INVESTIGATION OF THE ACTION OF AFLATOXIN B_1 *in vitro* AND *in vivo*

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The direct and transplacental action of aflatoxin B_1 was compared in organ cultures of embryonic lung tissue of strain A mice, BD-IX rats, and golden hamsters. It was shown to have a toxic action on the culture but no carcinogenic effect. In experiments on mice transplacental penetration of aflatoxin B_1 led to an increased frequency of mammary gland tumors only in the progeny.

KEY WORDS: *Aflatoxins; organ cultures; transplacental action.*

Considerable experimental evidence of the toxicity and carcinogenic action of the aflatoxins in the postnatal period has now accumulated [7, 10, 12-14]. However, aside from isolated studies [8, 11], virtually no investigations have been made of their transplacental action.

In this investigation the direct and transplacental action of aflatoxin B_1 was studied *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

The *in vitro* experiments were carried out on organ cultures of embryonic lung tissue from strain A mice, BD-IX rats, and golden hamsters. During the last third of pregnancy the animals received two, three, or four subcutaneous injections of aflatoxin B_1 in a dose of 0.01 mg/g body weight per injection. The lungs of the embryos of the experimental animals were explanted for organ culture by the method adoped in the writers' laboratory [2]. The direction action of aflatoxin B_1 was studied on organ cultures of embryonic lung tissue of strain A mice with the toxin in a concentration of 0.03 and 0.015 µg/ml medium. Organ cultures of embryonic lung tissue of intact animals served as the control.

The *in vivo* experiments were carried out on strain A mice which received one, two, or three subcutaneous injections of aflatoxin B_1 in a dose of 0.01 mg/g body weight per injection in the last third of pregnancy. The experimental mothers (F_0) and their progeny (F_1) were sacrificed at the times indicated and examined macroscopically.

EXPERIMENTAL RESULTS

The results showing the effect of the direct action of aflatoxin are given in Table 1.

When the toxin was used in a concentration of 0.015 µg/ml medium the frequency of degenerative changes in the experimental cultures was significantly lower (18%) than in the control (32.4%; $P < 0.001$). Destruction of the organotypical structure, sometimes with signs of hyperplasia, was observed in 25.85% of the experimental cultures. The frequency of these changes was virtually independent of the duration and time of administration of the test substance.

Aflatoxin B_1 in a concentration of 0.03 µg/ml, if acting for 1-3 days, caused a marked increase in the frequency of extensive necrosis (14.2%) compared with the control (1.63%). Disturbance of the organotypical structure with very slight evidence of hyperplasia was ob-

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TABLE 1. Direct Action of Aflatoxin B_1 on Organ Cultures of Embryonic Lung Tissue of Strain A Mice

Concentration, μg/ml medium	Duration of exposure (days)	Duration of culture (days)	Number of explants: total	unchanged	with disturbance of organotypical structure	with degenerative changes	with extensive necrosis
0,015	1—10	8—25	604	337(57,0)	256(25,85)	109(18)	10(1,65)
0,03	1—3	8—25	430	175(40,7)	25(5,8)	161(31,4)	61(14,2)
Control	—	8—25	738	485(67,5)	2(0,27)	239(32,4)	12(1,63)

Legend. Here and in Table 2 figures in parentheses are percentages.

TABLE 2. Transplacental Action of Aflatoxin B_1 on Organ Cultures of Embryonic Lung Tissue of Mice, Rats, and Golden Hamsters

Species and strain of experimental animals	Dose of substance, mg/g body weight	Number of explants: total	unchanged	with disturbance of organotypical structure	with degenerative changes	with total necrosis on 1st day of culture
Mice, strain A	0,04	340	—	—	—	340(100,0)
	0,03	517	43(8,3)	38(7,35)	36(6,95)	400(77,3)
	0,02	635	92(14,5)	42(6,6)	376(59,1)	125(19,7)
	Control	738	485(65,7)	2(0,27)	251(34,0)	—
Rats, BD-IX	0,02	1075	338(31,4)	18(1,67)	276(24,48)	452(42,0)
	Control	456	309(68,0)	3(0,6)	144(31,4)	—
Golden hamsters	0,02	240	102(42,5)	62(25,8)	16(6,65)	60(25,0)
	Control	128	94(73,5)	18(14,0)	16(12,5)	—

served in only 5.8% of the experimental cultures.

A study of the transplacental action of aflatoxin B_1 showed (Table 2) that when injected into the pregnant female in a dose of 0.04 mg/g body weight it had a powerful toxic action on organ cultures of the embryonic lung tissue of strain A mice. All the explants died during the first 2 days of culture. In doses of 0.03 and 0.02 mg/g body weight of the donor 77.3 and 19.7% of the explanted fragments of embryonic lung tissue respectively died during this period. As a result of the transplacental action of aflatoxin B_1 in a dose of 0.02 mg/g complete necrosis of the tissue during the first stage of the experiment was observed in 42% of explants of embryonic lung tissue of BD-IX rats and 25% of explants from golden hamsters.

The transplacental administration of aflatoxin B_1 in a dose of 0.02 mg/g body weight of the donor thus had the strongest toxic action on the embryonic lung tissue of the rats and the weakest on the embryonic lung tissue of the mice ($P < 0.001$), to correspond to the sensitivity of these animals to its toxic and carcinogenic action.

In the surviving experimental explants during further cultivation marked degenerative and necrotic changes were observed together, in some cases, with a distrubance of the organotypical structure and the development of histiotypical growth with slight features of hyperplasia. Unlike other carcinogens studied previously by the writers in experiments with *in vitro* organ cultivation of embryonic rodent lung tissue [2, 3, 5, 6], however, in this case no marked hyperplastic preadenomatous changes were observed in the epithelium, still less any adenomas of the lungs. This could be attributed to the fact that aflatoxins unlike, for example, urethane and benz(a)pyrine are powerful hepatocarcinogens, and in the present case lung tissue is not the target for carcinogenesis even in a system so sensitive as organ cultures of embryonic lung tissue of strain A mice, with a high predisposition to adenoma.

The results of the study of the action of aflatoxin B_1 *in vivo* in doses of 0.01, 0.02, and 0.03 mg/g body weight on experimental female strain A mice and on their progeny showed that in none of the doses used did the substance cause any appreciable increase in the frequency of adenoma of the lungs or in the number of adenomas, or did it accelerate their development compared with the intact control (Table 3). Aflatoxin B_1 under these circumstances likewise did not exhibit any hepatotropic action. The possible reason for this may have been the duration of its action. In the present experiments this was limited to the

TABLE 3. Action of Aflatoxin B_1 on Experimental Females and Their Progeny

Dose of aflatoxin B_1, mg/g body weight	Age of mice, months	Number of animals	Number of mice with tumors					
			of lungs		of mammary glands		elsewhere	
			absolute	%	absolute	%	absolute	%
Experimental females (F_0)								
0,01—0,03	12—24	47	16	34,1	13	27,6	—	
Experimental progeny (F_1)								
0,01	1	60(26♂+34♀)	—	—	—		—	
	5	57(29♂+28♀)	1♀	3,57	—		—	
	12	26(21♂+5♀)	2(1♂+1♀)	7,7	5♀	100,0	—	
	24	6(5♂+1♀)	3♂	50,0	1♀	100,0	1 (liver)	
0,02	1	56(21♂+35♀)	—		—		—	
	5	70(29♂+41♀)	—		—		—	
	12	35(22♂+13♀)	7(2♂+5♀)	20,0	—		1 (liver)	
	24	8(5♂+3♀)	4(3♂+1♀)	50,0	—		—	
0,03	1	56(21♂+35♀)	—		—		—	
	5	41(24♂+17♀)	2♀	5,0	—		—	
	12	2♂	—		—		—	
Control	1	80(39♂+41♀)						
	5	91(51♂+40♀)	1♀	1,1	1♀	2,4	—	
	12	282(140♂+142♀)	56(24♂+32♀)	19,8	26♀	18,3	1 (kidney)	
	24	86(38♂+48♀)	29(13♂+16♀)	35,0	8♀			

fetal period of embryogenesis and did not exceed 3-7 days; however, the frequency of liver tumors depends more on the duration of its action than on the total dose of aflatoxin given [11]. Mice, on the other hand, are most resistant to the toxic and carcinogenic action of aflatoxins [14]. Meanwhile a statistically significant increase in the number of mammary gland tumors was observed in the experimental (F_1) progeny (Table 3).

Aflatoxin B_1 thus did not cause the development of tumors in adult mice, but its carcinogenic action was manifested in their progeny. Similar results were observed in previous experiments [4, 6]. Observations of a like nature have also been made in clinical practice: Young girls and women whose mothers have taken estrogens during pregnancy have developed carcinoma of the vagina [9]. The mammary gland tumors developing in the present experiments in the progeny of the experimental animals are interesting also because the mammary gland tissue is not the proper target for the carcinogenic action of aflatoxin. In this case aflatoxin B_1, by injuring the liver tissue, may perhaps disturb its detoxicating function relative to estrogens. Consequently hyperestrogenemia develops and causes tumors of the mammary glands. It will be recalled that more than 20 years ago the development of mammary gland tumors in CC57Br mice after chronic poisoning with CCl_4 was observed in this same laboratory [1].

The experimental observations described above are evidence of distinguishing features of the carcinogenic action of the natural substance aflatoxin B_1. Animals not only of different species (and also different organs of the same animals) but also during different periods of life (postnatal and prenatal) differ in their sensitivity to its action.

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SYNCHRONIZATION OF CELL PROLIFERATION IN SARCOMA 37 OF MICE BY HYDROXYUREA

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The synchronizing action of hydroxyurea on the passage of sarcoma 37 cells through the S phase and mitosis was investigated in mice, allowing for diurnal fluctuations in mitotic activity and the index of labeled nuclei. The degree of synchronization was estimated from changes in the number of proliferating or labeled cells and the rate of change of synchronization. The tumor consisted of at least two cell populations in which variations in the number of cells both in the S period and also, probably, in mitosis were out of phase. The degree of artificial synchronization of the cells in mitosis based on the rate of change of synchronization was much higher than the natural level in the tumor not divided into separate populations. However, the number of cells undergoing artificial synchronization was not significantly different not only from the number of cells in the tumor undivided into separate populations, but also from the number of cells naturally synchronized in one of the populations. A possible explanation of this fact is that hydroxyurea acted on only one group of cells, for fluctuations in the number of DNA-synthesizing cells in the separate populations also were out of phase.

KEY WORDS: *Sarcoma 37; mitosis; DNA synthesis; hydroxyurea; synchronization of cell proliferation.*

Cell systems synchronized with respect to proliferative processes can yield more accurate information about the mitotic cycle and its changes under different conditions. For this reason, besides the analysis of natural synchronization, in recent years artificial synchronization has been used, especially in systems whose natural synchronization is weak. Hydroxyurea, which temporarily blocks the passage of cells from the G_1 to the S phase and DNA synthesis, is an effective synchronizer of cell proliferation in tumors *in vivo* [6, 8].

In this investigation the synchronizing action of hydroxyurea on cell proliferation was studied in sarcoma 37, making allowance for diurnal variations in mitotic activity and in the number of DNA-synthesizing nuclei. The need for investigating diurnal rhythms during experimental procedures directed toward cell division is partly explained by the unequal action of certain cytostatics when administered at times of maximal or minimal proliferative activity [1, 2, 5].

EXPERIMENTAL METHOD

The tumor chosen for this investigation was sarcoma 37, inoculated into albino mice in which the synchronization of cell proliferation had been studied in the esophageal epithe-

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