# EFFECT OF CORTISONE AND INSULIN ON HEXOKINASE ACTIVITY OF RAT BLOOD LYMPHOCYTES

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Intramuscular injection of large doses (1.2 mg/100 g body weight) of cortisone acetate into albino rats for 6 days lowers the hexokinase activity of the blood lymphocytes on the average by half. Simultaneous injection of insulin prevents the inhibitory action of cortisone.

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Intramuscular injection of a large dose of cortisone acetate into rabbits inhibited the hexokinase (HK) activity in the blood leukocytes on the average by 32%, and this effect was abolished if insulin was injected simultaneously with the cortisone [1].

The peripheral blood leukocytes are cells which differ considerably in their origin, structure, and functions. Differential analysis of the regulatory effect of hormones on metabolism and, in particular, on activity and synthesis of enzymes in the various blood cells is therefore of great importance.

HK activity in lymphocytes isolated from rat blood was studied in this investigation.

## EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 190-300 g, deprived of food for 16-18 h before the experiment.

Activity of the enzyme was first determined in control animals (series I). Cortisone acetate was injected intramuscularly in a dose of 1.2 mg/100 g body weight daily for 4 (series II) or 6 (series III) days. In the experiments of series IV, the same dose of cortisone was injected daily for 6 days together with insulin: 0.025 unit for the first 5 days and 0.25 unit on the 6th day per 100 g body weight.

The animals were decapitated 2 h after the last injection of hormones, and the blood escaping after decapitation (7-10 ml) was collected and defibrinated.

Lymphocytes were isolated by Teodorovich's method [2] modified by ourselves. Defibrinated blood was diluted with 0.7% gelatin solution in 0.85% NaCl in the ratio of 1:1 and centrifuged (centrifugation I) for 3 min at 200 g. The supernatant was again centrifuged (centrifugation II) for 3 min at 700 g; the residue was kept and the supernatant (II) added to residue (I) to extract lymphocytes from it more completely. The suspension thus formed was again centrifuged (centrifugation III) for 3 min at 200 g. The residue (III) was discarded, and supernatant (III) was added to residue (II) and again centrfigued for 3 min at 700 g. The supernatant (IV) was discarded, and the residue (IV) was treated with 2 ml 2% gelatin solution in physiological saline and allowed to stand at room temperature for 45-60 min.

The residue precipitated during this period (V) contained most of the erythrocytes; lymphocytes and a small proportion of erythrocytes were suspended in the supernatant (V). This suspension was diluted with physiological saline (1:2) and centrifuged (VI) for 3 min at 700 g. The resulting supernatant (VI) was discarded, while residue (VI) was suspended in 2.2 ml physiological saline and used for incubation and subsequent analysis of KH activity.

The total number of cells and the number of lymphocytes and erythrocytes separately were counted in a Goryaev's chamber, and stained films were examined under the microscope. Such suspensions usually contained from 10 to 25 million lymphocytes and from 2 to 12 million erythrocytes per ml.

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TABLE 1. HK Activity of Lymphocyte Suspension from Rat Blood

| Experimental conditions | Series no. | Decrease in glucose content (in μg) | P |
|---|---|---|---|
| Control | I | 1.08±0.20 (21) | |
| Cortisone for 4 days | II | 0.55±0.36 (11) | <0.1 |
| Cortisone for 6 days | III | 0.46±0.16 (10) | <0.01 |
| Cortisone and insulin for 6 days | IV | 1.13±0.29 (13) | <0.02 |

Note. Number of experiments given in parentheses.

A special series of 8 control experiments showed that HK activity could not be detected by the method used in this investigation (7 experiments) or was very slight (0.4 unit of activity in one experiment) in a suspension of rat erythrocytes (25-30 million/ml).

HK activity was determined by the decrease in glucose concentration [1, 3]. Incubation of 1 ml of lymphocyte suspension was carried out for 90 min at 37° in the presence of 0.045 M $NaHCO_3$ solution, 0.02 M $MgCl_2$, 0.0039M ATP, 0.0049 M NaF, and glucose at the rate of 0.09 mg/ml incubation mixture. The total volume of the incubation mixture was 1.7 ml.

Glucose was determined by the Hagedorn–Jensen method after precipitation of the proteins with cadmium hydroxide.

HK activity was expressed as decrease in glucose content (in $\mu g/10^6$ lymphocytes/90 min; units of activity).

## EXPERIMENTAL RESULTS

HK activity of lymphocytes taken from the blood of normal rats (experiments of series I) varied from traces of activity (in 5 of 21 experiments) to 2.6 units, with a mean value of 1.08 unit (100%; Table 1). When the mean values were calculated, "traces of activity" were conventionally taken as zero.

Injection of cortisone for 4 days (11 experiments) as a rule (in 8 of 11 experiments) led to a considerable decrease in HK activity. In one experiment, however, activity of the enzyme was actually higher than in the rats of the control series (3.6 units). The mean decrease in HK activity compared with the controls was not significant ($P > 0.05$).

After 6 injections of cortisone acetate, HK activity fell by a greater and significant degree (by 58%, experiments of series III, $P < 0.01$).

Injection of insulin simultaneously with cortisone completely prevented the inhibition of HK activity in lymphocytes produced by cortisone alone (series IV). The blood sugar at the time of decapitation of the animals in the experiments of series IV varied from 12 to 35 mg%, mean 24 mg%.

Insulin had no effect in vitro on the HK activity of lymphocytes (supplementary series of 6 experiments).

Prolonged (6 days) administration of cortisone thus significantly depressed HK activity. Simultaneous intramuscular injection of insulin under the same experimental conditions prevented the inhibitory action of cortisone.

Insulin had no effect in vitro on HK activity of lymphocytes.

## LITERATURE CITED

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