than those of the Mg-containing pyroxenes but smaller than those in pyroxmangite, which are a mixture of Mn, Fe, Ca, and Mg.

A *Neunerketten* silicate chain in ferrosilite III suggests the possibility of larger repeat units as octahedral cation size decreases. Pigeonites and even enstatite are likely candidates. Recently Perrotta and Stephenson (*13*) reported the existence of a triclinic enstatite phase (termed "high clinoenstatite") which is apparently stable in the temperature range previously attributed to protoenstatite. This phase, whose stability field is enlarged by the presence of some Ca, may possibly contain pyroxenoid-type silicate chains with repeat units larger than nine. If this is true, the correct unit cell will be very difficult to determine by powder diffraction techniques because ϵ, the angle between **c** and $\mathbf{c}_m$, decreases (to about 4° to 5° for a 15-unit chain) as the repeat unit increases.

As chain offsets become less frequent they may occur less regularly, leading to the concept of a "disordered" chain. Perhaps, in addition to irregular stacking of octahedral and tetrahedral layers, the "disordered enstatites" reported by Brown and Smith (*14*) contain irregular chains. Although ferrosilite III would be classified as a pyroxenoid on the basis of the proposed structure, the existence of phases containing longer chain repeat units, or even the postulated variable repeat units, will render the structural distinction between pyroxenes and pyroxenoids obsolete.

CHARLES W. BURNHAM*
Geophysical Laboratory,
Carnegie Institution of Washington,
Washington, D.C.

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17. I thank D. H. Lindsley for providing single crystals of ferrosilite III, and for many helpful discussions concerning the synthesis and phase relations of the $FeSiO_3$ polymorphs, and C. T. Prewitt for numerous valuable discussions on pyroxenoid crystal chemistry. D. R. Peacor and C. T. Prewitt reviewed the manuscript.
* Present address: Hoffman Laboratory, Harvard University, Cambridge, Mass. 02138.

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Reaggregation of Insect Cells as Studied by a New Method of Tissue and Organ Culture

Abstract. *Cells of the dissociated pupal fat of saturniid moths reaggregated when maintained in vitro in a new type of chamber that permits the culture to "respire" through a film of polyethylene. The fat-body cells are mutually cohesive, but depend on ameboid hemocytes to bring them together. The hemocytes are also capable of causing the reaggregation of particles of diethylaminoethyl-Sephadex.*

In the course of experiments performed for other purposes, we have discovered an improved technique for culture of insect tissues and organs in vitro. The same experiments revealed a phenomenon well known to students of vertebrate embryology but, as far as we are aware, not previously demonstrated in the postembryonic development of organisms higher than sponges—namely, the reaggregation in vitro of dissociated cells to form a discrete tissue. The experiments in question were carried out as follows.

Through a scalpel incision in the facial region, blood was expressed from a pupa of the silkworms *Antheraea polyphemus* or *Hyalophora cecropia* on about the 5th day of adult development. The expressed blood, as is always the case during early adult development, contained great numbers of individual cells of the fat body, which, at this particular phase of the life history, are subject to dissociation by the slightest mechanical disturbance.

Immediately after collecting the blood, we added to it crystals of streptomycin sulfate and phenylthiourea, a potent inhibitor of tyrosinase. Drops of blood were transferred into the depression of a culture chamber formed by cementing a Lucite plastic washer (19 mm outside diameter, 13 mm inside diameter, 1.0 mm thick) onto a thin (0.05 mm) disc of polyethylene. The blood-filled depression was then capped by placing a glass microscope slide across the washer and sealing it in place with melted wax. The result was a bubble-free, optically flat preparation. Polyethylene, rather than a glass cover slip, was used because of its paradoxical permeability to oxygen and carbon dioxide and impermeability to water or water vapor (*1*). It occurred to us that the polyethylene film might constitute a lung-like surface and permit the culture to "breathe" by the diffusion of respiratory gases.

In cultures of this type we were amazed to witness the rapid reassembly of the individual fat-body cells into large aggregates. Within an hour or two, the process was conspicuous; in less than a day, virtually all of the cells had reaggregated into a number of large masses (Figs. 1 and 2).

No reaggregation took place when the polyethylene film was covered or replaced by a glass cover slip which was sealed in place with melted wax. The importance of the availability of oxygen was further demonstrated by placing freshly prepared "polyethylene cultures" in an atmosphere of carbon dioxide or nitrogen. Reaggregation was blocked, but was promptly restored when the cultures were returned to air. Reaggregation was also prevented when a crystal of 2,4-dinitrophenol was added to the culture, thereby indicating the importance of the contemporaneous formation of adenosine triphosphate.

The reaggregation of the fat-body cells was reminiscent of the self-reconstruction of dissociated tissues prepared from vertebrate embryos (*2*). The important difference in this case was that the fat-body cells show no intrinsic motility or locomotion. Under phase-contrast optics one could see that the fat-body cells were being drawn together and reassembled into aggregates by a totally different cell, the ameboid "plasmatocyte" (*3*), a type of hemocyte present in the blood in great numbers. This conclusion was confirmed by time-lapse cinematography in which the plasmatocytes were seen to crawl around, frequently adopting a spindle shape with their processes adhering to two or more fat-body cells or groups of cells which were then dragged together

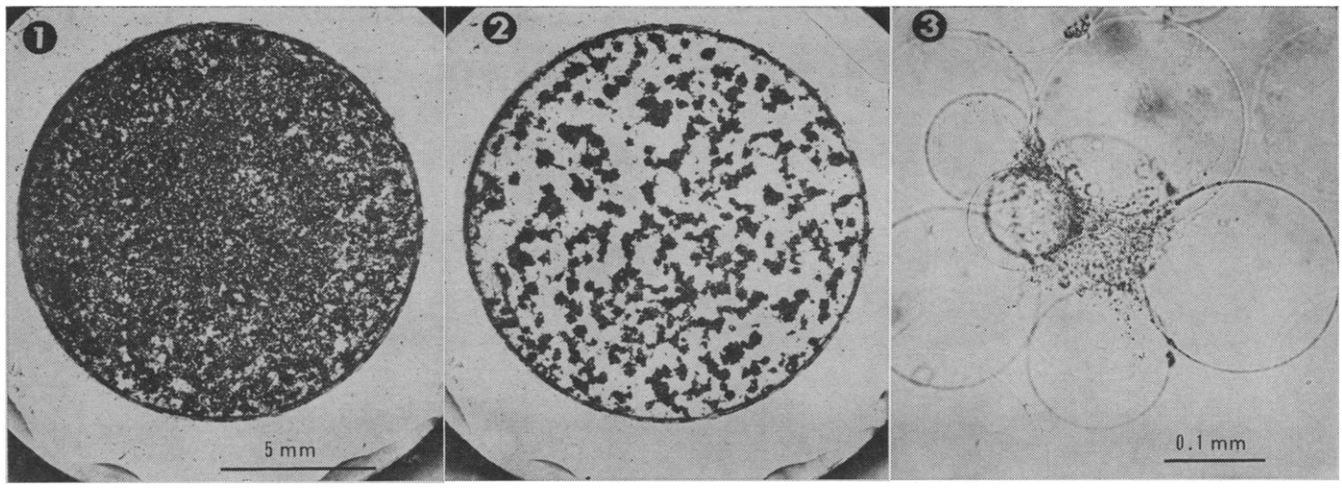

Fig. 1. A freshly made culture of blood from a developing pupa of *A. polyphemus* containing great numbers of dissociated fat-body cells. Fig. 2. The same culture as that shown in Fig. 1 after 18 hours. The fat-body cells have reaggregated into large, compact masses. Fig. 3. A cluster of beads of DEAE Sephadex held together by a small clump of hemocytes which have begun to spread themselves over the surface of the beads. Photographed through a compound microscope, with bright-field optics.

by the active contraction of the plasmatocytes. By histological sections it was possible to show that the contracted plasmatocytes were incorporated into the reassembled cell masses.

The role of the hemocytes in the reaggregation of the nonmotile dissociated cells was further demonstrated by subjecting samples of fresh blood to vigorous agitation in a Vortex mixer—a procedure which fragmented nearly all the hemocytes while doing little damage to the fat-body cells. After this treatment, the fat-body cells failed to reaggregate unless they were first collected by gentle centrifugation and resuspended in fresh blood containing intact hemocytes.

Cellular reaggregation was completely blocked by the addition of ethylenediaminetetraacetate (EDTA) to chelate the divalent cations. This treatment resulted in the prompt immobilization of the plasmatocytes, but was fully reversible when the cells were collected by centrifugation and resuspended in fresh cell-free plasma.

With the exception of the divalent cations, the precise ionic composition of the blood seemed to be of little importance. The blood medium can be diluted with as much as three volumes of Ringer solution (*4*) without affecting normal reaggregation. Moreover, reaggregation was not disturbed when the hemocytes and fat-body cells were collected by gentle centrifugation and resuspended in cell-free plasma derived from diapausing pupae of the same or even of different species.

We studied the mutual affinities between the plasmatocytes and fat-body cells by a simplification of the cellular system in vitro. To this end, diapausing pupae were subjected to an integumentary injury to obtain blood replete with hemocytes but devoid of fat-body cells (*5*). This blood was then used to prepare cultures in which the fat-body cells were replaced by inert beads of Sephadex which are about the same size.

The result was strikingly dependent on the charge carried by the Sephadex particles. The plasmatocytes showed no affinity whatever for the uncharged beads of G-Sephadex and only scant affinity for the negatively charged beads of CM- or SE-Sephadex. By contrast, they readily adhered to the positively charged beads of diethylaminoethyl (DEAE)-Sephadex and promptly drew them together into clusters as in the case of the fat-body cells (Fig. 3). Here again, the process was completely blocked when the plasmatocytes were immobilized by the addition of dinitrophenol or EDTA.

We carried out further studies on the mutual affinities between the cells and particles by utilizing Moscona's (*6*) technique of "rotation-mediated aggregation." For this purpose, fat-body cells or Sephadex beads were suspended in blood and placed in 10-ml erlenmeyer flasks on a gyratory shaker. The continuous, gentle, random collisions between the suspended particles substituted for the motility which the plasmatocytes supply to static cultures.

In experiments of this type, the clumping of DEAE-Sephadex particles continued to depend on the presence of intact, active hemocytes; when the latter were fragmented or immobilized by the addition of dinitrophenol or EDTA, no clumping took place. Microscopic examination of the clumps revealed that the individual particles were covered with, and held together by, hemocytes (Fig. 3).

The mutual adhesion of fat-body cells in gyrating suspensions continued to require the presence of divalent cations but did not require the presence of hemocytes. Large clumps readily formed even in flasks where the hemocytes had been disrupted mechanically or poisoned by dinitrophenol. These findings show that the dissociated fat-body cells possess an intrinsic adhesiveness which the particles of DEAE-Sephadex lack.

DAVID R. WALTERS
CARROLL M. WILLIAMS
Biological Laboratories, Harvard University, Cambridge, Massachusetts

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