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Received Oct. 9, 1967. P.S.E.B.M., 1968, Vol. 127.

### Effect of Aflatoxin on Human Leukocytes (32740)

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(Introduced by J. Reilly)

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Aflatoxin B<sub>1</sub>, a mycotoxin produced by *Aspergillus flavus*, is carcinogenic in rats (1-3) and trout (4). Studies in tissue culture and in cell-free systems indicate that aflatoxin primarily affects the synthesis of nucleic acid (5,6). Chromosome abnormalities have been produced by this compound in seedling roots of vetch (*Vicia faba*) (7) and in a cell line derived from the kidney of a rat kangaroo (*Potorous tridactylis apicalis*) (7,8). The experiments reported in this paper were designed to investigate the effect of aflatoxin on human leukocyte culture and to characterize the type of response.

**Materials and Methods.** Two preparations of aflatoxin were used: an aflatoxin mixture (495) containing 15% B<sub>1</sub>, 9% G<sub>1</sub>, and less than 1% B<sub>2</sub> and G<sub>2</sub>; and a crystalline B<sub>1</sub> preparation<sup>1</sup> (6).

Leukocyte cultures (72 hours) were set according to a modification of the method of Moorhead *et al.* (9), using plasma inocula from three healthy females. Aflatoxin 495 was added to produce final concentrations of 1.0, 5.0, 10.0, 25.0, and 50.0  $\mu\text{g/ml}$  and aflatoxin B<sub>1</sub> at a final concentration of 40.0  $\mu\text{g/ml}$  to replicate cultures.

<sup>1</sup> Aflatoxin was obtained from Dr. A. D. Campbell, Division of Food Chemistry, Food and Drug Administration.

Our preliminary work had shown that aflatoxin is toxic when added initially at the time of plasma inoculation. Therefore, exposure was delayed until 22 hours after inoculation. Cells were exposed to aflatoxin for either 8 or 48 hours and were harvested after 70-hours total incubation. A dose effect in percent was determined by scoring number of metaphase cells with chromosomal aberrations per total number of analyzable metaphase cells. Photomicrographs were made of all cells with microscopically detectable abnormalities, and the abnormalities were further scored as to type (break, gap, translocation, deletion, fragment), site (long arm, short arm, centromere) and karyotypic group according to the Denver Report (10).

**Results and Discussion. Mitotic rate.** A definite inhibition of mitosis was found after 8-hours exposure to aflatoxin (Table I). The mitotic inhibition was greater after 48-hours

TABLE I. Mitotic Index of Aflatoxin-Treated Cells at Various Exposure Times.

Length of exposure (hours)	Aflatoxin concentrations ( $\mu\text{g/ml}$ )					
	0	1	5	10	25	50
8	4.2	4.8	3.6	2.6	3.8	3.0
48	4.2	3.4	2.8	2.2	1.9	1.4

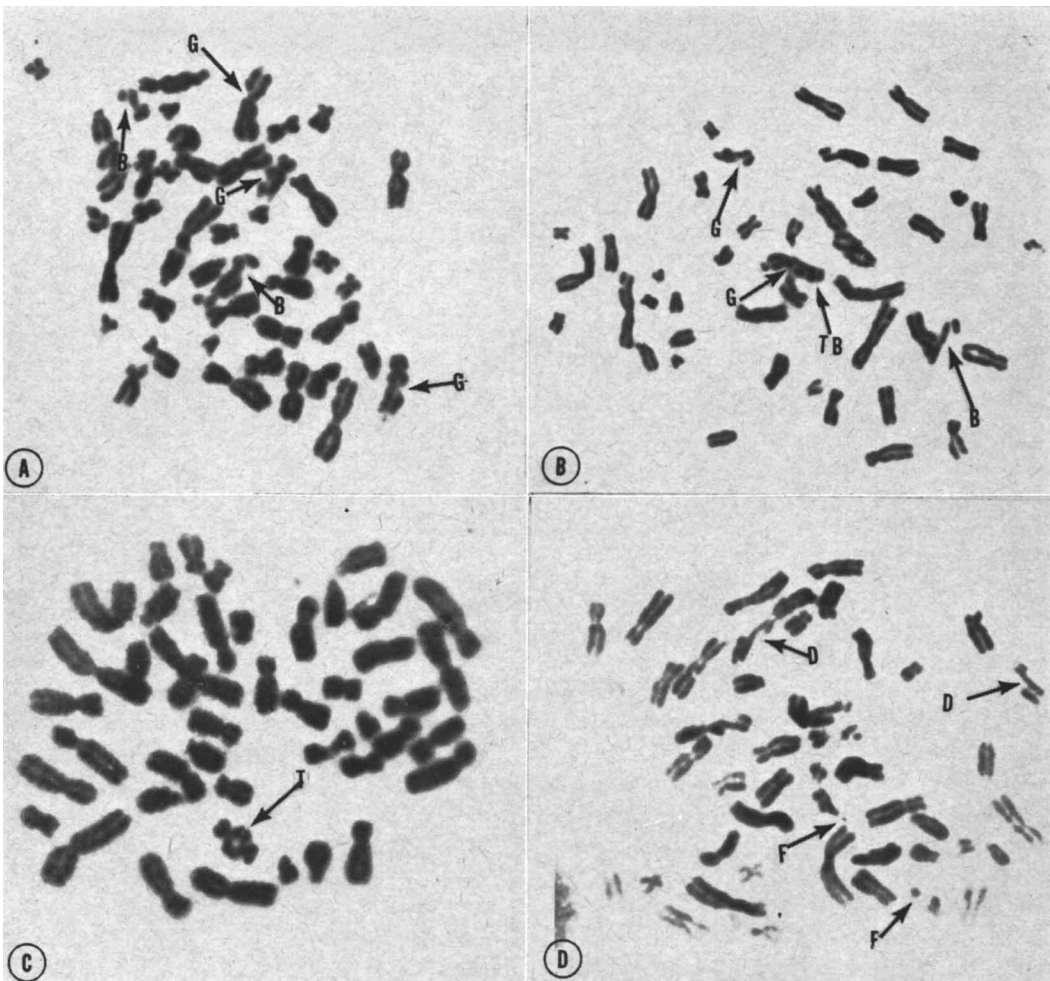


FIG. 1. Chromosome aberrations produced by aflatoxin. A. Aflatoxin 5 ppm at 8-hour exposure. B. Aflatoxin 25 ppm at 8-hour exposure. C. Aflatoxin 25 ppm at 48-hour exposure. D. Aflatoxin 50 ppm at 48-hour exposure. Abbr.: G = gap; F = fragment; D = deletion; TB = terminal bud; and T = translocation.

exposure to the chemical and the effect was proportional to the concentration of aflatoxin.

*Cytogenetic studies.* Chromosomal aberrations included gaps, breaks, fragments, deletions, and translocations, and each aberration usually affected only one chromatid. In general, gaps were more prevalent than breaks, and the breaks exhibited a definite discontinuity in chromatin with no connecting strand between the broken ends (Fig. 1A). A few isochromatid gaps were observed at the distal ends of the long arms resulting in terminal "buds" (Fig. 1B). Other isochromatid breaks and gaps were infrequently seen. Aflatoxin

induced occasional exchange configurations between apparent homologues suggestive of somatic pairing (Fig. 1C). Stickiness of chromosome ends resulting in chromatin bridges between chromosomes was also observed. Acentric fragments were apparent at the highest concentrations of aflatoxin used (Fig. 1D). Treated cells exhibited a somewhat greater incidence of aneuploidy than controls, but this increase was not statistically significant.

The percentage of cells with breakage increased both with increasing concentrations of aflatoxin and with lengthened exposure

TABLE II. Distribution and Test for Randomness of Obvious Chromosome Gaps and Breaks.

Aflatoxin ( $\mu\text{g/ml}$ )	Prepa- ration	Number of breaks by chromosome group									Mean no. of breaks per cell	
		A			B	C	D	E	F	G		Total
		1	2	3								
Control			1	1		1					3	0.06
1.0	495	1	1	1	4	7	1	2	0	0	17	1.00
5.0	495	5	8	4	10	26	9	4	1	1	68	1.50
10.0	495	14	5	5	6	24	4	8	0	0	66	1.55
25.0	495	20	16	6	18	37	9	5	0	0	111	2.40
40.0	B <sub>1</sub>	1	0	2	5	11	3	1	3	1	27	2.00
50.0	495	6	9	4	7	16	3	1	0	3	49	3.80
Total observed <sup>a</sup>		47	39	22	50	121	29	21	4	5	338	
Expected		29.10	27.24	22.84	40.70	129.42	33.73	28.87	15.18	10.85	337.94	
$[(O-E)^2]/E$		11.01	5.08	.03	2.12	.55	.66	2.14	8.23	3.15		

$X^2 = 32.97$   
 $p < .0005; df = 8$

<sup>a</sup> Exclusive of control.

time (Fig. 2). Exposure to 1–50  $\mu\text{g/ml}$  aflatoxin for 48 hours produced 1.1–24.4% cells with breakage. Removal of aflatoxin after 8-hour exposures and addition of fresh media resulted in a decrease in breakage frequency; 0.0–8.3% at the same concentrations. Controls exhibited 0.07% breakage. The mean number of breaks per cell increased with increasing concentrations of aflatoxin (Table II). These figures represent maximum exposure to aflatoxin.

Aflatoxin did not affect the chromosomes in a random pattern based on the relative quantity of chromatin as presented in the Denver Report (10) (Table II:  $X^2 = 32.97$ ,

$p < .0005; df = 8$ ). The largest chromosomes, i.e., Groups A and B, showed an excessive amount of breakage based on DNA content, with chromosome no. 1 having the largest amount. Groups C and D were within the expected range while the smaller groups, E, F, and G, were deficient in breakage. The long arms of the chromosomes were more frequently attacked in all karyotypic groups than either the short arms or the centromere (Table III).

TABLE III. Compilation of Breakage Sites on Chromosomes.

	Long arms (%)	Short arms (%)	Centromere (%)
Aflatoxin	65	22	13
Control	67	33	0

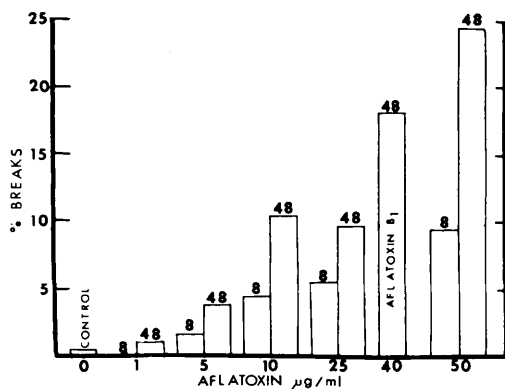


Fig. 2. Chromosome breaks with increasing concentrations of aflatoxin.

*Summary.* Aflatoxin inhibits mitosis and causes breakage and translocations in the chromosomes of cultured human leukocytes. The nonrandom pattern of breakage was confined mainly to the largest chromosomes of the human complement. Chromosome no's. 1 and 2 were particularly affected, while groups E, F, and G exhibited a lower than expected frequency of breaks.

The effect of aflatoxin on human leukocytes is of the delayed type which is characteristic

of chromosome breaking compounds such as alkylating agents (11,12).

The authors wish to acknowledge the technical assistance of Miss Regina Austin and Mrs. Holdine Roginski.

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Received Oct. 11, 1967. P.S.E.B.M., 1968, Vol. 127.

### Immunosuppressive Activity of Rabbit Antisera Directed against Mouse Lymphocytic Leukemia L1210\* (32741)

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In the course of studies on the *in vivo* localizing properties of antibodies directed against the lymphocytic leukemia L1210, it was found that the antibodies exhibited marked localization in spleens of the injected mice as did antibodies directed against homogenates of mouse spleen and lymph nodes. Such localization in spleen was seen with anti-L1210 sera prepared against cells grown in continuous culture as well as those in the ascites form.

In view of the fact that antibodies against lymphocytic cells are known to suppress antibody formation(1), it was of interest to determine whether the antibodies to cultured leukemic cells and to ascites cells would cause immunosuppression in mice.

The effect on the immune response was evaluated by determining the concentration of plaque forming cells in the spleen and by determining the titer of humoral hemagglutinins 4 days following a single injection of sheep erythrocytes into mice. The results are reported here.

\* Supported in part by Contract #AT-(30-1)-2651 from the U. S. Atomic Energy Commission.

*Materials and Methods.* The mice used throughout this study were 2 months old, male inbred DBA/2.

The L1210 cells grown in a suspension cell culture were obtained from Dr. G. E. Moore. Some of the characteristics of these cells were described(2). The ascites form of the L1210 cells was maintained in male DBA/2 mice and was transplanted weekly by intraperitoneal injections.

Antisera were prepared in rabbits against the following materials: (i) L1210 leukemia cells grown in cell culture, (ii) L1210 leukemia cells grown *in vivo* as ascites tumors in DBA/2 mice, and (iii) homogenates of DBA/2 spleens and lymph nodes.

The leukemia cells from either source were washed 3 times with 50 volumes of Ringer's solution. Three rabbits were injected with cultured L1210 cells and 4 rabbits with ascites L1210 cells. Each rabbit received 2-3 injections at 2-week intervals of a cell suspension in Freund's adjuvant equivalent to 0.25-0.4 ml of packed cells. The injections were made intradermally (in the footpads), subcutaneously, and intramuscularly. The