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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