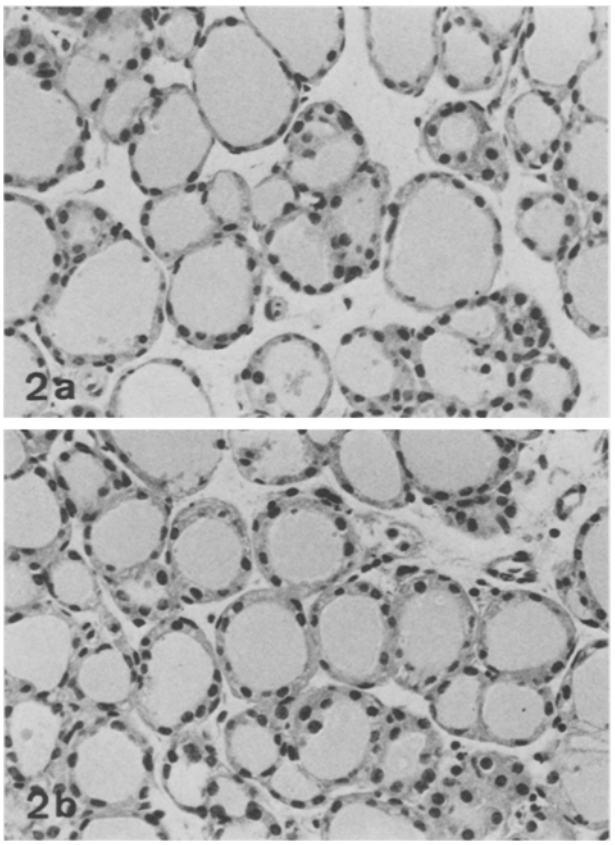

Figure 3. Mouse thyroid tissue after 3-h stimulation without TSH (*a*) or with 10 mU/ml of TSH (*b*). ×280

and Tg from perifused mouse thyroids. It is of interest that the TSH-induced release of Tg is delayed when compared with that of T_3 and T_4. For the mechanisms by which Tg crosses the closed follicular wall and reaches the circulation as an intact molecule, several possibilities have been discussed in the past: inflammation of the thyroid, temporary relaxation of tight junctions [1] and transepithelial vesicular transport of Tg [14]. Although the precise mechanism of TSH-induced Tg release has not been clarified yet, it is strongly suggested by the present study that the TSH-induced release mechanism is quite different between Tg and T_3 or T_4. This perifusion system may contribute to further elucidation of the mechanism of secretion of Tg.

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Spasmin-like proteins in various ciliates revealed by antibody to purified spasmins of *Carchesium polypinum*

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Summary. It was found that some ciliates, *Stentor, Spirostomum* and *Blepharisma,* which can contract rapidly like the stalks of Vorticellidae, have Ca^{2+}-binding proteins that are very similar to spasmins, in the immunological sense. The presence of spasmins in other Protozoa and in some Metazoa was also investigated.
Key words. Spasmin-like proteins; *Carchesium; Vorticella; Stentor; Spirostomum; Blepharisma;* immunoblotting.

Peritrich ciliates (*Vorticella, Carchesium* and *Zoothamnium*) have contractile stalks which are mainly composed of the spasmoneme and the surrounding sheath. Through investigations of the glycerinated spasmoneme [1–4], it has been shown that contraction of the spasmoneme is brought about by Ca^{2+} binding to some contractile elements composed of Ca^{2+}-binding proteins in the organelle. However, the molecular mechanism of the contraction is not known yet. Amos et al. [5] extracted two Ca^{2+}-binding proteins from isolated spasmonemes of *Zoothamnium geniculatum* and termed them spasmin a and spasmin b. Yamada and Asai [6, 7] reported that there are at least three kinds of Ca^{2+}-binding proteins in the stalks of *Carchesium polypinum,* and these Ca^{2+}-binding proteins are entirely different from calmodulin or troponin-C. Thus, the stalks contain new types of Ca^{2+}-binding proteins, and some of them are directly related to the contraction of the organelle. In this paper we use the term spasmins to refer to the Ca^{2+}-binding proteins extracted from the stalks of *C. polypinum.*

To elucidate the contractile mechanism of the spasmoneme, it is important to know which of the Ca^{2+}-binding proteins is essential to the spasmoneme-type contraction. It is not

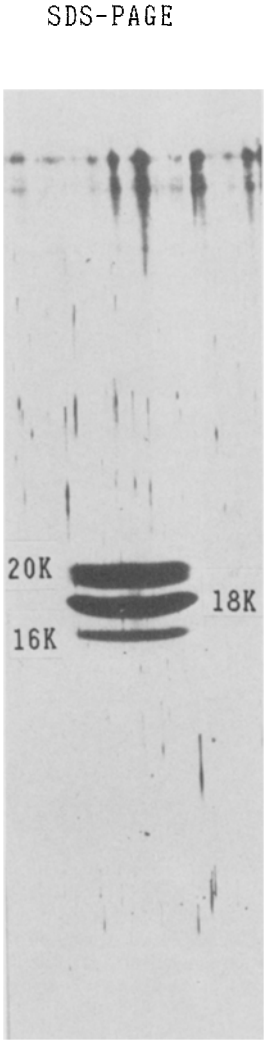

Figure 1. SDS gel electrophoretic pattern of spasmins (20, 18, 16 kDa) purified by the procedure described in text. A 12% polyacrylamide-SDS gel was used according to Laemmli and Favre [12]. Silver-staining was employed. The molecular weights were determined by using crude extract of skeletal muscle of a rabbit as a standard.

known what kinds of protozoan cells have spasmoneme-type contractile organelles. We have prepared an antibody to the spasmins extracted and purified from the stalks of *Carchesium* and investigated its cross-reactivity with various ciliates. In the present paper we show that the ciliates *Stentor, Spirostomum* and *Blepharisma,* which can contract rapidly like the stalks of Vorticellidae, have Ca^{2+}-binding proteins that are very similar to spasmins in the immunological sense. Spasmins were extracted with 8 M urea solution from the stalks of *Carchesium polypinum* (about 10 g) glycerinated for about a month. After removal of the urea by dialysis, the extracts were applied to a gel filtration column (TSK-G2000SWG, Toyo Soda). The fractions containing spasmins were combined and applied to a phenyl-Sepharose column (Pharmacia). Spasmins were eluted from the phenyl-Sepharose column in a Ca^{2+}-dependent manner (data not shown). The spasmin fractions thus obtained were further applied to a Mono Q ion exchange column (FPLC, Pharmacia). The fraction of spasmins contained three kinds of Ca^{2+}-binding proteins whose molecular weights are 16, 18 and 20 kDa (fig. 1). These proteins correspond to spasmin B in Yamada and Asai's report [6]. We mixed this fraction with

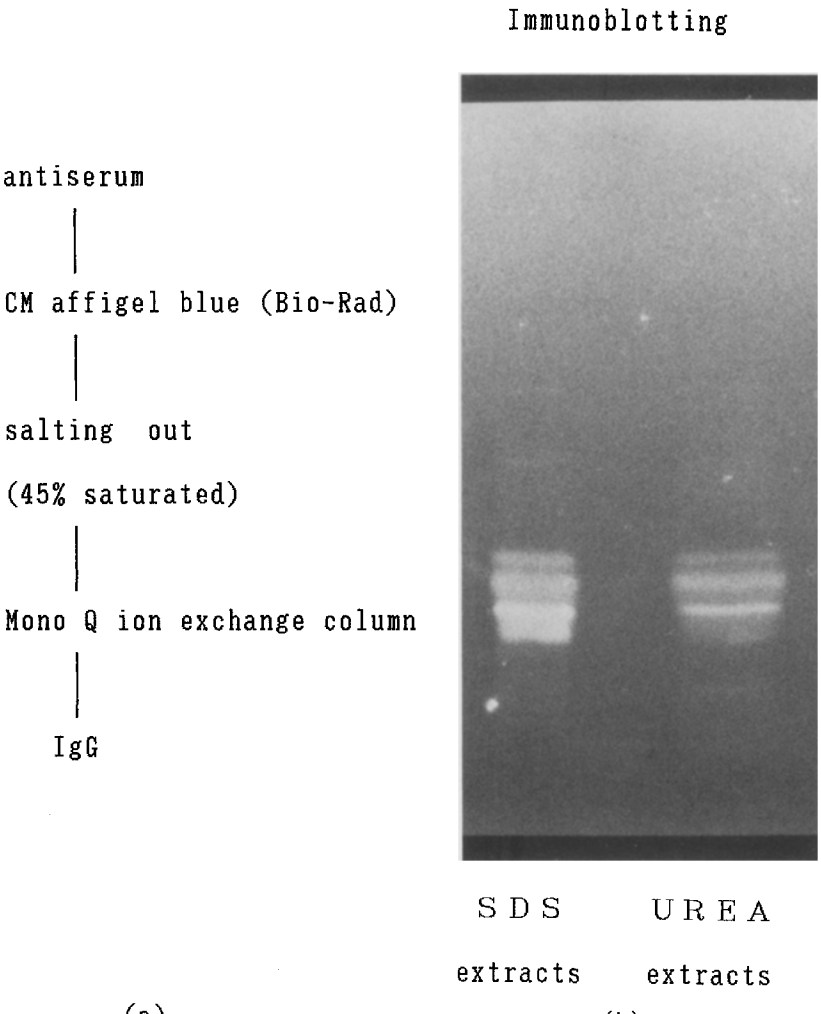

Figure 2. *a* Purification of anti-spasmins antibodies. *b* Immunoblotting pattern of 8-M urea extracts and that of SDS extracts of *Carchesium* against the anti-spasmins antibody (IgG). The procedure of immunoblotting was as follows. Proteins separated by SDS-PAGE were transferred from the polyacrylamide gel to nitrocellulose sheets, then the nitrocellulose sheets were incubated for at least 2 h in 10 mM phosphate-buffered saline (PBS), pH 7.3, containing 5% bovine serum albumin (BSA) at room temperature. The nitrocellulose sheets were next incubated for 2 h in PBS containing 1% BSA and anti-spasmins immunoglobulin G (IgG) (final dilution 1:200). The sheets were washed for 100 min with five successive 100-ml portions of PBS, and final incubation was carried out for 2 h in the dark in PBS containing 1% BSA and FITC-labeled swine anti-rabbit immunoglobulin G (diluted 1:100).

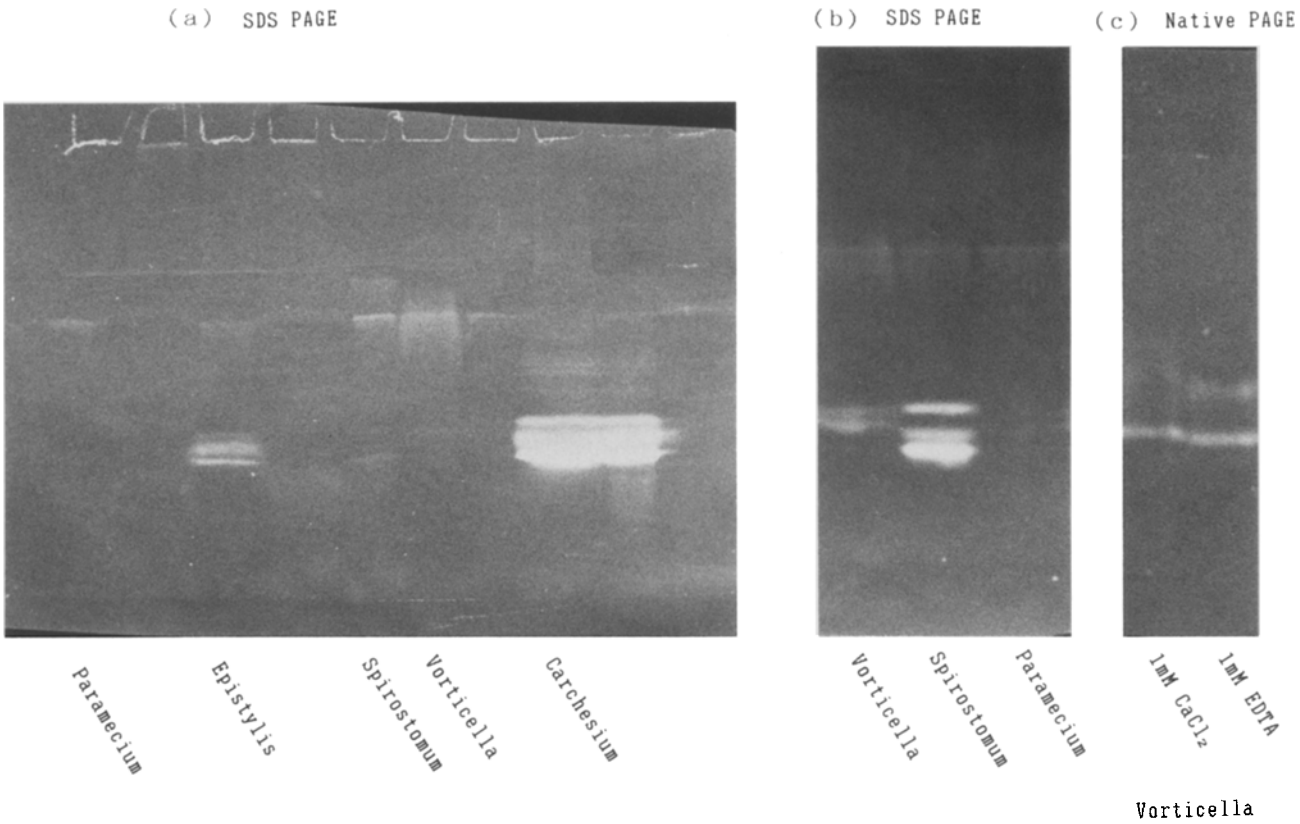

Figure 3. *a* Immunoblotting patterns of SDS extracts of *Carchesium, Vorticella, Spirostomum, Epistylis* and *Paramecium* against the anti-spasmins antibody (pure IgG). *b* Immunoblotting patterns of concentrated extracts of *Spirostomum, Vorticella* and *Paramecium*. *c* Immunoblotting patterns of 8-M urea extracts of *Vorticella*. It can be seen that the electrophoretic mobility in alkaline gel (12% polyacrylamide, pH 8.6) is reduced in the presence of 1 mM $CaCl_2$ as compared with in the presence of 1 mM EDTA.

Freund's complete adjuvant and injected the mixture directly into the lymph nodes of a rabbit. A booster injection was given one month after the first injection, and then blood was collected within a week. The antiserum obtained was purified by a conventional method as shown in figure 2a.

The specificity of the antibody to spasmins was checked by immunoblotting[8]. When 8 M urea extracts of the spasmoneme were used as a test material for the immunoblotting, three bands were seen at the positions corresponding to the molecular weights of the three kinds of antigens which were injected into the rabbit (fig. 2b). Therefore, it is clear that the antibody obtained has high specificity for spasmins. When SDS extracts of the spasmoneme instead of 8 M urea extracts were used as a test material for the immunoblotting, we observed four bands whose molecular weights are 15, 16, 18 and 20 kDa (fig. 2b). The band of 15 kDa may be a degradation product of one of the above three bands. The other bands of 16, 18 and 20 kDa correspond to those of the original antigens.

Cross reactions between the anti-spasmin antibody and SDS extracts of various ciliates are shown in figure 3 (a, b, c) and figure 4, and summarized in the table. It was found that ciliates which can contract rapidly like the family Vorticellidae have a spasmin-like 20 kDa. The bands seen near the top of the gel in some figures are an artifact because these patterns were sometimes seen even when the SDS-sample buffer without proteins was run as a control.

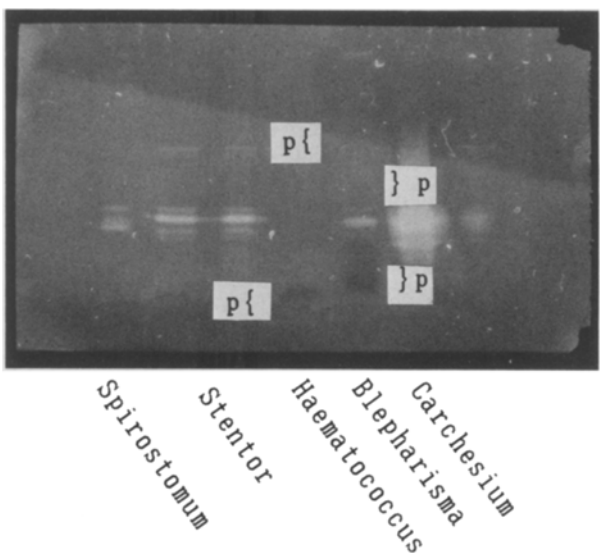

Figure 4. Immunoblotting pattern for SDS extracts of *Spirostomum, Stentor, Haematococcus, Blepharisma* and *Carchesium*. P indicates pigments originated from the cell bodies of *Haematococcus* and *Blepharisma*.

Summary of cross reactions between the anti-spasmins antibody and SDS extracts of various Protozoa

	Species	part	Number of bands	Molecular weight (k daltons)	Contractility
Ciliata	Holotrichia				
	Carchesium polypnium	Stalk	4	20 18 16 15	C
	Vorticella convallaria	Stalk	2	20 18	C
	Epistylis sp.	Stalk	3	18 17 16	N
	Paramecium caudatum	Total cell	0		N
	Tetrahymena pyriformis	Total cell	0		N
	Colpidium sp.	Total cell	0		N
	Spirotrichia				
	Blepharisma americanum	Total cell	2	20 18	C
	Spirostomum ambiguum	Total cell	3	20 17 16	C
	Stentor coeruleus	Total cell	4	20 18 16 15	C
	Stylonychia sp.	Total cell	0		N
Flagellata	*Haematococcus* sp.	Total cell	0		N
	*Tetraselmis** sp.	Total cell	1	20	C

* from Salisbury et al.[11]

The stalks of *Epistylis*, which belongs to the family of Vorticellidae, has no spasmoneme and cannot contract. Probably, the three kinds of spasmin-like proteins of 18, 17 and 16 kDa in *Epistylis* were extracted from the extracellular elastic sheath. These spasmin-like proteins may be independent of the spasmoneme contraction. The 20-kDa spasmin was not present in stalks of *Epistylis* sp. Therefore, the 20-kDa spasmin may be a key protein for understanding the spasmoneme contraction.

Stentor has at least three kinds of spasmin-like proteins very similar to those of *Carchesium* in the immunological sense. A *Stentor* protein of 15 kDa may be a proteolytic product of one of the above three bands, as suggested in the case of *Carchesium*. It is well known that *Stentor* has a contractile structure named the myoneme. It is most likely that the three kinds of spasmin-like proteins of *Stentor* originate from the myoneme. *Blepharisma* and *Spirostomum*, which belong to the *Spirotrichia*, like *Stentor*, also contain the 20-kDa spasmin and other spasmin-like proteins (fig. 3). These findings may support the idea that the family of peritrich Vorticellidae belongs to *Spirotrichia* rather than *Holotrichia*. *Haematococcus*, which was used as food for *Stentor*, does not contain spasmins. It has been reported that *Tetraselmis* has a spasmin-like protein with a molecular weight of 20 kDa[11]. On the basis of these findings, we propose here the hypothesis that the 20-kDa spasmin is essential for spasmoneme contraction, although the stalk has several kinds of Ca^{2+}-binding proteins.

We have investigated by the same procedure whether spasmin-like proteins are present in cells from Metazoa (*Hydra*, *Planaria*, sperms of *Coturnix*, gills of oysters, gills of asari clams, various organs of a rat). The results were all negative (data not shown), so it is not clear yet whether Metazoa have spasmin-like proteins or not. However, we have established that the contractile Ca^{2+}-binding protein of 20 kDa is not specific to the family of Vorticellidae, but is distributed widely in Protozoa.

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