SERUM INORGANIC PHOSPHATE AND "ALKALINE" PHOSPHATASE ACTIVITY IN HYPOPHYSECTOMIZED RATS*

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In 1932, Thompson and Collip (11) in a review of the influence of the endocrine glands on the calcium and inorganic phosphate content of blood serum made no mention of the pituitary. Since that time several observations suggest that it should be considered also. Schour and van Dyke (7) found significant defects of calcification of the teeth of hypophysectomized rats which were corrected more or less completely by replacement therapy. Pugsley and Anderson (6) found that the negative calcium balance of hypophysectomized rats maintained on a low calcium diet could be changed to a positive balance by the administration of anterior pituitary growth hormone.

Mortimer (5) presented a thorough radiological investigation of the effect of hypophysectomy and hormone administration upon the bones of the rat using the skull for particular study. Hetherington and Weil (3) employing the same experimental animal found that hypophysectomy resulted in a negative calcium balance, while phosphorus was reduced but maintained a positive balance. Snyder and Tweedy (10) in 1941, using both rats and guinea pigs, found a slight increase in inorganic phosphate with no change in calcium following daily injections of alkaline anterior pituitary preparations.

In 1935 a case of acromegaly in which there was an increased urinary excretion of calcium accompanied by marked osteoporosis was described by Sriver and Bryan (8). Bauer and Aub (1) (1941)

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in reporting calcium-phosphorus metabolism studies on five cases of acromegaly conclude that "the pituitary gland must be considered as one of the factors capable of altering calcium and phosphorus metabolism."

TABLE I

<table>
<thead>
<tr>
<th>Date</th>
<th>Weight</th>
<th>Alkaline phosphatase</th>
<th>Inorganic phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>gm.</td>
<td>units</td>
</tr>
<tr>
<td>Apr. 3</td>
<td>C., H.† 140-155</td>
<td>150</td>
<td>20-55</td>
</tr>
<tr>
<td>&quot; 15</td>
<td>C. 160-180</td>
<td>170</td>
<td>49-73</td>
</tr>
<tr>
<td>H. 137-144</td>
<td>141</td>
<td>40-85</td>
<td>72</td>
</tr>
<tr>
<td>&quot; 21</td>
<td>C. 167-184</td>
<td>176</td>
<td>56-76</td>
</tr>
<tr>
<td>H. 135-142</td>
<td>138</td>
<td>55-144</td>
<td>113</td>
</tr>
<tr>
<td>&quot; 28</td>
<td>C. 187-200</td>
<td>192</td>
<td>64-94</td>
</tr>
<tr>
<td>H. 130-140</td>
<td>134</td>
<td>85-154</td>
<td>109</td>
</tr>
<tr>
<td>May 6</td>
<td>C. 189-205</td>
<td>197</td>
<td>57-98</td>
</tr>
<tr>
<td>H. 125-140</td>
<td>130</td>
<td>60-130</td>
<td>109</td>
</tr>
<tr>
<td>&quot; 13</td>
<td>C. 193-210</td>
<td>203</td>
<td>57-81</td>
</tr>
<tr>
<td>H. 122-142</td>
<td>129</td>
<td>82-181</td>
<td>114</td>
</tr>
<tr>
<td>&quot; 20</td>
<td>C. 202-223</td>
<td>210</td>
<td>63-89</td>
</tr>
<tr>
<td>H. 122-142</td>
<td>132</td>
<td>80-163</td>
<td>123</td>
</tr>
<tr>
<td>&quot; 27</td>
<td>C. 208-225</td>
<td>212</td>
<td>79-194</td>
</tr>
<tr>
<td>H. 120-141</td>
<td>129</td>
<td>79-194</td>
<td>128</td>
</tr>
<tr>
<td>June 16</td>
<td>C. 2 rats</td>
<td>49, 78</td>
<td>64</td>
</tr>
<tr>
<td>H. 3</td>
<td>110-130</td>
<td>122</td>
<td>4.0-4.8</td>
</tr>
</tbody>
</table>

* Hypophysectomy, April 5.
† C. represents a control group of six rats; H. a hypophysectomized group of eight rats.

Recently "alkaline" phosphatase activity of blood serum has come to be recognized as a valuable supplement to calcium and inorganic phosphate determinations. The present study was undertaken to follow the effect of hypophysectomy upon phosphatase activity and inorganic phosphate concentration in the blood of the white rat.
The animals used were laboratory white female rats from three litters. The first litter contained six, three of which were hypophysectomized when 84 days old; the second contained five, three of which were operated on when 83 days old; the third litter contained three, two of which were hypophysectomized when 70 days old. This gave a total of eight hypophysectomized animals and six controls. Stock diet and water were given ad libitum and approximately 20 cc. of milk fed each animal daily.

Records were kept of the weight changes for each animal. The control rats gained approximately 70 gm. each; the hypophysectomized animals all showed a slight loss. At intervals approximately 0.2 cc. of blood was obtained from the tail by means of a small collecting capsule. Phosphatase activity and inorganic phosphate determinations were made on this blood by a microprocedure previously described (9). The values obtained by averaging the results of the phosphatase and inorganic phosphate determinations for each group are given in Table I.

Five animals (two control, three operated) were sacrificed 70 days after hypophysectomy. Blood chemistry studies included serum calcium (Tisdall (12)) and total protein (Kagen (4)). Completeness of hypophysectomy was confirmed by examination of

\[ \text{H indicates the animal was hypophysectomized; C represents control.} \]

\[ \text{1 The stock diet consisted of yellow corn-meal 67 per cent, linseed oil meal 12 per cent, casein with arginine 16 per cent, alfalfa meal 3 per cent, sodium chloride 1 per cent, and calcium carbonate 1 per cent.} \]

### Table II

**Chemical Findings at Autopsy**

<table>
<thead>
<tr>
<th>Rat No.*</th>
<th>&quot;Alkaline&quot; phosphatase units</th>
<th>Serum inorganic phosphate mg. per cent</th>
<th>Calcium mg. per cent</th>
<th>Total protein per cent</th>
<th>Femur Ca:P</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-131</td>
<td>110</td>
<td>4.4</td>
<td>10.0</td>
<td>6.57</td>
<td>1.92</td>
<td>Hypophysectomy complete</td>
</tr>
<tr>
<td>H-135</td>
<td>125</td>
<td>4.8</td>
<td>13.1</td>
<td>5.12</td>
<td>1.77</td>
<td>&quot;</td>
</tr>
<tr>
<td>H-138</td>
<td>130</td>
<td>4.0</td>
<td>10.3</td>
<td>5.75</td>
<td>1.65</td>
<td>&quot;</td>
</tr>
<tr>
<td>C-131</td>
<td>49</td>
<td>7.0</td>
<td>9.4</td>
<td>6.20</td>
<td>1.76</td>
<td>&quot;</td>
</tr>
<tr>
<td>C-135</td>
<td>78</td>
<td>8.3</td>
<td>9.7</td>
<td>6.15</td>
<td>1.87</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* H indicates the animal was hypophysectomized; C represents control.
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the sella. The femur was analyzed for the Ca:P ratio. The data obtained are given in Table II.

DISCUSSION

The elevation of the alkaline phosphatase activity of eight hypophysectomized rats was definitely greater than that of six control litter mates. The study involved 111 determinations over a 10 week period. The individuals within both groups showed marked differences in phosphatase activity (Table I). This individual variation was also noted by Chen, Freeman, and Ivy (2) in a series of analyses of rat serum and tissue phosphatase activity. Since the controls were from the same litters as the hypophysectomized animals, comparison of the averages for each group was made. This comparison shows the average in the hypophysectomized group to be 20 to 96 per cent higher than that of the controls, which is a significant difference. The explanation of this difference will require additional investigation.

The rise of phosphatase activity in the control group may be related to growth. During the test period each of these animals gained approximately 70 gm. in weight. The rats in this investigation were observed from about the age of 70 days to 154 days. Weil (14) found a rise in plasma phosphatase activity during growth of normal rats from birth to 44 days.

The lowered inorganic phosphate of the hypophysectomized group confirms the reported findings of Hetherington and Weil (3) and Snyder and Tweedy (10). This reduced phosphate is clearly shown by the serial determinations. Here again individual differences are found as in the phosphatase activity. This wide range of values illustrates how some of the contradictory findings reported may have arisen (13). A difference in calcium was suggested by the five determinations done at autopsy. Serial determinations would be essential to follow the calcium and phosphate changes in relation to each other.

On gross examination the bones of the hypophysectomized groups were found to be smaller and thinner. This was very marked in the skull and has been described in detail by Mortimer (5). The Ca:P ratio, however, did not reveal any chemical difference between the two groups of animals (Table II).
SUMMARY

1. Eight hypophysectomized rats were found to have an elevation of phosphatase activity above that obtained for six control litter mates. This elevation persisted for at least 10 weeks after operation.

2. The inorganic phosphate level of the control animals remained consistently above that of the hypophysectomized group.

3. At the end of 10 weeks the serum calcium of three of the hypophysectomized animals was higher than that of two controls.

We are indebted to Dr. T. S. Sutton, Department of Animal Husbandry, for providing us with the animals used for this study and the stock diet fed during the experimental period, and to Dr. H. L. Reinhart, Pathologist of the University Hospital, for his criticisms and suggestions.

BIBLIOGRAPHY

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