These results demonstrate that intraendothelial channels represent the main transport pathway for the drainage of large molecules (lipids) from the interstitium into the lymphatic vessel lumen. Apart from the mechanism reported above, a small amount of lipid is carried to the lumen through phagocytic vacuoles. Intracytoplasmic vesicles and diffusion mechanisms play a significant role in the transport of fluids and small molecules. It has also been pointed out that specialized junctional complexes were observed to be unmodified during the functional and experimental conditions studied. Therefore the finding of 'open junctions' in endothelial wall must be considered to be a consequence of injury or pathological conditions[19-22].

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Existence of an α-chymotrypsin-like immunoreactivity in bovine mast cells

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Summary. Using an indirect fluorescent antibody technique, we detected an α-chymotrypsin-like immunoreactivity, but no trypsin-like immunoreactivity in bovine mast cells.

An α-chymotrypsin-like and/or trypsin-like enzyme has been detected in mast cells. In the mast cells of humans[2], dogs[2], rabbits[2], rats[2-7] and mice[2], α-chymotrypsin-like activity was demonstrated, while in these cells in humans[8-10], dogs[8-10] and cats[10], a trypsin-like activity was reported. Aprotinin, which inhibits the activities of chymotrypsin and trypsin, is present in bovine mast cells[11-13], but it is unknown whether an α-chymotrypsin-like and/or trypsin-like enzyme is also present in these cells. We now report the existence of an α-chymotrypsin-like immunoreactivity in bovine mast cells.

Materials and methods. The following reagents were used: trypsin (EC 3.4.21.4) and α-chymotrypsin (EC 3.4.21.1) – both crystallized 3 times – (Miles Laboratories, USA); trypsinogen, type 1, and α-chymotrypsinogen-A, type 2, (Sigma, USA); acrylamide, N,N′-methylenebisacrylamide and Alcian blue 8 GX (Wako Pure Chemicals, Japan); Nuclear Fast Red (E. Merk, FRG); tosyl-L-arginine methyl ester hydrochloride (TAME) and benzoyl-L-tyrosine ethyl ester (BTEE) (Protein Research Foundation, Japan); goat antirabbit IgG conjugated fluorescein isothiocyanate (FITC) (Medical and Biological Laboratories Ltd, Japan); complete Freund's adjuvant (Difco Laboratories, USA).

Production of antibody to trypsin or α-chymotrypsin. Polyacrylamide disc-gel electrophoresis was carried out with 7% gel in 0.4 M glycine-HCl buffer (pH 4.0), first at 1 mA per tube for 10 min and then at 3 mA per tube for 30 min. Amido black was used to stain protein in the gel. The small gel segments containing enzyme activity (trypsin activity was measured with TAME as substrate by the method of Simlot and Feeney[14] at 37 °C, and chymotrypsin activity was determined with BTEE as substrate by the method of Hummel[15]) were pooled, and homogenized with 0.9% NaCl. An equal amount of complete Freund's adjuvant was added; the mixture was thoroughly emulsified and injected s.c. into Japanese white rabbits. Similar injections were repeated every 2 weeks. After 6 injections, the serum was harvested. Immunoglobulin was precipitated from the serum at 50% saturation with ammonium sulfate.

Immunocytochemistry. Fresh bovine lung, obtained from a local slaughter house, was fixed in 10% formaldehyde, embedded in paraffin, and sectioned in the usual way. After removel of the paraffin, the location of enzyme in the tissue was determined by the indirect Coons technique[16] with FITC-labeled goat antirabbit IgG. After observation of the fluorescence, the sections were stained with 1% Alcian blue in 3% acetic acid (pH 2.5). The nucleus was stained with Nuclear Fast Red.

Results and discussion. In the immunized rabbits, all antiserum titers were similar. In the double diffusion test, their serum gave a clear precipitin line with a 16-fold diluted solution of 1 mg/ml of trypsin or α-chymotrypsin, whereas serum from nonimmunized rabbits or from immunized rabbits treated with excess antigen did not show any precipitate. Immunological cross-reaction between bovine trypsin and α-chymotrypsin has been reported[17]. In the present study, at a concentration of 1 mg/ml of α-chymotrypsin, a precipitin line was produced with antitrypsin serum, but trypsin even up to 15 mg/ml did not react with anti-α-chymotrypsin serum. Trypsinogen or α-chymotrypsinogen-A also gave an antigen-antibody reaction. The optical concentration in the reaction was the same as that of the original antigen, and the same antigenicity as that of the original antigen was apparent.

The site of the antigen-antibody reaction in bovine lung was determined by the indirect fluorescent antibody techni-

que. Fluorescence was observed using the IgG fraction of anti-α-chymotrypsin serum in mononuclear cells in the connective tissue. The cytoplasmic granules of these cells could be stained with Alcian blue. This finding suggests that mast cells contain an α-chymotrypsin-like immunoreactivity. However, the cells with fluorescence numbered about half those stained with Alcian blue, and the extent of the reaction varied in different sections. It is possible that some mast cells contain too little antigen to be detectable by the present indirect immunofluorescent technique or that some antigen is eluted from the sample during the processes of fixation and section.

Fluorescence due to an α-chymotrypsin-like immunoreactivity was observed when the sections were fixed with 10% formaldehyde, but not in the case of fixation with 95% ethanol or Bouin's solution. Regardless of the fixation, fluorescence was not demonstrated with various dilutions of antibody to trypsin. The fluorescence observed in this study was attributed to an α-chymotrypsin-like immunoreactivity and not to a trypsin-like immunoreactivity because 1. the 2 antibodies have similar titers; 2. fluorescence was observed with the antibody of α-chymotrypsin, not with that of trypsin; 3. in the antigen-antibody reaction in the gel, trypsin did not react with anti-α-chymotrypsin serum, even at higher concentrations. The α-chymotrypsinogen also gave an antigen-antibody reaction. Its optical concentration in the reaction and its antigenicity were the same as those of the original antigen. Thus, it is uncertain whether the fluorescence detected was due to an α-chymotrypsin-like or its zymogen form of immunoreactivity. However, in mast cells, an α-chymotrypsin-like enzyme is present in the active form, not as a zymogen[6,7], and it is bound ionically to heparin[7].

The physiological function of the mast cell protease is not clear. The enzyme might be involved in the secretory activity of the mast cells or be secreted by the mast cells and act on some component of the connective tissue[4]. Attempts to show that cells stained with fluorescent antibody also have an α-chymotrypsin-like activity were not successful. Difficulty in the demonstration of the activity in bovine mast cells may be due to the existence of an aprotinin which inhibits trypsin, chymotrypsin and kallikrein. Aprotinin is an intracellular inhibitor[18], and it readily binds to heparin because of its high basicity in vitro[19,20]. Thus in bovine mast cells, the α-chymotrypsin-like immunoreactive materials might be regulated by aprotinin and/or heparin.

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Gonadectomy and survival of *Ancylostoma caninum* (Nematoda) filariform larvae in mice

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Summary. Gonadectomy reversed the susceptibility response of male and female Swiss mice to *Ancylostoma caninum* infection. Orchiectomy decreased the survival of filariform larvae ($p > 0.001$ to < 0.25), whereas ovariectomy increased it significantly ($p > 0.001$ to < 0.01).

Numerous investigators have reported sex differences in infection with various parasitic worms, both in natural and experimental conditions[2]. This communication describes the influence of gonadectomy, i.e. removal of the source of sex hormones of the host, on the susceptibility of mice to *Ancylostoma caninum* infection.

Materials and methods. In 2 experiments 100 pairs of Swiss albino mice, 4 months old, weighing 28 ± 2 g, were used. Half of them were gonadectomized and remaining half were sham-operated a month prior to infection. All were infected with 1000 ± 25 filariform larvae of *Ancylostoma caninum,* cultured in the laboratory using the Petri dish technique of Sen et al.[3]. Animals were necropsied from 3 to 40 days post-infection, under ether anaesthesia. Larvae were recovered and counted after digestion of visceral organs and muscle in artificial gastric juice, followed by the Baermann technique, according to Soh[4]. Student's t-test was used for determining statistical significance.

Results and discussion. Mortality was not greater than 6% in gonadectomized and control animals of both sexes. Orchiectomy in male mice depressed their susceptibility to parasitic infection, as is evident from the 4 to 31% decrease in the larval recoveries from castrated animals in comparison to sham-operated controls (table 1). On the 3rd and 7th day of infection this difference was insignificant, but on the 11th day it was significant ($p < 0.05$) and from the 15th day onwards until the end of experiment it was highly significant ($p > 0.001$ to < 0.001). The average number of larvae