Effect of Various High Protein Diets on Vitamin $B_{12}$ Status in Rats

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Various workers have suggested an increased Vitamin $B_{12}$ requirement in animals fed different high protein diets. From the literature studies, relatively little information has been available on the vitamin $B_{12}$ status of the animals maintained on high protein rations. An attempt was, therefore, made to find out the vitamin levels in these animals. Animals were fed two different high protein diets with varied ketogenesity, one having egg albumin as the protein source and the other soybean. Vitamin $B_{12}$ levels in blood, liver and kidney were found to be depleted considerably in high protein fed animals. Egg albumin which is known to contain more ketogenic amino acids than the soybean protein was found to cause more ketogenesis and more depletion of vitamin $B_{12}$ in the liver, kidney and blood in the animals. A possible role of high protein diet on vitamin $B_{12}$ status was discussed in the light of the observations on ketogenic potency of the protein. Glucose cyclo-acetoacetate hydrolysate, which was shown earlier to be a ketolytic compound, was found to prevent, to some extent, the depletion of vitamin $B_{12}$ levels in high egg albumin fed animals.

Effect of different dietary constituents on vitamin $B_{12}$ requirement in animals have been studied by various workers earlier (1, 2, 3). Numerous reports have been obtained to implicate vitamin $B_{12}$ in protein metabolism (4, 5). It was observed that young rats depleted of vitamin $B_{12}$ and receiving either vitamin-free casein or soybean protein as the protein source of an adequate diet, show superior weight gains when vitamin $B_{12}$ was administered daily by intramuscular injection. Similar observations were also made by the same authors in the case of animals fed with high levels of protein (6). Hartman et al. (7) reported that high protein diet causes a deleterious and sometimes a fatal effect on vitamin $B_{12}$ deficient rats. Peterson and Register (8) have obtained a great degree of vitamin $B_{12}$ deficiency in rats fed either high protein or high fat rations, as reflected from the studies on gain in body weight. They also noticed that high levels of fat increased the deficiency further when the rats were fed on high protein diet. Strikingly enough, little information is available on blood and tissue vitamin $B_{12}$ status in high protein fed animals.
Khanade and Nath (9) have observed an increased ketogenesis in liver and decreased utilization of ketone bodies in kidney in rats fed high fat-high protein diet. Recent investigations of Nath and Nath (10) evidenced a decrease in vitamin B$_{12}$ levels in rats injected with ketone bodies. Feeding different high fat diets was shown to cause a decrease in vitamin B$_{12}$ levels in rats, in accordance with their ketogenic potency (11).

In the light of these findings, it was felt worth investigating the levels of vitamin B$_{12}$ in blood and tissues of high protein fed animals. In addition, a comparative study on vitamin B$_{12}$ status in animals fed two different high protein diets (with varying content of ketogenic amino acids) was also contemplated.

Hydrolysed product of glucose cycloacetoacetate (GCAA) was reported to be an antiketogenic compound from this laboratory earlier (12). GCAA was also observed to potentiate the vitamin B$_{12}$ activity in E. coli 113-3 (13) and play a beneficial role in maintaining vitamin B$_{12}$ levels in ketone bodies injected rats (14). Hence, the role of this compound, on vitamin B$_{12}$ status in rats fed high protein diet was also investigated.

**EXPERIMENTAL**

Forty male albino rats (weighing about 150 g each) were divided equally into four groups and were caged individually. All groups were maintained on different diets as represented in Table 1. Group I was the control group, whereas animals from groups II and III were fed on high egg albumin and group IV on high soybean diet. Group III animals received daily 20 mg/100 g body weight of the hydrolysed product of glucose, GCAA, by subcutaneous injection. Water was provided *ad libitum*. Care was taken to prevent coprophagy.

After 11 weeks of feeding, animals were killed by decapitation and blood was collected in oxalated tubes. Liver and kidney were excised quickly, frozen in cracked ice, dried with filter paper, weighed accurately and homogenized in a previously chilled Potter-Elvehjem glass homogenizer. Vitamin B$_{12}$ contents were determined in liver, kidney and blood. Total ketone bodies, and total sulphydryls were estimated.

*Liver Ketone Bodies* — Total ketone bodies of liver were determined by the method of Greenberg and Lester (17).

<table>
<thead>
<tr>
<th>Components of diet</th>
<th>Group I (control)</th>
<th>Group II and III (high egg albumin)</th>
<th>Group IV (High soybean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>55</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Vitamin-free casein</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt mixture (Hawk and Oser)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Egg albumin</td>
<td></td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture (in 10 g sugar)$^a$</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ Vitamin mixture includes, thiamine hydrochloride 40 µg, riboflavin 80 µg, nicotinic acid 500 µg, p-aminobenzoic acid 250 µg, inositol 2 mg, pyridoxine hydrochloride 40 µg, choline chloride 5 mg, biotin 0.2 µg, folic acid 10 µg, calcium pantothenate 250 µg, vitamin K (menadione) 50 µg, vitamin A and D (Adexolin) 2 to 3 drops/rat/day.
Liver Total Sulfhydryl —— Total sulfhydryl content of liver were found out by the Buttler’s modification (18) of Phillips and Grunerts method.

Vitamin \textit{B}_{12} Assay —— Vitamin \textit{B}_{12} was assayed microbiologically by using \textit{Euglena gracilis var. bacillaris} as the test organism (19).

\textbf{RESULTS AND DISCUSSION}

Animals fed on either of the high protein diets show an increase in the liver ketone bodies with a corresponding decrease in vitamin \textit{B}_{12} levels (Table 2). Kidney was found to be affected most. The depletion in vitamin \textit{B}_{12} content in blood and tissue is more conspicuous in animals fed high egg albumin ration. Hydrolysed product of GCAA could improve the vitamin \textit{B}_{12} status in these animals significantly. With increased ketogenesis, a decrease in liver sulfhydryl levels is also evident from these studies.

It is known from the available data on the amino acid composition of proteins (20) that egg albumin contains higher amounts of ketogenic amino acids, such as leucine, isoleucine, tyrosine and phenylalanine than those present in soybean protein. It was observed by Lerner (21) and Edison (22) that in high protein fed animals certain amino acids and ammonia liberated during the deamination process are ketogenic. Our results are in accordance with the above observations, presenting higher ketogenesis in animals fed on high protein diets. Nath and Nath (10) have demonstrated a significant decrease in vitamin \textit{B}_{12} levels in ketone bodies injected animals. It was also found that the effect of various high fat diets on the levels of this vitamin varied in accordance with their ketogenic petency. The saturated fat diets with higher ketogenic potency caused a lowering of the vitamin \textit{B}_{12} levels more significantly than that caused by the lesser ketogenic high fat diet, the unsaturated ones (11). Similar results are indicated in our present investigation, where vitamin \textit{B}_{12} levels are the lowest in high egg albumin fed animals having the highest ketogenesis.

Peterson and Register (8) stressed over the large requirements of vitamin \textit{B}_{12} in high protein fed animals and thought it to be due to an important role played by this vitamin in the utilisation of protein in non-protein functions. Recently Nath (23) has postulated that ketone bodies hamper the sulfhydryl metabolism

\begin{table}
\centering
\caption{Vitamin \textit{B}_{12} Levels in Blood, Liver and Kidney of Experimental Animals}
\begin{tabular}{ |l|c|c|c|c|c|c| }
\hline
Group & Blood & Liver & Kidney & \multicolumn{2}{|c|}{Liver} \\
 & mg/g/ml & mg/g & \( \mu g/g \) & Totat ketone bodies & Total sulfhydryl \\
\hline
I. Normal & 0.80±0.1 & 120±8 & 0.92±0.1 & 0.75±0.2 & 1.2±0.2 \\
II. High egg albumin. & 0.52±0.2\(^a\) & 81±6\(^a\) & 0.45±0.1\(^a\) & 3.2±0.4\(^a\) & 0.74±0.1\(^a\) \\
III. High egg albumin + GCAA. & 0.69±0.1\(^b\) & 112±7\(^b\) & 0.72±0.2\(^b\) & 1.7±0.3\(^b\) & 0.92±0.1\(^b\) \\
IV. High-soybean. & 0.65±0.2\(^a\) & 104±8\(^a\) & 0.70±0.1\(^a\) & 1.2±0.1\(^a\) & 0.98±0.1\(^a\) \\
\hline
\end{tabular}
\label{table:2}
\end{table}

\(^a\) Indicates \( p \) less than 0.01 from group I using Student’s "t test".

\(^b\) Indicates \( p \) less than 0.05 from group II using Student’s "t test".
greatly, thereby inhibiting catalase activity, resulting in accumulation of hydrogen peroxide, which in turn brings about an inactivation of vitamin B₁₂. The vitamin B₁₂ depletion observed in high protein fed animals in the present study might be due to either of the reasons or due to both of them. But the pattern of ketogenesis and sulfhydryl distortion observed favour to some extent the latter explanation. Although it is premature, at this stage, to suggest any definite reason for the fall of vitamin B₁₂ levels in these animals, yet ketogenesis is reflected to be an important factor in assessing the vitamin status.

Earlier, hydrolysed product of GCAA was found to be an antiketogenic substance (12), which could protect the disturbances caused in serum protein levels (24), serum enzymes (25) and vitamin B₁₂ levels (14) in acetoacetate induced anaemia. Since our observations on GCAA partially preventing the fall in vitamin B₁₂ levels of high protein fed animals are in line with the earlier findings, the effect of GCAA can be accounted from its antiketogenic property.

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