A factor potentiating serotonin in the induction of germinal vesicle breakdown in surf clam oocytes[1]

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Summary. Simultaneous addition of an aliquot of body fluid obtained from the surf clam, *Spisula solidissima,* enhanced oocyte germinal vesicle breakdown induced with serotonin but not with KCl. When the body fluid and serotonin were added sequentially to the oocytes, potentiation did not occur. Body fluids of both males and females were effective at a 200-fold dilution. The factor is stable when treated with heat, acid, base, trypsin and pronase. It is hydrophobic and not dialyzable through tubing with a molecular weight cutoff of 1000 daltons. The factor is probably not a protein.
Key words. Spisula solidissima; oocyte maturation; germinal vesicle breakdown; serotonin; 5-hydroxytryptamine.

Naturally shed and ovarian oocytes of the surf clam, *Spisula solidissima,* retain intact germinal vesicles. Oocyte maturation is naturally induced by fertilization although KCl is also an effective inducer[3]. Matsutani and Nomura[4] reported that serotonin (5-hydroxytryptamine), a neurotransmitter, induces spawning of the marine bivalves, *Patinopecten yessoensis* and *Crassostrea gigas.* Many species of bivalves contain substantial concentrations of monoamines including serotonin in the central nervous system[5–10]. Matsutani and Nomura[11] determined the presence of serotonin and catecholamines in the central nervous system and gonads of the scallop, *Patinopecten yessoensis,* by a histochemical fluorescence technique. Recently, Hirai et al.[12] showed that serotonin injected into *Spisula solidissima* induced spawning. Also serotonin added to the suspending sea water at concentrations of 10–100 µM induces germinal vesicle breakdown (GVBD) of surf clam oocytes. Since the concentration of serotonin required for GVBD was relatively high, we searched for an endogenous substances which might potentiate the GVBD-inducing activity of serotonin.

In this communication, evidence will be presented showing that a serotonin-potentiating factor is present in the body fluid of *Spisula solidissima.* Some properties of the body fluid factor were determined.

Materials and methods. Serotonin hydrochloride and trypsin were purchased from Sigma Chemical Co. Pronase was obtained from Kaken Kagaku, Tokyo, Japan. *Spisula solidissima* collected during their breeding season were kept in a laboratory aquarium with cold running sea water at the Marine Biological Laboratory, Woods Hole. Oocytes were collected from the ovaries according to the method described by Allen[3]. Body fluid was obtained by cutting the adductor muscles and the mantle and collecting the mantle fluid.

Oocytes were washed thoroughly in filtered natural sea water (NSW) and transferred to a medium containing appropriate concentrations of serotonin and/or body fluid diluted with NSW. GVBD took place within 10–20 min after exposure to serotonin. After 40–50 min at room temperature, GVBD was scored by microscopic examination.

Results and discussion. Oocytes isolated from the ovaries of *Spisula solidissima* and suspended in NSW underwent GVBD when exposed to 5 µM or higher concentrations of serotonin, as reported by Hirai et al.[12]. In our search for endogenous substances modulating the serotonin-sensitivity of oocytes, we found that the body fluid, from either female or male clams, added to the medium potentiated the GVBD-inducing activity of serotonin. Although sensitivity of the oocytes to serotonin varied significantly depending upon the individual gonad, such potentiation was always observed. One of the typical examples is shown in figure 1. Body fluid itself did not induce GVBD at all. Thus, it is clear that the serotonin-potentiating effect is not due to the presence of serotonin in the body fluid. When oocytes were treated sequentially with body fluid and serotonin or vice versa, potentiation was not observed. Therefore, the serotonin-potentiating effect of the body fluid can be elicited only when added to the oocytes simultaneously with serotonin. It is conceivable that the active factor in the body fluid may modulate the interaction of serotonin with its receptors on the oocyte surface in a reversible manner.

Body fluid diluted 200-fold was still fully effective, regardless of the sex of its source (fig. 2). Although properties of the factor in the body fluids of male and female clams were the

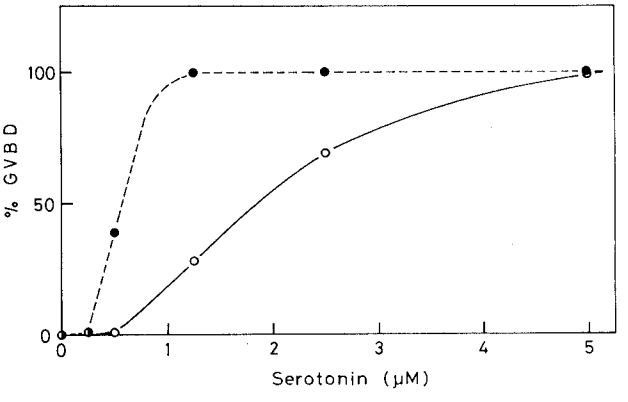

Figure 1. Effect of *Spisula* body fluid on serotonin-induced GVBD. Oocytes of the surf clam were incubated in filtered NSW containing varying concentrations of serotonin. Temperature, 20–22 °C; incubation time, 40–50 min. Suspending medium: 100 µl of NSW plus 100 µl of body fluid (●——●); 200 µl of NSW alone (○——○).

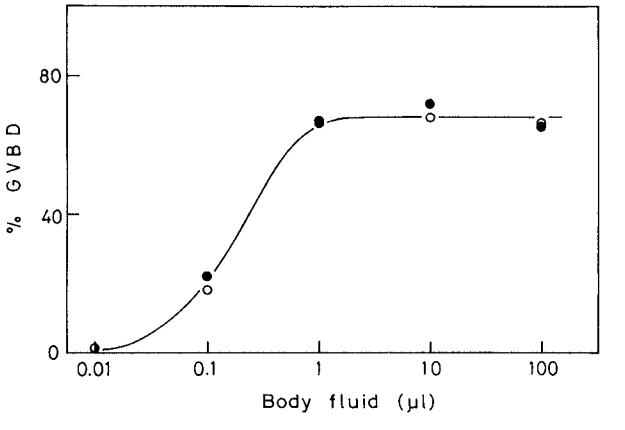

Figure 2. Effect of varying concentrations of *Spisula* body fluid on serotonin-induced GVBD. The experimental conditions were the same as described in fig. 1, except that the indicated amounts of body fluids obtained from male (●) or female (○) clams were present in 200 µl of the medium containing 1.25 µM serotonin in NSW.

same, structural elucidation is necessary to establish that the active factors(s) of both sexes are identical substances.

It is known that maturation of surf clam oocytes can be induced with higher concentrations of KCl[3]. However, the concentration of KCl necessary for inducing 100% GVBD was 45 mM both in the presence and absence of the body fluid. Thus, it is evident that the body fluid factor specifically potentiates serotonin action but not KCl action in the induction of GVBD.

The activity of body fluid in enhancing the serotonin-induced GVBD remained stable after exposure to heat, acid or base. No loss of the activity was detected after heating at 100 °C for 30 min in NSW, in 0.1 N HCl or in 0.1 N NaOH. Activity was retained after treatment with proteolytic enzymes, 0.07% trypsin or 0.2% pronase, at 30 °C for 1 h. The activity was stable in 95% ethanol.

The factor was retained after dialysis in tubing with a molecular weight cutoff of 1000 daltons and was partially lost after dialysis in tubing with a molecular weight cutoff of 10,000 daltons. The activity was completely adsorbed on active charcoal and partially recovered from the charcoal by treatment with acetone or 95% ethanol containing 1 N HCl. This finding indicates the highly hydrophobic nature of the body fluid factor. The present evidence suggests that the serotonin-potentiating factor in the surf clam body fluid is neither a peptide nor an inorganic ion.

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3 Allen, R.D., Biol. Bull. *105* (1953) 213.
4 Matsutani, T., and Nomura, T., Mar. Biol. Lett. *3* (1982) 353.
5 Welsh, J.H., and Moorhead, M., Science *129* (1959) 1491.
6 Welsh, J.H., and Moorhead, M., J. Neurochem. *6* (1960) 146.
7 Malanga, C.J., Wenger, G.R., and Aiello, E.L., Comp. Biochem. Physiol. *43A* (1972) 825.
8 Hiripi, L., and Osborne, N.N., Comp. Biochem. Physiol. *53B* (1976) 549.
9 Stefano, G.B., and Catapane, E.J., Experientia *33* (1977) 1341.
10 Smith, J.R., Comp. Biochem. Physiol. *71C* (1982) 57.
11 Matsutani, T., and Nomura, T., Bull. Jap. Soc. scient. Fish. *50* (1984) 425.
12 Hirai, S., Kishimoto, T., Koide, S.S., and Kanatani, H., Biol. Bull. *167* (1984) 518.

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Protective effect of vitamins against trichothecene toxicity towards *Saccharomyces cerevisiae*

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Summary. Several trichothecene mycotoxins were shown to inhibit the growth of *Saccharomyces cerevisiae*. This effect was most pronounced with the macrocyclic trichothecenes, especially verrucarin A. Much less growth inhibition was observed with T-2 toxin. Verrucarol, diacetoxyscirpenol, acetyl T-2 toxin, HT-2 toxin, T-2 tetraol and neosolaniol were inactive at a concentration of 75 µg of toxin per disc. Incubation of *S. cerevisiae* with verrucarin A together with vitamins resulted in a decrease in toxicity. Pyridoxine-HCl, Ca-pantothenate, thiamine-HCl and α-tocopheryl acetate were amongst the most potent of the vitamins tested which reversed growth inhibition, overcoming the inhibitory potential of the toxins.
Key words. Saccharomyces cerevisiae; trichothecenes; mycotoxins; vitamins.

Trichothecenes are a group of mycotoxins, produced by soil fungi, which contain 12, 13-epoxytrichothec-9-ene[3]. These toxins, widely distributed in nature, can cause tremendous damage to humans, animals and plants[3-6]. Their principal mode of action in eucaryotes is inhibition of protein synthesis[7]. Recent studies in our laboratory have suggested that these toxins are also active at the level of the cell membrane. T-2 toxin inhibited platelet aggregation[8], and phagocytosis by polymorphonuclear cells[9], and induced hemolysis in human red blood cells[10]. Acetyl T-2 toxin markedly reduced the intracellular pool size of soluble low molecular weight precursors in *Mycoplasma gallisepticum* during biosynthesis of macromolecules[11].

Schoental suggested that alkylation of coenzymes by the trichothecenes may be the biochemical basis of the effect of these hepatotoxins[12]. It was further suggested that B-vitamins may prevent acute liver lesions induced by these agents[12, 13]. Recently, we showed that, in vitro, α-tocopherol is effective in protecting erythrocytes against the hemolysing effect of T-2 toxin[10]. As vitamins B_1, B_3 and B_6 are essential constituents of coenzymes, their concentration may be of crucial importance in determining the toxicity of xenobiotic agents. This is supported by observations that the effects of the trichothecene mycotoxins are more acute in individuals suffering from malnutrition and B-vitamin deficiency[4].

Because of the severe health hazards posed to humans and animals by the trichothecenes, we thought it important to examine the influence of potential protective agents against their toxic actions. Trichothecenes are cytotoxic and cytostatic to several types of normal and malignant cells[5]. In view of their extreme toxicity, we selected a model system (the yeast *S. cerevisiae*) which would be extremely sensitive to the toxic effect of trichothecenes. Our main purpose was to screen for compounds that would inhibit the effect of these toxic agents. The information provided by such a study should prove of value in the planning of a rational treatment program for trichothecene intoxication. To obtain some insight regarding the beneficial effects of vitamins and other reducing agents against verrucarin A toxicity, we tested their influence on the growth initiation period in *S. cerevisiae*.

Materials and methods. Strains. Saccharomyces cerevisiae (bakers yeast, listed as cat. No 15-6250), and *Crithidia fasciculata* LPS 295 were obtained from the Carolina Biological Supply Company, Barlington, N.C., USA. *Ochromonas danica* (listed as cat. No 933/2) came from the Culture Collection of Algae and Protozoa, University of Cambridge, U.K. *Media. S. cerevisiae* was grown in a Sabouraud dextrose medium, (g per 100 ml): neopeptone, Difco, 1; dextrose 4; with or without agar 1.5. *C. fasciculata* was grown in the medium described by Wertlieb and Guttman[14], with or without 1.5%