

# CHANGES IN THE PLASMA LEVEL OF SOME HYDROLYTIC ENZYMES DURING THE RESORPTION OF OESTROGEN-INDUCED MEDULLARY BONE IN COCKS

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## INTRODUCTION

The biochemical mechanisms by which bone is resorbed are not fully understood. It is generally assumed, however, that the mineral matter is removed under the influence of locally-produced acids and that the organic matter is solubilized by enzymic mechanisms. Acid hydrolases of lysosomal origin have been implicated in the process of bone resorption in organ culture (VAES, 1965), and it was considered that a study of the activity of some lysosomal enzymes in plasma during the resorption of oestrogen-induced medullary bone might provide *in vivo* evidence for or against the participation of these enzymes in the process of bone resorption, on the assumption that changes in the activities of these enzymes in the bone might be reflected in their activities in the plasma. Formation of medullary bone is readily induced in cocks by oestrogen administration and resorption occurs when the hormone is withdrawn (URIST, 1959).

## MATERIALS AND METHOD

### 1. *Birds and treatments*

Fifteen medium hybrid cockerels (*Thorner 404*) 12-weeks of age and weighing 1.6-2.0 kg, were used in this experiment. Eight of the birds received 11 intramuscular injections of oestradiol dipropionate (Ovocyclin P, Ciba) at a level of 1 mg/kg body weight administered on alternate days, while the remaining 7 birds, acting as controls, were injected with the carrier oil.

Blood samples (5 ml) were taken from the wing vein into a heparinized syringe on day of

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(the day before the first injection) and on days 7, 14 and 21, during which time medullary bone formation was taking place. The last injection was on day 21 and further blood samples were taken on days 25, 29, 33 and 39.

One control and one oestrogen-treated bird were killed on day 21 and another of the oestrogen-treated cocks was killed on day 29. One femur from each of these birds was taken for histological examination.

## 2. Analytical methods

The blood was centrifuged immediately and the plasma analyzed for total calcium (HERTELENDY and TAYLOR, 1961), acid and alkaline phosphatase (BELL and SILLER, 1962) aryl sulphatases A and B (ROY, 1953) and  $\beta$ -glucuronidase (FISHMAN, SPRINGER and BRUNETTI, 1948).

## RESULTS

### 1. Bone histology

Large amounts of medullary bone were observed in the femur of the bird killed at the end of the period of oestrogen administration but none was present in the femur of the control cock. The bird killed 8 days after the last injection of hormone still possessed substantial amounts of medullary bone but the spicules were thinner, presumably due to resorption, and it was evident that there was less bone tissue per unit area than in the cock killed 8 days earlier.

### 2. Plasma calcium

The level of total calcium in the plasma of the oestrogen-treated birds rose from a mean of 12.0 mg/100 ml to 87.6 mg/100 ml at day 14 and it remained at this level until the end of the injection period. Little variation occurred in the control group. Four days after the last injection the mean level was 35.1 mg/100 ml and normal levels were not restored until day 33 (table 1).

TABLE I

*Mean levels of total plasma calcium (mg/100 ml)  
in cocks before, during and after 21 days of  
oestrogen treatment (Means  $\pm$  S. E.)*

Day of experiment	Control 7 birds (1)	Oestrogen-treated 8 birds (2)
0	11.3 $\pm$ 0.3	12.0 $\pm$ 0.3
7	11.4 $\pm$ 0.3	48.7 $\pm$ 2.6
14	11.7 $\pm$ 0.3	87.6 $\pm$ 4.6
21	11.7 $\pm$ 0.4	88.8 $\pm$ 3.8
25	11.5 $\pm$ 0.4	35.1 $\pm$ 4.7
29	11.5 $\pm$ 0.3	17.5 $\pm$ 1.3
33	11.4 $\pm$ 0.3	13.3 $\pm$ 0.6
39	11.4 $\pm$ 0.3	12.1 $\pm$ 0.3

(1) One killed on day 21.

(2) One killed on day 21 and another on day 29.

It must be assumed that the birds were under the influence of oestrogen until the plasma calcium had fallen to a level not significantly different from the pre-injection level and that bone resorption was not proceeding for certain until that time.

### 3. Plasma alkaline phosphatase

The activity of this enzyme varied slightly from bleeding to bleeding, but at no time did the mean value differ significantly from the value at day 0. Somewhat greater fluctuations occurred in the treated group from the day of the last injection, but even the highest mean value (on day 29) did not differ significantly from the initial value.

In order that the changes in the plasma levels of this enzyme (and of the acid hydrolases also) might be compared in the different physiological conditions, the mean values for days 7, 14 and 21, when the birds were under the influence of oestrogen have been calculated and these values are presented in table 2 together with the values for the two final bleedings when bone resorption can be assumed to have been taking place. All these means are expressed as percentages of the mean values at the start of the experiment (day 0).

### 4. Plasma acid hydrolases

The activity of all the acid hydrolases studied decreased significantly during the period of oestrogen treatment in relation to the pre-injection levels and the reduction in  $\beta$ -glucuronidase and aryl sulphatases was greater than the reduction in acid phosphatase (table 2). After the withdrawal of the hormone the activity of all three enzymes increased sharply, but only in the case of acid phosphatase did the level rise above the pre-injection level.

TABLE 2

*Changes in the activities of plasma enzymes during oestrogen treatment and during the bone resorption occurring when oestrogen was withdrawn*

Values are means for 7 or 8 birds expressed as percentages of the pre-treatment levels of activity. The control birds were not injected with oestrogen

Physiological state	Oestrogen treatment		Bone resorption			
	7, 14 and 21		33		39	
Enzyme	Control	Oestrogen	Control	Oestrogen	Control	Oestrogen
Alkaline phosphatase	101	96	92	116	92	95
Acid phosphatase	104	67 <sup>(1)</sup>	83	149 <sup>(1)</sup>	86	156 <sup>(1)</sup>
Aryl sulphatases	105	31 <sup>(1)</sup>	85	77	72	80
$\beta$ -glucuronidase	82	28 <sup>(1)</sup>	79	58	75	92

<sup>(1)</sup> Significantly different from corresponding control value ( $p < 0.001$ , 't' test).

## DISCUSSION

Of the hydrolases studied only the sulphatases are located solely in the lysosomes. Acid phosphatase and  $\beta$ -glucuronidase, besides being present in the lysosomes, are found elsewhere in the cell. Nevertheless, the activity in the plasma of all these enzymes was substantially reduced under the influence of oestrogen and it is possible that this was due to a stabilizing effect of the hormone on the lysosomal membranes (WILLMER, 1961). The decline in acid phosphatase was much less than that of the other two acid hydrolases studied, which suggests that, under normal conditions, much of the plasma acid phosphatase arises from sources other than the lysosomes. It is not possible to relate these changes specifically to the formation of new bone since oestrogens induce the most profound changes in the metabolism of the liver as well as the bone.

Once the effects of the oestrogen had worn off and the level of plasma calcium had returned to normal, the most important physiological respect in which these birds differed from normal was in the resorption of medullary bone. The only plasma component which showed a significant difference between the oestrogen-treated and control birds at this time was acid phosphatase and it would appear that bone resorption is associated with the release of this enzyme into the blood, thus confirming the observations of TAYLOR, WILLIAMS and KIRKLEY (1965) and MORRIS and TAYLOR (1970) with laying hens. The bone cells most likely to liberate acid phosphatase are osteoclasts and mature osteocytes both of which are rich in lysosomes. The biochemical role of acid phosphatase in bone resorption is not clear, however.

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## SUMMARY

Plasma levels of acid and alkaline phosphatases, aryl sulphatases and  $\beta$ -glucuronidase were studied in 8 cocks before, during and after the intramuscular injection of oestrogen. Alkaline phosphatase did not show any significant changes but the levels of all three acid hydrolases were significantly depressed during the period of oestrogen treatment. Following the withdrawal of the hormone the activities of the acid hydrolases increased, but only in the case of acid phosphatase was the level raised above the pre-injection level, suggesting that this enzyme is released into the blood during bone resorption.

## RÉSUMÉ

VARIATION DE LA CONCENTRATION PLASMATIQUE DE QUELQUES ENZYMES  
HYDROLYTIQUES DURANT LA RÉSORPTION DE L'OS MÉDULLAIRE INDUIT PAR LES  
ŒSTROGÈNES CHEZ LE COQ

Les concentrations plasmatiques des phosphatases acides et alcalines, des arylsulphatases et de la  $\beta$ -glucuronidase sont étudiées chez 8 coqs avant, durant et après l'injection intramusculaire d'œstrogènes. La phosphatase alcaline ne change pas, mais les niveaux des trois hydrolases acides sont significativement réduits durant le traitement aux œstrogènes. L'activité de ces hydrolases remontent dès que le traitement œstrogénique est arrêté mais le niveau ne devient supérieur à celui avant injection que pour la phosphatase acide, ce qui suggère que cet enzyme est libéré dans le sang durant la résorption osseuse.

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