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 [14 C]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 [14 C]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 [14 C]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

interaction and might unmask DNA. Thus any effect of oestradiol-17 β on the phosphokinase would be of interest.

The protein phosphokinase activity decreased after ovariectomy and was elevated after an intraperitoneal injection of 10 μ g. of oestradiol-17 β , whereas similar preparations from liver and kidney were not stimulated. When uterus from unovariectomised rats was separated into epithelial and stromal components (Smith, Martin & King, 1969, preceding abstract), the phosphokinase activity was higher in the epithelium than in the stroma but after ovariectomy the epithelial activity decreased to about half that of the stroma. The stromal activity did not change. This raised the possibility that the oestradiol-17 β -binding protein and the protein phosphokinase might be identical. A 0.3M-KCl extract of uterine nuclei, labelled *in vivo* with [6,7-³H]oestradiol-17 β , was prepared. The sedimentation profile of this extract on a 5–20% (w/v) sucrose gradient indicated that it contained the 4.5S oestradiol-17 β -binding protein whereas the phosphokinase had a sedimentation coefficient of about 6S, indicating that they were not identical. It is possible that in chromatin the two activities are related but were dissociated by 0.3M-KCl. The hormone sensitivity of phosphokinase suggest that protein phosphokinase activation may represent an early step in cell activation by this hormone.

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Incorporation of [2-¹⁴C]Thymidine into Deoxyribonucleic Acid of Rat Brain during Postnatal Development: Effect of Thyroid Hormone

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Cerebral synthesis of DNA during maturation has mainly been studied by radioautographic techniques. Quantitative results on the fate of labelled thymidine in the brain are scarce. The incorporation of [2-¹⁴C]thymidine into DNA in different regions of rat brain was therefore investigated (7–35 days).

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It was previously observed that treatment with thyroid hormone (tri-iodothyronine) in infancy leads to premature termination of the postnatal increase in the number of cells in the brain (Balázs, Cocks, Eayrs & Kovács, 1969). The mechanism of this effect may be studied by comparing the rates of incorporation of labelled thymidine into cerebral DNA of hyperthyroid and normal rats.

Male rats received a subcutaneous injection of [2-¹⁴C]thymidine (54.5 mc/m-mole; 20 μ c/100 g. body wt). The uptake of [¹⁴C]thymidine by the immature brain was substantial. [¹⁴C]Thymidine was incorporated into cerebral DNA, and the ¹⁴C content in other constituents of the acid-insoluble fraction was negligible. The distribution of ¹⁴C between the acid-soluble and the DNA fractions was equal in the cerebrum and hypothalamus at about 2 hr. after injection, but before 0.5 hr. in the cerebellum. The specific radioactivity of DNA increased linearly in the cerebrum and hypothalamus in the first hour after injection, and reached a plateau at about 2 hr.; the plateau was attained earlier (about 1 hr.) in the cerebellum.

To compare the rate of [¹⁴C]thymidine incorporation into DNA of different brain regions at different ages the specific radioactivities were related to the concentration of ¹⁴C in the acid-soluble fraction. The highest relative specific radioactivities were observed at 7 days; at 35 days the relative specific radioactivities were 10 and 2.5% of the values observed at 7 days in the cerebrum and cerebellum respectively. The relative specific radioactivity of DNA was much higher (two- to four-fold) in the cerebellum than in the cerebrum of 7-day-old animals, but little difference was observed at 14 days. The more precipitous fall in the relative specific radioactivity of the cerebellum is probably related to the age-dependent change in the proportion of cells not involved in proliferation.

Incorporation of [¹⁴C]thymidine was not affected by tri-iodothyronine in the brain of 14-day-old rats. On the other hand, it was previously found that the labelling of DNA was considerably decreased in the brain and especially in the cerebellum of 21-day-old rats (Balázs, Kovács, Cocks & Eayrs, 1969). The cerebral content of DNA of hyperthyroid rats compared with normals is also markedly lower at 21 than at 14 days. The results suggest that the decreased DNA content of the brain of tri-iodothyronine-treated rats is due to a premature decrease in cell multiplication.

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