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The long-term influence of sterigmatocystin on mouse and rat liver nuclear deoxyribonucleases

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Normally the highest acid DNAase (deoxyribonuclease) cellular activity levels are found in lymphatic and neoplastic tissues while the lowest levels are found in spermatozoa and erythrocytes which do not replicate.[1] In contrast to the pattern in normal tissue, a lack of correlation between growth rate and acid DNAase activity was found in a series of malignant animal tissues.[2–4] Considerable increase in activity of pancreatic DNAase was found in *in vitro* experiments after the administration of various potent carcinogens.[5,6]

The present study reports sequential changes in activity of nuclear acid and alkaline DNAases throughout the period of administration of a potent hepatocarcinogen, sterigmatocystin,[7,8] to a susceptible (rat) and resistant (mouse) animal species. The experimental animals were random-bred Onderstepoort Albino mice and Wistar-derived Albino rats from our own colony. Due to the difficulty of dosing mice, the long-term administration (371 days) of sterigmatocystin was achieved by mixing the toxin with the standard laboratory mashed ration at a level of 0·2 mg per mouse daily. Groups of animals were killed by decapitation at predetermined intervals; each group consisting of four treated animals and four controls. Specimens of hepatic tissue were obtained for histological examination and thereafter the livers were weighed and homogenized.

Albino male rats with body weights of 100 ± 5 g received sterigmatocystin, dissolved in dimethylsulphoxide (DMSO) per gastric tube. The experiment lasted 335 days and the animals were dosed once per week with 20 mg sterigmatocystin per kilogram of body weight except between days 140 and 170. Controls received the same volume of DMSO only. Two treated and two control animals were killed by decapitation at 3-weekly intervals. The livers were excised, a sample placed in 10% buffered formalin for histological examination and the remainder homogenised.

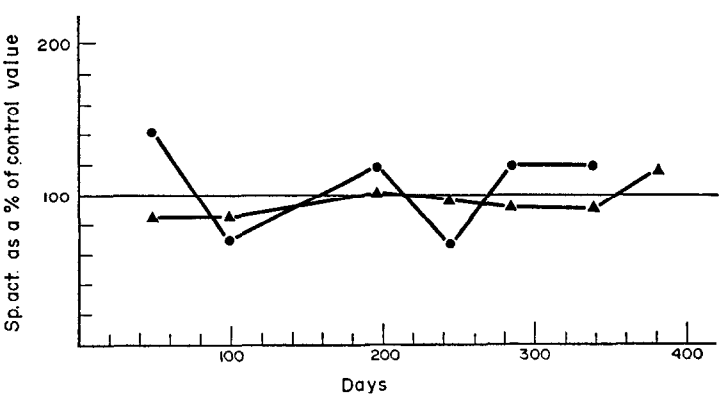

Fig. 1. Long-term influence of sterigmatocystin on the specific activities (in units/milligram of protein) of mouse liver nuclear acid DNAase (▲) and alkaline DNAase (●).

The isolation of liver nuclei was performed according to a modification of the method by Mahler and Cordes.[9] Activity assays of the acid and alkaline DNAases were performed with modifications on the nuclear sap and the acidic fraction of the liver nuclei.[10,11] For acid and alkaline DNAase, 1 unit (U) was defined as that amount of enzyme which produces one A_{260} U/hr. The specific activity (S.A.) was expressed as units/milligram of protein. The protein determination was carried out according to the method of Lowry *et al.*[12]

Results obtained from the eight groups of mice killed during the 371 days period indicated that long-term exposure to sterigmatocystin had virtually no effect on the specific activities of mouse liver nuclear acid and alkaline DNAases (Fig. 1). No histological changes could be detected in the livers of these mice.

Administration of sterigmatocystin to rats at a level of 20 mg/kg body weight weekly resulted in considerable changes in the activity of acid and alkaline DNAases (Fig. 2). Hyperplastic nodules were first detected macroscopically in the livers of rats killed on day 230. Microscopic examination of these livers revealed nodular regeneration while the internodular parenchyma exhibited cloudy swelling and marked narrowing of the sinusoids. Kupffer cells were very prominent while the chromatin of hepatocytes was clumped within pleomorphic nuclei. At day 335 nodular hyperplasia and foci of adenomata were observed in the liver sections of these animals.

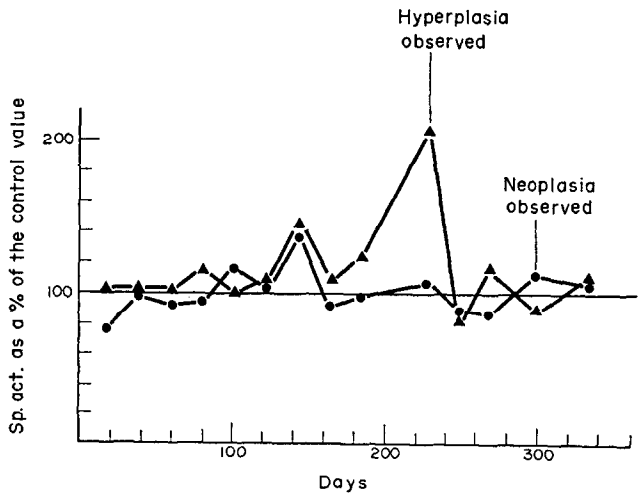

FIG. 2. Long-term influence of sterigmatocystin on the specific activities (in units/milligram of protein) of rat liver nuclear acid DNAase (▲) and alkaline DNAase (●).

The specific activity of nuclear acid DNAase decreased practically to normal (control) values when neoplasia commenced. This enhancement of acid DNAase activity in the precancerous phase may be of diagnostic value.

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Binding of amines to purified bovine adrenal medullary storage vesicle membranes*

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THE MEMBRANES of adrenal medullary storage vesicles play an active role in the maintenance of catecholamine stores. The transport of amines into the vesicles is stimulated by ATP and magnesium and inhibited by reserpine or *N*-ethylmaleimide;[1,2] the latter also reduces the ATPase activity of the membranes as well as the incorporation of ^{32}P from labeled ATP into membrane components.[3,4] Recent studies have suggested that "uptake" and "storage" of amines in the vesicles are two separate and distinct processes: Taugner[5,6] has shown that, when suspended in isotonic medium, isolated vesicle membranes can re-form into "empty" vesicles which still accumulate amines by an ATP and magnesium-stimulated mechanism, but which do not store amines stably. Similarly, amine uptake without stable storage can occur in newly formed storage vesicles *in vivo*.[7,8] Although serotonin is stored less stably than catecholamines in isolated vesicles, it is taken up to a greater extent, which suggests that serotonin has a greater affinity for "uptake" but a lower affinity for "storage".[9] Metaraminol, on the other hand, is both taken up and stored to a lesser extent than are catecholamines.[7,9,10] Studies of the uptake of small molecules by other systems[11] indicate that a membrane-bound macromolecular carrier is involved in the transport of the small molecule across the membrane. The studies reported here show that epinephrine and other amines which are taken up by isolated storage vesicles of the adrenal medulla are bound to the vesicle membranes and suggest that this binding is to a carrier involved in the uptake process.

Bovine adrenal glands were obtained from a local slaughterhouse and the cortices were stripped from the medullae. The latter were homogenized in 10 vol. of isotonic sucrose and purified storage vesicles were obtained by a combination of differential and discontinuous sucrose density gradient centrifugation as described by Smith and Winkler.[12] The vesicles were lysed by resuspension in a small volume of distilled water, dialysed overnight against 3 l water, and sedimented by centrifugation at 130,000 *g* for 30 min. The pellet was washed and resedimented three times, resuspended in a small volume of water, and layered over 1 M sucrose. After 1 hr at 100,000 *g*, the purified membranes were collected from the interface of the water and sucrose layers and washed once with distilled water. The final suspension contained 2 mg protein[13] per ml.

Incubations used in the binding studies each contained 0·1 ml of the membrane preparation and 0·9 ml of 10 mM Tris buffer (pH 7), with bivalent cations, adenine nucleotides, radioactive amines (1–5 μCi epinephrine-7-^{14}C, metaraminol-7-^{3}H or serotonin-2-^{14}C) and drugs in varying concentrations. Blanks were treated identically but contained an additional 0·1 ml Tris instead of the membrane preparation. Incubations were for 15 min at 30° unless otherwise indicated. The membranes were sedimented at 26,000 *g* for 10 min and the supernatant was saved for the determination of the specific activity of the labeling medium. The pellet was washed and resedimented twice, and then dissolved

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