Fractionation of Chromatin from Dimethylbenzanthracene-Induced Rat Mammary Adenocarcinoma

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It has been suggested that oestradiol-17 β activates nuclear function by converting heterochromatin into euchromatin (King & Inman, 1966). It was therefore of interest to locate oestradiol-17 β in different chromatin fractions and this has been studied in the oestrogen-sensitive dimethylbenzanthracene-induced rat mammary adenocarcinoma.

Purified nuclei (Frenster, Allfrey & Mirsky, 1963) were ruptured by sonication for 10sec. and the nuclear components fractionated by differential centrifugation. The fractions were: (1) 1000g for 10min., nucleoli and unbroken nuclei; (2) 9000g for 60min., heavy chromatin (15% of the chromatin DNA); (3) 100000g for 60min., light chromatin (35% of chromatin DNA); (4) 200000g for 16hr., very light chromatin (30% of chromatin DNA). Longer sonication (30–60sec.) results in a considerable increase in the amount of chromatin sedimenting at higher centrifugal force. By analogy with the results of Frenster *et al.* (1963) the light fractions were presumed to be mainly euchromatin, but the various fractions did not differ in appearance in the electron microscope, except for the size of the clumps. The ratio of protein to DNA was similar (4.0) in all the chromatin fractions. Additional DNA with little associated protein could be precipitated by addition of 15mM-CaCl₂ to the 200000g sedimentation.

The thermal denaturation temperatures of all the chromatin fractions were similar (T_m 83° in 15mM-NaCl–1.5mM-sodium citrate) except that the light fractions also contained a ribonuclease-resistant component with T_m 55°. This component was not extracted from nuclei with 10mM-tris. The possibility that this is a DNA–RNA or RNA–RNA hybrid, such as occurs between ribosomal and synthetic messenger RNA (McLaughlin, Dondon, Grunberg-Manago, Michelson & Saunders, 1968), is being investigated.

The binding of [6,7-³H]oestradiol-17 β was 3 times greater relative to the DNA content to the light chromatin fractions than to the other fractions, suggesting a localization of the oestradiol receptor in this type of chromatin. Preliminary results suggest that this fraction also has a higher template ability for RNA synthesis. The lighter fractions also contained higher activities of protein phosphokinase, which may be involved in oestradiol action (King, Gordon & Steggles, 1969).

These results suggest an enrichment in the light chromatin fractions of oestradiol-17 β binding and

of some metabolic activities that appear to be involved in early oestradiol action.

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The Use of Protamine for a Simple Test of Oestradiol-17 β Binding

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Oestradiol-17 β -binding proteins are precipitated, with their bound oestradiol-17 β , by protamine (King, Gordon & Steggles, 1969), and this has been developed as a simple test for specific tissue binding of [³H]oestradiol-17 β . Equal volumes of tissue extract and protamine sulphate (7.5mg./ml.) were mixed and stood at 4° for 10min. The ³H in the precipitate was measured as the bound oestradiol-17 β . When uteri were labelled *in vitro* with 1.0nM-[6,7-³H]oestradiol-17 β , sucrose-gradient analysis of the 10⁵g supernatant showed no unbound oestradiol-17 β and 88% of the ³H radioactivity was precipitated by protamine. By labelling the 10⁵g supernatant with different amounts of [6,7-³H]-oestradiol-17 β we constructed Scatchard (1949) binding plots. The protamine method gave values for the dissociation constant and number of binding sites of 500 pmoles/mg. of protein and 30 pmoles/mg. of protein respectively, which were in reasonable agreement with values of 300 pmoles/mg. of protein and 15 pmoles/mg. of protein respectively obtained from the sucrose-gradient data. About 5–17% of the ³H radioactivity was precipitated when the supernatant was equilibrated with either 1.0nM-[7 α -³H]progesterone, [1,2-³H]cortisol or [7 α -³H]-testosterone. Pretreatment with 6.0nM-oestradiol-17 β did not affect these values, but it depressed the subsequent precipitation of [6,7-³H]oestradiol-17 β (1.0nM) to 29%.

After labelling of the 10⁵g supernatant from rat brain cortex, muscle, liver and spleen with 1.0nM-[6,7-³H]oestradiol-17 β , 19, 16, 38 and 37% of the ³H radioactivity respectively was precipitated by protamine. The reason for the high amounts

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