THE INFLUENCE OF VITAMIN B₁₂ ON CARBOHYDRATE AND LIPIDE METABOLISM*

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In previous communications (1, 2) evidence has been presented to show that vitamin B₁₂ deficiency causes derangement in carbohydrate and lipide metabolism in the animal body, and a marked diminution of glutathione concentration in the blood. The present report describes further studies on the effect of vitamin B₁₂ on carbohydrate and lipide metabolism, and a possible relationship between these metabolic changes and the glutathione concentrations in the blood and tissues.

EXPERIMENTAL

Experimental Animals and Composition of Diets—The vitamin B₁₂-deficient rats used in this investigation were raised in our laboratory as described before (1). Diets A and B, the composition of which has been reported previously, were used in the early part of this work. However, observations in this (3) and other laboratories (4) indicate that a high carbohydrate and low fat diet accelerates the development of vitamin B₁₂ deficiency; hence, in the later experiments, one such ration, Diet HCLF-7, was used. This diet consists of 70 per cent of a commercial soy bean product,¹ 26 per cent sucrose, 4 per cent Salts IV (5), and vitamin supplements (6), and, by analysis, it contains approximately 25 per cent soy protein, 1 per cent fat, and 68 per cent carbohydrate.

Determination of Reducing Sugars in Blood and Urine—Reducing sugar in samples of whole blood or filtered urine was determined by Somogyi’s method (7). Both iodometric titration and Nelson’s photometric analysis (8) were used according to the estimated amounts of sugar in the samples.

Isolation and Estimation of Phospholipides—Total lipides from body tissues were isolated and estimated as described previously (1). The ex-

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† Present address, Department of Biochemistry, Jefferson Medical College, Philadelphia 7, Pennsylvania.
¹ Mead Johnson and Company, laboratory product No. 376, which contains soy bean flour, arrowroot starch, Dextri-Maltose, calcium phosphate (dibasic), and sodium chloride.
traction of lipides from whole blood was carried out according to the method of Boyd (9), and the extract was rectified with purified petroleum ether. Total phospholipides were isolated by precipitation with acetone and magnesium chloride as described by the same author. Estimation of phospholipides in both ether-soluble and ether-insoluble fractions was made by phosphorus determination after hydrolysis with 10 N H₂SO₄ and oxidation with hydrogen peroxide. The phosphate was then estimated by the method of Fiske and Subbarow (10) with Elon as the reducing reagent.

Studies on Possible Relationship between Carbohydrate Metabolism and Glutathione Concentration in Blood and Tissues—The results of our earlier studies indicate that both derangement in carbohydrate metabolism and diminution in blood glutathione content occur in vitamin B₁₂ deficiency. Since many of the enzymes involved in carbohydrate metabolism are sulfhydryl enzymes, it appears probable that there may be an interrelationship between the impairment in carbohydrate metabolism and changes in concentration of glutathione which occur simultaneously in a deficiency of this vitamin. If vitamin B₁₂ is required for the utilization of carbohydrate as well as for the formation of glutathione in the animal body, an increased burden of carbohydrate metabolism would accelerate the depletion of stores of this vitamin in the tissues, and this might be reflected by changes in the blood sugar level and glutathione content. To test this hypothesis, six normal male rats, approximately 2.5 months old and with an average weight of 202 (197 to 207) gm., were placed on a soy bean diet (HCLF-7) and given additional glucose by injection. The dosage of glucose and frequency of injection were so adjusted that the food intake of the experimental animals was not appreciably decreased. Alternating intraperitoneal and subcutaneous injections of 5 to 10 ml. of a 25 per cent sterile glucose solution to each rat fulfilled this purpose. The level of blood glutathione was determined as described elsewhere (2). The results of these studies are presented in Figs. 2 and 3.

Results

Blood Sugar Levels and Glucose Tolerance Curves—In the preliminary experiments it was observed that the blood sugar levels of growing young rats after a short period of fasting (12 to 24 hours) were lower in the vitamin B₁₂-deficient group than in the vitamin B₁₂-treated controls. But when the fasting period was extended to 48 hours, several of the deficient rats had abnormally high blood sugars (170 to 498 mg. per cent), showing a tendency toward "hunger diabetes," whereas none of the vitamin B₁₂-treated controls had such an abnormality. The urinary excretion of reducing sugar during a fasting period declined much more rapidly among the vitamin B₁₂-deficient rats than among the normal animals. One possi-
ble explanation for the above findings is that the vitamin B₁₂-deficient animals have less carbohydrate reserve in their body. In Fig. 1 are shown the glucose tolerance curves of vitamin B₁₂-deficient rats and controls receiving the vitamin. After fasting overnight, the rats received glucose

![Glucose Tolerance Curves](image)

**Fig. 1.** Glucose tolerance curve of vitamin B₁₂-deficient and vitamin B₁₂-injected rats. The dash line represents the average value of four vitamin B₁₂-deficient rats while the solid line represents the average value of three normal rats.

### Table I

**Phospholipide Content of Body Tissues of Rats**

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B₁₂-deficient (5 rats)</th>
<th>Vitamin B₁₂-deficient, vitamin B₁₂-treated (5 rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (average), gm.</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Final body weight (average), gm.</td>
<td>60</td>
<td>147</td>
</tr>
<tr>
<td>Weight of tissues analyzed, * gm.</td>
<td>33.6</td>
<td>76.4</td>
</tr>
<tr>
<td>Total phospholipides per rat (average), mg.</td>
<td>190 ± 17</td>
<td>564 ± 68</td>
</tr>
<tr>
<td>Phospholipide per unit tissue weight, %</td>
<td>0.56 ± 0.03</td>
<td>0.74 ± 0.09</td>
</tr>
</tbody>
</table>

* Vitamin B₁₂-deficient weanling rats fed Diet B for a period of 6 weeks before sacrifice. The sampling of tissues assayed is described in the text.

by intravenous injection in a dose of 750 mg. per kilo of body weight. It will be noted that the average blood sugar level of the deficient rats remained unusually high at the end of 60 minutes after injection, while that of the vitamin B₁₂-treated rats had returned to normal.

**Phospholipide Content of Blood and Tissues in Vitamin B₁₂ Deficiency**

In Table I are presented data on the phospholipide content of tissues in vitamin B₁₂-deficient and B₁₂-treated young rats. The fat was isolated
from the lean tissues of the carcass and did not include skin, subcutaneous adipose layer, or viscera, and, therefore, the values represent chiefly the intercellular and intracellular lipides, a major portion of which constitutes an integral part of the cellular constituents. Even though these rats were litter mates and had approximately the same average initial body weights and were raised under the same dietary regimen, the injection of vitamin $B_{12}$ resulted in a 3-fold increase of the total phospholipides in the lean tissues of the carcass as well as in a higher phospholipide content per unit weight of tissues.

### Table II

**Phospholipide Content of Blood in Pernicious Anemia**

<table>
<thead>
<tr>
<th>Date, 1951</th>
<th>Vitamin B$_{12}$, orally</th>
<th>Hematocrit</th>
<th>Total phospholipides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg. per 100 ml blood</td>
</tr>
<tr>
<td>F. G., male; age 56 yrs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept. 1</td>
<td></td>
<td>17</td>
<td>57</td>
</tr>
<tr>
<td>&quot; 1</td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>&quot; 8</td>
<td></td>
<td>24</td>
<td>138</td>
</tr>
<tr>
<td>&quot; 17</td>
<td></td>
<td>33</td>
<td>253</td>
</tr>
<tr>
<td>H. J., male; age 63 yrs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug. 29</td>
<td></td>
<td>34</td>
<td>185</td>
</tr>
<tr>
<td>&quot; 29</td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Sept. 5</td>
<td></td>
<td>37</td>
<td>189</td>
</tr>
<tr>
<td>&quot; 17</td>
<td></td>
<td>38</td>
<td>236</td>
</tr>
<tr>
<td>&quot; 24</td>
<td></td>
<td>37</td>
<td>245</td>
</tr>
</tbody>
</table>

Pernicious anemia is considered a state of vitamin $B_{12}$ deficiency in man, and such patients are known to have an abnormally low blood phospholipide content when in relapse (11, 12). Table II contains the results of the determination of the blood phospholipide levels of two patients with pernicious anemia before and after vitamin $B_{12}$ therapy. Both patients had significantly low blood phospholipide before treatment, in proportion to the severity of the anemia, but the levels rapidly returned to normal after treatment with the vitamin.

**Effect of High Carbohydrate Intake on Blood Sugar and Glutathione Levels and Influence of Glutathione or Vitamin B$_{12}$ Administration on Hyperglycemia**—When a group of six normal, young, male adult rats was fed Diet HCLF-7 and given injections of a 25 per cent glucose solution at the intervals indicated in Fig. 2, hyperglycemia and a concomitant decrease of glutathione content of the blood resulted. The average fasting blood sugar
level was elevated from its initial value of 106 mg. per cent to 152 mg. per cent 10 days after the start of glucose administration and reached 249 mg. per cent on the 24th day, i.e. 2 days after the termination of glucose injection. The fasting blood sugar level remained at 154 mg. per cent 11 days after the last parenteral dose of glucose. The average blood glutathione was 410 $\mu$M per 100 ml. of blood cells at the beginning, but the value decreased to 328 $\mu$M 6 days after the cessation of glucose injection, a 20 per cent reduction from its initial level, with a $P$ value of less than 0.02.

To study the effect of glutathione on the blood sugar levels of these hyperglycemic rats, 25 mg. of reduced glutathione were given to each rat by subcutaneous injection, and the blood sugar levels were followed for 3 hours. Administration of glutathione produced a transient, but significant, drop in the blood sugar in these animals, from an average of 134 to 112 mg. per cent in 3 hours. Blood sugar levels of these rats measured at the same hours (2 and 5 p.m.) on the following day showed that it had risen from 143 to 153 mg. per cent without injection of glutathione.

The effect of vitamin $B_{12}$ on induced hyperglycemia was next studied with the same group of rats. The results are illustrated in Fig. 3. A few days after the termination of the above experiment, injection of glucose was resumed while the animals were maintained on the same diet. This treatment again resulted in a rise in blood sugar levels from an average of 117 to 181 mg. per cent in 9 days. At this point, the animals were divided randomly into two groups of approximately the same average blood sugar levels. To one group was given vitamin $B_{12}$ (1.0 $\gamma$ per rat per day) by
subcutaneous injection, while the glucose injection to both groups of animals was continued. 1 week after the administration of vitamin B$_{12}$ was begun, the treated group gained an average of 12 gm. in body weight, and the blood sugar levels decreased to an average of 174 mg. per cent, while the untreated group lost an average of 2 gm. in body weight and had an average blood sugar of 198 mg. per cent. Attempts were made to produce an even greater difference in blood sugar levels between the two groups of rats by increasing the frequency of glucose injection (daily instead of every other day) to both groups while the injection of vitamin B$_{12}$ to one group was maintained on the same daily dosage. With the increased dose of glucose, both groups of rats lost weight, the loss averaging 5 gm. for the vitamin B$_{12}$-treated group and 14 gm. for the untreated group during the ensuing 8 day period, but the average blood sugar for the vitamin B$_{12}$-treated group remained at 209 mg. per cent, while that of the latter group had risen to 367 mg. per cent. The blood sugar determination was repeated 4 days later, and a difference of the same order of magnitude was observed.

**DISCUSSION**

In this communication are presented data which provide two additional points of evidence demonstrating the importance of vitamin B$_{12}$ in carbohydrate and lipide metabolism. In the first place, the abnormally high blood sugar levels of vitamin B$_{12}$-deficient rats in the glucose tolerance test (Fig. 1) indicate that they suffer from derangement in their capacity to utilize carbohydrate. As a result the deficient animals have a smaller carbohydrate reserve and consequently are more sensitive to fasting. This
is further evident from the fact that they had consistently lower blood sugar levels after a short period of fasting, but developed "hunger diabetes" sooner than the control animals. The "hunger diabetes" is considered as a sign of alarm reaction due to increased gluconeogenesis from tissue protein. The sharp drop in the urinary excretion of reducing sugar by the deficient rats during fasting may be taken as another sign of a poor carbohydrate reserve.

In the second place, data presented in Tables I and II demonstrate that vitamin B₁₂ deficiency entails disturbance in lipide metabolism, which can be corrected by administration of the vitamin. It is well known that a major part of the carbohydrates utilized is converted into lipides and, according to Stetten and Boxer (13, 14), this conversion would amount to approximately 30 per cent of the available body glucose in normal rats placed on a high carbohydrate diet. The rapidity with which our vitamin B₁₂-deficient rats lost body weight and developed hyperglycemia under the influence on a high carbohydrate-low fat diet, and the reported increased requirement of vitamin B₁₂-deficient rats for this vitamin when raised on such a diet (3, 4), further suggest that the deficient animals have lost part of their ability to transform carbohydrate into lipides. In harmony with this hypothesis is the fact that abnormally small amounts of phospholipides were found in the tissues of vitamin B₁₂-deficient rats and in the blood of patients with pernicious anemia in relapse. Administration of this vitamin to the above resulted in marked increases in phospholipide content of blood and tissues. Muller (11) and Kirk (12) have observed that in pernicious anemia in relapse the blood phospholipide and cholesterol were abnormally low, and that both of these substances increased after an effective liver therapy, owing presumably to the action of vitamin B₁₂.

In the latter part of this investigation, a possible relationship between blood glutathione concentration and carbohydrate utilization was brought to light. It was found that the blood glutathione levels in normal rats could be lowered by feeding a high carbohydrate-low fat diet coupled with glucose injection for a relatively short period of time (Fig. 2), with a concomitant rise in blood sugar levels which persisted long after the cessation of glucose injection. Since vitamin B₁₂ deficiency likewise causes the lowering of blood glutathione, these results are suggestive that a high carbohydrate intake brought about a rather rapid depletion of vitamin B₁₂ stores in the animal body, owing to increased requirement for the vitamin in carbohydrate metabolism. Furthermore, injection of glutathione resulted in a transient, but significant, drop in blood sugar levels of these rats within 2 to 3 hours, indicating the existence of a close relationship between blood glutathione concentration and carbohydrate utilization. However, considering the extremely rapid turnover of glutathione in the animal body,
as shown by the studies of Waelsch and Rittenberg (15, 16), one would not expect that the derangement in carbohydrate metabolism in vitamin B\textsubscript{12} deficiency can be fully corrected by the injection of glutathione, owing to the impossibility of maintaining an adequate supply from temporary exogenous sources. Therefore, an adequate supply of the peptide to meet the physiological requirement can be maintained only when the glutathione synthesis in the animal body is restored to normalcy by the administration of vitamin B\textsubscript{12}. In such a situation, it would be expected that hyperglycemia would be corrected also owing to the increase in glutathione synthesis. This belief is substantiated by our data, presented in Fig. 3, which demonstrate that injection of vitamin B\textsubscript{12} to these deficient animals effectively prevented the hyperglycemia and loss of body weight induced by such a diet and glucose injection.

Although the mechanism by which vitamin B\textsubscript{12} aids in the utilization of carbohydrate is still unknown, experimental evidence from these studies indicates that the maintenance of an adequate concentration of glutathione in the blood and tissues may be at least one of several important factors in the functioning of this vitamin. There are several plausible explanations of the mechanism of action by which glutathione could improve the utilization of carbohydrates, such as the activation of sulfhydryl enzymes and the protection of the \( \beta \)-cells in the pancreas (17-19) from the deleterious effect of certain metabolic products, alloxan and dehydroascorbic acid for instance. Although these substances are usually found only in minute quantities in the animal body, their concentration can increase to a considerable extent under certain conditions (20, 21). For example, Banerjee \textit{et al.} (22) found that dehydroascorbic acid was present in considerable amounts in scorbutic guinea pig tissues and that the glutathione content was markedly diminished in these tissues, and particularly so in the pancreas. However, the rapidity with which glutathione acts to lower the blood sugar after its injection suggests that this substance may be directly involved in the utilization of carbohydrate. This supposition is substantiated by the observations of Cavallini (23) on the role of glutathione in the coupled oxidative decarboxylation of pyruvate and of Racker (24) on the mechanism of glyoxalase function and by the discovery of Krimsky and Racker (25, 26) that glutathione is the prosthetic group of 3-phosphoglyceraldehyde dehydrogenase.

Yacowitz \textit{et al.} (27) reported that vitamin B\textsubscript{12} exerted pantothenic acid-sparing action for the growth of chicks, and that this vitamin seems to increase the conversion of pantothenic acid to coenzyme A in the liver. This finding further establishes a relationship between vitamin B\textsubscript{12} and another soluble sulfhydryl compound, coenzyme A. Since coenzyme A is an important participant in the formation of fatty acids, phospholipides, cholesterol, and acetylcholine, it furnishes additional evidence that vita-
min B₁₂ also plays an essential rôle in lipogenesis and the metabolism of choline.

SUMMARY

The effect of vitamin B₁₂ on carbohydrate and lipide metabolism was studied by measurement of blood sugar levels, by glucose tolerance tests, and by estimation of the phospholipide content of blood and tissues. Its possible relationship to the changes in blood glutathione content was also investigated.

Experimental results indicate that vitamin B₁₂ deficiency entails a derangement in carbohydrate utilization and decreases the phospholipide content of blood and tissues.

Administration of glutathione or vitamin B₁₂ lowered the blood sugar levels of rats with hyperglycemia induced by a high carbohydrate-low fat diet and by glucose injections.

It is concluded that vitamin B₁₂ plays an important rôle in carbohydrate and lipide metabolism and that the effect of this vitamin on blood glutathione concentration may be of significance in its rôle in metabolism.

BIBLIOGRAPHY

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