longer responded to the dishabituatory stimulus.

The ability of the foot shock to repeatedly dishabituate gnawing was demonstrated (Fig. 2). In accordance with the classical model of habituation (*12*), foot shock gradually lost its dishabituatory power, that is, "habituation of dishabituation" occurred.

We next wished to determine whether the habituatory decrement demonstrated in the first three experiments would modify the interaction between stimulation-induced readiness to gnaw and physiologically induced hunger. Subjects were totally deprived of food for 6 days. On the seventh and eighth days of deprivation each subject was placed in the test chamber and allowed to eat from a dish of wet mash for 30 seconds. On day 1 of experiment 4 (the ninth day of deprivation) each subject was stimulated for 10 minutes in the test chamber with gnawing blocks available. On day 2 the food dish was also introduced and, after the subject had eaten for 15 seconds, stimulation was administered for 10 minutes. One observer operated microswitches to record time eating and time gnawing; another observer recorded latencies for (i) gnawing after stimulation began, (ii) returning to eat during stimulation, and (iii) returning to eat after stimulation ceased. Day 3 procedures were identical to those of day 1.

Less gnawing occurred when food was present (day 2) than when food was absent (average of days 1 and 3) (correlated $t = 2.79$, $P < .05$). On day 2 five animals (*13*) left the food dish and began to gnaw within 4.5 seconds (mean = 3.3 seconds) after stimulation began (Fig. 3). These animals returned to the mash and resumed eating within 82 seconds (mean = 60.4 seconds) after onset of stimulation. Four subjects never gnawed after they resumed eating. One animal vascillated between gnawing and eating but devoted the last 180 seconds of the stimulation interval exclusively to eating. These results suggest that at the beginning of the stimulation interval the stimulation-induced readiness to gnaw was stronger than the physiologically induced hunger, but as habituation progressed the tendency to gnaw could not compete with the more stable physiologically induced motive.

An unexpected finding was that animals stopped eating upon termination of stimulation. One subject did not resume eating during the 15 minutes he was left in the test chamber after stimulation. The other four subjects resumed eating between 77 and 271 seconds after stimulation. This poststimulation inhibition of eating could be explained by assuming that, along with inducing a readiness to gnaw, the stimulation induced a readiness to eat (stimulation-induced hunger) which summated with physiological hunger. Total hunger might then habituate, as did the readiness to gnaw. In this situation, stopping the stimulation might further reduce hunger to below threshold level.

The results of these four experiments indicate that a stimulation-induced readiness can habituate and that this habituation can alter the interactions between competing motivational tendencies. In light of these results, previous evidence for the plasticity of stimulation-induced behaviors might be reevaluated. If hypothalamic stimulation can simultaneously elicit two behavioral tendencies, the behavior manifested may depend upon which of two types of goal objects are available. When both types are available the manifest behavior may depend upon which readiness is prepotent. If the initially prepotent readiness habituates, another behavior with a flatter habituation gradient may become manifest independent of environmental manipulations. For example, an animal may show preference for eating over drinking when first stimulated. But a change in preference after long periods of stimulation may not depend upon a conditioned preference for either food or water but may result from a differential habituation of stimulation-induced hunger and stimulation-induced thirst. We do not question the functional plasticity of neural mechanisms underlying hypothalamically controlled behavior, but wish only to emphasize that there are several processes which may produce alteration of these mechanisms, for example, habituation, sensitization, classical conditioning, and instrumental conditioning. The relative importance of these processes in modifying the behavioral effects of intracranial stimulation is still to be determined by carefully controlled investigations.

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References and Notes

1. S. Larsson, *Acta Physiol. Scand.* **32** (Suppl. 115), 8 (1954); N. E. Miller, *Fed. Proc.* **19**, 846 (1960); B. Andersson and S. M. McCann, *Acta Physiol. Scand.* **35**, 191 (1955); W. Roberts and R. Carey, *J. Comp. Physiol. Psychol.* **59**, 317 (1965); W. W. Roberts and H. O. Kiess, *ibid.* **58**, 187 (1964); E. Vaughan and A. E. Fisher, *Science* **137**, 758 (1962).
2. W. Roberts and R. Carey, *J. Comp. Physiol. Psychol.* **59**, 317 (1965).
3. E. S. Valenstein, V. C. Cox, J. W. Kakolewski, *Science* **159**, 1119 (1968).
4. ———, in *Reinforcement and Behavior*, J. T. Tapp, Ed. (Academic Press, New York, 1969), p. 242.
5. R. A. Wise, *Science* **162**, 377 (1968).
6. "Habituation" is used here to refer to those temporary response decrements induced by continuous or repeated stimulation independent of response-induced decrements (that is, fatigue).
7. J. D. Harris, *Psychol. Bull.* **40**, 385 (1943).
8. S. E. Glickman and S. M. Feldman, *Electroencephalogr. Clin. Neurophysiol.* **13**, 703 (1961); H. Ursin, K. Wester, R. Ursin, *ibid.* **23**, 41 (1967).
9. All points used in this study were within 1 mm of a point A15.5, V0.0, L2.8 in the atlas by R. H. Carlson and J. N. Kott [*Acta Anat.* **77**, 321 (1970)].
10. In order to minimize polarization of electrodes, all stimulation was carried out with a wave form devised by J. C. Lilly, G. M. Austin, W. W. Chambers [*J. Neurophysiol.* **15**, 319 (1952)].
11. P. M. Groves and R. F. Thompson, *Psychol. Rev.* **77**, 419 (1970); I. Kupfermann, V. Castellucci, H. Pinsker, E. Kandel, *Science* **167**, 1743 (1970).
12. R. F. Thompson and W. A. Spencer, *Psychol. Rev.* **73**, 16 (1966).
13. In the wild, prairie dogs spend considerable time firming up the walls of their burrows and the mounds at the opening of their burrows by a species-characteristic response called nose tapping. Nose tapping can sometimes be produced by electrical stimulation if wet dirt is available to the animal. In experiment 4, one of the subjects stopped eating and began nose tapping rather than gnawing when stimulation was administered. The data from this animal are omitted from the results summarized in Fig. 3.
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Agglutinin Interaction with Embryonic and Adult Cell Surfaces

Concerning the agglutinability of certain chick embryo cells by concanavalin A (Con A) and wheat germ agglutinin (WGA), Moscona (*1*) states that the previous experiments of Burger and Pollack and Burger (*2*) and Inbar and Sachs (*3*) were carried out with "adult cells from established cell culture lines." This statement is not completely consistent with the experimental facts. A substantial portion of these studies was carried out with the 3T3 line of cells, which was derived from a Swiss mouse embryonic culture (*4*), and with its virus-transformed variants. Thus, the failure of Con A or WGA

to agglutinate untransformed 3T3 cells is not a consequence of an adult origin, but rather it is the result of some acquired membrane property in the transition of these mouse embryo cells from a strain to a line.

I have found that cells, in secondary or subsequent passages, from either Swiss mouse embryo cultures or from Sprague-Dawley rat embryo cultures are sensitive to agglutination by Con A at 5 μg/ml and by WGA (*5*) at 200 μg/ml after they are harvested with 0.02 percent disodium ethylenediaminetetraacetate in phosphate-buffered saline. Under these conditions, SV40-transformed 3T3 cells are agglutinated moderately and 3T3 cells not at all. A similar examination of adult mouse spleen cells revealed that they, too, were agglutinated by Con A at 20 μg/ml and by WGA at 80 μg/ml. Aub (*6*) had observed a similar phenomenon with a cruder WGA preparation. In the absence of the agglutinins, no cellular aggregation occurs with cells from any of the sources, except with SV40-transformed 3T3 cells, which aggregate slightly. Unlike the chick embryo cells described by Moscona (*1*), the mouse embryo and adult spleen cells were agglutinated by both Con A and WGA without trypsin treatment. Moreover, adult spleen cells isolated in the presence of soybean trypsin inhibitor (1.0 mg/ml) or tosyllysinechloromethyl ketone (0.5 mg/ml) were just as agglutinable as cells isolated in the absence of these protease inhibitors. These experimental conditions reduce, but do not eliminate, the possibility that a cell surface protein was removed by enzymatic activity during the isolation procedure. The results of the rodent embryo cell experiments are in contrast to those of Inbar and Sachs (*3*), who reported that secondary cultures of mouse, rat, and hamster embryo cells, in their hands, were not agglutinable by Con A at 500 μg/ml.

Thus, it appears that in the development of the 3T3 line, a population of cells was obtained that has a cell membrane property unlike the original embryo cells from which they were derived. Whether this membrane component that masks the agglutinin binding sites of the original embryo cells is formed as a result of a mutational event, the selection of a cell type with this particular property, or as a consequence of the aneuploidy of the line is not evident (*7*). The 3T3 cell line, whose study forms the basis for a substantial portion of the developing litany dealing with agglutinin receptors on cell surfaces, cannot be considered either adult or normal. Therefore, considerable caution should be exercised in the interpretation of the agglutinin studies when the neoplastic properties of cells and the relation of these properties to embryonic differentiation is investigated with established heteroploid cell lines as "normal" unagglutinable reference standards.

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References

1. A. A. Moscona, *Science* **171**, 905 (1971).
2. M. M. Burger, *Proc. Nat. Acad. Sci. U.S.* **62**, 994 (1969); R. E. Pollack and M. M. Burger, *ibid.* **62**, 1074 (1969).
3. M. Inbar and L. Sachs, *ibid.* **63**, 1418 (1969).
4. G. Todaro and H. Green, *J. Cell Biol.* **17**, 299 (1963).
5. Fraction 3 of the wheat germ agglutinin preparation as described by M. M. Burger and A. R. Goldberg, *Proc. Nat. Acad. Sci. U.S.* **57**, 359 (1967).
6. J. C. Aub, B. H. Sanford, M. N. Cote, *ibid.* **54**, 396 (1965).
7. R. Pollack, S. Wolman, A. Vogel, *Nature* **228**, 938 (1970).

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Sivak's comments mention various unpublished findings which lead him to disagree with work from the laboratories of Sachs and Burger, and to express annoyance at the "developing litany dealing with agglutinin receptors," and so forth. One must await the detailed publication of Sivak's work, with full descriptions of techniques, measurements, and evaluation methods, to see how his actual data measure up to his present summary statements and polemic criticisms.

Whether or not 3T3 cells are "adult," is largely a semantic side-issue which takes nothing away from my work, nor does it add to it, and it should not divert attention from the real problems and facts. The notion that normal postembryonic and adult tissue cells are generally less readily agglutinated by Con A (unless trypsinized) than embryonic cells does not depend only on 3T3 cells; it would be rash to reject its wider implications at this time, simply because of Sivak's futile concern with the "adulthood" of 3T3 cells. These 3T3 cells originated years ago in a near-term mouse fetus (not in an embryo) and their tissue origin and state of differentiation, when isolated, was not determined. However, it is a fact that, unlike embryonic tissue cells, 3T3 cells are not agglutinated by Con A, unless trypsinized; they may have been postembryonic to begin with, or may have since "matured," or somehow acquired "adult-like" surface features.

Be that as it may, this matter is quite beside the essential aspects of my findings concerning agglutination of embryonic cells with Con A; Sivak's mentioned results contain nothing contradictory to my own data, regardless of the foreboding style of his comments. As I have reported, early embryonic cells freshly isolated with EDTA from chick embryo neural retina are readily agglutinated by Con A. I suggested as a working hypothesis that, with development and differentiation, Con A receptors on these cells become masked with a trypsin-sensitive material, so that in postembryonic and adult stages these cells are no longer agglutinated by Con A, unless the cells are trypsinized. This suggestion has now been substantiated, and the data will be reported.

Such developmental changes in cell surfaces are, in my opinion, of interest with respect to growth and differentiation and also in considerations of neoplastic transformation. Naturally, the generality of these findings remains to be explored, and the purpose of my report in *Science* was to arouse interest in such exploration. It is known, of course, that certain kinds of normal cells from adult organisms are agglutinated by Con A, whereas certain tumor cells are not; the study of such exceptions may yield important clues to the significance of lectin receptors in cell surfaces, and Sivak's work may do this. Similarly, a detailed survey of various embryonic tissue cells (from different species, tissues, stages of differentiation, and after cultivation in vitro) may also reveal differences in reactions with lectins, and these too may be potentially useful for the exploration of embryonic cell surfaces.

The study of cell-lectin interactions is a relatively young, difficult, but potentially important area of cell biology in which no one should at present pretend to a monopoly of knowledge. It requires more information and analytical data; it needs testable working hypotheses and a constructive diversity of approaches, not semantic quibbling in letters to editors; and it can benefit from cooperation among those working in it. Sivak's contentions might well be reexamined in this light.

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