(Ord & Stocken, 1972), confirming that this membrane effect is not dependent on catecholamines.

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Prolactin-Stimulated Deoxyribonucleic Acid Synthesis in Rabbit Mammary Tissue

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In an earlier communication Fiddler & Falconer (1969) reported that prolactin stimulated the incorporation of thymidine into DNA in rabbit mammary gland. This work has been extended with the aim of investigating the role of DNA synthesis in cyto-differentiation in the mammary gland.

Pseudo-pregnancy was induced as described previously (Fiddler & Falconer, 1969). At 12 days after the induction of pseudo-pregnancy an individual mammary gland was injected with prolactin (125 µg). Further glands were injected at 6h intervals. At 24h after the first prolactin injection all injected glands and one un-injected gland were excised. Tissue slices from the glands were incubated for 2h in [Me^3H]thymidine (1 µCi/ml). DNA was extracted from the tissue by a method based on that of Munro & Fleck (1966). Measurement of the specific radioactivity of the DNA showed that prolactin markedly stimulated the incorporation of thymidine into DNA, with the greatest stimulation occurring 18–24h after the injection. Addition of chloramphenicol (25 µg/ml) and streptomycin (25 µg/ml) to the incubation medium had no effect on the incorporation of thymidine into DNA. The incorporation of thymidine in both prolactin-stimulated and unstimulated tissue was inhibited by hydroxyurea (50 µg/ml).

Radioautographic studies indicated that prolactin caused an increase in the number of cells that incorporated thymidine into their nuclei. Further, prolactin-stimulated tissue that had been treated with

colchicine contained more arrested metaphases than did unstimulated tissue. These results suggest that prolactin stimulates DNA synthesis and cell division in mammary tissue.

Partial characterization of the radioactive DNA from prolactin-stimulated and unstimulated tissue has been carried out. The DNA was extracted by a detergent–chloroform method and centrifuged in a CsCl density gradient. The gradients were fractionated and the specific radioactivity of each fraction was determined. In prolactin-treated tissue the DNA at the heavy side of the peak, corresponding in position to the genes coding for rRNA (Birnstiel *et al.*, 1972), was much more heavily labelled than was the bulk of the DNA. In unstimulated tissue the same region of the peak was slightly more heavily labelled than was the bulk of the DNA. This suggests that prolactin may also stimulate amplification of the cistrons coding for rRNA. This suggestion is currently being tested by using hybridization techniques.

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Role of Proteins in the Condensation of Hen Erythrocyte Chromatin

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Chromatin in hen erythrocytes is condensed and genetically inactive, whereas rat liver chromatin contains both inactive condensed and active uncondensed fractions. Chromatin isolated from erythrocytes is swollen in media of low cation concentration, but condenses as the unbound cation concentration is raised to the values that occur in the intact cell (Leake *et al.*, 1972). We report here some observations on the influence of chromatin proteins on this condensation.

Proteins were extracted from hen erythrocyte chromatin or nuclei with increasing concentrations of NaCl. They were separated from the residual chromatin either by centrifugation or by gel filtration on Sepharose 4B. Proteins were extracted from the residual chromatin with 0.25M-HCl. All protein fractions were characterized by polyacrylamide-gel electrophoresis (Johns, 1967). The proportion of condensed chromatin at different Mg^{2+} concentrations was determined by its sedimentation on low-speed centrifugation (Leake *et al.*, 1972).

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