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.

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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

Table 1. Percentage increase in large cells and cells in mitosis relative to control culture (no additive).

Subject	Sex	Age (yr)	Duration of symptom (yr)	Immune globulins* (% of normal)			Phytohemagglutinin	Streptolysin S	Specific antigens†
				γ_2	γ_{1A}	γ_{1M}			
"Congenital" agammaglobulinemia									
J.J.	M	15	12	3.5	0	0	89		0–1
R.H.	M	14	10	<1.0	0	0	93	79	0–3
"Acquired" agammaglobulinemia									
T.S. ‡	M	39	10	12.0	0	15.5	87	72	0–2
V.P.	F	35	14	12.5	0	4.0	90	84	0–4
D.T.	M	37	9	18.0	0	0	71.6	58.8	0.1
Normal									
100 subjects		4–45		100 ±12	100 ±36	100 ±29	80–95	40–95	5–40

* 0 indicates less than minimum detectable level (2.5 percent of normal). † Diphtheria, tetanus, typhoid, and streptolysin O. ‡ The lymphocytes of patient T.S. also failed to respond to penicillin despite a history of penicillin sensitivity preceding the development of agammaglobulinemia.

demonstrable antibody to streptolysin O, and a proportion of the lymphocytes in the culture invariably responds to streptolysin O by transforming to large lymphocytes, some of which further differentiate to plasma cells. Nonetheless, the lymphocytes of both normal and agammaglobulinemic individuals when exposed to the nonspecific mitotic stimuli provided by streptolysin S (*9*) and phytohemagglutinin showed similar mitotic rates and similar high degrees (50 to 99 percent) of differentiation to large cells, of which 10 to 15 percent morphologically resembled plasma cells. In normal cells, phytohemagglutinin induces a marked increase in production of RNA (*10*) and of protein (including the three types of immune globulins) (*11*). Lymphocytes from the agammaglobulinemic patients did not produce detectable quantities of γ-globulin on stimulation with phytohemagglutinin, despite undergoing morphologic differentiation.

Ancillary data relevant to the failure of synthesis of globulin are provided by studies of heterozygous female carriers of sex-linked agammaglobulinemia. Recent data suggest that one of the two X chromosomes in mammalian female cells is genetically inactive (*12*). Our studies of the mothers of agammaglobulinemic patients indicate that the lymphocytes of these heterozygous carriers are functionally of two types (*13*) whose relative proportions differ in different carriers. When stimulated with phytohemagglutinin, one type makes γ-globulin, whereas the other type does not, despite the fact that both differentiate to plasma cells and are not distinguishable morphologically.

These data strongly suggest that lack of ability to respond to specific environmental stimuli (antigens) is the cause rather than the result of the deficiency in plasma cells in the usual forms of agammaglobulinemia. [A lack of plasma cell precursors, however, is not excluded in the rare alymphocytic form ("Swiss type") (*14*) of agammaglobulinemia.] The production of γ-globulin by normal lymphoctyes is blocked by addition to the culture of actinomycin D (*11*), a substance known to inhibit formation of new messenger RNA (mRNA). Hence the postulated genetic defect in synthesis in one or another of the polypeptide chains of the immune globulins in agammaglobulinemia (*6*) may be attributable to mutations resulting in (i) formation of appropriate mRNA but in insufficient quantities, (ii) formation of altered mRNA incapable of coding for normal γ-globulin, or (iii) formation of faulty mRNA capable of binding to and "blocking" the ribosomes responsible for synthesis of the various polypeptide chains of γ-globulin.

In any event, the resultant failure of γ-globulin synthesis would preclude the usual morphologic concomitant of such synthesis, namely, cellular differentiation to plasma cells. The ability of these cells to respond to nonspecific stimuli (phytohemagglutinin, streptolysin S), however, demonstrates that cellular differentiation is not impaired in this group of diseases.

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