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Hormonal Control of RNA Polymerases in Rat Bone Marrow Nuclei. The Action of Erythropoietin and Testosterone*

Control hormonal de RNA polimerasas en núcleos de médula ósea de rata. Acción de eritropoyetina y testosterona

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The cytodifferentiation of stem cells to mature cells in bone marrow is an appropiate system to study biochemical aspects of hormonal action. We have used this system to analyze the way how erythropoietin and testosterone regulate erythropoiesis at the molecular level.

Experiments designed to correlate the biochemical action of both hormones and to determine their differential action on rat bone marrow nuclei DNA-dependent RNA polymerases are reported.

The effect of both hormones on the synthesis of RNA by isolated nuclei derived from normal rats was studied. Erythropoietin enhances the activity of RNA polymerase type II while testosterone stimulates polymerase type I activity.

Gel-electrophoresis analysis of nuclear RNA shows that erythropoietin enhances the synthesis of RNA species with sedimentation coefficients of 30S, 22S, 15S and 9S. Testosterone estimulates the synthesis of the 28 and 18S RNA as well as 4S RNA.

A model is postulated to explain the action of erythropoietin and testosterone on RNA synthesis by isolated rat bone marrow nuclei.

The molecular events involved in bone marrow cell differentiation are still not well understood.

Mouse fetal erythropoiesis and murine erythroleukemia cell induced by dimethyl sulfoxide have been used as suitable models for studying erythroid differentiation at the molecular level (1). However many aspects of the process are still unknown in adult bone marrow mainly due to the several limitations involved in the use and handling of normal erythropoietic cells.

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It has been demonstrated by several authors that erythropoietin stimulates the synthesis of several classes of RNA (2, 3, 4). One of these RNA, with a high molecular weight may represent the precursor for functional mRNA (5, 6). Testosterone also enhances the synthesis of nuclear RNA, however the effect seems to be on ribosomal RNA precursors with sedimentation coefficients in the range of 45S (7).

These observations strongly suggest that both erythropoietin and testoserone act on the bone marrow nuclei at the transcriptional stage, but in a different manner (5).

It is known that RNA polymerase activity is under hormonal control (8). Valladares *et al.* (9) found that testosterone enhances preferentially RNA polymerase I activity in isolated rat bone marrow nuclei, while Piantadosi *et al.* (10) showed in the spleen from phletoric mouse that an early effect of erythropoietin is the stimulation of the activity of RNA polymerase II. Perretta *et. al.*(5) demonstrated in isolated rat bone marrow nuclei that erythropoietin stimulates RNA polymerase II activity.

The present experiments were undertaken to investigate in rat bone marrow the effect of erythropoietin and testosterone on *in vitro* nuclear RNA synthesis. The results suggest hat both hormones exert their action at different biochemical levels during transcription. While erythropoietin principally enhances RNA polymerase II activity, increasing the synthesis of 30S, 22S, 15S and 9S RNA, testosterone stimulates polymerase I activity producing a great increase of 28 and 18S RNA.

The results presented enable us to postulate that both hormones act in the control of the erythropoietic process.

MATERIALS AND METHODS

1. *Animals*

Female Wistar rats (200-280 g) were used for all the experiments. Anemia was induced by a daily subcutaneous injection of phenylhydrazine hydrochloride (1 mg/10 g body weight) for three consecutive days. Animals were used 5 days after the last injection when hemoglobin concentration ranged between 6 to 8 g/100 ml. Polycythemia was induced by one intraperitoneal injection of 10 ml suspension of isologous heparinized blood cells in 9% NaCl (3:1 v/v). Rats were used after 72 h when hemoglobin concentration ranged between 19 to 25 g/100 ml.

Normal animals received no treatment and their hemoglobin concentration was 14 to 16 g/100 ml. Hemoglobin was measured according to Cannan (11).

Accordingly with the experiment, normal rats were intravenously injected either with 0.5 ml of normal rat plasma containing 4.5 units of erythropoietin or with 0.5 ml of a testosterone solution of 0.5 mg/ml. Control rats were treated with 0.5 ml of normal rat plasma. Animals were killed by bleeding three hours later and bone marrow cells removed from both femura and tibiae. The cells were suspended in ice cold saline.

2. *Preparation of nuclear fraction*

The preparation of the nuclear fraction was performed as previously described (7). Nuclei were resuspended in 60 mM Tris HCl buffer, pH 8.1 and aliquots were taken for measurement RNA synthesis and DNA content (12).

a) *RNA synthesis assays*

^{3}H-UTP incorporation into acid insoluble material was measured at low ionic strength (LIS) in a reaction mixture containing 60 mM Tris-HCl buffer pH 8.1; 5 mM $MgCl_2$; 30 mM KCl; 10 mM mercapto-ethanol; 0.2 mM GTP, ATP and CTP; 1 uM UTP and 1 μCi ^{3}H-UTP and a proper volume of the nuclear suspension (10-50 μg DNA) in 0.5 ml. For assays performed at high ionic strength (HIS) the mixture also contained 0.4 M ammonium sulphate and 5 mM $MnCl_2$.

Enzyme reaction (15 min at 37°C) was terminated by the addition of 2 ml of 10% (w/v) trichloroacetic acid containing 1 mM sodium pyrophosphate. Mixtures were chilled

and the precipitates were filtered on Whatman GF/C filter and washed consecutively (5 ml × 4) with 5% trichloroacetic acid and 10 ml of ethanol. Filters were dried and incubated at 37° overnight with 0.5 ml of Protosol. After adding 50 ul of glacial acetic acid and 10 ml of Liquifluor radioactivity was measured in a liquid scintillation spectrometer Nuclear Chicago Mark I with an efficiency of 25%. Proper blanks were run for each determination. RNA synthesis was estimated in terms of dpm ^{3}H-UMP incorporated into acid-insoluble material/mg DNA.

b) *Isolation and characterization of IN VITRO synthesized bone marrow RNA*

The reaction mixture described above was used but ATP, GTP and CTP concentrations were 0.1 mM; 1 uM UTP and 2 uCi of ^{3}H-ATP, ^{3}H-CTP and ^{3}H-UTP.

Reaction was terminated by the addition of 5 ml of 1% sodium dodecylsulphate containing 0.1% diethyl pyrocarbonate and 1 mM $MgCl_2$ and 1 volume of a phenol-cresol-H_2O (7:1:2) mixture containing 0.1% hydroxyquinoline. Then the procedure continued following the method described by Nicol *et al.* (13). The final RNA preparation was solubilized in 36 mM-Tris-HCl buffer containing 30 mM NaH_2PO_4 and 1 mM-EDTA (disodium salt) pH 7.8, containing 0.2% sodium dodecyl sulphate (w/v) and 5% sucrose (w/v). RNA determination was done by the method of Dische (14).

Polyacrylamide gels were prepared as described by Loening (15). Gels were prerun 30 min at 5°C at 7 V/cm, 5 mA/gel. Bone marrow RNA solutions (20-30 μg) were loaded on top of gels and run for 5 h at the same conditions. Gel slices (2.5 mm) were solubilized with Protosol at 40°C for 18 h. Radioactivity was measured after adding 10 ml Liquifluor (5).

Solutions of RNA markers (Calbiochem, USA) with sedimentation coefficients of 25, 18 and 4S were run under identical conditions. The migration of these markers on the gel was in agreement with calculated values according to Lewicki and Sinskey (16).

3. *Chemicals*

Erythropoietin (step III) specific activity of 5.9 units/mg protein was obtained from Connaught Laboratories Limited. Ontario, Canada. Testosterone propionate, ATP, CTP, GTP, UTP and DNase were from SIGMA Chemical Company. Mo. USA. α-amanitin, 25S and 18S RNA were from Calbiochem, California, USA. 4S tRNA was purchased from Miles Laboratories In. Kankakee. USA. Acrylamide and biscrylamide were from UCB, Bruselles, Belgium. ^{3}H-UTP (35 Ci/m mol), ^{3}H-ATP (26 Ci/m mol), ^{3}H-CTP (26.2 Ci/m mol), Protosol and Liquifluor were obtained from New England Nuclear Corporation, Mass., USA. All other chemicals were reagent grade.

RESULTS

I. *RNA synthesis by bone marrow nuclei from normal, polycythemic and anemic rats*

a) *Effect of ionic strength*

In these kinds of animals the levels of RNA polymerase activities in nuclei isolated from bone marrow cells are shown in Table I.

Between 2-2.5 fold increase in polymerase action is observed at HIS in comparison with LIS conditions. This ionic dependence of RNA synthesis seems to reflect that different RNA polymerases are involved in the process, while polymerase I activity is optimal at LIS, polymerases II and III are preferentially expressed at HIS.

At HIS, the ^{3}H-UMP incorporation diminished 33% in the polycythemic animals in comparison with normal rats, while in anemic nuclei there is an increase of 30%. At LIS condition it is observed that polycythemic nuclei presented a slight decrease of 10% while anemic samples showed a stimulation of 56%.

When bone marrow nuclei are incubated in the presence of α-amanitin at low (1 μg/ml) and high/100 μg/ml concentration there is an inhibition of 40% and 90% respectively in the group of animals at HIS. These results suggest that at low dose of α-amanitin it is still

TABLE I

RNA synthesis by isolated bone marrow nuclei from normal, polycythemic and anemic rats at high and low ionic strength (HIS and LIS). Effect of two doses (100 μg/ml and 1 μg/ml) of α-amanitin (α-A)

Conditions	dpm/^{3}H-UMP incorporated/mg DNA	
	HIS	LIS
Normal	23190 ± 1920	8280 ± 100
+ α − A (1 μg/ml)	13910 ± 630*	6790 ± 320
+ α − A (100 μg/ml)	2940 ± 250*	5760 ± 380
Polycythemic	15600 ± 1380	7440 ± 300
+ α − A (1 μg/ml)	9670 ± 420*	5950 ± 280
+ α − A (100 μg/ml)	1800 ± 90*	5280 ± 260
Anemic	30100 ± 1540	12880 ± 1150
+ α − A (1 μg/ml)	16010 ± 980*	11080 ± 890*
+ α − A (100 μg/ml)	2750 ± 180*	9270 ± 620*

Model experiment from a series of 4 similar.
The figures indicate the mean of 3 samples ± S.E.
*Significantly different from their controls at the $P < 0{,}05$ by t test.
Conditions for incubation and measurement of ^{3}H-UTP incorporation into RNA were done as described in Materials and Methods.

expressing a 60% of the total polymerase activity in the three groups, what it means that activity is ascribed to polymerase III because at HIS conditions and low dose of α-amanitin polymerase II is also restrained. The effect of high dose of the toxin at the same ionic strength allows the expression of 10% of the total polymerase activity in the three groups. In this condition, polymerase II and III are completely inhibited wherefore it is assumed that the remaining activity is due to a resistant action of polymerase I or to an inespecific incorporation of labeled precursor.

At LIS the α-amanitin inhibition is rather different. Low concentration of toxin provoked a disminution of 20% in each sample of the respective group, while high concentration only a 30%. These figures indicate that at this salt conditions remain some polymerase activity represented probably by the type III enzyme.

The results of Table I are aggreed with others of the literature (9) and proved that the method even does not permit to measure the RNA polymerase activities separately, it is suitable to estimate the changes in the level of activities of the polymerases present in the nuclei under several stimulus.

b) *Characterization of nuclear RNA*

In order to gain insight into the types of RNA synthesized by bone marrow nuclei under *in vitro* conditions a separation by polyacrylamide-gel electrophoresis was performed. RNA isolated from normal polycythemic and anemic bone marrow nuclei show the profiles depicted in Fig. 1.

The electrophoretic patterns of nuclear RNA synthesized at HIS conditions (Fig. 1, a) are similar for RNA isolated both from normal and anemic animals. Main radioactive peaks are observed at regions were the sedimentation coefficients are 30, 22, 15, 9 and 5S. RNA synthesized by nuclei derived from polycythemic rats show a similar pattern, however radioactive peaks at 30S and 9S are absent.

When RNA synthesized at LIS conditions is subjected to electrophoretic separation a clearly different situation is observed in comparison with the profile obtained at HIS conditions (Fig. 1, b). Both nuclear RNA from normal and anemic rats show similar electrophoretic patterns. RNA from polycythemic rats, however lacks radioactive

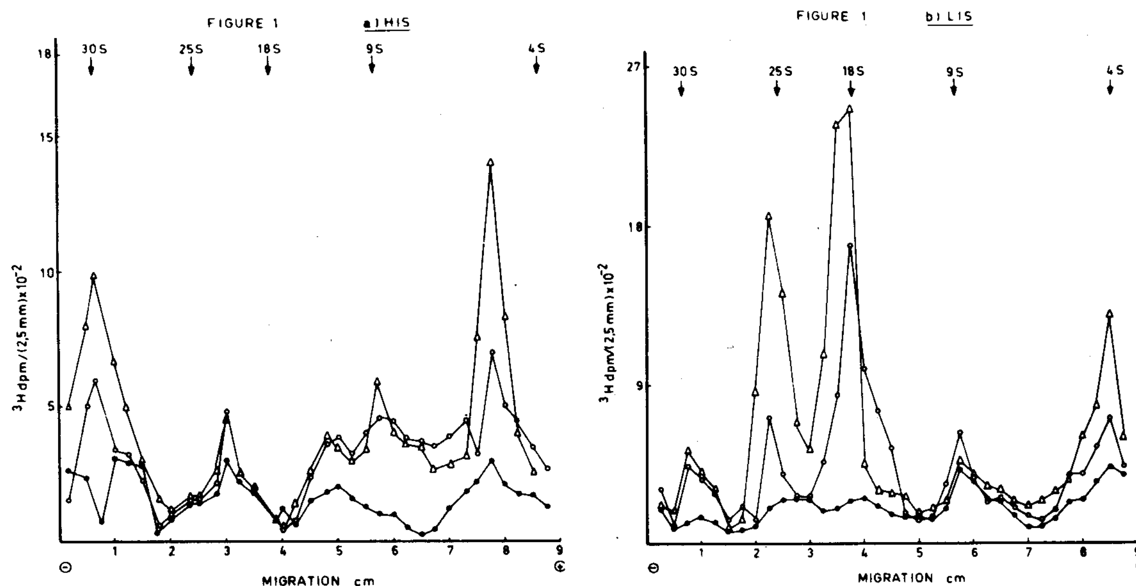

Fig. 1. Polyacrylamide-gel electrophoresis patterns of nuclear RNA obtained from nuclei form normal, polycythemic and anemic animals at high (HIS) and low (LIS) ionic strength conditions. a) HIS and b) LIS.

Specific activities (dpm/μg RNA) of total nuclear RNA used were:
a) at HIS: normal = 2701; polycythemic = 2053 and anemic = 3511;
b) at LIS: normal = 1080; polycythemic = 883 and anemic = 1650;
20 μg of RNA were run at HIS conditions and 30 μg of RNA at LIS;
Arrows show calculated sedimentation coefficients (16);
○—○ normal; ●—● polycythemic; △—△ anemic;
For details, see Materials and Methods.

peaks with sedimentation coefficients higher than 18S.

The different sizes of the profiles shown in the figures are due to the fact that nuclear RNA with different specific activities were applied, accordingly with the erythropoietic capacity of the animal groups. Nuclei from both normal and anemic rats show pronounced capacity to synthesized rRNA whereas generally low synthesis was observed with polycythemic rat nuclei.

II. *The effect of erythropoietin and testosterone on IN VITRO RNA synthesis by bone marrow-nuclei*

The effect of erythropoietin and testosterone alone or in combination on RNA synthesis by nuclei from normal rats is presented in Table II. At HIS conditions erythropoietin produces a 20% enhancement of RNA synthesis as compared to controls. Testosterone does not produce any alteration. Both hormones produce a significative 28% stimulation of the RNA synthesis. However, at LIS conditions, testosterone alone or in combination with erythropoietin produce an increase of 25 and 38% respectivelly on RNA synthesis. Erythropoietin produces no increase.

When the nuclei were incubated with a low dose of α-amanitin, in the presence of erythropoietin, testosterone and erythropoietin plus testosterone, a same rate of inhibitions is produced in rat at HIS and LIS conditions, respectively. However, in bone marrow nuclei treated with α-amanitin plus testosterone and testosterone plus erythropoietin, there is an increase of 23-26% in the ^{3}H-UTP incorporation into RNA, in

TABLE II

Effect of erythropoietin (EP) and testosterone (T) on RNA synthesis by normal rat bone marrow nuclei at high and low ionic strength (HIS and LIS) conditions. Effect of α-amanitin (α-A): 1 μg/ml

Conditions	dmp ^{3}H-UMP incorporated/mg DNA	
	HIS	LIS
Control	18020 ± 750*	7480 ± 450
Plus α-A	10810 ± 380	6830 ± 110
EP	21860 ± 730*	8280 ± 260
Plus α-A	9970 ± 770	6790 ± 40
T	20000 ± 980	9350 ± 530*
Plus α - A	10320 ± 320	8420 ± 300
EP + T	23030 ± 1020*	10330 ± 230*
Plus α-A	10060 ± 820	8630 ± 200

Model experiment from a serie of 4 similar.
The figures indicate the mean of 3 samples ± S.E.
*Significantly different from their controls at $P < 0.05$ by t tests. For details, see Materials and Methods.

comparison to controls containing the toxin alone.

III. *Characterization of nuclear RNA synthesized under the effect of erythropoietin and testosterone*

RNA species synthesized by normal bone marrow nuclei under the effect of erythropoietin and testosterone alone or in combination were resolved and its sedimentation coefficients characterized by gel-electrophoresis. Results of such studies are presented in Figs. 2, 3 and 4.

Erythropoietin stimulates the RNA species 30S, 22S, 9S and 5-6S when the nuclei are incubated at HIS while at LIS it is not observed any activation, at a exception of a 4S RNA. Testosterone at LIS exhibits a great efficiency to activate 26, 18 and 4S RNA, while erythropoietin alone and plus testosterone only show a light increase in 9 and 4S RNA.

Table III shows the main classes of RNA affected by the action of both hormones, summarizing the data presented in Figs. 2, 3 and 4.

DISCUSSION

RNA polymerase activities were measured in isolated rat bone marrow nucleic obtained from normal, polycythemic and anemic animals. Eukaryotic cells contain at least three classes of RNA polymerases whose enzymatic characteristic can be aproximatelly measured and distinguished employing appropiate conditions of ionic strength, different concentrations of α-amanitin and presence of specific divalent cations (17).

Under polycythemic and anemic conditions the erythropoietic process is almost supressed and activated, respectively. The metabolism of total RNA follows similar pattern in bone marrow cells being the RNA synthesis very diminished in polycythemic animals and augmented in anemic conditions (18, 19).

Erythropoietin and testosterone act in a direct manner in rat bone marrow activating selectively the synthesis of different types of nuclear RNA. This effect may produce specific RNA leading to the synthesis of hemoglobin.

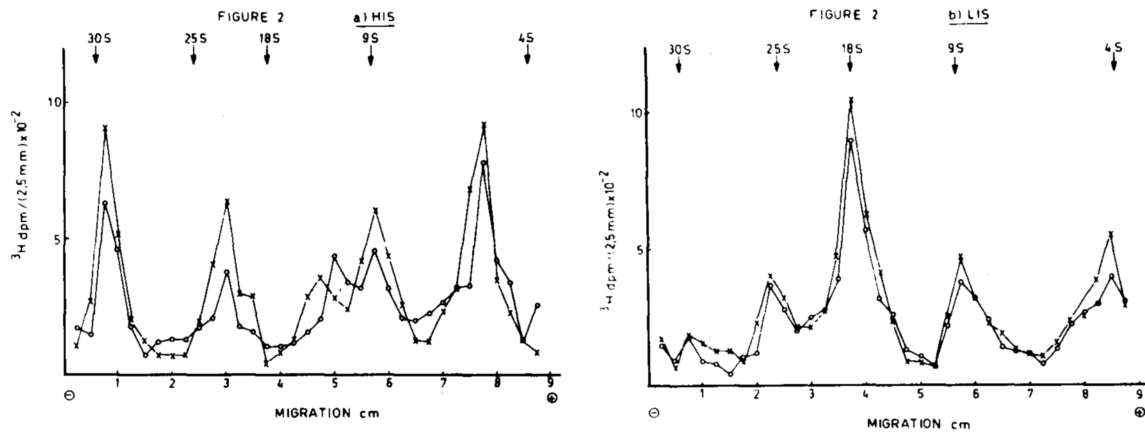

Fig. 2. Effect of Erythropoietin (EP) — a) HIS and b) LIS. Polyacrylamide-gel electrophoresis of nuclear RNA obtained from isolated normal rat bone marrow nuclei. The rats were treated *in vivo* with 4.5 units of EP for 3 h.

Specific activities (dpm/μg RNA) of total nuclear RNA applied were:

a) at HIS: control = 2659 and treated with EP = 3137;

b) at LIS: control = 1037 and treated with EP = 1172;

Arrows show calculated sedimentation coefficients (16);

o—o control x—x treated with EP;

For details, see Materials and Methods.

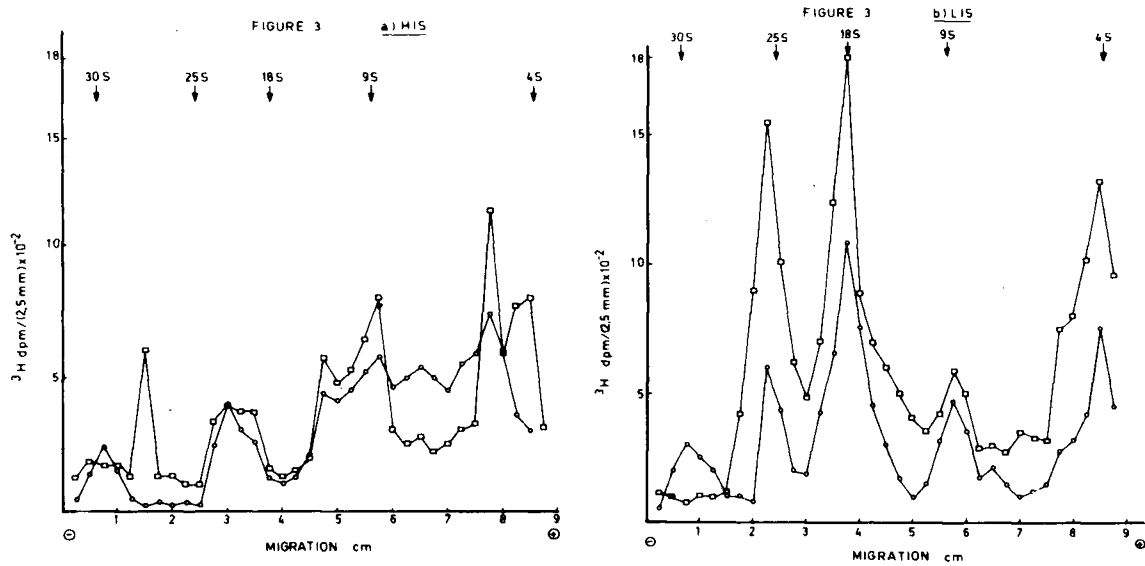

Fig. 3. Effect of Testosterone (T) — a) HIS and b) LIS. Polyarylamide-gel electrophoresis of nuclear RNA obtained from isolated normal rat bone marrow nuclei. The rats were treated *in vivo* with 250 μg of T for 3 h.

Specific activities (dmp/μg) of total nuclear RNA applied were:

a) at HIS: control = 2595 and treated with T = 2826;

b) at LIS: control = 1115 and treated with T = 1349;

Arrows show calculated sedimentation coefficients (16);

o—o control; □—□ treated with T.

For details, see Materials and Methods.

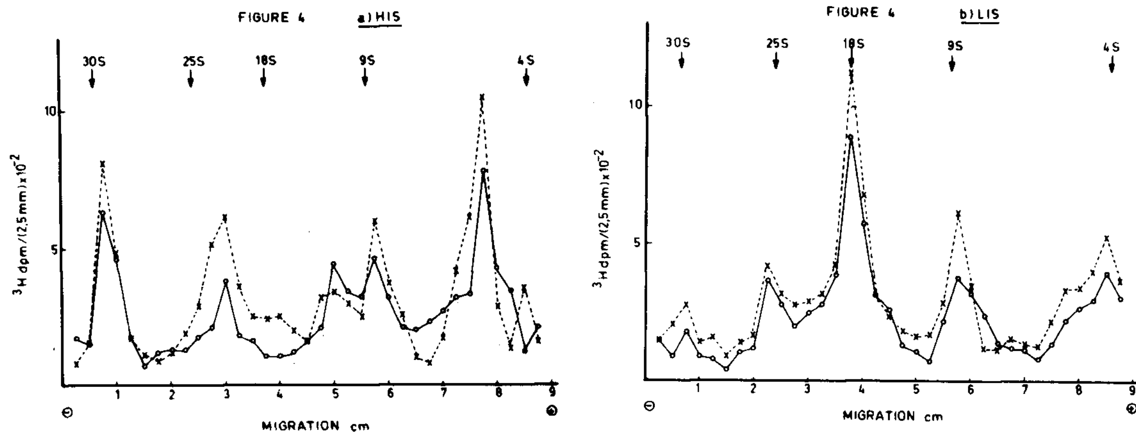

Fig. 4. Effect of Erythropoietin plus Testosterone—a) HIS and b) LIS. Polyacrylamide-gel electrophoresis of nuclear RNA obtained from isolated normal rat bone marrow nuclei. The rats were treated *in vivo* with EP (4.5 U) and T (250 μg) for 3 h.

Specific activities (dmp/μg RNA) of total nuclear RNA applied were:

a) at HIS: control = 2659 and treated = 3191;

b) at LIS: control = 1037 and treated = 1296;

Arrows show calculated sedimentation coefficients (16);

o—o control x----x treated rats.

For details, see Materials and Methods.

TABLE III

Effect of erythropoietin (EP) and testosterone (T) alone or in combination on the synthesis of different species of RNA from normal rat bone marrow-nuclei.

RNA Coefficients Sedimentation	Change of RNA radioactive peaks observed in the presence of:					
	EP		T		EP + T	
	HIS	LIS	HIS	LIS	HIS	LIS
30	+	–	–	–	+	+
26	–	–	+	+	–	+
22	+	–	–	+	+	–
18	–	–	–	+	–	+
9	+	–	+	+	+	+
5-6	+	–	+	–	+	+
4	–	+	+	+	+	+

The data summarize those of figures 2,3 and 4. A comparative study was done for each RNA specie resolved at proper S values calculating the difference of radioactivity between hormone treated and controls;

+ : denotes differences of radioactivity higher than 20%.

– : denotes no differences of radiactivity.

Here, we demonstrate that erythropoietin stimulates RNA polymerase II which synthesizes RNA species with sedimentation coefficients of 30S, 22S, 15-16S and 9S. The first three ones may correspond to intermediate messengers precursors for the functional hemoglobin 9S messenger RNA. The action of EP and EP plus testosterone at HIS resembles the profile obtained from anemic rats, in which the blood level of EP is

very augmented. Recently, Bastos & Aviv (20) have demonstrated in cultures of dimethyl sulfoxide-treated Friend erythroleukemic cells, the existence of three RNA species containing base sequences common to globin messenger RNA and with sedimentation coefficients of 27, 15 and 10S.

The last one which accumulates in the cytoplasm may represent the active RNA in translation at ribosomes level. In the other hand, Scherrer and Marcaud (21) found in duck erythroblast ribosomes RNA species consisting of 9S RNA and a class of polydisperse RNA with sedimentation coefficients within the range of 6 and 28S. The polydisperse material have a base distribution similar to that of heterogeneous nuclear RNA (hnRNA). Likewise, Labrie (22) has detected in rabbit reticulocytes the presence of a 10S RNA with characteristics of globin messenger RNA.

The characterization of the different clases of RNA synthesized in the presence of erythropoietin showed in this paper may represents the pattern of RNA maturation in rat bone marrow nuclei, thereafter, we can conclude that erythropoietin is implicated in the induction of the synthesis of messenger RNA whose multistep maturation process seems to follow a typical pattern represented by the appearance of specific RNA types.

The increase synthesis of 5-6S RNA observed in the presence of EP would be the consequence of a enlargement of the RNA polymerase III activity. A direct effect of the hormone sems very difficult to occurs, because in the presence of α-amanitin, in conditions in which only is inhibited polymerase II the activity is not altered under the action of erythropoietin alone or with testosterone. It also could be explained by an augmentation in the levels of the enzyme, concomitant to a greater rate in the proliferation and differentiation process triggered by EP. Similar variations of polymerase III have been detected in others high proliferative and differentiated tissues. In sum, the stimulated synthesis of 5-6S RNA seems to be an indirect consequence of the previous specific action of erythropoietin.

The rate of synthesis of several RNA species increases under the effect of testosterone at HIS and LIS and according to our findings and others of the literature (9, 5) the action of the hormone can be explained in differents ways. At HIS and LIS conditions, the steroid seems to activate maturation processes while at LIS the hormone specifically enhances the synthesis of a 30S RNA that could be the precursor RNA of 28 and 18S ribosomal RNA. The activation of 4S RNA may reflect a direct action of testosterone on RNA polymerase III activity or an stimulation of the processing rate of precursors transfer RNA (23). The testosterone profile at LIS is very similar to that obtained at LIS with anemic rat shown in Fig. 1.

In summary, testosterone stimulates the incorporation of ^{3}H-nucleotides tri phosphate into RNA in isolated rat bone marrow nuclei confirming previous reports of the *in vitro* effect of the hormone on nuclear RNA synthesis (7). We then conclude that testosterone activates RNA polymerase I which is responsible of 26S and 18S RNA synthesis that might correspond to 28 and 18S ribosomal species, the typical ribosomal of superior eukaryotic cells.

It could be argued that the increased incorporation of nucleoside triphosphates by the action of both hormones could be due to modifications at nucleus membrane level so that the entry of these molecules into the organelle is greatly facilitated. Nevertheless, experiments made with broken nuclei show that testosterone stimulates RNA synthesis at a same extent as in complete nuclei (9). By the other hand, it hasn't been possible to establish if changes in polymerase activity reflect the modulation of a constant bulk of enzyme molecules or conformational chromatin changes that help RNA template capacity. In the same way Perretta and Romero (24) have found that isolated bone marrow nuclei are able to synthesize DE NOVO purine bases, so that these metabolic pathways may constitute another site of a direct action of hormones in RNA synthesis, one of the most prematurely detected molecular event in the erythropoietic model.

It has been described in eukaryotic cells that the RNAs initially produced by gene transcription are large molecules not identical

to the functionally competent nature forms of RNA. These functional RNA arise form precursors RNA through a processing that comprises molecular alterations, such specific cleavage of the transcripts, selective degradation of non-conserved portions, formation of 5' terminal cap structures and 3' terminal poly A segments, and the methylation of particular bases residues. Processing or maturation steps may represent another level of regulation in which hormones may participate. In fact, it is shown (7, 23) that testosterone could modulate maturation modifying specific endonuclease activity in nucleus rat bone marrow.

Finally, we suggest that both hormones perform their action in a different molecular way in rat bone marrow. While erythropoietin activates RNA polymerase II, testosterone enhances the activity of RNA polymerase I and possibly the maturation mechanism of some RNA species. Thus, the necesary biosynthesis machinery to produced the globins is conformed which will be assembled with the HEM group to form the hemoglobin which is the molecule that characterizes the erythrocyte function.

The proposed model (Fig. 5) interprets the results present in this work and represents a more complete version of that suggested y Minguell, Perretta *et al.* (25) because it gives more concrete evidence about the differential action of both hormones at transcription level.

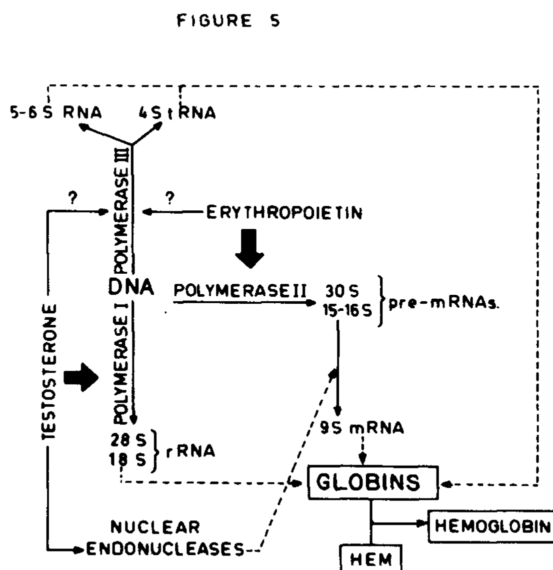

Fig. 5. Diagramatic representation of the action of erythropoietin and testosterone on erythropoietic process. A proposed molecular model.

RESUMEN

La citodiferenciación de células basales a maduras en médula ósea representa un sistema apropiado para estudiar aspectos bioquímicos de la acción hormonal. Hemos utilizado este modelo para analizar a nivel molecular como eritropoyetina y testosterona regulan la eritropoyesis.

Se estudia la acción bioquímica de ambas hormonas determinando su acción diferencial sobre la actividad de las RNA polimerasas de médula ósea de rata.

Se analiza el efecto de ambas hormonas sobre la síntesis de RNA en núcleos aislados provenientes de ratas normales, encontrándose que la eritropoyetina estimula la actividad de la RNA polimerasa II, mientras que testosterona aumenta la de la Pol I.

Electroforesis en geles de poliacrilamida muestran que la eritropoyetina activa la síntesis de RNA con coeficientes de sedimentación de 30, 22, 15 y 9S. Testosterona provoca un aumento en los RNA 28, 18 y 4S.

Se postula un modelo que explica la acción de ambas hormonas en la síntesis de diferentes tipos de RNA en núcleos aislados de médula ósea de rata.

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