tential occurred at about the time of the development of competence for bone marrow graft rejection in genetically resistant infant mice (Table 1) (*4, 5*).

Responding cells of B6 × C3H mice repeatedly injected with parental C3H spleen cells retained the ability to generate both antiparental B6 and antiallogeneic DBA/2 cytotoxic effector cells (Table 2). The F_1 cells harvested from mice first treated with parental B6 cells lost their cytotoxic reactivity against B6 stimulating cells while retaining reactivity against allogeneic DBA/2 cells. Hence, the specific abrogation of hybrid resistance resulting in phenotypic susceptibility to B6 marrow grafts (Table 1) also resulted in unresponsiveness in vitro.

These experiments demonstrate that cell-mediated cytotoxicity is inducible in strain combinations for which in vitro reactions to classical *H-2K* and *H-2D* alloantigens and in vivo skin graft rejections are precluded. Such combinations are characterized, however, by strong reactivity in vivo against *Hh-1* incompatible hemopoietic grafts. No exception was noticed so far to this positive correlation, although genetic polymorphism, immunologic immaturity, and induced specific unresponsiveness were introduced in the experimental design as sources of variation to possibly dissociate resistance to bone marrow grafts and competence for in vitro cytotoxicity.

Since a population of radioresistant macrophages has been implicated as the effector of bone marrow graft rejection in vivo, presumably in cooperation with thymus-independent lymphocytes (*6, 7*), the in vitro bioassay should be instrumental in establishing to what extent the mechanisms of cytotoxicity against *Hh* differ from classical T cell–mediated lymphol-ysis. However, it is still an open question whether reactions against *Hh* are mediated by the same cell types in vitro and in vivo. Irrespective of the mechanistic aspects of this problem, this straightforward bioassay makes it possible to investigate hemopoietic histoincompatibility in species other than mice, and the role of the *Hh* system in leukemogenesis (*13*). If successful with human cells and in allogeneic combinations, the in vitro model will improve matching procedures for clinical bone marrow transplantation.

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Differentiation of Erythroleukemic Cells in the Presence of Inhibitors of DNA Synthesis

Abstract. *Erythroid differentiation can be readily induced by butyric acid in cultured erythroleukemic cells in the presence of inhibitors of DNA synthesis and in the absence of cell division. This result appears to rule out more complex models for globin gene expression which require gene replication or cell division (or both).*

A variety of arguments have been put forward that suggest that DNA synthesis, accompanied by an appropriate number of mitoses, is necessary for the differentiation of erythroid cell precursors (*1*). Recent studies have explored this question with the use of cultured erythroleukemic cells which undergo erythroid differentiation in the presence of dimethyl sulfoxide (*2, 3*). The results of these studies suggest that two rounds of mitosis are required for erythroid induction in the presence of dimethyl sulfoxide (*4*), or that dimethyl sulfoxide must be present during, or immediately after, DNA synthesis (*5*). Such interpretations fit well with the data and with the teleologic argument that DNA synthesis results in the rearrangement of regulatory elements by exposing or activating the required set of genes.

Our own studies of this question, in which we used inhibitors of DNA synthesis and the dimethyl sulfoxide induced–erythroleukemic cell system, initially led to ambiguous results. Erythroid differentiation was invariably reduced by inhibitors of DNA synthesis, but it was never entirely quenched. In addition, it was clear that such reagents had pleiotropic effects on the inhibition of RNA and protein synthesis which were particularly severe in the presence of dimethyl sulfoxide. Thus, the observed inhibition of erythroid differentiation might have been a consequence

Table 1. Butyric acid–induced erythroid differentiation in log and stationary phase erythroleukemic cells. A cloned line of erythroleukemic mouse cells, T3C12 (*3*), was grown in F-12 medium supplemented with 10 percent calf serum (*7*). Cells in the log phase of growth were passed to a concentration of 1×10^4 cell/ml; stationary phase cells, to a concentration of 3.2×10^5 cell/ml, the usual growth limit of this cell line under these conditions. Butyric acid, 1 m*M*, was added on day zero, as indicated, and cells were assayed on day 5 for percent of benzidine-positive cells (*7*). A 0.1- to 0.2-ml sample of cells was added to a well in a Microtiter dish and mixed with 0.1 volume of freshly prepared benzidine reagent containing 0.15 percent H_2O_2, 4 percent CH_3COOH, and 0.015 percent benzidine. Approximately 200 cells of each duplicate sample were scored for benzidine-staining (positive) cells.

Cells	Cell count		Benzidine-positive cells (%)
	Initial	Final	
Log phase	1×10^4	1.2×10^5	32
Stationary phase	3.2×10^5	2.9×10^5	21

Table 2. Effect of cytosine arabinoside and hydroxyurea on the incorporation of [^{3}H]thymidine into DNA in erythroleukemic cells. Log phase T3C12 cells, (1×10^5)/ml, were incubated in the presence or absence of the indicated antibiotic for 15 minutes. [^{3}H]Thymidine was added to each culture to a specific activity of 1 μc/ml, and the incubation was continued for 1 hour. Portions of cells were taken at zero time (immediately after addition of [^{3}H]thymidine) and at the conclusion of the labeling period. The cells were then centrifuged and washed in phosphate-buffered saline. The pellet was then washed with 5 percent trichloroacetic acid (TCA), centrifuged, and dissolved in 0.5*N* KOH. After incubation for 30 minutes at 37°C, the samples were neutralized, and TCA was added to 10 percent. The precipitate was collected on Millipore filters and the radioactivity was counted by liquid scintillation.

Addition	[^{3}H]Thymidine incorporated (count/min per 10^3 cells)
None	5509
Ara-C, 1 μg/ml	710
Ara-C, 2 μg/ml	588
Hydroxyurea, 0.1 m*M*	652
Hydroxyurea, 0.2 m*M*	632
Zero time control	680

of either or both of these effects, or a generalized toxic effect on the erythroleukemic cells.

Our recent observation that butyric acid induces erythroid differentiation at concentrations less than a hundredth of that required for dimethyl sulfoxide (*6*) has allowed us to overcome these serious experimental difficulties. Butyric acid is less toxic to cultured cells during prolonged incubation and, in contrast to dimethyl sulfoxide, effectively induces stationary phase cells to undergo erythroid differentiation (Table 1).

As a further test of the requirement for DNA synthesis and cell division, 1β-D-arabinofuranosylcytosine (ara-C) and an unrelated inhibitor of DNA synthesis, hydroxyurea, were added to cultures of control and butyric acid–induced cells. Both antibiotics were added at concentrations that profoundly inhibited DNA synthesis (Table 2). No further cell division was observed. In both cases, in vitro differentiation, as measured by the appearance of benzidine-positive (containing hemoglobin) cells, occurred as efficiently as in control cultures (Table 3). That the percent of benzidine-positive cells provides a quantitative estimate of the extent of erythrodifferentiation has been established by our previous studies (*7*), which showed that the percent of positive cells is directly correlated with the globin messenger RNA accumulated by erythroleukemic cells and the percent of cells containing oxyhemoglobin, as determined by microspectrophotometry. The degree of benzidine staining of individual cells observed in these studies was no different in control cultures and those treated with antibiotics.

Table 3. Effect of cytosine arabinoside and hydroxyurea on butyric acid–induced erythrodifferentiation. Cells were grown, and butyric acid and antibiotics (where indicated) were added at day zero; cells were assayed for benzidine staining as described in the legend of Table 1.

Addition	Benzidine-positive cells (%) at butyric acid concentration:	
	None	1 m*M*
None	0	38
Ara-C, 1 μg/ml	0	39
Hydroxyurea, 0.1 m*M*	0	34

It can be argued that a small portion or a specific region of the genome is being replicated in the presence of either ara-C or hydroxyurea and this possibility cannot be ruled out entirely. Nevertheless, the two antibiotics have different mechanisms of action (*8*) and both quickly inhibit [^{3}H]thymidine incorporation as well as cell division in treated cells. In addition, the ability of nondividing, stationary phase cells to undergo erythroid differentiation in the presence of butyric acid lends additional support to the conclusion that neither division nor DNA synthesis is required for the expression of hemoglobin genes under these conditions.

Our results seem to eliminate the complicating assumptions that were needed to explain the regulated expression of globin genes that required mitosis or, at least, gene replication in erythroleukemic cells. Thus the globin system becomes formally more analogous to those prokaryotic operons in which control operates on nonreplicating DNA.

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Prostaglandin $F_{2\alpha}$ Production by the Brain During Estrogen-Induced Secretion of Luteinizing Hormone

Abstract. *The arteriovenous difference in the concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) across the brain of the anestrous sheep was measured before and during the induction of luteinizing hormone secretion with 17β-estradiol. The results indicate that (i) the brain in vivo is a significant source of $PGF_{2\alpha}$, (ii) the release of $PGF_{2\alpha}$ from the brain occurs in pulses with a circhoral rhythm, and (iii) the process through which estrogen exerts its negative and positive feedback effects on luteinizing hormone secretion may involve amplitude modulation of $PGF_{2\alpha}$ output from the brain.*

Although the role of estrogen in the regulation of luteinizing hormone (LH) secretion by negative and positive feedback actions is firmly established (*1*), the underlying mechanisms are not fully understood. Recent reports have shown that prostaglandins (PG's) can elicit release of LH from the anterior pituitary gland in vitro (*2*) and in vivo (*3, 4*). Our studies have led us to suggest that PG's of central origin, acting at a site (or sites) proximal to the anterior pituitary, may play a role in the neuroendocrine mechanism which translates an increase in the circulating level of estrogen into a signal for the release of LH (*4, 5*). We now extend this evidence by demonstrating that the brain releases prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in circhoral pulses which undergo pronounced modulations during the induction of LH secretion by the intravenous infusion of 17β-estradiol (17β-E_2).

Anestrous Merino sheep were used as experimental subjects because, in response to the administration of 17β-E_2, these animals exhibit a very pronounced release of LH (*5, 6*) after a short latent period (8 to 12 hours). Eighteen hours before each experiment was begun, the left carotid artery and left jugular vein, exteriorized in a loop