tensities of the red light are corrected for the *photopic* transmission by the red filter. These results seem to lend no support to the view that rods alone serve as receptors for the steady-state "photopupil" response (photopupil refers to narrowing of the pupil in response to stimulation of the retina by light).

We made these measurements by sampling all 30 frames obtained from 10 to 40 seconds (one frame per second) after allowing the eye to be adapted to each new degree of brightness, and not, as Bouma suggests, by selecting only those parts of the response in which the temporal changes of pupil size are minimum. Bouma introduced the hypothesis that his sampling procedure would give results different from those which contradict the "rods alone" theory. His hypothesis would predict for our subject that when the oscillations in the size of pupil were minimal the pupil would dilate to the diameter held by it in total darkness. We tested this prediction by exposing the eye to a bright (4.3 $\log_{10}$troland) field and measuring the size of the pupil every second for 5 minutes. At no time during this 5-minute period did the pupil dilate to within 2 mm of the diameter that it had had in the dark. Moreover, the pupil diameters around which the minimum fluctuations occurred were precisely those values obtained by the sampling procedure used to obtain the measurements in Fig. 1*b* under the same conditions.

No evidence among all the psychophysical, electrophysiological, neuro-ophthalmological, or clinical measurements made by us on M.L. supports the idea that the nerve connections of his retina differ in any way from those of the normal eye. In fact, everything we know about his eyes strongly suggests that his defects are due only to disturbed photopigment kinetics in his rods and cones. Thus, we have clear evidence that in an eye lacking functioning rods there can be quite unequivocal steady-state photopupil responses. We infer that in a normal retina both rods and cones serve as photoreceptors for steady-state photopupil response in the same way that they serve for the transient photopupil response.

JURRIAAN TEN DOESSCHATE
University of Utrecht,
Utrecht, Netherlands

MATHEW ALPERN
University of Michigan, Ann Arbor

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Primary Immune Reactions in Organ Cultures

Abstract. *Primary immune reactions were initiated and maintained in vitro. Spleen explants from mice treated with either phytohemagglutinin or adjuvant were stimulated to form hemagglutinins and hemolysins, whereas no treatment of spleen donors was necessary to invoke a "graft-versus-host" response which led to specific splenomegaly.*

Interest in the mechanism of antibody formation has led to repeated attempts to induce primary immune responses in vitro (*1*). Probably two types of cells are involved, macrophages and lymphocytes, and an interaction between these two cell types may be essential in the development of an immune response (*2*). However, in previous experiments both the yield of antibodies and the frequency of positive cultures were quite low (*2*). A possible explanation for the difficulties encountered in the initiation of immune reactions in vitro was the fact that lymphocytes fail to proliferate under the usual conditions of culturing, while lymphoid proliferation may be important in the early stages of antibody formation. Furthermore, the tissue culture methods used in previous studies were not designed to encourage cellular differentiation, which may also be a key element in the initiation of immune reactions.

In our experiments an organ culture method was used which permits lymphoid maintenance, differentiation, and proliferation (*3*). Spleen fragments, 0.2 to 0.3 mm in thickness and 0.5 to 1.0 mm^2 in surface area, were explanted in the well of a Millipore filter assembly prepared by gluing a TH Millipore filter (porosity 0.45 μ, thickness 25 μ) to the underside of a plastic mount (*4*). The assembly was placed in an organ culture dish (Falcon 3020) containing 1.0 ml of a tissue culture medium consisting of Eagle's basal medium supplemented with 10 percent of horse serum (Difco 0569; or A gamma-globulin, Hyland 117-100, for studies of agglutinins), 5 percent chick-embryo extract (9-day embryos), erythromycin (50 μg/ml), and mycostatin (50 μg/ml). Cultures were incubated at 37.5°C in a water-saturated atmosphere of 95 percent oxygen and 5 percent carbon dioxide. Mice of strains Balb/cHeAu, C_3H/HeAu and F_1 ($C \times C_3H$) were used in all experiments.

In "graft-versus-host" reactions embryonic intact spleens from 17-day embryos and spleen fragments from 1- to 4-day-old F_1 ($C \times C_3H$) mice were used as host tissues. Cultures were set up in pairs, and the size of explants was carefully matched for each pair. Spleen cell suspensions were prepared from C_3H (parental) or F_1 ($C \times C_3H$) (syngeneic) adult mice by passing minced tissue through syringe needles of progressively smaller bore. Approximately 5×10^5 nucleated cells were added to the explanted "host" spleens, and cultures were scored after 3 to 4 days. Explants grown in the presence of parental cells were compared to explants grown in the presence of syngeneic cells (40 cases) or to explants grown without any cells added (25 cases). The presence of parental cells led to marked splenomegaly (1.5- to 3-fold enlargement) in all 65 cases (Fig. 1*a*). The histological changes occurring within the explanted spleen tissues were similar to those in vivo in typical graft-versus-host reactions (*5*). The sequence of histological changes included lymphoid cell accumulation and dispersion, increased mitotic activity associated with active granulocytopoiesis, and ultimate lymphoid-cell degeneration (*6*). Experiments with allogeneic as well as parental cells indicate that immunological maturation of the spleen occurs within 3 days after birth, at which time a typical "discriminant spleen assay" (*7*) can be obtained in vitro.

To stimulate antibodies to sheep red blood cells, spleen fragments were obtained from adult C_3H mice which were free of natural agglutinins as concluded from tests performed on serum samples. The sheep blood cells (Gibco)

were added to the explants as 1 percent of a 0.01-ml suspension of washed, packed cells in tissue culture medium. Samples of medium were taken at different times from 1 to 15 days, and tissues were tested for the presence of hemolysins, by use of the plaque technique (*8*). However, no antibody formation could be detected in any of the cultures (Table 1). The explants gradually decreased in size during the period of the experiment (2 months), and restimulation with antigen at different time intervals did not lead to antibody formation.

Since in these cultures lymphoid cell proliferation did not occur, we tested the possible effect of phytohemagglutinin (PHA), an agent known to stimulate lymphoid-cell proliferation (*9*). In line with observations of Mekori *et al.* (*10*), 0.2 ml of PHA (Difco-PHA-M) was injected intraperitoneally into adult mice one day before removing the spleen. Five to ten fragments were tested from each spleen, sheep red cells being added as in the earlier experiments. Media collected during the 1st week showed no agglutinins. When tissues were transferred to new filter wells after 5 to 7 days and a fresh suspension of the sheep red cells was added (Fig. 1*b*), erythrocytes agglutinated around some of the explants (Fig. 1*c*, Table 1). This agglutination was observed repeatedly within 1 to 2 days whenever fresh sheep cells were added to rinsed explants. Agglutinating activity could be detected during the 2nd week in the tissue culture medium of six out of 17 of these cultures. The titer of antibodies in the media reached the maximum peak of 1:16, then decreased, becoming undetectable after 18 days. However, agglutination around the explants was observed during 2 months. At least two fragments from each test spleen showed positive agglutinating ability, while no such agglutination was ever observed in spleens not treated with PHA. In no case did PHA-treated spleens show agglutinating activity except after antigenic stimulation.

Table 1. Incidence of agglutination of sheep red blood cells by spleen explants.

Initial treatment of donor*	Spleens		Ratio of No. of cultures reacting to total
	Tested (No.)	With reacting cultures (No.)	
None	8	0	0/36
Phytohemagglutinin	8	8	34/56
Adjuvant	2	2	4/10
Sheep red blood cells	2	2	10/10

* Donor animals injected one day prior to removal of spleen.

To test whether the agglutinating material represents PHA which remains in the tissue and is liberated in the presence of erythrocytes, media containing agglutinins were tested in parallel against mouse erythrocytes. No trace of agglutination was detected, although such erythrocytes have been found to be agglutinated by PHA.

Similar results were obtained when spleen-donor animals were injected intraperitoneally with an 0.5-ml emulsion of equal volumes of adjuvant (complete adjuvant, Difco) and saline (Table 1) rather than with PHA.

Further assays were carried out to test for hemolysin production in these cultures. Spleen explants were transferred to fresh filter wells and overlaid with a 30-percent suspension of sheep cells in 0.7-percent agar prepared in tissue culture medium with 1-percent diethylaminoethyldextran (DEAE) added. After 2 more days of incubation, the medium was removed and complement (Difco, diluted 1:12 in Kolmer's saline) was added. Hemolysin plaques appeared within 1 hour around those test explants that previously showed agglutinin production (Fig. 1*d*), whereas no such plaques were observed in cultures that showed no agglutination or in cultures of spleens from untreated mice.

To determine whether the observed agglutination and hemolysis were manifestations of an immune response, the specificity of the reaction was tested. Explants which agglutinated the sheep cells were rinsed in medium, dissected into three equal-sized fragments, and then individually cultured in agar containing either sheep, chicken, or mouse red blood cells. Hemolysis occurred only around tissues incubated with sheep red blood cells.

In order to compare the immune reaction induced in vitro with a primary immune response induced in vivo, spleens were explanted from mice that had been injected intraperitoneally 24 hours before with 0.5 ml of a 5-percent suspension of sheep red cells. Cultures were treated as above, and agglutination around the tissues was noticed after 2 days in nine out of ten cultures, the remaining culture becoming positive after 3 days. However, agglutinins in the medium were detected only on the 7th day in three

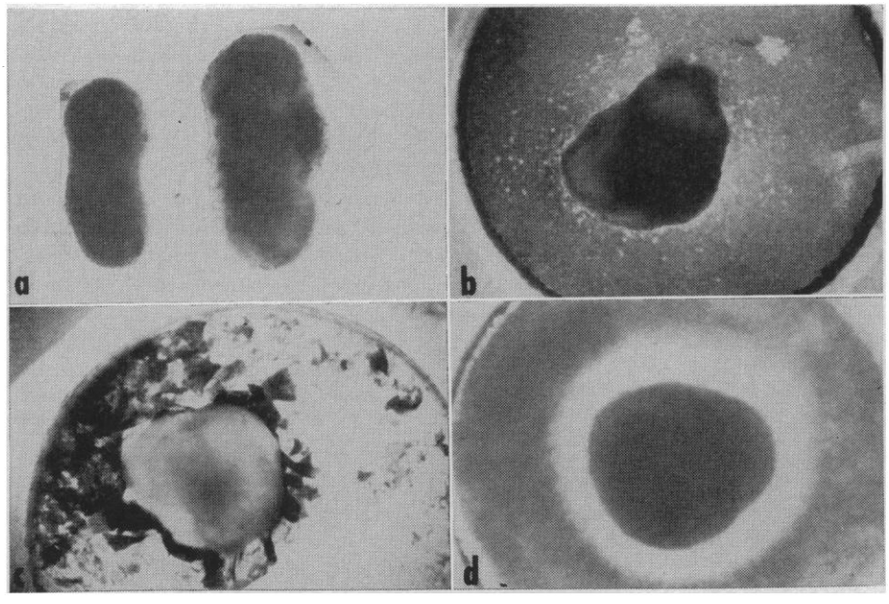

Fig. 1. (*a*) Embryonic spleens (17-day) from F_1 (C × C_3H) mice; left, cultured with syngeneic spleen cells; right, cultured with parental strain (C_3H) spleen cells. The spleen is markedly enlarged. (*b*) Spleen fragment from adult C_3H mouse, cultured on sheep red blood cells. (*c*) Spleen fragment as in *b*, stimulated in vitro for 5 days with sheep cells (1 percent), then transferred to medium containing 5 percent of sheep cells. Agglutination around the explant is observed. (*d*) Spleen fragment as in *c* imbedded in agar containing sheep red cells and complement. There is a plaque around the tissue.

out of ten and on the 10th day in six out of ten cultures at a maximum peak titer of 1:16, which subsequently decreased, becoming undetectable on the 18th day. However agglutination around the tissues continued, indicating that the agglutination around tissue is the more sensitive assay for demonstrating antibodies in this system.

Thus immune responses can be induced and maintained entirely in vitro. One might argue that this response is actually due to stimulation of preexisting natural antibodies that are undetectable. However, the fact that spleens from untreated normal mice did not respond to antigenic stimulation in vitro, the similarity of the kinetics of agglutinin formation in explants stimulated in vitro and those induced in vivo, and the observation that spleens from immunized mice respond to a secondary stimulation in vitro with no treatment of PHA or adjuvant (*11*) place this reaction with those described as primary immune responses. While treatment of donor-spleen tissue with PHA was essential for demonstrable agglutinin or hemolysin production no treatment was required to elicit the graft-versus-host reaction in vitro. The fact that PHA in itself is not involved in the immune response is suggested both by the specificity of the observed reaction and by the fact that adjuvant can elicit a similar reactivity of spleen explants. That no prior stimulation is necessary for evoking a graft-versus-host response is consistent with observations made on human lymphocytes in vitro (*12*). It may well be that spleen cells provide both antigenic stimulation and a stimulus to cell proliferation. Alternatively, this reaction may not require proliferation by lymphocytes at all, or it may simply represent a more sensitive assay of an immune process.

Why only some fragments from each spleen produce detectable antibodies is not clear. Whether the reason is technical or whether the explanation lies in a limited total number of competent cells present in an unimmunized spleen cannot be decided from our experiments. Since explants, once positive, remain positive for a period of up to 2 months, careful study of the period immediately after explantation is required.

AMIELA GLOBERSON*
ROBERT AUERBACH
Department of Zoology
University of Wisconsin, Madison

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Brucite in Carbonate Secreted by the Red Alga Goniolithon sp.

Abstract. *X-ray diffraction studies, chemical analyses, infrared-absorption studies, and nuclear-magnetic-resonance spectrum analysis demonstrate the existence of the mineral brucite, $Mg(OH)_2$, in the skeletal carbonate secreted by the red marine alga* Goniolithon *sp. Electron microprobe examination of the carbonate shows that the brucite is concentrated in certain areas of the skeletal structure. These results explain the anomalously high solubility and large cell size of the* Goniolithon *"carbonate" observed by earlier investigators.*

Recent investigations of magnesian calcites secreted by marine organisms have directed particular attention to the skeletal carbonate secreted by the red alga *Goniolithon*. This interest is drawn in part by the exceptionally high magnesium content of the carbonate ($MgCO_3$, 24 to 29 mole percent) and also by its anomalous physical and chemical properties. First, the results of both saturometer and "equilibrium-*p*H" measurements indicate that the carbonate is considerably more soluble than extrapolation from the solubilities of solid solutions of calcite less rich in magnesium predicts (*1*). Second, the cell size of the carbonate, determined by x-ray powder diffraction, is much larger than its chemical composition would require, assuming that the magnesium is present in ideal solid solution in the calcite lattice (*2*). Noting that calcites less rich in magnesium show variations in cell size that closely approximate the ideal solid-solution pattern of Vegard's law, Goldsmith *et al.* (*3*) suggested that the skeletal material secreted by *Goniolithon* consists of a physical mixture of two phases: a solid-solution magnesian calcite and minor amounts of some phase richer in magnesium in amounts that are too small or too poorly crystallized to be detected by x-ray.

During the past 2 years, skeletal material deposited by *Goniolithon* has been carefully examined in three laboratories. These investigations were independently conceived and entirely different techniques were used. Although no single study provided sufficient evidence to justify a positive conclusion, the evidence provided by all three combined overwhelmingly supports only one conclusion. I will discuss these studies in logical sequence, with no suggestion of precedence (*4*).

In order to determine whether the skeletal material secreted by *Goniolithon* constitutes an homogeneous single phase, several specimens (*5*) were subjected to microprobe analysis (*6*). Partial wet-chemical analysis of the carbonate yielded an alkali-earth cation ratio that corresponded to a calcite of ideal solid solution containing nearly 29 mole percent of $MgCO_3$. X-ray diffraction studies, however, showed only a single phase (magnesian calcite), with a cell size that corresponded to an ideal solid solution containing approximately 18 mole percent of $MgCO_3$ ($d_{10\cdot4}$, 2.975 Å) (*7*). Organic matter was removed from the samples with Clorox or H_2O_2, or both, and they were then mounted and polished for examination by electron microprobe (*8*); typical images are shown in Fig. 1.

Electron-back-scatter images (Fig. 1*a*) show the lattice-like structure of the skeletal material. The field of the photograph, showing a longitudinal section of a single algal "branch," is approximately 180 μ in diameter. The dark areas, depressions in the polished surface of the specimen, represent transected cellular openings in the structure. An image of the same field generated by electron-excited calcium-Kα fluorescence (Fig. 1*b*) indicates that the open cells are calcium-deficient areas sur-