ability to form plaques on *E. coli* K12 or on *M. xanthus* FB.

Generation of chloramphenicol-resistant bacteria by infection with P1CM phage shows that DNA carrying the chloramphenicol transacetylase gene can enter myxobacteria and can be replicated through many cell divisions. Loss of drug resistance from bacteria propagated in the absence of chloramphenicol suggests that the CM gene has not stably integrated into the myxobacterial chromosome and that it may be replicating as a plasmid more slowly than the cells are replicating. The clearing effect of P1 on FB growing on solid medium may be due to P1 genes whose expression is toxic to myxobacterial cells. However, there is no evidence for replication of these P1 genes, and no infective phage results from the infection. P1 might be used to transfer other genes from *E. coli* into myxobacteria for which specialized transducing particles exist, such as the genes for lactose metabolism (*10*) and for proline synthesis (*11*). It may be possible to select mutants of either P1 or *M. xanthus* (or both) that would permit P1 to mediate gene transfer between two myxobacterial cells (*12*).

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Concanavalin A: Its Action in Inducing Oocyte Maturation-Inducing Substance in Starfish Follicle Cells

Abstract. *Maturation of starfish oocytes is induced by 1-methyladenine produced in follicle cells under the influence of a gonad-stimulating hormonal peptide released from the nervous system. Concanavalin A stimulates isolated follicle cells to produce 1-methyladenine, which suggests that it has the same capacity as the peptide hormone to indirectly induce oocyte maturation.*

Oocyte maturation and spawning in starfish are caused by 1-methyladenine (*1*), which is secreted by the follicle cells (*2*). The production and secretion of 1-methyladenine are induced by gonad-stimulating substance (GSS) (*3*), which is released from the nervous tissue (*4*). Recently it has been reported that the lectin phytohemagglutinin M (Difco) induces maturation of starfish oocytes (*5*), although this activity was subsequently shown to be due to contaminating 1-methyladenine in the lectin preparation (*6*). We show here that another lectin, concanavalin A (Con A), induces oocyte maturation by stimulating production and release of 1-methyladenine.

When isolated starfish (*Asterina pectinifera*) oocytes with their follicular envelopes were kept in artificial seawater (ASW) (*7*) containing Con A (*8*) at various concentrations (0.04 to 2 mg/ml) for 1 hour, oocyte maturation revealed by the breakdown of germinal vesicles was found to occur at Con A concentrations above 0.12 mg/ml. Experiments to determine if the maturation-inducing activity of Con A was due to contamination with 1-methyladenine showed that this compound was not present in our Con A preparations (*9*).

The maturation-inducing action of Con A was found to be an indirect effect mediated by lectin interaction with follicle cells. When isolated starfish oocytes with intact follicular envelopes were incubated in ASW containing Con A at 25°C for 1 hour, they underwent oocyte maturation. When oocytes whose follicle cells had been removed by treatment with calcium-free ASW were placed in ASW containing Con A, they failed to mature at lectin concentrations up to 2 mg/ml. Treatment with calcium-free ASW does not reduce the capacity of oocytes to mature (*10*). These results indicate that the follicle cells are involved in oocyte maturation induced by Con A.

We next sought to determine whether Con A binding to follicle cells induces the production of a maturation-inducing substance (MIS). Incubation mixtures containing follicle cells (*11*) isolated from approximately 10,000 eggs in ASW, Con A (5 mg/ml) in ASW, and follicle cells plus Con A together in ASW were centrifuged after 60 minutes at 25°C. The supernatants were assayed for MIS with isolated oocytes with their follicular envelopes removed. Maturation-inducing substance was found only in the supernatant of the incubation media containing both follicular cells and Con A, and this activity was equivalent to that of $1.23 \times 10^{-7}M$ 1-methyladenine. These results suggest that Con A can stimulate follicular cells to produce MIS.

Subsequent experiments showed that the MIS induced by the action of Con A on follicle cells was 1-methyladenine. Follicle cells from 4.5×10^6 eggs were incubated at a concentration corresponding to 30,000 eggs per milliliter in ASW with Con A (5 mg/ml) for 2 hours at 25°C. After centrifugation

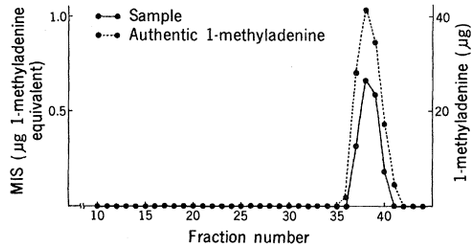

Fig. 1. (Solid line) Fractionation of the pooled active fractions of follicle cells and Con A on a Sephadex G-15 column equilibrated with 0.5*M* pyridine acetate buffer (*p*H 8.5), which is also used as eluant; fraction size, 3 ml; flow rate, 20.6 ml/hour. A part of each fraction (0.1 ml) was dried and the residue was redissolved in ASW and assayed with isolated oocytes without follicles, using authentic 1-methyladenine as the standard reference. (Dotted line) For comparison, the dried residue of 18 ml of 0.03*M* borate buffer (*p*H 8.5) was dissolved in 1 ml of $10^{-3}M$ 1-methyladenine and 1 ml of 0.5*M* pyridine acetate buffer and fractionated on the same column under the same conditions.

to remove the cells, the supernatant was treated with ethanol and subsequently with chloroform (*12*). The resulting sample (4 ml) was applied to a column (1.5 by 48.8 cm) of Sephadex G-15 equilibrated with 0.03*M* sodium borate buffer, *p*H 8.5, and eluted with the same buffer into 3-ml fractions. The active fractions (42 to 47, a total of 18 ml) were pooled and concentrated to dryness by a rotary evaporator (below 40°C). The residue was dissolved in 2 ml of 0.5*M* pyridine acetate buffer, *p*H 8.5, and chromatographed with the same buffer on a column (1.5 by 47.3 cm) of Sephadex G-15. Each fraction was assayed for MIS activity with isolated oocytes (*13*). When authentic 1-methyladenine was applied to the same column, the elution pattern was identical to that of the sample (Fig. 1). To confirm this finding the active fractions were pooled, concentrated to dryness, dissolved in a small amount of water, and applied to thin-layer chromatographic plates of microcrystalline cellulose (*14*) with and without authentic 1-methyladenine. The plates were developed with a mixture of isopropanol, hydrochloric acid, and water (65 : 16.7 : 18.3 by volume) or with a mixture of isopropanol and 5 percent ammonium sulfate (5 : 95). A spot corresponding to that of 1-methyladenine was detected under ultraviolet light. Further, the MIS activity was found to be confined to this spot when ASW extracts of material from the spot and from other areas were assayed with isolated oocytes.

The release of 1-methyladenine from the follicle cells stimulated by Con A (5 mg/ml) was found to be dependent on the number of cells in the incubation medium. Control incubation without Con A did not result in 1-methyladenine release (Fig. 2). Also, blocking the saccharide-binding sites of Con A with methyl α-D-mannopyranoside (α-MM) (*15, 16*) inhibited release of 1-methyladenine. In an incubation medium containing follicle cells from 30,000 eggs per milliliter and Con A (5 mg/ml) the production of 1-methyladenine was almost completely inhibited by concentrations of αMM and methyl α-D-glucopyranoside greater than 10 m*M* (Fig. 3). However, D-galactose (28 m*M*) only slightly inhibited 1-methyladenine release under equivalent conditions. When follicle cells preincubated in ASW containing 50 m*M* αMM for 2 hours at 25°C were washed with ASW and then incubated with Con A for an additional 2 hours, the inhibitory effect of αMM was no longer observed, which indicates that the inhibitory effect was due to αMM occupying the binding sites of Con A. Methyl α-D-mannopyranoside (50 m*M*) had no effect on the production of 1-methyladenine in follicle cells induced by GSS (200 μg of dry nerve per milliliter) under the same conditions.

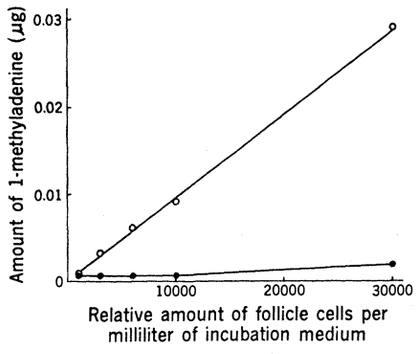

Fig. 2. Effect of follicle cell density on Con A–induced 1-methyladenine production in *Asterina pectinifera*. Follicle cells were incubated in ASW containing Con A (5 mg/ml) for 1 hour at 25°C. Number of follicle cells is expressed as the number of oocytes from which they were obtained. Amount of 1-methyladenine was determined by bioassay with isolated oocytes without follicles, using authentic 1-methyladenine as reference standard (*13*). (Open circles) Experiment, (closed circles) control without Con A.

Recently Con A has been found to mimic insulin effects on isolated adipocytes (*16, 17*). Here Con A was found to mimic the action of a gonad-stimulating hormone peptide (GSS) in inducing the production of 1-methyladenine in isolated follicle cells. Further studies on the action of Con A (a lectin with well-defined binding properties) on follicle cells may yield information on the mechanism of hormone-stimulated 1-methyladenine production.

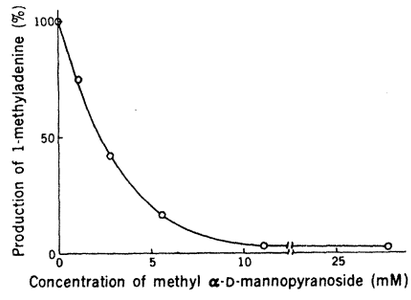

Fig. 3. Inhibition of 1-methyladenine production by methyl α-D-mannopyranoside (αMM). Artificial seawater (0.9 ml) containing Con A (5 mg) and αMM (various concentrations) was incubated for 5 minutes; then 0.1 ml of follicle cell suspension (from 30,000 eggs) was added and the mixture was incubated for 2 hours at 25°C. After centrifugation, the media were assayed.

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9. Since 1-methyladenine can be extracted by 85 percent ethanol, ethanol was added to Con A solution (25 mg/ml in 0.5*M* NaCl) to make its final concentration 85 percent. The solution was homogenized and centrifuged. The supernatant was designated as the Et-spnt fraction. The precipitate was dissolved in deionized water and centrifuged, and the supernatant was designated as the Et-ppt fraction. When both fractions were evaporated (below 40°C) to dryness, dissolved in ASW, and assayed with isolated oocytes, maturation occurred only in the Et-ppt fraction. Further, all the activity contained in the original Con A solution was recovered from the Et-ppt fraction. Heat treatment of Con A solution (100°C for 20 minutes) completely abolished its maturation-inducing activity; 1-methyladenine is known to be quite stable under such heat treatment.
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12. Ethanol was added to the concentrated supernatant (final concentration, 90 percent) and the sample was centrifuged. The supernatant was washed with chloroform and the water phase concentrated to dryness. The residue was dissolved in a small amount of deionized water and its *p*H was adjusted to 8.4. Borate buffer (0.03*M*, *p*H 8.5, 0.5 ml) was added to the sample (total volume, 4 ml).
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