# EFFECT OF CORTISONE ON TRANSCRIPTION OF RAT LIVER DNA OF VARIOUS DEGREES OF REPETITION

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## 1. Introduction

It has been shown previously [1–3] that induction of specific enzymes in liver by cortisone involves synthesis of new species of hybridizable nuclear RNA. However, hybridization conditions employed in these studies (low nucleic acid concentration and short incubation times) provided evidence on the hybridization of RNA only with the rapidly reassociating repetitive sequences of DNA [4, 8].

The present work was undertaken in order to investigate the effects of cortisone on the transcription of rat liver DNA of various degrees of repetition as well as single-copied DNA. For this purpose hybridization of nuclear liver RNA from cortizone-treated rats with DNA was studied over a wide range of $C_0t$ values.

The results obtained are interpreted to mean that cortisone induces preferential activation of transcription from repetitive DNA sequences involving additional DNA sites. The stimulation of transcription of slowly reassociating DNA sites is also observed, these sites being transcribed in considerable amounts before hormone treatment.

## 2. Materials and methods

100–120 g male rats were used. Cortisone-acetate in 1 ml of 0.14 M NaCl (5 mg per 100 g body weight) was injected intraperitoneally 3 hr before the animals were sacrificed. Control rats received 1 ml of 0.14 M NaCl. 40 min before slaughter [$^{14}$C]orotic acid (300 $\mu$Ci/100 g body weight) was administered to both control and cortisone-treated rats.

DNA was isolated by the phenol–detergent method [2] and treated with RNAase (Calbiochem) and pronase (Serva).

Nuclear RNA was extracted with hot phenol at the temperature interval of 55–65°C [5] and treated with DNAase (Worthington, RNAase-free) and pronase. This RNA has an apparent base composition similar to that of DNA [5]. Specific radioactivity of RNA preparations was 1500–2000 counts/min/$\mu$g.

DNA was sheared in 0.14 M NaCl by sonication to fragments of 300 nucleotide pairs, sedimenting at 5S.

After denaturation (15 min in boiling water bath) and reassociation to $C_0t = 100$ in 0.12 M phosphate buffer, pH 6.7 (PB) at 60°C DNA was separated into single- and double-stranded fragments on hydroxyapatite columns according to Britten's standard procedure [4]. Double-stranded DNA, containing repetitive fraction reassociated at $C_0t = 100$, was eluted with 0.5 M PB, while slowly reassociating DNA, which had not renatured at $C_0t = 100$, was eluted with 0.12 PB.

DNA–RNA hybridizations were run at 67°C in 0.24 M PB at various $C_0t$ values using excess of rat liver DNA [6]. The values of equivalent $C_0t$'s were calculated for 0.12 PB [7]. Hybridization samples were treated with RNAase (20 $\mu$g/ml, 10 min). Thereafter hybrids were isolated on Sephadex G-100 columns [8] or were collected on membrane filters (HUFS, Czechoslovakia) after precipitation with cold 5% trichloroacetic acid. All measurements were carried out on triplicate samples.

## 3. Results and discussion

The renaturation curve of rat liver DNA is shown in fig. 1. About 35% of DNA reassociates at $C_0t = 100$. This DNA contains the repetitive fraction [6]. The rest of the DNA, including single-copied and low repetitive sequences, was isolated as described above and allowed to renature. It was shown to reassociate with $C_0t_{1/2}$ about 500. The curve presented corresponds well to the published data [6].

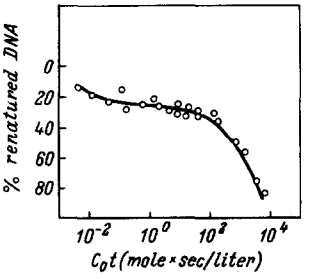


Fig. 1. Renaturation of rat liver DNA. $C_0t$ is the product of initial concentration (moles necleotides per liter) and the time of incubation in seconds.

To investigate the effect of cortisone on the extent of hybridization of rat liver RNA both the repetitive DNA fraction, renaturing at $C_0t = 100$, and the low repetitive and unique fractions were used.

Fig. 2(a) shows the results of hybridization of liver nuclear RNA from control and cortisone-treated rate with repetitive DNA. The hybridization capacity of nuclear RNA isolated from cortisone-stimulated livers is considerably higher (about 2 times) than that of control RNA. Hybridization in DNA excess provides data on the composition of the RNA population. Thus the higher extent of hybridization of RNA from cortisone-treated rats with the repetitive DNA fraction suggests that cortisone induces an increase in the relative amount of RNA classes transcribed from repetitive DNA sequences in liver cells.

Fig. 2(b) illustrates the hybridization of slowly reassociating DNA sequences with control RNA and RNA from cortisone-stimulated livers. RNA from cortisone-treated rats appears to react to a somewhat greater extent at $C_0t$ values from 3000 to 12 000 and to a lesser extent at $C_0t$ values more than 12 000, corresponding to single-copied DNA sequences. This difference in hybridization values has been reproducibly demonstrated using three different cortisone-stimulated and control RNA preparations. This suggests that under the action of cortisone the relative content of RNA transcribed from unique sequences is slightly decreased.

The above data thus seem to suggest that cortisone induces preferential activation of transcription of repetitive DNA sequences in liver cells, resulting in an increase in the content of transcripts from repetitive DNA and a decrease of the relative content of RNA transcribed from unique sequences.

The observed changes in the RNA population under the action of cortisone might result from the involvement in transcription of additional DNA

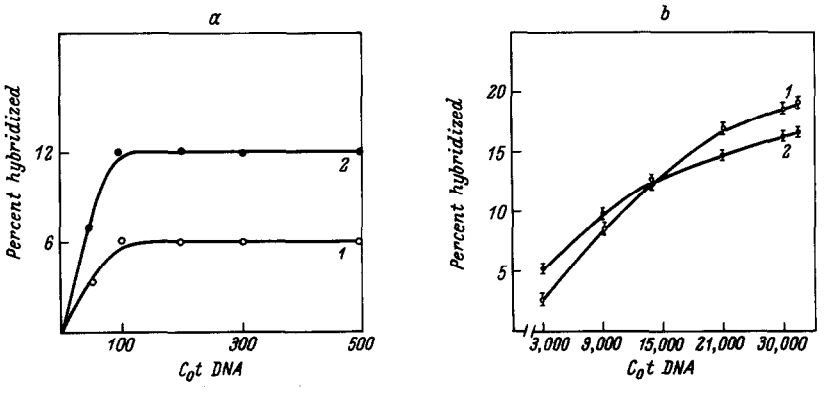


Fig. 2. Hybridization capacity of nuclear [$^{14}$C] RNA isolated from livers of control (1) and cortisone-treated rats (2) with (a) the fraction of repetitive DNA (renaturing at $C_0t = 100$) and (b) slowly renaturing DNA fraction. Abscissa: equivalent $C_0t$ value. Ordinate: percent of hybridization.

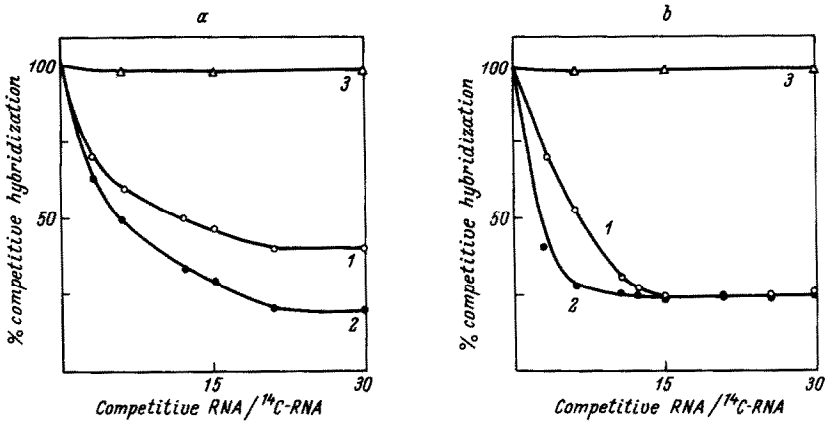


Fig. 3. Competitive hybridization of [$^{14}$C] RNA, isolated from cortisone-treated rats with (a) repetitive DNA fraction and (b) slowly reassociating DNA fraction; (a) DNA $C_0t = 100$; (b) DNA $C_0t$ in hybridization sample = 12 000. (1) Competitive RNA isolated from control animals; (2) competitive RNA was extracted after cortisone administration; (3) unspecific competitor–RNA from the cells of *Tetrahymena pyriformis*. Abscissa: the ratio of the amount of competitive RNA to [$^{14}$C] RNA in a hybridization sample. Ordinate: percent of competitive hybridization (hybridization in the absence of competitor is taken for 100%).

sequences or from changing the rate of transcription of DNA sites that are transcribed in the control.

To investigate possible qualitative changes in the population of RNA synthesized on both repetitive and unique sequences of DNA, competitive hybridization experiments were performed. In these experiments hybridization of [$^{14}$C] RNA from cortisone-treated rats was studied in the presence of increasing levels of competing unlabelled RNA from control and cortisone-stimulated livers.

The hybridization curves obtained with a repetitive DNA fraction differ significantly (fig. 3a). This difference demonstrates that cortisone induces the appearance of new species of RNA transcribed from repetitive sequences which are not detectable before hormone treatment. This result is in good agreement with those previously reported for hybridization of RNA from cortisone-treated rats with unfractionated DNA on membrane filters involving rapidly renaturing DNA sequences [1, 2].

The changes in the population of RNA transcribed from slowly reassociating DNA are less pronounced. As can be seen from fig. 3b, hybridization curves with the increase of concentration of competitive RNA at first differ and then coincide. This can be interpreted meaning that cortisone induces an increase in content of some classes of molecules in the RNA population that are present in considerable amounts before hormone treatment in liver cells.

Thus, in liver nuclei cortisone induces preferential activation of transcription of repetitive DNA sequence involving additional DNA sites. Along with these changes the stimulation of transcription on the part of slowly reassociating DNA sites is observed, these sites being transcribed in the controls in considerable amounts.

It seems early to interpret the observed changes in transcription of repetitive DNA sequences at present. The function of the RNA newly synthesized under the action of cortisone remains to be investigated. These RNA's may serve as regulator RNA or messengers for regulator proteins. There are no data at present suggesting that structural genes may be present in multiple copies in the genome with the exception of genes for histones). Nevertheless the possibility that RNA complementary to repetitive DNA sequences can serve as messengers for cortisone-inducible proteins seems worth investigation.

## References

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