THE RÔLE OF COPPER IN CARBOHYDRATE METABOLISM

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Copper has been designated as one of the dietary essentials by a number of investigators and appears to occupy a unique position as a necessary supplement to iron for the synthesis of hemoglobin within the animal body. However, our present knowledge concerning the function of copper, other than as a hematopoietic agent, is limited. In view of the fact that this element is found by analysis in many organs, one is led to believe that its presence is not accidental, but perhaps is required to promote normal activity in that portion of the body containing it. Any attempt to disclose specific organic effects on living rats receiving copper would be difficult. It therefore occurred to us that an investigation of some major physiological process might yield information indicative of another rôle of copper in nutrition. With this idea in mind a series of glucose metabolism experiments was initiated.

Gettler and Lindeman (1) have reported abnormally high blood sugar in pernicious anemia. They interpret the results to mean either a failure in glucose oxidation or a disturbance of the glycogen-glucose equilibrium. Glucose tolerance tests performed by Rennie (2) on patients with pernicious anemia, after a 10 to 12 hour fasting period, showed higher values than were obtained on the controls. No correlation could be drawn between hemoglobin and blood sugar levels. The work presented in this paper consists primarily of a comparison of glucose utilization by anemic rats, before and after copper and iron supplementation, with that of normal animals. Sugar tolerance tests have been selected as a means of determining the rate of glucose metabolism in an attempt to answer the following questions: (1) Does copper influence other physiological processes aside from that of hemoglobin formation? (2) Can the rate of dis-
appearance of glucose from the blood stream be correlated with hemoglobin content in nutritional anemia? (3) Is the fasting blood sugar level higher in anemic than in normal animals?

EXPERIMENTAL

Anemic rats maintained upon a milk ration low in copper served as test animals because hemoglobin and stored copper could be markedly reduced simultaneously. The blood sugar changes in these animals were then studied first from the standpoint of anemia and next after copper administration. The experiments to be described comprise 78 anemic and eighteen normal rats equally divided with respect to sex. Six animals of the same sex were housed together in a galvanized iron wire cage resting on glass rods over a pan containing wood shavings. All animals were selected from stock colony mothers at 30 days of age. Those to become anemic were fed upon whole milk—from pure-bred Holstein cows—collected in glass containers, in order to eliminate copper and iron contamination. A copper and iron depletion period of from 8 to 10 weeks was prescribed for the anemic rats, while the normal controls were fed a stock diet for the same length of time. Hemoglobin determinations were made periodically by the Newcomer acid hematin method.

After the hemoglobin had fallen to a sufficiently low level the experiments were started by fasting the rats for 20 hours previous to bleeding. Preliminary tests served to eliminate those showing either signs of nervousness during the tail bleeding operation or an unwillingness to drink readily from a pipette. Only rats demonstrating consistent glucose values were continued on experiment, since it is well known that fear and emotion will cause fluctuations in blood sugar determinations. The regular procedure was to determine first the blood sugar level after a 20 hour fast. Each animal was then given orally a pure glucose solution in an amount affording exactly 200 mg. of glucose per 100 gm. of body weight. Individual blood analyses were carried out at ½, 1, and 2 hours after glucose ingestion. Previous experience had shown 10 minute intervals to be very annoying to the unanesthetized animal; besides, the blood sugar curve rose steadily during the first 30 minutes after which it declined at a similar rate.

Blood sugar determinations were made by the method of Folin
A slight modification was introduced because of the difficulty encountered in obtaining the 0.1 cc. of blood, required by the Folin pipette, from the tail of the rat. Newcomer hemoglobin pipettes proved much better for our method of sampling. Two 20 c.mm. volumes of blood from an individual were accurately measured and delivered into a 15 cc. centrifuge tube containing 4 cc. of Folin's dilute tungstic acid solution. After thoroughly mixing and centrifuging, the supernatant liquid was poured into a Pyrex tube marked at a 25 cc. volume content. This protein-free filtrate represented 40 c.mm. of blood and equaled the amount taken for a single determination in the Folin and Malmros method. Their procedure was followed from this point. Table I offers a comparison of glucose values obtained with the Folin and also the Newcomer pipette from the same blood sample. The blood was collected from normal rats after a 9 hour fast. They were stunned and immediately bled into beakers containing a little sodium oxalate to prevent clotting.

Copper as CuSO₄·5H₂O was fed orally at a level of 0.1 mg. daily in the milk. The copper salt was made by dissolving a Hilger spectrograph electrode in dilute HNO₃; the resulting Cu(NO₃)₂ was filtered, converted to CuSO₄ by digesting with H₂SO₄, and finally crystallized from a dilute solution. Selected crystals dissolved in copper-free water formed the solution which was fed.

### Table I

**Effect of Sampling on Accuracy of Method**

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Glucose per 100 cc. blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Folin pipette</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
</tr>
<tr>
<td>1</td>
<td>113</td>
</tr>
<tr>
<td>2</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>111</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>7</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>89</td>
</tr>
<tr>
<td>Average</td>
<td>104.3</td>
</tr>
</tbody>
</table>

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Iron as FeCl$_3$ was administered at a rate of 1.0 mg. daily in the milk. Pure FeCl$_2$ was made by dissolving Baker and Adamson's standardization iron wire in a 1:1 HCl solution. The filtered crystals were placed in a solution of 2 per cent HCl and freed of copper by bubbling H$_2$S through the liquid for 30 minutes; after filtration, the iron was oxidized to FeCl$_3$ by boiling with HNO$_3$ and was converted to Fe(OH)$_3$ upon the addition of NH$_3$. Ferric hydroxide, thus obtained, was washed well on a Buchner filter and put into solution as FeCl$_3$ by bubbling HCl through the suspension. The solutions as fed were analyzed for iron by the KCNS method to insure quantitative administration. Both CuSO$_4$ and FeCl$_3$ solutions, made in the manner described, proved to be free from contamination of each other. They were tested separately on anemic rats and found to produce no hematopoietic response.

DISCUSSION

The blood sugar tolerance curves shown in Figs. 1 to 4 represent composite data obtained from a number of rats grouped according to individual hemoglobin values at the beginning of the experiment. Fig. 1 depicts the performance of forty-eight anemic and eighteen normal animals. Average hemoglobin values for the anemic males and females were 4.8 and 5.1 per cent respectively. It can be seen that a high fasting level and peak exist in nutritional anemia, although the rate of return to initial conditions is practically the same as in normal controls having a hemoglobin content of 15 per cent. Intestinal absorption is not impaired in anemia as evidenced by the uniform time of maximum rise in blood sugar. Sugar metabolism appears to go on at the same rate in both sexes, due to the fact that ovulation does not occur in anemic rats.

Six males were tested (Fig. 2) first at a hemoglobin level of 4.1 per cent as shown in the upper curve. They were then fed 0.1 mg. of copper together with 1.0 mg. of iron daily in the milk for 15 days, when their hemoglobin values averaged 11.55 per cent; at this point glucose tolerance was determined. Results of this experiment make up the lower curve (Fig. 2). That the glucose utilization approaches a normal state is manifested by both a lower fasting level and peak. A comparison of the following curves—Fig. 2 (upper), Fig. 2 (lower), and Fig. 1 (lower) with corresponding hemoglobin values of 4.1, 11.55, and 15.0 per cent-establishes a corre-
Figs. 1 to 4. Effect of anemia, copper, and iron on blood sugar level and glucose tolerance of rats. In Fig. 1, solid upper curve is composite for twenty-four anemic males; upper dotted curve, composite for twenty-four anemic females; lower curve, data on eighteen normal animals receiving stock ration. Fig. 2 shows the results obtained on six males before and after feeding 0.1 mg. of Cu + 10 mg. of Fe for 15 days; Fig. 3, for six males and six females before and after a 10 day Cu feeding period; Fig. 4, for six males and six females before and after feeding 1.0 mg. of Fe for 10 days.
lation between amount of hemoglobin and reducing power of the blood.

The top curve (Fig. 3) was derived from data obtained on six male and six female rats, whose hemoglobin values averaged 5.4 per cent. After a 10 day copper feeding period their glucose tolerance changed markedly as is shown by the lower curve (Fig. 3). It can be clearly seen that copper ingestion has lowered the maximum point by some 30 mg. while the fasting level remained practically stationary. Copper administration did not cause regeneration of hemoglobin in these animals; therefore, the effect must have been due to this element alone. Furthermore, the fasting level has been shown to remain high as long as anemia persists (Figs. 1 and 2). Other data, not included in this paper, substantiate these results.

We have no direct experimental evidence as to the mode in which copper acts to reduce the glucose peak in anemic rats. The most logical explanation seems to involve an improvement in liver function to bring about an acceleration of glycogen formation and thus a rapid removal of glucose from the blood. Unpublished data, obtained in this laboratory from anemic rat urine, show no impairment of pancreatic activity. The possibility of a direct oxidation catalyzed by copper either in the blood stream or in muscular tissue is overruled, provided that glucose is assumed to be the only reducing agent in anemic blood. And if this assumption is correct, then a lowered fasting value should result from copper administration. Pure iron alone does not affect glucose tolerance (Fig. 4). The uppermost curve produced from data on twelve anemic rats divided equally as to sex falls almost upon the lower curve obtained from the same rats after 1.0 mg. of iron had been fed daily for 10 days. Hemoglobin values averaged 6.2 per cent and were not altered by iron feeding.

SUMMARY

1. Oral administration of copper alone to anemic rats produces a different type of glucose tolerance curve than that obtained from the same animal before the mineral ingestion. A significant lowering of the maximum point demonstrates a rôle of copper in nutrition aside from hemoglobin formation. The fact that hemoglobin values were unaltered proves that the effect was due not to ordinary physiological oxidative processes, but must have arisen from a hitherto undescribed property of copper.
2. Pure iron alone does not improve glucose utilization as shown by sugar tolerance tests.

3. Any increase of hemoglobin in anemic rats produces a proportional increase in sugar tolerance coupled with a lowered glucose level in the blood after a 24 hour fast.

4. Blood sugar values determined after 20 hours of fasting are consistently higher in animals suffering with nutritional anemia as compared to normal controls of the same age; whether or not this reducing substance is all glucose has not been determined. Both sexes perform similarly on glucose tolerance experiments when the hemoglobin is at an anemic level.

BIBLIOGRAPHY

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