EFFECT OF TESTOSTERONE ON THE BINDING OF PROSTATE HISTONE TO DNA *in Vitro*

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There are indications that histones may be involved in the regulation of gene activity (Bonner and Ts'o,1964). We have now obtained evidence that the bonds between histone and DNA in rat prostate gland are weakened in the presence of testosterone. This suggests that the hormone can regulate gene activity in this gland by directly influencing DNA-histone interactions. The ability of lysine-rich histone, derived from rat prostate, to raise the melting-out temperature (T_m) of DNA, was significantly diminished when testosterone was added *in vitro*. In contrast, testosterone did not lower the T_m of DNA when the latter was combined with lysine-rich histones from rat liver, rat spleen or calf thymus.

Lysine-rich histones were derived from heat-treated rat liver, spleen and prostate and from calf thymus as described by Sluyser *et al.* (1965), and Sluyser and Bos (1966). These authors have shown that brief heat-treatment (5 min at 80°) of the tissues destroys proteolytic activity and thus breakdown of histones during their subsequent extraction is prevented. After isolation the histones were dialyzed overnight but not freeze-dried. DNA was isolated from the prostate

glands of 4 months-old rats according to the procedure of Kay et al. (1952) or from the livers of 3 weeks-old rats. The livers were homogenized in 0.25 M sucrose-0.018 M $CaCl_2$ solution and, after centrifugation at 15.000 x g for 10 min, DNA was isolated from the sediment.

Initial experiments revealed that the addition of testosterone to reconstituted prostate nucleohistone caused a lowering of the T_m when melting-out was performed in 0.002 M NaCl. On the other hand, testosterone did not lower the T_m of reconstituted liver nucleohistone under similar conditions.

It seemed of interest to compare the effect of the hormone on the binding of histones from various tissues, with one type of DNA. Rat-liver DNA was therefore combined with these histones and the melting-out studied. In order to ensure binding under conditions in which some degree of equilibrium could occur, these experiments were carried out as follows. 40 µg of liver DNA and 14 µg of histone were mixed in 3 ml of 2 M NaCl-0.015 M citrate. Different amounts of testosterone were then added in 0.05 ml ethanol. The mixtures were dialyzed for 16 h against 3 l of distilled water which contained the same concentration of testosterone as the material inside the dialysis bag. Fig.1 shows the result of these experiments. Testosterone again lowered the T_m of nucleohistones when the histone component had been derived from prostate gland. No lowering of the T_m was observed when histones from the other tissues were used.

Further studies on the binding of lysine-rich and arginine-rich histones to different types of DNA will be presented elsewhere.

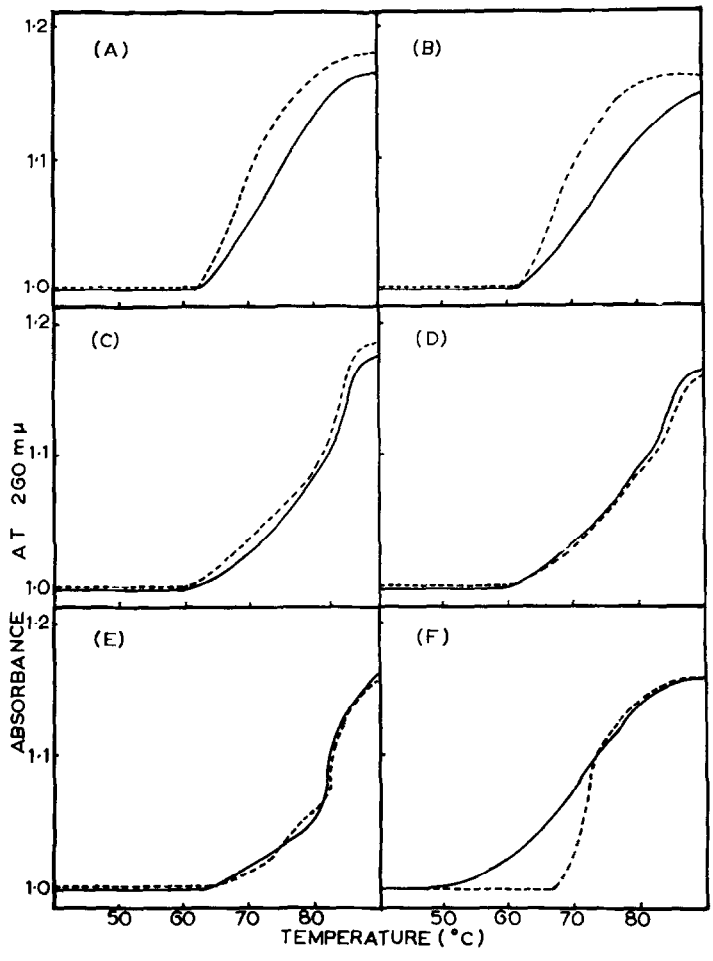

Figure 1. Melting-out of rat-liver DNA with lysine-rich histones from various tissues, in the presence (---) and in the absence (——) of testosterone.
40 μg DNA was mixed with 14 μg histone in 3 ml of 2 M NaCl-0.015 M citrate solution. Testosterone was added in 0.05 ml ethanol while the controls received 0.05 ml ethanol. Each mixture was dialyzed overnight without stirring against 3 l distilled water containing the same concentration of testosterone as the material inside the dialysis bag.
(A) rat-prostate histone, 1 μg testosterone/3 ml;
(B) rat-prostate histone, 5 μg testosterone/3 ml;
(C) rat-liver histone, 1 μg testosterone/3 ml;
(D) rat-liver histone, 5 μg testosterone/3 ml;
(E) rat-spleen histone, 5 μg testosterone/3 ml;
(F) calf-thymus histone, 1 μg testosterone/3 ml.

Absorbance is expressed as $A_{temp}/A_{40^{\circ}}$.

The T_m of DNA alone (dissolved in dialyzed 2 M NaCl-citrate buffer) or of DNA plus testosterone was 67°.

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REFERENCES

Bonner,J. and Ts'o,P.O.P., Eds. The Nucleohistones. Holden-Day Inc., San Francisco, London, Amsterdam, 1964

Kay,E., Simmons,N. and Dounce,A., J.Am.Chem.Soc.,74,1724 (1952).

Sluyser,M., Thung,P.J. and Emmelot,P.,Biochim.Biophys.Acta, 108,249(1965).

Sluyser,M. and Bos,T., Biochim.Biophys.Acta, in press (1966).