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Letter to the Editor

Role of Podophyllotoxin in the Bedding and Dietary Zearalenone on Incidence of Spontaneous Tumors in Laboratory Animals

The incidence, in laboratory animals, of tumors considered "spontaneous" can vary greatly in different laboratories and even in the same laboratory at various times (3, 6, 8, 13), and this fact is of great concern to the experimental oncologist.

Genetic, viral, and immunological factors have been considered to be responsible. The effect of environmental factors on the incidence of spontaneous tumors came to light recently in a striking way.

An interesting observation has been reported from Australia by Sabine *et al.* (14). C3H*A^{vy}fB* mice, which have a very high incidence of mammary and hepatic tumors in the NIH Laboratories in the United States (18), have been found to develop fewer tumors when imported to Australia; the incidence declined to almost zero in subsequent generations, bred in the new environment. However, when the mice in Australia were given the diet, provided in the United States, the incidence of mammary tumors increased. It was restored to the NIH level when the Australian bedding, consisting of shavings of Douglas fir (*Pseudotsuga* spp.), was replaced by shavings of red cedar wood, *Juniperus virginiana* L., the bedding used at NIH. The Australian authors suggested that the bedding and diet used in the United Stated may contain carcinogens.

Carcinogens in Bedding. I have drawn attention to the carcinogenic hazard to laboratory animals of wood shaving beddings before the appearance of this publication (15). Wood has been implicated in the etiology of nasal tumors among wood workers (1) and of nasopharyngeal tumors among certain communities in Africa and Asia (17). Substituted cinnamaldehydes and related constituents of lignins have been suggested as the possible carcinogenic agents, as the *p-O*-methyl ether of sinapaldehyde (3,4,5-trimethoxycinnamaldehyde) has been found to be an effective carcinogen (16).

However, the observations of Sabine *et al.* (14) implicate specifically the shavings from *J. virginiana* L. In this instance, I suggest that the lignan podophyllotoxin may contribute to the carcinogenic action of this bedding.

Podophyllotoxin and related lignans have been found in *J. virginiana* L. and certain other *Juniperus* species in the course of an investigation into the antitumor action of crude extracts from dried juniper needles (4). Podophyllotoxin is better known as the active constituent of podphyllin, a crude preparation from *Podophyllum peltatum* L., which has been traditionally used as a cathartic, as a remedy for condyloma acuminata, for tumors, and for various other conditions. The composition and biological effects of podophyllin were well reviewed in 1954 by Kelly and Hartwell (7). It is a mitotic poison and can cause striking hyperplastic lesions.

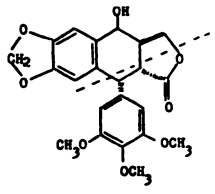
Since then, O'Gara (12) found that the prolonged ingestion of podophyllin in diet by BALB/c male mice induced, among 12 animals that survived for more than 1 year, 3 hepatomas, 3 lymphomas, and 2 polyps (of the cecum and colon). Five mice had "considerable hyperplasia of reticulum cells of liver, spleen and lymph nodes." However, additional testing of podophyllotoxin would be desirable in order to establish its carcinogenic potentialities.

It may be significant that the structure of podophyllotoxin (Chart 1) contains moieties related to the carcinogenic 1′-hydroxysafrole (2) and to 3,4,5-trimethoxycinnamaldehyde (16).

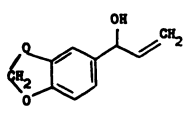
Various lignans are present in plants and woods (5). These compounds contain methylenedioxy and polymethoxy substituents, and their testing for carcinogenic activity would be of interest.

Mice, that are kept in close contact with the bedding all their lives would obviously be more affected by its constituents than rats that usually are housed on wire bottoms.

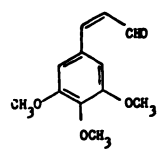
Estrogens in Diet. The mammary tumors in the C3H *A^{vy}fB* mice appear, on Australian evidence, to be related to the ingestion of the diet used in the United States, and indicate the presence in the diet of estrogens that are active when administered p.o. Disregarding the possibility that synthetic estrogens as food additives could be present, I suspect natural estrogens and, in particular, zearalenone (Chart 2) and its congeners. These effective estrogens are secondary metabolites of common fungi, the fusaria, which like *Fusarium graminearum*, sometimes contaminate cereals (9) and have been responsible for outbreaks of vulvovaginitis in pigs, sterility in cattle, etc. (10). It remains to be shown whether zearalenone



Podophyllotoxin



1′-Hydroxysafrole



3,4,5-trimethoxycinnamaldehyde

Chart 1. Structures of podophyllotoxin, 1′-hydroxysafrole, and 3,4,5-trimethoxycinnamaldehyde.

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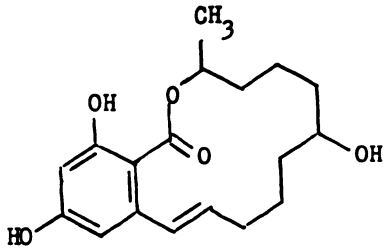

Zearalenol

Chart 2. Structure of zearalenol (reduced zearalenone).

and its reduction products have a carcinogenic action on the target organs that is similar to that of diethylstilbestrol and the steroidal estrogens (11). Synthetic zearalenols are already available commercially under the brand name, Ralgro, to be used as additives to livestock fodder in order to accelerate the growth and fattening up of young animals (10). Mice that were given the NIH diet and bedding attained significantly higher body weight than those kept under the conditions usual in Australia (14).

In 1953 (19), certain commercial laboratory animal rations had already been reported to possess estrogenic activity.

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