mately 20 to 30 percent of the nucleated cells were nonviable at 6 to 8 hours, while more than half of the cells were nonviable at 24 hours. At the same time, there was a marked reduction in the number of antibody plaques formed. As an example, after 2 hours of incubation there was a 35 to 65 percent reduction in the number of antibody plaques per total number of viable lymphoid cells compared to control incubation mixtures containing immune cells from NIH mice and Hanks solution only. After 8 hours there was a 50 to 80 percent decrease in plaque count. In contrast, there was no significant decrease in plaque counts per number of viable cells when spleen cells from immunized NIH mice were incubated with nonsensitized, normal C$_3$H cells, or heated, nonviable "killer" C$_3$H cells (Fig. 1 and Table 1). Similarly, there was no demonstrable decrease in plaque formation after incubation of immune NIH cells with normal, isologous NIH cells for varying periods of time from 2 to 24 hours. In several instances there was an indication of increased antibody plaque formation at 24 hours or longer when immune NIH cells were incubated with normal NIH cells instead of Hanks solution.

Suppression of plaque formation was closely related to the relative concentration of immune NIH and "killer" C$_3$H cells in the incubation suspensions. An initial ratio of two C$_3$H "killer" cells for each NIH "target" cell resulted in a 60 to 80 percent reduction in the relative number of plaques formed after 2 hours incubation (Table 1). A lower ratio resulted in less suppression. One sensitized C$_3$H cell for each ten "target" cells resulted in only slight inhibition of plaque formation.

The results indicate that the antibody plaque forming capability of immune lymphoid cells may be inhibited by prior incubation in vitro with lymphoid cells from homograft sensitized mice. Significant inhibition may be detected after only a few hours incubation of the two sets of cells. Such inhibition apparently reveals tissue incompatibility between "target" and "killer" strains. Inhibition does not occur if "killer" cells have been rendered nonviable by heating. Other experiments indicate that serum samples from homograft sensitized "killer" mice are much less effective inhibitors of plaque formation than lymphoid cells (*9*). However, serum specimens from rabbits hyperimmunized either with mouse γ-globulin (*7S* or *19S*) or with mouse spleen cell suspensions are markedly effective in preventing plaque formation when incorporated into agar together with plaque-forming cells and sheep RBC (*9*). These observations and those reported here lend support to the belief that the Jerne technique detects new antibody formation rather than the release of preformed antibody.

The experiments have been limited to spleen cells from "target" mice primed only with red blood cell antigens. Attempts to couple protein antigens, such as bovine serum albumin or egg albumin, to red cells by tannic acid or bisdiazotized benzidine methods have not been successful in our laboratories as far as the antibody plaque technique is concerned. However, preliminary experiments in which red cells have been coated with polysaccharide antigens derived from Gram-negative bacteria (*Shigella* microorganisms) have resulted in plaque formation with spleen, lymph node, and peripheral leukocytes from immunized mice (*9*). This method should be of use in studies concerned with suppression of plaque formation with peripheral leukocytes obtained from mice immunized with bacteria and plated with red cells coated with bacterial antigen.

HERMAN FRIEDMAN
Departments of Microbiology, Albert Einstein Medical Center, and *Temple University School of Medicine, Philadelphia, Pennsylvania*

References and Notes

1. H. S. Lawrence, *Physiol. Rev.* **39**, 811 (1959); F. T. Rappaport, L. Thomas, J. M. Converse, H. S. Lawrence, *Ann. N.Y. Acad. Sci.* **87**, 217 (1960).
2. L. Brent and P. B. Medawar, *Brit. Med. J.* **2**, 269 (1963).
3. F. Celada and R. R. Carter, *J. Immunol.* **89**, 161 (1962).
4. P. Teraski, *Am. Surgeon*, **25**, 896 (1959); R. L. Walford, R. Gallagher, J. R. Sjaarda, *Science* **144**, 868 (1964).
5. F. Bach and K. Hirschhorn, *Science* **143**, 813 (1964); A. C. Rubin, K. H. Stenzel, K. Hirschhorn, K. Bach, *ibid.*, p. 815.
6. N. K. Jerne and A. A. Nordin, *ibid.* **140**, 405 (1963); N. K. Jerne, A. A. Nordin, C. Henry, *Cell-Bound Antibodies* (Wistar Institute Press, Philadelphia, 1963), p. 109.
7. J. S. Ingraham, *Compt. Rend.* **256**, 5005 (1963); J. S. Ingraham and A. Bussard, *J. Exptl. Med.* **119**, 667 (1964).
8. H. Friedman, *J. Immunol.* **89**, 257 (1962).
9. ———, in preparation.
10. I thank Mrs. Leony Mills for technical assistance. Supported in part by grants from the National Science Foundation.

28 May 1964

Physiological Studies on Fruit Development by Means of Ovule Transplantation in vivo

Abstract. *Young fertilized ovules belonging to different species, genera, and families were transplanted onto pepper placenta and grown in vivo to mature, viable seeds. This suggests that the physiological requirements for growth of these ovules may be similar between plant species that are quite different taxonomically.*

The principal factors that control fruit growth are not understood. An extensive search is in progress (*1*, *2*) to determine whether each species or genus may require specific growth factors for the development of its own fruit. We have performed transplantation experiments with young ovules in vivo which suggest that the chemical control of the developing fruits may be similar for some varieties, species, genera, and families.

Explants consisting of young fertilized ovules (hereinafter referred to as ovules) of several types of fruits were inserted in the placenta of growing, attached fruits of pepper (*Capsicum frutescens* L. var. California Wonder). The morphology of the pepper fruit makes it particularly suitable for this technique (*3*).

A small triangular opening, each side measuring 1 cm, was made in the pericarp of the pepper (Fig. 1). Through this opening the operation was performed on the pendant placenta by means of surgical instruments. To

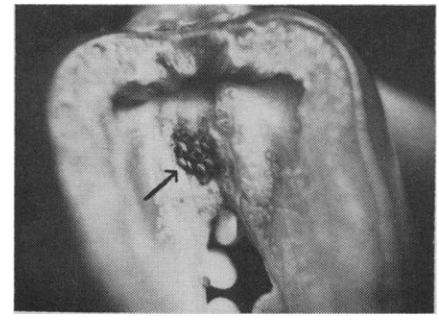
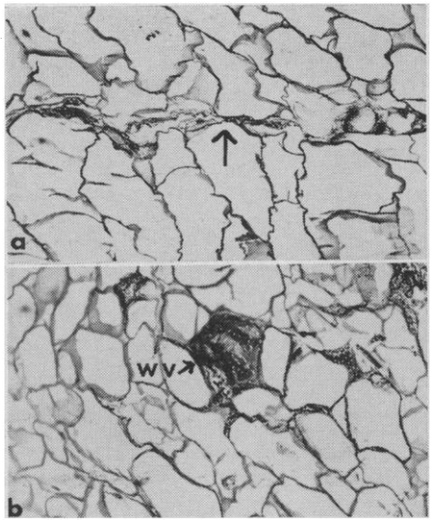


Fig. 1. Pepper fruit attached to parent plant showing the window (cut larger for photograph) made in the pericarp wall. Transplanted strawberry receptacle with achenes can be seen nearing maturation (arrow).



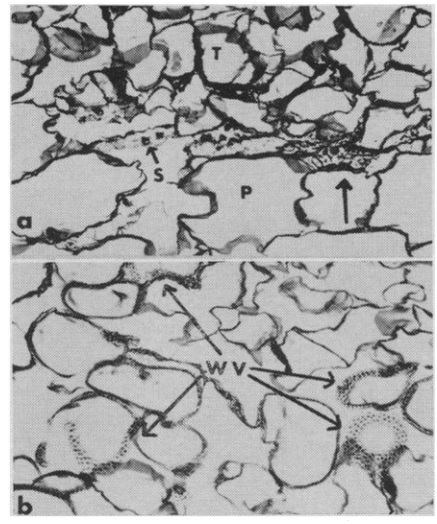


Fig. 2 (left). Transverse section of the area of placental union between pepper and pepper (× 100). (*a*) Arrow indicates region of union. (*b*) Wound vessel (*WV*) differentiated in host tissue. Fig. 3 (right). Transverse section of the area of placental union between tomato (*T*) and pepper (*P*) (× 100). (*a*) Starch grains (*S*) apparent along interface. Arrow indicates region of union. (*b*) Several wound vessels (*WV*) differentiated in pepper tissue.

prepare the receptor fruit, young ovules were removed from a small area of the placenta with fine forceps, and a small wedge-shaped incision was made there with a surgeon's cataract knife. An explant consisting of one or two ovules with a small piece of placenta was removed from the donor fruit. The placental tissue of the explant was trimmed to fit the incision already prepared in the receptor fruit. After the insertion of the explant, the entire fruit was immediately covered with a perforated plastic bag. Observations on the progress of the transplant were made through the "window." Little or no infection problem was encountered throughout the period the donor ovule was grown in the placenta of the host fruit.

Table 1. Results of transplantations between different species, genera, and families. The fruit of the pepper plant (*Capsicum frutescens* L. var. California Wonder) was used as host in every case. The fruit was 15 to 20 days from pollination when used.

Donor plant	Age of ovule when transplanted (days after pollination)	Time ovule remained on pepper after transplantation (days)	Remarks
	*Intervarietal**		
C. frutescens L. var. Wisconsin Lakes	15	30	Seeds germinated and seedlings grew to normal plants.
	*Interspecific**		
C. annuum L. (Chili pepper)	17	30	Seeds germinated and seedlings grew to normal plants.
	*Intergeneric**		
Lycopersicum esculentum Mill var. Roma (Tomato)	20	30–35	Seeds germinated only after period of dormancy—seedlings grew to normal plants.
Solanum melongena L. var. Black Beauty (Eggplant)	20	30–35	Seeds harvested at maturity; viable but dormant.
Solanum pseudocapsicum L. (Jerusalem cherry)	20	30–35	Seeds harvested at maturity; viable but dormant. Entire fruit was also cultured.†
	*Interfamilial**		
Fragaria virginiana (Strawberry Hort. var. Sparkle)	7	20-30	Achenes germinated and seedlings were obtained.

* Nature of transplantation. †Whole young fruit (20 days from pollination) was grown in the pepper by inserting the stalk of the fruit into the pepper placenta. The fruit matured 30 days after transplantation.

Young ovules ranging from 7 to 20 days from pollination were transplanted into the pepper. These ovules were obtained from families which were as different as Solanceae and Rosaceae. The seeds which developed from these transplanted ovules were collected 20 to 35 days after transplantation (depending upon the specific ovule used) and germinated on moist filter paper in petri dishes. The seedlings grew to mature plants and bore normal flowers and fruits. A summary of transplantations between various species of plants and the results obtained is presented in Table 1.

In the experiments with pepper on pepper, the area of the embryo was measured from median section of 10 μ in thickness. At the time the transplants were made, the average area of the embryos was about 0.0035 mm^2, at seed maturation, the average area was 0.4500 mm^2. Mitotic figures were observed at the time the ovules were transplanted, indicating that cell division was still in progress. The cells of the graft unions were parenchymatous in nature. Some wound tracheids were distributed among the parenchyma cells of the host, at the interface of the graft union (Fig. 2).

In the experiments with tomato on pepper, the average area of the embryo increased from 0.0025 mm^2 to 0.2000 mm^2 while it was on the pepper placenta. The graft union consisted of parenchymatous cells, and the newly differentiating xylem elements in the host parenchyma were more numerous at this interface than in the experiments with pepper on pepper. There was an accumulation of starch grains in the parenchymatous cells along the margins of the host tissue (Fig. 3). The responses of the tissues from the two placentae at the point of contact have not yet been examined for other genera and families.

In these experiments, the ovules were transplanted while the cells were still dividing. It would be of interest to know the chemical structure of some of the special growth factors, such as hormones, which arise during this crucial period of the growth of the ovule but which are as yet unidentified. It is possible that these substances only set in motion those processes of differentiation in the several areas of the reproductive structure which result in complete seeds and fruits. The physiological and biochemical apparatus which is provided for the differentiating tissues

may be common to all of the fruit parts.

There are some interesting similarities between the results obtained in our experiments in vivo and those reported in the literature on ovule culture in vitro. However, there are also points of contrast that should be borne in mind when comparisons are drawn between the two types of experiments. In our experiments the supporting medium is the growing pepper placenta through which all of the chemical substances are supplied to the developing ovule.

An important difference is that the developing fruit is a dynamic entity in which the nature and quantity of nutrients and growth factors change constantly (*2*). The materials so received by the explants enable them to increase in volume and also to grow and differentiate from the globular embryo stage into mature viable seeds. The technique of ovule culture is comparatively recent (*4*). Maheshwari, (*5*) using Nitsch's medium (*6*) supplemented with vitamins and such growth factors as kinetin, indoleacetic acid, and combinations of various amino acids has obtained mature viable seeds from young ovules. They reported successful results starting with ovules in two-cell pro-embryo stage in *Papaver* (*5*) and *Zephyranthes* (*7*) and globular stage in Gynandropsis (*4*). We are aware of no other instance where viable seeds have been obtained from cultures of ovules in vitro during globular or earlier stages without the use of unknown growth factors such as coconut milk, yeast extract, or casein hydrolysate.

Our experiments demonstrate that a common parent is able to supply the necessary growth factors to support the growth and development of young ovules which belong to different species, genera, and families. Since the ovules matured normally and produced seeds and fruits, it is possible that the physiological requirements for the growth of fruits are the same between plant species that are quite different taxonomically. Our results also indicate that it may not be necessary to search for many new and different growth factors in the fruits of each species of plant.

VIJAYA L. MOOSAD MELNICK
LEROY HOLM
B. ESTHER STRUCKMEYER
Department of Horticulture,
University of Wisconsin, Madison

References and Notes

1. L. C. Luckwill, in *Cell, Organism, and Milieu: 17th Symp. Soc. for the Study of Development and Growth,* D. Rudnick, Ed. (Ronald, New York, 1959), p. 223; J. P. Nitsch, in *Proc. of the Plant Science Symp.* (Campbell Soup Co., Camden, N.J. 1962), p. 5.
2. J. Van Overbeek, *ibid.*, p. 5.
3. V. L. Moosad, thesis, Univ. of Wisconsin (1961).
4. R. N. Chopra and P. S. Sabharwal, *Plant Embryology;* Symp. held at Univ. of Delhi, November 1960 (Council of Scientific and Industrial Research, New Delhi, 1962).
5. N. Maheshwari, *Science* **127**, 342 (1958).
6. J. P. Nitsch, *Am. J. Botany* **38**, 566 (1951).
7. M. Kapoor, *Phytomorphology* **9**, 313 (1959).
8. We thank Emma L. Fisk for her comments on the manuscript and Leo Rake for technical assistance. This work was supported in part by NIH grant GM-09064-03.

1 June 1964

Agammaglobulinemia: The Fundamental Defect

Abstract. *Addition of phytohemagglutinin and of streptolysin S to in vitro cultures of leukocytes of normal and agammaglobulinemic subjects resulted in mitosis of lymphocytes and their differentiation to plasma cells. In contrast, specific antigens induced mitosis and differentiation of lymphocytes of normal but not of agammaglobulinemic donors. The data suggest that the absence of plasma cells in agammaglobulinemia is not in itself responsible for failure of antibody production, but is rather the morphologic concomitant of the primary defect (failure of antibody production on exposure to antigenic stimulus).*

The idiopathic agammaglobulinemias, both the so-called "congenital" (*1*) and "acquired" (*2*) varieties, appear to be genetically determined. Most authorities assume that the primary defect in these disorders is the absence of plasma cells in lymph nodes, bone marrow, and other antibody-forming tissues and that inability to synthesize γ-globulin (and antibody) is a result of the absence of plasma cells (*3*).

However, a genetic defect directly responsible for the absence of one or another cell line seemed to us less likely than a genetic defect which prevents the elaboration of those cellular products normally formed in response to appropriate environmental stimuli (*4*). A more tenable explanation for the deficiency of plasma cells in agammaglobulinemia might be that differentiation of lymphocytes into antibody-producing plasma cells is concomitant with, rather than a prerequisite for, antibody formation. If the progressive morphologic change in immunologically competent cells after antigenic stimulation is attributable to cellular differentiation, during which γ-globulin (antibody) is produced (*5*), the primary genetic defect responsible for the various types of agammaglobulinemia could then be attributed to failure of synthesis of one or another of the polypeptide chains of the immune globulins (*6*). This might result from one or more of several mechanisms, including (i) mutations at regulator gene loci controlling quantitative aspects of synthesis of the polypeptide chains of the immune-globulin (an X-linked) regulator, for example, in the case of "typical" sex-linked agammaglobulinemia in which all three immune globulins are deficient, (ii) loss or duplication of genetic material due to unequal homologous crossing over during meiosis or mitosis of the chromosomes bearing the structural genes for the polypeptide chains of γ-globulin (*7*), or (iii) mutation at these structural loci. Regardless of the mechanism responsible for failure of immune globulin synthesis, the absence of plasma cells is envisioned here as merely a morphologic manifestation, secondary to failure of response to specific stimuli.

To test this hypothesis, the lymphocytes of 100 normal and five agammaglobulinemic subjects were studied in vitro in the culture system described elsewhere (*8*). Of the subjects with idiopathic agammaglobulinemia, two had "typical" sex-linked agammaglobulinemia and three the "acquired" form of the disease (Table 1). Leukocytes were obtained for culture 1 week after the second of two injections (1-week interval between injections) of a variety of antigens including diphtheria and tetanus toxoids (four subjects) and typhoid antigen (three subjects).

Lymphocytes from the patients with agammaglobulinemia (in contrast to lymphocytes from normal donors) failed to differentiate or to produce γ-globulin when challenged in the culture by the immunizing antigens. Especially pertinent is the failure of differentiation in vitro of agammaglobulinemic cells upon addition of streptolysin O to the culture medium. This is of special significance since in almost 100 percent of normal individuals older than 1 year the serum contains