Additive Effects of Ultraviolet B and Crude Coal Tar on Cutaneous Carcinogen Metabolism: Possible Relevance to the Tumorigenicity of the Goeckerman Regimen

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The effect of cutaneous exposure to ultraviolet B (UVB) radiation alone, to crude coal tar (CCT) alone, and to the combination of UVB and CCT on the inducibility of the microsomal cytochrome P-450-dependent carcinogen-metabolizing enzyme aryl hydrocarbon hydroxylase (AHH) and other monooxygenases such as 7-ethoxyresorufin O-deethylase (ERD) and 7-ethoxycoumarin O-deethylase (ECD) activities in the skin of neonatal rats was studied. Exposure of the animals to UVB (400–1600 mJ/cm^2) alone resulted in a dose-dependent increase in cutaneous enzyme activities. At a UVB dose of 1200 mJ/cm^2 increases in AHH, ECD, and ERD were 194%, 115%, and 244%, respectively. A single topical application of CCT (10 ml/kg) 24 h before sacrifice resulted in significant induction of AHH (350%), ECD (921%), and ERD (796%) activities. Treatment of animals with the same dose of CCT followed by UVB exposure resulted in additive and/or synergistic effects on AHH (858%), ECD (1229%), and ERD (1166%) activities in the skin. In contrast, exposure of animals to UVB prior to CCT application had effects no different from those of CCT alone. Epoxide hydrolase and glutathione S-transferase activities in skin from all experimental groups were not different from those of controls. High-pressure liquid chromatographic analysis of the metabolism of benzo[a]pyrene (BP) by cutaneous microsomes prepared from animals treated with UVB alone, CCT alone, and the combination of UVB and CCT revealed increased formation of all the metabolites in each experimental group. The largest increase in metabolite formation occurred in animals receiving CCT followed by UVB exposure. The inducibility of *trans*-7,8-diol formation by UVB alone and CCT alone was 203% and 435%, respectively, whereas with CCT followed by UVB it was 1065%. The differential responses in AHH activity were found to parallel the capacity of skin microsomal enzymes to enhance the binding of [^{3}H]-BP to DNA. These studies indicate that the sequence of exposure to the components of the Goeckerman regimen in rodents greatly influences matabolic activity in skin. When applied in the same sequence employed in the Goeckerman regimen (CCT followed by UVB exposure) the additive effect upon catalytic activity essential for cancer initiation suggests a possible mechanism for the enhancement of human skin cancer in individuals exposed to this therapeutic regimen. *J Invest Dermatol 87:348–353, 1986*

The Goeckerman regimen is widely used for the treatment of psoriasis, a common dermatologic disease of the human population. This modality consists of the application of various formulations containing crude coal tar (CCT) to skin followed by exposure to ultraviolet B (UVB) radiation [1], both of which are known to be carcinogenic for the skin of experimental animals and of humans [2,3]. It is now clear that patients undergoing repeated Goeckerman therapy are at increased risk for the development of cutaneous cancer [4] whereas patients receiving intermittent infrequent courses of this modality have little or no enhanced risk of developing skin cancer [5]. Recent epidemiologic studies by Stern et al [6] confirm that psoriatic patients who receive multiple courses of Goeckerman therapy have an increased incidence of skin carcinoma compared to age- and sex-matched control populations.

CCT is an exceedingly complex mixture of chemicals that is rich in polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BP) [7]. The carcinogenic and mutagenic effects

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Abbreviations:
AHH: aryl hydrocarbon hydroxylase
BP: benzo[a]pyrene
CCT: crude coal tar
7-EC: 7-ethoxycoumarin
ECD: 7-ethoxycoumarin O-deethylase
EH: epoxide hydrolase
ERD: 7-ethoxyresorufin O-deethylase
GSH: glutathione (reduced)
GST: glutathione S-transferase
HPLC: high-pressure liquid chromatography (-ic)
NADPH: nicotinamide adenine dinucleotide phosphate (reduced)
3-OHBP: 3-hydroxybenzo[a]pyrene
7-OHC: 7-hydroxycoumarin
PAH: polycyclic aromatic hydrocarbon
RF: resorufin

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of CCT and certain of its constituent chemicals in experimental systems have been well documented [3,7–9]. Saperstein and Wheeler [10] have demonstrated in vitro mutagenic responses to CCT. In subsequent studies Wheeler et al [11] have shown the presence of unidentified mutagenic substance(s) in the urine of patients treated with topically applied 5% CCT. Recent studies by Storer et al [12] have shown that following topical application of 2% CCT to the skin of human volunteers certain PAHs including phenanthrene, anthracene, pyrene, and fluoranthene can be identified in blood extracts.

The carcinogenic PAHs themselves are relatively inert biologically and essentially act as precarcinogens that must first undergo metabolic activation by mammalian enzymes which convert them into their biologically active ultimate carcinogenic metabolites [13–15]. These metabolites are highly reactive, unstable moieties that can bind covalently to cellular RNA, DNA, and proteins, as well as to synthetic copolymers of nucleic acids [13–15]. It is generally agreed that the covalent interaction of reactive metabolites of the PAHs with DNA may represent an essential initial step in tumor induction [13–15]. Studies during the past decade have identified one of the isomeric metabolites of BP known as (+)-7β,8α-dihydroxy-9α-10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene as the ultimate carcinogenic species of this PAH [13–15]. The formation of this metabolite is catalyzed by 3 successive enzymatic steps: first, hydroxylation by aryl hydrocarbon hydroxylase (AHH), a cytochrome P-450-dependent mixed-function oxidase, followed by epoxide hydrolase (EH) and again by AHH [13–15]. The reactive epoxide intermediates are detoxified by conjugation with glutathione, a reaction catalyzed by glutathione (GSH) transferases [13–15]. These enzymatic pathways for the metabolic activation and inactivation of PAHs have been identified in the skin [16,17].

Studies from our laboratory have shown that topical application of CCT to animals [18–20] and to humans [21] results in the induction of cutaneous AHH activity. We have recently shown that the use of CCT-containing shampoo induces AHH activity and the DNA binding of PAHs in human hair follicles [22]. Highly inducible AHH activity appears to correlate with enhanced risk of tumor susceptibility to PAHs in several inbred mouse strains [23]. Since each of the major components of the Goeckerman regimen (CCT and UVB) are known to be carcinogenic in mammalian skin we wondered whether the combination might have an effect upon carcinogen metabolism. In the study described here, we have attempted to mimic the Goeckerman therapy of psoriasis in the skin of experimental animals. Our results indicate that skin exposure to the components of the Goeckerman regimen, in the sequence in which they are used therapeutically, may have an additive enhancing effect on the metabolic activation of PAH carcinogens that could explain the cancer-causing effect of this regimen.

MATERIALS AND METHODS

Chemicals ^{3}H-Labeled BP-4,5-oxide (sp act 289 mCi/mmol) and unlabeled BP-4,5-oxide were provided by the Cancer Research Program of the National Cancer Institute, Division of Cancer Cause and Prevention (Bethesda, Maryland). [G-^{3}H]Benzo[a]pyrene (sp act 25 Ci/mmol) and [7,10-^{14}C]BP (sp act 58.5 mCi/mmol) were purchased from Amersham Searle (Chicago, Illinois). Gold label BP, 7-ethoxycoumarin (7-EC) 7-hydroxycoumarin (7-OHC), resorufin, and phenol (>99% pure) were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). 7-Ethoxyresorufin was a product of Pierce Chemicals. Protease (Type XI), m-cresol, 8-hydroxyquinoline, nicotinamide adenine dinucleotide phosphate (reduced) (NADPH), calf thymus DNA, and ribonuclease A (Type III-A) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Standard coal tar solution (USP) was used. All other chemicals were obtained from commercial sources in the purest form available.

Radiation Source The UVB radiation source consisted of a bank of 4 Westinghouse FS-40-T-12 fluorescent sunlamps which delivered an average of 3.0 J/cm^2/s over the wavelength range 290–315 nm. Dosimetry of the radiation source was calibrated weekly with an International Light Spectroradiometer System (IL 700/760/780) containing a PM 270 D-CM 149 detector with a spectral range of 240–810 nm.

Animals Sperm-positive pregnant Sprague-Dawley rats of known insemination date were obtained from the Holtzman Rat Farm (Madison, Wisconsin). Neonatal rats born in situ were allowed to suckle until day 4 after birth; they were then withdrawn from their mothers and used in these experiments. The advantages of using neonatal rodents for studies on cutaneous drug and carcinogen metabolism have been described previously [17,18].

Treatment of Animals In each experiment 120 neonatal rats were divided into 6 groups of 20 each and treated in the following manner: *group 1* received a single topical treatment of acetone (10 ml/kg) and served as control, *group 2* received UVB alone (1200 mJ/cm^2), *group 3* received a single topical application of CCT alone (10ml/kg), *group 4* received UVB followed immediately by CCT, *group 5* received UVB followed by acetone (this group was considered necessary because animals in group 6 received CCT followed by UVB), and *group 6* received CCT followed by UVB. For the UVB exposures animals were exposed to the radiation source for a period of 4 h to deliver the desired dose. Each treatment was designed in such a way that all animals were killed 24 h after receiving CCT or acetone. The dose of CCT was selected based on its maximum enzyme induction effects [18–20]. The UVB dose selected was based on its own maximal inducing effects (see Fig 1).

Enzyme Assays Aryl hydrocarbon hydroxylase activity in cutaneous microsomes was determined by a modification of the method of Nebert and Gelboin [24], the details of which have been described earlier [16,17]. The quantitation of phenolic BP metabolites was based on comparison with the fluorescence of a standard solution of 3-hydroxybenzo[a]pyrene (3-OHBP). 7-Ethoxycoumarin O-deethylase (ECD) activity was determined according to a slight modification of the procedure of Greenlee and Poland [25], the details of which have been described earlier [16,17]. The amount of the product formed was calculated in comparison with an authentic standard of 7-OHC. 7-Ethoxyresorufin O-deethylase (ERD) activity was determined by a modification of the method of Pohl and Fouts [26], the details of which were described earlier [27]. Quantitation of the deethylated metabolite was based on comparison with the fluorescence of a standard solution of resorufin (RF). Epoxide hydrolase activity in the microsomal fractions was assayed using BP-4,5-oxide as substrate according to the thin-layer chromatographic technique of Jerina et al [28], the details of which were described previously [16]. Cytosolic GSH-transferase activity was assayed with BP-4,5-oxide as substrate as described previously [17]. Protein was determined, after precipitation with trichloroacetic acid, by the procedure of Lowry et al [29] using bovine serum albumin as a reference standard.

In Vitro BP-DNA-Binding Studies The incubation system for the in vitro [^{3}H]-BP DNA-binding studies was similar to that described by Hesse et al [30]. After incubation, the reaction mixtures were centrifuged at 105,000 *g* to isolate the microsomes, and digested with sodium dodecyl sulfate to extract any traces of protein. The extraction procedure was similar to that described by Lesca et al [31]. Details of these procedures were described earlier [32]. Extracted calf thymus DNA was then dissolved in 5 ml of 0.1 M sodium chloride, pH 7.0, and estimated by measuring its absorption at 260 nm. The purity of the DNA was assessed by the absorbance ratios of A 260/280 $\geq$ 1.98 and A 260/230 $\geq$ 2.21 [33]. Aliquots of extracted DNA were counted on a Packard TriCarb 460 CD liquid scintillation spectrometer to determine the amount of [^{3}H]-BP covalently bound to calf thymus DNA.

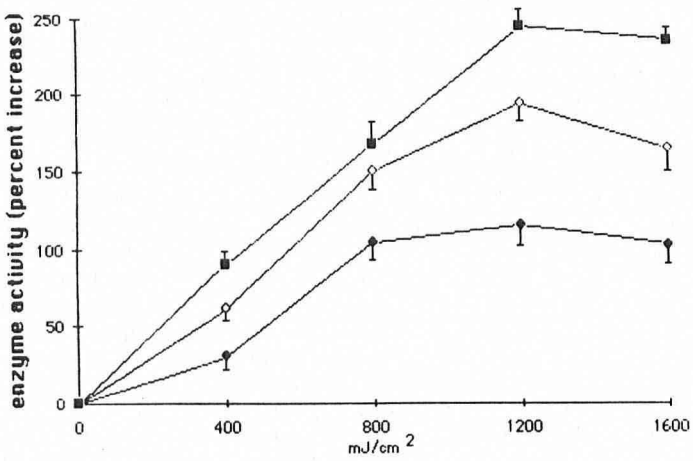

Figure 1. UVB dosimetry for studies on cutaneous monooxygenase activities. Animals were exposed using a bank of 4 Westinghouse FS-40-T-12 fluorescent sunlamps at a distance of 9 inches to deliver the dosage indicated. Following UVB exposure animals were immediately killed and skin microsomal fractions prepared and enzyme activities determined. *Open diamonds,* ECD; *solid diamonds,* AHH; *solid squares,* ERD. Data represent mean of 4 different preparations. For each preparation 20 neonatal rats were pooled.

In Vitro Incubation System for BP Metabolism The incubation mixture in a final volume of 1.0 ml contained 0.5 mg skin microsomal protein (prepared from control or treated animals), 0.10 mmol of phosphate buffer, pH 7.4, 3 μmol of $MgCl_2$ and 1.3 μmol of NADPH. The reaction was initiated by the addition of 80 nmol of [^{14}C]BP in 40 μl acetone. The samples were incubated for 30 min in the dark at 37°C in a Dubnoff metabolic shaker. The reaction was terminated by the addition of 1 ml of cold acetone followed by 2 ml of ethyl acetate. The mixture was vortexed for 1 min to extract any unreacted BP as well as the metabolites into the organic phase. The organic and aqueous layers were separated by centrifugation at 1500 rpm for 10 min. The radioactivity in the aqueous phase ranged from 0.03–0.4% of the total radioactivity and was proportional to the extent of BP metabolized. The organic phase was dried under a stream of nitrogen and dissolved in 100 μl of methanol for high-pressure liquid chromatographic (HPLC) analysis as described earlier [32]. All operations were performed under yellow light.

High-Pressure Liquid Chromatographic Analysis of Formation of Metabolites A Waters Associates Model 204 liquid chromatograph, fitted with a Waters Associates C_{18} μBondapak column (4.6 mm × 25 cm) was used for the analysis of radiolabeled metabolite mixtures of BP. Identification of metabolites was based on reference standards. The column was eluted at ambient temperature with a 30-min linear gradient of methanol:water:tetrahydrofuran (40:27:3, v/v) to methanol at a solvent flow rate of 0.8 ml/min [34]. The eluates were monitored at 254 nm, fractions of approximately 0.2 ml were collected dropwise, and the radioactivity of each fraction was determined on a Packard Tri-Carb 460 CD liquid scintillation spectrometer.

Statistical Analysis The statistical significance of differences between control and treated animals were evaluated by the Student's *t*-test. A p value of 0.05 or less was considered as statistically significant.

RESULTS

Effect of Skin Exposure to UVB on Cutaneous Monooxygenase Activities The effect of exposure of animals to varying doses of UVB alone on cutaneous AHH, ECD, and ERD activities is shown in Fig 1. Ultraviolet B exposure alone resulted in dose-dependent increases in all 3 monooxygenase activities. At a UVB dose of 1200 mJ/cm^2 increases in ERD, AHH, and ECD activities were 244%, 194%, and 115% as compared with control animals kept in the dark. Since there appears to be a plateau or decline in enzyme activities beyond a UVB dose of 1200 mJ/cm^2 this was selected for further studies.

Effect of Exposure of Animals to UVB and CCT, Alone and Combined, on Cutaneous Monooxygenase, EH and Glutathione S-Transferase (GST) Activities The effect of exposure of animals to UVB and CCT, alone and combined, on cutaneous AHH, ERD, and ECD activities is shown in Table I. All 3 monooxygenase activities in skin were found to be induced by the exposure of animals to UVB alone (127–167%) and CCT alone (350–921%). Cutaneous exposure to UVB followed by treatment with CCT was no more effective than exposure to CCT alone. The treatment of animals with acetone followed by exposure to UVB (group 5) was slightly less effective than UVB exposure alone. Nevertheless, in each case there was statistically significant induction as compared with the enzyme activities in unexposed animals. CCT treatment followed by UVB exposure (group 6) produced maximum inducing effects on enzyme activities. The observed enzyme induction effects by the combination of CCT followed by UVB were either additive or synergistic. Cutaneous exposure to UVB alone and treatment with CCT alone resulted in 127% and 350% increases, respectively, in AHH activity. However, in those animals where CCT treatment was followed by UVB exposure, the observed increase was 858% as compared with untreated animals. The increase in AHH activity in this group when compared with those exposed to UVB alone or to UVB followed by acetone was 322% and 412%, respectively. These responses in enzyme activities were quite similar to those observed in animals receiving CCT treatment alone (350%). Similar patterns of effects were observed for ERD and ECD activities. Cutaneous EH and GST activities remained unchanged

Table I. Effect of UVB and CCT, Alone and Combined, on Cutaneous Monooxygenase Activities in Neonatal Rats

	AHH Activity		ERD Activity		ECD Activity	
Treatment	pmol 3-OHBP/min/mg Protein	Percent Increase over Control	pmol RF/min/mg Protein	Percent Increase over Control	pmol 7-HC/min/mg Protein	Percent Increase over control
Control	1.75 ± 0.12		1.92 ± 0.14		0.34 ± 0.01	
UVB alone	3.97 ± 0.34[a]	127	4.64 ± 0.27[a]	142	0.89 ± 0.07[a]	162
CCT alone	7.85 ± 0.66	350	17.20 ± 2.41[b]	796	3.47 ± 0.25[b]	921
UVB + CCT	8.00 ± 0.64[b]	358	17.89 ± 2.84[b]	832	3.67 ± 0.27[b]	979
UVB + acetone	3.27 ± 0.21[a]	87	3.59 ± 0.31[a]	87	0.82 ± 0.08[a]	141
CCT + UVB	16.74 ± 1.06[c]	858	24.30 ± 2.97[c]	1166	4.52 ± 0.34[c]	1229

Data represent mean ± SEM of 4 separate experiments. Enzyme activities in control (acetone-treated) group were no different from the untreated group. For treatment conditions see *Materials and Methods.*

[a]Statistically significant from control ($p < 0.01$).

[b]Statistically significant from control and from UVB ($p < 0.01$).

[c]Statistically significant from control, from UVB and from UVB + CCT ($p < 0.01$).

Table II. Effect of UVB and CCT, Alone and Combined, on Cutaneous Microsomal Enzyme-Mediated Binding of [^{3}H]BP to Calf Thymus DNA

Treatment	Covalent Binding (pmol [^{3}H]BP/mg DNA)	Percent Increase over Control
Control	3.84 ± 0.24	
UVB alone	6.96 ± 0.61[a]	81
CCT alone	10.10 ± 1.16[b]	163
UVB + CCT	10.77 ± 1.12[b]	180
UVB + acetone	5.29 ± 0.26[a]	38
CCT + UVB	12.86 ± 1.44[c]	235

Data represent mean ± SEM of 4 separate experiments.
[a]Statistically significant from control ($p < 0.01$).
[b]Statistically significant from control and from UVB ($p < 0.01$).
[c]Statistically significant from control, from UVB, and from UVB + CCT ($p < 0.01$).

following exposure of animals to UVB alone, to CCT alone, or to the combination of these 2 modalities (data not shown).

Effect of Exposure of Animals to UVB and CCT, Alone and Combined, on Cutaneous Microsomal Enzyme-Mediated Binding of [^{3}H]BP to DNA Skin microsomes prepared from animals treated with UVB alone and CCT alone caused 81% and 163% increases, respectively, in the binding of [^{3}H]BP to calf thymus DNA as compared with microsomes prepared from control animals (Table II). Microsomes prepared from the skin of animals exposed to UVB followed by treatment with CCT resulted in a 180% increase in macromolecular binding. This increase in the binding activity was not different from that of CCT treatment alone. Consistent with the effects observed on monooxygenase activities, treatment of animals with topically applied CCT followed by UVB exposure resulted in the highest increase (235%) in the binding of [^{3}H]BP to calf thymus DNA.

Effect of Exposure of Animals to UVB and CCT, Alone and Combined, on Cutaneous Microsomal Metabolism of BP In further experiments, the effect of exposure of animals to UVB and CCT, alone and combined, on the pattern of skin microsomal metabolism of BP was assessed. The quantitation of BP metabolites was studied using HPLC analysis. As shown in Table III, skin exposure of animals to UVB alone resulted in an overall 121% increase in formation of BP metabolites. Treatment of animals with CCT alone resulted in 352% increased formation of BP metabolites. Exposure of animals to UVB followed by CCT treatment had effects no different from that of CCT treatment alone. However, topical application of CCT followed by exposure to UVB resulted in the highest enhancement of BP metabolite formation. The increase in BP metabolite formation by skin microsomes prepared from groups of animals treated with CCT followed by UVB was 834%, 322%, and 373%, respectively, as compared with the control group, and groups treated with UVB alone, and with UVB followed by acetone. As shown in Table III, formation of diols, quinones, and phenols increased similarly. Formation of BP-*trans*-7,8-diol, the precursor of the ultimate carcinogenic metabolite of BP [13–15], increased 1065% in skin microsomes prepared from animals treated with CCT followed by UVB exposure as compared with untreated controls (Fig 2). This increase was higher than that occurring for any other metabolite. Interestingly, skin exposure of animals to UVB alone resulted in the highest increases in the formation of BP-*trans*-diols including BP-*trans*-7,8-diol formation. This increase in diol formation is significantly higher than that which occurred for total metabolite formation (Fig 2).

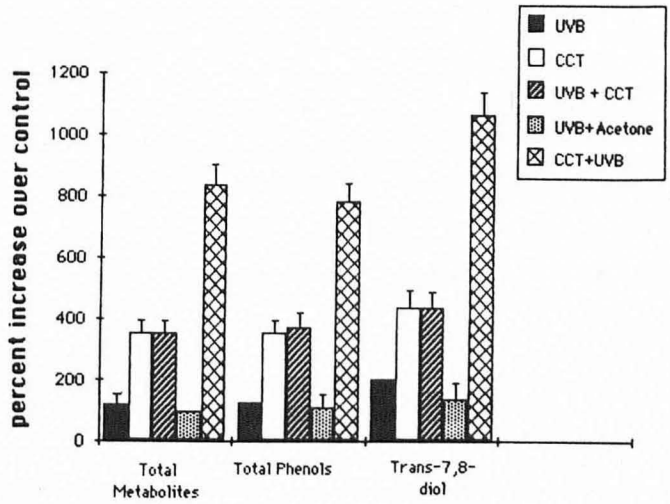

Figure 2. Effect of UVB and CCT, alone and combined, on generation of specific metabolites of BP by cutaneous microsomal enzymes. For other details see *Materials and Methods*.

DISCUSSION

The skin is a major interface between the body and its environment and is therefore at particular risk for the development of malignancy. It is known that individuals repeatedly exposed to CCT and UVB employed in the Goeckerman regimen for psoriasis are at increased risk for the development of cutaneous cancer but the reason for this is unknown [4]. The major purpose of this study was to determine whether differences in the metabolism of carcinogenic PAHs could explain the enhanced skin tumorigenicity evoked by the combination of skin exposure to CCT and UVB radiation. While the individual cutaneous oncogenicity of CCT, and of UVB, are beyond dispute, there is a paucity of

Table III. Effect of UVB and CCT, Alone and Combined, on Cutaneous Microsomal Metabolism of BP in Neonatal Rats

Metabolite[a]	Control	UVB Alone	CCT Alone	UVB + CCT	UVB + Acetone	CCT + UVB
Trans-9,10-diol	1.3	3.1	5.4	5.3	2.4	12.5
Trans-4,5-diol	4.7	9.8	18.4	16.4	8.6	54.3
Trans-7,8-diol	4.0	12.1 (203)	21.4 (435)	20.8 (420)	9.4 (135)	46.6 (1065)
1,6-Quinone	17.2	36.4	79.7	72.5	32.4	162.7
3,6-Quinone	16.5	34.9	74.7	79.7	28.7	152.4
6,12-Quinone	22.4	48.9	98.6	100.4	44.5	210.4
9-OH	12.4	31.4 (123)	50.3 (353)	48.6 (372)	28.6 (108)	96.7 (783)
3-OH	29.6	62.2	139.8	149.7	58.7	274.3
Total metabolites	108.1	238.8 (121)	488.3 (352)	493.4 (356)	213.3 (97)	1009.9 (834)

[a]Picomoles of metabolite formed/30 min/mg protein. Eighty nmol [^{14}C]BP (sp act 58.5 mCi/mmol) were incubated with skin microsomes (0.5 mg protein) in a final volume of 1.0 ml. Numbers shown are average values of triplicate samples which agree within 10% of the indicated values. These values were within 10–20% of the values obtained from experiments using different skin microsomal preparations. Other details are provided in *Materials and Methods*. Percent increase over control is given *in parentheses*.

knowledge concerning the manner in which these 2 carcinogens interact to evoke cancer in the skin.

Crude coal tar is a complex mixture of chemicals generated by the destructive distillation of coal. It contains thousands of chemicals some of which are PAHs, of which several are known to be carcinogenic probably because of their ability to structurally modify DNA. Similarly UVB radiation is carcinogenic and this may also be for the same reason. The effect of exposure of human and rodent skin to CCT on cutaneous carcinogen matabolism has been studied. Bickers and Kappas [21] showed that CCT is capable of inducing human skin AHH in vivo and that the induction response varies somewhat among individuals, perhaps on a genetic basis. Our subsequent studies [18] have demonstrated that CCT applied topically to neonatal rats can induce cutaneous and extracutaneous AHH activity. In addition, topical application of CCT to animals results in the induction of hepatic EH activity and cytochrome P-450 levels [20]. In another study, Mukhtar et al [19] evaluated the effects of topical application of a number of defined constituent PAHs present in a standard CCT mixture for their capacity to modulate cutaneous and hepatic drug and carcinogen metabolism. These studies indicated that several PAHs present in coal tar have induction effects on drug and carcinogen metabolism in skin and liver and that there are tissue-specific differences in responses to some of these hydrocarbons. Since the inducibility of AHH in target tissue appears to correlate with tumor induction in that tissue, the observation that CCT could induce cutaneous AHH is consistent with data indicating that CCT is carcinogenic for skin.

Excessive sun exposure is also known to be a causative factor in human skin cancer as first proposed by Unna in 1894 [35]. He recognized that neoplasms occurred in the preferentially chronically sun-exposed skin of sailors. Classical studies in experimental animals by Blum defined the carcinogenic action spectrum as being almost exclusively in the UVB range of 290–320 nm [36]. These wavelengths are at the lower, more energetic end of the solar spectrum that reaches the surface of the earth. Numerous investigators have clearly shown that rodents offer an excellent experimental animal model for studies of UV skin carcinogenesis [2]. Furthermore the initiation-promotion concept of chemical carcinogenesis is also applicable to UV carcinogenesis. Thus, it has been shown that a single dose of UV radiation followed by croton oil promotion produces skin tumors in hairless mice [37]. Although there are some differences in the biologic behavior of UV-induced skin tumors as compared with chemically induced cutaneous lesions, the initiation and promotion concept of carcinogenesis appears to be valid for both CCT and UV tumorigenesis.

Very few studies have been conducted to assess the effects of UV radiation on drug and carcinogen metabolism in skin or other tissues of experimental animals. Tredger and Chhabra [38], while studying drug metabolism in liver and intestine, observed circadian variations in enzyme activities. They postulated that the light cycle could be related to these circadian changes. Pohl and Fouts [39] found increased ECD activity in the skin of female hairless mice exposed to short-wave UV radiation (254 nm) and to a sunlamp (280–750 nm). Goerz et al [40] exposed female hairless Ng/mice to UV radiation for 16 h daily for a period of 24 weeks (mean daily doses: UVA = 106 J/cm^2, UVB = 0.62 J/cm^2), and observed increases in the acitivities of AHH, aminopyrine N-demethylase, ECD, and in the contents of the cytochrome P-450 in liver of exposed animals. However, these investigators detected no effect of such exposure on skin enzyme activity.

The manner in which chemical agents may influence UV carcinogenesis is a subject of considerable interest. Such agents include chemical promoters, photosensitizers, and carcinogens. A number of substances including CCT and select PAHs present in CCT are both phototoxic and carcinogenic. In 1937, Bungeler [41] observed that by injecting tar subcutaneously into white mice prior to exposure to sunlight the yield of both benign and malignant tumors was greatly increased. Urbach [42] demonstrated that CCT enhanced the number of ear tumors from carefully measured midwavelength(280–360 nm) exposure.

Several investigators have examined the effects of combined treatment of UV radiation and chemicals, including tar, on tumor development in experimental animals. Findlay [43] painted a small area of depilated mouse skin prior to exposure to a quartz mercury vapor lamp 4 times a week. In these studies skin cancers developed more rapidly in mice so treated than in mice treated with tar alone. Subsequent investigators obtained conflicting results from similar studies. Experiments in which skin painting with BP [44] or 20-methylcholanthrene [45] was followed by exposure to visible light or in which topical application of BP, dimethylbenzathracene, and 20-methylcholanthrene was followed by UV radiation [46] have either failed to demonstrate an increase in carcinogenesis or have resulted in fewer tumors than did the chemical carcinogen alone. Other workers have reported increased [47], decreased [48], or no effect [49,50] from studies on the combination of UV radiation and PAH. None of these studies has attempted to mimic Goeckerman therapy in assessing the interaction of UV radiation and PAHs on skin cancer formation.

Our data indicate that both UVB alone and CCT alone each substantially enhances cutaneous microsomal cytochrome P-450-dependent AHH, ECD, and ERD activities. Of the 3 monooxygenases studied, ERD is specifically dependent upon cytochrome P-448. In general, the specificity for cytochrome P-448 is in the order of ERD > AHH > ECD. It is interesting to note from the data in Fig 1 that the degree of observed increases in enzyme activity followed the specificity of cytochrome P-448. It is important to emphasize that this isoenzyme of cytochrome P-450 participates in the activation of many precarcinogens to their ultimate carcinogenic metabolite. In addition our data indicate that both UVB alone and CCT alone substantially enhance the metabolism of the carcinogenic PAH, BP, and the binding of metabolites of BP to epidermal DNA. When CCT was applied to skin previously irradiated with UVB additive effects were observed on these parameters in skin. These results indicate that exposure of rodent skin to CCT followed by UVB radiation additively augments the effect of each on carcinogen metabolism thought essential for tumor induction. Perhaps irradiation of CCT-treated skin yields larger amounts of reactive metabolites of PAHs. Pretreatment with UVB followed by CCT does not exhibit additive effects. The explanation for this major difference may relate to photochemical changes produced in skin by UVB which somehow alters the cutaneous response to CCT. It remains to be seen whether greater increases in skin AHH and other parameters following cutaneous treatment with CCT followed by exposure to UVB results in increased carcinogenicity than that occurring with CCT treatment alone. These studies are currently under way in our laboratory.

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