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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precipitated from liver and spleen is unknown. The values obtained with cortex and muscle probably represent the lowest sensitivity of the method with tissue extracts, as no specific binding of [6,7-³H]oestradiol-17 β to cortex was detected by gradient centrifugation; anterior hypothalamus, contained a 9S binding protein and, with it, more ³H radioactivity was precipitated by protamine (25%) than with cortex. After labelling of the 10⁵g supernatant from muscle with 1.0nM-[7 α -³H]-dehydroepiandrosterone sulphate or [7 α -³H]testosterone sulphate, 5% of the ³H radioactivity was precipitated by protamine, indicating the virtual absence of non-specific interaction between the polycation and steroid sulphates. After equilibration of 1.0nM-[6,7-³H]oestradiol-17 β with albumin, serum or phosphovitin, less than 5% of the ³H radioactivity was precipitated by protamine.

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Acetoacetate Formation in Bovine Tissues

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Studies with preparations *in vitro* have demonstrated that the liver and the epithelial lining of the rumen wall are important sites of ketogenesis in the healthy and the ketotic ruminant (see Hibbitt & Baird, 1967). In the cow it has also been claimed, on the basis of observations made in the intact animal, that the mammary gland may also be ketogenic, at least during ketosis (Kronfeld, 1963).

Krebs, Wallace, Hems & Freedland (1969) have shown, in rat liver, that measurement of ketone-body production from slices may not give a true indication of ketogenic potential. An alternative to the use of slices and tissue pieces is to assay the activity of the four enzymes involved in acetoacetate formation from acetyl-CoA (acetoacetyl-CoA thiolase, HMG-CoA* synthase, HMG-CoA lyase and acetoacetyl-CoA deacylase) in homogenates of the tissues concerned. In the present study these enzymes were assayed by the methods of Williamson, Bates & Krebs (1968) in homogenates of bovine liver, rumen epithelium, mammary gland and kidney.

HMG-CoA lyase was assayed in only liver and rumen epithelium from healthy cows. The other three enzymes were assayed in each of the four tissues derived from healthy lactating cows and

from cows suffering from spontaneous ketosis. No significant differences were found in the activities of these three enzymes in the ketotic tissues as compared with the healthy tissues.

The activities of the enzymes in bovine liver were similar to those reported for the corresponding enzymes in rat liver, indicating that in the cow, as in the rat, acetoacetate formation proceeds mainly via the HMG-CoA pathway.

Rumen epithelium was found to contain thiolase, HMG-CoA synthase and HMG-CoA lyase, but no deacylase. It is therefore clear that acetoacetate formation must take place exclusively by way of HMG-CoA in this tissue. This observation is of some interest and demonstrates that the HMG-CoA pathway for acetoacetate synthesis is not confined to the liver in ruminants, as it appears to be in non-ruminants.

Thiolase and deacylase were both present in bovine kidney tissue, but not HMG-CoA synthase. Neither HMG-CoA synthase nor acetoacetyl-CoA deacylase was present in bovine mammary gland, although there was some thiolase activity.

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Lipolytic Activity in the Pancreas

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The major lipolytic activity of the pancreas is attributed to the pancreatic lipase, but the organ also contains a relatively high content of lipoprotein lipase, which preferentially degrades the triglyceride moiety of lipoproteins, in contrast with pancreatic lipase, which acts on emulsified triglyceride. Although different reactivity to inhibitors has been reported for the two enzymes, LeVeen, Giordano & Zinberg (1961) and Kessler, Finkel, Dreiling & Janowitz (1963) have been unable to ascertain whether the lipoprotein lipase activity of the pancreas is a secondary property of the conventional lipase or is due to the presence of a separate enzyme.

In the present study the lipolytic activities of two different preparations from the pancreas have been measured by using as substrates a commercial triglyceride emulsion both in the free and albumin-activated state for determination of triglyceridase and lipoprotein lipase activity respectively.

Each preparation exhibited the properties of both enzymes, being inhibited by NaF and activated by

*Abbreviation: HMG-CoA, β -hydroxy- β -methylglutaryl-CoA.