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bisdioxopiperazines, have been synthesized as potential intracellularly activated chelating agents (A. M. Creighton, unpublished work).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 $\mu\text{C}/\text{ml}$. amounts of [$6\text{-}^3\text{H}$]thymidine (15 c/m-mole), [$5\text{-}^3\text{H}$]uridine (5 c/m-mole), or L-[$4,5\text{-}^3\text{H}$]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 ($\pm$)-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 $\mu\text{g}/\text{ml}$.) 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 ($\pm$)-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

normal dog prostatic tissue. Equimolar concentrations of oestradiol-17 β and the disulphate and diphosphate of diethylstilboestrol had approx. 20% of the inhibitory capacity of diethylstilboestrol. Dibutylidihydrostilboestrol, synthesized by a modification of the method of Dodds, Golberg, Lawson & Robinson (1939), was nearly as effective an inhibitor as diethylstilboestrol with the human and dog enzyme systems. Only slight inhibition (<7%) was found with the *cis* and *trans* isomers of dibutylstilboestrol. It was observed that testosterone and 17 β -hydroxy- Δ -androstane-3-one stimulated the DNA polymerase isolated from certain of the human hyperplastic prostatic tissues, whereas testosterone was shown to have no effect on the DNA polymerase prepared from calf thymus tissue.

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Effects of Oestradiol-17 β and Progesterone on Nuclear-Protein Synthesis in the Mouse Uterus

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Oestradiol-17 β on its own stimulates mitosis in the epithelium but not in the stroma of the mouse uterus; after progesterone pretreatment oestradiol-17 β causes mitosis in the stroma but not in the epithelium (Martin & Finn, 1968). Changes in uterine nuclear-protein synthesis were followed *in vitro* after hormone treatment. A method was devised for isolating stromal and epithelial nuclei separately. Irrespective of hormone treatment, epithelial nuclei contained more acidic protein/mg. of DNA than did stromal nuclei, but the same amount of histone. After 1 hr. incubation of uteri *in vitro* with [¹⁴C]lysine, the specific radioactivities of both types of nuclear protein were two- to five-fold greater in epithelial than in stromal nuclei.

In the epithelium incorporation into acidic nuclear proteins was increased sixfold 4 hr. after treatment with oestradiol-17 β (0.05 μ g.), declining rapidly thereafter. Only a small increase was observed in the stroma. In both tissues incorpora-

tion into histones was increased 2-2.5-fold by 6 hr., and remained high up to 18 hr. These changes were reflected in increased amounts of both types of protein/mg. of DNA.

Treatment with progesterone (1 mg./day) for 3 days did not affect the amounts of either type of nuclear protein/mg. of DNA in either tissue. However, in the epithelium it depressed the incorporation of [¹⁴C]lysine into both proteins by 30-60%, whereas in the stroma it increased incorporation into histones (by 10-20%) but had no effect on the acidic nuclear proteins.

After progesterone pretreatment, in the stroma incorporation of [¹⁴C]lysine into acidic nuclear proteins was increased fivefold 4 hr. after oestradiol-17 β treatment, declining rapidly thereafter. Incorporation into histones was maximal at 6-8 hr. and had returned to control values after 18 hr. Similar changes were observed in the epithelium, but incorporation into both types of nuclear protein was lower than after treatment with oestradiol-17 β alone. Incorporation into epithelial histones also returned to control values at 18 hr., which differed from the persistent increase with oestradiol-17 β alone.

These results show that increased nuclear-protein synthesis is an early effect of oestrogen on the mouse uterus. Acidic-nuclear-protein synthesis increases before histone synthesis. Progesterone treatment accentuates the response in stromal cells but depresses it in epithelial cells.

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The Protein Phosphokinase Activity of the Oestradiol-17 β -Binding Fraction from Uterine Nuclei and its Stimulation by Oestradiol-17 β

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The binding of oestradiol-17 β to an acidic protein from chromatin (King, 1967) and its ability to form complexes with polycations prompted the suggestion that oestradiol-17 β might affect the histone-acidic protein interaction and thus alter DNA transcription (King, Gordon & Steggles, 1969). A 0.3 M-KCl extract of uterine nuclei contained the oestradiol-17 β -binding protein, protein phosphokinase and phosphate acceptor protein. The enzyme effected phosphorylation of dephosphorylated phosphvitin, but not of histones. Phosphoprotein phosphatase activity was very low. As the bonds between histone and nuclear acidic protein are predominantly ionic, the introduction of a phosphate group into the acidic protein should decrease the