THE EFFECT OF ESTRADIOL INJECTIONS UPON CHICKEN LIVER NUCLEI RIBONUCLEIC ACID POLYMERASE

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In our previous work on the biological role of RNA-polymerase in animal tissues (Busch et al., 1962 ; Chambon et al., 1962 ; Mandel et al., 1962), we showed that this enzyme in rat liver nuclei is stimulated under conditions where protein synthesis is enhanced. Since estradiol (Clavert et al., 1944 ; Mandel et al., 1947) increases protein synthesis in bird liver, we investigated the effect of this hormone on RNA-polymerase in liver nuclei of the chick, after having established that the enzyme exists in this species.

Material and methods. We used young chicks of both sexes, weighing around 500 grams. Some were injected daily with 1.5 mg of a solution of estradiol benzoate in mineral oil. Preparation of nuclear extracts according to a modification of the method of Weiss (1960), incubation conditions and measurement of incorporated radio-activity with C^{14}-ATP as substrate were carried out as previously described (Busch et al., 1962). In some cases, we used C^{14}-CTP instead of C^{14}-ATP, omitting KCl and NaF from the incubation medium.

Properties of enzyme from normal chick. Nuclear extracts of livers of normal chicks incorporate radioactive triphosphates into an acid-insoluble product much as do extracts from rat liver ; the optimal pH and Mg^{++} requirements are the same, and incorporation is completed in 10 minutes ; the activity is sharply reduced

when one of the four nucleoside triphosphates is omitted or when the enzyme preparation is preincubated with DNAase (Table 1). However the specific activity is lower than in rat liver : about 33 μμmoles of C^{14}-ATP are incorporated in 10 minutes per milligram of protein, against 80 μμmoles in the case of the rat. The incorporation of C^{14}-CTP is 64 μμmoles per mg of proteins. Thus the ratio of incorporation of CTP to that of ATP is 1.93 in extracts of chicken liver nuclei, while we found a ratio of 1.5 in extracts of rat liver nuclei.

Table 1. Incorporation of C^{14}-ATP into RNA by extracts of chick liver nuclei.
Reactions carried out at 37° during 10 minutes (20 minutes when ammonium sulphate was added) in a final volume of 0.25 ml containing (in μmoles) : Tris-phosphate buffer pH 7.5, 25 ; Mg^{++}, 7.5 ; 2-mercaptoethylamine, 0.25 ; KCl, 15 ; NaF, 5 ; C^{14}-ATP, 0.25 ; non-labeled GTP, UTP and CTP, each 0.25 ; enzyme (2 mg of protein).

System	μμmoles incorporated per mg protein
Complete	33
+ammonium sulphate (10 p.100 saturated)	100
After DNAase pretreatment	7
-GTP	7
-CTP	8
-GTP, CTP and UTP	2

Goldberg (1961) observed a stimulation of HeLa cell RNA-polymerase by addition of ammonium sulphate to the incubation medium. This increase also occurs with extracts of chicken liver (Table 1).

Effect of estradiol injections. Figure 1 shows the pattern of increase of RNA-polymerase activity in liver nuclei of the female chick. With male chicks, the increase is somewhat higher after 24 hours (1.8-fold) but does not change much thereafter (2.0-fold after 3 days). The ratio of incorporation of CTP to

that of ATP, as well as the rate of stimulation by ammonium sulphate are the same in nuclear extracts of liver of the normal and injected chicken.

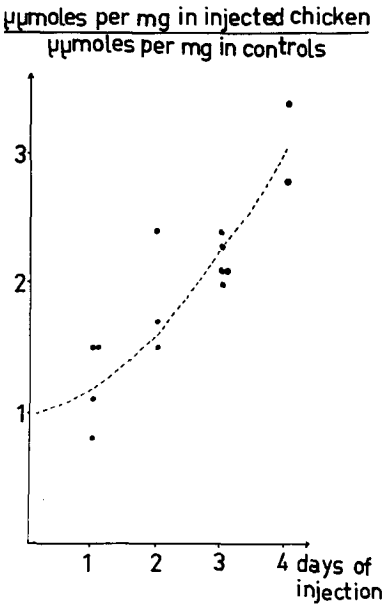

Figure 1. Variation of activity of RNA-polymerase after estradiol injection.
Ordinates show the ratio of activity per mg of protein in the liver of estradiol-injected chicks to that of controls.

Discussion. The observed increase in C^{14}-ATP or C^{14}-CTP incorporation seems to be due to an enhanced activity of the same RNA-polymerase which is present in normal animals ; it behaves in the same way toward DNAase, to omission of single nucleoside triphosphates, etc. The increase cannot be due to the disappearance of a soluble inhibitor or to a decrease in nuclease activity since by incubating mixtures of enzymes from normal and injected chicks we find exactly the sum of individual enzyme activities (Table 2).

The hypertrophy of the liver and the increase in serum proteins become apparent only on the third day of estradiol injec-

Table 2. Mixed incubations of liver nuclei RNA-polymerase from normal and estradiol injected chicken.

Source of enzyme	µµmoles incorporated in 10 minutes
Normal chicken	115
Injected chicken	388
Expected value for the mixture	503
Actual mixture	474

tion ; since the increase of RNA-polymerase activity measured in vitro occurs as early as 24 hours after the first injection, it is possible that this effect of estradiol represents a stimulation of messenger RNA synthesis. Another case has been described where RNA-polymerase is increased in a parallel manner to androgen-stimulated growth of the prostate (Hancock et al., 1962), and recently it has been shown (Gall and Callan, 1962) by autoradiography that administration of gonadotropic hormone accelerates chromosomal RNA synthesis in an amphibian.

The mechanisms by which RNA-polymerase activity is affected by the hormone or one of its derivatives is unknown ; in preliminary experiments where estradiol was added directly to the incubation medium no stimulation was observed. Stimulation due to estradiol injection occurs by a different mechanism from that due to ammonium sulphate in vitro since both effects are additive.

After estradiol injection, no change in composition of the synthesized RNA could be detected through comparison of C^{14}-ATP and C^{14}-CTP incorporations. Finally, the ratio of these incorporations is different from the CMP to AMP ratio in chicken liver DNA (Chargaff, 1955).

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Abbreviations

RNA and DNA : ribo- and deoxyribonucleic acids ; DNAase : deoxyribonuclease ; ATP, GTP, UTP and CTP : adenosine, guanosine, uridine and cytidine triphosphates.