# STEROID EFFECTS ON RAT THYMUS ACID RIBONUCLEASE*

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Glucocorticoid treatment of lymphosarcoma P1798 which results in tumor regression is preceded by a large increase in acid ribonuclease (RNase) activity (MacLeod, et al., 1963; Ambellan and Hollander, 1966). The lymphosarcoma also shows a reduction in RNA synthesis (MacLeod, et al., 1963). When rats are injected with glucocorticoid, their thymus also shows a reduction in size (Doughtery, et al., 1964) and decreased RNA synthesis (Feigelson, M., 1964; Wiernik and MacLeod, 1965) as well as lowered protein synthesis (Feigelson, 1964; Pena, et al., 1964) and RNA polymerase activity (Nakagawa and White, 1966; Pena, et al., 1966). The similarity between the two lymphoid systems suggested that acid RNase activity might also increase during thymus involution. Pena, Dvorkin and White (1966), as well as Wiernik and MacLeod (1965) have reported, however, that there was no change in the activity of this enzyme at 3 hrs or 4 hrs following steroid treatment.

Experiments in our laboratory have, on the other hand, consistently demonstrated a significant increase in the specific activity of acid RNase of rat thymus as early as 2 1/2 hrs following intraperitoneal injection of 9-α fluoroprednisolone (9FP) into rats. The experimental conditions used and possible reasons for these different results are presented below.

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Holtzman male rats about 6 wks old (170-190 g) were given a single intraperitoneal injection of 10 mg of 9FP (supplied by Microbiological Associates through the courtesy of the Cancer Chemotherapy National Service Center). At the times specified, animals were sacrificed by exsanguination (in some cases ether was used) and 7.5% homogenates were prepared in 0.25 M sucrose solution and centrifuged at 80,000 x g (av.) for 60 min to prepare a high speed supernatant fraction which was used in all experiments except when otherwise indicated. The assay system for RNase activity has been described (Ambellan and Hollander, 1966a). Final buffer concentration in incubation mixtures used was 0.034 M $K_2HPO_4$, 0.001 M $NaH_2PO_4$, 0.012 M citric acid, 0.002 M ethylenediamine tetracetic acid (EDTA), pH 5.9. The pH was adjusted when necessary by addition of NaOH or HCl to concentrated stock buffer and appropriate dilutions made to maintain similar concentrations of phosphate, citrate and EDTA. Total ionic strength was 0.18 with maximum variation of 5% after adjusting the pH. The assay is linear in time and concentration.

In over 20 different experiments we have found an increase in acid RNase activity assayed at pH 5.9 in rat thymus following injection of animals with 9FP. The magnitude of the increase in enzyme activity was related to time after injection, but there was considerable variation among different experiments. The increase was 100-300%, 18 hrs after treatment; 60-100% at 3 hrs; and 35-100%

Table 1

Effects of 9FP Treatment on Acid RNase Activity in Rat Thymus

| Experiment | Treatment (hrs) | No. of rats | Specific Activity acid RNase[a] | Increase (%) |
|---|---|---|---|---|
| #1 | 0 (control)[b] | 3 | 72 ± 3.0 | |
| | 2 1/2 | 4 | 126 ± 12.5 | 75 P < 0.01 |
| #2 | 0 (control) | 4 | 57 ± 9.5 | |
| | 3 | 4 | 125 ± 14.5 | 120 P < 0.01 |

a) Absorbance 260 mμ/μg protein/30 min, ± standard error of mean.
b) Control animals were injection with steroid suspending medium.

at 2 1/2 hrs. Table 1 which shows a typical experiment indicates that the increased enzyme activity observed at 2 1/2 and at 3 hrs after treatment is significant. In the period 45-120 min after treatment, the results were quite variable and the precise time of onset of the increased enzyme activity has not yet been definitely established.

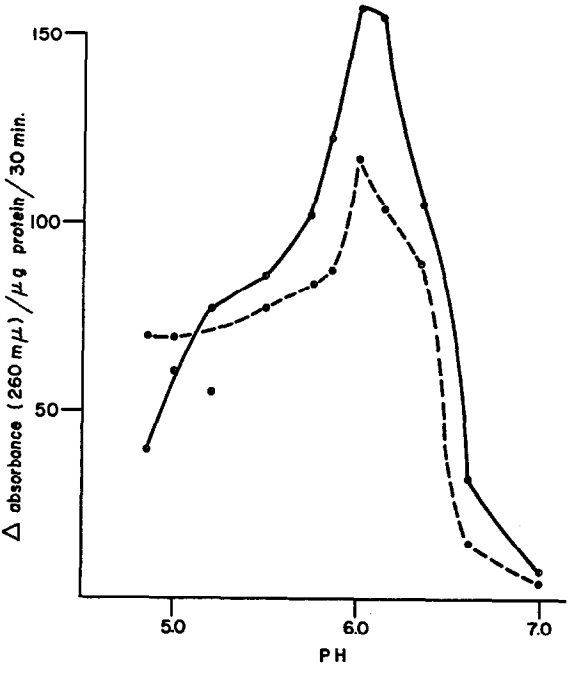


Figure 1. pH curve of RNase activity of rat thymus high speed supernatant fractions (80,000 x g, 60 min) prepared from a 7.5% homogenate in 0.25 M sucrose 2 1/4 hrs after intraperitoneal injection of animals with 10 mg 9FP.
Solid line = 9FP treated
Dotted line = controls.

Figure 1 shows the pH curve of RNase activity in high speed supernatant fractions 2 1/4 hrs after 9FP treatment. It should be noted that the peak of enzyme activity for both control and treated thymus preparations is approximately at pH 6.0 and that at this pH there is a 35% increase in RNase activity of thymus of treated rats. There was no difference in RNase activity between thymus of treated and control animals when the assay was carried out at pH 5.0 Since Pena, et al. (1966), failed to detect changes in rat thymus activity at

pH 5.0, 3 hrs after treatment of animals with cortisol when they used the supernatant fraction of 12,000 x g centrifugation (30 min), we also assayed a fraction prepared by this centrifugation 3 hrs after treatment with 9FP. The results are shown in Figure 2. There was a 45% increase in RNase activity measured at pH 6.0, but no difference from controls were observed at pH 5.0.

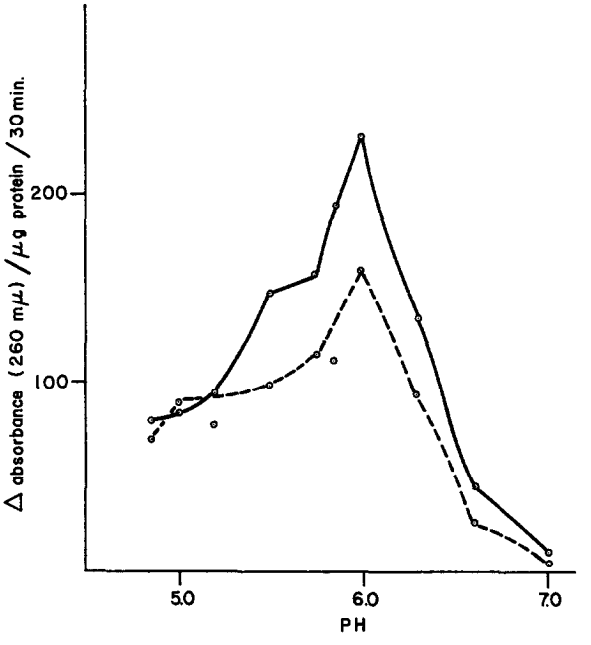


Figure 2. pH curve of RNase activity of rat thymus supernatant fraction prepared from a 5% homogenate by centrifugation at 12,000 x g for 30 min.
Solid line = 9FP treated
Dotted line = controls.

It appears, therefore, that the inability of Pena, et al. (1966) to find an increase in acid RNase following steroid treatment may be due to the pH at which their assay was carried out. There is also the possibility that 9FP is more effective than cortisol as a lympholytic agent. Another difference is that Pena, et al. (1966) used $Mg^{++}$ in their homogenizing medium and omitted EDTA from the enzyme assay. Wiernik and MacLeod (1965) did not detect changes in acid RNase (acetate buffer, pH 5.7) of rat thymus 4 hrs after 9FP treatment in homogenates which were frozen and thawed 3 times. With respect to this result, it

should be noted that (Ambellan and Hollander, 1966) differences between control and treated tissue samples in other systems such as lymphosarcoma P1798, which were evident in freshly prepared samples were no longer evident after freeze-thaw treatment.

The pertinence of the present report is that there still remains a positive correlation between increased acid RNase activity and lymphoid regression following various treatments in all cases so far investigated (Ambellan and Hollander, 1966; Erbe, et al., 1966). In lymphosarcoma P1798 the enzyme increase precedes tumor regression. Thus the possibility of a causal relationship between increased acid RNase and lymphoid tissue regression merits further investigation. This, together with an investigation of the effects of steroid treatment on RNase inhibitor, is the subject of present research in our laboratory.

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