those of young women who are not genetically susceptible. If we can determine how susceptibility genes for breast cancer are expressed, we can begin to investigate how to alter this expression to modify preclinical developments in the natural history of breast cancer.

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2. All families from the registry with at least three cases of breast cancer in mothers and daughters or sisters, for whom cancer diagnosis could be confirmed by pathology review, and with relatives from at least three generations available for sampling, were included in the investigation. Prior to genetic analysis, the 21 large families who met these requirements were classified into three groups on the basis of two epidemiologic criteria: ages at diagnosis of breast cancer patients and sites of other cancers present at higher than expected frequencies. Group 1: 11 families reported herein with breast and ovarian cancer or only breast cancer in excess (average age at diagnosis, 48 years); group 2: eight families with frequent postmenopausal breast cancer and endometrial cancer (average age at breast cancer diagnosis, 66 years); group 3: two families with breast cancer in very young women (average age, 34 years) and high frequencies of adrenal-cortical carcinoma, leukemia, brain tumors, and sarcomas. The occurrence of ovarian cancer among close relatives of breast cancer patients in four of the group 1 families led us to postulate a common susceptibility to breast and ovarian cancer in those families. More than 80 percent of breast cancers and all ovarian cancers were verified by pathology review. [H. T. Lynch, R. E. Harris, H. A. Guirgis, K. Maloney, L. L. Carmody, J. F. Lynch, *Cancer* **41**, 1543 (1978).]
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4. The risk of breast cancer to a genetically susceptible woman in these 11 families before age 35 is about 12 percent, before age 50 about 50 percent, and before age 80 about 87 percent. Women in the pedigree who do not carry a susceptibility allele are at no increased risk of breast cancer (M. C. King, R. C. P. Go, R. C. Elston, H. T. Lynch, N. L. Petrakis, *Prev. Med.*, in press).
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Survival of Mice Receiving Melanoma Transplants Is Promoted by Hydroquinone

Abstract. *In BALB/c female mice with melanoma transplants, the incidence of "takes" is decreased and survival is increased by hydroquinone, a melanocytolytic agent. The mechanism of drug action is suggested to be via DNA. The significant and high degree of positive response to hydroquinone treatment in vivo is encouraging for the clinical management of melanoma with melanocytolytic agents.*

Melanoma occurs in all known human populations, in both sexes and in all age groups; the incidence is highest in the Caucasoid population (*1*). Among the most malignant of human tumors, melanoma composes 1 to 2 percent of the total cancer incidence and about 20 percent of all skin cancer. Generally, the prognosis is poor; the best available clinical procedures, individually or in combination, offer only a limited degree of temporary relief (*2*). However, a number of agents have been found to selectively destroy normal integumental melanocytes in vivo (*3–7*). These melanocytolytic (depigmentary) agents may detrimentally affect abnormal melanocytes (melanoma cells); encouraging findings have been reported for some of these agents (*8, 9*). Here we report the effects in vivo of one such melanocytolytic agent, hydroquinone, on mice receiving melanoma transplants.

The NIH Harding Passey melanoma was grown in BALB/c female mice as follows. On day zero, 0.1 ml of melanoma brei (12 g of melanoma plus 10 ml of 0.9 percent saline) was injected subcutaneously in the right axilla to form the transplants. Following an NIH screening procedure (*10*), we injected subcutaneously either hydroquinone or 0.9 percent saline vehicle once daily on days 1 through 9. The animals were injected at 15 to 17:30 hours daily to avoid possible diel variation. The control and experimental groups are indicated in Table 1. After the initial 9 days of chemotherapy the mice received no further treatment. They were examined daily for the 140-day experimental period, after which time the surviving mice were killed. Autopsies were performed on all mice, and the melanomas were weighed. For statistical analysis of survival we used the log-rank method of life table analysis (*11*) which has been applied previously to clinical trials in cancer chemotherapy (*12*). Other data were analyzed by Student's t-test.

Comparison of the median survival times (Table 1) reveals that all of the control groups not implanted with melanoma have median survival times exceeding 140 days (the length of the study). Further, only one of the melanoma-bearing groups (injected with 80 mg of hydroquinone per kilogram of body weight) has a median survival exceeding 140 days. Median survival for the other melanoma-bearing groups ranges from 94 to 96 days.

Comparison of the survival curves of the melanoma-bearing groups treated with 16 or 80 mg of hydroquinone per kilogram revealed a statistically significant difference in survival rates ($\chi^2 = 12.12$, d.f. = 1, $P < .0005$). The estimated probability of survival to 140 days was .725 for the group that received the high dose and .350 for the group that received the low dose.

The two melanoma-bearing control groups not receiving chemotherapy (no treatment; 0.9 percent saline) were not significantly different in survival ($\chi^2 = 0.02$, d.f. = 11, not significant). Data for these two control groups, therefore, were combined for subsequent analyses. A comparison of each of the two hy-

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droquinone-treated, melanoma-bearing groups with the combined melanoma-bearing control groups revealed that survival of the group that received the 16-mg dosage of hydroquinone was not significantly different ($\chi^2 = 0.69$, d.f. = 1, not significant); however, survival of the group that received 80 mg/kg was significantly different ($\chi^2 = 22.51$, d.f. = 1, $P < .0001$) from the controls. Even more interesting, comparison of the survival curve of normal female mice with that of the 80-mg group that received the dosage of hydroquinone (Fig. 1) revealed that the difference between these survival curves was of borderline significance ($\chi^2 = 3.95$, d.f. = 1, $P = .047$) at 140 days.

After tumor transplantation, the melanoma "takes" in the otherwise untreated and saline-injected groups were not statistically different, the incidence being 91.7 percent. In contrast, the incidence of successful transplantation was reduced with hydroquinone treatment, being 55.6 percent in the group that received 16 mg/kg and 23.7 percent in the group that received 80 mg/kg. However, the tumor weights of all groups (Table 1) were not significantly different. Thus, the growth of the melanoma cells that survived was similar to that of the untreated cells.

In these experiments hydroquinone treatment decreased melanoma incidence and increased host survival. The mechanism of hydroquinone action is unknown. However, radioactively labeled hydroquinone is rapidly taken up (< 30 minutes) and turned over (biological half-life < 2 hours) by the skin (*13*). Further, the drug affects integumental melanocytes leaving other cell types morphologically unaffected (*6, 7*). Melanoma melanocytes also are destroyed by hydroquinone both in vivo (*4*) and in vitro (*8, 9*).

These data suggest that the drug is rapidly taken up by melanocytes, both normal and abnormal. The lability of hydroquinone in solution further suggests a brief temporal period of drug action on these cells. The melanocyte response to hydroquinone may be rapid or slow, depending on the state of the cell at the time of exposure as well as on the parameter studied. Clearly, a rapid and dramatic alteration in a variable critical to melanocyte survival would provide mechanistic insight and a means of drug evaluation. In this regard, hydroquinone depresses Harding Passey melanoma DNA synthesis in vitro within 5 minutes, and at 4 hours DNA synthesis is 4 percent of that of controls (*9*). The total DNA synthesis in these hydroquinone-treated melanoma cells remains depressed at the same level during the entire experimental period (5 minutes to 4 hours), revealing the sensitivity of DNA to this quinone. The other reported (*9*) measures of macromolecular biosynthesis in this system are not affected as rapidly nor to the same degree as DNA. Thus, the effectiveness of such hydroquinone therapy appears to result from rapid and specific drug action on melanocytes and is detected as an interaction with DNA. A number of other anticancer drugs classified as quinones also interfere with DNA synthesis. This suggestion in regard to hydroquinone does not preclude the presence of additional mechanisms (*9, 14*). However, in view of the rapid and profound DNA effects, such additional mechanisms would seem to be secondary.

We found that hydroquinone (80 mg/kg) prolonged the survival of the melanoma-bearing mice almost to that of normal mice during the 140-day period of study. This increased survival may have resulted in part from the decreased incidence of tumors in the drug-treated group; however, two reports (*15*) indicate that several melanomas do not respond in vivo to a monomethyl ether derivative of hydroquinone. This derivative is insoluble in isotonic saline so that solubility may affect its action and use of a solubilizing vehicle may not completely overcome this problem after dilution of the injected material in vivo. Significantly, however, a number of soluble melanocytolytic agents originally described by Chavin and Schlesinger (*3*) have been used in different laboratories on different model melanocyte systems

Table 1. The effects of hydroquinone on female BALB/c mice that received transplants on day 0 of the Harding Passey melanoma. The observation period extended to 140 days after transplantation. The hydroquinone was injected once daily on days 1 to 9. The median lethal dose of hydroquinone is 160 mg/kg (*16*).

Treatment	Number of animals	Survival, 50th quantile (days)	Melanoma weight (g)*
	Control groups		
None	39	> 140	
Saline (0.9 percent)	10	> 140	
Hydroquinone (16 mg/kg)	10	> 140	
Hydroquinone (80 mg/kg)	10	> 140	
	Groups with melanoma transplants		
None	40	95	13.3 ± 1.4
Saline (0.9 percent)	40	94	14.7 ± 2.7
Hydroquinone (16 mg/kg)	40	96	15.8 ± 3.6
Hydroquinone (80 mg/kg)	40	> 140	14.2 ± 3.9

*Mean ± standard error.

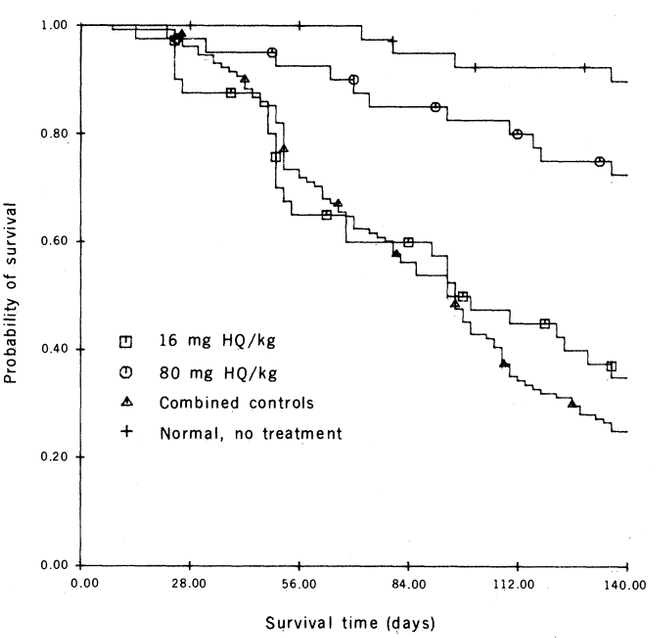

Fig. 1. Comparison of survival curves of female BALB/c mice receiving Harding Passey melanoma transplants (day 0) with and without a daily course of hydroquinone (HQ) chemotherapy (days 1 to 9), and untreated normal female mice. The group of mice receiving the low dose of hydroquinone (16 mg/kg) is not significantly different from the tumor-bearing control groups. However, the group of mice receiving the higher dose (80 mg/kg) shows both a significantly increased survival time and a lower tumor incidence than the tumor-bearing control groups. The survival of the group that received 80 mg/kg was almost comparable to that of the normal female mice. The data for all groups were obtained on a daily basis; the symbols are used only to identify the data of a given group.

and have been reported to be effective in their melanocytolytic action both in vivo and in vitro (*3–9*). It is clear, therefore, that melanocytolytic agents, if administered appropriately, may be helpful in the clinical management of malignant melanoma.

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Somatomedin C: Restoration in vivo of Cycle Traverse in G_0/G_1 Blocked Cells of Hypophysectomized Animals

Abstract. *DNA synthesis and mitosis in frog lens epithelium are abolished by hypophysectomy and restored by somatomedin C. Both growth hormone and triiodothyronine also restore lens cell proliferation in vivo but not in vitro. Somatomedin-like activity in frog serum is diminished by hypophysectomy and is restored by growth hormone and triiodothyronine.*

The somatomedins (*1*) are a family of growth hormone-dependent peptides, ancestrally related to insulin (*2*) and capable of inducing DNA synthesis and mitosis in cell culture systems (*3*). There have been no reports that these effects could be achieved in vivo since (i) the somatomedins are available in limited quantities and (ii) there has been no animal model in which DNA synthesis and mitosis could be totally but reversibly eliminated.

Three weeks after frogs are subjected to hypophysectomy, both mitotic and DNA synthetic activity disappear completely in the lens epithelium (*4*). Microspectrophotometric and autoradiographic analyses have shown that in the hypophysis-deprived frog almost all of the epithelial cells come to reside in the G_0/G_1 segment of the cell cycle (*5*). In organ cultures of lenses from frogs the addition of 15 to 20 percent normal frog serum to the medium (Medium-199) triggers division (*5, 6*); however, the addition of serum from hypophysectomized frogs does not stimulate cell proliferation (*5*).

When administered in vivo, growth hormone, frog prolactin, or triiodothyronine (T_3) restore or maintain the proliferative process in lenses of hypophysectomized animals (*7, 8*). However, these hormones fail to promote cell division in organ culture (*5, 7*). Growth hormone injected into hypophysis-deprived frogs rebuilds the mitogenicity of the serum, as judged by lens organ culture tests (*5*). The foregoing information suggests that a pituitary-dependent mitogen, such as somatomedin C (*2*), may be effective in frog lens epithelium. Since no division or thymidine incorporation occur 3 to 4 weeks after hypophysectomy and since every cell in a whole-mount preparation of the tissue can be observed by light microscopy, the frog lens is an ideal system with which to evaluate pertinent growth factors.

To assess the effects of human somatomedin C, we used newly postmetamorphic *Rana catesbeiana* (8 to 21 g); the blood volume of each frog was estimated to be 0.25 to 0.40 ml. For assays of somatomedin-like activity of serum, we used adult bullfrogs (*R. catesbeiana*) and leopard frogs (*R. pipiens*). We estimated the somatomedin-C content of various frog serums by a heterologous radioimmunoassay using a rabbit antiserum against human somatomedin C and human ^{125}I-labeled somatomedin C as the tracer (*9*). For the receptor assays of somatomedin C we used ^{125}I-labeled somatomedin C as the tracer and a human placental cell membrane preparation as the source of receptor (*10*).

Somatomedin C was purified from Cohn fraction IV of human serum (*11*). The weight of somatomedin C in this preparation was based on the somatomedin C content determined by radioimmunoassay and the known specific activity of the pure peptide. Surgical hypophysectomy was carried out by the procedure of Hogben (*12*). Human growth hormone (Calbiochem), purified somatomedin C, or Earle's balanced salt solution (the medium in which the hormones were dissolved) and [^{3}H]thymidine (specific activity 6.7 Ci/mmole) were injected into the dorsal lymph sac in a volume of 100 μl according to schedules to be detailed. On the day after the final injection the animals were killed and the enucleated eyes were fixed in Carnoy's fluid. Whole-mount preparations were made from the lenses (*13*). The preparations were autoradiographed (Kodak NTB3) and after 14 days of exposure were stained with Harris hematoxylin. Corneal endothelium was separated from the stroma and corneal epithelium. Both corneal endothelium and lens epithelium are avascular and share the aqueous humor as their principal, if not sole, source of nutrients. The serum for radioimmunoassay was derived from *R. catesbeiana* by puncture of the conus arteriosus or ventricle. The blood was allowed to clot and was then centrifuged; the samples were frozen at −20°C.

As shown in Fig. 1, normal (intact) adult bullfrog serum is only about 5 percent as potent as is human serum in competing with ^{125}I-labeled somatomedin C for binding to antibody. Furthermore, bullfrog and human serum give nonparallel displacement curves, although displacement curves of various samples of frog serum are parallel to each other. Serum from animals that had been hypophysectomized 1 month previously was 50 percent as potent as normal frog

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