tion of a single wavelength or of all the wavelengths together.
4. T. Seto and C. M. Pomerat, *Copeia No. 4* (1965), p. 415.
5. G. G. Rose, *Texas Rep. Biol. Med.* **12**, 1074 (1954).
6. H. Eagle, *Science* **130**, 432 (1959).
7. It was also noted that the condensed interphase chromatin could be altered without dye sensitization, and the chromatin appeared to be more sensitive to the laser light than the mitotic chromosomes.
8. M. W. Berns and A. D. Floyd, in preparation.
9. Supported by NSF grant GB-24457 and University of Michigan grant IN-40K from the American Cancer Society. We thank D. Rounds, of the Pasadena Foundation for Medical Research, for his helpful comments.

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Embryonic and Neoplastic Cell Surfaces: Availability of Receptors for Concanavalin A and Wheat Germ Agglutinin

Abstract. *Embryonic tissue cells dissociated with ethylenediaminetetraacetate are readily agglutinated by the carbohydrate-binding protein concanavalin A. In this property, they resemble transformed, neoplastic cells; and they differ from untransformed adult cells, which are agglutinated by concanavalin A only after their receptors are unmasked by proteolytic treatment. Receptor sites for wheat germ agglutinin are also present on the surface of embryonic cells, but in a masked form, as on untransformed adult culture cells; they can be unmasked by treatment of the cells with trypsin. Concanavalin A binding sites on embryonic cells may function in cell contact and cell organization during embryonic morphogenesis and differentiation and later become masked in adult cells. The unmasking of these sites in neoplastic cells may represent a return, in this respect, to a condition resembling that of embryonic cells and may be related to cell mobility associated with infiltration and metastasis.*

Changes in the properties of cell surfaces represent an important aspect of embryonic differentiation, morphogenesis, neoplastic transformation, and metastasis. Studies of the histogenetic reaggregation of dissociated cells in vitro and of the self-assembly of tissues from cell suspensions have contributed to the understanding of the surface properties of cells (*1*). Another valuable approach to this problem is the use of exogenous proteins that specifically bind to various carbohydrates on the cell surface and cause cell agglutination. Neoplastic cells (transformed by carcinogens or viruses) are agglutinated by a glycoprotein from wheat germ (*2*) and by concanavalin A (Con A), a jack-bean globulin (*3*); but these proteins do not agglutinate untransformed cells. Untransformed cells do possess binding sites for these agglutinins, but in a masked form; thus treatment of untransformed cells with trypsin renders them agglutinable by exposing the carbohydrate-containing binding sites (*2*, *3*). On the other hand, treatment of untransformed cells with ethylenediaminetetraacetate (EDTA, Versene) does not render them agglutinable with these proteins. These findings suggest that the surfaces of normal cells differ from those of neoplastic cells by the inaccessibility of their sites for interaction with the above agglutinins and by the exposure of such sites through treatment of the cells with trypsin, but not with EDTA.

The above agglutination experiments were performed with adult cells from established cell culture lines; to further test the validity of their conclusions, we examined in the present study the question of whether embryonic cells, isolated from normal tissues, resembled untransformed or transformed adult, cultured cells in their possession of masked or exposed surface receptors for Con A and wheat germ agglutinin (WGA). The cells used in these experiments were freshly obtained from neural retina and liver tissues of 10-day chick embryos by dissociating these tissues into suspensions either with trypsin or with EDTA. The trypsinization procedure yields single cell suspensions after 20 minutes of incubation (38°C) in a 0.5 percent to 1 percent solution of crystalline trypsin in calcium- and magnesium-free Tyrode solution (CMF) (*4*); the trypsinized cells were thoroughly washed with Tyrode solution containing deoxyribonuclease (5 μg/ml) (*5*). For dissociation with EDTA, tissue fragments were incubated for 40 minutes in CMF with 0.02 percent disodium EDTA, washed with CMF, and then disrupted by pipetting; the resulting mixture of cells and tissue fragments was filtered through sieves which allowed passage of only single cells and of small clumps of cells.

To test for agglutination, 0.2 ml of cell suspension (1.5 to 2.5 × 10^5 cells) prepared either with trypsin or with EDTA was added to Erlenmeyer flasks (25 ml) which contained 3 ml of Tyrode solution with deoxyribonuclease (5 μg/ml) and various concentrations of Con A (Calbiochem) or WGA (*6*). The flasks were immediately placed on a gyratory shaker (70 rev/min), at 38°C and the presence and size of agglutinates was checked at various times with a microscope.

Embryonic retina and liver cells dissociated by trypsin were agglutinated by Con A in the concentration range of 100 to 1000 μg/ml. The magnitude of the effect depended directly on the concentration of Con A. Agglutination began within 10 minutes after the start of incubation on the shaker, and the agglutinates reached maximum size within 30 minutes to 1 hour (Fig. 1). The largest of these agglutinates, obtained with the highest concentrations of Con A, contained 100 to 200 cells. Control cultures without Con A contained no agglutinated cells at any time; after 30 minutes of incubation, control cells had begun to aggregate histogenetically, as normally expected, and had formed clusters of 20 to 40 cells. However, these aggregates of normal cells were quite different in morphology and size from the agglutinates formed in Con A cultures. As in previous studies (*3*), the agglutination of cells by Con A was inhibited by α-methyl-D-glucopyranoside, a carbohydrate that strongly binds to Con A; it was not inhibited by *N*-acetylglucosamine.

To determine whether the agglutination by Con A of embryonic tissue cells dispersed by trypsin was due to unmasking by the protease of receptor sites for Con A, as in the case of adult tissue culture cells, embryonic tissue cells dispersed by EDTA were tested next. Con A at concentrations of 50 to 1000 μg/ml rapidly agglutinated EDTA-dissociated retina cells, even though they had not been exposed to trypsin. Moreover, the agglutination of the EDTA-dispersed cells was considerably more pronounced than that of trypsin-dissociated cells, in that the "lag period" before the onset of noticeable agglutination was shorter and the 30-minute agglutinates were many times larger and contained thousands of cells. Even though EDTA-dissociated embryonic cells tend to adhere to each other more rapidly than do trypsin-dissociated cells also in

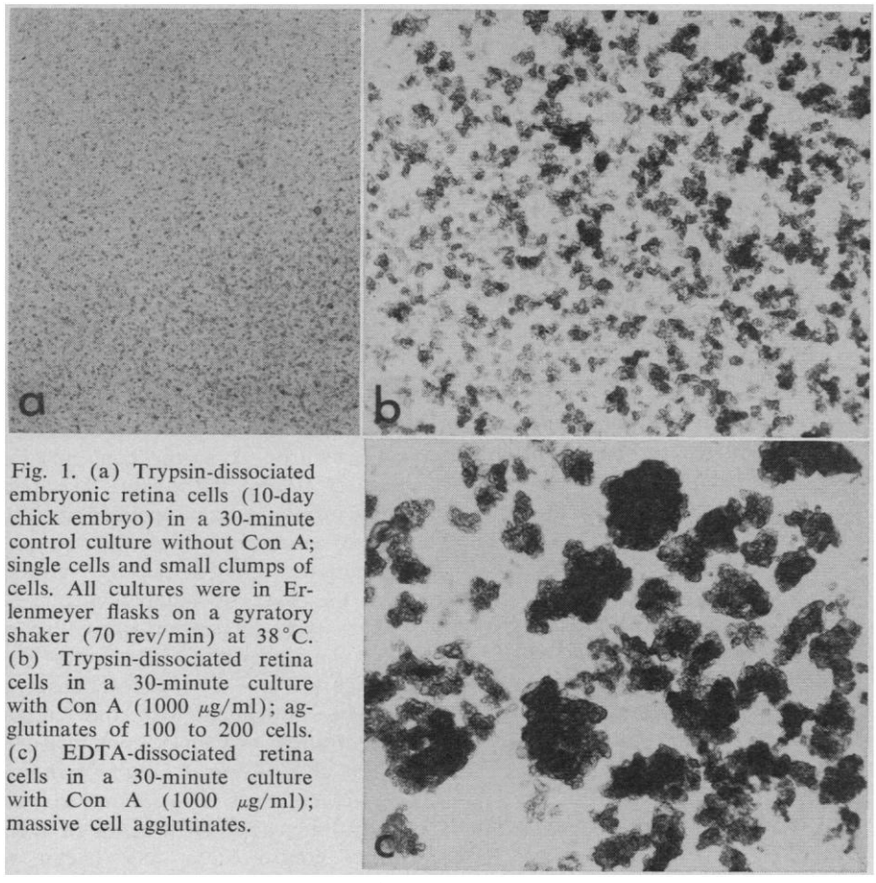

Fig. 1. (a) Trypsin-dissociated embryonic retina cells (10-day chick embryo) in a 30-minute control culture without Con A; single cells and small clumps of cells. All cultures were in Erlenmeyer flasks on a gyratory shaker (70 rev/min) at 38°C. (b) Trypsin-dissociated retina cells in a 30-minute culture with Con A (1000 μg/ml); agglutinates of 100 to 200 cells. (c) EDTA-dissociated retina cells in a 30-minute culture with Con A (1000 μg/ml); massive cell agglutinates.

the absence of Con A (*7*), the agglutination of EDTA-dissociated cells by Con A was strikingly faster and grossly more massive than that of cells dissociated with trypsin. Embryonic liver cells dissociated by EDTA were also agglutinated by Con A. The agglutination of EDTA-dissociated cells by Con A was inhibited by competition with α-methyl-D-glucopyranoside, but not with *N*-acetylglucosamine. Therefore, in contrast to culture cells from adult cell lines, embryonic cells from two different tissues can react with Con A without proteolytic treatment and thus possess unmasked binding sites for Con A on their surfaces.

A possible factor in this difference between embryonic tissue cells and culture cells in the accessibility of receptors for Con A could be the exposure of the cultured cells to conditions of cultivation in vitro, which may have resulted in the masking of receptors for Con A. To test this, suspensions of 10-day embryonic retina cells were plated into culture dishes and maintained as monolayers for 24 to 48 hours in serum-containing medium; the cells were then detached and dispersed by mild trypsinization, by treatment with EDTA, or by scraping with a rubber policeman. After thorough washing in Tyrode solution, the agglutinability of the cells with Con A was tested. The most pronounced agglutination was obtained with cells detached and dispersed mechanically or by EDTA; the trypsin-treated cells formed smaller agglutinates. Therefore, exposure of embryonic cells for 48 hours to the conditions of cultivation in a monolayer did not result in masking of the binding sites for Con A. Although this does not completely exclude the possibility that prolonged cultivation might reduce the accessibility of binding sites for Con A, a more likely alternative is that these sites are normally accessible on the surface of embryonic cells and masked on normal adult cells.

The fact that trypsin-dissociated embryonic cells are less agglutinable by Con A than their EDTA-dissociated counterparts is, in all likelihood, due to partial degradation by the protease of the Con A binding sites on the cell surface. It is not due to destruction of Con A by residual trypsin on the cells because thorough washing of the cells with trypsin inhibitor (soybean) before the addition of Con A did not increase the agglutinability of the cells; conversely, prolonged incubation of the cells in trypsin increased the "lag period" which precedes visible agglutination with Con A.

In contrast to the effect of Con A, WGA did not result in agglutination of EDTA-dissociated embryonic retina cells; however, after dissociation with trypsin these cells were readily agglutinated by WGA (concentrations: 100 to 500 μg/ml); this agglutination proceeded faster and resulted at 30 minutes in larger agglutinates than with Con A. This agglutination was competitively inhibited by *N*-acetylglucosamine in accordance with previous findings (*2*). Addition of WGA to trypsin-dissociated cells that had received prior treatment for 15 minutes with Con A resulted in an additive effect of both agglutinins. It, therefore, appears that embryonic cells have different receptor sites for Con A and for WGA; those for Con A are accessible without trypsinization, but those for WGA are masked and become exposed only after treatment of the cells with a protease. It is not yet known whether WGA receptors ever exist on embryonic cells in an unmasked form.

The existence on embryonic cell surfaces of exposed sites containing carbohydrates, which are detectable by their affinity for Con A, raises possibilities that these sites may function in morphogenetic cell contacts, cell mobility, and tissue organization during embryonic development, and that they become masked when morphogenesis is complete, cell movements cease, and the cells reach an "adult" state. Neoplastic transformation, by causing these sites to become exposed again, would represent, in this respect, a "retrogression" to a condition found in embryonic cells; this retrogression may be related to the increased mobility of transformed cells and may be associated with their propensity to infiltrate and metastasize. The masked nature of WGA receptors on embryonic cells, as well as on normal adult cells, contrasts them with the Con A receptors and suggests that the unmasking of WGA receptors in transformed cells may have a special significance in neoplasia. Elucidation of the nature and properties of receptors for Con A and for WGA on embryonic cells and the normal role of these receptors might lead to information about morphogenetic processes and differentiation in embryonic development and in neoplastic cells.

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References and Notes

1. A. A. Moscona, in *In Vitro*, M. M. Sigel Ed. (Williams & Wilkins, Baltimore, 1968), vol. 3, pp. 13–21; *Develop. Biol.* **18**, 250 (1968); J. E. Lilien and A. A. Moscona, *Science* **157**, 70 (1967); J. E. Lilien, in *Current Topics in Developmental Biology*, A. A. Moscona and A. Monroy, Eds. (Academic Press, New York, 1969), vol. 4, p. 169.
2. M. M. Burger and A. R. Goldberg, *Proc. Nat. Acad. Sci. U.S.* **57**, 359 (1967).
3. M. Inbar and L. Sachs, *ibid.* **63**, 1418 (1969); *Nature* **223**, 710 (1969).
4. A. A. Moscona, *Exp. Cell Res.* **22**, 455 (1961).
5. ——— and M. H. Moscona, *ibid.* **41**, 697 (1966).
6. I thank Dr. Max M. Burger for a sample of purified wheat germ agglutinin.
7. S. Kleinschuster and A. A. Moscona, in preparation.

8. Supported by PHS grant HD-01253.

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Laser Stimulation of Nerve Cells in Aplysia

Abstract. *Laser radiation at 488 nanometers selectively stimulates neurons in the abdominal ganglion of the marine mollusk* Aplysia californica. *The laser radiation can be scanned over the surface of the ganglion and can be effectively utilized in mapping cellular interconnections. The laser appears to cause these changes through some mechanism other than damage.*

A method for stimulating selectively and reversibly any of a large array of neurons within a short time period would be highly useful in studies of individual cells and especially in the mapping of cellular interconnections. I report here the development of such a technique in which laser radiation is applied to the abdominal ganglion of the marine mollusk *Aplysia californica*. This preparation possesses some cells that have light-sensitive properties (*1*) and has a number of identified neurons as well as some unidentified neurons whose locations have not yet been specified (*2*). [The notation identifying various cells is given in (*2*).]

Intracellular recordings were obtained from identified cells by impaling them with conventional microelectrodes filled with 2*M* KCl and then illuminating either those cells or the surrounding regions of the ganglion with a blue (488 nm), green (515 nm), or near-infrared (1060 nm) laser beam having a minimum spot size (*3*) of about 10 μm. The beam penetrated the ganglion with an attenuation of ~50 percent. The experimental arrangement is shown in Fig. 1.

The laser stimulation of the cells with blue or green light produced firing with the light pulse "on" in some cases and with the light pulse "off" in others. An example of light-induced firing in a silent cell (cell R2) is shown in Fig. 2. The irradiation caused an initial depolarizing component which gradually returned to near rest potential. With the light pulse "off" the membrane potential dropped momentarily and then moved above the critical firing threshold, causing spiking. The change in membrane potential was typical of the responses seen in seawater at room temperature (~ 21°C). In some instances, particularly with the addition of ouabain, firing occurred during the light pulse whereas in others firing occurred only as a rebound to turning the laser beam off. These effects could be reproducibly obtained at levels of intensity which did not cause significant damage (abrupt irreversible depolarization) (*4*). With this technique it was possible to obtain firing in each of a representative sample of 12 identified cells located on the dorsal surface of the ganglion (cells L1, L2, L3, L4, L6, L7, L8, L9, L11, R1, R2, and R15). None of these cells was selected for any unusual photoreceptive properties; presumably this technique will be effective in stimulating almost any cell in the ganglion.

The capacity to activate individual cells without impaling them could pro-

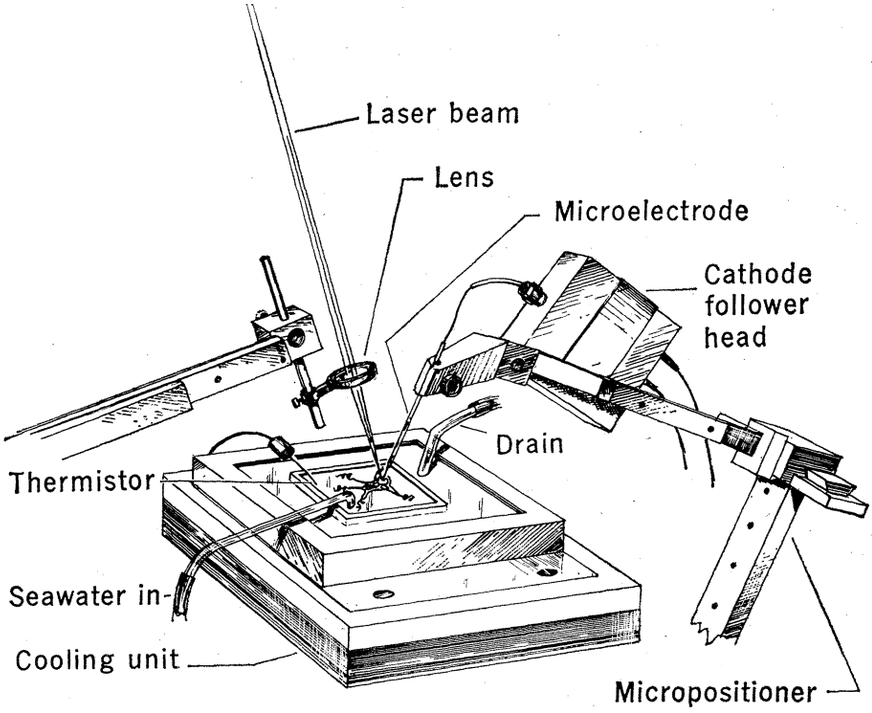

Fig. 1. Experimental arrangement. The lens is mounted on a micropositioner which permits scanning of the beam across the ganglion as well as focusing in the vertical direction.

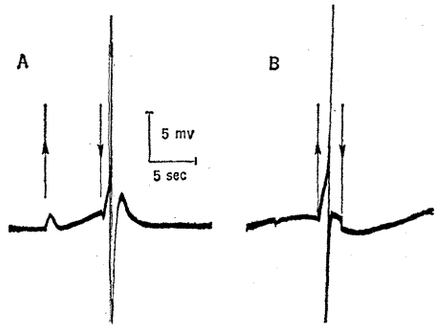

Fig. 2. Typical response of a silent cell (cell R2) to 488-nm light (A) in normal seawater (12.5 mw of beam power), and (B) in seawater and 1.37 m*M* ouabain (4.5 mw of beam power). The arrow pointing upward indicates that the light pulse is "on," and the arrow pointing downward indicates that the light pulse is "off." In normal seawater the initial depolarization was followed by an approximate return to rest potential with firing with the light pulse "off." In ouabain solution less than half the light intensity causes firing with the light pulse "on." The full nerve spikes (not shown) were ~ 90 mv peak-to-peak for the various recordings presented.