Studies on Vitamin B₁₂ Status in Blood and Tissue of Alloxan Diabetic Animals

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Studies were performed to find out the vitamin B₁₂ status in blood tissues of alloxan diabetic animals. It was found that diabetic animals maintaining higher levels of blood ketone bodies had an increased requirement of vitamin B₁₂. The maximum requirement for the vitamin was found in diabetic animals getting a higher dose of acetoacetate. Hepatic constituents also changed adversely in order of level of ketone bodies in the diabetic animals. Some direct or indirect interrelationship between vitamin B₁₂ with ketone bodies are suggested.

Although the role of vitamin B₁₂ in relation to diabetes mellitus is not fully understood, there are evidences to substantiate that this micronutrient plays an important role directly or indirectly in carbohydrate metabolism (1). It has been shown to relieve hyperglycemia and glycosuria (2) under various experimental conditions. A decrease in the activities of glycolytic enzymes and some other SH-enzymes in vitamin B₁₂ deficiency was also reported by Biswas and Johnson (3).

The vitamin B₁₂ levels of serum and urine in diabetic patients with or without retinopathy, has been a subject of conjecture as controversial reports have been obtained by different workers on the subject (4,5). According to some, diabetics with retinopathy excrete significantly more of the vitamin than non-diabetics, while diabetics without retinopathy excrete considerably less amounts of the vitamin than the nondiabetics (4). On the other hand, Bookman et al. (5) found a lack of correlation with vitamin B₁₂ excretion in diabetics (with or without retinopathy) and normal subjects. Serum levels of vitamin B₁₂ in diabetics with retinopathy was found to be much higher than those without retinopathy (6). However, others failed to find significant difference in serum vitamin B₁₂ levels between diabetics and normal persons (7). However, a decreased blood vitamin B₁₂ in diabetes mellitus was evidenced by some other workers (8). In view of the above observations it was thought worthwhile to investigate into the true picture of vitamin B₁₂ status in diabetes.

In the earlier reports it has been shown that the vitamin B₁₂ levels of the serum and liver decrease with higher ketone bodies in the animal system (9,10). In the present investigation, therefore, vitamin B₁₂ levels of serum and liver were estimated in alloxan diabetic animals under different levels of ketone bodies.
Methods and Materials

Fifty male albino rats weighing 100-150 g were fed on laboratory stock diet. They were divided equally into five different groups. Group I animals were normal controls. Animals from rest of the groups were injected subcutaneously with alloxan (10 mg/100 g body weight). Group III animals were again injected on the subsequent day with alloxan (8 mg/100 g body weight). Animals from group IV and V were simultaneously injected with acetoacetate 10 and 15 mg/100 g body weight, respectively, daily for 30 days. Blood sugar and ketone bodies were assayed every week by the standard procedures (11, 12). At the end of 30 days animals from all the groups were sacrificed and blood collected. Tissues such as liver and kidney were removed, chilled and prepared for vitamin B₁₂ assay. Blood and tissue vitamin B₁₂ levels were estimated microbiologically by the standard method (13). Hepatic constituents such as liver sulfhydryl, RNA, DNA, and protein were estimated by the usual procedures (14-17).

Results

From Figure 1 it becomes clear that acetoacetate injection along with alloxan made the animal more susceptible to diabetes as indicated by the high blood sugar levels. It is interesting to note that although blood sugar of animals from group II comes down considerably at the end of 30 days, animals from the rest of the diabetic groups maintained a high level of blood sugar till their sacrifice. This is in confirmation with the earlier works of Nath and his coworkers who had shown increased susceptibility to diabetes by acetoacetate in animal (18). Animals from group III, getting alloxan for two subsequent days, maintained a higher levels of ketone bodies throughout the experiment (see Fig. 2). Animals of group IV and V

![Fig. 1 Progressive Blood Sugar Levels in the Experimental Animals](image1)

![Fig. 2 Progressive Blood Ketone Bodies in the Experimental Animals](image2)
TABLE 1

<table>
<thead>
<tr>
<th>Status in the Blood and Tissue of the Alloxan Diabetic Rats</th>
</tr>
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<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>I. Control (10)*</td>
</tr>
<tr>
<td>II. Alloxan (8)</td>
</tr>
<tr>
<td>III. Alloxan (7)*</td>
</tr>
<tr>
<td>IV. Alloxan + acetoacetate (10 mg/100 g)</td>
</tr>
<tr>
<td>V. Alloxan + acetoacetate (15 mg/100 g)</td>
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</tbody>
</table>

*a Figure in parenthesis represents the number of rats sacrificed.
*b Injected with alloxan for two consecutive days.
* Significantly altered from group I at P<0.01 by using Student’s t-test.

TABLE 2

<table>
<thead>
<tr>
<th>Alteration in Certain Hepatic Constituents in Alloxan Diabetic Rats</th>
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<tr>
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</tbody>
</table>

* Significantly decreased from group I at P<0.01 by using Student’s t test.
* Indicates group of animals injected with alloxan for two consecutive days.

showed higher levels of blood ketone bodies maintained throughout the experiment when compared to those of group II (Diabetic control).

It is evident from Table I that although alloxan diabetic animals (group II) getting a single dose of alloxan did not have any significant alteration in the vitamin B12 levels of either blood or tissue of those diabetic animals, which were having a high level of ketone bodies maintained for a prolonged period, showed a significant decrease in blood and tissue vitamin B12 levels. The maximum deleterious effect was found in animals from group V. This was followed by animals from group IV and III. The above findings suggest that diabetic animals would cause an increased requirement of vitamin B12 only when the animals maintained high levels of ketone bodies during the diabetic state per se.

The picture of hepatic constituents of animals from various groups are described in Table 2. Hepatic RNA, protein and total sulfhydryl were decreased considerably in animals of group III, IV and V. The animals affected most were those of group V. Hepatic DNA, however, did not show appreciable alteration in any of the groups.

DISCUSSIONS

Although contradictory evidences on vitamin B12 status were reported by the earlier workers (4,5) in diabetes, little efforts were made by them to supply with a possible explanation for the above differences on the subject. Our results clearly
show that although in diabetic animals the vitamin B$_{12}$ status did not change on one injection of alloxan, the maintenance of ketone bodies in the diabetic state caused much decrease in blood and tissue vitamin B$_{12}$ at the end of 30 days. The decrease was also found to be in proportion to the blood ketone bodies in the animals. The low serum vitamin B$_{12}$ levels in diabetics found by earlier workers (8) were probably only in cases, where a state of ketosis prevailed for a considerable amount of time. In this context, it is worth mentioning that our preliminary findings on vitamin B$_{12}$ levels in diabetic patients showed a considerable decrease in serum vitamin B$_{12}$ levels only when the patient had severe ketosis for a prolonged period. From the present investigation it becomes evident that vitamin B$_{12}$ has some close relationship with ketone bodies.

REFERENCES