floor mineralogically unchanged. This supports the possibility of short-lived vaterite preservation in the initial phase of its incorporation into tropical marine sediments at shallow depth. To determine whether vaterite-bearing hard parts become transient constituents of the depositional environment under these conditions will require careful search for them in shallow-water marine deposits.

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8. The seawater sample, collected off the Palos Verdes peninsula, Los Angeles, California, had a salinity of 33.7 per mil and a *p*H of 8.22. Room temperatures ranged from 20° to 28°C.
9. The reference vaterite was precipitated at room temperature by dropwise addition of 20 ml of 2*M* $CaCl_2$ to 25 ml of 2*M* Na_2CO_3 at a rate of one drop every 3 seconds.
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Immunoglobulin-Positive Cells: Their Appearance in the Mixed Lymphocyte Reaction

Abstract. *In mixed lymphocyte cultures prepared with thoracic duct lymphocytes from allogeneic rats, approximately 20 percent of all blast cells that appeared at the end of 72 hours of incubation had surface receptors for rabbit antibody to rat immunoglobulin.*

It is generally accepted that in the mixed lymphocyte reaction (MLR) small lymphocytes from allogeneic donors transform into blast cells, synthesize DNA, and proliferate (*1, 2*). Most of the evidence to date indicates that the responding lymphocytes and the resulting blast cells are thymus-derived (T) cells (*3*). However, evidence suggesting that bone marrow–dependent cells (B cells) with immunoglobulin (Ig) receptors on their surfaces may also undergo blastogenesis in the MLR (*4*). In view of the fact that the MLR represents a cell-mediated immune response (*5*) and that such responses may possibly involve both T and B cells (*6*), our study was undertaken to further explore the possibility that B cells respond in the MLR.

To prepare MLR's, equal numbers (5×10^6) of cells from the thoracic duct lymph (TDL) of young adult Lewis male rats and F_1 hybrids of the Lewis and Brown Norway (BN) strains were mixed together in 5 ml of culture medium (*2*). Under these conditions, the MLR response is considered to be unidirectional with parental Lewis cells reacting against the BN component of the F_1 hybrid (*7*). For controls, nonmixed cultures were prepared with 10×10^6 TDL cells from either the Lewis or F_1 donor. After 72 hours at 37°C, cells from each culture were treated with 10 μg of ^{125}I-labeled rabbit antibody to rat Ig for 30 minutes at 4°C (*8*). The cells were then washed, and smears were prepared for light microscopic radioautography. The antibody to Ig (Cappel Laboratories, Inc.) was iodinated by the method of Greenwood *et al.* (*9*) and had a specific activity of 7.0 μc/μg. Although the purity of the antibody to Ig was assured by immunoelectrophoresis, two additional tests were carried out in order to further define the specificity of the antibody. First, 10×10^6 Lewis rat thymocytes were treated with ^{125}I-labeled antibody to Ig. Since less than 0.3 percent of the cells were labeled, we concluded that the antibody to Ig would not bind to cells lacking surface receptors for Ig. Second, the ^{125}I-labeled antibody to Ig was incubated with purified rat Ig prior to its exposure to MLR cells. Because lymphocytes and blast cells failed to label, we concluded that the Ig had reacted with the antibody to Ig, thereby inhibiting the binding of the latter to cell surfaces.

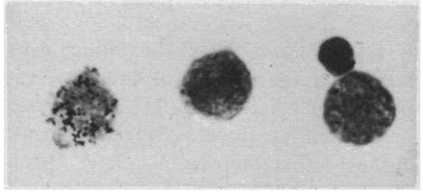

Fig. 1. Light microscopic radioautograph of a blast cell (left) treated with ^{125}I-labeled antibody to Ig. Two unlabeled blasts (center and bottom right) and a typical small lymphocyte (top right) are also shown (× 1000).

The percentage and types of labeled cells in control and MLR cultures were determined by counting at least 1000 cells per slide. Lymphoid cells were classified as either small lymphocytes (SL) or large and medium lymphocytes (LML) (*10*). Blast cells were defined as those cells greater than 14 μm in diameter and having a nucleus with a fine chromatin pattern, observable nucleoli, and intensely basophilic cytoplasm.

Results indicated that TDL cells from either Lewis or F_1 (Lewis × BN) animals consisted of only SL's and LML's before culture and at the end of 72 hours of incubation. When Lewis and F_1 cells were mixed, the cells present after 72 hours included SL's and LML's as well as a significant percentage (23 percent) of blast cells. Since the total number of cells surviving after 72 hours has been shown to be approximately the same for both mixed and nonmixed cultures

(5), it was concluded that the lower percentage of SL's in MLR's as compared to nonmixed cultures reflected a lower number of such cells.

After being exposed to ^{125}I-labeled antibody to Ig, cells from both mixed and nonmixed cultures were found to be labeled or Ig-positive (Ig+). In nonmixed cultures, more than 90 percent of the Ig+ cells were SL's, the remainder being LML's. As shown in Table 1, these Ig+ SL's and Ig+ LML's accounted for approximately 10 percent of the total population of SL's and 33 percent of the total population of LML's, respectively. In MLR's, Ig+ SL's were also present but, unlike nonmixed controls, Ig+ blasts (Fig. 1) together with Ig+ LML's comprised over half the total population of Ig+ cells. As indicated in Table 1, these Ig+ SL's, Ig+ blasts, and Ig+ LML's represented approximately 10 percent of all SL's, 20 percent of all blasts, and 53 percent of all LML's, respectively, that were present in MLR's. By considering the fact that, in MLR's as compared to nonmixed controls, the total population of SL's was less, and the total populations of blasts and LML's were greater, these percentage values indicate that (i) there were fewer Ig+ SL's in MLR's than in nonmixed cultures and (ii) the numbers of Ig+ blasts and the numbers of Ig+ LML's had increased in MLR's over the numbers present in nonmixed controls.

From the foregoing experiments it is evident that the response of SL's in the MLR resulted in the appearance of a significant number of Ig+ blast cells. Although the B or T cell origin of such blasts could not be determined by the methods used in this study, it seems reasonable to propose that they represented transformed B cells rather than transformed T cells for several reasons.

1) In MLR's containing Ig+ blast cells there was a lower proportion of Ig+ SL's than in nonmixed cultures. This observation may indicate that the population of Ig+ SL's became reduced as such cells became transformed into Ig+ blast cells.

2) Since B cells have the capacity to undergo blastogenesis, as in the response to pokeweed mitogen (*11*), it is not unreasonable to predict that such cells might also be able to transform in response to allogeneic cells.

3) For T cells to have become Ig+ blast cells, surface receptors for antibody to Ig must have been revealed or acquired during the process of blastogenesis. Although this possibility cannot be excluded, it is believed unlikely that the number of T cells which became labeled with antibody to Ig could account for the proportion of Ig+ blasts that we observed.

Table 1. Percentages of small lymphocytes (SL), large and medium lymphocytes (LML), and blast cells labeled with ^{125}I-antibody to Ig. Nonmixed cultures contained either Lewis or F_1 (Lewis × BN) TDL cells. Mixed cultures were prepared with equal numbers of Lewis and F_1 (Lewis × BN) TDL cells. All cultures were incubated for 72 hours at 37°C before being exposed to antibody to Ig.

Labeled cell	Percentage labeled	
	Nonmixed	Mixed
SL	10	11
LML	33	53
Blasts	0	20

Finally, the conclusion that Ig+ blasts arose from B cells is consistent with several other studies which have suggested that B cells respond in the MLR (*4*).

Unlike these previous studies, however, results of the present investigation indicated by means of direct visual observation that B cells from the TDL of rats transformed in the MLR and that such transformation made a significant contribution to the total MLR response.

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Induction of Neural Differentiation in Cultures of Amphibian Undetermined Presumptive Epidermis by Cyclic AMP Derivatives

Abstract. *Induction of neural differentiation in cultures of undetermined presumptive epidermis from three amphibian species was achieved by the addition of 1 millimolar dibutyryl adenosine 3′,5′-monophosphate, 8-bromoadenosine 3′,5′-monophosphate, or adenosine C′,E′-monophosphate together with theophylline. Adenosine 5′-monophosphate, adenosine 2′,3′-monophosphate, dibutyryl guanosine 3′,5′-monophosphate, and butyrate at 1 millimolar are ineffective. These results suggest that the action of the primary inductor or inductors may be mediated via adenosine 3′,5′-monophosphate.*

Since the report of Spemann (*1*) that the dorsal blastoporal lip of developing amphibian embryos acts as an inductor (primary organizer/primary inductor) to determine the differentiation of the neural plate, the identity(s) and mode(s) of action of the inductor(s) have been the subject of intensive research [for review, see (*2*)]. The dorsal lip as well as tissue extracts and numerous other substances have been shown to induce neural differentiation in explants and in cells in culture. However, the best evidence to date suggests that the primary inductors are likely to be proteins [for review, see (*3*)]. In this respect, these inductors resemble polypeptide hormones in that they are proteins that serve the function of intercellular messengers. Since the protein hormones are generally believed not to enter the target cell but to act only on the membrane of the target cells, and since adenosine 3′,5′-monophosphate (c-AMP) is known to be the second messenger of a variety of hormones, we undertook to test whether the primary inductor(s) also acts via the second messenger c-AMP. We wish to report here that dibutyryl adenosine 3′,5′-monophosphate (dibutyryl-c-AMP), 8-bromoadenosine 3′,5′-monophosphate (8-Br-c-AMP), and c-AMP with theophylline can indeed induce undeter-