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Effect of sterigmatocystin on rat liver nuclear RNA

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STERIGMATOCYSTIN, a carcinogenic mycotoxin[1, 2] isolated from three fungal species,[3] is chemically related to aflatoxin B_1.[4, 5] In primary monkey epithelial cells sterigmatocystin inhibits mitosis and causes a decrease in the size of the nucleolus together with separation of the nucleolus into fibrillar, granular and dense areas.[6]

Actinomycin D, and several carcinogens including aflatoxin B_1, 3'-methyl-4-dimethylaminoazobenzene and 4-nitro-quinoline-*N*-oxide, produce a similar effect on the nucleoli of various cell types[7–10] and have an inhibitory effect on the synthesis of nucleolar RNA.[10] The present investigation was performed to determine the effect of sterigmatocystin on the RNA synthesis in rat liver nuclei.

Three groups of twenty-one Wistar rats, each weighing about 150 g, from our own colony, were used. The first group was injected intraperitoneally (i.p.) with sterigmatocystin (50 mg/kg) as a suspension (25 mg/ml) in a 1·5% solution of cellofas B (sodium carboxymethyl cellulose) in water. The sterigmatocystin was isolated from a culture of *Bipolaris* sp. on maize meal and purified. After recrystallization it was found to be 99 per cent pure. The second group was injected i.p. with a corresponding volume of 1·5% cellofas B in water. Three animals in each of these two groups were sacrificed ½, 1, 2, 3, 4, 16 and 48 hr after treatment. The rats in the third group were used as untreated controls. Two further groups of nine rats were injected with dimethylsulphoxide (DMSO) and sterigmatocystin in DMSO, respectively.

Fifteen minutes before the animals were killed by decapitation, each rat was injected i.p. with 5 μCi of 6-^{14}C-orotic acid (60·8 mCi/mM obtained from the Radio Chemical Centre, Amersham, England). The livers were removed and chilled in ice-cold 0·25 M sucrose + 3·3 mM $CaCl_2$. Pure nuclei were prepared according to the method described by Muramatsu *et al.*[11]

The nuclear pellet from 4 g of liver was resuspended in 5 ml 0·25 M sucrose and lysed with 5 ml of a 0·2% sodium lauryl sulphate solution in a Dounce homogenizer. 1-ml aliquots of the lysate were precipitated with 8 ml of 0·4 N perchloric acid at 0°, and after 15 min the precipitates were centrifuged. RNA was determined on the precipitates by mild alkaline hydrolysis;[12] an acidified hydrolysate with an absorbance of 34·2 at 260 mμ was taken to contain 1 mg of RNA nucleotides per ml.[13]

For the determination of ^{14}C-orotic acid incorporation into RNA, 1 ml of the above acidified hydrolysate was added to 15 ml of Bray's cocktail[14] and counted in a Beckman Scintillation Counting System for 10 min. The quenching was determined by adding an internal standard of 1-^{14}C-*n*-hexadecane with a specific activity of 1·06 μCi/g (obtained from the Radio Chemical Centre, Amersham, England). The samples were recounted and the quenching calculated. The results are expressed as DPM/μg RNA.

DMSO had a marked inhibitory effect on the incorporation of orotic acid in nuclear RNA 1 hr after exposure, which masked any effect produced by sterigmatocystin. For this reason sterigmatocystin was administered as a suspension in cellofas B solution.

The i.p. injection of sterigmatocystin had no effect on the total RNA of the liver nuclei up to 48 hr after administration. There was no difference between the incorporation of orotic acid in the untreated animals and that in the cellofas B-treated animals. The i.p. injection of sterigmatocystin, however, had an inhibitory effect on orotic acid incorporation (Fig. 1). The maximum inhibition was found 1 hr after treatment with the toxin, when the RNA-incorporation of the toxin treated rats was only 40 per cent of the RNA-incorporation of the control rats. Four hours after treatment, the incorporation was similar to that of the controls and remained at this level up to 48 hr.

The reduction in orotic acid incorporation after administration of sterigmatocystin may be the result of cellular damage rather than a direct effect of the toxin. Liver necrosis increases in severity up to 4 days after a single dose of sterigmatocystin,[15] whereas the incorporation of orotic acid returns to normal 4 hr after administration, indicating that the two effects of sterigmatocystin are independent of one another. Whether these effects are due to the action of sterigmatocystin itself or to

one of its metabolic degradation products is not known, but work on the metabolism of sterigmatocystin is now in progress. The reduction of orotic acid incorporation, however, appears to be the result of the direct action of the toxin or a metabolite of the toxin.

Sterigmatocystin inhibits RNA synthesis for 4 hr after a single i.p. dose as indicated by the decrease in orotic acid incorporation. Simard and Bernhard[16] postulate that compounds which inhibit RNA synthesis, cause segregation of the various components of the nucleolus. The effects of sterigmatocystin on RNA synthesis and nucleolar morphology are in agreement with this postulate. The

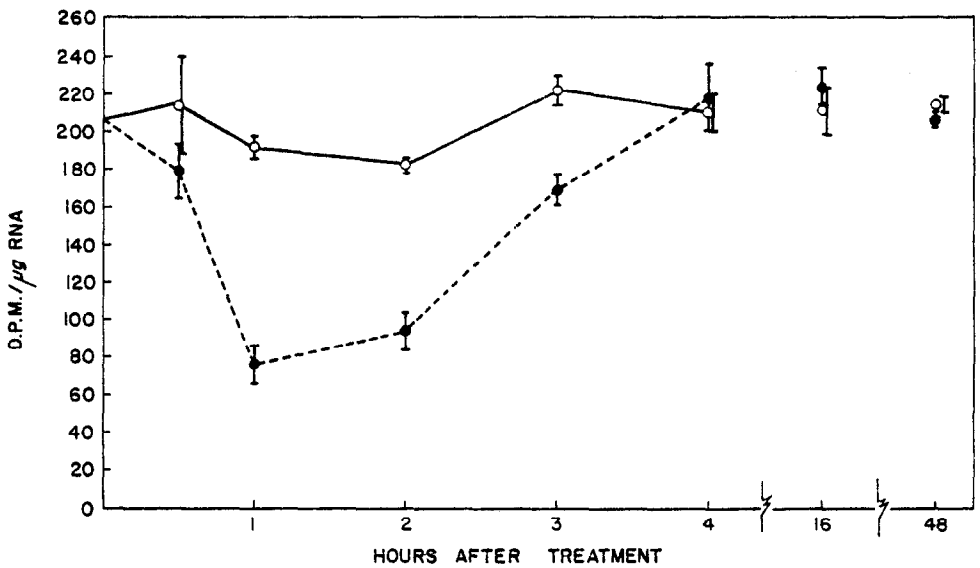

FIG. 1. Incorporation of ^{14}C-orotic acid into nuclear RNA of normal liver from rats treated with cellofas B (○—○) and liver from rats treated with sterigmatocystin suspended in cellofas B (●—●). The mean value obtained from untreated controls (group 3 in this text) was 205 DPM.

inhibition of RNA synthesis could be due to blocking of RNA polymerase[16] or to the interaction of sterigmatocystin with the acidic proteins from the nucleus, because it has been shown by several workers[17, 18] that acidic proteins from the nucleus have an effect on the syntheis of RNA. Several other carcinogens, including aflatoxin B_1, 3′-methyl-4-dimethylaminoazobenzene and 4-nitroquinoline-*N*-oxide, produce nucleolar changes in various cell types and an inhibition of RNA synthesis in rat liver nuclei. It appears, therefore, that these compounds produce some of their effects by interfering with genetic material and may be carcinogens by virtue of this fact.

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REFERENCES

1. F. DICKENS, H. E. H. JONES and H. B. WAYNFORTH, *Br. J. Cancer* **20**, 134 (1966).
2. I. F. H. PURCHASE and J. J. VAN DER WATT, *Fd. Cosmet. Toxicol.* **6**, 555 (1968).
3. C. W. HOLZAPFEL, I. F. H. PURCHASE, P. S. STEYN and L. GOUWS, *S. Afr. med. J.* **40**, 1100 (1966).
4. J. E. DAVIES, D. KIRKALDY and J. C. ROBERTS, *J. Chem. Soc.* 2169 (1960).

5. E. BULLOCK, J. C. ROBERTS and J. G. UNDERWOOD, *J. Chem. Soc.* 4179 (1962).
6. J. C. ENGELBRECHT, to be published.
7. T. S. RO, N. K. SHANKAN and H. BUSCH, *Cancer Res.* **26**, 780 (1966).
8. W. BERNHARD, C. FRAYSSINET, C. LAFARGE and E. LE BRETON, *C.r. Acad. Sci.* **261**, 1785 (1965).
9. S. S. LAZARUS, V. G. VETHAMANY, S. H. SHAPIRO and D. AMSTERDAM, *Cancer Res.* **26**, 2229 (1966).
10. L. R. FLOYD, T. UNUMA and H. BUSCH, *Exptl Cell Res.* **51**, 439 (1968).
11. M. MURAMATSU, K. SMETANA and H. BUSCH, *Cancer Res.* **23**, 510 (1963).
12. A. FLECK and H. N. MUNRO, *Biochim. biophys. Acta.* **55**, 571 (1962).
13. J. F. SCOTT, A. P. FRACCASTORO and E. B. TAFT, *J. Histochem. Cytochem.* **4**, 1 (1956).
14. J. A. BRAY, *Analyt. Biochem., N. Y.* **1**, 279 (1960).
15. I. F. H. PURCHASE, Personal Communication (1969).
16. R. SIMARD and W. BERNHARD, *Int. J. Cancer.* **1**, 463 (1966).
17. J. PAUL and R. S. GILMOUR, *J. molec. Biol.* **40**, 137 (1969).
18. J. PAUL and R. S. GILMOUR, *J. molec. Biol.* **34**, 305 (1968).