

succinic anhydride of the free ϵ -amino groups of immunogenic hapten-PLL conjugates destroys their antigenicity for all guinea pigs (2). These observations suggest that this trait involves an essential, lysine-specific, single metabolic operation upon the antigen in the pathway to form the inducer.

BERNARD B. LEVINE
BARUJ BENACERRAF

New York University School of
Medicine, New York

References and Notes

1. F. S. Kantor, A. Ojeda, B. Benacerraf, *J. Exptl. Med.* **117**, 55 (1963).
 2. B. B. Levine, *Proc. Soc. Exptl. Biol. Med.* **116**, 1127 (1964).
 3. ———, A. Ojeda, B. Benacerraf, *Nature* **200**, 544 (1963).
 4. ———, *J. Exptl. Med.* **118**, 953 (1963).
 5. Subscripts refer to average numbers of hapten and lysine residues per molecule of conjugate.
 6. B. B. Levine and B. Benacerraf, *J. Exptl. Med.* **120**, 965 (1964).
 7. We thank Miss V. Levytska for assistance. Supported by the Health Research Council of the City of New York under contracts I-138, I-240, and U-1297, and by PHS grant E-2094.
- 6 November 1964

Blood of a Cockroach: Unusual Cellular Behavior

Abstract. *In blood smears of a cockroach a small cell occurs which is frequently paired with a larger anucleate cytoplasmic body. The larger body in such pairs is free of nucleic acid, but contains polysaccharides, as demonstrated by the periodic acid-Schiff reaction. At one extreme of a series of these associations, the cell is completely distinct from the cytoplasmic body. At the other extreme the nucleus of the cell is within and apparently a part of the larger body. A graded series of pairs suggests a mechanism resembling phagocytosis in which the cytoplasmic body either gains or regains cell status by retaining the nucleus of the ingested member.*

Cells in the hemolymph of the adult cockroach *Gromphadorhina portentosa* Schaum, from Madagascar, seem to fit classifications established from hematologic studies of other insects (1). However, an unusual feature of this hemolymph is that it contains numerous, membrane-limited, anucleate cytoplasmic structures which are relatively large and crescent-shaped. Each structure is intimately associated with a single small cell, but the degree of association varies among the pairs. A comparison of many pairs indicates that the cytoplasmic body is phagocytic, and that the nucleus of the ingested small cell may survive and, per-

haps, function in the "alien" cytoplasm. When the general realm of living things is considered, survival of an anucleate cytoplasm is in itself a rare phenomenon. Of course, limited cytoplasmic endurance following nuclear extrusion is well recognized from determinations of the life span of the mammalian erythrocyte. It is also known that amoebae carry on some vital activities, including food ingestion, for a period up to 15 days after enucleation (2). Energy production persists for considerably longer periods in anucleate fragments of newt eggs (3), sea urchin eggs (4), and *Acetabularia* (5).

More than 300 preparations of

roach blood have been examined. Blood was obtained in all instances from the dorsal surface of the insect's abdomen following inter-tergal puncture. Instant clot formation allowed easy manipulation of the sample with forceps. Standard and histochemical procedures were applied to whole as well as sectioned material. Thin preparations, necessary for optimum resolution by phase-contrast microscopy, were made by compressing a clot between a slide and a No. 0 coverglass. The degree of compression required in no way altered the appearance of cells; this was confirmed by examining blood samples prepared by other techniques, but few such samples were suitable for photography. For example, small clots were examined without the application of a coverglass. Larger clots were dissociated by opening the tips of the forceps while dragging the clot over a short distance of the slide. Such preparations have thick and thin portions, and hemocytes remain positioned at every angle. Hence, the cytological phenomenon reported cannot be considered an artifact induced by compression with the coverglass. That the forces of coagulation itself do not contribute to inter- and intracellular disorganization was confirmed by examining samples of hemolymph withdrawn from insects previously injected with heparin. Cocaine hydrochloride and potassium oxalate were additional anticoagulants applied to check the effect of heparin. It is perhaps noteworthy that whatever direct or indirect effect *Gromphadorhina* hemocytes may have had on clot formation, no cell lysis occurred, whereas lysis does occur in the hemocytes of some other insects when clotting takes place (6).

Therefore, the numerous paired structures consisting of a single small cell and a larger body resembling a cell but lacking a nucleus, and seen in varying degrees of intimate relationship, do not represent nuclear extrusion resulting from either clot formation or handling. Furthermore, nuclear egestion, in contrast to phagocytosis, is discounted on the basis of the evidence presented in Fig. 1. In this figure, two anucleate cytoplasmic bodies (Fig. 1a) appear to have been engaged in ingesting a single cell, an event observed only twice. The binding force of opposed cytoplasmic extensions on the cell in Fig. 1b also suggests ingestion rather than extrusion. Asymmetric cytoplasmic

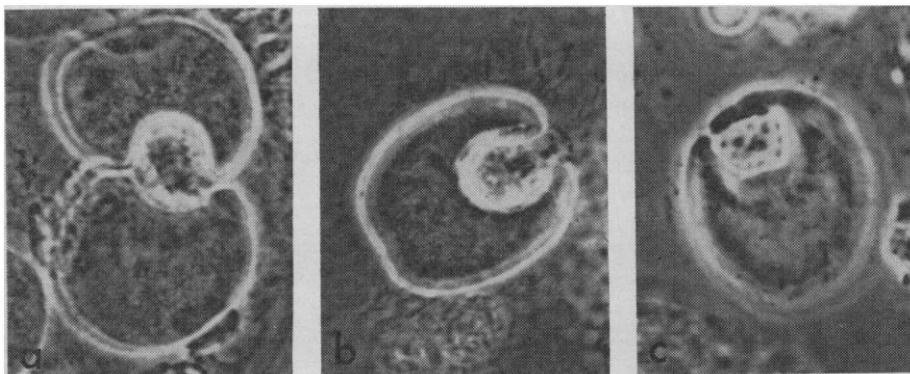


Fig. 1. Evidence suggesting cell ingestion by the crescent body. Phase-contrast, $\times 1000$. a, Two crescent bodies associated with a single cell. b, Opposed cytoplasmic extensions of the crescent body appear to have exerted a binding force on the related cell. c, One extension of the crescent body exceeded the "growth" of the opposing member in the final stage of cell ingestion.

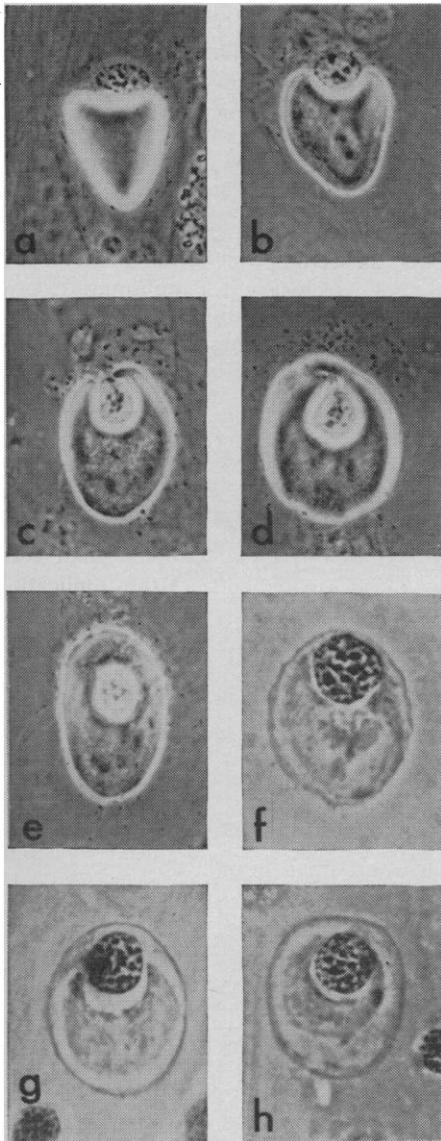


Fig. 2. Cell-crescent body associations representing a graded series in *Gromphadorhina* hemolymph ($\times 760$). *a-e*, Phase-contrast studies of stages representing phagocytosis. *f-h*, Cells, stained with galloycyanin-chromalum, showing progressive decrease of inner crescent substance.

extensions are frequently observed in the final phase of cell ingestion. An example of this is shown in Fig. 1c. Were this an extrusion phenomenon, one might expect to see cytoplasmic swelling and evidence of a ruptured membrane.

In the majority of cases, associated bodies in the hemolymph are juxtaposed in such manner that the anucleate member of the pair (averaging 25μ in diameter and 5μ at the thicker edge) partly surrounds the smaller nucleated member (averaging 8μ in diameter) with broad pseudopod-like extensions. The anucleate cytoplasm

thus has the shape of a crescent (Fig. 2b). In these associations, approximately half of the partially ingested cell, which is round, flat, and scanty in cytoplasmic content, projects beyond the two "pseudopodia" forming the lesser curvature of the crescent body. In other associated pairs either "pseudopod" extensions have not yet formed, or the cell-juxtaposed face of the crescent body is no more than a slight concavity (Fig. 2a). The variety of associated pairs seen suggests an ordered sequence resembling phagocytosis (Fig. 2, *c-e*).

Once incorporated within the cytoplasm of the crescent body, the nucleus of the former cell persists in a somewhat enlarged state. Newly formed cells of this type may be seen to undergo breakdown of the inner crescent form (Fig. 2, *f-h*)—an area of more dense granulation within the crescent body which conforms to the shape of the cytoplasmic membrane without contacting it. Fixed and stained cells are represented in Fig. 2 (*f-h*) because of enhanced contrast afforded in stages which are apparently participating in the reorganization of the inner crescent substance.

When tested by the periodic acid-Schiff (PAS) reaction, the crescent body substance was strongly positive and the intensity of staining was almost uniform. Negative results were obtained with the diastase reaction. Lipid material could not be demonstrated with Sudan Black B. Staining procedures selective for nucleic acid identification (Feulgen, galloycyanin-chromalum, toluidine blue, pyronin Y, acridine orange) gave negative results. Stains were used with various fixatives and with ribonuclease.

What is the fate of newly formed cells? If the degree of PAS sensitivity is indicative of high polysaccharide content, then one might regard this feature as representing an energy store available for the possible fulfillment of subsequent cytological events. But evidence for functional integration between the ingested nucleus and its adopted cytoplasm is lacking, even though cellular disintegration does not seem to occur. Another unsolved question relates to the origin of the phagocytic crescent bodies.

HOPE RITTER, JR.

Department of Biology,
State University of New York at
Buffalo, New York 14214

References and Notes

1. V. B. Wigglesworth, *Ann. Rev. Entomol.* **4**, 1 (1959).
2. J. F. Danielli, *Nature* **170**, 1042 (1952).
3. H. Tiedemann and H. Tiedemann, *Naturwissenschaften* **41**, 535 (1954).
4. H. M. Malkin, *J. Cellular Comp. Physiol.* **32**, 481 (1954).
5. J. Brachet, H. Chantrenne, F. Vanderhaeghe, *Biochim. Biophys. Acta* **18**, 544 (1955).
6. C. Gregoire, *Arch. Biol. Liège* **66**, 103 (1955).
7. Supported by NSF research grant GB-988. I thank L. R. Cleveland, J. F. Danielli, and T. S. Hauschka for their interest and assistance with the manuscript. Cockroaches were kindly supplied by Thomas Eisner. I am grateful for the technical assistance of F. Roe and A. Penny.

14 December 1964

Cross-Correlation Analysis of Electroencephalographic Potentials and Slow Membrane Transients

Abstract. *Cross-correlation analysis reveals a close correlation between the waves in an electroencephalogram and slow membrane transients of single neurons of the sensorimotor cortex of cats during spontaneous activity, augmenting and recruiting responses, and after local application of strychnine. Time-series correlation coefficients up to 0.7 have been computed. It is suggested that the waves of the electroencephalogram reflect an integration of the changes of membrane potentials in both the cell bodies and dendrites of cortical neurons.*

In addition to spike activity, intracellular recordings of the electrical activity of neurons reveal slow changes of the membrane potential which are referred to as postsynaptic potentials. Similarities in the time course of afterpotentials of motoneurons and the periodicity of α -waves in the electroencephalogram (EEG) had led to the assumption (i) that the EEG consists of a summation of postsynaptic potentials (1). Because of the characteristics of nonrefractory and graded responses it was assumed (ii) that the EEG originates in the apical dendrites or might be a summation of dendritic postsynaptic potentials (2). Other theories of the origin of the EEG consider (iii) somadendritic dipoles, (iv) autorhythmicity of neuronal elements, (v) modulation of cortical d-c potentials, and (vi) activity of glial cells to be the important factors (3, 4).

The striking similarities in the time course of EEG potentials and slow membrane transients, particularly de-