THE EFFECT OF INSULIN AND OF PANCREATECTOMY ON THE DISTRIBUTION OF PHOSPHORUS AND POTASSIUM IN THE BLOOD.

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INTRODUCTION.

The simultaneous fall in the concentration of glucose and of inorganic phosphate in the blood after the administration of insulin, together with the discovery by Embden and his coworkers (1-3) of hexose phosphoric esters in striated muscle, has led a number of investigators (4, 5) to suggest that the function of insulin may be to cause a combination of the glucose and phosphoric acid to form an ester similar to or identical with Embden's lactacidogen.

The organic acid-soluble phosphorus compounds which are present in blood corpuscles in considerable quantity have been shown by Kay and Robison (6) to consist of at least two phosphoric esters, one of which is readily hydrolyzed by an enzyme present in ossifying cartilage. The possibility that one of these compounds might be a hexose phosphoric ester, possibly the lactacidogen of muscle, was tested by Kay and Robison (6). They attempted to observe whether any hydrolysis of the esters occurred in the presence of muscle enzymes, which are known to hydrolyze at least one of the phosphoric acid groups of the diphosphoric ester. The results were inconclusive, and failed to prove the presence of the hexose diphosphoric ester in normal blood. Other experiments however, on the effect of insulin on the distribution of phosphorus compounds in blood, led them to conclude that insulin causes the synthesis of esters of phosphoric acid in the red blood corpuscles from the glucose and inorganic phosphate of the blood. The possibility that the esters thus synthesized may be identical with one of the hexose phosphates found in muscle by Embden makes further study of this problem desirable. Experiments conducted in this laboratory have failed however to confirm the findings of Kay and Robison. In view of the importance of their conclusions, the results of our experiments are presented.

The work of other investigators bearing indirectly on this subject should first be mentioned. Goodwin and Robison (7) isolated from blood a phos-
Phosphorus and Potassium in Blood

Phosphoric ester which had reducing properties, was optically active, and was hydrolyzed by the bone phosphatase. They did not state whether or not it may be hydrolyzed by muscle enzymes. Greenwald (8) isolated from blood a non-reducing organic phosphorus compound, possibly a salt of the diphosphoric ester of l-glyceric acid. Jost (9) found that the greater part of the organic acid-soluble phosphorus of the corpuscles is in the form of the diphosphoric ester of glyceric acid. Cuthbertson (10) found a decrease in the organic acid-soluble phosphorus of whole blood during fatigue, but failed to record the proportion of red corpuscles, which contain nearly all the organic acid-soluble phosphorus. His findings therefore may have been due merely to a fall in hematocrit. Hynd (11) found that one-sixth of the sugar disappearing from the blood after insulin may be accounted for by conversion to a non-reducing complex stored in the corpuscles, which on hydrolysis again yields reducing substances. Kay and Robison, as already stated, had reported an increase in the organic acid-soluble phosphorus of blood corpuscles after insulin administration. Calculated on the basis of 1 molecule of phosphoric acid to each molecule of glucose, the increase of organic phosphorus accounted for about 40 per cent of the sugar which disappeared from the blood. In view of the important rôle attributed to the corpuscles by their conclusions, it seemed worth while to reinvestigate this question.

The experiments reported in this paper were therefore performed with the purpose of determining whether or not the organic phosphorus of the red blood corpuscles is affected by insulin. The experiments divide themselves into two groups: (1) the effect of insulin on the distribution of phosphorus in the blood of normal dogs; and (2) the changes in the phosphorus distribution caused by removal of the pancreas. If the phosphoric esters of the corpuscles are synthesized by the action of insulin, then the complete withdrawal of insulin from the body might be expected to cause a decrease in these esters.

The concentration of total acid-soluble phosphorus and inorganic and lipid phosphorus in both serum and corpuscles is reported. The distribution of potassium between serum and corpuscles was also investigated, since potassium is known to disappear from the serum simultaneously with inorganic phosphorus and glucose (12, 4).

EXPERIMENTAL.

Dogs were used for the experiments rather than rabbits so that at least two fairly large blood specimens might be taken from each animal. Thus the use of control animals was avoided. Blood
samples were taken by heart puncture after injecting cocaine subcutaneously at the site to be punctured, with the result that pain and struggling were eliminated. The blood was aspirated from the heart into a cylindrical separatory funnel, and there defibrinated by gentle stirring with a glass rod. In two experiments (Nos. 5- and 6-Ins.) lithium oxalate was used to prevent clotting. The use of anticoagulants was avoided because of possible effects on the phosphorus distribution and interference with the potassium determinations. To remove any shreds of fibrin which failed to cling to the glass rod, the blood was filtered through acid-washed gauze directly into 50 cc. centrifuge tubes, with a minimum of disturbance, avoiding excessive loss of CO₂. Samples of whole blood were immediately taken for determinations of water, hematocrit, lipid phosphorus, and for the preparation of the trichloroacetic acid and tungstic acid filtrates. The rest of the blood was centrifuged at 4000 R.P.M. for 25 minutes. At the end of this period the column of corpuscles had the appearance of laked blood, indicating complete separation of serum from the corpuscles (13). Hematocrit tubes (10 cm. in length) were centrifuged in duplicate together with the blood. The serum was next aspirated as completely as possible into another vessel, and the corpuscles freed from the remaining traces of serum by wiping with strips of filter paper. There was no noticeable hemolysis except for the one case which is noted in the tables.

The serum and corpuscles were each analyzed directly for total acid-soluble phosphorus and potassium. Whole blood and serum were analyzed for inorganic phosphorus, lipid phosphorus, and glucose, the corpuscle composition being calculated from these after determination of the percentage of corpuscles in the whole blood. Water determinations were made on whole blood, serum, and corpuscles.

Since it is difficult to measure accurately samples of corpuscles by volume, it was considered best to weigh the samples of corpuscles, and those of whole blood and serum as well, in order to report all results in terms of mg. per 100 gm. of material rather than per 100 cc. Hence in preparing the tungstic acid, trichloroacetic acid, and lipid filtrates, the samples were first weighed in the volumetric flasks in which the precipitation of protein was to be made.
Phosphorus and Potassium in Blood

Analytical Methods.

Water was determined in whole blood, serum, and corpuscles by drying 1 cc. portions at 110° for 24 hours. The samples were measured quickly into small weighing bottles containing cones of fluted filter paper, the bottles and paper having been dried for 24 hours, cooled, and weighed just previous to use.

The percentage of corpuscles by weight rather than volume is required for calculating the composition of corpuscles from analyses of whole blood and serum, if results are to be expressed in mg. per 100 gm. This value may be calculated accurately by means of the following formula, as explained in a previous publication (14).

\[
\text{Percentage corpuscles} = \frac{100 \times (H_{2}O_{s} - H_{2}O_{wb})}{H_{2}O_{s} - H_{2}O_{c}}
\]

Wherever corpuscle composition was calculated from the analysis of whole blood and serum, the following formula was used:

\[
X_{c} = X_{s} - \left[ \frac{100 \times (X_{s} - X_{wb})}{\text{Per cent}} \right]
\]

where \(X\) represents the number of mg. per 100 gm. of the substance in question. The percentages of inorganic phosphorus, lipid phosphorus, and glucose in corpuscles were calculated in this way.

Blood sugar was determined by the method of Benedict (15). Inorganic, total acid-soluble, and lipid phosphorus were determined by the method of Fiske and Subbarow (16). The precipitation of blood proteins and the determination of inorganic phosphorus were carried out without delay in order to avoid changes in the inorganic phosphorus. The loss of some CO₂ during defibrination undoubtedly renders the blood slightly more alkaline, causing the conversion of a very small amount of inorganic phosphorus into an organic ester (17). The values for organic phosphorus were obtained by subtracting inorganic from the total acid-soluble phosphorus. The corpuscle filtrate was prepared by weighing accurately about 13 gm. of corpuscles in a 200 cc. volumetric flask, taking with water, precipitating the proteins by addition of sufficient 20 per cent trichloroacetic acid to bring the
final concentration up to 8 per cent, and diluting to the mark with water after bubbles ceased to form. Hydrolysis of any of the organic phosphorus during laking introduces no error, since inorganic phosphate is not determined in the corpuscle filtrate, but calculated from whole blood and serum analyses.

Potassium was determined by the method of Kramer and Tisdall (18) with the modifications described by Kerr (14). By use of stronger permanganate and oxalate in the titration of the potassium cobaltit-nitrite, the method may be given greater flexibility and permit the determination of potassium over a wider range of concentration than in the original method. The following procedure gave good results.

To the washed precipitate of potassium cobaltit-nitrite in the centrifuge tube are added 2 cc. of 0.03 N permanganate and 1 cc. of 4 N sulfuric acid. The tube is placed in boiling water for 1 minute, the precipitate being stirred with a fine glass rod. 2 cc. of 0.025 N sodium oxalate are introduced with a pipette, the contents of the tube stirred until the permanganate is decolorized, and the tube reheated for about 15 seconds. The excess of oxalate is then titrated with 0.03 N permanganate from a micro burette.

The determination of potassium in blood filtrates was accompanied in every case by a control determination of the potassium in an artificial solution of blood salts of known composition.

All analyses for inorganic, total acid-soluble, and lipid phosphorus, potassium, and blood sugar were done in duplicate or triplicate.

In the second group of experiments, 24 hour urine specimens were collected, preserved with toluene, and analyzed for glucose and total nitrogen in order to calculate the dextrose-nitrogen ratios.

Serum proteins were calculated from the refractive indices by the method of Neuhausen and Rioch (19).

Experiments on Overdosage with Insulin.

In order to determine the effects of overdosage with insulin on normal dogs, the normal blood was taken after 18 to 24 hours fasting. Insulin was then injected subcutaneously. The second blood specimen was taken during the period of depression, gener-
**TABLE I.**

*Experimental Details, with Data on Hematocrit and Blood Dilution.*

Ins. denotes experiments on the effect of overdosage with insulin on normal dogs; DP., depancreatized dogs.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Weight of dog</th>
<th>Time (a.m.)</th>
<th>Insulin injected (units)</th>
<th>Blood taken (cc.)</th>
<th>Specimen</th>
<th>H₂O in whole blood (per cent)</th>
<th>H₂O in serum (per cent)</th>
<th>H₂O in corpuscles (per cent)</th>
<th>Corpuscles by weight (per cent)</th>
<th>Corpuscles by volume (per cent)</th>
<th>Serum proteins (g.)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Ins. 18.0</td>
<td>8</td>
<td>50</td>
<td>60</td>
<td>Normal.</td>
<td>77.66</td>
<td>90.20</td>
<td>50.14</td>
<td>47.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>60</td>
<td></td>
<td>Normal. After insulin.</td>
<td>78.25</td>
<td>90.77</td>
<td>47.8</td>
<td>47.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Ins. 12.5</td>
<td>8</td>
<td>50</td>
<td>65</td>
<td>Normal.</td>
<td>80.77</td>
<td>89.64</td>
<td>38.1</td>
<td>35.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>75</td>
<td></td>
<td>Normal. After insulin.</td>
<td>82.60</td>
<td>90.42</td>
<td>32.3</td>
<td>30.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Ins. 15</td>
<td>7.10</td>
<td>80</td>
<td>80</td>
<td>Normal.</td>
<td>81.70</td>
<td>92.32</td>
<td>39.9</td>
<td>39.2</td>
<td>7.5</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>9.45</td>
<td>80</td>
<td></td>
<td>Normal. After insulin.</td>
<td>80.98</td>
<td>92.28</td>
<td>43.6</td>
<td>41.9</td>
<td>7.5</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>8-Ins. 15</td>
<td>7.25</td>
<td>80</td>
<td>80</td>
<td>Normal.</td>
<td>81.33</td>
<td>91.72</td>
<td>41.1</td>
<td>38.8</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>9.40</td>
<td>80</td>
<td></td>
<td>Normal. After insulin.</td>
<td>81.82</td>
<td>91.98</td>
<td>40.9</td>
<td>38.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-Ins. 19.5</td>
<td>9</td>
<td>80</td>
<td>90</td>
<td>Normal.</td>
<td>80.97</td>
<td>92.18</td>
<td>43.3</td>
<td>42.0</td>
<td>7.4</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>12.30</td>
<td>90</td>
<td></td>
<td>Normal. After insulin.</td>
<td>81.55</td>
<td>92.44</td>
<td>42.4</td>
<td>40.0</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lactating female, fasted 20 hrs. No signs of depression at 11 a.m. Lithium oxalate used as anticoagulant.

Fasted 18 hrs. No signs of depression at 11 a.m. Lithium oxalate used as anticoagulant.

Fasted 19 hrs. Depressed and unable to stand at 9.30 a.m. Defibrinated blood.

Depressed and unable to stand at 9.40 a.m. Defibrinated blood.

Fasted 22 hrs. Depressed at 11 a.m. Defibrinated blood. Some hemolysis in second sample.
<table>
<thead>
<tr>
<th></th>
<th>11 Ins.</th>
<th>17-DP.</th>
<th>19-DP.</th>
<th>22-DP.</th>
<th>24-DP.</th>
<th>26-DP.</th>
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<tr>
<td></td>
<td>11</td>
<td>July 18</td>
<td>Aug. 4</td>
<td>July 19</td>
<td>July 26</td>
<td>Aug. 11</td>
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<tr>
<td>10 Ins.</td>
<td>6.5</td>
<td>19.5</td>
<td>15.5</td>
<td>8.0</td>
<td>7.0</td>
<td>6.3</td>
</tr>
<tr>
<td>11</td>
<td>7.25</td>
<td>July 18</td>
<td>80</td>
<td>July 19</td>
<td>July 26</td>
<td></td>
</tr>
<tr>
<td>11.40</td>
<td>70</td>
<td>80</td>
<td>Normal.</td>
<td>90</td>
<td>90</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>Normal.</td>
<td>80</td>
<td>Normal.</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>110</td>
<td>70</td>
<td>Normal.</td>
<td>80</td>
<td>Normal.</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>After insulin.</td>
<td>110</td>
<td>After insulin.</td>
<td>70</td>
<td>After insulin.</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>65.84</td>
<td>65.18</td>
<td>75</td>
<td>80</td>
<td>65.5</td>
<td>70</td>
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<tr>
<td></td>
<td>47.0</td>
<td>43.5</td>
<td>75</td>
<td>36.2</td>
<td>53.5</td>
<td>36.4</td>
</tr>
<tr>
<td></td>
<td>45.4</td>
<td>42.1</td>
<td>75</td>
<td>36.6</td>
<td>34.7</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>9.2</td>
<td>8.7</td>
<td>75</td>
<td>6.1</td>
<td>7.2</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>depessed at 11, muscles twitching at</td>
<td>onset of convulsions, 3 hrs. after the</td>
<td>onset of convulsions, 1½ hrs. after the</td>
<td>and emaciated. Died after taking the</td>
<td>taken during period of depression after</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.30. Defibrinated blood.</td>
<td>insulin.</td>
<td>insulin.</td>
<td>blood specimen.</td>
<td>insulin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tbody>
</table>

S. E. Kerr
<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Specimen</th>
<th>Whole blood</th>
<th>Serum</th>
<th>Corpuscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Ins.</td>
<td>Normal</td>
<td>50.1</td>
<td>88.4</td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>47.8</td>
<td>34.5</td>
<td>16.2</td>
</tr>
<tr>
<td>6-Ins.</td>
<td>Normal</td>
<td>38.1</td>
<td>13.6</td>
<td>4.41†</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>32.2</td>
<td>12.8</td>
<td>2.44†</td>
</tr>
<tr>
<td>7-Ins.</td>
<td>Normal</td>
<td>39.9</td>
<td>86.6</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>43.8</td>
<td>42.7</td>
<td>2.44†</td>
</tr>
<tr>
<td>8-Ins.</td>
<td>Normal</td>
<td>41.1</td>
<td>87.9</td>
<td>5.24</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>40.2</td>
<td>51.3</td>
<td>1.26</td>
</tr>
<tr>
<td>9-Ins.</td>
<td>Normal</td>
<td>43.3</td>
<td>87.6</td>
<td>5.37</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>42.4</td>
<td>51.3</td>
<td>2.28</td>
</tr>
<tr>
<td>10-Ins.</td>
<td>Normal</td>
<td>47.0</td>
<td>76.8</td>
<td>3.96</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>42.1</td>
<td>41.8</td>
<td>1.67</td>
</tr>
<tr>
<td>Averages</td>
<td>Normal</td>
<td>43.3</td>
<td>85.5</td>
<td>5.09</td>
</tr>
<tr>
<td></td>
<td>After insulin</td>
<td>41.4</td>
<td>44.3</td>
<td>1.92</td>
</tr>
</tbody>
</table>

* Determined in corpuscle filtrate.
† Not included in the averages.
ally 2 to 3 hours after the insulin injection, before the onset of convulsions. Further experimental details are given in Table I. In this table are also found the values for water content of the whole blood, serum, and corpuscles, the volume hematocrit, the percentage of serum proteins, and the percentage of corpuscles by weight, as calculated from the water determinations. The volume hematocrits were included for the sake of a rough check on the weight hematocrits.

The changes in phosphorus and potassium distribution are found in Table II, which presents the results of the analyses of whole blood, serum, and corpuscles. The values for the corpuscle content of glucose, inorganic phosphorus, and lipid phosphorus were calculated from the figures for whole blood and serum, as already explained.

The results in Table II show the simultaneous fall in the glucose, inorganic phosphate, and potassium of serum, already reported by a number of investigators (4, 12). The inorganic phosphate of serum decreases greatly after insulin, the total acid-soluble phosphorus falling simultaneously, since it is composed almost entirely of inorganic phosphate. The small amount of organic phosphorus which is found in the serum increases as a result of the insulin in all cases except Experiment 8-Ins. The quantities present are however so small that these results are of doubtful significance.

The greater part of the total acid-soluble phosphorus of blood is the organic phosphorus of the corpuscles, which forms the subject of chief interest in this investigation. The results show practically no change in the total acid-soluble phosphorus in two cases, but a decrease in four cases. When the inorganic phosphorus is subtracted, the organic phosphorus is found to remain practically unaffected by insulin, the average of the six experiments showing a very small decrease. As a check on these direct determinations of total acid-soluble phosphorus in the corpuscle filtrates, determinations were also made on whole blood in four of the six experiments, the corpuscle composition being calculated from the whole blood and serum values. The results agreed within 1 per cent of those obtained by direct analysis. There can be no doubt therefore that insulin has no effect on the total amount of organic phosphorus in the corpuscles of the dog.
Phosphorus and Potassium in Blood

After insulin there is a small but consistent decrease in the lipid phosphorus of the serum, with a slight increase in the corpuscles in all but one case. The slight increase in the water content of serum which is noted to occur always after insulin (Table I) is not sufficient to account for the decrease in the lipid phosphorus. The change in the water content is probably due at least in part to dilution of the blood by tissue fluids after withdrawal of the first blood sample.

The concentration of potassium in the serum is greatly decreased after insulin. In the corpuscles the changes are very small; a fall in three cases, and a distinct rise in one (Experiment 7-Ins.), with the average for the six experiments showing no change. In a former investigation (20) experiments on three dogs showed slight increases in corpuscle potassium after insulin. There is no reason to doubt the accuracy of those experiments, but the evidence in the present case indicates without question that the potassium which disappears from the serum does not enter the corpuscles.

Experiments on the Effect of Pancreatectomy, and Subsequent Overdosage with Insulin.

Although it has been shown that even overdosage with insulin does not cause an increase of the organic acid-soluble phosphorus or of the potassium of corpuscles, the possibility remains that a total withdrawal of insulin from the body might cause losses of these compounds from the corpuscles.

A second group of experiments was therefore conducted to determine whether removal of the pancreas would result in changes in the distribution of phosphorus and potassium. A number of dogs were placed on a diet of lean meat (20 gm. per kilo of body weight), which was maintained throughout the experiment. After 10 days on this diet, blood was taken for analysis, this representing the normal period. The pancreas was then completely removed from each dog under ether anesthesia. In order to permit healing of the wounds the animals were given moderate doses of insulin twice daily for 7 to 10 days after the operation. The insulin was then withdrawn and the dogs allowed to become completely diabetic. The D:N ratio was determined in the urine each day after the withdrawal of insulin. After 5 days (11 days
## Protocol 1.

Record of Experiments on Depancreatized Dogs.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>17</th>
<th>19</th>
<th>22</th>
<th>24</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of dog, kg</td>
<td>19.5</td>
<td>15.5</td>
<td>8</td>
<td>8</td>
<td>6.3</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Daily ration of lean meat, gm</td>
<td>390</td>
<td>310</td>
<td>160</td>
<td>160</td>
<td>128</td>
</tr>
<tr>
<td>Meat diet started</td>
<td>July 8</td>
<td>July 8</td>
<td>July 8</td>
<td>July 14</td>
<td>Aug. 7</td>
</tr>
<tr>
<td>Normal blood sample taken</td>
<td>“ 18</td>
<td>“ 18</td>
<td>“ 19</td>
<td>“ 26</td>
<td>“ 11</td>
</tr>
<tr>
<td>Deapancreatized</td>
<td>“ 21</td>
<td>“ 20</td>
<td>“ 22</td>
<td>“ 26</td>
<td>“ 6*</td>
</tr>
<tr>
<td>Postoperative recovery period (insulin given twice daily)</td>
<td>“ 20-</td>
<td>“ 20-</td>
<td>“ 22-</td>
<td>“ 26-</td>
<td>“ 6-</td>
</tr>
<tr>
<td>Diabetic period (no insulin)</td>
<td>July 30-</td>
<td>July 30-</td>
<td>July 30-</td>
<td>Aug. 3-</td>
<td>Aug. 11-</td>
</tr>
<tr>
<td>Diabetic blood sample taken</td>
<td>Aug. 4</td>
<td>Aug. 4</td>
<td>Aug. 11</td>
<td>Aug. 16</td>
<td>Aug. 16</td>
</tr>
<tr>
<td>Insulin given (after bleeding, in 3 doses), units</td>
<td>30</td>
<td>30</td>
<td>Died</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Overdosage with insulin, blood sample taken during the insulin shock</td>
<td>Aug. 5</td>
<td>Aug. 5</td>
<td>Aug. 17</td>
<td>Aug. 17</td>
<td></td>
</tr>
<tr>
<td>Amount of insulin given, units</td>
<td>80</td>
<td>80</td>
<td>120</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

### D:N ratios during the diabetic period.

<table>
<thead>
<tr>
<th>After stopping insulin.</th>
<th>1st day</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>8th</th>
<th>9th</th>
<th>10th</th>
<th>11th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.01</td>
<td>3.90</td>
<td>3.09</td>
<td>2.65</td>
<td>2.81</td>
<td>1.80</td>
<td>2.26</td>
<td>1.50</td>
<td>1.50</td>
<td>1.60</td>
<td>2.17</td>
</tr>
</tbody>
</table>

* The normal blood specimen for Dog 26 was taken on the 4th day after the removal of the pancreas, insulin having been given daily during this period.
### TABLE III.
Effect of Removal of Pancreas and of Overdosage with Insulin on Distribution of Phosphorus and Potassium in Blood of Dogs.
Mg. per 100 gm.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Specimen</th>
<th>Whole blood.</th>
<th>Serum.</th>
<th>Corpuscles.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-DP.</td>
<td>Normal.</td>
<td>48.6</td>
<td>60.6</td>
<td>5.26</td>
</tr>
<tr>
<td></td>
<td>Diabetic.</td>
<td>37.0</td>
<td>338.0</td>
<td>5.21</td>
</tr>
<tr>
<td></td>
<td>Insulin.</td>
<td>33.5</td>
<td>40.6</td>
<td>2.61</td>
</tr>
<tr>
<td>19-DP.</td>
<td>Normal.</td>
<td>52.1</td>
<td>81.7</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>Diabetic.</td>
<td>42.2</td>
<td>274.7</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td>Insulin.</td>
<td>38.0</td>
<td>49.9</td>
<td>3.58</td>
</tr>
<tr>
<td>22-DP.</td>
<td>Normal.</td>
<td>43.5</td>
<td>71.9</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>Diabetic.</td>
<td>13.8</td>
<td>185.8</td>
<td>3.89</td>
</tr>
<tr>
<td>24-DP.</td>
<td>Normal.</td>
<td>45.6</td>
<td>63.4</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>Diabetic.</td>
<td>35.8</td>
<td>226.0</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>Insulin.</td>
<td>36.1</td>
<td>40.2</td>
<td>1.05</td>
</tr>
<tr>
<td>26-DP.</td>
<td>Normal.</td>
<td>38.6</td>
<td>164.8†</td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td>Diabetic.</td>
<td>34.7</td>
<td>254.1</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>Insulin.</td>
<td>38.7</td>
<td>37.7</td>
<td>1.73</td>
</tr>
<tr>
<td>Averages.</td>
<td>Normal.</td>
<td>45.3</td>
<td>69.4</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>Diabetic.</td>
<td>33.7</td>
<td>355.7</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>Insulin.</td>
<td>36.6</td>
<td>42.1</td>
<td>2.24</td>
</tr>
</tbody>
</table>

* Postoperative "normal."
† Not included in averages.
in Experiments 22 and 24) a second sample of blood was taken, this representing the completely diabetic period. After the taking of this second sample, 30 units of insulin were given in three doses during the day. On the following day a large overdose of insulin was administered with the hope of reversing whatever changes had occurred during the diabetic stage. A third sample of blood was taken 2 or 3 hours later, just before the onset of convulsions. Details of the experimental procedure, together with the D:N ratios, are recorded in Protocol 1.

Due to several fatalities it was necessary to include in the experiment one dog (Experiment 26) whose blood had not been analyzed before the removal of the pancreas.

The results of the analyses are given in Table III. The water content of whole blood, serum, and corpuscles, from which the percentage of corpuscles by weight is calculated are found in Table I.

The change from the normal to the severe diabetic condition is marked by a slight but definite increase in the organic acid-soluble phosphorus of the corpuscles in all but one of the five dogs studied, Dog 17 showing a decrease. In Experiment 22 the dog received no insulin for 13 days, was extremely weak and emaciated when the second blood sample was taken, and died a few hours later. In this case the increase of organic phosphorus amounts to 28 per cent of the original value. The average increase for the five experiments is 4.3 mg., or about 8.6 per cent.

Large doses of insulin administered to the diabetic dogs cause insignificant changes in the organic phosphorus of the corpuscles. The level of inorganic phosphorus in serum and corpuscles is not significantly changed due to the removal of the pancreas.

The changes in potassium concentration in the corpuscles are not the same in the five experiments. The only striking change is the increase of potassium during the severe diabetic period in Experiment 22. A similar result is observed in Experiment 24, but the reverse occurs in Experiment 17. The conclusion seems justified that the potassium content of corpuscles is not affected directly by the presence or absence of insulin. Although overdoses of insulin cause a fall in the concentration of potassium in the serum (Experiment 19 excepted), the removal of the pancreas produces no consistent changes.
The blood of one human diabetic patient was available for study during the course of these experiments. Determinations of potassium, inorganic phosphorus, and total acid-soluble phosphorus were made before treatment had begun, and again after several days of treatment with insulin, in order to learn whether insulin caused the same changes as those found in the dog. The analyses, presented in Table IV, show that an injection of 40 units of insulin made no appreciable change in the organic phosphorus or in the potassium concentration of the corpuscles. Human corpuscles resemble those of rabbits rather than those of dogs in their potassium content.

**TABLE IV.**

*Effect of Insulin on Organic Phosphorus and Potassium in Blood of a Human Diabetic.*

<table>
<thead>
<tr>
<th>Date</th>
<th>Remarks</th>
<th>Serum.</th>
<th>Corona.</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 22</td>
<td>Untreated diabetic.</td>
<td>667</td>
<td>2.2</td>
</tr>
<tr>
<td>&quot; 22-28</td>
<td>Insulin daily.</td>
<td>2.72</td>
<td>17.0</td>
</tr>
<tr>
<td>&quot; 28, 8 a.m.</td>
<td>40 units insulin.</td>
<td>45.9</td>
<td>2.86</td>
</tr>
<tr>
<td>&quot; 28, 10.15 a.m.</td>
<td>Blood sample taken.</td>
<td>43.0</td>
<td>64.5</td>
</tr>
</tbody>
</table>

After injecting the diabetic animals with insulin there is a noticeable increase in the water content of both serum and corpuscles. The changes are, however, not large enough to affect the interpretation of the results in Table III. Serum protein determinations were made because of the information they might give as to the extent of blood dilution by tissue fluids. The serum proteins fall after removal of the pancreas, and decrease still further after injection of insulin (except in Experiment 24). The changes in water content and serum proteins may be due in part to the drawing of fairly large samples of blood for analysis, with subsequent dilution of the blood by tissue fluids. The malnutrition associated with severe diabetes probably also plays a part in
the lowering of the serum proteins (21). The changes in hematocrit are of little significance, since on defibrination varying proportions of corpuscles are removed with the fibrin. This of course in no way changes the corpuscle composition. The hematocrit determinations were made solely for the purpose of calculating the corpuscle content of glucose and inorganic and lipid phosphorus, from the analyses of whole blood and serum.

**DISCUSSION.**

Examination of the results of analysis shows that the organic acid-soluble phosphorus of the corpuscles of the dog is not increased by insulin. On the contrary it increases slightly when insulin is withdrawn. These results are contradictory to those of Kay and Robison (6). It should be pointed out, however, that these authors worked with rabbits, and that the corpuscles of the rabbit differ distinctly from those of the dog in having a higher content of organic phosphate and of potassium. It seems improbable, however, that insulin should have a different effect on rabbit blood than on dog blood. Some possible reasons for the discrepancy between our results and those of Kay and Robison may be mentioned. These authors present the averages of eleven experiments, comparing the blood of normal control rabbits with that of others which had received insulin. The organic acid-soluble phosphorus in the blood of different rabbits may, however, vary as much as 12.6 mg. per 100 cc. according to data presented by these same investigators (22). This variation is most probably due to differences in hematocrit, since the organic phosphorus is found almost entirely in the corpuscles, but individual differences in the phosphorus content of the corpuscles of various rabbits may likewise occur, if we may judge by our experience with dogs. Kay and Robison do not give the hematocris for either group of rabbits. The results of the individual experiments together with hematocris or hemoglobin determinations such as the one given in their Table VI (6) would be much more convincing.

Secondly, their analyses, as recorded, actually show a decrease of 1 per cent in the organic phosphorus of whole blood after insulin, but this they interpret as an increase of 14 per cent, since the blood volume is said to increase by "some 15 per cent" under
Phosphorus and Potassium in Blood

the conditions of the experiment. In view of the fact that the corpuscles contain practically all of the organic acid-soluble phosphorus of the blood, the necessity of reporting the exact hematocrits in each case is again apparent. This was done in a single experiment (Table VI) in which hemoglobin determinations show the extent of blood dilution. Furthermore, the amount of organic phosphorus found after insulin was corrected for blood dilution by multiplying by the factor \( \frac{\text{Hb before injection}}{\text{Hb at death}} \). This however gives a true correction only when the diluting fluid is phosphorus-free. The dilution of the blood must be due in part at least to an inflow of tissue fluids, which contain quantities of phosphorus similar to plasma, and hence the corrected figures are too high. The results obtained by Kay and Robison in the one experiment recorded in their Table VI seem nevertheless to show an increase in organic phosphorus in whole blood even when this factor and the blood dilution are taken into consideration.

The assumption by Kay and Robison of a blood dilution of 15 per cent was based on the work of Haldane, Kay, and Smith (23), but these authors state definitely that “the individual variations from this figure are in some cases large.” They also conclude that “comparative quantitative determinations in blood before and after the giving of insulin, particularly in the case of substances not equally distributed between the corpuscles and plasma, can only be accepted if this volume change is taken into account.”

For these reasons, the conclusions of Kay and Robison in regard to changes in the organic phosphorus of corpuscles must be questioned, although it is possible that the effect of insulin on rabbits may be different from that on dogs, due to the difference in corpuscle composition. Insulin certainly does not increase the organic acid-soluble phosphorus of the corpuscles of dogs, nor can any of the sugar which disappears from blood be accounted for by the synthesis of phosphoric esters in corpuscles from the inorganic phosphorus of blood.

The presence in the corpuscles of a phosphoric ester with reducing properties (7), however, makes it possible that a carbohydrate group is present in its molecule. The observation of Hynd (11) that insulin causes a synthesis of a non-reducing complex in corpuscles which on hydrolysis again yields a reducing sugar is also
significant. It is quite possible that insulin may cause a synthesis of a hexose phosphoric ester in the corpuscles, the blood sugar combining with an organic phosphate compound already in the corpuscles, without increasing the total organic phosphorus; the inorganic phosphorus going at the same time to the tissues. There is, however, no evidence that this is the case, and it seems more reasonable to believe that the phosphoric esters of the corpuscle, even though they may contain hexose groups, are not concerned with the action of insulin.

SUMMARY AND CONCLUSIONS.

1. Experiments are described showing the effect of insulin overdosage on the distribution of inorganic phosphorus, organic acid-soluble phosphorus, lipid phosphorus, and potassium between the serum and red blood corpuscles of the dog. A second group of experiments shows the effect of removal of the pancreas, and subsequent overdosage with insulin, on the organic phosphorus and the potassium of blood.

2. The organic acid-soluble phosphorus content of corpuscles is not significantly altered by overdosage with insulin. It is slightly increased on removal of the pancreas.

3. The changes found in the organic phosphorus of corpuscles do not support the hypothesis that insulin causes a synthesis of phosphoric esters in the corpuscles.

4. Lipid phosphorus is not significantly affected by insulin.

5. The potassium which disappears from serum after insulin does not enter the corpuscles.

The writer desires to express his thanks to Mr. V. H. Krikorian and Mr. D. Berberian for assistance with the experimental work, and to Dr. A. Bejjian and Dr. W. F. Dodd for performing the surgical operations on the dogs.

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THE EFFECT OF INSULIN AND OF PANCREATECTOMY ON THE DISTRIBUTION OF PHOSPHORUS AND POTASSIUM IN THE BLOOD
Stanley E. Kerr


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