functions of the yttrium concentration.

The trend toward an enormous reduction of θ_D becomes evident with as little as 1 percent Y, while γ remains almost unchanged throughout most of the range. The rapid decrease in lattice stiffness is in parallel to a rapid decrease in the melting temperature of Ir, as observed in most Ir-rare earth systems, as well as to a change in physical properties. The relative constancy of γ suggests little change in the electronic structure from that of iridium. The change in lattice stiffness is thus predominantly responsible for this extraordinary enhancement of superconductivity, an effect which is not limited to iridium metal (*5*).

Many of the results in the past for binary superconductors that did not fit with either the electron-to-atom ratio or crystallographic considerations are now readily understood by the presence of superconducting eutectics and their changed phonon structures. Enhancement of superconducting transition temperatures has been observed by other investigators, but never to the extent found in the iridium-yttrium system. Of particular interest are the investigations of the Ti-Mo (*6*) and Zr-Mo (*7*) systems. The well-known In-Sn and Pb-Bi alloys also fall in this category. In all of these systems the existence of a superconducting eutectic is the probable reason for their enhanced superconducting T_c.

B. T. MATTHIAS
Institute for Pure and Applied Physical Sciences, University of California, San Diego, La Jolla 92093

G. R. STEWART
A. L. GIORGI
J. L. SMITH
Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545

Z. FISK
Institute for Pure and Applied Physical Sciences, University of California

H. BARZ
Bell Laboratories, Murray Hill, New Jersey 07974

References and Notes

1. R. A. Hein, J. W. Gibson, B. T. Matthias, T. H. Geballe, E. Corenzwit, *Phys. Rev. Lett.* **8**, 408 (1962).
2. B. T. Matthias, Z. Fisk, J. L. Smith, *Phys. Lett. A* **72**, 257 (1979).
3. J. L. Smith, unpublished data.
4. T. H. Geballe, B. T. Matthias, V. B. Compton, E. Corenzwit, G. W. Hull, Jr., L. D. Longinotti, *Phys. Rev. A* **137**, 119 (1965).
5. B. T. Matthias, *Phys. Lett. A* **25**, 226 (1967).
6. J. C. Ho and E. W. Collings, *ibid.* **29**, 206 (1969); E. W. Collings and J. C. Ho, *ibid.*, p. 306.
7. K. Yasohama and T. Ogasawara, Jr., *Phys. Soc. Jpn.* **36**, 1349 (1974).
8. B.T.M. is also at Bell Laboratories, Murray Hill, N.J. 07974. Work at Los Alamos was performed under the auspices of the Department of Energy. Work at La Jolla was supported by grant NSF/DMR77-08469 from the National Science Foundation.

3 March 1980

Tumor-Promoting Phorbol Esters Stimulate Hematopoietic Colony Formation in vitro

Abstract. *Tumor-promoting phorbol esters stimulated mouse bone marrow cells to form myeloid colonies in agar cultures without added colony-stimulating factors. The colony-stimulating ability of various phorbol esters correlated well with their ability to promote skin tumors in vivo. These results suggest that phorbol esters mimic the action of specific colony-stimulating factors that regulate growth.*

Tumor-promoting phorbol diesters such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) have the ability to promote tumor formation in the skin of mice previously treated (initiated) with a suboptimal dose of a chemical carcinogen (*1*). Phorbol esters exhibit a wide variety of effects in vitro on cultured cells, such as stimulation of DNA synthesis and cell proliferation (*2–5*), either inhibition (*6–8*) or induction (*9–11*) of terminal differentiation, induction of plasminogen activator and other enzymes (*5, 12*), and changes in cell membrane properties (*13–15*). In many studies the phorbol esters seem to produce effects that are normally induced by the action of a natural growth regulator (*14*). For example, TPA mimics a number of the biologic effects produced by epidermal growth factor (EGF) and apparently does so by altering the function of cell surface receptors for EGF (*14, 15*).

One culture system in vitro that has a well-characterized requirement for a growth-regulating factor is the soft agar cloning technique for granulocyte-macrophage colony-forming cells, also called colony-forming unit culture (CFU-C) (*16*). In this system, mouse bone marrow cells form colonies of granulocytes and macrophages (or monocytes) only in the presence of added growth regulating factor, called colony-stimulating factor (CSF). Biologically active CSF has been purified from medium conditioned by L cells and appears to be a glycoprotein of molecular weight 65,000 (*17*). Many other active CSF preparations have been reported and partially characterized [for review, see (*18*)], but little is known about structural details of CSF molecules or their active sites. We report here that tumor-promoting phorbol esters (but not their inactive analogs) stimulate mouse bone marrow cells to form hematopoietic colonies in agar culture without added CSF.

Because of the suggestion that phorbol esters imitate the action of growth regulators (*14*), we tested their effect on agar cultures of mouse bone marrow cells. Figure 1 shows that freshly isolated mouse bone marrow cells formed colonies in soft agar in the presence of $5 \times 10^{-8} M$ TPA (without added CSF). The TPA-stimulated colonies were quite uniform, discrete aggregates of 50 to 500 cells; by contrast, colonies stimulated by L cell–derived CSF (LC-CSF) (*17*) were more loosely arranged aggregates of 50 to 10,000 cells. Figure 1 shows that with increasing numbers of marrow cells plated, there were increasing numbers of colonies formed, although for TPA-stimulated colonies, linearity was observed over a more narrow range of cell numbers than for LC-CSF–stimulated colonies. Figure 1 also shows that the target cell for the TPA effect was abundantly present in the marrow nonadherent cell fraction. Depleting the marrow population of cells that adhere to plastic at 37°C enriched the sample for TPA-stimulated colony-forming cells to about the same extent as for LC-CSF colony-forming cells, consistent with the possibility that the target cell for the TPA effect is the same as that for LC-CSF.

To characterize the cells in the TPA-stimulated colonies we removed intact single colonies by aspiration into micropipettes and transferred them to glass slides. After air-drying and staining them with aceto-orcein, we examined the colony cells microscopically (*19*). In addition, using other cultures, we dehydrated the agar matrix and stained the colony cells in situ and then examined them under an inverted microscope (*20*). In three independent experiments, 80 to 90 percent of colonies were entirely composed of cells with the characteristics of mature macrophages, and 10 to 20 percent of colonies also contained a variable percentage of monocytes and neutrophils.

To harvest large numbers of colony cells for further examination, we also cultured fresh marrow cells in methylcellulose medium (*21*) containing $10^{-7} M$ TPA. Wright-stained preparations were made of cells harvested from these cultures by dilution and washing. In differential counts of 200 cells on slides prepared from six cultures we found that 81 ± 4 percent were of the monocyte-macrophage type; remaining cells were neutrophils or immature myeloid cells. When harvested colony cells were tested further, more than 60 percent showed firm adherence to plastic petri dishes in 1 hour at 37°C. Colony cells were also

0036-8075/80/0425-0402$00.50/0

phagocytic for latex particles with 74 percent showing phagocytosis after 1 hour and 90 percent after overnight incubation at room temperature. These functional properties are typical of the monocyte-macrophage cells that make up LC-CSF-stimulated colonies. Therefore, although the TPA-stimulated colonies differ from LC-CSF-stimulated colonies in size and shape, the cells within TPA-stimulated colonies appear to be morphologically and functionally identical to the cells of LC-CSF-stimulated colonies.

Unfractionated and nonadherent marrow cells were also cultured in soft agar in the presence of a wide range of concentrations of TPA and related phorbol compounds with varying tumor-promoting ability. Figure 2 shows that TPA-stimulated colony formation over a wide dose range with effects observed at concentrations as low as $10^{-11}M$. In addition, although the parent compound, phorbol, and 4α-phorbol-12,13-didecanoate (4α-PDD) were inactive, other phorbol esters were capable of stimulating colony formation over similar dose ranges (Fig. 2). Phorbol-12,13-didecanoate (PDD) was only slightly less active than TPA, phorbol-12,13-dibenzoate (PDB) had intermediate activity, and phorbol-12,13-diacetate (PDA) had slight activity but only at $10^{-6}M$. The relative activity of the phorbol esters in stimulating marrow colony formation (TPA > PDD > PDB > PDA > 4α-PDD, phorbol) correlates well with their tumor-promoting potency in vivo and with their activity in other systems in vitro (*4, 5, 10, 11, 14, 15*).

These studies show that unfractionated and nonadherent murine bone marrow cells generate colonies of cells when cultured in semisolid media in the presence of low concentrations of TPA and other phorbol esters with tumor-promoting activity. The colony cells appear to be predominantly phagocytic mononuclear cells of the monocyte-macrophage type. Comparison with mouse marrow colony formation in the presence of L cell-derived CSF suggests that the cells forming colonies in the presence of TPA are of the CFU-C type, or a subpopulation of CFU-C primarily committed to macrophage differentiation. Supporting this interpretation, we found (*22*) that TPA-stimulated colony formation is inhibited by low concentrations of prostaglandin E_1, a substance that has recently been shown to specifically inhibit monocyte-macrophage colony formation by mouse bone marrow cells stimulated by LC-CSF and other sources of CSF (*23*). Also, TPA and other active analogs are mitogenic for mouse peritoneal macrophages (*24*), indicating effects on mature progeny as well as progenitor cells.

There are at least three possible mechanisms by which the tumor-promoting phorbol esters might stimulate bone marrow colony formation. One possibility is that these compounds may have direct colony-stimulating activity; that is, that they act directly on progenitor cells to induce cellular proliferation and differentiation. If this were the case, the known structures of these compounds might provide valuable clues to the conformation of the more complex glycoprotein CSF molecules. A second possible mechanism is that the active phorbol esters may interact with marrow-derived cells to induce the elaboration and secretion of glycoprotein CSF which then stimulates progenitor cells to proliferate. This seems unlikely because we have found that marrow cells in liquid culture fail to generate significant amounts of CSF when incubated for 1 to 7 days in appropriate concentrations of the active phorbol esters.

A third possibility is that the active phorbol esters may alter the responsiveness of progenitor cells to CSF which might be present in suboptimal concentration in the culture medium. Colony-stimulating factor is thought to act by binding to cell surface receptors and initiating regulatory signals (*25*). Our results suggest that the phorbol esters may interact with cell receptors to facilitate events that are triggered by the action of CSF on these receptors. The effects of the phorbol esters on CSF receptors need not be direct, of course, since more general alterations in cell surface membranes could alter receptor function. If such changes resulted in a marked increase in CSF receptor numbers or in their affinity for CSF, colony formation might be induced by trace amounts of CSF (present in the serum-containing medium) which under usual conditions

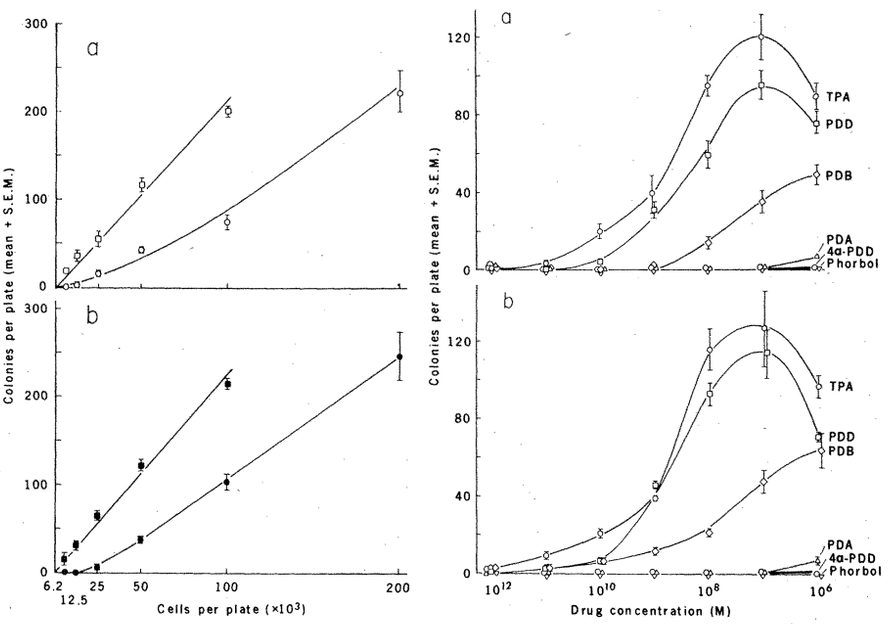

Fig. 1 (left). Effect of TPA on colony formation by mouse marrow cells. Unfractionated (a) or nonadherent (b) femoral marrow cells from 12-week-old B6D2F$_1$ female mice were suspended in supplemented McCoy's medium containing 0.3 percent agar and plated in 1.0-ml portions in 35-mm petri dishes containing TPA (final concentration of $5 \times 10^{-8}M$) or LC-CSF (0.1 ml of a 1:2 dilution, previously shown to cause maximum stimulation of colony formation). After 7 days of incubation at 37°C in a humidified atmosphere containing 5 percent CO_2, colonies of 50 or more cells were scored by means of a dissecting microscope at a magnification of ×25. The TPA, phorbol, and esters were stored at −70°C in dimethyl sulfoxide (DMSO), diluted in phosphate buffered saline (PBS) (*p*H 7.4) prior to use, and added to each plate in 0.1-ml portions. Solvent (DMSO) controls gave no colony formation. Nonadherent cells were obtained by allowing marrow cells in supplemented McCoy's medium to adhere to plastic petri dishes at 37°C for 1 hour. Nonadherent cells were collected by washing and subjected to a second adherence step in the same manner. Symbols: ○ and ●, TPA; □ and ■, LC-CSF. The results shown are the means and standard errors (*S.E.M.*) of triplicate determinations. Fig. 2 (right). Effect of phorbol and its esters on colony formation by mouse marrow. Unfractionated (a) and nonadherent (b) marrow cells were plated in supplemented McCoy's 0.3 percent agar medium in 35-mm petri dishes. The various compounds were diluted in PBS and added to the plates at the final concentrations indicated. After 7 days, colonies of 50 or more cells were scored. Symbols: ○, TPA; □, phorbol-12,13-didecanoate (*PDD*); ◇, phorbol-12,13-dibenzoate (*PDB*); △, phorbol-12,13-diacetate (*PDA*); ⬡, 4α-phorbol-12,13-didecanoate (4α-*PDD*); ▽, phorbol. The results shown are the means and standard errors of triplicate determinations.

are insufficient for colony formation. Such a hypothesis is also consistent with the work of Dicker and Rozengurt (*26*) who showed that there is a marked synergistic interaction between TPA and growth-promoting polypeptides (EGF, insulin, fibroblast-derived growth factor) in stimulating DNA synthesis in mouse and human fibroblasts in serum-free medium.

The hypothesis that tumor-promoting phorbol esters interact with (or at least alter the function of) cell surface receptors for regulatory growth factors is attractive because it suggests a possible basis for tumor promotion in vivo. The phenotypic expression of a carcinogenic event depends on the transformed cell's ability to proliferate. If tumor promoters render cell surface receptors more sensitive to physiologic growth factors—including proliferative stimuli—then previously initiated cells are more likely to express their carcinogenic potential in the presence of a promoter. In addition, if such a mechanism can be further substantiated, the tumor promoters may prove valuable in understanding the role of growth regulators in the control of normal cellular proliferation.

ROBERT K. STUART
Oncology Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
JOHN A. HAMILTON
Developmental Hematopoiesis Section, Sloan-Kettering Institute, New York 10021

References and Notes

1. T. J. Slaga, A. Sivak, R. K. Boutwell, Eds., *Mechanisms of Tumor Promotion and Cocarcinogenesis* (Raven, New York, 1978); R. K. Boutwell, *Crit. Rev. Toxicol.* **2**, 419 (1974).
2. A. Sivak, *J. Cell. Physiol.* **80**, 167 (1972).
3. A. L. Baynton, J. F. Whitfield, R. J. Isaacs, *ibid.* **87**, 25 (1976); J. I. Whitfield, J. P. McManus, D. J. Gillan, *ibid.* **82**, 151 (1973).
4. R. Peterson, S. Mondal, D. W. Brankow, W. Thon, C. Heidelberger, *Cancer Res.* **37**, 3223 (1977).
5. S. H. Yuspa, V. Lichti, T. Ben, E. Patterson, H. Hennings, T. J. Slaga, N. Coburn, W. Kelsey, *Nature (London)* **262**, 402 (1976).
6. G. Rovera, T. G. O'Brien, L. Diamond, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2894 (1977); L. Diamond, T. G. O'Brien, G. Rovera, *Nature (London)* **269**, 247 (1977).
7. H. Yamasaki, E. Fibach, U. Nudel, I. B. Weinstein, R. A. Rifkind, P. A. Marks, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3451 (1977); D. N. Ishii, E. Fibach, H. Yamasaki, I. B. Weinstein, *Science* **200**, 556 (1978).
8. R. Cohen, M. Pacific, N. Rubinstein, J. Biehl, H. Holtzer, *Nature (London)* **266**, 538 (1977).
9. R. M. Miao, A. H. Fieldsteel, D. W. Fodge, *ibid.* **274**, 271 (1978).
10. E. Huberman and M. F. Callaham, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1293 (1979).
11. G. Rovera, T. G. O'Brien, L. Diamond, *Science* **204**, 868 (1979).
12. M. Wigler and I. B. Weinstein, *Nature (London)* **259**, 232 (1976).
13. C. Wenner, J. Hackney, H. Kimelberg, E. Mayhew, *Cancer Res.* **34**, 1731 (1973).
14. L.-S. Lee and I. B. Weinstein, *Science* **202**, 313 (1978).
15. M. Shoyab, J. E. DeLarco, G. J. Todaro, *Nature (London)* **279**, 387 (1979).
16. T. R. Bradley and D. Metcalf, *Austr. J. Exp. Biol. Med. Sci.* **44**, 287 (1966); D. H. Pluznik and L. Sachs, *J. Cell. Comp. Physiol.* **66**, 319 (1965).
17. E. R. Stanley and P. M. Heard, *J. Biol. Chem.* **252**, 4305 (1977). The L cell-derived CSF used in our experiments was partially purified from L cell-conditioned medium by Sephadex G-50 gel filtration.
18. D. Metcalf, *Hematopoietic Colonies: In Vitro Cloning of Normal and Leukemic Cells* (Springer-Verlag, New York, 1977), pp. 67–79.
19. ———, *ibid.*, pp. 69–70.
20. The contents of the culture dish were fixed by adding 1.0 ml of buffered 5 percent glutaraldehyde for 6 minutes, and dehydrated overnight by adding 2 ml of absolute methanol. The resulting thin disk was washed with distilled water and stained with hematoxylin.
21. P. A. Chervenick and D. R. Boggs, *Blood* **37**, 131 (1971). Marrow cells were plated in supplemented McCoy's medium containing 0.8 percent methylcellulose instead of agar and stimulated by $10^{-7}M$ TPA. After 7 days of incubation, as for agar cultures, the colony cells were harvested by diluting the contents of the dish with medium and washing the cells.
22. R. K. Stuart, J. A. Hamilton, L. L. Sensenbrenner, M. A. S. Moore, in preparation.
23. N. Williams, *Blood* **53**, 1089 (1979).
24. J. A. Hamilton and S. Dientsman, in preparation.
25. E. R. Stanley and L. J. Guilbert, in *Mononuclear Phagocytes—Functional Aspects*, R. van Furth, Ed. (Martinus-Nijkoff, The Hague, in press).
26. P. Dicker and E. Rozengurt, *Nature (London)* **276**, 723 (1978).
27. We thank M. A. S. Moore for encouragement and financial support, A. Bootes for technical assistance, and R. Eger for providing partially purified CSF. Supported by NIH-NCI core grant CA06973, NSF grant PCM7709114, NIH-NCI grant CA19052, and NIH-NCI core grant CA08748.

8 November 1979; revised 14 January 1980

Hydralazine-Pyrimidine Interactions May Explain Hydralazine-Induced Lupus Erythematosus

Abstract. *Hydralazine, the prototypic drug that induces systemic lupus erythematosus, reacts with thymidine and deoxycytidine. Analysis of a reaction mixture of therapeutic concentrations of hydralazine with labeled thymidine reveals at least four labeled products. At higher concentrations, hydralazine reacts with labeled deoxycytidine to form at least three labeled products. Formation of these products is markedly enhanced by exposure to ultraviolet light. The reaction of hydralazine with thymidine and deoxycytidine may be in part responsible for initiating drug-induced systemic lupus erythematosus.*

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown origin that occurs in humans and predominantly affects females (*1*). The clinical manifestations of SLE include fever, photosensitivity, polyarthralgia, polyserositis (pleuritis and pericarditis), anemia, and cutaneous, renal, and neurologic abnormalities (*1*). About 10 percent of the cases of SLE are induced by one of the following drugs: hydralazine, procainamide, isoniazid, chlorpromazine, or the hydantoins (*1–4*). Drug-induced SLE may be clinically and serologically similar to idiopathic SLE (*2, 5*). In general, however, renal and neurologic disease are rare in drug-induced SLE. Cessation of drug therapy is frequently followed by complete remission. Similarities between drug-induced and idiopathic SLE are much greater than differences, however, and it has been argued that they are the same disease (*3, 6*). Study of possible molecular mechanisms of drug-induced SLE may thus be important for understanding the entire spectrum of this disorder.

Tissue injury in idiopathic and drug-induced SLE is presumed to be mediated by antibodies to either nucleic acids or nucleoproteins or to both (*7, 8*). The mechanism of production of antibodies

Table 1. Hydralazine-thymidine reaction. Hydralazine ($10^{-5}M$; Sigma Chemical) and [methyl-^{3}H]thymidine ($1.6 \times 10^{-6}M$; New England Nuclear) were mixed in a solvent of $1.5 \times 10^{-3}M$ NaCl in a buffer of $1.5 \times 10^{-4}M$ sodium citrate (*p*H 7.4) and incubated in total darkness at 35°C without agitation or aeration. Separation of the reaction mixture after 24, 48, 72, 96, and 120 hours by thin-layer cellulose chromatography with a solvent of *n*-butanol, acetic acid, and water (5:2:3 by volume) yielded at least five radioactive spots with R_F values of 0.70, 0.74, 0.78, 0.83, and 0.88. Thymidine has an R_F of 0.70. The reaction was studied at *p*H 5.9, 6.4, 6.9, 7.4, 7.9, and 8.4 at a 10^{-1} molar concentration of hydralazine and $1.6 \times 10^{-6}M$ thymidine to determine its *p*H optimum. The yield of product was optimal at *p*H 6.9 and 7.4; N.D., not detectable.

Reaction time (hours)	Product (%)			
	1 R_F 0.74	2 R_F 0.78	3 R_F 0.83	4 R_F 0.88
12	0.4	N.D.	N.D.	N.D.
24	1.1	0.1	0.2	0.1
48	1.3	0.1	0.2	0.1
72	1.8	0.2	0.3	0.2
96	3.1	0.9	0.7	0.4
120	5.3	2.0	1.2	1.1

0036-8075/80/0425-0404$00.50/0