

amounts of leukotactic factor will not be detected, even though the cells presumably differ by strong isoantigens. If, on the other hand, differences in isoantigenicity are absent or minor, little or no leukotactic activity will be elaborated, despite the fact that both components of the cell mixture are competent. However, to produce the factor, the components of cultivated cell mixtures need not both be immunologically competent. Mixtures composed of node cells and cells carrying foreign transplantation antigens will be just as effective. The results would also indicate that competent cells recognize isoantigenic differences within a surprisingly short period of time. Since the intensity of leukocytic infiltration reflects degrees of immunogenetic differences, this should provide a means for quantitative measurement of histocompatibility.

Although final judgment on the nature of the cellular interaction leading to the production of leukotactic factor would be premature, the available evidence indicates that it is an immunologically specific process. While the results offer a possible explanation of the nature of skin reactions in lethally irradiated hamsters after inoculation of genetically dissimilar cells, their more general implications for homograft reaction and cellular hypersensitivity remain to be elucidated.

H. RAMSEIER

Department of Medical Microbiology,
University of Zürich,
8006 Zürich, Switzerland

References and Notes

1. R. E. Billingham and W. K. Silvers, *Annu. Rev. Microbiol.* **17**, 531 (1963); J. L. Gowans and D. D. McGregor, *Progr. Allergy* **9**, 1 (1965); L. Brent and P. B. Medawar, *Brit. Med. Bull.* **23**, 55 (1967).
2. G. Möller and E. Möller, *Nature* **208**, 260 (1965); G. Möller, V. Beckman, G. Lundgren, *ibid.* **212**, 1203 (1966).
3. H. Ramseier and J. W. Streilein, *Lancet* **1965-I**, 622 (1965).
4. H. Ramseier and R. E. Billingham, *J. Exp. Med.* **123**, 629 (1966).
5. Four identical skin sites, each measuring 1 cm in diameter, were cut into small fragments and trypsinized in 10 ml of 0.25 percent trypsin (Difco) at room temperature. Samples were removed after 10, 15, and 20 minutes with leukocyte pipettes and 2 percent acetic acid as a diluent. Samples were counted in a hemocytometer by phase-contrast microscopy. The mean of three counts was used to estimate the number of PMN cells present per skin reaction.
6. R. E. Billingham *et al.* *J. Nat. Cancer Inst.* **28**, 365 (1962); R. E. Billingham and W. K. Silvers, in *The Thymus*, V. Defendi and D. Metcalf, Eds. (Wistar Institute Press, Philadelphia, 1964), p. 41.
7. R. E. Billingham, G. H. Sawchuck, W. K. Silvers, *Proc. Nat. Acad. Sci. U.S.A.* **46**, 1079 (1960).
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Subcellular Structure of Endosperm Protein in High-Lysine and Normal Corn

Abstract. *Optical microscopy shows that the protein network in endosperm cells of normal corn is composed of an amorphous matrix in which granules averaging about 2 microns in diameter are embedded. That these granules are rich in zein is demonstrated by their solubility in 80 percent ethanol. High-lysine corn, with submicroscopic granules clearly resolved only in the electron microscope, has a much lower content of zein than normal corn. The small size of subcellular protein granules in high-lysine maize as compared with normal corn correlates with the reported difference in zein content of the two types of corn.*

A marked change in the composition of endosperm protein of maize was first revealed by Mertz *et al.* (1). They discovered that the protein in a strain of flourey maize, homozygous for the mutant gene *opaque-2* (o_2), contained about twice as much lysine as normal maize and only one-third as much zein (1).

To establish a structural basis for this change in protein composition, we compared the subcellular structure of the endosperm of high-lysine mutants with that of normal corn. Microscopically, cells of the maize endosperm show starch granules embedded in a protein network. If the starch is digested with α -amylase, the protein network can be exposed for microscopic examination. In normal corn, this network consists of a matrix protein in which globular protein granules, a little less than $2\ \mu$ in diameter (average), are embedded (Fig. 1). These granules may appear as strings of beads.

Unlike normal corn, endosperm cells

of the high-lysine mutant o_2 gave little evidence of protein granules when examined with a light microscope. However, the electron microscope showed that granules were actually present in the matrix protein (Fig. 2); their diameter is approximately 1/20 that found in normal corn.

The proteinaceous character of the network was demonstrated by digestion with the protease, pronase; both components, the granules and matrix, were solubilized. A positive staining reaction indicative of protein was obtained in both components on treatment with ninhydrin-Schiff or chloramine-T Schiff reagent (2). Treatment with 80 percent ethanol dissolved the granules and left the matrix protein intact. In view of the fact that zein is defined as the alcohol-soluble fraction of corn endosperm, the effect from treatment with ethanol provided direct evidence that the granules were the primary site of zein deposition in corn. Duvick (3) had previously suggested

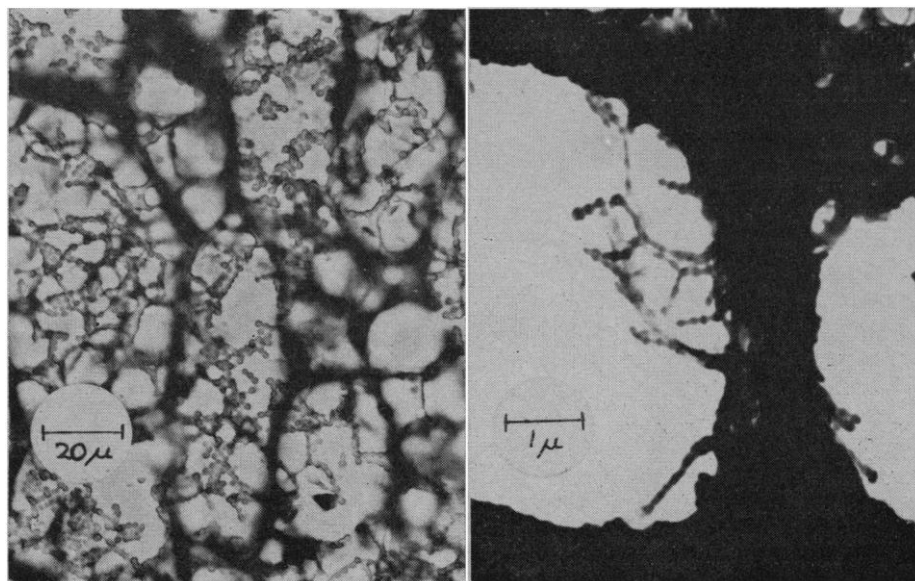


Fig. 1 (left). Destarched section of normal maize endosperm, showing the spherical protein granules (about $2\ \mu$ in diameter) embedded in a protein network. The dark lines are cell walls. Fig. 2 (right). Destarched *opaque-2* maize endosperm, showing spherical protein granules in beadlike arrangement (average diameter $0.1\ \mu$).

that these granules were the major site of zein storage. *Floury-2* mutant, also known to be low in zein and high in lysine (4), presented a subcellular pattern of storage protein deposition similar to that of *o₂*; that is, mainly amorphous matrix protein was present. However, no zein granules were detectable in the electron microscope.

A change in size of the protein granules represents a corresponding change in zein content, and the protein granules are largely the site of zein storage in maize endosperm. Our studies provide a visual basis for the observations of Mertz *et al.* (1) that changes in protein composition of opaque and floury maize mutants as compared with normal corn are accompanied by a reduction in the amount of zein deposited. Our work also suggests that, while the mecha-

nism for deposition of subcellular bodies in which zein is stored is greatly impaired in high-lysine mutants, deposition of nongranular storage protein high in lysine content is simultaneously increased.

M. J. WOLF, U. KHOO
H. L. SECKINGER

Northern Regional Research
Laboratory, Agricultural Research
Service, U.S. Department of
Agriculture, Peoria, Illinois 61604

References and Note

1. E. T. Mertz, L. S. Bates, O. E. Nelson, *Science* **145**, 279 (1964).
2. M. S. Burstone, *J. Histochem. Cytochem.* **3**, 32 (1955).
3. D. N. DuVick, *Cereal Chem.* **38**, 374 (1961).
4. O. E. Nelson, E. T. Mertz, L. S. Bates, *Science* **150**, 1469 (1965).
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positions of the two pools "Zone 2" of Ricketts and Calvin (5).

In 1964, 141 urchins were marked and placed in the experimental pool; 72 with test diameters between 3 and 7 cm came from the pool itself, 7 large animals with test diameters between 7 and 9 cm were collected at about 0 tide from a boulder field 45 m north-east of the experimental pool, and 62 (1.2 to 7 cm) came from the control pool. After they were marked, the animals were measured to the nearest 0.01 cm with knife-edge vernier calipers. The five possible diameters were measured from the center of an ambulacrum to the opposite interambulacrum. Urchins under 2 cm in diameter were measured only three times. Standard errors of measurement (S/\sqrt{N} , where S is the standard deviation and N is number) in all cases were about 0.01 cm and were independent of size. After 1 year, 71 marked individuals were recovered from the experimental pool, and all were measured five times. Standard errors of measurement after 1 year were inversely proportional to size: about 0.02 cm in animals 2 cm wide and 0.01 in animals 4 cm wide and larger. The increase in error in small urchins was apparently due to a slower growth rate of the marked ambulacrum relative to other areas of the test. The change in test diameter is expressed as a function of the original diameter (Fig. 1) with all points derived from the means of the original measurements and the five measurements after 1 year. Not all 71 points are on the graph; those not shown fall within the major cluster. The least-squares regression, however, included all points. The fate of the 70 animals not recovered after 1 year is only partially known. Migration did not appear to be significant. Some early mortality occurred after the marking; but after the 1st month, tag loss and mortality could not be separated.

A striking feature of Fig. 1 is the large number of individuals which decreased in size during the year. That this was a real negative change is evident from the very small standard errors of measurement. Since only hard

Negative Growth and Longevity in the Purple Sea Urchin *Strongylocentrotus purpuratus* (Stimpson)

Abstract. Purple sea urchins were tagged, measured, and placed in a tidepool at Sunset Bay, Oregon. After 1 year, many animals had decreased in size. Accordingly, size is not necessarily a reliable index of age in *Strongylocentrotus purpuratus*; however, it is possible to conclude that these animals are relatively long-lived.

Critical studies of the growth of echinoids under natural conditions have been few. Most have been based on shifts in size distributions over periods of one or more years (1); these shifts can be fairly accurately observed and used for determining the growth of small animals, but they become questionable for large individuals. The development of a suitable marking method for urchins (2) has allowed information concerning growth to be gathered in the field on all but very small individuals.

Since 1962, an intertidal population of the purple sea urchin *Strongylocentrotus purpuratus* (Stimpson) has been studied at the south side of Sunset Bay, Coos County, Oregon, 43°21' north latitude. This side of the bay, formed of tipped beds of sandstone dipping sharply to the east and striking north and south, has been differentially weathered to produce a series of ridges, flat areas, and channels. Growth of urchins in two pools on one of these flat areas was examined. One pool was at approximately 3.5 feet (1.1 m) above 0 tide and had a surface area of 1.2 m², and a maximum depth

of 0.25 m. This was used to hold tagged individuals and will be called the "experimental" pool. The other, the "control," was larger (approximately 5 m²), 7 m south of the experimental pool and intertidally 0.9 m lower; it had a maximum depth of 0.65 m. Size distributions were examined for 3 years in this pool, and shifts in the modal classes of small animals were used to check for gross effects of marking on small urchins. Within the pools, urchins lived in cavities of their own construction (3) and apparently moved about only rarely. The dominant vegetation surrounding the pools was the eel grass *Phyllospadix Scouleri* Hook. Maximum tidal excursion at this region of the Oregon coast is roughly 9.5 feet (2.9 m) in June (4) which then makes the relative intertidal

Table 1. Analysis of size distribution of urchins in the control pool. Means and standard deviations were determined graphically (9). S.D., standard deviation; N, number of urchins.

Year	Mode I (cm)	S.D.	N	Mode II (cm)	S.D.	N
1964	1.65	.48	200	5.13	.59	111
1965	2.44	.69	145	5.70	.52	93
1966	3.50	.57	72	5.37	.57	34