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Toxic Responses in Gerbils Treated Topically With Aflatoxin B_1 and Dimethylformamide

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It has been demonstrated that aflatoxin B_1 (AFB_1), a toxic metabolite produced by certain isolates of *Aspergillus flavus*, is a potent liver carcinogen in a number of animals including fish (AYRES *et al.* 1971), rats (NEWBERNE and WOGAN 1968), and fowl (ASPLIN and CARNAGHAN 1961). In the work reported by NEWBERNE and WOGAN in 1968, AFB_1 caused a high level of hepatic carcinomas when 0.015 ppb of this toxin was included in the diet fed to rats. AFB_1 also has been found to reach maximum concentration levels in the liver of rats within one-half hour following intraperitoneal injections (WOGAN 1968). It has been reported that acute liver lesions were produced in rabbits following application to AFB_1 to rabbit skin (WEI *et al.* 1970). It was generally assumed that these toxic and precarcinogenic responses were due to the percutaneous absorption of AFB_1. A more recent study has indicated that lesions in rats were not due to percutaneous absorption of AFB_1, but subsequent ingestion of the applied agent (PURCHASE and STEYN 1973).

With percutaneous absorption under consideration, studies were undertaken with adult Mongolian gerbils. These were planned to determine if liver lesions could be induced in both sexes and if the commonly used solvent for AFB_1, dimethylformamide (DMF), suppressed or potentiated toxic responses (LLEWELLYN *et al.* 1974).

MATERIALS and METHODS

Young adult, Mongolian gerbils, *Meriones unguiculatus*, of both sexes, having an approximate body weight of 65 grams were studied. All animals were from our stock colony and were allowed to acclimate to their new cages prior to treatment (LLEWELLYN *et al.* 1975). They were given free access to laboratory chow and tap water. Bedding was changed twice weekly. All studies were completed in a photoperiod having 14 hours of light. Animals were maintained at 25±3°C.

The following treatment groups containing five

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animals each were studied: absolute controls for both sexes (ACM,ACF); DMF administered once weekly for both sexes (DMF-1XM, DMF-1XF); DMF administered twice weekly for both sexes (DMF-2XM, DMF-2XF); AFB_1 and DMF administered once weekly for both sexes (DMF-AFB_1-1XM, DMF-AFB_1-1XF); and AFB_1 and DMF administered twice weekly for both sexes (DMF-AFB_1-2XM, DMF-AFB_1-2XF). All animals were shaved over a two-cm^2 area located between the shoulders. This area was painted with DMF alone and/or AFB_1 dissolved in DMF. Applications were measured using calibrated syringes and spread with pre-moistened brushes. Each topical application consisted of 0.1 ml DMF. AFB_1 applications consisted of 500 ug AFB_1/0.1 ml DMF for each painting. The groups painted once a week received a total of 500 ug AFB_1 and the twice weekly total was 1000 ug AFB_1. All applications were administered over a period of 35 days. Animals were maintained for a total of 174 days during which time change in body weights and general health were recorded. At the termination of the study animals were sacrificed by ether anesthesia and autoposies were made. The liver, kidney, bladder and treated skin were fixed in buffered formalin. Histological examinations were made of paraffin sections of tissue stained with hematoxylin and eosin.

To prepare the topical solution, AFB_1 (Calbiochem, LaJolla, Cal.) was dissolved in DMF and stored in darkness. AFB_1 was confirmed both qualitatively and quantitatively using thin layer chromatography and the visual dilution technique (HORWITZ *et al.* 1975). Fecal samples were collected twice weekly and subjected to similar analyses. Safety procedures, in addition to laboratory apparel, included the use of sodium hypochlorite to decontaminate work areas (YANG 1972).

RESULTS and DISCUSSION

No animals succumbed during the period of study. Histopathological analysis of the liver, kidney and other organs were normal. Livers failed to show necrotic areas previously associated with ingested AFB_1 liver damage (HASTINGS and LLEWELLYN 1973) or ingested DMF (LLEWELLYN *et al.* 1974). Fecal samples from AFB_1 treatment and other groups contained no toxin or were any related metabolites found. There seems to have been little or no AFB_1 absorbed or consumed. The thin layer chromatography detection procedures for AFB_1 used herein are sensitive below to 2 ppb.

If body weight changes are representative for some aspects of toxicity, then several such responses should

be noted from this study. Generally it was found that ACM and ACF animals continued to show slight gains in body weight (TABLES 1 & 2). The male and female animals initially had the same body weights. The male animals were originally younger and expected to exceed the weight of the females. They did. In the male treatment groups the ACM animals showed the highest gain in body weight. The DMF-AFB_1-1XM animals failed to gain weight initially, showed fluctuations over the duration of the experiment, but by 120 days had attained body weights similar to the ACM and the DMF-AFB_1-1XM.

The mean gain in body weight followed the same trend as follows at 42, 100, 127 and 174 days: ACM>DMF-AFB_1-2XM>DMF-1XM>DMF-AFB_1-1XM>DMF-2XM. Only the DMF-2XM animals appeared to have a substantially different rate of weight gain as compared to the remaining four groups.

Aflatoxins have been applied to the skin of mice, incombination with other agents. In one study, AFB_1 was painted on mice in benzene at 10 ug/ml following pretreatment with 7,12-dimethylbenz (a)anthracene (VAN DURREN *et al.* 1969). In this case both actinomycin D and AFB_1 inhibited tumor induction.

In another study with mice (LINDERFELSER *et al.* 1974) AFB_1 and T-2 toxin were placed on the back of mice, in benzene at 25 ug/dose. Incombination, the two compounds caused an increase in lethality over either of the toxins when applied singly.

To further evaluate the studies relating to percutaneous absorption, the influence of AFB_1 on membranes must be taken into consideration. For example, studies report that AFB_1 causes an interruption or a difference in membrane transport such as that of acetate into human skin lipid (CLIFFORD and REES 1967); LO and BLACK 1972; KINIMOTO *et al.* 1974). WILLIAMS and RABIN (1969) indicated that AFB_1 and other carcinogens occupy or destroy the membrane binding site for steroid hormones. AFB_1 seems to have an effect on membranes but certainly it must be absorbed if it is to function as a toxin or a liver carcinogen. Recently it has been proposed that AFB_1 can or must be activated, possibly to an epoxide, for it to show toxic and carcinogenic action (CAMPBELL and HAYES 1976).

From the data reported herein and that of PURCHASE and STEYN (1973), gerbils and rats probably do not absorb appreciable levels of AFB_1 via the skin, therefore not allowing substantial levels of activated products or the

TABLE 1

MEAN CHANGE IN BODY WEIGHT IN GRAMS FOR MALE ANIMALS

Time Elapsed (DAYS)	Control (ACM)	DMF-1XM	DMF-AFB_1-1XM	DMF-2XM	DMF-AFB_1-2XM
12	1.2	-0.7	0.2	1.4	-1.2
42	4.4	3.6	2.2	0.8	4.0
53	5.5	5.5	1.9	1.6	3.7
63	7.3	5.8	2.6	-0.1	4.5
74	7.0	6.3	3.6	-0.1	4.8
82	5.7	4.5	3.8	-1.6	3.4
92	7.5	6.9	5.0	1.6	5.1
100	8.3	6.7	5.7	1.6	7.1
106	9.1	6.9	6.3	2.3	7.6
113	9.4	6.3	6.1	2.7	8.6
120	10.6	7.8	6.6	4.4	9.6
127	9.5	7.5	6.7	4.7	9.1
174	9.0	8.1	8.6	4.0	7.7

TABLE 2

MEAN CHANGE IN BODY WEIGHT IN GRAMS FOR FEMALE ANIMALS

Time Elapsed (DAYS)	Controls (ACF)	DMF-1XF	DMF-AFB_1-1XF	DMF-2XF	DMF-AFB_1-2XF
12	1.1	0.8	0.9	-2.8	0.8
42	1.3	0.4	1.2	-2.1	0.5
53	1.6	0.7	2.5	-2.6	1.1
63	1.8	0.4	3.5	-1.3	0.2
74	1.4	0.5	3.8	-0.8	0.8
82	1.6	1.3	1.5	1.5	1.5
92	2.2	2.9	5.1	0.0	2.3
100	3.3	3.2	3.7	1.0	1.3
106	3.1	1.9	4.7	1.1	2.6
113	4.7	2.5	5.0	-0.6	4.2
120	4.0	3.6	4.8	1.8	4.4
127	4.7	3.4	3.5	-1.3	4.2
174	5.2	4.0	3.3	-3.8	5.0

AFB_1 itself to function as a toxic or carcinogenic agent. Chronic studies may yield a different toxic and carcinogenic response for both the skin and liver tissue. There is little doubt that both rats and gerbils do respond to AFB_1 given *per os*.

Optimistically, it is hoped that human skin does not respond to AFB_1 like the rabbit or as proposed by LO and BLACK (1972). If it does, then handlers of groundnuts and grains may encounter occupational dermatoses. No reports of such cases have been documented to date.

SUMMARY

Male and female Mongolian gerbils failed to show observable pathological responses in the liver to topically applied AFB_1 and DMF indicating that percutaneous absorption of AFB_1 is absent in this species. In males, some responses in weight changes were attributed, proportionally, to increased doses of DMF. Unexplained and currently under investigation is the possibility that AFB_1 may have reduced the response of some animals to DMF. This response may relate in some way to the modification of absorption of DMF in the gerbil since AFB_1 is known to have an influence on cell membranes.

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