THE EFFECT OF HYPOPHYSECTOMY AND ADRENO-
CORTICOTROPIC HORMONE ON THE ALKALINE
PHOSPHATASE OF RAT PLASMA*

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Effect of Hypophysectomy

It is well known that the phosphatase content of bone, kidney, liver,
and blood may change under varying conditions of growth, diet, and
disease (1). Among these tissues, blood perhaps furnishes the over-all
changes of this enzyme activity. Weil (2) found a rise in plasma phos-
phatase activity from birth to maturity during the growth of normal
rats. Kinard and Chanutin (3) have reported that the phosphatase
content of the whole rat increases from the time of birth. There is ample
evidence that certain bone diseases cause a marked alteration in plasma
phosphatase (4). Furthermore, phosphatase has been shown to play a
part in bone regeneration (5).

The effects of hypophysectomy on bone histology have been described
recently by Becks, Simpson, and Evans (6). The immediate reaction is
a thinning of the cartilage plate, and after longer postoperative intervals
the bony trabeculae become coarser and less numerous. Since the phos-
phatase activity is intimately related to changes in bone, one may expect
that changes may occur in plasma phosphatase after hypophysectomy. In
a survey of the literature, there appears only one report (7) on serum
phosphatase activity in hypophysectomized rats.

EXPERIMENTAL

Male rats used were of the Long-Evans strain. Hypophysectomy was
performed at 40 days of age by the parapharyngeal approach. The
completeness of the operation was ascertained at autopsy by examination
of the sella turcica. The animals were maintained on the usual diet of
this laboratory ad libitum. Blood was taken from the inferior vena cava
after the animals were anesthetized by the intraperitoneal administration
of sodium amytal. 4 per cent sodium citrate solution (0.5 cc. per sample)
was used as the anticoagulative agent. The alkaline phosphatase in the
plasma was determined immediately or within 24 hours. The plasma
samples were always kept frozen at $-15^\circ$.

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from the Rockefeller Foundation, New York.
The alkaline phosphatase activity was estimated by a modification of the method of King and Armstrong as described by Binkley, Shank, and Hoagland (8); at least three determinations were carried out on each plasma sample. The Cenco-Sheard-Sanford photometer with orange filter was employed for recording the color intensity.

The micro-Kjeldahl method was used for nitrogen determination. The phosphatase potency was expressed either in units$^1$ per 100 mg. of nitrogen or per 100 cc. of plasma.

**Plasma Phosphatase Content of Normal Male Rats**—Results on the plasma alkaline phosphatase activity of male rats of different ages are summarized in Table I. It will be noted that the phosphatase activity increases from 21 to 40 days of age and remains practically constant up to 90 days of age when the enzyme activity is expressed on the basis of 100 cc. of plasma. When the phosphatase potency is expressed in units per 100 mg. of N, the activity seems to decline after 40 days of age.$^2$ It has been recorded by Weil (2) that "the high plasma phosphatase activity of the rat 1 month old decreases gradually with the maturity of rat, but remains above the original low enzyme value." The same conclusion was also arrived at by Gould (9).

It is of interest to note that the nitrogen concentration in the plasma increases gradually from 7.0 mg. to 9.56 mg. per cc. as the rats grow from 21 to 40 days of age. Further, the nitrogen content of the plasma is 6.2 mg. per cc. These values were secured from one sample of pooled plasma. It appears that the enzyme activity of these young rats seems to be lower than that of the 40 day-old animals.

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**Table I**

*Phosphatase Content in Plasma of Male Rats at Different Ages*

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of rats</th>
<th>Body weight (gm.)</th>
<th>N per cc. plasma (mg.)</th>
<th>Phosphatase (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>10</td>
<td>47.5 ± 1.65$^*$</td>
<td>7.00 ± 0.28</td>
<td>4.17 ± 0.45</td>
</tr>
<tr>
<td>40</td>
<td>11</td>
<td>142.0 ± 3.07</td>
<td>7.71 ± 0.14</td>
<td>5.67 ± 0.45</td>
</tr>
<tr>
<td>40†</td>
<td>10†</td>
<td>126.3 ± 3.50</td>
<td>7.94 ± 0.11</td>
<td>4.89 ± 0.35</td>
</tr>
<tr>
<td>55</td>
<td>10</td>
<td>211.5 ± 6.64</td>
<td>8.43 ± 0.11</td>
<td>4.90 ± 0.40</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>257.6 ± 5.94</td>
<td>9.56 ± 0.17</td>
<td>4.52 ± 0.61</td>
</tr>
</tbody>
</table>

$^*$ Mean ± standard deviation.

† Fasting 48 hours.

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1 A unit of alkaline phosphatase is that amount of activity which will liberate 1 mg. of phenol in 30 minutes in barbiturate buffer of pH 9.7.

2 From nine rats 7 days of age with an average body weight of 17.0 gm., we have obtained the plasma alkaline phosphatase activity of 4.75 units per 100 mg. of nitrogen. The nitrogen content of the plasma is 6.2 mg. per cc. These values were secured from one sample of pooled plasma. It appears that the enzyme activity of these young rats seems to be lower than that of the 40 day-old animals.
21 days to 90 days. The latter value agrees with that reported by Levin (10) who found that serum in adult male rats eating ad libitum contains 6.04 per cent protein or 9.66 mg. of nitrogen per cc.

The effect of fasting on the plasma phosphatase has been studied by Weil and Russell (11) and Gould (9). They found that normal rats show a decrease of enzyme activity after fasting for 24 hours. However, Gould's experiments with fat-fed animals indicated that 1 day of fasting caused little change in the serum phosphatase level. As shown in Table I, there is a definite lowering of plasma alkaline phosphatase after 48 hours of fasting, but less marked than that observed by Weil and Gould.

**Plasma Phosphatase Content of Hypophysectomized Male Rats**—From Table II, it is evident that hypophysectomy decreases the alkaline phos-

<table>
<thead>
<tr>
<th>Post-operative</th>
<th>No. of</th>
<th>Body weight</th>
<th>N per cc. plasma</th>
<th>Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>rats</td>
<td>Initial gm.</td>
<td>Final gm.</td>
<td>mg.</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>142.0 ± 3.10*</td>
<td>130.6 ± 2.82</td>
<td>7.71 ± 0.14</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>144.7 ± 3.12</td>
<td>133.3 ± 3.88</td>
<td>8.77 ± 0.16</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>154.5 ± 4.45</td>
<td>124.6 ± 2.02</td>
<td>8.96 ± 0.24</td>
</tr>
<tr>
<td>15</td>
<td>17</td>
<td>147.1 ± 1.98</td>
<td>124.6 ± 2.02</td>
<td>8.67 ± 0.16</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

phatase activity of male rat plasma. In the 4th day after the operation, the phosphatase level changes from 43.8 to 37.1 units per 100 cc. of plasma. The enzyme level continues to fall but becomes constant after 8 days after hypophysectomy.

It is very well known that hypophysectomy causes atrophy of the adrenal, thyroid, and reproductive organs as well as the cessation of body growth. Therefore, the influence of hypophysectomy on the plasma phosphatase may be attributable to over-all changes in the function of the adrenal, thyroid, and reproductive organs as well as to the cessation of body growth. For instance, a lowering of serum phosphatase has been observed by Watson (12) after injection of adrenal cortical extract and an increase of the enzyme upon the administration of testosterone propionate (13). Moreover, thyroxine is known to increase the phosphatase content of the bone (14). Since, as already mentioned, rapidly growing rats have a higher phosphatase
ALKALINE PHOSPHATASE OF PLASMA

level in the serum, it may be expected that a loss of body weight may produce a lowering of the enzyme activity. The depression of alkaline phosphatase in male rat plasma after hypophysectomy may therefore be explained by the loss of growth, on lessening in function of thyroid or gonad, or the combination of these factors. On the other hand, from the lowering in adrenal function due to the removal of the pituitary one would expect an elevation of the plasma phosphatase level. It is clear from the data just presented that the influence of lessened adrenal function is completely overshadowed by the effect from the other deficiencies which result from hypophysectomy.

It is of interest to compare our results with those obtained by Jones and Shinowara (7). They found that hypophysectomy causes an elevation of serum phosphatase activity. Their observations were made with female rats. It was thought that the disagreements between our findings and theirs may be due to the sex difference of the animals employed. We have therefore investigated the alkaline phosphatase content of hypophysectomized female rats as compared to their normal female controls.

Table III summarizes the results obtained with normal and hypophysectomized female rats. The animals were operated upon when 26 to 28 days of age; two groups of different postoperative periods were used: 11 to 18 days and 20 to 24 days. No evident difference was observed in the phosphatase level between these two groups of hypophysectomized rats. The normal animals employed were 41 to 42 days old and their plasma phosphatase activity served for comparison. It is clear that, as found in male rats, the plasma alkaline phosphatase content in hypophysectomized female rats is definitely lower than that in normal animals.

**Effect of Adrenocorticotropic Hormone**

The relationship of the adrenals to phosphatase in animal tissues has been studied by a number of investigators. Williams and Watson (15)
have shown that corticosterone reduces the phosphatase content in rat femurs, while desoxycorticosterone acetate produces an increase. In an earlier paper Watson (12) reported that adrenal cortical extract lowers the serum phosphatase but desoxycorticosterone acetate has no effect. Furthermore, adrenalectomy produces a marked lowering of phosphatase in the cat kidney (16). It would appear of considerable interest to investigate the plasma alkaline phosphatase level in animals with adrenals hypertrophied by the administration of adrenocorticotropic hormone.

In Table IV, results obtained with hypophysectomized male rats are summarized. The adrenocorticotropic hormone was isolated from sheep pituitaries by the method previously described (17). Male rats hypophysectomized at 40 days of age were injected intraperitoneally immediately after operation with 0.2 mg. of adrenocorticotropic hormone daily (except Sunday) for 15 days. As was shown in an earlier report (17), this daily dose is sufficient to maintain the weight of adrenals at 25.0 mg., while the hypophysectomized controls have adrenal weights of 10.5 mg. The data indicate that, when alkaline phosphatase is expressed either in units per 100 mg. of N or per 100 cc. of plasma, the enzyme level is significantly lower in the hormone-treated group than in the control.

Similar experiments were carried out in 40 day-old normal male rats. A total daily dose of 1.0 mg. of adrenocorticotropic hormone was employed; intraperitoneal injections were instituted three times daily, twice on Saturday, and once on Sunday for 15 days. The results in Table V show that the averaged adrenal weights of six injected animals were 50.3 mg., while those of the controls were 29.3 mg. The increase of adrenal function is further displayed by both the inhibition of body growth and the

### Table IV

**Effect of Adrenocorticotropic Hormone on Phosphatase Content in Plasma of Hypophysectomized Male Rats**

<table>
<thead>
<tr>
<th>Experiment on 17 rats</th>
<th>Body weight</th>
<th>Adrenals</th>
<th>N per cc. plasma</th>
<th>Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Per 100 mg. N</td>
<td>Per 100 cc. plasma</td>
</tr>
<tr>
<td></td>
<td>gm.</td>
<td>gm.</td>
<td>mg.</td>
<td>units</td>
</tr>
<tr>
<td>Injected*</td>
<td>144.6 ± 2.52†</td>
<td>112.7 ± 2.49</td>
<td>25.0 ± 1.10</td>
<td>2.61 ± 0.24</td>
</tr>
<tr>
<td>Control...</td>
<td>147.1 ± 1.98</td>
<td>124.6 ± 2.02</td>
<td>10.5 ± 0.45</td>
<td>3.46 ± 0.22</td>
</tr>
</tbody>
</table>

* Rats hypophysectomized at 40 days of age; injections with 0.20 mg. of hormone daily began on the day of operation once daily for 15 days except Sundays; i.e., thirteen injections in 15 days.
† Mean ± standard deviation.
reduction of thymus weight in the injected group as compared with the controls. The alkaline phosphatase content in the plasma is convincingly reduced by the hormone; the hormone-treated animal has an average of 28.4 units of phosphatase per 100 cc. of plasma, whereas the untreated male rats of 55 days of age possess 41.3 units.

From bone studies, it was found that adrenocorticotropic hormone caused a retardation in both chondrogenesis and osteogenesis in the region of the proximal epiphysis of the tibia of normal rats (18). Moreover, the inhibiting action of adrenocorticotropic hormone on the growth of young and adult male rats has been clearly demonstrated (19); it may therefore be concluded that the reduction of alkaline plasma phosphatase activity by the hormone is probably due to the lowering of the enzyme content in the osseous tissues. To verify this presumption data on tibia phosphatase activity should be known. Such studies are planned for future investigations.

A comparison of the results herein reported with those obtained by Watson (12, 15) when adrenal cortical hormones were employed seems to indicate that the adrenals hypertrophied by adrenocorticotropic hormone secrete mainly, if not wholly, steroids which have an oxygen atom on C11, rather than substances akin to desoxycorticosterone. This deduction is in agreement with the conclusion of Fraenkel-Conrat et al. (20) from studies of liver arginase.

**SUMMARY**

The alkaline phosphatase in the plasma of male rats at various ages (21 to 90 days) has been determined. After hypophysectomy, the enzyme

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**TABLE V**

*Effect of Adrenocorticotropic Hormone on Phosphatase Content in Plasma of Normal Male Rats*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of rats</th>
<th>Body weight (gm.)</th>
<th>Adrenals (mg.)</th>
<th>Thymus (mg.)</th>
<th>N per cc. plasma</th>
<th>Phosphatase Per 100 cc. Plasma units</th>
<th>Per 100 cc. Plasma units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected*</td>
<td>6</td>
<td>130.7±5.83</td>
<td>50.3±2.91</td>
<td>123.8±11.75</td>
<td>0.02±0.34</td>
<td>9.02±0.15</td>
<td>28.4±0.15</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>142.0±3.07</td>
<td>29.3±0.85</td>
<td>410.1±10.23</td>
<td>8.43±0.11</td>
<td>4.90±0.40</td>
<td>41.3±0.40</td>
</tr>
</tbody>
</table>

* 40 day-old male rats; 1.0 mg. of hormone divided into three injections daily. On Saturday two injections and once only on Sunday, for 15 days.
† Mean ± standard deviation.
activity in the plasma of male rats 40 days of age decreases with successive postoperative periods (4 to 15 days). A similar lowering of the plasma phosphatase values in female rats occurs after removal of the pituitary.

In normal and hypophysectomized male rats the administration of adrenocorticotropic hormone in doses causing hypertrophy of the adrenals produced a significant decrease in the alkaline phosphatase level of the plasma.

BIBLIOGRAPHY

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