in phagocytosis. The C′3 receptor compensates for the inability of γM antibodies to react with monocytes and for the inhibition of the reaction of γG antibodies by free γG (*12*). The γG receptor, on the other hand, compensates for the lower complement-binding capacity of γG antibodies relative to γM antibodies (*13*).

HEINZ HUBER
Department of Medicine, University of California School of Medicine, San Francisco Medical Center, San Francisco

MARGARET J. POLLEY
Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California

WILLIAM D. LINSCOTT
H. HUGH FUDENBERG
Department of Medicine, University of California School of Medicine, San Francisco Medical Center

HANS J. MÜLLER-EBERHARD
Department of Experimental Pathology, Scripps Clinic and Research Foundation

References and Notes

1. H. Huber and H. H. Fudenberg, *Int. Arch. Allergy and Applied Immunol.*, in press.
2. A. F. LoBuglio, R. S. Cotran, J. H. Jandl, *Science* **158**, 1582 (1967).
3. A. Berken and B. Benacerraf, *J. Exp. Med.* **123**, 119 (1966).
4. H. J. Müller-Eberhard, *Advan. Immunology* **8**, 1 (1968).
5. W. E. Bennett and Z. A. Cohn, *J. Exp. Med.* **123**, 145 (1966).
6. The isolated monocytes in a concentration of 10^6 cell/ml were allowed to settle in Leighton tubes containing a cover slip. After 2 hours at 37°C, the cell layer was washed three times with warm Hanks balanced salt solution, which removed contaminating lymphocytes and proteins present in the culture medium used in the earlier steps of the separation (*1*). The final cell layer consisted of at least 95 percent of phagocytic cells, as judged by their ingestion of polystyrene particles or red cells optimally sensitized by γG antibodies. Unsensitized, sensitized, and complement-coated red cells were added to the monocytes in a final concentration of 0.5 percent in warm, balanced salt solution. Unless otherwise stated, this incubation medium was free of serum proteins.
7. H. J. Müller-Eberhard, A. P. Dalmasso, M. A. Calcott, *J. Exp. Med.* **123**, 33 (1966). The nomenclature of complement components is that recommended at the Complement Workshop [*Immunochemistry* **3**, 495 (1966)]. Complexes of erythrocytes (E), antibody (A), and components of complement (C′) are designated according to the reaction step achieved: for example, EAC′1, EAC′1,4, EAC′1,4,2, EAC′1,4,2,3.
8. After the monocytes had reacted with the red cells for 60 minutes, the coverslip with the monocyte layer was removed from the Leighton tubes and washed twice in warm balanced salt solution. To distinguish attachment of the red cells to the monocytes from intracellular localization, hypotonic lysis (0.06*M* NaCl for 45 seconds) was applied to some of the monocyte preparations, which lysed the bound, but not the ingested red cells. The preparations were finally fixed and stained. At least 200 monocytes were evaluated on each slide (*1*). With unsensitized red cells, the range of ingestion was 0 to 5 percent (*1*). Under optimum conditions more than 90 percent of the monocytes contained at least one sensitized red cell. At a lesser degree of sensitization this percentage decreased, reaching an end point comparable to that obtained by hemagglutination caused by specific antiserums (*1*).
9. Red cells sensitized by γG antibody were added to monocytes and incubated at room temperature for 30 minutes. Attachment to most of the monocytes, but almost no ingestion, was demonstrable. Then, γG in a final concentration of 1 mg/ml was added, and the cells were incubated at 37°C for 60 minutes; after this time most of the monocytes contained intracellular red cells. In control tubes, in which γG was added to the monocytes together with red cells, no attachment or ingestion was observed.
10. K. Nishioka and W. D. Linscott, *J. Exp. Med.* **118**, 767 (1963); D. S. Nelson, *Advan. Immunol.* **3**, 131 (1963); J. J. van Loghem and M. van der Hart, *Vox Sang.* **12**, 539 (1962); D. S. Nelson and G. Uhlenbruck, *ibid.*, p. 43.
11. N. R. Cooper and H. J. Müller-Eberhard, *Immunochemistry* **5**, 155 (1968).
12. LoBuglio *et al.* (*2*) showed that inhibition by γG-globulin is diminished at hematocrit values in excess of 75 percent, comparable to those encountered in the splenic red pulp.
13. T. Boros and H. J. Rapp, *Science* **150**, 505 (1965).
14. Supported by PHS grants AI-07007 and H-5997. Dr. Huber is supported by a grant from the Max Kade Foundation, Inc., New York. We thank Mrs. M. Gumbrecht for her assistance.

21 August 1968

Zymogen Granules in Enzyme Liberation and Activation in Pea Seeds

Abstract. *Pea seeds have zymogen-like granules that contain an inactive form of the enzyme amylopectin-1,6-glucosidase. The enzyme can be liberated from the particles in the inactive form and can then be activated by limited proteolysis with trypsin.*

A particulate fraction of cells of pea seeds contains amylopectin-1,6-glucosidase (E.C. 3.2.1.9) which is released into the soluble fraction of the cell during germination (*1*). We now describe the particles and demonstrate that activation of the enzyme does not involve synthesis of new protein.

A particulate fraction was prepared by centrifugation at 30,000*g* for 30 minutes from homogenates (*1*) of seeds soaked for 4 hours. The precipitate was resuspended, layered on a gradient with a concentration of 20 percent Ficoll (Pharmacia) at the top of the tube and 50 percent Ficoll at the bottom and centrifuged for 1 hour at 10,000*g* at 0°C; the fractions at various densities were then recovered. Since the amylopectin-1,6-glucosidase in the particulate fraction can be activated and released by addition of supernatant fractions of the same homogenate (*1*), the activities of the fractions were assayed either immediately on recovery, or after suitable incubation with the supernatant fraction obtained from the initial homogenate of the seeds. The fraction in equilibrium with 20 percent Ficoll contained all the amylopectin-1,6-glucosidase activity (Table 1).

Table 1. Density-graient fractionation (in Ficoll medium) of particles from which the amylopectin-1,6-glucosidase is released. Control is supernatant plus buffer. Incubation is at 26°C.

Ficoll (%)	Activity (IDC unit/ml) after addition of supernatant at	
	0 time	2½ hr
0	0.175	0.182
20	.192	1.740
30	.225	0.215
40+50	.287	.325
Control	.165	.170

To study the appearance of the particulate fraction, we rinsed it with buffer in the presence of lauric acid (*2*) to remove the Ficoll. The particles were then fixed with glutaraldehyde, treated with osmic acid, embedded in Luft's Epon (*3*), and sectioned. The sections were stained with uranyl acetate and examined under the electron microscope, before and after activation (Fig. 1). The intact, purified particulate fraction is composed of well-defined particles with granular content. The particles lose their granular content, and subsequently also their structure, after treatment with trypsin which releases the amylopectin-1,6-glucosidase. It is therefore legitimate to regard the structural changes and the activation as related processes and to refer to these particles as zymogen granules. It was now necessary to establish whether release and activation of the enzyme involved synthesis of new protein.

Seeds were germinated for 72 hours in the presence of ^{35}S-labeled K_2SO_4 (*4*) and homogenized; the supernatant was recovered after centrifugation of the homogenate at 30,000*g*. The supernatant was subjected to electrophoresis on agar gel. Amylopectin-1,6-glucosidase, α-amylase, and β-amylase were located on the gel. Radioactivity in the various sections of the gel was determined in parallel. No radioactivity was

incorporated into amylopectin-1,6-glucosidase, while radioactivity was present in α-amylase and β-amylase (Fig 2). Seeds were also soaked for 4 hours in the presence of the labeled K_2SO_4 and the zymogen granules were isolated. Of the total radioactivity absorbed by the seeds (110×10^6 count/min), only 100 count/min could be recovered from the purified granules. The granules were then exposed to a short activation treatment with trypsin (*1*), and the soluble protein that was released was recovered. This fraction that contained the enzyme activity showed only a trace of radioactivity (28 count/min). Thus the release and activation of the amylopectin-1,6-glucosidase from the particles is not accompanied by protein synthesis or incorporation of ^{35}S. The presence of ^{35}S in the two amylases serves as an internal control, showing that newly formed enzymes do incorporate ^{35}S from radioactive sulphate.

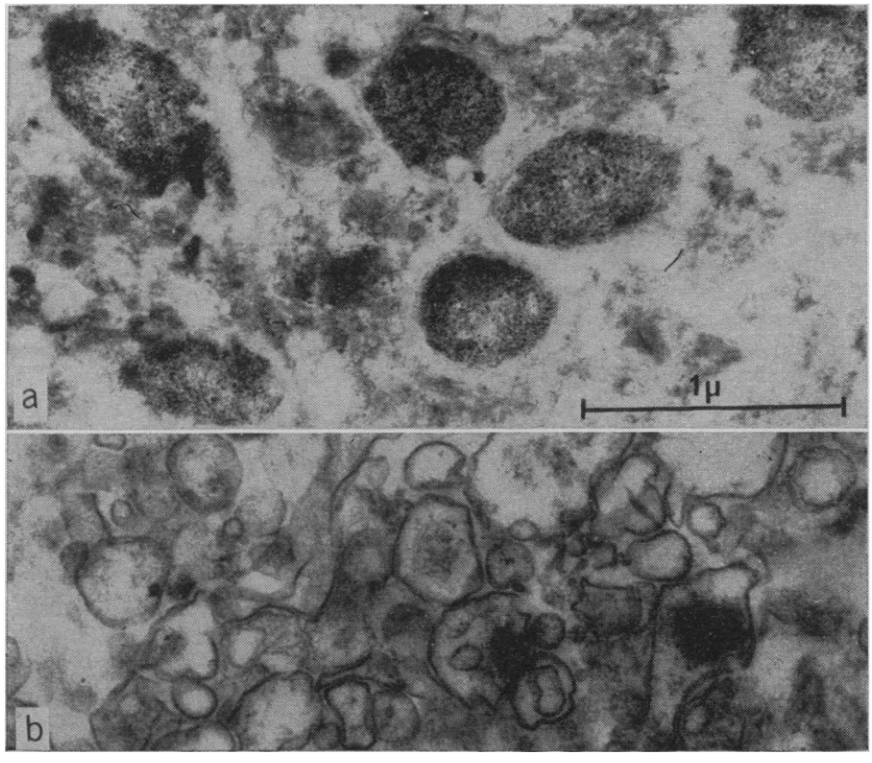

Fig. 1. Electron micrograph of the particles from which amylopectin-1,6-glucosidase is released. (a) Appearance of particles after isolation. (b) The same particles after prolonged treatment with trypsin ($\times$ 46,372).

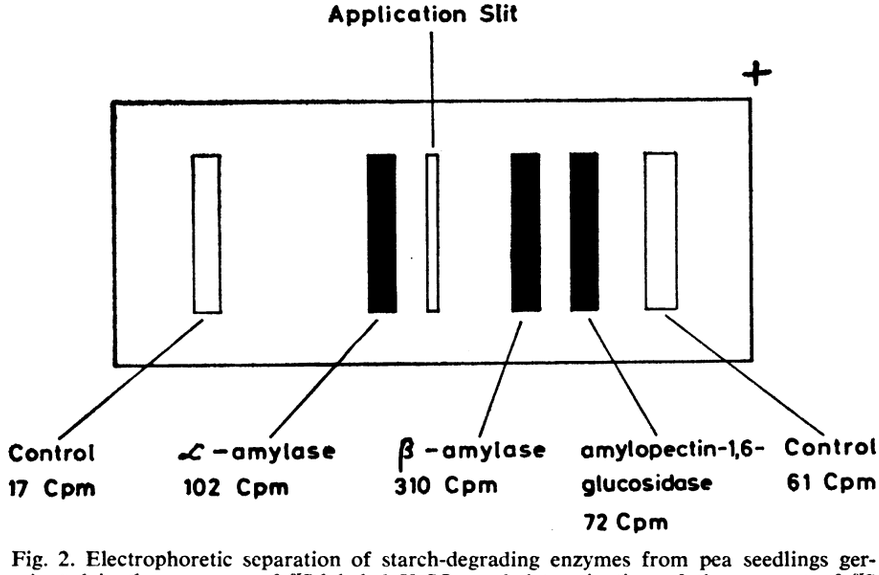

Fig. 2. Electrophoretic separation of starch-degrading enzymes from pea seedlings germinated in the presence of ^{35}S-labeled K_2SO_4 and determination of the amount of ^{35}S incorporated into each enzyme. [After being stained for enzyme activity (*1*), portions of the gel that showed activity were removed and dried in a scintillation bottle. Activity was counted in the scintillation liquid (*5*).]

A particulate fraction was prepared from pea seeds soaked for 4 hours in water. The particulate fraction was disrupted with ultrasound and the suspension was centrifuged at 30,000*g* for 30 minutes. The resultant particulate and supernatant fractions were assayed for amylopectin-1,6-glucosidase activity before and after activation with trypsin. The residual particulate fraction contained 0.135 iodine dextrin color (IDC) unit per milliliter both before and after activation. In contrast, activity in the supernatant fraction rose from 0 to 1.250 IDC unit/ml after trypsin treatment. Thus release and activation are distinct processes. The solubilized, inactive enzyme prepared by sonic disruption was precipitated with ammonium sulphate, and the precipitate was resuspended and dialyzed overnight. Activity was again determined before and after treatment with trypsin. The amylopectin-1,6-glucosidase could be recovered in an inactive form by ammonium sulphate fractionation and subsequently be activated.

We believe that these experiments are the first to demonstrate the presence of zymogen granules in a plant tissue. No information is as yet available on the relation of these granules to cytological structures in the intact cells of the seed. The occurrence of such granules in germinating seeds and the demonstration that enzymes are activated during germination may be important for understanding the mechanism of germination and for determining the ways in which the processes of germination are controlled.

A. M. MAYER
Y. SHAIN

Botany Department, Hebrew University, Jerusalem, Israel

References and Notes

1. Y. Shain and A. M. Mayer, *Physiol. Plant.* **21**, 765 (1968).
2. M. Schramm, R. Ben-Zvi, A. Bdolah, *Biochem. Biophys. Res. Commun.* **18**, 446 (1965); M. Schramm, B. Eisenkraft, E. Barkai, *Biochim. Biophys. Acta.* **135**, 44 (1967).
3. J. H. Luft, *J. Biophys. Biochem. Cytol.* **9**, 409 (1961).
4. Y. Shain and A. M. Mayer, *Phytochemistry* **7**, 1491 (1968).
5. G. A. Bruno and J. E. Christian, *Anal. Chem.* **33**, 1216 (1961).
6. Supported in part by grant FG-Is-239 from the U.S. Department of Agriculture.

20 August 1968; revised 11 October 1968