Uptake of Aflatoxin B_1 by the Skin of Rats

Aflatoxin B_1, a metabolite produced by some strains of *Aspergillus flavus*, has been shown to be an extremely potent hepatotoxin to most animal species either given orally or intraperitoneally[1]. WOGAN et al.[2] reported that 0.5 h after administration, aflatoxin B_1 was present at maximum levels in liver and kidneys of the rat. The concentration was 5–15 times greater in liver than in other tissues, and at the end of 24 h, the liver contained an amount equal to the content of the remainder of the carcass. This has been used to explain the fact that the histologic and biochemical manifestations of the toxic action appear almost exclusively in liver. To our knowledge, no epidermal carcinogenesis caused by aflatoxin has been reported.

The aim of the present study was to investigate the uptake of aflatoxin in rat skin painted with aflatoxin B_1 solution and the pathological changes of skin and liver produced by the treatment.

Materials and methods. Rats of the Sprague-Dawley strain aged 4 weeks and weighing about 55 g each were used. They were maintained on a commercial diet[3] with free access to water.

For histological studies, 12 rats were divided into 2 groups. The experimental animals were painted on the dorsal skin, an area of about 2 cm^2 which was previously shaved to remove the fur, with 100 µg of aflatoxin B_1, dissolved in 10 µl of N,N-dimethyl formamide-propylene glycol (1:1, v/v), once a day for 50 days. The control animals were similarly treated with the vehicle. The body weight of the animals were recorded daily. At the 51st day the animals were exsanguinated. The skin and liver were removed and fixed in 10% neutral formalin for histological studies.

To determine the absorption rate of aflatoxin B_1, another 14 rats were used. The skin of each animal was painted similarly with a single dose of 6.5 µg of aflatoxin B_1-^{14}C, prepared according to ADYE and MATELES[4] with a specific activity of 6.5 µc/µmole. After 0, 0.5, 1, 3, 6, 10 and 24 h, each group of 2 animals was exsanguinated. The skin was carefully dissected off the underlying tissue, fascia and muscle. The painted skin sample, totally about 1 cm^2 in size, was placed in a counting vial and dried at 100–105 °C for 1 h. To dissolve the skin, the dry sample was moisted with 0.3 ml of distilled water for a short period and then was mixed with 3 ml of Solubilizer (Nuclear-Chicago Corp., Des Plaines, Ill.; N.C.C.). The solution was then mixed with 10 ml of diluted Liquifluor (N.C.C.) and counted in a liquid scintillation counter (model 6850, N.C.C.). The counting efficiency was determined by an internal standard of benzoic acid-^{14}C.

Results. As shown in Figure 1, the growth rate of the experimental rats was markedly retarded as compared with the controls. This growth retardation was similar to the animals treated with aflatoxin B_1 i.p. or orally[5]. The morphologic changes of the aflatoxin B_1-painted rats were found mainly in the liver. The skin of the painted area showed little change. Histological studies of the liver of the experimental animals revealed scattering focal necrosis, portal round cells infiltration with ductular and bile duct proliferation; the number of large nuclear and binucleolar cells was significantly increased. Meanwhile, abnormal nodular lesions, which appeared as round nests of clear cytoplasmic hepatic cells, were universally present. Those clear hepatic cells were confirmed with α-amylase to be rich in glycogen.

The rate of uptake of aflatoxin B_1-^{14}C is shown in Figure 2. It indicates that a significant portion of the applied radioactivity disappeared from the skin as early as 0.5 h after painting, and by 1 h about half of the ^{14}C has been absorbed. The rate declined rapidly in the succeeding hours. About 20% of radioactivity was still present in the skin at 24 h.

Discussion. The results reported herein demonstrated that the dermis of rats is capable of taking up aflatoxin B_1. Since the main target organ of the toxin is shown to be liver and the toxic effect was absent on the painted skin area, the present studies also indicate that the toxicity of aflatoxin B_1 is tissue specific.

It has been suggested[6] that aflatoxins are carcinogenic per se, and that they can exert their carcinogenic action

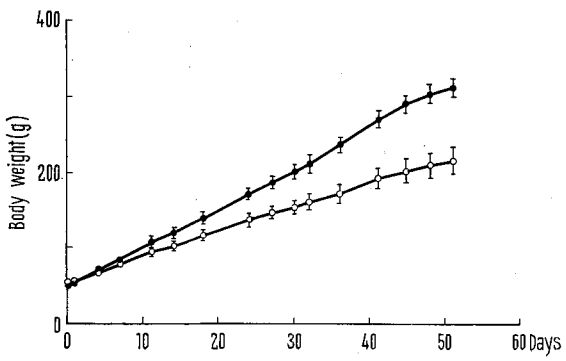

Fig. 1. Growth of rats painted on the skin with aflatoxin B_1 solution (○—○), or only with vehicle (●—●). The vehicle used was N,N-dimethyl formamide-propylene glycol (1:1, v/v). Data are expressed as mean values ± S.E.M. from 6 rats/group.

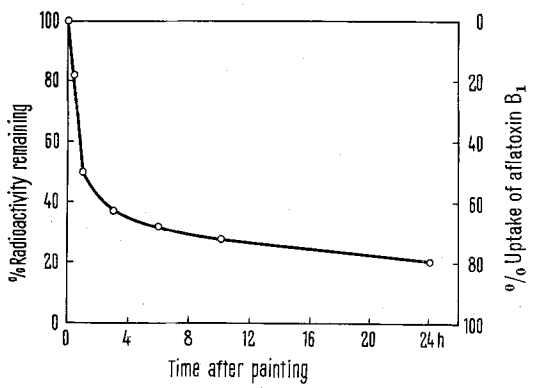

Fig. 2. Uptake of aflatoxin B_1-^{14}C by the skin of rats at various time intervals after painting. The method is described in the text. Values are averages of 2 experiments.

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in various organs of the body to which they are carried by the blood stream. Rao et al.[7] recently indicated that aflatoxin B was capable of binding with plasma albumin in vitro. Therefore a possible in vivo transport mechanism for aflatoxin as an albumin complex has been suggested.

The marked increase of glycogen content of the hepatic cells is rather surprising. The result is in contrast to the report of Shank and Wogan[8] who found that liver glycogen was not affected by a 5 daily oral administration of sublethal dose of the toxin to rats. Whether or not this is due to the different routes of administration or the duration of treatment remains to be studied[9].

Zusammenfassung. Nachweis der Absorption von Aflatoxin B_1 durch die Rattenhaut. Die toxische Leberwirkung nach der Hautbepinselung entsprach den von anderen Autoren gefundenen Verhältnissen bei peroraler oder intraperitonealer Verabreichung.

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Enhanced Phagocytic Function in vivo After Whole Body Irradiation

Cells of the reticuloendothelial system (RES) are generally considered to be very radioresistant. They usually shown no morphological damage after even high doses of radiation[1] and little change in phagocytic capacity when tested in vitro[2,3]. The results are far more divergent, however, when the phagocytic activity of the RES as a whole is studied in irradiated animals: depression, no change and increase of activity have been reported[4]. This is probably partly due to differences in species, nature of particles used for measuring phagocytosis, dose of radiation, and time after the exposure, among the different authors. One thing is, however, common to most of these in vivo studies, namely that RES function was tested only within the first few days after irradiation.

Experiments described below were designed to test the phagocytic activity of the RES, with either inert or immunogenic particles, at later times after sublethal or lethal irradiation.

Clearance of colloidal carbon. CBA/H mice, 4–5 months old, were irradiated with 600, 800 or 1000 rads of X-rays[5]. 7 or 14 days later they were injected i.v. with colloidal carbon (16 mg/100 g body wt., Pelikan C11/1431a) and the rate of clearance was measured as described before[5], except that blood samples were obtained from the orbital venous plexus.

Colloidal carbon was cleared exponentially from peripheral blood of all mice, but faster in irradiated than in control ones. At 7 days after 1000 rads and 14 days after 800 rads, carbon disappeared about twice as fast as in unirradiated controls ($T_{0.5}$ = 29.6 and 34.0 min respectively as compared to 60.5 min). The rate of clearance, K (also known as the phagocytic index), was significantly higher in all irradiated groups than in the control group and increased with increase in the radiation dose (Table I).

The rate of clearance of particles from the blood is considered to be a measure of the overall phagocytic activity in vivo in which the liver is known to play a major part[6]. The present results could therefore be interpreted as indicating enhanced phagocytic function of the RES after irradiation. However, in view of the suggested increase in vascular permeability after irradiation[7] it could be argued that faster disappearance of colloidal particles from peripheral blood is a result of general leakage from the vascular system and not necessarily caused by an increased activity of the macrophage system. These 2 possibilities could be distinguished by measuring the uptake of particles by those organs known to play a major role in the removal of particles from the blood stream by RES activity. A general increase in vascular permeability would lead to a reduced uptake in specific organs while an increased uptake in an organ like the liver would suggest greater activity of the macrophage system. In order to study the organ uptake of particles, radioactively labelled bacteria were used in the next experiment.

Clearance and uptake of bacteria. E. coli, strain B/r, were grown to stationary phase, killed by heating to 60 °C for 30 min and labelled with ^{51}Cr (sodium chromate solution, 216 μCi/1 μg Cr, Amersham) as described by Howard et al.[8]. There was less than 3% free ^{51}Cr in the final preparation. CBA/H male mice, about 5 months old,

Table I. Rate of clearance of colloidal carbon from peripheral blood of irradiated and control mice[a]

Radiation dose (rads)	Rate of clearance ($K \times 100$) at times after irradiation[b, c] 7 days	14 days
600	1.46 ± 0.08 (5)	1.32 ± 0.03 (5)
800	1.80 ± 0.11 (15)	2.25 ± 0.17 (20)
1000	2.41 ± 0.13 (9)	–
Controls		1.17 ± 0.03 (32)

[a] All values are means ± standard errors. [b] Number of mice per group is given in brackets. [c] All means are significantly different from the control at 0.05–0.01 level.

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