THE PARADOXICAL EFFECT OF HYDROCORTISONE AND ACTINOMYCIN ON THE ACTIVITY OF RABBIT LEUCOCYTE ALKALINE PHOSPHATASE[1]

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Alkaline phosphatase activity of polymorphonuclear leucocytes from humans shows a wide range of activity from very low present in chronic myelogenous leukemia[1] to very high activities present during pregnancy[2]. This wide range of activity suggested that alkaline phosphatase from this tissue source would be inducible, similar to the increase in alkaline phosphatase of heteroploid cells following prednisolone addition[3]. A study of the increase in leucocyte alkaline phosphatase (LAP) activity following the administration of various steroid intermediates to rabbits was carried out. Progesterone was found to be the most effective in increasing the activity of LAP[4]. In the present study a comparison of the increases in LAP activity following the administration of progesterone was compared to the increases following hydrocortisone or prednisolone alone or simultaneously with actinomycin D. The results showed that progesterone and prednisolone increased LAP activity while hydrocortisone resulted in no increase in activity. When the steroids were administered simultaneously with actinomycin D, the progesterone mediated increases were blocked by actinomycin. In contrast, prednisolone or hydrocortisone plus actinomycin resulted in increased LAP activity.

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METHODS:

Mature male New Zealand white rabbits were injected intraperitoneally with progesterone 5 mg/K suspended in glycerol, alone or with actinomycin D 10 μg/K or with puromycin 10 mg/K; hydrocortisone 5 mg/K alone or with actinomycin D 10 μg/K; prednisolone 5 mg/K alone or with actinomycin D 10 μg/K. Blood samples were obtained at zero hour and at varying times as described in each experiment.

Blood was obtained by cardiac puncture, using heparinized plastic syringes, and the leucocytes isolated by the method of Bertino[5]. Details of enzyme extraction from leucocytes and the assay procedure were as previously described[4]. The product βnaphthol liberated from the substrate βnaphthyl acid phosphate was measured fluorometrically. The activity of the enzyme was expressed as μM βnaphthol formed /mg. protein /hr.

RESULTS:

1. Leucocyte alkaline phosphatase activity following progesterone alone or with actinomycin or puromycin.

Following the intraperitoneal injection of 5 mg/K of progesterone suspended in glycerol to a group of 6 rabbits, the base line activity of leucocyte alkaline phosphatase (LAP) rose from 2.1 μM βnaphthol /mg. prot./hr. to 20.0 μM βnaphthol /mg. prot. at 24 hours. When actinomycin D 10 μg/K was administered simultaneously with progesterone to a group of 6 rabbits at zero hour and actinomycin D only given again 6 hours later, the enzyme activity decreased to 1.7 μM βnaphthol /mg. prot./hr. at 6 hours and to 3.4 μM βnaphthol /mg. prot./hr. at 24 hours. When puromycin 10 mg./K was administered simultaneously with progesterone to a group of 6 rabbits the average activity of LAP was 1.8 μM βnaphthol /mg. prot./hr. at zero hour, 2.1 at 6 hours and 2.0 at 24 hours. This is shown in Fig. 1. These results showed that since the increase in enzyme activity following progesterone was suppressed by both actinomycin D and puromycin that the increase was likely due to de novo synthesis of new enzyme

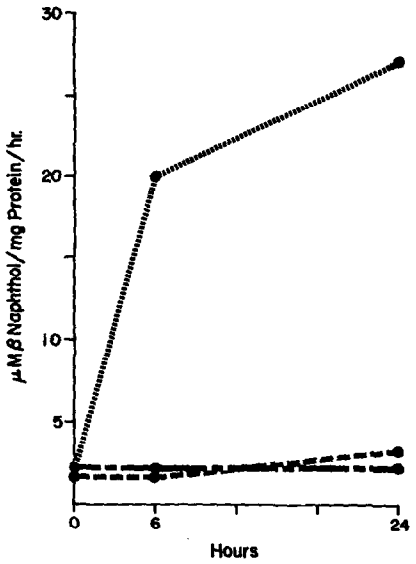

Figure 1

Progesterone, progesterone plus actinomycin or progesterone plus puromycin were administered. Blood samples obtained and leucocytes isolated as in text. •--------• progesterone, •_ _ _• actinomycin plus progesterone, •——• puromycin plus progesterone. Enzyme assay as in text.

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protein.

2. Effect of hydrocortisone 5 mg./K alone or with actinomycin D 10 μg/K.

In initial studies which compared the effectiveness of various steroids to increase leucocyte alkaline phosphatase activity, it was consistently noted that after the administration of hydrocortisone 5 mg./K, the average 24 hour value of 0.4 had not increased above the base line of 1.1 μM βnaphthol/mg. prot./hr. When actinomycin D 10 μg/K was given simultaneously with hydrocortisone, enzyme levels increased from average base line value of 4.7 to 16.4 μM βnaphthol /mg. prot./hr. at 24 hours. These studies were repeated and samples taken at 6 and 24 hours. Six hours after hydrocortisone 5 mg./K to 6 rabbits, the average value was 1.7, unchanged from the average base line of 2.0, and after 24 hours 2.2 μM βnaphthol/mg. prot./hr. Average values after hydrocortisone plus actinomycin D 10 μg/K were zero

hour 3.7, 6 hours 9.8 and 24 hours 6.1 μM βnaphthol /mg. prot./hr. A study with samples obtained at 0.3 and 6 hours was made with 6 rabbits. Average values after hydrocortisone 5 mg./K were zero hour 3.0, 3 hours 2.6, 6 hours 2.5 μM βnaphthol /mg. prot./hr. Following hydrocortisone plus actinomycin D 10 μg/K average values were zero hour 4.4, 3 hours 12.8 and 6 hours 12.2 μM βnaphthol /mg. prot./hr. This is shown in Figs. 2 and 3. Actinomycin D alone did not increase LAP activity. As shown in Fig. 4 , studies with hydrocortisone plus 50 μg/K actinomycin D resulted in even larger increases of LAP activity from a base line of 3.7 to a 3 hour of

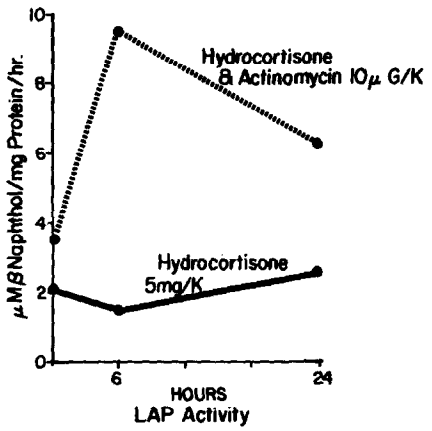

Figure 2

LAP activity at 6 and 24 hours following hydrocortisone 5 mg/k or hydrocortisone plus actinomycin in 10 μG/k. Leucocyte isolation and enzyme assay as in text.

60 and a 6 hour value of 76 μM βnaphthol /mg. prot./hr.

From this series of studies it appeared that hydrocortisone alone was not effective in increasing rabbit leucocyte alkaline phosphatase, but combined with actinomycin a large increase in enzyme activity occurred.

3. Effect of prednisolone alone or with actinomycin D on leucocyte alkaline phosphatase activity.

Prednisolone which is an effective inducer of alkaline phosphatase

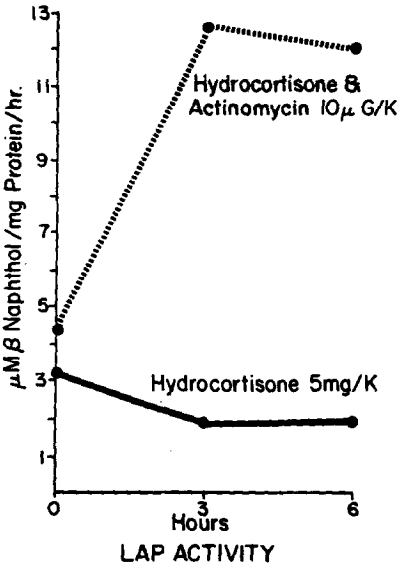

Figure 3

LAP values 3 and 6 hours following hydrocortisone 5 mg/k or hydrocortisone plus actinomycin 10 μG/k. Leucocyte isolation and enzyme assay as in text.

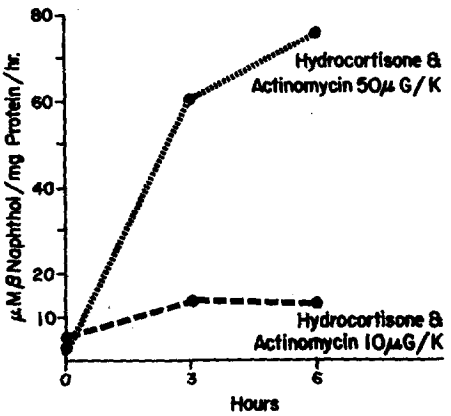

Figure 4

LAP activity following hydrocortisone 5 mg/k plus 10 μG/k or 50 μG/k Actinomycin D. Leucocyte isolation and enzyme assay as in text.

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in tissue culture was given to 6 rabbits. Following the administration of prednisolone 5 mg./K the average values for LAP increased from zero hour value of 2.2 to a 3 hour value of 10.7 and a 6 hour value of 7.4 μM

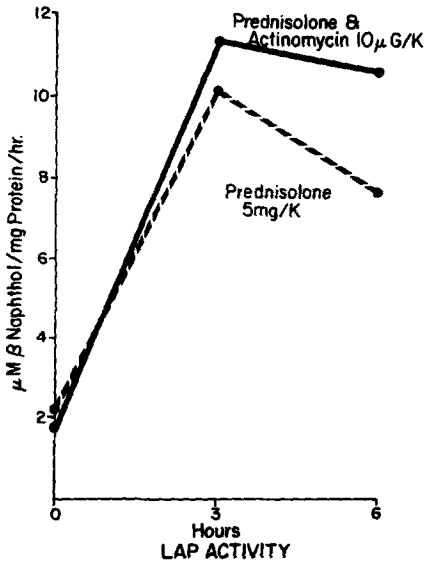

Figure 5

LAP activity following prednisolone 5 mg/k alone or plus actinomycin 10 μG/k. Leucocyte isolation and enzyme assay as in text.

βnaphthol /mg. prot./hr. Following prednisolone plus actinomycin D 10 μg/K the average values were: zero hour 1.8, 3 hours 11.6 and 6 hours 11.0μM βnaphthol /mg. prot./hr. This is shown in Fig. 5. To determine whether prednisolone exerted its effect prior to actinomycin when the two were administered simultaneously, actinomycin 10 μg/K was given 2 hours before prednisolone. The average zero hour LAP activity was 1.5 μM βnaphthol /mg. prot./hr. This increased to 6.4 μM βnaphthol /mg. prot./hr., 3 hours after prednisolone. The results indicated that the increase in activity was not due to prednisolone stimulation before actinomycin was effective.

DISCUSSION:

In studies involving both carbohydrate[6] and amino acid[7] metabolism where steroid administration increased various enzyme activities, the simultaneous administration of actinomycin D abolished these increases. Decreased synthesis of DNA directed RNA following actinomycin D was found in a number of studies[8,9].

Several reports have been published of increased enzyme activity following actinomycin administration. Rosen[10] showed that the repeated administration of sub-lethal doses of actinomycin D greatly increased the activity of four enzymes of amino acid metabolism. Moog[11] noted a greater increase in alkaline phosphatase activity of the developing mouse duodenum given actinomycin D. Papaconstantinou[8] noted an increase in the crystallin proteins in the fiber cells of the differentiating lens (to which actinomycin was added) but not in epithelial cells.

The mechanism of the observed increase in enzyme activity in the present study is not known. One theoretical model would be that if the synthesis of new messenger RNA were inhibited by actinomycin and the messenger RNA for alkaline phosphatase were long lived while the repressor was short lived, synthesis of new enzyme would occur. Other possible mechanisms may involve stabilization of the ribosomal template or the increased availability of substrates for LAP synthesis in the actinomycin treated animal. The results add to the growing body of data which indicates that actinomycin may act in a more complex manner within the cell than only inhibiting DNA directed RNA synthesis.

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