INDUCTION OF SOMATIC MOSAICISM IN DROSOPHILA AND OF DNA REPAIR SYNTHESIS IN MAMMALIAN LIVER CELL CULTURES BY MYCOTOXINS

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The genotoxic properties of chemical carcinogens are nowadays being brought to light at an increasing rate. Injuries to the DNA and mutations in various systems are used by 85-90% of carcinogenic polycyclic hydrocarbons, nitroso compounds, and other agents [9]. So far as the carcinogenic mycotoxins are concerned, the results of their investigation in express tests are contradictory [6, 8, 12, 14, 16].

It was accordingly decided to investigate the genotoxic properties of four mycotoxins in two test systems not previously used for this purpose. In the first test the indicator of activity was induction of somatic mutagenesis (the total of somatic recombination, point mutations, and elimination of one of the X chromosomes) in imaginal disk cells of drosophila larvae, in the second it was induction of DNA repair synthesis in cultures of human embryonic or adult rat liver cells.

EXPERIMENTAL METHOD

Citrinin, isolated from a culture of Penicillium citrinum AUA 523 by Dr. S. Kneist (Department of Mycology, Dermatology Clinic, Medical Academy, Erfurt, East Germany), stachbotryotoxin, isolated from a culture of Stachbotrys atre by Dr. M. Palyusik (Veterinary Research Institute, Hungarian Academy of Sciences, Budapest), and patulin (from Arzneimittelwerk, Dresden, East Germany), were generously provided by Dr. R. Thust. Aflatoxin B_1 was obtained from Calbiochem (USA).

Experiments were carried out by the method described previously [2] on D. melanogaster larvae, heterozygous for two recessive genes y and sn^3, located in the X chromosome. The Y (yellow) gene determines the yellow color of the body and bristles in the homozygous state, whereas the sn^3 (singed^3) gene determines the twisted shape of the bristles, and heterozygous $sn^3/+$ individuals have black, straight bristles. Heterozygous $y+/+sn^3$ larvae were obtained by mass crossing of virgin females of the $y+^{sn}/y+^{sn}$ genotype with males of the wsn^3/y genotype in test tubes 2 cm in diameter, each containing 10 ml of standard nutrient medium. After 3 days the parents were transferred to fresh tubes, and 0.3 ml of an aqueous solution or suspension of the test mycotoxins with 5% dimethyl sulfoxide (DMSO) or a 5% solution of DMSO in water was applied to the surface of the nutrient medium on which the larvae were developing. At the first flight of the flies females were studied under the MB F-4 microscope and the presence of mosaic patches (yellow or sn^3 bristles) was recorded; their frequency was calculated as a total for all types of patches (single yellow, singed, and double) per 100 individuals.

Liver cells from male Wistar rats were isolated and cultured by G. A. Bannikov's method in petri dishes on coverslips coated with collagen [4]. A culture of human embryonic liver cells at 7-12 weeks of development was obtained by Eraizer's method [3]. Mycotoxins, dissolved in medium L-15 (from Flow Laboratories, England) with 1% acetone was added to the rat liver cultures 1-2 days after seeding, and to human liver cells 3-4 days after seeding, i.e., toward the time of formation of a complete monolayer. DNA repair synthesis was determined

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TABLE 1. Induction of Somatic Mutations in D. melanogaster by Mycotoxins

Mycotoxin	Concentration, M	Number of females examined	Survival rate, % of control	Frequency of mutation, %	P
Control	—	1815	100	0,61	
Citrinin	$8 \cdot 10^{-2}$	359	107,9	0,55	>0,05
	$6 \cdot 10^{-3}$	754	159,4	0,92	>0,05
	$4 \cdot 10^{-4}$	597	90,0	1,17	>0,05
	$1,4 \cdot 10^{-4}$	873	92,2	1,26	>0,05
Stachbotryotoxin	$1,1 \cdot 10^{-2}$	282	49,7	1,41	>0,05
	$4 \cdot 10^{-3}$	1239	87,3	0,72	>0,05
	$4 \cdot 01^{-4}$	395	93,2	0,76	>0,05
Patulin	$3,2 \cdot 10^{-2}$	511	108	1,36	>0,05
	$3,2 \cdot 10^{-3}$	1286	90,6	1,86	<0,01
	$4 \cdot 10^{-4}$	747	99,4	0,67	>0,05
Aflatoxin B_1	$3,2 \cdot 10^{-3}$	0	0	—	
	$3,2 \cdot 10^{-4}$	0	0	—	
	$3,2 \cdot 10^{-5}$	0	0	—	
	$1 \cdot 10^{-6}$	172	22,7	5,23	<0,001
	$3,2 \cdot 10^{-7}$	1094	60,7	6,58	<0,001
	$3,2 \cdot 10^{-8}$	1154	78,2	2,34	<0,001

autoradiographically. Immediately after exposure to the mycotoxins the cells were incubated with a solution of [^{3}H]thymidine (12 Ci/mmole, 10 μCi/ml) for 1 h and exposed with NTB-Kodak photographic emulsion for 14 days. During this procedure cells in which replicative DNA synthesis was taking place (S cells) contained more than 100 grains of silver above their nuclei, whereas cells with DNA repair synthesis contained from 4 to 100 grains of silver, after deducting the background [1]. In each experimental group 100 nuclei were examined in succession, and the intensity of labeling and the percentage of cells repairing DNA were determined. During evaluation of repair synthesis, induced in the cell culture by agents inhibiting replicative DNA synthesis, all the most intensively labeled cells, equal in number to the number of S cells in the control, were regarded as S cells. Inhibition of replicative DNA synthesis, used as an indicator of toxic action, was determined from the decrease in the number of intensively labeled S cells (with more than 100 grains of silver per nucleus) in cultures treated with mycotoxins.

EXPERIMENTAL RESULTS

Somatic Mutagenesis in Drosophila. Citrinin in concentrations up to 8×10^{-2} M did not affect the survival of the larvae or their conversion into adult flies. The frequency of mutation was a little higher than the control level but the difference was not statistically significant (Table 1).

Stachbotryotoxin was found to be more toxic than citrinin, but in a toxic concentration (1.1×10^{-2} M) it had no significant effect on the frequency of mutation. All flies which developed in the presence of 1.1×10^{-2} M stachbotryotoxin were small and had morphoses of Delta type. In some individuals a reduplication of the thorax and scutellum, and anomalies or absence of legs, and so on, were observed. Furthermore, melanotic pseudotumors were found in the thorax or abdomen of 3.5% of females. If the concentration of the agent was reduced to 4×10^{-3} M the number of morphoses fell sharply, but pseudotumors were still found in 2% of individuals.

Patulin, which within the range of doses tested did not affect either survival rate or metamorphosis, caused a small but statistically significant increase in the mutation level in a concentration of 3.2×10^{-3} M.

Judging from its LD_{50} value aflatoxin B_1 is about 30,000 times more toxic for drosophila larvae than stachbotryotoxin. Flies which developed in the presence of aflatoxin B_1 had low viability, developed slowly, and were smaller than controls but morphoses were observed among them in only 5% of cases and were manifested as disturbances of wing shape; a pseudotumor was found in only one individual. The mutagenic activity of this agent was much higher than that of the others, and it was still present in dilutions of about 3.2×10^{-8} M, which is 100,000 times lower than the concentration of patulin with which some increase in the mutation level was obtained.

DNA Repair Synthesis in Liver Cell Cultures. The results of this part of the investigation were generally similar to those of the previous part. Only aflatoxin B_1 was found to be capable of inducing DNA repair synthesis in rat liver cell cultures, in agreement with previous observations [15]. The remaining mycotoxins did not induce this effect, even in toxic concentrations at which replicative DNA synthesis was completely or partially inhibited in human embryonic liver cells (Table 2).

TABLE 2. DNA Repair Synthesis in Liver Cell Cultures Treated with Mycotoxin

Mycotoxin	Concentration, M	Percent of labeled cells outside S phase	
		rat	human
Citrinin	$8 \cdot 10^{-4}$*	3±3	3±1
	$6 \cdot 10^{-4}$*	1	14±8
	$4 \cdot 10^{-4}$	3	16±17
	$2 \cdot 10^{-4}$	1±1	9±3
Control	—	2±1	9±10
Stachbotryotoxin	$3 \cdot 10^{-4}$	3±4	—
	$5 \cdot 10^{-5}$*	5±1	2
	$1 \cdot 10^{-5}$	6±2	8
	$6 \cdot 10^{-6}$	5±3	27
Control	—	9±12	3
Patulin	$1{,}6 \cdot 10^{-3}$*	2±3	2
	$8 \cdot 10^{-4}$	4±6	—
	$1{,}6 \cdot 10^{-4}$*	—	3
	$8 \cdot 10^{-5}$	3±2	—
	$1{,}6 \cdot 10^{-5}$	4±4	9
Control	—	9±12	3
Aflatoxin B_1	$5 \cdot 10^{-4}$	95±2	39±7
	$8 \cdot 10^{-5}$	82	—
Control	—	19±15	9±5

Legend. Cells were treated for 3-5 h. M ± m given for two or three experiments. Asterisk indicates decrease in number of intensively labeled S cells in culture of human hepatocytes by more than 90% compared with the control.

On the whole the results obtained with the powerful carcinogen aflatoxin B_1, which was used as a control compound, are evidence of the reliability of the test systems used.

Consequently, citrinin and stachbotryotoxin have no real genotoxic activity in the test systems used, and such activity in the case of patulin is only just perceptible. This agrees in general with the oncologic characteristics of these mycotoxins. Citrinin induces tumor formation only when combined with other compounds [7, 11]; carcinogenic properties of stachbotryotoxin are not mentioned in the literature, although this agent has been studied for a long time both experimentally and in clinical veterinary medicine [17]. Patulin is a weak carcinogen [5, 10], which is easily inactivated in the cell [13] without reaching the critical macromolecules.

On the whole the results confirm data of the writers' previous investigations, conducted on the same objects with carcinogens of other classes [1, 2] and they allow a parallel to be drawn between the mutagenic and carcinogenic properties of chemical compounds.

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LITERATURE CITED

1. I. V. Budunova and G. A. Belitskii, Vopr. Onkol., No. 11, 53 (1982).
2. L. M. Shabad, E. M. Khovanova, E. G. Logvinenko, et al., Dokl. Akad. Nauk SSSR, 231, 997 (1976).
3. T. L. Éraizer, K. N. Grinberg, and S. G. Vorsanova, Byull. Éksp. Biol. Med., No. 5, 123 (1975).
4. G. A. Bannikov, L. Saint Vincent, and R. Montesano, Br. J. Cancer, 42, 596 (1980).
5. F. Dickens and H. E. H. Jones, Br. J. Cancer, 15, 85 (1961).
6. A. Korte, Mutat. Res., 78, 41 (1980).
7. P. Krogh, B. Hald, and E. J. Pedersen, Acta Pathol. Microbiol. Scand. B., 81, 689 (1973).
8. M. H. Kuczuk, P. M. Benson, H. Heath, et al., Mutat. Res., 53, 11 (1978).

9. R. Montesano, H. Bartsch, and L. Tomatis (editors), Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, Suppl. 2, Lyon (1980), p. 85.
10. H. Osswald, H. K. Frank, D. Komitowski, et al., Food Cosmet. Toxicol., 16, 243 (1978).
11. Y. Shinobara, M. Arai, K. Hirao, et al., Gann, 67, 147 (1976).
12. R. Thust and S. Kneist, Mutat. Res., 67, 321 (1979).
13. R. Thust, S. Kneist, and J. Mendel, Mutat. Res., 103, 91 (1982).
14. M. Umeda, T. Tsursui, and M. Saito, Gann, 68, 619 (1977).
15. G. E. Williams, Cancer Res., 37, 1845 (1977).
16. A. Wright and S. von Lindroth, Mutat. Res., 58, 211 (1978).
17. T. D. Wyllie and L. G. Morehouse (editors), Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, Vol. 3, New York (1979), pp. 87, 327, and 462.