Early skin responses of hibernating and nonhibernating ground squirrels to topical applications of DMBA

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Summary. Skin patches on hibernating and nonhibernating ground squirrels were treated with multiple topical applications of the carcinogen 7, 12-dimethylbenza(a)anthracene. Nonhibernators showed blistering, peeling, drying, hair loss, increased vascularization and hyperpigmentation in proportion to DMBA concentration. The latter was apparently due to a) an increased number of dermal and epidermal melanocytes and b) the appearance of melanocytes with large coarse cytoplasmic granules. Notably, hibernators remained free of gross skin changes and were histologically similar to untreated controls.

Hibernation, a unique and complex phenomenon involving alterations in the physiological state of an animal, confers protection from the environment. Specifically, hibernating ground squirrels are less susceptible to the damaging effects of ionizing radiation; i.e., hemopoetic, GI and CNS injury, than their nonhibernating counterparts[2-4]. In fact, ground squirrels irradiated while hibernating survive longer after arousal than ground squirrels irradiated while in the nonhibernating state. This observed radio-protection may be due to a combination of hypothermia, hypoxia and general metabolic depression. Jaroslow et al.[5] suggest that the initial radiation-induced injury is the same in hibernating and nonhibernating ground squirrels but the net recovery is different based on the very different physiological demands of the animals during the post-irradiation recovery period.

That hibernating ground squirrels are less radio-sensitive suggests that hibernation may afford similar protection against exposure to carcinogens. The polycyclic aromatic hydrocarbon 7,12-dimethylbenza(a)anthracene (DMBA) is one of the most potent carcinogens known[6,7] and as such has been conclusively linked to a variety of predominantly localized neoplasms when topically applied to the skin of mice[8-11], rabbits[10,11] and rats[11]. In addition, changes in dermal melanocytes were noted following application to gerbils[12,13]. None of the aforementioned mammals is capable of hibernation. The purpose of this preliminary study was to determine whether the naturally occurring metabolic alterations of seasonal hibernators affect topical sensitivity of the integument to a chemical carcinogen.

Materials and methods. Two populations of 13-lined ground squirrels *(Spermophilus tridecemlineatus)* were used in this study: nonhibernating animals, maintained at room temperature (thermoregulating near 37 °C) and hibernating animals, maintained in a dark cold room at 3 °C (thermoregulating near 6 °C). The dorsal trunk skin was shaved in both groups of animals to expose 1×2 cm patches of epidermis. A series of 5 topical applications (0.2 ml each) of either 3%, 1% or 0.5% DMBA in dimethylsulfoxide (DMSO) were made over an 11-day period. Skin patches treated with DMSO alone or which were left untreated served as controls. Patches were observed daily for gross changes.

On day 12 all animals were anesthetized with 0.2 ml sodium pentobarbitol (65 mg/ml) and skin patches were dissected free of the underlying tela subcutanea. Patches were fixed in 10% neutral buffered formalin, dehydrated and embedded for histological sectioning. Cross sections (10 µm) were stained with hematoxylin and eosin for routine microscopic evaluation, and 5-µm sections stained with silver-methenamine borate and light green for specific localization of melanin granules[14].

Results. Grossly, nonhibernator skin treated with DMSO and untreated control skin appeared and remained pale grey and supple (fig. 1a). Nonhibernators treated with 0.5% DMBA, however, showed slight hyperpigmentation. This became more pronounced in the 1% group in which concomitant drying of the skin was noted. After only 2 topical applications of 3% DMBA (day 6) nodular elevations (≤ 3 mm across) which ultimately blistered and peeled became visible. This was accompanied by a progressive change in the skin from pale grey and supple on day 1 to black and leathery by day 11 (fig. 1b). Hair regrowth was evident in all shaved areas except in those treated with 1% or 3% DMBA.

Skin patches on hibernating animals remained free of gross changes throughout the study and appeared identical

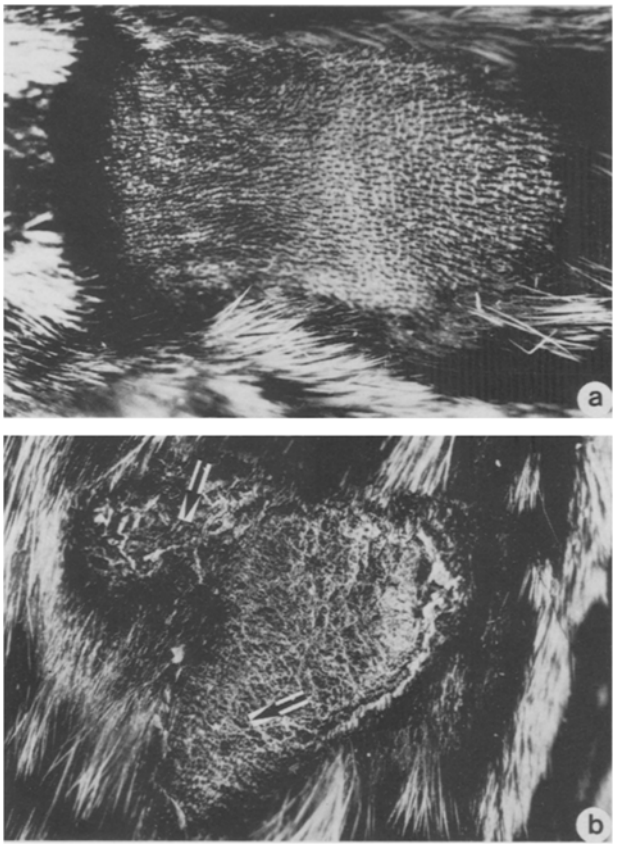

Figure 1. *a* Gross appearance of untreated control skin from a nonhibernator. The superficial epidermis is pale grey and supple and shows evidence of hair regrowth. This is grossly identical to nonhibernator skin treated with 5 topical applications of DMSO over an 11-day period (solvent controls) and to all carcinogen-treated and control patches of skin from hibernating animals. *b* Gross appearance of nonhibernator skin after 5 topical applications of 3% DMBA in DMSO over an 11-day period. Note hyperpigmentation, dryness, scaling and absence of hair regrowth. Original area of application was rectangular. Occasional runoff was sufficient to dissolve hair and produce hyperpigmentation, etc. in unshaved areas as well (arrows).

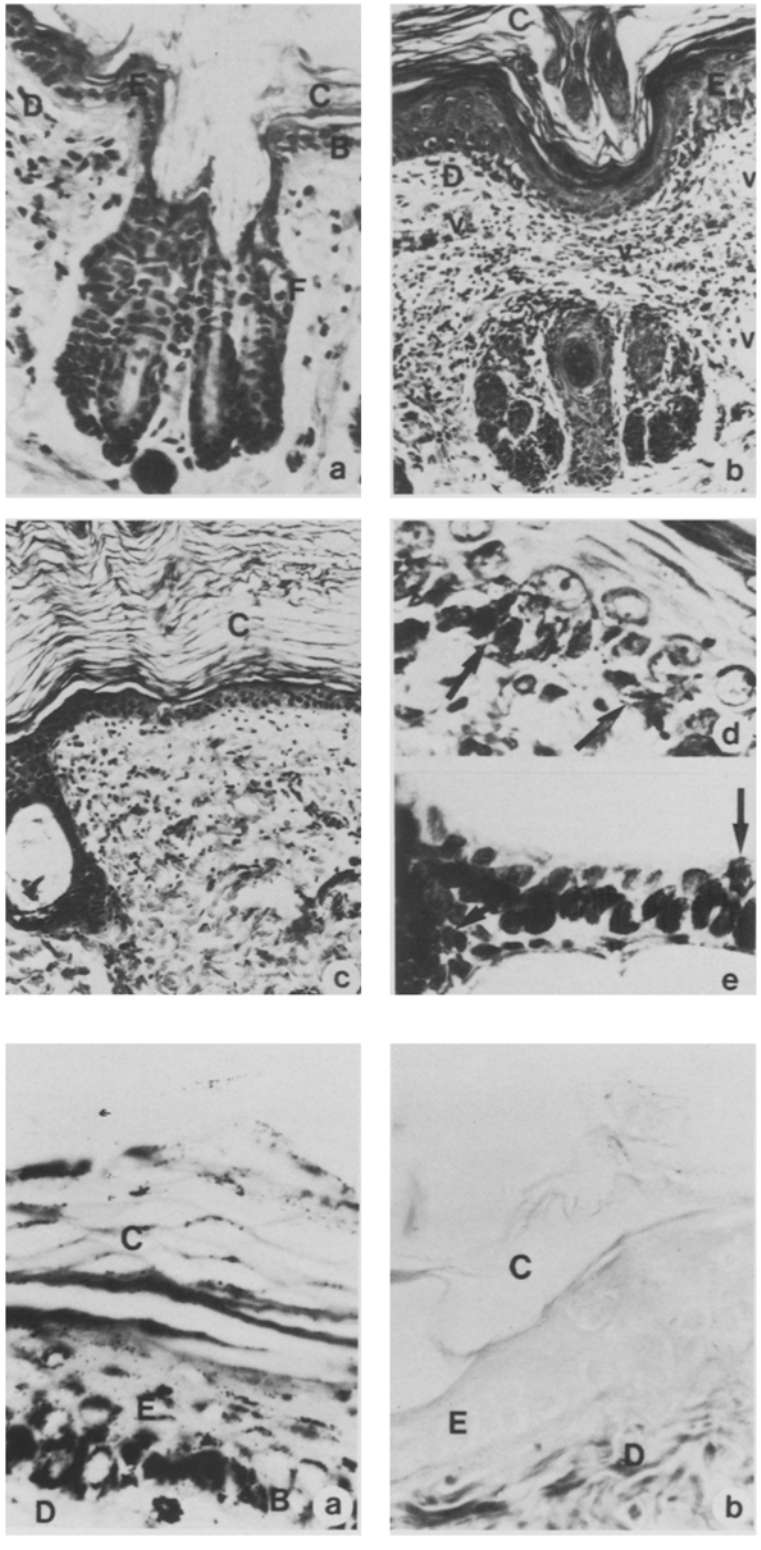

Figure 2. *a* Histological section of untreated control skin from a nonhibernator. The cellular epidermis (E) and stratum corneum (C) are of comparable thickness. Typical melanocytes with small cytoplasmic granules are located primarily in the outer root sheaths of hair follicles (F) and occasionally in the epidermal stratum basale (B) and papillary layer of the dermis (D) (not shown). This is identical in appearance to nonhibernator skin treated with 5 topical applications of DMSO over an 11-day period (solvent controls). H and E, × 133.
b Histological section of nonhibernator skin after 5 topical applications of 3% DMBA in DMSO over an 11-day period. Atypical melanocytes with large coarse granules extend not only throughout the cellular epidermis (E) and stratum corneum (C) but are also located in the papillary layer of the dermis (D) and in the outer root sheaths of hair follicles (not shown). Local blood vessels (V) are more numerous. The thickened, irregular stratum corneum is analogous to the grossly visible sloughing seen in fig. 1b. H and E, × 66.
c Histological section of hibernator skin after 5 topical applications of 3% DMBA in DMSO over an 11-day period. Except for the considerably thicker stratum corneum (C) all carcinogen-treated and control patches from hibernators are histologically identical to control patches from nonhibernators. H and E, × 66.
d Atypical melanocytes from the epidermal stratum basale of nonhibernator skin treated with 3% DMBA demonstrating large coarse granules in the cytoplasm (arrows). H and E, × 267.
e Typical melanocytes from the outer root sheath of a hair follicle in untreated nonhibernator control skin demonstrating small uniform cytoplasmic granules (arrows). H and E, × 267.

Figure 3. *a* Histological section of nonhibernator skin after 5 topical applications of 3% DMBA in DMSO over an 11-day period. Note the abundance of melanin granules throughout both the cellular epidermis (E) (particularly in the stratum basale (B)) and the stratum corneum (C). Granules are also present in the dermis (D). Silver-methanamine borate, × 267.
b Histological section of hibernator skin after 5 tropical applications of 3% DMBA in DMSO over an 11-day period. Note the absence of melanium granules in the cellular epidermis (E), stratum corneum (C) and the dermis (D). Silver-methenamine borate, × 267.

to untreated patches in nonhibernating animals (fig. 1a). Hair regrowth was observed in all patches.

Histologically, skin patches of nonhibernators showed a thin cellular epidermis and a comparably thin overlying layer of keratin (stratum corneum). In both DMSO and untreated patches epidermal pigmentation was minimal and consisted of melanocytes with small cytoplasmic granules (fig. 2e). These were confined to the outer root sheaths of hair follicles and to the stratum basale. Few dermal melanocytes were detected in the papillary layer of the dermis (fig. 2a). Pigmentation of the epidermal stratum basale first occurred in nonhibernators treated with 0.5% DMBA. In skin patches treated with 1% or 3% DMBA melanocytes were distributed throughout the thickness of the epidermis and contained large coarse cytoplasmic granules (fig. 2d). In the 3% group melanocytes

were noted extensively within the stratum corneum and there was a noticeable increase in the occurrence of local blood vessels. The number of melanocytes in the papillary layer of the dermis increased proportionately to the applied DMBA concentration (figs 2b and 3a).

Skin patches from hibernators demonstrated a thin cellular epidermis and a relatively thick stratum corneum. Both epidermal and dermal pigmentation were minimal and appeared histologically identical to untreated patches from nonhibernating animals. Melanocytes with large coarse cytoplasmic granules were absent and local blood vessels appeared similar to those from untreated patches (figs 2c and 3b).

Following sacrifice gross inspection indicated that internal organs were unaffected by treatment of the integument with DMBA in both groups of animals.

Discussion. Although the literature contains reports of tumor induction in various species of mammals exposed to topical applications of DMBA[8-13] this is the first study involving exposure of hibernating and nonhibernating mammals. As such, it was not the intent of this preliminary work to discern why but rather *if* naturally occurring physiological changes can protect an organism from carcinogen-associated gross and microscopic alterations. Indeed, the data obtained from this study confirm the speculation that the altered physiology of hibernation can confer such protection. Early, rather dramatic skin changes were consistently noted in nonhibernators in proportion to DMBA concentration. These included blistering, peeling, drying, hyperpigmentation, hair loss and peripheral vasodilation. Notably, hibernating animals remained unaffected by all treatments.

The reasons for this dichotomous reactivity between hibernators and nonhibernators remain unclear. It is possible that the noticeably thicker stratum corneum in hibernator skin may serve as a natural mechanical barrier to protect the underlying dermis during the protracted period of hibernation. That this mechanical barrier could have reduced the amount of DMBA entering the cellular strata and dermis cannot be discounted. However, this may be somewhat minimized by the fact that DMSO as a solvent has a proven ability to rapidly penetrate skin[15].

In addition, hibernating animals exhibit both a lower mitotic index and severe metabolic depression[16]. These factors may partially account for the observed difference in skin responses between these 2 groups of animals. With fewer cells undergoing mitosis less likelihood exists of incorporating DMBA-damaged DNA[17] into the genome. Insofar as metabolism is concerned, damaged DNA can be repaired more efficiently in cells having to perform at low rather than high levels of routine maintenance[5]. Equally plausible is the possibility that DMBA is not sufficiently catabolized to its active constituents in hibernators due to alterations in the microsomal enzymes[18] responsible for its breakdown.

Apparently physiological factors can render animals sensitive or resistant to a chemical carcinogen. This may provide insight into the stimulation or inhibition or the body's own mechanisms to arrest and control endogenously as well as exogenously-generated aberrant processes. Further study of skin reactivity in hibernating and nonhibernating animals following topical application of DMBA is in progress.

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A scanning electron microscopic study of the air and blood capillaries of the lung of the domestic fowl *(Gallus domesticus)*

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Summary. Air and blood capillaries of the lung of the domestic fowl constitute the functional gas exchange units. They anastomose profusely and interlace with each other in 3 dimensions. Air capillaries are not blind-ending tubules as has occasionally been suggested.

A cross-current relationship between bulk parabronchial gas and pulmonary blood flow has been experimentally demonstrated[2] and anatomically confirmed[3]. There is no doubt that this relationship is of prime importance in the superior arterialisation of blood in the avian lung, compared with that of mammals[4]. Nevertheless the actual gas exchange in the avian lung takes place in the exchange tissue mantle which surrounds the parabronchial lumina, the ultimate functional units being the air capillaries and blood capillaries.

The size, shape and spatial relationships of these structures are undoubtedly of great functional significance, but very little is known about their anatomy. Even the question of the extent to which the air capillaries anastomose with each