A Study of Inorganic Phosphorus Release Accompanying Glycolysis Of Blood In Cancer
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When blood undergoes glycolysis at 37° C, in addition to the loss of blood sugar, there are accompanying changes in the inorganic phosphorus. This relationship has been investigated and reported by a number of authors (2, 8, 9, 10). They agree that during the first 4 hours of glycolysis, the inorganic phosphorus of the blood in normal individuals either remains at a constant level, or is only slightly diminished. After 6 to 8 hours when glycolysis is practically complete, there is a progressive rise in the inorganic phosphorus and after the 15th hour, the concentration may reach as high as 252 mgm. per cent (6).

The inorganic phosphorus of normal blood keeps at a fairly constant level, and is elevated only in conditions such as uremia or when the calcium is greatly diminished.

Increase in the inorganic phosphorus during glycolysis is at the expense of organic soluble phosphorus of the blood cells (ester phosphorus). The explanation for the liberation of the inorganic phosphorus under these conditions, offered by Engelhardt and Braunstein (3), Roche and Roche (7), and generally concurred in by most investigators, is that blood glycolysis is accompanied by a constant synthesis and hydrolysis of organic phosphate. These reactions, namely, synthesis of hexose-phosphoric acid esters as a first step in glycolysis, and secondly the hydrolysis of these esters are interrelated, although the hydrolysis may proceed independently of the synthesis. When hydrolysis exceeds synthesis inorganic phosphorus is liberated.

Glycolysis can be inhibited by the introduction of sodium fluoride as an anti-coagulant. When blood taken in fluoride was incubated for 6 hours at 37° C, a release of inorganic phosphorus could be demonstrated, in spite of the fact that no glycolysis had occurred.

The results of the determinations of glucose and inorganic phosphorus made before incubation and at 6 hours and 24 hours after incubation using fluoride and heparin as anti-coagulants, are given in Table I. A comparison of the inorganic phosphorus values at the end of the 24 hours incubation period shows a greater release of inorganic phosphorus in those blood specimens subjected to glycolysis.

Inhibition of glycolysis can also be induced by inactivation of heparinized blood at 57° C, for 30 minutes. Accompanying this inactivation there is also a release of inorganic phosphorus. In a series of 7 cases the increase ranged from 0.7 to 3.7 mgm. per cent. This is apparently the release of a heat labile phosphorus fraction.

At the end of the inactivation period, the blood samples were placed in an incubator at 37° C, for 6 hours. Glucose values showed no change during this period, but there was a further increase in the inorganic phosphorus of from 1.0 to 4.4 mgm. per cent. It is apparent that at least two different enzymatic processes are involved in the release of inorganic phosphorus. One, in which glycolysis plays the major role, and another which proceeds independently of glycolysis.

During a study of the role of glycolysis of the blood in cancer (to be published), observations were made at the same time on the release of inorganic phosphorus. As far as we are aware, no investigations of the rate of hydrolysis of phosphate esters during glycolysis in cancerous individuals have been reported. In view of this, we have made a study of the inorganic phosphorus during glycolysis in 196 individuals of whom 64 were cancerous and 132 were noncancerous.

PROCEDURE

Blood was obtained by veni-puncture after an over-night fast. A solution of heparin1 (Lederle, 100 mgm. per 1 ml.) was used as an anti-coagulant in the amount of 0.1 ml. per 10 ml. of blood. Immediately after withdrawal of the blood, the inorganic phosphorus was determined by the method of Fiske and Subbarow (5) with a Klett-Summerson colorimeter. The remaining blood was kept in a closed container and placed in an incubator at 37° C, for a period of 6 hours. This time period was chosen because glycolysis was then practically

* Under a grant from the Kolb Fund.
completed. Inorganic phosphorus was again determined on this incubated specimen. The glucose was determined by the method of Benedict (1).

A loss of 70 per cent or more of blood sugar from the initial blood sugar value, in 4 hours incubation at 37°C, was considered to be an accelerated glycolytic rate.

Normal controls.—In 25 normal individuals, the increase in inorganic phosphorus varied between —2.1 and +3.2 mgm. per cent, after 6 hours' incubation, with a mean value of 0.93 mgm. per cent.

Cancer cases.—In 64 individuals suffering from some form of neoplastic malignant disease, the increment of inorganic phosphorus after 6 hours of glycolysis varied between —1.8 and +5.6 mgm. per cent, with a mean value of 2.22 mgm. per cent.

Non-cancer cases.—In 107 patients free from neoplastic disease, the inorganic phosphorus increment varied between —1.3 and 6.4 mgm. per cent, with a mean value of 1.53 mgm. per cent.

Table I: Effect of Glycolysis on Inorganic Phosphorus

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Before incubation</th>
<th>6 hours after incubation</th>
<th>24 hours after incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>H* F*</td>
<td>H F</td>
<td>H F</td>
<td>H F</td>
</tr>
<tr>
<td>1</td>
<td>93 86</td>
<td>2.6 2.8</td>
<td>5 89</td>
</tr>
<tr>
<td>2</td>
<td>91 91</td>
<td>3.1 3.0</td>
<td>27 89</td>
</tr>
<tr>
<td>3</td>
<td>83 84</td>
<td>4.5 4.5</td>
<td>4 83</td>
</tr>
<tr>
<td>4</td>
<td>98 99</td>
<td>4.2 4.0</td>
<td>4 85</td>
</tr>
<tr>
<td>5</td>
<td>80 79</td>
<td>4.7 4.2</td>
<td>19 80</td>
</tr>
</tbody>
</table>

* H—Heparin, F—Fluoride, (all values given in mgm. per cent)

Table II: Changes in Inorganic Phosphorus of the Blood Accompanying Glycolysis in Normal and Pregnant Individuals, and in Patients with Nonmalignant Diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
<th>Cases showing an increase greater than 1.53 mgm./%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>25</td>
<td>cases under 70%</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>4</td>
<td>cases over 70%</td>
</tr>
<tr>
<td>G. I. ulcers</td>
<td>13</td>
<td>cases under 70%</td>
</tr>
<tr>
<td>Liver diseases</td>
<td>7</td>
<td>cases over 70%</td>
</tr>
<tr>
<td>Hypertension and cardio-vascular disease</td>
<td>15</td>
<td>cases under 70%</td>
</tr>
<tr>
<td>Thyroid disturbances</td>
<td>5</td>
<td>cases over 70%</td>
</tr>
<tr>
<td>Benign growths</td>
<td>19</td>
<td>cases under 70%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6</td>
<td>cases over 70%</td>
</tr>
<tr>
<td>Bacterial disease, acute</td>
<td>16</td>
<td>cases under 70%</td>
</tr>
<tr>
<td>Bacterial disease, chronic</td>
<td>13</td>
<td>cases over 70%</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>9</td>
<td>cases under 70%</td>
</tr>
<tr>
<td>Totals</td>
<td>132</td>
<td>cases over 70%</td>
</tr>
</tbody>
</table>

some form of neoplastic malignant disease, the inorganic phosphorus increased after 6 hours of glycolysis varied between —1.8 and +5.6 mgm. per cent, with a mean value of 2.22 mgm. per cent.

If we consider all of the 196 cases reported regardless of diagnosis from the standpoint of the mean value of 1.53 mgm. per cent or more, in the inorganic phosphorus, we find that 96 showed the increase, while 101 did not. A breakdown of the data into cancerous and noncancerous groups, shows that in 46 of 64 cancer patients (72 per cent), there was an increase of inorganic phosphorus greater than 1.53 mgm. per cent, while of the 132 noncancerous patients studied, only 50 or (38 per cent), showed a similar increase.

Table II records the results of our observations on 132 individuals who were free from neoplastic malignant disease. The glycolytic rate is shown concomitantly with the increase in inorganic phosphorus. If we consider the release of inorganic phosphorus in conjunction with the glycolytic rate, we find that in 47 of 49 cases showing a rise greater than 1.53 mgm. per cent, there was also an acceleration of the glycolytic rate of 70 per cent or more. In 29 cases in which there was also an acceleration of the glycolytic rate, the phosphorus release was under 1.53 mgm. per cent. In the 54 instances in
which the glycolytic rate was under 70 per cent, the phosphorus release was less than 1.53 mgm. per cent.

The data given in Table III shows the results in 64 individuals with neoplastic malignant disease. Of these, in 46 cases in which a phosphorus release greater than 1.53 mgm. per cent was observed, 15 did not have an accelerated glycolytic rate. Only 1 patient with a glycolytic rate of over 70 per cent, showed a phosphorus release lower than 1.53 mgm. per cent. In the other 17 cases in which the glycolytic rate was under 70 per cent, the phosphorus release was less than 1.53 mgm. per cent.

CONCLUSIONS

It has been shown that phosphorus release in the blood may occur with or without glycolysis; however glycolysis seems to influence the rate of liberation.

Liberation of inorganic phosphorus from the blood, (after glycolysis is complete), has been studied in 196 individuals, of whom 64 were suffering from some form of malignant neoplasm. The blood of those patients having a malignant tumor shows a greater tendency towards an increased liberation of inorganic phosphorus. The increase is not entirely due to glycolysis.

A comparison of the mean value for the inorganic phosphorus increase of the blood, between patients with malignant and nonmalignant conditions, is statistically significant.

REFERENCES

NOTICE

The Index to Literature of Experimental Cancer Research, 1900 to 1935, prepared by the Donner Foundation, is now available. It is a bound volume of over 1000 pages. The Donner Foundation has generously distributed copies to the principal medical libraries of the United States and Canada, and to some European libraries. Individuals wishing a copy for their personal use may obtain one by addressing the Donner Foundation, 2136 Land Title Building, Philadelphia 10, Pa. The price is $10, which is less than the cost of publication.

ERRATUM

In the paper by Bernhard, Rosenbloom and Eichen, entitled "A Study of Inorganic Phosphorus Release Accompanying Glycolysis of Blood in Cancer," Vol. 8, No. 6, p. 295, in the last line of the first paragraph, "252 mgm. per cent" should read "25 mgm. per cent."
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