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# EFFECT OF DEXAMETHASONE ON RNA SYNTHESIS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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The action of dexamethasone on lymphoid tissue in a culture of partially purified peripheral blood lymphocytes was biphasic in character. After incubation of lymphocytes in vitro with the hormone for 6 h stimulation of RNA synthesis was found. Sedimentation analysis of labeled RNA fractionated on a column containing poly-V-sepharose indicated an increase in the mRNA content and enrichment of cytoplasmic RNA with polyA sequences. Meanwhile $Mn^{++}$-dependent RNA-polymerase, sensitive to $\alpha$-amanitine, was activated. After cultivation of the lymphocytes with the hormone for 24 h, RNA synthesis was inhibited. The biphasic character of the action of the steroid also was observed in the rosette-formation test.

KEY WORDS: lymphocytes; RNA synthesis, dexamethasone.

It was in 1967 that Kidson [9] postulated and obtained experimental evidence for the relatively biphasic character of the action of glucocorticoids on lymphoid tissue. Munck [11] suggested that one of the first manifestations of the action of glucocorticoids on lymphoid tissue should be stimulation of the synthesis of "specific forms of RNA. However, the view is still held that the mechanisms of hormonal regulation of lymphocytes and of a parenchymatous organ such as the liver are in principle different, if not opposite. Under the influence of glucocorticoids, metabolic processes in the liver are stimulated, whereas in lymphoid tissue the synthesis of RNA and protein, on the contrary, is depressed.

In the investigation described below various stages of RNA synthesis were studied during exposure of small human lymphocytes to dexamethasone.

## EXPERIMENTAL METHOD

Small lymphocytes were separated from the total leukocyte pool of blood by sedimentation on a nylon column by the writers' modification of the method of Shapot and Gorozhanskaya [3]. The leukocytes were retained on the nylon column for 30 min at 37°C in an atmosphere containing 5% $CO_2$. The suspensions studied consisted to the extent of 95-98% of small lymphocytes. The concentration of cells in the suspension was adjusted to $6 \times 10^6$ cells/ml. The lymphocytes were incubated in medium No. 199 with 20% autologous plasma. Dexamethasone was added simultaneously with the beginning of incubation up to a final concentration of 60 $\mu$g/ml. The nuclei were isolated from the lymphocytes in a medium of 0.32 M sucrose with 0.01 M $MgCl_2$ and Triton X-100 in a final concentration of 0.5%, followed by disintegration of the cells in a Potter-Elvehjem homogenizer. The

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TABLE 1. Composition of Cells of Blood Leukocyte Pool

| Cells | Original suspension | After passage through column |
|---|---|---|
| Polymorphs | 39% | 1—3% |
| Monocytes | 3% | — |
| Lymphocytes | 58% | 99—97% |
| Red cells | Many | Single cells |
| Platelets | * | * |

TABLE 2. Action of Dexamethasone on RNA Synthesis in Lymphocytes

| | Specific radioactivity of RNA (in counts/min/mg) | |
|---|---|---|
| | nucleus | cytoplasm |
| Control | 740 000 | 23 000 |
| Experiment | 602 000 | 2 305 000 |

TABLE 3. Action of Dexamethasone on Adsorption of PolyA-Containing RNA in Lymphocytes on Sepharose 4B Columns

| | Specific radioactivity of RNA (in counts/min/mg) | |
|---|---|---|
| | nucleus | cytoplasm |
| Control | 1 625 000 | 78 500 |
| Experiment | 20 500 | 130 000 |

TABLE 4. Action of Dexamethasone on DNA-Dependent RNA-Polymerase Activity of Lymphocyte Nuclei

| | Specific radioactivity of DNA (in counts/min/mg) | |
|---|---|---|
| | RNA-polymerase A | RNA-polymerase B |
| Control | 23 000 | 13 600 |
| Experiment | 11 000 | 16 000 |

purity of the nuclei was verified in the phase-contrast microscope. DNA was determined by Burton's method [1] and activity of DNA-dependent RNA-polymerase (EC 2.7.7.6) by measuring the incorporation of the labeled precursor into RNA [8]. Partial purification of the DNA-dependent RNA-polymerase of the nuclear extracts was carried out as described previously [8]. Cytoplasmic and nuclear RNA were separated by the usual method [2] and additionally purified by passage through a column containing Biogel P-2. The preparations of nuclear RNA were fractionated in a linear sucrose density gradient. RNA containing polyA sequences was adsorbed on poly-V-sepharose columns by the method described earlier [13]. Uridine-$^3$H, added 45 min before the end of incubation, was used as one of the radioactive precursors of RNA. The radioactivity of the labeled preparations was measured on a Mark II Nuclear Chicago scintillation counter in toluene scintillator (PPO 4 g/ml; POPOP 0.2 g/liter, with Triton X-100 310 mg/liter). Rosette formation was analyzed by Biozzi's method [6] in Pukhal'skii's modification [5].

## EXPERIMENTAL RESULTS

To obtain a more homogeneous culture of lymphocytes, the lymphocyte fraction was subjected to specific purification and enrichment (Table 1). The lymphocyte preparations used for the subsequent work were thus

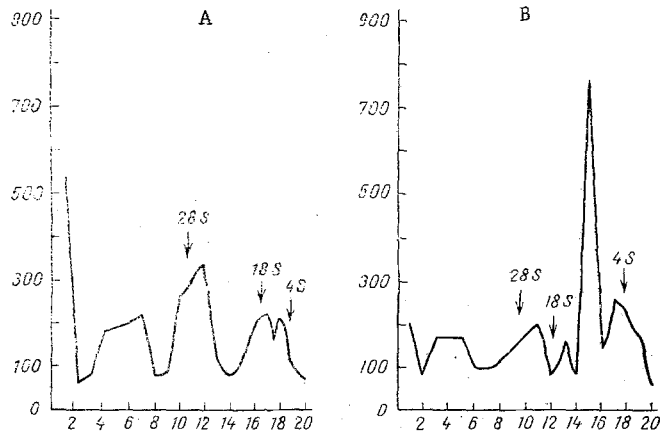


Fig. 1. Sedimentation profiles of nuclear RNA of human blood lymphocytes. Centrifugation in sucrose density gradient (5-20%) on Spinco L-50 ultracentrifuge in SW-39 rotor at 37,000 rpm for 200 min. Dexamethasone (60 μg/ml) added from beginning of incubation. Uridine-$^3$H (100 μCi/ml) added for 45 min. A) Incubation of lymphocytes for 6 h; B) incubation of lymphocytes with dexamethasone for 6 h. Abscissa, No. of fractions; ordinate, radioactivity (in counts/min).

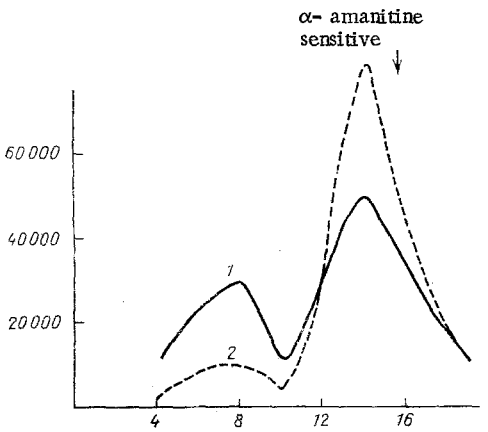


Fig. 2. Sedimentation profiles of DNA-dependent RNA-polymerase of nuclear extracts of human blood lymphocytes. Centrifugation in sucrose density gradient (5-20%) on Spinco L2-65B ultracentrifuge in SW-65 rotor at 60,000 rpm for 24 h. Dexamethasone (60 μg/ml) added from beginning of incubation. 1) Incubation of lymphocytes for 6 h; 2) incubation of lymphocytes with dexamethasone for 6 h. Abscissa, No. of fractions; ordinate, specific radioactivity (in counts/min/mg protein).

sufficiently homogeneous as regards cell composition; moreover, the resulting cells retained their immunoreactivity even after purification, as was shown by their ability to form rosettes.

Lymphocytes obtained by the method described above were incubated with dexamethasone for 5 h. After 5 h uridine-$^3$H was added to the culture for 45 min, after which the nuclear and cytoplasmic RNA which had incorporated the labeled precursors were again analyzed (Table 2). Stimulation of RNA synthesis was observed after 6 h. It was seen most clearly in the cytoplasmic RNA. In the nuclear RNA, on the other hand, no significant changes in specific radioactivity were found. Additional characteristics of RNA which was induced 6 h

after addition of the hormone were obtained by centrifugation of this RNA in a sucrose density gradient (Fig. 1). Most of this RNA had a sedimentation coefficient within the 10-15 S zone. Analysis of the cytoplasmic RNA by passage through a Sepharose 4B column with poly-V fixed to it, after the action of the hormone for 6 h, showed that the specific activity of RNA enriched with polyA sequences was sharply increased (Table 3). The results of these experiments suggest that the induced forms of RNA belongs to the messenger class.

The effect of stimulation of RNA synthesis under the conditions described was reflected not only in an increase in the label in RNA. As Table 4 shows, by the sixth hour of incubation $Mn^{++}$-dependent RNA-polymerase in the nuclei was activated. Similar results were obtained by analysis of partially purified RNA-polymerase from lymphocytes by centrifugation in a sucrose density gradient. It was the $Mn^{++}$-dependent, $\alpha$-amanitine-sensitive fraction of RNA-polymerase that was sharply activated during this period of interaction between the steroid and the cells. Meanwhile, incubation of the steroid with lymphocytes for 24 h led, on the contrary, to inhibition of the partially purified polymerase B preparation responsible for RNA synthesis (Fig. 2). Investigation of lymphocytes from 32 donors showed that the addition of dexamethasone in a dose of 60 $\mu$g/liter increased the number of rosettes by $1.73 \pm 0.26$ times compared with the control. Differences between the experimental and control samples were statistically significant by Student's criterion ($P < 0.05$).

According to data in the literature glucocorticoids inhibit the synthesis of RNA, DNA, and protein in lymphoid tissue [10]. This has been shown particularly clearly with respect to rodent thymus cells. So far as the blood lymphocytes are concerned, the facts here are very contradictory and many investigators consider that the small lymphocytes of the blood, in the resting state, are generally unable to respond to glucocorticoids [12]. At the same time, during blast transformation of the blood lymphocytes under the influence of PHA inhibition of synthesis of RNA, DNA, and protein by glucocorticoids is observed. In organs such as the liver, glucocorticoids activate the synthesis of RNA, DNA, and protein by inducing the formation of many enzymes [4]. The impression is thus obtained that lymphocytes and liver tissue possess mechanisms capable of distinguishing between steroid molecules in the early stages of interaction of the steroid with the cells.

Results obtained in the present investigation showed that after incubation of the lymphocytes with the hormone for 6 h, the synthesis of cytoplasmic RNA containing polyA sequences was stimulated. At the same time, tests on similar preparations after cultivation of lymphocytes with the hormone for 24 h showed inhibition of RNA synthesis.

The change in the rate of RNA synthesis under the influence of glucocorticoids in human blood lymphocytes is evidently biphasic in character. First, the synthesis of cytoplasmic RNA containing polyA sequences is stimulated through activation of $Mn^{++}$-dependent RNA-polymerase, but later the synthesis of these RNA is inhibited. In fact, similar views regarding the biphasic character of the action of glucocorticoids on liver cells were experimentally confirmed by an investigation of DNA synthesis by Dresser [7].

In conclusion it must be emphasized that, in the writers' opinion, the first stage in the action of glucocorticoids on the liver and lymphoid tissue has certain common features and is expressed as an increase in the rate of synthesis of "specific" forms of RNA, whereas the second stage is evidently different and is substantially more sharply expressed in lymphoid tissue than in the liver.

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