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

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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

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to nucleic acid and nucleoproteins is obscure. There are at least two possible explanations for the formation of these antibodies in drug-induced SLE. Drugs or their products (i) may modify host nuclear material and increase its immunogenicity (*9, 10*) or (ii) may interact primarily with nuclear material in the cells of the host's immune system, mutating the DNA and thereby overcoming immunological tolerance. Chemical interaction of an SLE-inducing drug with DNA could be the basis for the pathogenesis of drug-induced SLE and some instances of idiopathic SLE. We have, therefore, examined interactions of hydralazine with the nucleosides of DNA.

Substances may interact with DNA either noncovalently or covalently, or in both ways. Hydralazine interacts noncovalently with DNA (*10–12*), but its structure suggests that its hydrazine group also should react covalently. Hydrazine specifically reacts with the pyrimidine bases of DNA but not with the purine bases (*13, 14*). The rate and extent of the reaction of hydrazine and DNA are optimal under anhydrous conditions, but the reaction proceeds in hydrazine hydrate (*15*). Pyrimidines incubated with 5*M* hydrazine in aqueous medium (*p*H 8.0 at 21°C) with a copper catalyst and continuous aeration disappeared from the reaction mixture at the following half-times: thymine, 4 minutes; uracil, 10 minutes; and cytosine, 24 hours (*15*). The primary product formed from thymine appeared to be dihydrothymine.

[Methyl-^{3}H]thymidine was mixed with therapeutic concentrations of hydralazine (*16*) (Table 1). Thin-layer chromatography of the reaction mixture at 12 hours revealed at least two radioactive spots with R_F values of 0.70 and 0.74 (Table 1). Unreacted thymidine was the slower moving spot. The number of products detected increased with the duration of incubation. Thin-layer chromatography at 24 hours showed at least five radioactive spots with R_F's of 0.70, 0.74 (product 1), 0.78 (product 2), 0.83 (product 3), and 0.88 (product 4) (Table 1). The number of radioactive products did not change from 24 to 120 hours, but the amount of labeled thymidine converted to radioactive products increased markedly. When measured at 120 hours, 5.3 percent of [methyl-^{3}H]thymidine was converted to product 1, 2.0 percent to product 2, 1.2 percent to product 3, and 1.1 percent to product 4 (Table 1). Thus, hydralazine at therapeutic concentrations reacts with thymidine to form substantial quantities of modified nucleoside products.

Hydralazine-induced SLE is frequently exacerbated and perhaps in some instances initiated by exposure to ultraviolet light (*1, 17*). Further, in approximately 50 percent of patients idiopathic SLE flares up with exposure to the sun (*1*). When a hydralazine-thymidine mixture was exposed to sunlight for 3 hours, the yield of product increased substantially from that obtained in darkness (Table 2). The relative quantity of product 1 increased by a factor of 3.2, product 2 by 3.8, product 3 by 3.6, and product 4 by 5.3.

The wavelength dependence of this photochemical reaction was examined by irradiating mixtures of hydralazine and thymidine with light generated from lasers (Table 2). Approximately 1.8×10^{18} photons were absorbed at each of the wavelengths studied except those at 488 and 632 nm, since hydralazine absorbed a minimum of light above 350 nm. The yields of products 1, 2, 3, and 4 were increased substantially when the reaction mixtures were irradiated with light at wavelengths from 285 to 315 nm (Table 2). The relative efficiency of the photoreaction was greatest at the lowest wavelength used (285). No effect could be demonstrated when the reaction mixture was irradiated with light of 488 and 632 nm. We have, therefore, shown that the hydralazine reaction with thymidine is significantly enhanced by incident ultraviolet light.

Hydrazine hydrate reacts to a limited extent with cytosine and not at all with purines (*15*). The reactivity of hydralazine with deoxycytidine and deoxyadenosine was tested. No reaction of deoxyadenosine ($1.0 \times 10^{-7}M$) with hydralazine ($1.0 \times 10^{-1}M$) could be demonstrated when the mixture was incubated for 28 days at 35°C with or without irradiation from fluorescent light. The conditions

Table 2. Photochemistry of the hydralazine-thymidine reaction. Absorbance characteristics of hydralazine were studied in the spectra extending from 240 to 600 nm. Hydralazine absorbs maximally at 260 nm. Absorption of approximately one-half that magnitude is observed at 306 and 315 nm. There is minimal, if any, absorption of hydralazine from 350 to 600 nm. Thymidine absorbs maximally at 260 nm and minimally above 300 nm. Hydralazine ($10^{-2}M$) and thymidine ($1.6 \times 10^{-6}M$) were mixed in a solvent, as given in Table 1, at 23°C for 3 hours.

Wavelength (nm)	Product (%)			
	1 R_F 0.74	2 R_F 0.78	3 R_F 0.83	4 R_F 0.88
		Control (darkness)		
	1.8	0.6	0.5	0.3
		Sunlight		
	5.8	2.3	1.8	1.6
		Light generated from lasers		
285	6.5	3.7	2.9	2.6
290	6.7	3.3	2.5	2.2
295	6.3	3.1	2.6	2.0
300	5.0	2.5	2.5	2.4
306	5.1	2.3	1.9	1.3
310	5.1	2.5	1.8	1.4
315	5.7	3.3	2.2	1.5
488	1.7	0.7	0.6	0.3
632	1.6	0.6	0.4	0.3

Table 3. Hydralazine-deoxycytidine reaction. Hydralazine ($10^{-1}M$) and [5-^{3}H]deoxycytidine ($1.0 \times 10^{-6}M$; New England Nuclear) were mixed in a solvent, as given in Table 1, at 35°C without agitation or aeration. Mixtures were reacted in total darkness (D) or in the presence of fluorescent light (L) (Ken Rad F15T8/CW). The mixture was separated in a solvent of *n*-butanol, acetic acid, and water (5:2:3). Thin-layer chromatography after 96 hours of incubation demonstrated at least four spots with R_F values of 0.66, 0.81, 0.85, and 0.92. Deoxycytidine had an R_F of 0.66.

Reaction time (days)	Condition	Product (%)		
		1 R_F 0.81	2 R_F 0.85	3 R_F 0.92
4	D	0.12	0.08	0.10
	L	0.16	0.13	0.23
11	D	0.18	0.19	0.24
	L	0.23	0.20	0.30
21	D	0.19	0.19	0.31
	L	0.23	0.21	0.32
28	D	0.19	0.20	0.31
	L	0.24	0.22	0.32

were the same as described for deoxycytidine (Table 3). However, products could be detected when hydralazine ($1.0 \times 10^{-1}M$) and [5-^{3}H]deoxycytidine ($1.0 \times 10^{-6}M$) were incubated for 4 days (Table 3). Thin-layer chromatography of the reaction mixture demonstrated at least four radioactive spots with R_F values of 0.66, 0.81, 0.85, and 0.92. The slowest moving spot was deoxycytidine. This reaction of deoxycytidine with hydralazine proceeded at a considerably slower rate than that of hydralazine with thymidine (Table 1). The reaction with deoxycytidine was similar to the reaction with thymidine in that the rate was increased by light irradiation (Table 3).

We have shown that hydralazine alters the pyrimidine bases of DNA. Modified DNA can be highly immunogenic (*18*). Thus, it is suggested that the interaction of SLE-inducing drugs with DNA can lead to a marked enhancement of the immunogenicity of DNA. An increase in the immunogenicity of DNA could explain drug-induced antibodies to nucleic acids and perhaps even to DNA. It is also possible that these drugs modify DNA of B cells or suppressor cells, thus altering the antibody response.

Studies on drug-induced SLE may provide clues to understanding idiopathic SLE. It is possible that some cases of idiopathic SLE may be induced by the unsuspected and undetected ingestion of drugs or exposure to chemical agents in the environment. For example, hydrazines are found in tobacco, tobacco smoke (*19*), and mushrooms (*20*); synthetic hydrazines are used in industry, agriculture, and medicine. Synthetic and naturally occurring compounds containing hydrazine that are present in the environment may induce disease in people with a proper genetic predisposition.

LEWIS M. DUBROFF
ROBERT J. REID, JR.
Department of Medicine,
State University of New York,
Syracuse 13210

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Allele Increasing Susceptibility to Human Breast Cancer May Be Linked to the Glutamate-Pyruvate Transaminase Locus

Abstract. *The patterns of the occurrence of breast cancer in 11 high-risk families were evaluated by segregation and linkage analysis. These patterns were consistent with the hypothesis that increased susceptibility to breast cancer was inherited as an autosomal dominant allele with high penetrance in women. The postulated susceptibility allele in these families may be chromosomally linked to the glutamate-pyruvate transaminase (E.C. 2.6.1.2, alanine aminotransferase) locus. Confirmation of this linkage in other families would establish the existence of a gene increasing susceptibility to breast cancer. Since there is no association in the general population between a woman's glutamate-pyruvate transaminase genotype and her cancer risk, the glutamate-pyruvate transaminase linkage cannot be used as a screening test for breast cancer.*

Probably the single factor most dramatically increasing the risk of breast cancer is the presence of the disease in the immediate family, particularly if more than one relative has had breast cancer, or if the relative was affected bilaterally or at a young age (*1*). However, this increased risk may be due to social, dietary, or environmental factors that predispose the family to breast cancer, or to inherited factors that increase susceptibility to breast cancer. Whether the pattern of breast cancer in some families is consistent with Mendelian segregation of a "breast cancer susceptibility" gene, and whether this gene is physically (chromosomally) linked to a known, clinically innocuous, genetic marker locus have not previously been determined.

We have surveyed a registry of families that were recruited, or that had volunteered, for studies in cancer genetics because breast cancer was frequent in each. We have assumed that in these families the incidence of breast cancer was high and have tested the consistency of this elevated incidence with the pattern expected for a genetically influenced disease (*2*). Four models for transmission of susceptibility to breast cancer were tested to determine which best fit the observed patterns of breast cancer. These models postulated Mendelian inheritance of increased susceptibility to breast cancer through an autosomal dominant allele, an autosomal recessive allele, an X-linked dominant allele, or higher risk of breast cancer for all women in a family due solely to shared environmental factors. For each model, we assumed that men could not be affected with breast cancer, although they could carry and transmit susceptibility (*3*).

The autosomal dominant model for inheritance of breast cancer susceptibility best fit the observed distribution of breast cancer in each of the 11 families ($P > .05$ for rejection by the likelihood ratio test). The environmental model was clearly rejected ($P < .03$ for each family). The autosomal dominant model was up to 1500 times more likely than the recessive model and up to 7000 times more likely than the X-linked model. However, since neither the autosomal recessive nor the X-linked dominant models could be consistently rejected, all three genetic models were used in subsequent linkage analysis. The good fit of the autosomal dominant model corresponds to several observations that clinicians had made about these 11 families: occurrence of breast cancer in several generations, apparent transmission of susceptibility by (unaffected) fathers to their daughters, little family history of breast cancer among most individuals marrying into the pedigree, virtual absence of unaffected mothers with affected daughters, and appearance of breast cancer in about 50 percent of the daughters of breast cancer patients (*4*).

The consistency of a genetic model with the observed pattern of breast cancer in a family has to be interpreted cautiously, since a number of nongenetic

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