rats had almond flavor paired with the glucose–digestive juice mixture and the banana flavor with nothing. This pairing was reversed for the other four rats. There was no significant difference between the two groups [$F(1, 7) < 1.0$, not significant] (Fig. 5). There was also no significant interaction of glucose intake with days [$F(8, 56) = 1.65$, $P > .05$]. The mixture of glucose and digestive juice, which was injected intragastrically, seems to have no rewarding effect. Because the taste of glucose is highly palatable and glucose itself is rapidly absorbed, neither of these two factors seems responsible for the rewarding effect of the milk–digestive juice mixture. Further, the glucose mixture would also cause stomach distention; therefore, its lack of effect makes an explanation in terms of stomach distention even more implausible.

Our results show that some rewarding signals are rapidly generated by the upper gastrointestinal tract when nutrients arrive there. Why whole milk, unmixed with stomach digestive juices, had no such effect remains a problem for further research. However, it seems that the upper gastrointestinal tract can, at least under some conditions, recognize some components of food and signal their arrival rapidly to the central nervous system.

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Lens Culinaris Lectin Immobilized on Sepharose: Binding and Sugar-Specific Release of Intact Tissue Culture Cells

Abstract. *Lens culinaris lectin (LCL) covalently linked to 2B Sepharose binds tissue culture cells to the matrix. This is prevented by hapten sugars specific for LCL. Unlike other immobilized lectins, lens culinaris lectin allows the removal of bound cells from the matrix on addition of the specific sugars in a concentration-dependent manner. Binding and release occur under physiological conditions. Released cells continue to grow.*

Lectins have proved to be an excellent tool for discovering cell surface changes that occur during neoplastic transformation (*1*) or during the cell cycle (*2*). Cell separation procedures utilizing these differences in the cell surface architecture would be of great interest. So far, immobilized lectins have been shown to induce specific, receptor-mediated binding of cells to solid supports. However, a hapten sugar–induced replacement of the cells under physiological conditions, even with mechanical aid, has been difficult if not impossible, as shown for concanavalin A (Con A) (*3*) and wheat germ agglutinin (*4*). Probably for the same reason, affinity chromatography with immobilized Con A yields only low amounts of glycoproteins upon elution with the specific sugar. This problem has been overcome to some degree by the use of lens culinaris lectin (LCL) (*5*).

We investigated the ability of immobilized LCL to induce cultured cells to bind to large agarose beads and to release them specifically on treatment with

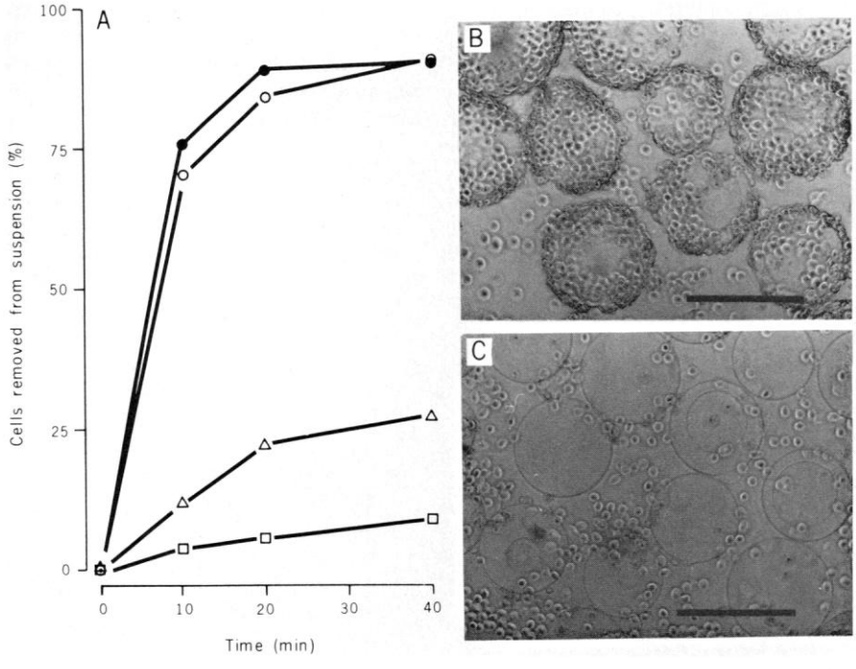

Fig. 1. Binding of HeLa cells to LCL-beads in the presence of different sugars (0.2*M*). (A) Time course of removal of cells from the supernatant in phosphate-buffered saline (•), D-galactose (○), methyl-α-D-glucopyranoside (△), and methyl-α-D-mannopyranoside (□). At the times indicated, two 100-μl portions were removed from the suspension and counted with a Coulter counter, model B. Maximum deviations do not exceed the symbols. (B and C) Phase contrast pictures taken from the D-galactose group (B) and the mannopyranoside group (C) at 20 minutes. Scale bar, 300 μm. HeLa cells were cultivated in Eagle's minimum essential medium supplemented with 10 percent calf serum. Suspensions were prepared from subconfluent cultures 1 to 3 days old. Dishes were washed two to four times with PBS-BSA without divalent cations but containing 2 m*M* EDTA until the cells came off. In the last wash as well as in all following steps a small amount of deoxyribonuclease I was included (about 20 to 30 units per milliliter). Before incubation the cells were washed with PBS-BSA. The cells (3×10^6 per group) were incubated with 0.5 ml of settled LCL-beads in a total volume of 5 ml in the presence of 0.2*M* sugars at room temperature. For the addition of the particular sugars, portions of 0.3*M* sugar solutions (physiological with regard to the osmotic pressure) containing 0.1 percent bovine serum albumin, 1 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, and 0.1 m*M* $MnCl_2$ were mixed with PBS-BSA in ratios necessary to obtain the final concentrations. The LCL-beads were equilibrated with the particular solution for 30 minutes and then mixed with the cells, which had been suspended in the same solution 5 minutes before combining.

hapten sugars in a batch procedure. Lens culinaris lectin is known to bind the sugars methyl-α-D-glucopyranoside and methyl-α-D-mannopyranoside like Con A, but with approximately 50 times lower binding constants (*6*). It is able to differentiate between transformed and nontransformed cells (*7*). According to the data available, LCL seems to bind to the same glycopeptide at the cell membranes as Con A but to different parts of the molecule (*8*).

Lens culinaris lectin was isolated according to a procedure described by Hayman and Crumpton (*5*) and modified by us as described elsewhere (*9*) and was coupled to large agarose beads (> 200 μm, separated by means of a gauze of defined pore size from 2B Sepharose; Pharmacia) which were activated with cyanogen bromide (CNBr) as described by March *et al.* (*10*). Batches with about 2.5 mg of immobilized LCL per milliliter of settled beads were employed. Suspensions were prepared from stationary HeLa cells and SV 3T3 cells by use of ethylenediaminetetraacetic acid (EDTA). Cells and beads were separately washed twice with phosphate-buffered saline containing 0.1 percent bovine serum albumin, 1 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, and 0.1 m*M* $MnCl_2$ (PBS-BSA). For binding studies, cells and beads were combined in a 5-cm plastic petri dish (Nunc or Falcon) in a final volume of 5 ml and incubated at room temperature on a rocking platform (15 rev/min). For details see the legends of Figs. 1 and 2. Unbound cells were separated from bound cells with a gauze of 100-μm pore size (*9*).

Untreated 2B Sepharose and CNBr-activated Sepharose without protein failed to bind cells. However, LCL-Sepharose is covered with cells within 10 to 30 minutes at room temperature or at 37°C. At 4°C it takes about twice as long to bind the same amount of cells. The loading capacity of 0.5 ml of settled beads was determined to be in the range 6×10^6 to 8×10^6 cells. As shown for HeLa cells in Fig. 1, the presence of hapten sugars prevented the binding of cells to LCL-beads to different degrees. In this respect 0.2*M* methyl-α-D-glucopyranoside is less powerful than the same concentration of methyl-α-D-mannopyranoside (Fig. 1A). The latter prevents almost any cell binding, as checked microscopically (Fig. 1, A and C) (some cells are removed from suspension, however, by attachment to the dish). D-Galactose does not interfere with the cell-bead binding (Fig. 1, A and B). These results indicate that the cells are induced by LCL to bind specifically to the agarose beads.

The next question was whether cells could be liberated by specific sugars in a short period of time under physiological conditions to maintain their morphological integrity as well as their viability. For the specific release of cells from LCL-beads, a binding time of about 1 hour was needed to improve the mechanical stability of the cell-bead bond. A rocking speed of about 30 to 40 rev/min during the removal supports the chemical release of the cells by mild mechanical forces. A typical experiment with HeLa cells (Fig. 2A) shows that cells bound to LCL-Sepharose are specifically dislodged by methyl-α-D-glucopyranoside and methyl-α-D-mannopyranoside. It is also seen than the mannopyranoside is more efficient than the glucopyranoside, a result that is in accord with the binding data shown in Fig. 1A. It should be noted that the results are in accord with the lower binding constant of LCL for the glucopyranoside (*6*). From a comparison of Fig. 2A and Fig. 2B, it is also evident, however, that in some experiments a small number of cells are unspecifically removed in the presence of D-galactose. Besides the release of cells by distinct sugars, varying concentrations of the same sugar liberate cells from LCL-beads at different rates, as shown in Fig. 2B. Recovery studies showed that within the limitation of 1 hour of binding followed by 1 hour of release 75 to 90 percent of the cells were regained by the use of physiological solutions. For recycling of LCL-beads the rest of the cells were removed with an unphysiological concentration (0.5*M*) of the mannopyranoside. The loading capacity of LCL-beads that had been recycled up to ten times did not change. Similar data on specific binding and release were obtained with SV 3T3 cells. However, their tendency to aggregate on being brought into motion, even in the absence of immobilized LCL, prevented the use of an electronic particle counter. These experiments were scored microscopically [the data are presented elsewhere (*9*)].

Besides specificity, cell integrity and viability are requirements for a cell fractionation procedure that utilizes chemical properties of cell surfaces. The integrity of the cells was checked by their abil-

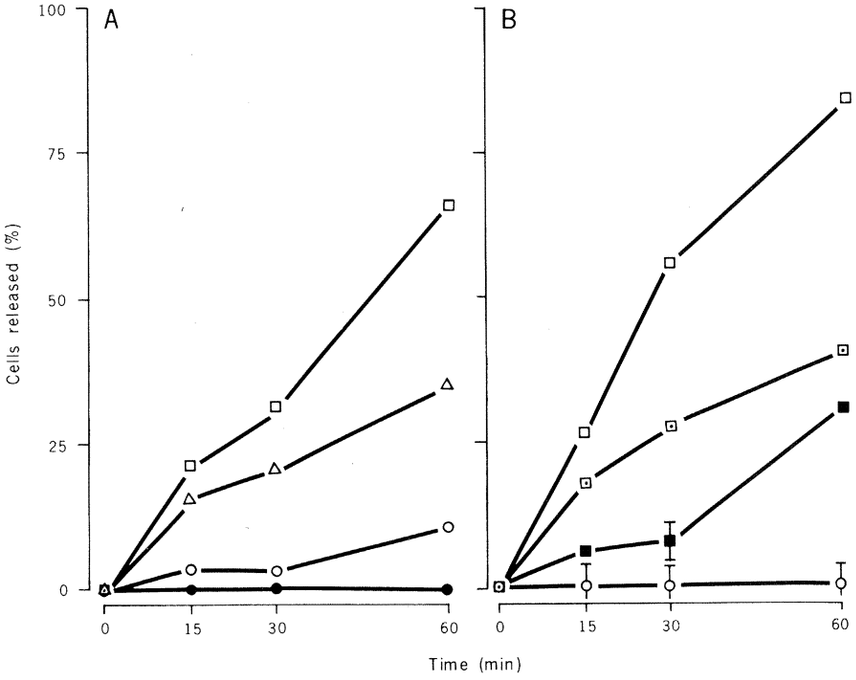

Fig. 2. Release of HeLa cells from LCL-beads in the presence of different sugars (A) or different concentrations of methyl-α-D-mannopyranoside (B). HeLa cells were allowed to bind to 0.5-ml portions of settled LCL-beads in a total volume of 5 ml in the presence of 0.2*M* D-galactose at room temperature for 1 hour. The supernatants were then replaced by the particular sugar solutions (for preparation see Fig. 1). (A) For the symbols of different sugars see Fig. 1. (B) Different concentrations of methyl-α-D-mannopyranoside: 0.2*M* (□), 0.02*M* (⊡), and 0.002*M* (■). The latter groups were substituted with D-galactose to final sugar concentrations of 0.2*M*. The lowest curve represents 0.2*M* D-galactose (○). At the times indicated two 100-μl portions were removed from the suspension and counted. A value of 100 percent equals that portion of cells present on the beads at the start of release [4.2×10^6 cells in (A) and 1.75×10^6 cells in (B)], calculated by subtracting the portion of unbound cells from the total input. Maximum deviations exceeding the symbols are plotted.

ity to exclude trypan blue and to multiply subsequently in the presence of complete culture medium. A binding and release procedure taking a total of 2 hours increased the percentage of trypan blue–positive cells by about 5 percent over control values, whereas control cells kept under the same conditions but without LCL-beads showed an increase of only about 1 percent over the initial value. During the binding it was also observed that, in general, the percentage of trypan blue–positive cells in the unbound fraction increased. Released cells multiplied in complete medium 1.54-fold in 24 hours, whereas control cells increased 1.65-fold. The multiplication rate during the following 2 days was almost identical in both groups. Sterility was controlled by antibiotics.

In summary, the results demonstrate that immobilized LCL induces tissue culture cells to bind to a solid matrix. This is prevented by hapten sugars specific for LCL. The cell-bead bond seems to be strong enough to resist appreciable mechanical removal of cells. The binding occurs sufficiently fast that the release procedure can be commenced before considerable secondary interactions between cell and bead have taken place, as suggested by Edelman *et al.* (*3*). Unlike immobilized Con A and wheat germ agglutinin, LCL allows the sugar-specific release of cells within a period of time short enough to maintain the viability of nearly all cells. It remains to be evaluated whether immobilized LCL permits cell separations on the basis of cell surface differences, such as those carried out with other lectins.

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23 January 1976

Facial Muscle Patterning to Affective Imagery in Depressed and Nondepressed Subjects

Abstract. *When subjects imagine happy, sad, and angry situations, different patterns of facial muscle activity are produced which can be measured by electromyography. These subtle, typically covert, facial expression patterns differentiate depressed from nondepressed subjects. Facial electromyography can provide a sensitive, objective index of normal and clinical mood states.*

It is a common human experience that thoughts or memories can elicit specific emotions or feeling states. The ability to experience various emotions depends in part on the person's mood and it is not unusual, for example, for a person who is depressed to describe having difficulty imagining happy situations and generating a positive feeling state. In the past researchers have relied almost exclusively on self-report as the means of assessing a person's differing moods. We report here the results of an experiment with both nondepressed and depressed subjects illustrating a psychophysiological procedure for indexing subtle emotional states.

The face has long been associated in both lower animals and man with the concept of innate, fundamental emotions (*1*). Recent cross-cultural data (*2*, *3*) document that at least six distinct emotions can be recognized in the human face: happiness, sadness, anger, fear, surprise, and disgust. In light of the unique speed and sensitivity with which the facial musculature responds (*4*) we reasoned that it would be possible to record discrete patterns of low-level muscle activity during the generation of affective imagery, even though the facial adjustments might be too small or rapid to be visually detected by the average observer. Prior research using electromyographic procedures has shown that covert muscle changes often accompany cognitive processes that are associated with different motor movements [for example, when subjects engage in silent reading, small muscle changes can be recorded from the lip region (*5*)]. This phenomenon has not been systematically studied in relation to different emotions elicited by imagery.

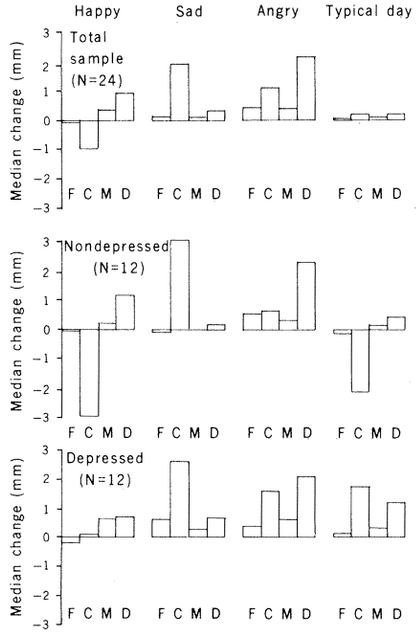

Fig. 1. Median EMG change scores from resting baselines for frontalis (*F*), corrugator (*C*), masseter (*M*), and depressor (*D*) regions during imagery conditions for the total sample and the nondepressed and depressed subsamples. One millimeter equals 45 μv per 30 seconds.

We recorded electromyographic (EMG) activity from selected regions of the face using miniature Beckman Ag-AgCl electrodes placed adjacent to each other in pairs with interelectrode resistance reduced to less than 3000 ohms. Surface electrodes have certain advantages over intramuscular fine wires in that they minimize stress to the subject, introduce little risk of infection, and can be readily placed over the same area for repeated measurement. The limitation of surface electrodes is that the recording is potentially affected by cross talk from adjacent muscles, making inferences about the activity of anatomically specific muscles subject to qualification (*4*). Although for convenience specific muscles are referred to in this report, the data actually reflect activity from muscle areas or regions. Since the method proves to be internally consistent in its