provides useful data for monitoring α2M values during such examinations.

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