(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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Removal of F1 histones had only slight effect on the Mg^{2+} concentration required for half-condensation of the chromatin. Also, after extraction of F1 histones, erythrocyte nuclei still showed the usual reversible swelling and shrinking with changes in Mg^{2+} concentration. However, after removal of F2c histone (peculiar to nucleated erythrocytes), as well as the F1 histone, the residual chromatin remained uncondensed with increased Mg^{2+} concentrations. Reassociation of F1 and F2c histones to residual chromatin by salt-gradient dialysis in 5M-urea gave a product that condensed like the original chromatin. Reassociation of the residual erythrocyte chromatin with histone F1 from rat liver had a similar effect.

A fraction was also prepared from rat liver chromatin, by differential centrifugation, that, like erythrocyte chromatin, condensed with increasing Mg^{2+} concentrations. Removal of histone F1 from this chromatin caused a change similar to that caused by removal of F1 and F2c histones from erythrocyte chromatin.

We conclude that lysine-rich histones, including histone F2c in hen erythrocytes, are involved in maintaining chromatin in a condensed state at the cation concentrations of the living cell (Siebert & Langendorf, 1970). It would seem that in chromatin that is uncondensed in the living cell either molecules are present that counteract the action of lysine-rich histones or their structure is somehow modified. We hope to test the second possibility by reconstitution of hen erythrocyte chromatin with F1 histones from an uncondensed fraction of liver chromatin (Leake *et al.*, 1972).

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Changes in the Composition of Rat Liver Chromatin Fractions during Diethylnitrosamine Carcinogenesis

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Earlier experiments (Gronow, 1971) had indicated that 70 days after feeding rats with the potent liver carcinogen diethylnitrosamine in the drinking water at 25–30 p.p.m. (3 mg/day per kg body wt.) resulted in alterations in the composition of the non-histone proteins of the liver nuclei. To investigate these

changes further, attempts were made to prepare heterochromatin and euchromatin fractions from these nuclei. By using the sonication technique of Frenster *et al.* (1963) it was found, as has been reported by other workers (e.g. Harbers *et al.*, 1968), that the balance between breakage of the bulk of the nuclei and the composition of the sedimented fractions was critical.

It was found that pretreatment of rat liver nuclei in a pressure cell enabled rupture of 90% of the nuclei to be achieved with a short sonication time. After removal of unbroken nuclei with a 500g spin for 10 min, three fractions containing DNA were obtained. Fraction I sedimented at 3000g for 30 min, fraction II at 75000g for 60 min and fraction III by the addition of 10mM- Ca^{2+} to the supernatant from fraction II. The percentages of DNA in these fractions were 12, 64 and 24 respectively. When animals were given [3H]orotate to check the transcriptional activity of the fractions it was found that the same weights of DNA in fractions I and II contained approximately the same amounts of 3H . Values for fraction III, however, were three to four times as great. Preliminary investigations indicated that no significant differences in the specific radioactivities of the fractions could be found in the liver chromatin of control and diethylnitrosamine-treated rats. If the chromatin fractions were labelled by injection of [3H]thymidine the specific radioactivity of that isolated from the treated animals was three times that obtained with the controls.

The non-histone proteins present in these fractions were examined by the sodium dodecyl sulphate–polyacrylamide-gel electrophoresis system of Weber & Osborn (1969). Apart from minor variations in the lower-molecular-weight components some 20 common bands were present in all fractions. The most prominent difference in the diethylnitrosamine-treated animals was the loss of a polypeptide from fraction III with a molecular weight of approx. 15 500.

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