FACTORS AFFECTING THE RIBOFLAVIN CONTENT OF THE LIVER

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Although the concentration of riboflavin in animal tissue tends to follow the intake, the available data are quite inadequate for establishing a consistent relationship which might aid in anticipating general requirements or the demand imposed by particular phases of metabolism. Physical exercise (1) and high fat diets (2) have been reported to increase the demand, and recorded data show that the riboflavin concentration in the liver may vary more than 100 per cent when calculated either on the basis of fresh tissue or the dry basis. No consistent correlation was found, however, between the concentration in muscle tissue and blood and the intake in rats (3) or humans (4). It is assumed, however, that a certain minimum concentration in the tissues is essential for maintaining life, but there has been no recorded evidence of a transient "mobilization" in the liver to meet immediate and particular requirements of assimilation and metabolism.

Since riboflavin is essential for physiological oxidation and because primary or partial oxidation of fatty acids, for example, is believed to be localized in the liver, a series of studies was designed to determine whether temporary variations in riboflavin concentration were induced under the stimulus of digestion and assimilation. The particular objective was to determine the riboflavin concentration in the liver following the ingestion of food and injection of riboflavin, thiamine, pyridoxine, and pantothenic acid following previous impoverishment in the animal of each of these factors.

EXPERIMENTAL

The experimental plan involved the depletion of large groups of white rats, 23 to 25 days old at weaning, of the particular factor whose influence it was desired to study; substantially 400 animals were used for obtaining the records presented here. The basal ration was identical for all groups, depletion of a particular factor being accomplished by omission of that substance from the primary supplements furnished as a known dosage per rat per day (Table I). The basal ration consisted of vitamin-free casein (Labco) 20, sucrose 69, hydrogenated vegetable oil (Crisco)
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3, Salt Mixture 40 (5) 4, powdered agar-agar 2, and medicinal cod liver oil 2 parts. Various groups of animals were prepared by depleting them of riboflavin, thiamine, vitamin B₆, or pantothenic acid; other groups received none of the primary supplements for a period of 3 or 4 weeks following weaning, it being impossible to maintain the animals for longer periods in the absence of all these factors. Still other groups were prepared for determining the influence of tissue saturation with thiamine. Such preparation involved depletion until the animals exhibited a medium degree of paralysis, at which time they received 100 γ of thiamine orally per day for 5 days. Additional groups were used for determining the influence of an increased amount of pantothenic acid. Such animals received 50 γ of

<table>
<thead>
<tr>
<th>Substance supplied</th>
<th>For riboflavin depletion</th>
<th>For thiamine depletion</th>
<th>For vitamin B₆ depletion</th>
<th>For pantothenic acid depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin, γ</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Thiamine, γ</td>
<td>0.75</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Rice polish concentrate (Labco), autoclaved, pH 8.5, mg.*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Rice polish factor 2 (Labco), mg†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B₆, γ</td>
<td></td>
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</tr>
</tbody>
</table>

* Contains no vitamin B₁, or riboflavin, but does contain 11 to 13 γ of pantothenic acid and 7 to 8 γ of vitamin B₆ per 100 mg.
† Contains no vitamin B₁, riboflavin, or vitamin B₆, but does contain 20 γ of pantothenic acid per 100 mg.
‡ Contains no vitamin B₁, riboflavin, vitamin B₆, or over 0.3 γ of pantothenic acid per 100 mg.

pantothenic acid for 13 weeks in addition to the other requisite primary supplements. Depletion of each of the factors was determined by the criteria prevailing at these laboratories (3, 6–8).¹

Following appropriate preparation, the requisite number of animals were fasted for a 24 hour period prior to the administration of 1 gm. of the basal ration and supplements other than the particular one under investigation. The food was given in semifluid form by forced feeding with a blunt hypodermic needle. (A degree of fluidity of the basal ration suitable for handling in this manner was obtained by mixing 50 gm. of the dry ration with sufficient water to make a 60 ml. volume.) The vitamin factors were administered singly or in combination as hereinafter noted, usually in 100

¹ See also Supplee, G. C., Bender, R. C., and Kahlenberg, O. J., unpublished data.
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γ quantities, in 0.5 ml. of physiological salt solution by injection directly into the heart. This procedure avoided certain uncontrolled features such as loss through excretion, variable absorption, and other unknown elements of control incident to oral feeding or injection at other sites.

Animals ready to receive a particular test substance were divided into two major groups with an equal number of each sex. One group which received 1 gm. of food only served as the negative control; data from this group are representative of the effect accruing from previous depletion or impoverishment of a particular factor. The second group received 1 gm. of food and also the injected vitamin; the data are representative of the effect of the vitamin as manifested during the following 24 hour period. From eight to twelve animals from each test group which received neither the 1 gm. of food nor the vitamin injection were sacrificed at the end of the preliminary 24 hour fast period and the liver immediately removed for analysis. All animals in the subgroups which were fed, or fed and injected, were returned to screen bottom metal cages and supplied with water only. At intervals of 4 hours, at least four animals from each group were sacrificed and the liver immediately removed for analysis. Glycogen was determined in one lobe of the liver by the method of Good, Kramer, and Somogyi (9); the results were used for calculating the glycogen content of the whole liver. Riboflavin determinations (7) were made on the remaining portion following desiccation and extraction of total lipids. All riboflavin values are expressed as micrograms per gm. of water-fat-glycogen-free liver tissue. In some instances the glycogen, riboflavin, and lipid determinations were made on pooled samples. In the greater majority of cases, however, determinations were made on individual specimens and the results averaged. For convenience of interpretation and comparison, the available results are presented in graphical form in Figs. 1 to 5.

Figs. 1 and 2 clearly reveal that the concentration of riboflavin in the water-fat-glycogen-free liver tissue increases during digestion and assimilation even in animals impoverished of this factor as a result of receiving a riboflavin-free diet for several weeks. (The records in Fig. 1 are from animals deprived of all the water-soluble vitamins for 3 weeks, whereas the results in Fig. 2 are from animals deprived of riboflavin only for 8 weeks.) This mobilization is temporary and the accentuated concentration tends to recede to the lower level prevailing prior to the demands imposed by assimilation of the test meal. The mobilization of riboflavin in the liver of impoverished animals not injected with this factor is relatively slow, the peak concentration being reached only after 12 to 16 hours; following this period the riboflavin level decreased rapidly to the prefeeding level. The animals which received the 100 γ injection showed a more rapid rise in the riboflavin level with less abrupt decline following the peak concen-
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...tration. The results from the animals receiving the 25 γ injection are analogous to those from the animals injected with the higher amount, but of somewhat lower magnitude.

It will be recalled that in each instance the data presented in Figs. 3, 4, and 5 were obtained from animals receiving 10 γ of riboflavin per day during the depletion of one or another of the factors under investigation, and accordingly extreme riboflavin impoverishment did not prevail.

The data from the animals impoverished of thiamine to the paralytic state (Fig. 3, Group 1) show only a slight riboflavin mobilization tendency in the liver during digestion; this tendency was not increased by the injection of 100 γ of thiamine. The magnitude of increase, 8 hours after feeding, in riboflavin concentration in the livers of the animals which had previously received 100 γ of thiamine per day orally for 5 days is markedly accentuated in comparison with the thiamine-impoverished animals; the injection of 100 γ did not greatly increase the riboflavin level above that of the controls (Fig. 3, Group 2).

The data from the pantothenic acid studies (Fig. 4) yield riboflavin concentration curves which seem to show a direct interacting influence between these factors in the animal body. The pantothenic acid-impoverished animals (Fig. 4, Group 1) showed no evidence of an increase in
riboflavin concentration in the liver during digestion and assimilation. However, the injection of 100 γ of pantothenic acid at the time of feeding caused a slight elevation in riboflavin level which prevailed throughout the 24 hour observation period.

The results from the animals (Fig. 4, Group 2) receiving 50 γ of pantothenic acid for 13 weeks in addition to the usual supplements are substan-

![Fig. 3](image1)

**Fig. 3.** Riboflavin concentration in the liver as influenced by the progress of assimilation and injection of thiamine. All the animals of Group 1 were fed 1 gm. of food at the beginning of the 24 hour observation period; supplements during depletion, 10 γ of riboflavin, 0.75 γ of thiamine, and 100 mg. of autoclaved (pH 8.5) rice polish concentrate; 100 γ of thiamine were injected. The average weight of the animals was 50.3 gm. The animals in Group 2 received the same treatment as those in Group 1 but with the exception that 100 γ of thiamine were supplied orally each day for 5 days preceding the 24 hour fast prior to the observation period. The average weight of the animals was 64.2 gm. Riboflavin is expressed as micrograms per gm. of lipid-glycogen-water-free liver tissue. Curve A, controls, not injected; Curve B, 100 γ of thiamine injected.

![Fig. 4](image2)

**Fig. 4.** Riboflavin concentration in the liver as influenced by the progress of assimilation and injection of pantothenic acid. All the animals in Group 1 were fed 1 gm. of food at the beginning of the 24 hour observation period; supplements during depletion, 10 γ of riboflavin, 12.5 γ of thiamine, 10 γ of vitamin B₆, and 100 mg. of autoclaved (pH 11) rice polish factor 2; 100 γ of pantothenic acid were injected. The average weight of the animals was 79.3 gm. The animals in Group 2 received the same treatment as those in Group 1 but with the exception that 50 γ of pantothenic acid were supplied daily in addition to the other supplements for 13 weeks prior to the observation period. The average weight of the animals was 152.5 gm. Riboflavin is expressed as micrograms per gm. of lipid-glycogen-water-free liver tissue. Curve A, controls, not injected; Curve B, 100 γ of pantothenic acid injected.

...tially different from those from the pantothenic acid-impoverished animals. The animals in Group 2 had an average weight more than twice that of the animals in any of the other groups. Inasmuch as the riboflavin supplement of 10 γ per day was maintained as a constant dosage irrespective of depletion time and final weight (groups in Figs. 1 and 2 excepted), the relatively lower initial concentration in the liver is probably explained by the greater tissue demand of the larger animals. It will be noted, however, that the...
concentration increases to a peak level in the controls 12 hours after feeding and declines thereafter (see the similar pattern for controls on the riboflavin-free ration, Fig. 2). Injection of 100 \( \gamma \) of pantothenic acid caused a more rapid (and somewhat phenomenal) rise in riboflavin concentration notwithstanding the limited supplementation of 10 \( \gamma \) per day than was manifested by any of the other groups, even including those injected with 100 \( \gamma \) of riboflavin (Fig. 2). An equally abrupt decrease followed 8 hours after injection and feeding. This particular reaction pattern indicates a specific influence of pantothenic acid in mobilizing riboflavin in the liver incident to the functional demands imposed by digestion and assimilation.

The results from the vitamin B₅ studies (Fig. 5) did not show extreme variations in riboflavin concentration in the liver in either the control or the injected animals; slight evidence of a temporary increase was shown by both groups 12 hours after feeding.

![Fig. 5. Riboflavin concentration in the liver as influenced by the progress of assimilation and injection of vitamin B₅.](http://www.jbc.org/)

The data presented illustrate the results obtained by methods designed to determine the influence of particular vitamin entities and their interaction on certain basic phenomena involved in metabolic processes. Although riboflavin is slowly lost through the excretions even on an impoverished dietary, the data lead to the conclusion that an involuntary mechanism excited by the ingestion of food induces a temporary mobilization of this factor in the liver to meet functional demands. Among the vitamin factors included in this study, pantothenic acid appears to have a more specific and direct effect upon those processes which cause this mobilization than does thiamine or pyridoxine. However, the evidence also indicates that thiamine, significantly but more indirectly, is also involved in maintaining this function.

**SUMMARY**

1. Injection of riboflavin directly into the blood stream causes an immediate increase in concentration in the liver.
2. The riboflavin concentration in the liver increases during digestion and assimilation, being mobilized therein presumably from other tissues; this transient concentration takes place even in animals whose tissue stores have been impoverished by a prolonged riboflavin-free dietary.

3. The mobilization of riboflavin in the liver during digestion and assimilation, by thiamine-depleted animals, is relatively slight. Thiamine replenishment of depleted tissues by oral feeding brings about a restoration of the riboflavin-mobilizing function.

4. Pantothenic acid appears to have a direct and specific function in the mechanism which causes the mobilization of riboflavin in the liver following ingestion of food.

5. The influence of vitamin B₆ on the concentration of riboflavin in the liver was found to be relatively slight in comparison with the apparent influence of the other factors studied.

BIBLIOGRAPHY

FACTORs AFFECTING THE RIBOFLAVIN CONTENT OF THE LIVER

G. C. Supplee, O. G. Jensen, R. C. Bender and O. J. Kahlenberg

J. Biol. Chem. 1942, 144:79-85.

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