mg), and codeine phosphate (30 mg) in man. Drugs were given with placebos in a "double-blind" investigation. Pain was induced by a high-frequency electronic stimulator applied to normal intact teeth. 2. Since the pain threshold end-point did not require activation of skeletal muscle, carisoprodol must have induced analgesia independently of its muscle relaxant action. 3. By this method, carisoprodol was 5 times more potent than acetylsalicylic acid in raising tooth pain threshold in man.

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Effect of Triethylene Thiophosphoramide (ThioTEPA) on Skin Pigmentation in Mice.* (26167)

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Hyperpigmentation of the skin of man and experimental animals following exposure to ultraviolet light or ionizing radiation is a commonly observed phenomenon. Radiation-induced hyperpigmentation has been shown to result from an increased amount of epidermal melanin associated with increased functional activity of the melanocytes in mouse(1) and human(2) skin. Similar results have been obtained in mice following local application of the carcinogenic hydrocarbon 5, 9, 10 - trimethyl - 1,2 - benzanthracene(3). During cancer chemotherapy studies in mice utilizing triethylene thiophosphoramide (ThioTEPA) it was observed that brown mice of the CBA and (RIII x CBA)F_1 hybrid strains undergoing long-term systemic administration of this drug developed a marked hyperpigmentation of the tail, ears, and extremities, similar to that described in mice exposed to chronic gamma-irradiation (1). The present study deals with the effects of ThioTEPA on skin pigmentation in various inbred strains of mice.

Methods and materials. ThioTEPA is the sulfur derivative of triethylene phosphoramide and is a member of the class of radiomimetic, tumor-inhibitory compounds known as alkylating agents. Dry crystalline ThioTEPA was dissolved in distilled water to make a concentration of 0.25 mg/ml and was refrigerated during the course of the experiment. Nine male and 9 female mice, 50 to 60 days old, of each of the following inbred strains were used:‡ DBA/2, Strong A, C3H, NH, Stoli, and (DBA/2 x NH)F_1 hybrids. Six males and 6 females of each strain were

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‡ Obtained from Kirschbaum Memorial Research Lab., Dept. of Anatomy, Baylor Univ. College of Medicine.

TABLE I. Effects of ThioTEPA on Skin Pigmentation in Various Strains of Mice.

Strain	Coat color	Sex	Gross hyperpigmentation	Microscopic hyperpigmentation of skin	
				Ear	Abdominal wall
DBA/2	Dilute brown (non-agouti)	♂	—	Moderate	—
		♀	—	"	—
Strong A	Albino	♂	—	—	—
		♀	—	—	—
(DBA/2 × NH) F_1 hybrid	Black	♂	+	Marked	—
		♀	+	"	—
Stoli	Dilute brown (agouti)	♂	—	—	—
		♀	—	Moderate	—
C3H	Brown (agouti)	♂	+	Marked	—
		♀	+	"	—
NH	Piebald (gray & white)	♀	—	Moderate	—

randomly selected to receive ThioTEPA, the remainder serving as controls. The treated mice received 0.002 mg/g body weight of ThioTEPA (approximately 0.05 mg), injected intraperitoneally, daily for 5 days per week until a total of 12 injections had been given. Each mouse was observed during this 16-day period for grossly detectable pigment changes. On the day following last injection of ThioTEPA all the animals were killed and specimens of ears, gonads, adrenals, and skin over the anterior abdominal wall were obtained for histologic study. These tissues were fixed in 10% neutral buffered formalin, and paraffin sections 6μ thick were made following routine histologic procedures. Sections were either stained with hematoxylin and eosin or studied unstained.

Results. All males of the NH strain treated with ThioTEPA died during the course of this study. Pigment changes noted in the mice receiving ThioTEPA are summarized in Table I. In no case were any changes observed in the controls. Darkening of the tail, ears, and extremities of the ThioTEPA treated C3H and (DBA/2 x NH)F_1 hybrid mice occurred from 10 to 14 days after injections were begun, and was observed in all treated mice of these strains. Microscopic examination of the ears of these animals revealed an increased amount of melanin pigment incorporated within epidermal cells as well as an apparent increase in number of melanocytes in the basal layer of the epidermis (Fig. 1). The number of dermal melanocytes was definitely increased as compared to controls. No significant difference was detected in the melanocyte activity of hair follicles. None of the treated mice of the other strains exhibited gross hyperpigmentation, although a moderately increased amount of melanin pigment within the epidermis was noted in histologic sections taken from DBA/2 mice and females of the Stoli and NH strains. No gross or microscopic pigmentary changes were seen in the anterior abdominal wall skin of any of the mice in this experiment. Histologic examination of the adrenals failed to demonstrate any abnormalities. However, pathologic changes were seen in the gonads of almost all animals subjected to ThioTEPA. The testes of all treated male mice showed severe destruction of the seminiferous tubules with a sparing of interstitial cells. Alterations in the ovaries of treated females were less consistent, varying from no changes in the Strong A strain to almost complete obliteration of graafian follicles and absence of normal ova in the C3H strain.

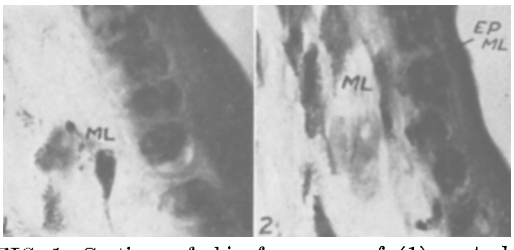

FIG. 1. Sections of skin from ears of (1) control and (2) ThioTEPA-treated C3H male mice showing melanocytes (ML) and melanin pigment deposited in cells of the epidermis (EP ML); × 507.

Discussion. ThioTEPA-induced hyperpigmentation of the extremities, tail, and ears of brown or black mice with sparing of the general body skin is similar to that described following total-body irradiation(1), both agents resulting in increased deposition of epidermal melanin and increased numbers of epidermal and dermal melanocytes. Presumably the greater number of melanotic melanocytes present in the extremities, tail, and ears(4) and the slower conversion of amelanotic to melanotic melanocytes in the general body skin(1) account for these findings. Szabó (5) has suggested that melanocytes of hair follicles can be stimulated with dimethylbenzanthracene and croton oil to function outside of their original environment. No significant increase in hair follicle melanocyte activity was detected in treated mice. The present study indicates, in addition, that similar microscopic changes occur to a lesser degree in mice of lighter coat color, but not in albinos. Further studies will determine whether ThioTEPA given in larger doses or for a longer time can cause gross hyperpigmentation even in these lighter coat-colored strains. The possible role of the adrenal or gonads in ThioTEPA-induced pigment changes is not clarified by these findings. All adrenals appeared normal histologically. All males and most females treated were apparently rendered sterile on the basis of histologic observations. It has been shown that ovariectomy will interfere with melanogenic activity(6).

Summary. Systemic administration of ThioTEPA caused the development of hyperpigmentation of the ears, tail, and extremities of brown or black mice, but not of mice of lighter coat color or albinos. These changes resulted from increased numbers of dermal and epidermal melanocytes and increased deposition of epidermal melanin. Similar microscopic changes were seen to a lesser degree in the lighter-colored mice, but not in albinos. Gross or microscopic evidence of hyperpigmentation of the general body skin was not found.

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Effect of Mycobacteria on Antibody Production Induced by Water-in-Oil Emulsions of Soluble Protein Antigens.* (26168)

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It has been well established that the sensitizing potency of tissue antigens in water-in-oil emulsions is augmented when mycobacteria are added. Thus, hypersensitivity states such as allergic encephalomyelitis, aspermatogenesis and thyroiditis are more readily produced when appropriate tissue antigens are incorporated into adjuvants containing mycobacteria than when the mycobacteria are omitted. On the other hand, the effect of mycobacteria on ability of antigens in emulsions to stimulate antibody formation is not clear. Addition of mycobacteria to water-in-oil emulsions containing viral, bacterial and

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