THE BIOMETRY OF CALCIUM AND INORGANIC PHOSPHORUS IN THE BLOOD PLASMA OF DAIRY CATTLE

APPLICATION OF RESULTS TO BONE MINERALIZATION*

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The problem of the nature of the calcium compounds of blood has long attracted the interest of biochemists, physiologists, and clinicians. The problem has many applications to both normal and pathological conditions.

Rona and Takahashi (1) concluded that calcium occurs in blood serum in two forms only, as calcium bicarbonate which is completely diffusible, and as a calcium-protein compound, the calcium of which is non-diffusible. The possibility of calcium occurring in the blood as calcium phosphate was also considered by Rona and Takahashi but rejected on the following grounds. They pointed out that the only form of calcium phosphate that could exist at the hydrogen ion concentration of blood would be an insoluble one. They reasoned that this compound would therefore have to be present in suspension and would thus be non-diffusible, whereas they found that the inorganic phosphorus of the serum is entirely diffusible.

According to Rona and Takahashi the calcium bicarbonate concentration of blood is expressed by the relation

$$\frac{(Ca^+) \cdot (HCO_3^-)}{(H^+)} = 350 \times 10^{-4}$$

From this it appears that at constant pH the calcium concentration of the blood varies, in part at least, inversely with the bicarbonate concentration.

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The solubility of calcium carbonate in blood has been considered extensively by Hastings, Murray, and Sendroy (2) and by Sendroy and Hastings (3) both from an experimental and from a thermodynamic standpoint. Their conclusion is that blood is not supersaturated with respect to calcium carbonate; it is probably undersaturated. Blood serum, in their studies, showed a stoichiometric solubility product for calcium carbonate of $10^{-6.40}$ compared with $10^{-7.40}$ for calcium carbonate in salt solutions of comparable ionic strength.

The relationship between calcium and protein in blood has been investigated by Salvesen and Linder (4), Marrack and Thacker (5), Loeb (6) (7), Loeb and Nichols (8) (9), Hastings, Murray, and Sendroy (2), and Peters and Eiserson (10). All of these studies support such a relationship. Salvesen and Linder found that decreases in serum calcium in non-uremic cases of Bright's disease without phosphate retention are paralleled by decreases in plasma protein. Marrack and Thacker investigated the dialysis of calcium from solutions of blood proteins and from blood serum and concluded that the calcium of body fluids is partly in the form of non-ionized protein compound, the formation of which accounts for the non-diffusible calcium of serum. Loeb, and Loeb and Nichols have studied the diffusibility of calcium from solutions of egg albumin, from serum globulin and from serum itself from the standpoint of the Donnan equilibrium theory and have concluded that essentially all the calcium is accounted for as ionized and non-ionized calcium-protein compounds. Hastings, Murray, and Sendroy have formulated the the equation, $[\text{Ca}] = 0.014 \times [\text{P}] - 1.4^1$ to express the relation between the total calcium and protein concentration in serum. The equation was based on their own data and the data of Salvesen and Linder. Peters and Eiserson have modified this equation to include the inorganic phosphorus of the blood. Their equation is $\text{Ca} = -0.255 \times \text{P} + 0.556 \times \text{protein} + 7^2$. It is not

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1 In this equation $[\text{Ca}] = \text{mM calcium per kilogram H}_2\text{O}$ and $[\text{P}] = \text{gram protein per kilogram H}_2\text{O}$. The minus sign is an error according to Peters and Eiserson (10).

2 In this equation the Ca and P are milligram per cent and protein is per cent.
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surprising that attempts made by Peters and Eiserson to calculate the calcium of blood by means of this equation led in some instances to errors of great magnitude. Casual inspection shows that the expression they have formulated has no biological significance when the calcium equals 7 mgm. per cent, because at this value the equation resolves itself into $P = 0.218$ protein. This relation is, to say the least, improbable. In fact the alignment chart given by Peters and Eiserson shows no such relation between blood phosphate and blood protein when calcium equals 7 mgm. per cent.

We have applied the Peters and Eiserson formula to several samples of normal cattle plasma with the results shown in table 1. The data show that the formula has no value for cattle blood.

<table>
<thead>
<tr>
<th>COW</th>
<th>CALCIUM FOUND</th>
<th>CALCIUM CALCULATED</th>
<th>DEVIATION OF CALCULATED FROM ACTUAL VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
</tr>
<tr>
<td>66</td>
<td>7.70</td>
<td>8.99</td>
<td>+1.29</td>
</tr>
<tr>
<td>82</td>
<td>9.35</td>
<td>9.40</td>
<td>+0.05</td>
</tr>
<tr>
<td>E33</td>
<td>8.09</td>
<td>9.56</td>
<td>+1.47</td>
</tr>
<tr>
<td>87</td>
<td>9.60</td>
<td>9.41</td>
<td>-0.19</td>
</tr>
<tr>
<td>60</td>
<td>7.70</td>
<td>10.14</td>
<td>+2.42</td>
</tr>
<tr>
<td>21</td>
<td>10.23</td>
<td>9.95</td>
<td>+0.28</td>
</tr>
</tbody>
</table>

The samples were picked because they showed considerable range of calcium.

Although, as already stated, Rona and Takahashi rejected the probability of calcium existing in the blood as phosphate, there is indirect evidence which is generally accepted as indicating a close relationship between calcium and inorganic phosphate in the blood. Binger (11), and Tisdall (12) showed that the injection of phosphates reduces blood calcium and causes tetany. It should be pointed out, however, that although the results of Binger were attributed to the effect of the phosphate ion on blood calcium, Tisdall's results were interpreted as due to a changed sodium-calcium ratio. Disodium phosphate was injected in
Tisdall's experiments. In addition to this indirect evidence there exist the extensive thermodynamic studies of Holt, La Mer, and Chown (13), Holt (14), and Sendroy and Hastings (3) (15), on the solubility of calcium phosphates in biological fluids. The former group of investigators conclude that, "serum is normally supersaturated with tertiary calcium phosphate to the extent of more than 200 per cent." The experiments of the latter group of workers also support a condition of supersaturation of tri-calcium phosphate in blood. On the other hand, Shear and Kramer (16), and Shear, Washburn, and Kramer (17) hold that the calcium phosphate of serum is di-calcium phosphate and that normal serum is either very slightly undersaturated or just saturated with respect to this compound.

The occurrence of calcium phosphate in blood in the form of CaHPO$_4$ or Ca$_3$(PO$_4$)$_2$ is generally regarded as necessary in order to account for the mineralization of bone. Even though compounds of this character account for only a portion of the total calcium of the blood, it would appear probable that a large part of the inorganic phosphate would be involved. In this case definite biometric relations should be found to exist between the calcium and inorganic phosphate of the plasma or serum.

The biometry of calcium and inorganic phosphorus in the blood serum of rabbits has been studied by Harnes (18) (19). In a series of 80 samples, representing 80 animals received at the laboratory in groups of 10 during 8 consecutive months from October to May, the coefficient of correlation of P·Ca was

$$ r_{P\cdot Ca} = -0.146 \pm 0.073 $$

In a second series of 170 samples of the blood from 10 rabbits taken at weekly intervals for 17 weeks from October to May, the animals being kept under laboratory conditions, the coefficient of correlation of P·Ca was

$$ r_{P\cdot Ca} = -0.124 \pm 0.051 $$

In neither series is the result mathematically significant, inasmuch as the correlation in each case is less than three times the probable error. It is of interest to note, however, that the corre-
lation is a negative one, thereby lending some support to the fact that there is frequently a tendency for the inorganic phosphate to vary inversely with the calcium in blood serum or plasma.

EXPERIMENTAL

The normal variations in the calcium and inorganic phosphate of the blood plasma of dairy cattle, secured at this laboratory, have

**TABLE 2**

*Mean, standard deviation, and coefficient of variation of calcium and inorganic phosphate in blood plasma of dairy cattle*

<table>
<thead>
<tr>
<th>Subscript</th>
<th>Mean Value</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca₁</td>
<td>13.25</td>
<td>1.81</td>
<td>13.67</td>
<td>60</td>
</tr>
<tr>
<td>Ca₂</td>
<td>12.56</td>
<td>1.46</td>
<td>11.61</td>
<td>60</td>
</tr>
<tr>
<td>Ca₃</td>
<td>12.76</td>
<td>1.71</td>
<td>13.44</td>
<td>60</td>
</tr>
<tr>
<td>Ca₄</td>
<td>12.86</td>
<td>1.69</td>
<td>13.17</td>
<td>180</td>
</tr>
<tr>
<td>P₁</td>
<td>4.94</td>
<td>2.85</td>
<td>57.77</td>
<td>60</td>
</tr>
<tr>
<td>P₂</td>
<td>4.75</td>
<td>2.52</td>
<td>53.09</td>
<td>60</td>
</tr>
<tr>
<td>P₃</td>
<td>4.85</td>
<td>2.72</td>
<td>55.98</td>
<td>60</td>
</tr>
<tr>
<td>P</td>
<td>4.85</td>
<td>2.70</td>
<td>55.74</td>
<td>180</td>
</tr>
</tbody>
</table>

1 The subscripts refer to the day in the three-day series.

**TABLE 3**

*Coefficient of correlation and probable error of calcium and inorganic phosphate in blood plasma of dairy cattle*

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Coefficient of Correlation</th>
<th>Probable Error</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>rCa₁Ca₂</td>
<td>+0.762</td>
<td>±0.036</td>
<td>60</td>
</tr>
<tr>
<td>rCa₂Ca₃</td>
<td>+0.625</td>
<td>±0.053</td>
<td>60</td>
</tr>
<tr>
<td>rCa₃Ca₄</td>
<td>+0.628</td>
<td>±0.053</td>
<td>60</td>
</tr>
<tr>
<td>rCa₄Ca₅</td>
<td>+0.628</td>
<td>±0.031</td>
<td>180</td>
</tr>
<tr>
<td>rP₁P₂</td>
<td>+0.970</td>
<td>±0.005</td>
<td>60</td>
</tr>
<tr>
<td>rP₂P₃</td>
<td>+0.954</td>
<td>±0.008</td>
<td>60</td>
</tr>
<tr>
<td>rP₃P₄</td>
<td>+0.931</td>
<td>±0.011</td>
<td>60</td>
</tr>
<tr>
<td>rP₄P₅</td>
<td>+0.946</td>
<td>±0.005</td>
<td>180</td>
</tr>
</tbody>
</table>

recently been published (20) (21). The data were secured from samples of blood taken on 3 consecutive days at monthly intervals. Twenty-four different animals furnished the blood. Five of these had only 1 period of 3 consecutive days, 10 animals had 2 periods,
1 animal had 3 periods, and 8 animals had 4 periods. There were, therefore, 60 sets of three-day samples.

The previous papers give the complete data for the calcium and inorganic phosphate for the consecutive days. The mean values, standard deviations, and coefficients of variation are given in table 2. The coefficients of correlation, calculated by the method of Harris (22), and their probable errors are given in table 3.

The original data also presented an opportunity to study the coefficients of correlation of the calcium and inorganic phosphate, and thus obtain further light on the probable importance of calcium phosphate in the blood. The results of these calculations are as follows:

\[
\begin{align*}
  r_{\text{Ca}P_{1}} & = +0.116 \pm 0.086 \ (n = 60) \\
  r_{\text{Ca}P_{2}} & = -0.109 \pm 0.086 \ (n = 60) \\
  r_{\text{Ca}P_{3}} & = +0.008 \pm 0.087 \ (n = 60) \\
  r_{\text{Ca}P} & = +0.022 \pm 0.050 \ (n = 180)
\end{align*}
\]

These results show even less correlation between calcium and inorganic phosphate in the blood plasma of dairy cattle than was found for rabbits by Harnes (18) (19). In the rabbit blood the coefficients did approach but did not reach mathematical significance, i.e., three times the probable error. In our calculations the summation correlation of Ca·P shows a probable error of nearly two and one-half times the correlation. The only conclusion that can be drawn from such a result is that the amount of calcium phosphate in the blood, at least of dairy cattle, is truly insignificant in relation to the other compounds of calcium and inorganic phosphate. We thus have mathematical proof for the conclusion of Rona and Takehashi (1) and of Loeb (6) (7), and Loeb and Nichols (8) (9) in so far as the occurrence of compounds of calcium and phosphate in the blood are concerned. It thus seems necessary to seek for an explanation of the mineralization of bone on other biochemical grounds than a mere precipitation of calcium phosphate from body fluid. Freudenberg and György (23) have proposed a theory which appears to deserve more recognition that it has received. According to this theory
calcification of bone consists of the following stages: (a) A calcium-protein compound is formed between the collagen of the cartilage and the diffusible calcium ions from the blood; (b) HPO₄²⁻ and HCO₃⁻ ions from the blood react with the calcium bound by the collagen; (c) Ca₃(PO₄)₂ and CaCO₃ split off, releasing the calcium binding groups of the protein, so that the process may be repeated.

SUMMARY AND CONCLUSIONS

A high coefficient of correlation exists between the phosphate of the blood plasma of dairy cattle on successive days.

A fairly high coefficient of correlation exists between the calcium of the blood plasma of dairy cattle on successive days.

No correlation whatever exists between the calcium and inorganic phosphate in the blood plasma of dairy cattle.

No biologically significant amount of calcium phosphate occurs in the blood.

The mineralization of bone is not explainable on the basis of a mere precipitation of bone salts from body fluid.

REFERENCES


(11) Binger, C. 1917 Toxicity of phosphates in relation to blood calcium and tetany. J. Pharmacol., x, 105-120.


(19) Harnes, A. R. 1929 Biometry of calcium, inorganic phosphorus, cholesterol, and lipid phosphorus in the blood of rabbits. II. Repeated observations on normal animals. J. Exp. Med., xlix, 287-301.


