THE PLASMA PHOSPHATASE IN RICKETS AND SCURVY

BY

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Robison in 1923 first described an enzyme which was concerned with the calcification and the growth of bone. He pointed out that the deposition of calcium phosphate in growing bone and its non-deposition elsewhere had not up to that time been explained. In 1904, Pfaundler had assumed a specific adsorption of calcium ions by the cartilage, with the consequent precipitation of insoluble salts. Later, Adler pointed out that an aqueous solution containing the same amounts of calcium phosphate and carbonate as are present in serum would be saturated with respect to these salts which would tend to be precipitated from the solution. The failure of this to occur in serum was attributed by Pauli and Samec to an increased solubility of calcium in the presence of the serum proteins, but the mechanism of specific adsorption of calcium from a solution such as is present by blood had not been made clear, and the work of Robison has suggested a chemical rather than a physical basis for calcification.

Robison's hypothesis may be briefly stated as follows:—The osteoblasts and hypertrophic cartilage cells secrete an active enzyme, a phosphatase, which by hydrolyzing the phosphoric esters of the blood brings about a local increase in the concentration of the various inorganic phosphate ions. The solubility product of tertiary calcium phosphate is thereby exceeded and deposition of this salt occurs in the ossifying zone. This enzyme is present in considerable quantity in ossifying cartilage and to an equal extent in teeth. It is present in greatest amount in bone at the time when deposition of bone is most active, and is absent from cartilages which do not calcify, as, for example, tracheal cartilage.

Robison's hypothesis has received further confirmation from the results of his experiments on calcification in vitro. He was able to demonstrate that a rachitic bone, split lengthwise and immersed in a solution of calcium glycerophosphate or calcium hexosemonophosphate, showed a deposition of calcium phosphate in the zones of preparatory calcification and hypertrophic cartilage cells, and in the periosteum.

Phosphatases are also found in the kidney, the intestinal mucosa and the blood plasma. The intestinal phosphatase is probably associated with the hydrolysis of the phosphoric esters in the food, while the renal phosphatase may be concerned with the appearance of inorganic phosphorus
in the urine. Although the relation of the renal and intestinal phosphatases
to bone phosphatase is not clear, it seems likely that the bone phosphatase
and the plasma phosphatase are identical.

Applying Robison's experimental work to clinical medicine, Kay and
others have shown that in certain generalized diseases of bone the plasma
phosphatase is markedly increased. The conditions which were first found
to be associated with an increased plasma phosphatase were osteitis fibrosa,
osteitis deformans, osteogenesis imperfecta, osteomalacia and rickets.

In view of these findings it was thought desirable to take a series of
children with rickets, and to trace the course of the plasma phosphatase and
its relation to the healing process. Later, the study was extended to other
conditions such as scurvy and fractures.

Method.—The method used was that of Kay adapted for use with small
quantities of plasma.

Blood is collected from a finger prick in an oxalated tube of the type described
by Martin and Lepper. 0.3 c.c.m. of the centrifuged plasma, 1.5 c.c.m. of N/100
hydrochloric acid, and 3 c.c.m. of sodium \(\beta\)-glycerophosphate (0.15 per cent.) are
mixed, and the carbon dioxide liberated from the plasma bicarbonate is extracted
under reduced pressure with a water pump. 0.8 c.c.m. of this solution is placed in
each of 5 Monax test tubes graduated to 5 c.c.m.; 0.5 c.c.m. of 20 per cent. trichloracetic
acid is added to tubes 1 and 2; a tiny drop of chloroform is placed in tubes 3, 4 and
5, and in tube 5 two drops of phenol red solution (0.02 per cent.). This tube (5) is
titrated to pH 7.6 at room temperature by the addition of N/50 caustic soda from
a micrometer burette (Trevan). An equal quantity of caustic soda is added to
tubes 3 and 4 which may be expected to reach the same pH as tube 5. Tubes
3, 4 and 5 are corked and incubated at 37°C. for 48 hours, when the pH of tube 5
is again read; the change of pH in this tube is usually slight. This adjustment
of the reaction is of considerable importance since the pH has a considerable
influence on the velocity of hydrolysis (Martland, Robison). 0.5 c.c.m. trichloracetic
acid is added to tubes 3 and 4, and the contents of tubes 1, 2, 3, and 4 made up to
3 c.c.m. with distilled water. After standing for 10 minutes, these tubes are filtered
and the phosphorus content of 2 c.c.m. filtrate estimated by the Briggs modification
of the Bell and Doisy method.

Results in normal infants and children.—In adults Kay has found that
the normal phosphatase varies from 0.1 to 0.21 units; he states that higher
figures are common in infancy. We have found that the average
phosphatase content in 14 infants under one year was 0.23 (range from 0.10
to 0.27). In the second year the average figure was 0.24, with a range of
0.11 to 0.33 units. The children in the first group were normal clinically and
radiologically. All cases of the second group were normal radiologically,
and as three had phosphatase values in the neighbourhood of 0.3, it
is probable that high values up to 0.3 units must be considered to be within
the normal range. The average plasma phosphatase of a series of seven
normal children, whose ages varied from six to twelve years, was 0.17, with
a range of 0.12 to 0.2 units.

Plasma phosphatase in rickets.—In all the cases one or more clinical signs
of active rickets were present, such as epiphyseal enlargement, beading of the
ribs, flabby muscles, delayed closure of the fontanelle, etc. Radiographically,
they all showed decalcification, enlargement of the epiphyses and irregularity of the epiphyseal line. The serum calcium and plasma phosphorus and phosphatase were estimated when each child was first seen in the active stage and at intervals while healing was in progress until this was complete. The progress of healing was checked both clinically and radiographically.

In a series of ten cases of uncomplicated rickets the calcium and phosphorus were both reduced to a greater or lesser degree; in two cases the calcium alone was lowered. The phosphatase in these cases averaged 0.76 units with a range of 0.4 to 1.4. In one case of early rickets the figure was only 0.3 units.

Two or three weeks after the commencement of treatment the calcium and phosphorus had risen to normal and maintained this level subsequently. The decline in the phosphatase on the other hand was much more gradual, and while the calcium and the phosphorus were normal soon after healing had begun, the phosphatase did not fall to normal until the healing process appeared complete.

These findings are illustrated by the following case:

C. C., female, admitted on January 20th, 1931. She had been fed since one month old on boiled milk and barley water. Orange juice had been given regularly but no additional fat-soluble vitamins. When first seen at 5 months, she was pale and much underweight for her age even allowing for the fact that she had been born prematurely and that at birth her weight was 5½ lb. On admission, her weight was found to be 8 lb. There was marked beading of the ribs and enlargement of the epiphyses, but there was no clinical evidence to lead one to suspect such fractures of the long bones as were revealed by the radiograms. The liver was slightly enlarged and the blood count on January 29th, showed a secondary anæmia.

TREATMENT AND PROGRESS.—The infant was fed on a humanized dried milk and a preparation of Vitamin D of which an amount equivalent to 2 mgrm. irradiated ergosterol was given twice daily from January 23rd to February 17th when, in view of the good progress in healing, the dose was reduced to 1 mgrm. twice daily. This dosage was continued until the child was discharged (see Table 1).

**TABLE 1.** Analyses in a case (C.C.) with active rickets.

<table>
<thead>
<tr>
<th>Age</th>
<th>Date</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Phosphatase</th>
<th>Radiological appearances</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>26-1-31</td>
<td>6.2</td>
<td>2.0</td>
<td>0.57</td>
<td>Acuterickets <em>i.e.</em> epiphyseal cupping and enlargement, decalcification. Green stick fractures of one ulna, tibia and fibula.</td>
</tr>
<tr>
<td></td>
<td>6-2-31</td>
<td>10.1</td>
<td>3.6</td>
<td>0.47</td>
<td>Healing commencing; calcification proceeding at bone ends; callus around sites of fractures.</td>
</tr>
<tr>
<td></td>
<td>17-3-31</td>
<td>3.6</td>
<td>0.44</td>
<td></td>
<td>Good progress in healing, sites of fractures scarcely discernible. Epiphyseal cupping very slight.</td>
</tr>
<tr>
<td>8</td>
<td>23-4-31</td>
<td>10.4</td>
<td>4.5</td>
<td>0.24</td>
<td>Rickets healed.</td>
</tr>
</tbody>
</table>
Plasma phosphatase in scurvy.—Six cases of scurvy were examined. In three of these the diagnosis was based on the history of scorbutic diet, the presence of spongy gums and petechial hæmorrhages on the tongue or palate, the demonstration of red blood cells in the urine and the characteristic radiological appearance of the bones. The other three cases had sub-periosteal hæmorrhages. In five of these cases seen in the acute stage, little or no alteration in the blood calcium and phosphorus was found, but the phosphatase in all the cases gave low figures, so that the average figure was 0·14 units as compared with the normal average of 0·23 at this age. The first three cases of scurvy examined had no subperiosteal hæmorrhages.
and no further investigations were made. It is unfortunate in the light of later events that these were omitted as subsequent analyses in the second three cases (those with subperiosteal haemorrhages) showed the phosphatase to be raised coincident with the calcification of the extravasated blood. Whether this increase of the enzyme is only associated with the calcifying blood clot, and is absent in cases of healing scurvy without such haemorrhages is uncertain, but it is hoped that further investigation will clear up this point.

Table 2 shows the changes in the phosphatase values and the correlated radiological appearances in one of these cases.
Table 2.

Analysis in a case of scurvy (V.C.) with subperiosteal haemorrhages.

<table>
<thead>
<tr>
<th>Age mths.</th>
<th>Date</th>
<th>Calcium mgm.</th>
<th>Pho-phorus mgm.</th>
<th>Phosphatase units</th>
<th>Radiological appearances</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>29-9-31</td>
<td>10-0</td>
<td>3-4</td>
<td>0.15</td>
<td>Typical acute scurvy both clinically and radiologically. Large tender swelling above right knee on admission; similar swelling appeared above left knee a few days later. Haemorrhage not visible in radiogram at this date.</td>
</tr>
<tr>
<td>13</td>
<td>10-31</td>
<td>9-3</td>
<td>4-4</td>
<td>0.52</td>
<td>Large calcifying haemorrhages en-sheathing both femora.</td>
</tr>
<tr>
<td>26</td>
<td>10-31</td>
<td>—</td>
<td>5-7</td>
<td>0.4</td>
<td>Haemorrhages more sharply defined.</td>
</tr>
<tr>
<td>9</td>
<td>11-31</td>
<td>—</td>
<td>—</td>
<td>0.43</td>
<td>Lamination visible.</td>
</tr>
<tr>
<td>24</td>
<td>11-31</td>
<td>—</td>
<td>—</td>
<td>0.41</td>
<td>Little difference noted in the size of the calcifying areas.</td>
</tr>
<tr>
<td>13</td>
<td>25-1-32</td>
<td>—</td>
<td>—</td>
<td>0.3</td>
<td>Opaque areas gradually diminishing.</td>
</tr>
<tr>
<td>15</td>
<td>4-4-32</td>
<td>—</td>
<td>—</td>
<td>0.251</td>
<td>Bone contour almost normal.</td>
</tr>
</tbody>
</table>
Plasma phosphatase in fractures.—Additional evidence of the role played by phosphatase in calcification and ossification has been obtained in six cases of fractures. Immediately after the injury the phosphatase was normal, but about 10 days later there was a considerable rise. This increase was maintained for some weeks, the elevation of the phosphatase corresponding to the amount of callus laid down and the length of time elapsing before healing was complete.

Fig. 3. W.C. 26-10-31. Fractured femur. Plasma phosphatase 0·17 units (v. Table 3).
Table 3 illustrates these findings in one of these six cases of fracture.

**TABLE 3.**

**Analyses in a case of fractured femur (occurring Oct. 25th 1931) in a child (W.C.) of six years.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Calcium mgm.</th>
<th>Phosphorus mgm.</th>
<th>Phosphatase units</th>
<th>Radiological appearances</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-10-31</td>
<td>11-4</td>
<td>—</td>
<td>0-17</td>
<td>Oblique fracture mid shaft of femur. No callus visible.</td>
</tr>
<tr>
<td>6-11-31</td>
<td>—</td>
<td>3-5</td>
<td>0-34</td>
<td>Considerable amount of callus present.</td>
</tr>
<tr>
<td>29-11-31</td>
<td>—</td>
<td>—</td>
<td>0-32</td>
<td>Increased callus in skiagrams.</td>
</tr>
<tr>
<td>19-12-31</td>
<td>—</td>
<td>—</td>
<td>0-30</td>
<td>Little change in skiagram.</td>
</tr>
<tr>
<td>23-1-32</td>
<td>—</td>
<td>—</td>
<td>0-30</td>
<td>Still little difference.</td>
</tr>
<tr>
<td>23-2-32</td>
<td>—</td>
<td>—</td>
<td>0-26</td>
<td>Bone resuming normal contour and structure.</td>
</tr>
<tr>
<td>18-4-32</td>
<td>—</td>
<td>—</td>
<td>0-23</td>
<td>Normal; no trace of fracture.</td>
</tr>
</tbody>
</table>
PLASMA PHOSPHATASE

Discussion.

It will be seen that the chemical changes in rickets and scurvy are to some extent contrasting, as might be expected from the other pathological findings in these conditions. In rickets, there is excessive proliferation of cartilage cells, increased vascularity, and the formation of a wide zone of osteoid tissue. The high phosphatase found in rickets is probably derived from these hypertrophic cartilage cells and osteoblasts which Robison has shown to be the source of the enzyme, and its presence in increased quantity supplies one of the factors essential for ossification.

In scurvy, on the other hand, the picture is one of cessation of growth and ossification is in abeyance. The cartilage cells are disorganized and are fewer in number and smaller in size than normal. Hence, in the acute stage, there is neither the source nor the need for the production of phosphatase. This holds good also in cases with subperiosteal haemorrhage before calcification begins. With the administration of antiscorbutic vitamin growth begins again and calcification of any haemorrhage present proceeds; the cartilage cells become active and the plasma phosphatase rises corresponding to a probable increase in the secretion of phosphatase in the bone. The findings in cases of fractures agree with these conclusions.

Conclusions.

1. The average normal plasma phosphatase in infants under one year was found to be 0.23 (range 0.10 to 0.27). In the second year the figures were slightly higher, average 0.24 (range 0.11 to 0.33), while the phosphatase content in older children up to 12 years of age averaged 0.17 (range 0.12 to 0.2).

2. The plasma phosphatase is increased in active rickets, the degree of elevation corresponding to the severity of the disease. The average phosphatase in nine cases of uncomplicated rickets was 0.75, with a range of 0.3 to 1.4. Whereas the blood calcium and phosphorus rapidly returned to normal with treatment, the decline in the phosphatase was much more gradual, and normal values were not regained until the healing process was complete.

3. The phosphatase was found to give low normal figures in the acute stage of scurvy whether subperiosteal haemorrhages were present or not. A rise of the phosphatase appeared coincident with the onset of calcification of the haemorrhages and this fell again with the absorption of these calcified areas.

4. The plasma phosphatase was normal in cases of fracture immediately after the injury, but rose at the same time as the callus appeared and fell to normal when the bone was completely healed.
The above investigations were carried out in the Mond Research Laboratory, and we wish to thank Dr. Eric Pritchard, the Medical Director, and the Staff of the Infants’ Hospital, for the facilities given for this work, and also those elsewhere who have so kindly allowed us their cases for investigation.

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