Binding of Concanavalin A and its Effect on the Differentiation of *Dictyostelium discoideum*

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(Received 13 October 1975)

Dictyostelium discoideum amoebae have two classes of binding sites for concanavalin A, one accessible to a competitive inhibitor of concanavalin A binding and one inaccessible. Uptake of concanavalin A occurs and is associated with a transfer from accessible to inaccessible binding sites. Low amounts of concanavalin A bound to cells produce a slight stimulation of differentiation, and this is associated with an increase in production of phosphodiesterase. Above a critical amount of concanavalin A bound, cell differentiation and phosphodiesterase production are inhibited. The relationship of concanavalin A-induced membrane changes to cell differentiation is discussed.

In the presence of a food supply, amoebae of the slime mould *Dictyostelium discoideum* grow as single cells. Starvation triggers their developmental cycle, the first stage of which involves the aggregation of individual cells into multicellular organisms. The ability of cells to aggregate, which is defined as aggregation competence (Bonner, 1967; Gerisch, 1968), develops after a few hours of starvation. Cells then form specific intercellular contacts (Gerisch, 1961; Beug *et al.*, 1973a) and migrate toward central collection points.

Cell movement appears to be oriented by an acrasin (Bonner, 1967) cyclic AMP (Konijn *et al.*, 1967; Barkley, 1969), which is rhythmically released by cells during starvation (Shaffer, 1962, 1975; Gerisch & Wick, 1975). Pulses of cyclic AMP also play an important role in the differentiation of amoebae into aggregation-competent cells (Darmon *et al.*, 1975). Both cellular and extracellular phosphodiesterases, which specifically hydrolyse cyclic AMP, have been demonstrated in *D. discoideum*, and are thought to play an important role in the reception (Malchow *et al.*, 1972) and/or the modulation (Bonner *et al.*, 1969; Gerisch *et al.*, 1972) of the chemotactic signal. The activity of the extracellular phosphodiesterase is itself modified by a tight-binding inhibitor, which is also excreted during starvation (Gerisch *et al.*, 1972).

When cells become aggregation competent, the total amount of phosphodiesterase synthesized and excreted by differentiating cells increases dramatically, and this rise is related to the developmental programme of the amoebae (Malchow *et al.*, 1972; Klein & Darmon, 1975). Gillette & Filosa (1973) however, have reported that, when cells are incubated with concanavalin A, cellular phosphodiesterase is induced and that this induction is associated with an

inhibition of cell differentiation. In an attempt to understand these seemingly contradictory results we undertook an extensive investigation of the effects of concanavalin A on the differentiation of *D. discoideum* amoebae.

Experimental

Strains and culture conditions

Experiments were performed on axenically grown Ax2 amoebae (Watts & Ashworth, 1970) in media specifically containing Oxoid Yeast Extract (Watts & Ashworth, 1970). Exponentially growing cells (2×10^6 – 4×10^6 amoebae/ml) were harvested by centrifugation at 400g for 10 min, washed, and resuspended in Volvic mineral water buffered with 17 mM-potassium phosphate, pH 6.2. The moment of transfer into buffer is considered as zero time of starvation. Unless indicated otherwise, all experiments used cells starved at a density of 2×10^7 cells/ml in spinner culture (Beug *et al.*, 1973a) at 22°C.

Criteria of aggregation competence

The behaviour of starved cells was examined by plating samples on to Petri dishes filled with 17 mM-phosphate buffer. Non-competent amoebae remain as individual cells and retain the regular rounded appearance of growing cells. Competent cells display an elongated morphology and establish polar contacts, which give rise to cell streams. Formation of cell contacts specific for differentiating amoebae depends on membrane structures that remain functional in the presence of high concentrations of EDTA (Beug *et al.*, 1973a). At various times, cell samples were dissociated in the presence of 10 mM-EDTA for 2 min. After such treatment, agglutinates of aggregation-

competent amoebae rapidly re-formed, whereas non-competent cells remained dissociated.

Binding of concanavalin A

Cells were incubated in buffer in the presence of various concentrations of ^{125}I -labelled concanavalin A ($10\mu\text{Ci}/\text{mg}$). At timed intervals, samples of cell suspensions were centrifuged in a Beckman Microfuge at $9000g$ for 1 min. The medium was removed, and duplicate portions ($100\mu\text{l}$) were counted in Bray's (1960) scintillation fluid to determine the amount of radioactivity remaining in the medium. Cell pellets were not washed, since it has been shown that washing with buffer can partially remove specifically bound concanavalin A (Phillips *et al.*, 1974), but were immediately frozen and thawed. The amount of cell-associated radioactivity was determined by counting radioactivity of duplicate $100\mu\text{l}$ samples of the cell lysate. Sites that bind lectin on the subsequent addition of α -methyl mannopyranoside, a competitive inhibitor of concanavalin A binding (Sharon & Lis, 1972), are referred to as inaccessible. Those sites for which the sugar successfully competes are referred to as accessible.

Calculation of the number of binding sites was performed by using 100000 as the mol. wt. of concanavalin A (Sharon & Lis, 1972). The amount of radioactive contamination was determined by incubating cells for 5 min with α -methyl mannoside before the addition of ^{125}I -labelled concanavalin A and determining the amount of label remaining in the cell pellet. When cells were preincubated with 0.2M - α -methyl mannoside, 4% of the total added radioactivity was found in the pellet. The data presented were not corrected for this value, since it was, at most, 10% of the radioactivity associated with cells that had not been preincubated with the sugar.

Enzyme assays

Phosphodiesterase activity was determined as described by Klein (1975). A unit of phosphodiesterase is defined as the enzyme activity that hydrolyses 1 nmol of cyclic AMP in 1 min at 30°C . The extracellular phosphodiesterase inhibitor was measured by the procedure of Gerisch *et al.* (1972). A unit of inhibitor is defined as the activity that neutralizes 1 unit of phosphodiesterase. *N*-Acetylglucosaminidase was assayed by the method of Loomis (1969). A unit of activity is defined as the amount of enzyme that liberates 1 nmol of *p*-nitrophenol/min under the described conditions.

Materials

Oxoid Yeast Extract was purchased from Oxoid Ltd. (London E.C. 4, U.K.). Concanavalin A, succinyl-concanavalin A (3 carboxypropionyl-concanavalin A) and ^{125}I -labelled concanavalin A (50 – $100\mu\text{Ci}/\text{mg}$) were generous gifts from Dr. M. Bornens

and Dr. E. Karsenty (Pasteur Institute, Paris). The concanavalin A was prepared by the method of Agrawal & Goldstein (1966); succinyl-concanavalin A was prepared as described by Gunther *et al.* (1973). ^{125}I -labelled concanavalin A was prepared by the chloramine-T method (Hunter & Greenwood, 1962). *p*-Nitrophenyl β -D-*N*-acetylglucosamine was purchased from Calbiochem (San Diego, CA, U.S.A.).

Results

Effect of concanavalin A on cell differentiation to aggregation competence

As shown in Table 1, concentrations of concanavalin A greater than $50\mu\text{g}/\text{ml}$ delay the appearance of aggregation competence of amoebae starved at a concentration of 2×10^7 cells/ml, and this delay is related to the concentration of concanavalin A tested. With $200\mu\text{g}/\text{ml}$, cell differentiation is completely inhibited for over 20 h. The effect of the lectin also depends on cell concentration, since differentiation of cells incubated at a density of $2 \times 10^6/\text{ml}$ is completely inhibited by $25\mu\text{g}$ of concanavalin A/ml. Similar results have been reported by Gillette & Filosa (1973).

The effect of concanavalin A on differentiation is influenced by the length of time for which cells are incubated with the lectin (Fig. 1). When 2×10^7 cells/ml are incubated with $100\mu\text{g}$ of concanavalin A/ml for less than 10 min, the expression of aggregation competence is not inhibited, but, on the contrary, is slightly stimulated. Longer incubation times result in relatively longer delays in cell differentiation.

In similar experiments, succinyl-concanavalin A produced no inhibition of aggregation even with concentrations as high as $400\mu\text{g}/\text{ml}$.

Table 1. *Effect of concanavalin A on development of aggregation competence*

Cells starved in spinner suspension were incubated with various concentrations of concanavalin A. Samples were examined after 5, 6, 10 or 20 h of starvation and tested for aggregation competence, as described in the Experimental section. + indicates that cells were aggregation competent; – indicates that cells remained undifferentiated.

Concanavalin A ($\mu\text{g}/\text{ml}$)	Time of incubation (h)	...	Aggregation competence			
			5	6	10	20
0			–	+	+	+
12			–	+	+	+
25			–	+	+	+
50			–	+	+	+
100			–	–	–	+
200			–	–	–	–

Fig. 1. Effect of the time of incubation with concanavalin A on aggregation

Cells starved in spinner suspensions at a density of 2×10^7 /ml were incubated with $100 \mu\text{g}$ of concanavalin A/ml. At various times samples were taken, the cells were centrifuged, washed and plated in buffer on Petri dishes. Plates were examined microscopically, and the time at which aggregation began was noted.

Cell agglutination

Concentrations of concanavalin A of less than $25 \mu\text{g}/\text{ml}$ do not provoke any cell agglutination. With higher doses agglutination is observed immediately on addition of lectin. The sizes of the cell clusters increase with increasing lectin concentration, becoming maximal with $100 \mu\text{g}/\text{ml}$. With succinyl-concanavalin A, no agglutination is observed even with doses of $400 \mu\text{g}/\text{l}$. On prolonged incubation, agglutinates formed in the presence of concanavalin A progressively disappear. For example, after 90 min, the agglutinates formed when cells are incubated with $100 \mu\text{g}$ of concanavalin A/ml are completely dissociated. It should be noted, however, that the cells resulting from this treatment differentiate after a long delay (see Table 1), indicating that agglutination is not in itself the factor that inhibits cell aggregation. The dissociated cells reagglutinate immediately when incubated in the presence of additional concanavalin A. The cell supernatant, however, is not able to agglutinate untreated cells. This result would be consistent with the idea that cells take up or destroy the lectin during the incubation.

Kinetics of concanavalin A binding

When cells are incubated with concanavalin A for 5 min before the addition of 0.2M - α -methyl mannoside, only 60% of the bound lectin is released. The inability of α -methyl mannoside to totally compete with concanavalin A binding has been observed in other systems (Phillips *et al.*, 1974), and is inter-

preted to be the result of lectin binding to two compartments. In our experiments these two compartments are referred to as accessible and inaccessible (as defined in the Experimental section). The amount of concanavalin A bound to cells incubated with $100 \mu\text{g}$ of concanavalin A/ml for increasing time-periods is shown in Fig. 2. The lectin initially binds to cells at a fairly rapid rate. After the first 30 min, binding continues progressively, but at a diminished rate. The initial rapid binding occurs in both accessible and inaccessible compartments. Although concanavalin A binding to the accessible compartment is completed after 30 min, binding to the inaccessible compartment continues, and does so at a rate that appears to parallel that of total binding.

Passage of concanavalin A from accessible to inaccessible compartments

The transfer of lectin from accessible to inaccessible compartments was studied by determining the

Fig. 2. Kinetics of concanavalin A binding

Cells were incubated at 22°C with $100 \mu\text{g}$ of ^{125}I -labelled concanavalin A/ml. At various times, samples of cells were either incubated for 5 min with α -methyl mannoside and centrifuged or were centrifuged immediately. Total lectin bound (●) and the amounts of concanavalin A bound in either accessible (■) or inaccessible (▲) compartments are plotted as a function of incubation time.

Fig. 3. Loss of accessible binding sites

Cells were incubated with $100 \mu\text{g}$ of ^{125}I -labelled concanavalin A/ml for 5 min, washed, and then resuspended in fresh buffer without concanavalin A. At the indicated times, cells were incubated with or without α -methyl mannoside for 5 min, and the amount of radioactivity released into the medium was determined. Total lectin bound (●) was calculated as the difference between the radioactivity present in the solution before and after the addition of amoebae. Lectin bound in the accessible compartment (■) was determined as the difference between the amount of concanavalin A released with and without treatment with α -methyl mannoside.

Fig. 4. Comparison of concanavalin A binding at 4°C and 22°C

Cells were incubated in phosphate buffer for 5 min with $100 \mu\text{g}$ of ^{125}I -labelled concanavalin A/ml at either 4°C (○) or 22°C (●). The number of molecules bound per cell was determined as described in the Experimental section.

amount of concanavalin A present in each compartment in cells which had been prelabelled with concanavalin A for 5 min, washed, and incubated in fresh medium. The amount of lectin bound to both compartments was determined at various times after

resuspension. The results of such experiments (Fig. 3) indicate that, although the total amount of lectin bound remains constant during the first 2 h, lectin bound to the accessible compartment progressively decreases. The decrease in the accessible compartment therefore indicates that a transfer of lectin from the accessible to the inaccessible compartment has occurred. This experiment also indicates that the initial binding in the inaccessible compartment cannot be accounted for by radioactive contamination (see the Experimental section), since in this experiment cells were washed after incubation with the lectin.

To determine whether or not the transfer from the accessible to the inaccessible compartment is an energy-dependent process, total binding to cells incubated at 22° and 4°C was compared. As shown in Fig. 4, after 15–20 min a maximum amount of concanavalin A is bound to cells incubated at 4°C . The fact that after 20 min the amount bound remains constant suggests that no movement from the accessible to the inaccessible compartment occurs at low temperature.

Determination of the number of concanavalin A-binding sites

To determine the number of lectin-binding sites, amoebae at a density of 1.5×10^7 cells/ml were incubated with various concentrations of concanavalin A for 5 min at 22°C . A 5 min incubation period was chosen to avoid corrections due to the transition of binding sites from accessible to inaccessible compartments. As shown in Fig. 5, saturation of concana-

Fig. 5. Number of concanavalin A-binding sites

Cells were incubated in phosphate buffer at a density of 1.5×10^7 cells/ml for 5 min with various concentrations of ^{125}I -labelled concanavalin A. The number of molecules of concanavalin A bound was determined as described in the Experimental section.

Fig. 6. Effect of 10min incubation with concanavalin A on phosphodiesterase and inhibitor

Cells were incubated in phosphate buffer with 100 μ g of concanavalin A/ml for 10min, washed, and resuspended in fresh buffer. At various times, samples were taken and activities of cellular (a) and extracellular (b) phosphodiesterase as well as of inhibitor (c) were determined. Concanavalin A-treated cells: ●, extracellular phosphodiesterase; ▲, cellular phosphodiesterase; ■, inhibitor. Control cells: ○, extracellular phosphodiesterase; △, cellular phosphodiesterase; □, inhibitor. The beginning of aggregation competence is noted by the arrows: concanavalin A-treated cells, $\blacktriangledown$; control cells, $\triangleleft$.

Fig. 7. Effect of long-term incubation with concanavalin A on phosphodiesterase and inhibitor

Cells were incubated in phosphate buffer with 100 μ g of concanavalin A/ml. At various times, samples were taken and activities of cellular and extracellular phosphodiesterase as well as of inhibitor were determined. Symbols are the same as in Fig. 6.

valin A-binding sites is reached with concentrations of lectin equal to or greater than 100 μ g/ml. At such

concentrations, the amount of lectin bound is 18 μ g/10⁷ cells or 1.1×10^7 molecules/cell. A Scatchard-plot analysis of these data indicates 1.8×10^7 binding sites/

cell. Experiments using succinyl-concanavalin A indicate that only 1.2×10^6 molecules of this compound bind/cell.

The number of binding sites for concanavalin A was approximately equal for vegetative and aggregation-competent amoebae. However, when competent cells are incubated with lectin for longer periods of time, binding of concanavalin A continues, but at a rate significantly lower than that seen with vegetative cells.

Effect of concanavalin A on phosphodiesterase activity

Short incubation. As shown in Fig. 6, when cells are incubated at a concentration of 2×10^7 /ml with $100 \mu\text{g}$ of concanavalin A/ml for 10 min, washed, and starved in fresh medium, a substantial peak of extracellular phosphodiesterase activity occurs during the first 4 h. This increase in extracellular phosphodiesterase activity can be accounted for by a decrease in the amount of extracellular phosphodiesterase inhibitor produced by the cells. Even when the amount of inhibitor produced by control cells is at a maximum, concanavalin A-treated amoebae have produced only one-half the amount of inhibitor produced by control cells.

During the first 4 h, no difference in the appearance of cellular phosphodiesterase between concanavalin A-treated and untreated cells is observed. After that period, an increase in phosphodiesterase activity occurs in cells incubated with lectin, and this increase precedes that seen in control cells. Under the conditions described above, aggregation competence is also advanced in concanavalin A-treated cells relative to untreated amoebae.

Continued incubation. Fig. 7 shows that continued incubation of amoebae with high doses of concanavalin A, conditions that totally inhibit cell differentiation, provokes: (1) a dramatic decrease in the appearance of the extracellular phosphodiesterase inhibitor; (2) a slight decrease in external phosphodiesterase (this effect is inconsistent, and in some

experiments no effect is observed); (3) an early increase in cellular phosphodiesterase, the activity of which starts to decline after 5 h of incubation. In control cells cellular phosphodiesterase activity is increasing at that time. After 8 h of incubation, lectin-treated cells contain only 35% of the amount of cellular phosphodiesterase present in the control cells. When cells are incubated with succinyl-concanavalin A at concentrations as high as $400 \mu\text{g}/\text{ml}$, no effect on phosphodiesterase is observed.

Since it is probable that cellular and extracellular phosphodiesterase activities represent various forms of one enzyme (Klein & Darmon, 1975; Klein, 1975; M. Darmon & C. Klein, unpublished work), the total amount of phosphodiesterase produced by cells is represented by the sum of both activities and the amount complexed to the inhibitor. After 6 h of starvation, more than 50% of the inhibitor produced by control cells is complexed with the extracellular phosphodiesterase (Klein & Darmon, 1975). Therefore the minimal amount of phosphodiesterase produced by control cells in the experiment described in Fig. 7 is 30 phosphodiesterase units (20 units of extracellular phosphodiesterase complexed to inhibitor, 5 units not complexed and 5 units of cellular enzyme). For lectin-treated cells, the maximal amount of enzyme produced is 9 units (3 units of extracellular enzyme complexed to inhibitor, 1 unit not complexed and 5 units of cellular enzyme). It is evident from these values that enzyme production is inhibited in cells incubated with concanavalin A. It appears therefore that the early rise in cellular phosphodiesterase, which occurs when cells are treated with concentrations of concanavalin A that inhibit cell differentiation, reflects an inhibition of enzyme excretion. This possibility is also supported by the observation that short incubations with lectin are sufficient to provoke an increase in cellular enzyme activity and a concomitant decrease in extracellular enzyme activity (Table 2). In these experiments cells were starved with concanavalin A for 10 min and both cellular and external

Table 2. *Effect of concanavalin A on cellular and extracellular phosphodiesterase*

Amoebae were starved in spinner suspensions at a density of 10^7 cells/ml. At the times indicated, $100 \mu\text{g}$ of concanavalin A/ml was added. After 10 min, the activity of cellular and extracellular phosphodiesterase was determined. Control cells were not treated with lectin. Numbers in parentheses indicate values obtained when cells were harvested by centrifugation, frozen, and thawed before incubation with lectin.

Time of starvation (h)	Phosphodiesterase activity (units/ 10^7 cells or /ml)					
	Concanavalin A-treated			Control		
	Cellular	Extracellular	Total	Cellular	Extracellular	Total
0	0.1	0.15	0.25	0.1	0.15	0.25
1.5	1.9 (1.7)	0.9	2.8	0.8 (1.8)	2.4	3.2
3	1.5	1.3	2.8	0.6	2.1	2.7

Table 3. *Effect of concanavalin A on cellular phosphodiesterase compartmentalization*

Cells were starved in spinner suspensions for either 1.5 or 8 h, after which time concanavalin A was added at a concentration of 100 μ g/ml. Cells were incubated with lectin for 10 min, and the activity of membrane-bound and cytoplasmic phosphodiesterase was determined. Control cells were not treated with lectin.

Time of starvation (h)	Phosphodiesterase activity (units/10 ⁷ cells)					
	Concanavalin A-treated			Control		
	Membrane	Soluble	Total	Membrane	Soluble	Total
1.5	0.3	0.7	1.0	0.15	0.5	0.65
8	7.9	2.6	10.5	4.5	3.7	8.2

Table 4. *Effect of concanavalin A on N-acetylglucosaminidase excretion*

Cells were incubated in phosphate buffer with 100 μ g of concanavalin A, and at various times samples were taken, and N-acetylglucosaminidase activity was determined as described by Loomis (1969).

Time of starvation (min)	N-Acetylglucosaminidase activity (units/10 ⁷ cells or /ml)			
	Concanavalin A-treated		Control	
	Cellular	Extracellular	Cellular	Extracellular
15	50	10	50	10
120	35	20	35	80

phosphodiesterase activities were immediately measured. Modification of the extracellular activity due to the presence of the extracellular inhibitor was minimized by starving cells for no longer than 3 h. The possibility that concanavalin A activates the cellular enzyme was examined by incubating cell lysates with various concentrations of concanavalin A. Under these conditions no increase in enzyme activity was detected.

The distribution of phosphodiesterase in cells treated with concanavalin A was examined by preparing crude membrane and cytoplasmic fractions (Malchow *et al.*, 1972) from cells that had been pre-incubated with the lectin. As shown in Table 3, the increase in cellular phosphodiesterase occurs mainly in the membrane fraction. This observation is compatible with the idea that the membrane-bound phosphodiesterase is an intermediate in the process of enzyme excretion (Klein & Darmon, 1975).

Effect of concanavalin A on excretion of N-acetylglucosaminidase

To determine if the effect of high concentrations of concanavalin A on cell excretion is specific for the parameters involved in the chemotactic signalling system, we determined the kinetics of excretion of N-acetylglucosaminidase, an enzyme normally excreted during starvation of *D. discoideum* and which presumably is not involved in chemotaxis. The results expressed in Table 4 show that after a 2 h incubation

with concanavalin A, the extracellular activity of this enzyme is decreased to about 25% of the control.

Discussion

The results described in this paper show that cells incubated in non-nutritive media have two classes of concanavalin A-binding sites, accessible (sugar-competitive) and inaccessible (non-competitive). At 22°C, cells gradually take up concanavalin A from the medium, and this is associated with a transfer of lectin from accessible to inaccessible binding sites. This transfer probably reflects the internalization of binding sites into the cell as reported in other systems (Barat & Avrameas, 1973; Oliver *et al.*, 1974). Internalization also occurs when *D. discoideum* is incubated with anti-membrane IgG (Beug *et al.*, 1973b). Fab univalent antibodies do not provoke this phenomenon (Beug *et al.*, 1973b), indicating that, as in leucocytes, only multivalent ligands induce their own internalization (Ryan *et al.*, 1974; Oliver *et al.*, 1974). However, some inaccessible binding sites do exist independently of any active process of internalization, since they are observed even during very short incubations of cells with lectin, and are probably present in cells incubated at 4°C. These may therefore reflect strong secondary attachments of concanavalin A to the membrane (Phillips *et al.*, 1974).

Approximately the same number of concanavalin A-binding sites are present in vegetative and aggrega-

tion-competent amoebae. However, uptake of lectin is decreased in aggregation-competent cells. This may indicate a decrease in the fluidity of the membrane of aggregation-competent amoebae, and may be the basis of the lowered concanavalin A agglutinability of aggregation-competent cells reported by Weeks & Weeks (1975).

The sensitivity of Ax2 amoebae to concanavalin A was found to vary, depending on cell-growth conditions (M. Darmon, unpublished work). When Difco Yeast Extract was used in the growth medium cells grew poorly, high background agglutination occurred immediately on suspension of amoebae in buffer, and concentrations higher than 200 µg of concanavalin A/ml were needed to observe any effect of the lectin. Poor growth conditions may account for the relative insensitivity of Ax2 amoebae to concanavalin A in the experiments reported by Weeks & Weeks (1975).

Analyses of the effects of lectin on cell differentiation suggest that the inhibitory action of concanavalin A on aggregation is related to the quantity of lectin bound to the cells. When cells are incubated with low concentrations of concanavalin A (50 µg/ml or less) or with very high concentrations for short periods of time (10 min or less) no inhibition of cell differentiation occurs, but, on the contrary, a slight stimulation is observed. Both these conditions correspond to a maximal binding of 1.5×10^7 molecules/cell. Above this critical number, the degree of inhibition of cell differentiation is proportional to the amount of lectin bound to the cells.

The different effects of low and high doses of concanavalin A on the differentiation of *D. discoideum* may be related to the redistribution of lectin receptors after lectin binding has occurred. It is probable that various other membrane components, for example cyclic AMP receptors (Malchow & Gerisch, 1974), are redistributed simultaneously with the concanavalin A-binding sites. Therefore, with low (stimulatory) or high (inhibitory) amounts of lectin bound to the membrane, surface components may be either exposed or first exposed but then immediately masked in the process of lectin uptake. The period necessary for cells to recover from the inhibitory doses of concanavalin A could indicate the time required for the appearance of new functional surface receptors.

The action of lectin on phosphodiesterase production reflects its effect on cell differentiation: conditions that advance aggregation competence stimulate phosphodiesterase production; phosphodiesterase production is decreased when cell differentiation is inhibited. In the latter case, experiments indicate that the increase in cellular enzyme activity is probably the result of a decrease in the rate of enzyme excretion. The effect on excretion is not specific for phosphodiesterase, but rather seems to reflect the general membrane changes induced by concanavalin A.

We thank Dr. M. Bornens and Dr. E. Karsenty for their assistance in interpreting results presented here. This research was supported by grants from the Centre National de la Recherche Scientifique. C. K. is a 'chercheur associé' in the C.N.R.S.

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