The Effect of Bisdioxopiperazines on the Synthesis of Deoxyribonucleic Acid, Ribonucleic Acid and Protein in Growing Mouse-Embryo Fibroblasts

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A series of derivatives of EDTA, the bisdioxopiperazines, have been synthesized as potential intracellularly activated chelating agents (A. M. Creighton, unpublished work). Several of these compounds show anti-tumour activity against sarcoma S180 and leukaemia L1210 tumours in mice (Creighton, Hellmann & Whitecross, 1969). To determine the effect of these drugs on the synthesis of DNA, RNA and protein in cultured mammalian cells, we measured the changes in the rates of incorporation of [³H]thymidine, [³H]uridine and [³H]leucine by mouse-embryo fibroblasts incubated with the drugs under various conditions.

Secondary mouse-embryo fibroblasts were cultured on squares of stainless steel (Birnie & Simons, 1967). After 24 hr. at 37°, an aqueous solution of the drug was added to the culture medium and, after further incubation, the cell cultures were pulsed for 1 hr. with 1 µC/ml. amounts of [6-³H]-thymidine (15 c/m-mole), [5-³H]uridine (5 c/m-mole), or L-[4,5-³H]leucine (22 c/m-mole). The amount of radioactivity incorporated into acid-insoluble material was measured as described by Birnie, Fox & Simons (1968).

Incubation of cell cultures with 4.5 µg. of (±)-1,2-bis-(3,5-dioxopiperazin-1-yl)propane (ICRF 159)/ml. for 1 hr. inhibited the subsequent incorporation of [³H]thymidine into DNA by 21%; this inhibition increased to 52% after 21 hr. of drug treatment. In contrast, after incubation with ICRF 159 (4.5 µg./ml.) for 21 hr., the incorporation of [³H]uridine into RNA and [³H]leucine into protein was inhibited by only 10% and 12% respectively. Even 0.6 µg. of ICRF 159/ml. inhibited [³H]-thymidine incorporation by 22% after 21 hr., and 60 µg. of the drug/ml. inhibited [³H]DNA synthesis by 70%.

Among analogues of ICRF 159, significant activity was confined to a group of compounds structurally very closely related to ICRF 159, including 1,2-bis-(3,5-dioxopiperazin-1-yl)ethane, *trans*-1,2-bis-(3,5-dioxopiperazin-1-yl)cyclobutane and *meso*-2,3-bis-(3,5-dioxopiperazin-1-yl)butane (ICRF 193), the most potent compound tested; incubation with 0.1 µg. of ICRF 193/ml. for 21 hr. inhibited subsequent incorporation of [³H]thymidine by 66%. In contrast, the stereoisomer (±)-2,3-bis-(3,5-

dioxopiperazin-1-yl)butane was inactive, as were a number of acyclic derivatives including some of the hydrolytic products of these compounds. The effect of these substances on [³H]thymidine incorporation correlated well with their activity against experimental tumours *in vivo* (Creighton *et al.* 1969).

Possible mechanisms of action of ICRF 159 are being investigated and will be discussed.

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The Effect of some Stilboestrol Compounds on Deoxyribonucleic Acid Polymerase from Human and Canine Prostatic Tissue

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The androgen-dependence of human prostatic cancer and its treatment by anti-androgen therapy is well established (Huggins, Scott & Hodges, 1941).

Studies on the action of steroid hormones provide valuable information about the control of hormone-dependent tumours. There are numerous reports concerned with the effects of steroid hormones on RNA polymerase and RNA synthesis, but little attention has been directed to their effect on DNA synthesis. Fahmy, Griffiths, Mahler & Williams, (1967) showed that certain oestrogens stimulated *in vitro* the DNA polymerase prepared from calf thymus. Fahmy & Griffiths (1968) also showed inhibition of this same enzyme system with diethylstilboestrol and some of its analogues.

The present study reports the effects *in vitro* of steroids on DNA polymerase prepared from normal dog prostatic tissue and from human hyperplastic and neoplastic prostatic tissue. The enzymes were prepared as described by Shepherd & Keir (1966). The effect *in vitro* of steroids on DNA polymerase was determined as described by Fahmy & Griffiths (1968).

The activity of the DNA polymerase, measured in terms of nmoles of [³H]thymidine 5'-monophosphate incorporated into DNA/mg. of protein, was inhibited by the addition of 10.2 nmoles of diethylstilboestrol. The degree of inhibition was in range 22–57% with enzymes prepared from seven human hyperplastic prostate specimens, 35–56% with preparations from neoplastic prostatic tissue and 36–43% with enzyme systems isolated from