Some anabolic aspects of protein metabolism in riboflavin deficiency in the rat*

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Flavin nucleotides are components of certain enzyme systems which are involved in the oxidation of nitrogenous compounds, including at least some of the amino acids. The oldest and best-known evidence for this relationship was derived from studies of biochemical systems in vitro, but it is also supported by the results of studies on intact animals. For example, Burch, Lowry, Padilla & Combs (1956) showed that when rats were deprived of riboflavin for 6 weeks the activities of xanthine oxidase and of several amino-acid oxidases in their livers were substantially reduced.

Kaunitz, Wiesinger, Blodi, Johnson & Slanetz (1954) observed that the deleterious effects of riboflavin deficiency on rats were more pronounced the higher the intake of protein, and concluded that riboflavin and protein are mutually limiting factors in nutrition. It has also been shown that on a diet low in protein the riboflavin content of the organs of rats decreases (McQuarrie & Venosa, 1945; Czaczkes & Guggenheim, 1946), regardless of the intake of the vitamin, and that the urinary excretion of it increases (Czaczkes & Guggenheim, 1946). With higher intakes of protein, however, the amount of the vitamin in organs was found to increase as its urinary excretion decreased (Czaczkes & Guggenheim, 1946). There have been other reports (Sarett & Perlzweig, 1943; Sherman & Ragan, 1947) that rats retain riboflavin in proportion to the intake of protein, and Sarett, Klein & Perlzweig (1942) showed that when positive nitrogen balance was induced in dogs and rats less riboflavin was excreted from test doses than when the nitrogen balance was negative.

None of these findings are necessarily at variance with the role of riboflavin in catabolic processes in protein metabolism. On the other hand, they do not contradict the concept that it is involved in anabolic processes, for which there is also direct evidence.

Kleiber & Jukes (1942) observed that chicks deficient in riboflavin stored about 30% less of their dietary nitrogen than pair-fed controls, and the difference was reflected in their body-weight, which would indicate that the nitrogen storage was

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associated with the synthesis of protein. Borgström & Hammarsten (1944) found that in rats the intake of riboflavin was directly related to the degree of nitrogen storage and to the ratio of gain in weight to food intake. Mayfield & Hedrick (1949) found essentially the same, but calculated the response in terms of the biological value of the dietary protein, which increased with increasing riboflavin intake. Frei & Ryser (1956) found that in riboflavin-deficient rats there was a substantial decrease in the amount of protein in liver mitochondria.

Some of the more recent work of Guggenheim & Diamant (1959) has provided information of particular importance for assessing this relationship. They found that in riboflavin deficiency in the rat the concentration of nitrogen in the liver was not affected, but that the liver was enlarged; and there was a decrease in the concentration of nitrogen in the carcass. Consequently the liver contained more nitrogen per unit of body-weight, and a higher proportion of the total nitrogen of the body. In comparison with various types of control animals, the proportion of nitrogen in the body did not differ importantly, but more of it was present in the liver. These observations point to an alteration of protein metabolism in riboflavin deficiency, characterized by what could be an impairment of the anabolic processes in muscle, and an enhancement of them in the liver. This effect is important, because there is much evidence that the protein of liver, or at least an important fraction of it, is particularly labile, and sensitive to dietary depletion and repletion (Addis, Poo & Lew, 1936a–c; Kosterlitz, 1944; Harrison & Long, 1945; Stanier, 1957). It could reasonably be expected, therefore, that this particularly active component of the body’s protein-producing system would be highly sensitive to a deficiency of any factor such as a vitamin that may be involved in an anabolic process. If it is so the findings of Guggenheim & Diamant (1959) do not indicate such a role for riboflavin.

The variations and conflict among these many diverse findings have produced a confused picture of the overall or resultant effect of riboflavin deficiency upon protein metabolism. That the question is of interest is attested by the amount of experimental work that has been devoted to it, and it is of importance because of the implication of a quantitative relationship between two nutrients.

We undertook the experiments herein described to obtain more information on this relationship, particularly concerning the importance to protein synthesis of an adequate supply of riboflavin. We measured the levels of liver nitrogen and of blood proteins in rats after growth impairment by deprivation of riboflavin. In some it was done after a short period of rapid protein repletion when the activity of the anabolic processes had been increased by previous protein depletion.

**EXPERIMENTAL**

The percentage composition of the diets used in the experiments was: vitamin-test casein 0, 16, and 40, with corresponding content of sucrose of 85, 69 and 45; cellulose flour 4; maize oil 7; salt mixture (Wesson’s (1932) modification of the Osborne & Mendel salt mixture, purchased as Salt Mixture W from Nutritional Biochemicals Corporation, Cleveland, Ohio) 4. To every 100 g of this mixture were added: cod-
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Liver oil concentrate (Ayerst, McKenna & Harrison Ltd (Montreal) Special Formula no. 33 101) 0.2 mg (40 i.u. vitamin A and 10 i.u. vitamin D), \( \alpha \)-tocopheryl acetate 3 mg, menaphthone 0.2 mg, choline chloride 100 mg, inositol 50 mg. Water-soluble vitamins were given to each rat daily by subcutaneous injection: thiamine hydrochloride 12.5 \( \mu \)g, pyridoxine hydrochloride 10 \( \mu \)g, calcium pantothenate 100 \( \mu \)g, nicotinic acid 20 \( \mu \)g, biotin 2 \( \mu \)g, folic acid 2 \( \mu \)g, vitamin B\(_12\) 0.01 \( \mu \)g. Those rats in control groups were given in addition 25 \( \mu \)g riboflavin.

Young male and female rats of the Wistar strain were used, sixty in each of three experiments, and 117 in a fourth. Their weights ranged between 70 and 90 (\( \sigma \)) or between 80 and 100 (\( \delta \)) g. They were divided equally among three groups so that the mean body-weight of those in each was the same. The animals in groups A and B were given riboflavin, and those in group C were not. The last showed varying degrees of anorexia with time, and impairment of growth when compared with the animals of group A, which were maintained on the same amount of food. The animals of group B were given smaller amounts of food, to keep their weights similar to those of group C. All animals were maintained individually in screen-bottomed cages. Water was supplied to them \( ad \ libr \). The rats were given the diet containing 16\% protein for 45–76 days, at which times the mean body-weight of group A differed from that of groups B and C by 10–15\%.

Expts 1 and 2 were terminated at this stage. In both, the livers and samples of blood, and in Expt 2 the kidneys also, were taken for analysis. At a corresponding stage in Expt 3 eight rats from each group, A, B and C, were killed. The rest were depleted of protein by dietary deprivation for 21 days, and moderate haemorrhage was imposed on the last day to deplete further the body proteins. On the following day four animals from each group were killed. The rest were put on the diet containing 40\% protein for a repletion period of 3 days, after which they were killed. After the animals were killed the livers and samples of blood were obtained for analysis. In Expt 4 the procedure was similar to that in Expt 3, but only half the animals were subjected to haemorrhage at the end of the period of dietary deprivation of protein. Two sets of results were thus obtained from this experiment, determined by whether haemorrhage was imposed (\( b \)) or not (\( a \)). The numbers of animals for which information was obtained at each stage, in the same order as noted for the preceding experiment, were five to seven, six, and eight to ten, for both (\( a \)) and (\( b \)).

Haemorrhage was accomplished by immersing the tail in warm oxalate solution after the end had been cut off. Blood equivalent to 2\% of the body-weight was removed, after which the animal was given intraperitoneally 5 ml physiological saline solution.

The rats were killed with pentobarbital sodium and diethyl ether. They were weighed, as were also the livers and kidneys, and weight relationships between the organs and the body were calculated. Blood was taken from the heart, and oxalated.

Haemoglobin was determined by a methaemoglobin method (Collier, 1944), and plasma protein by a biuret method (Gornall, Bardawill & David, 1949). Liver and kidneys were made into 'breis' with water, and their total nitrogen was determined by a titrimetric micro-Kjeldahl method (Sobel, Mayer & Gottfried, 1944; Hiller, Plazin &...
Van Slyke, 1948). In Expt 2 the proportion of solid material in liver was calculated from the weight of residues from portions of the brei heated to constant weight at 80° in a draught oven.

To evaluate the difference between some of the mean values obtained Student's $t$ test was used, as applied to small samples (Davenport & Ekas, 1936).

RESULTS

Tables 1 and 2 show that in the rat the concentrations of blood haemoglobin, plasma protein, and liver and kidney nitrogen are not affected by riboflavin insufficiency over a period long enough substantially to impair the growth of the animals. In the riboflavin-deficient rats there was a tendency for the liver to assume a greater proportion of the body-weight. Consequently the liver content of nitrogen in relation to body-weight tended to be greater in these animals than in those with adequate riboflavin. This finding is consistent with those of Guggenheim & Diamant (1959). If this

Table 1. Expt 1. Weight and nitrogen content of livers, and concentration of plasma proteins, of rats after 57 days on a diet containing 16% protein

<table>
<thead>
<tr>
<th>Nutritional state</th>
<th>No. of rats</th>
<th>Fresh weight as % of body-weight</th>
<th>Nitrogen content</th>
<th>Plasma protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver g/100 g</td>
<td>g/100 g body-weight</td>
<td>Liver mg/100 g</td>
</tr>
<tr>
<td>A (food controls)</td>
<td>20</td>
<td>3.3 (2.9 - 3.7)</td>
<td>3.4 (3.2 - 3.7)</td>
<td>113 (103 - 120)</td>
</tr>
<tr>
<td>B (weight controls)</td>
<td>20</td>
<td>3.1 (2.7 - 3.5)</td>
<td>3.5 (3.2 - 3.7)</td>
<td>110 (103 - 118)</td>
</tr>
<tr>
<td>C (riboflavin-deficient)</td>
<td>20</td>
<td>3.8 (3.2 - 4.9)</td>
<td>3.5 (3.2 - 4.4)</td>
<td>134 (111 - 159)</td>
</tr>
</tbody>
</table>

Significance of difference between A and C

- $P < 0.01$

Table 2. Expt 2. Weight and nitrogen content of livers and kidneys, and concentrations of blood haemoglobin and plasma proteins, of rats after 51 days on a diet containing 16% protein

<table>
<thead>
<tr>
<th>Nutritional state</th>
<th>No. of rats</th>
<th>Dry-matter content (%)</th>
<th>Fresh weight as % of body-weight</th>
<th>Nitrogen content</th>
<th>Blood haemoglobin content (g/100 ml)</th>
<th>Plasma protein content (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver g/100 g</td>
<td>g/100 g body-weight</td>
<td>Liver mg/100 g</td>
<td>Kidney g/100 g</td>
</tr>
<tr>
<td>A (food controls)</td>
<td>20</td>
<td>(28 - 32)</td>
<td>3.1 (2.8 - 4.4)</td>
<td>85 (81 - 117)</td>
<td>0.8 (0.7 - 1.0)</td>
<td>2.7 (1.9 - 3.0)</td>
</tr>
<tr>
<td>B (weight controls)</td>
<td>20</td>
<td>(27 - 32)</td>
<td>2.8 (2.5 - 3.0)</td>
<td>88 (80 - 106)</td>
<td>0.8 (0.7 - 1.0)</td>
<td>2.6 (1.8 - 3.1)</td>
</tr>
<tr>
<td>C (riboflavin-deficient)</td>
<td>20</td>
<td>(27 - 35)</td>
<td>3.5 (2.8 - 4.5)</td>
<td>97 (79 - 126)</td>
<td>0.9 (0.7 - 1.0)</td>
<td>2.5 (1.9 - 2.8)</td>
</tr>
</tbody>
</table>
increase were in non-protein nitrogen (NPN), it is likely that it would be reflected in an increased concentration of this fraction in the blood. In a similar experiment not described here we measured the blood NPN, and found mean values for groups A, B and C of 40.6, 33.8 and 39.7 mg/100 ml respectively. We therefore interpret the changes in liver nitrogen as reflections of changes in the protein.

Fig. 1 gives the results of the two experiments in which the regeneration of protein after protein depletion was studied. In these experiments the concentrations of the
protein fractions studied were affected in the same way regardless of the state of riboflavin nutrition of the animal. The peculiarity seen in the previous experiments was again evident in the livers of the riboflavin-deficient rats. Not only before the period of protein depletion, but also in the subsequent period of repletion, the ratio of liver-weight to body-weight, and consequently of liver content of nitrogen to body-weight, since the concentration of nitrogen was not affected, tended to be larger.

**DISCUSSION**

The proteins measured in these experiments are among the most important of the body proteins. That of the liver is probably the most labile and the most sensitive to depletion and repletion. In particular it would be expected that if protein synthesis is affected it would be evident in changes in this fraction when stress is put upon the anabolic system.

These experiments produced evidence that a deficiency of riboflavin in the rat does not impair the maintenance and synthesis of certain proteins of the body that are quantitatively and metabolically important. Previous findings that indicate that the state of riboflavin nutrition is directly related to nitrogen storage and to the maintenance of specific proteins do not therefore necessarily indicate that the relationship applies to all the proteins of the body. The tendency toward accumulation of more protein in the liver in riboflavin deficiency, even under conditions of active protein repletion, is particularly important. The evidence from these experiments strengthens the belief that anabolic processes in protein metabolism are not adversely affected by riboflavin deficiency.

Bartlett & Glynn (1950) found that in hypophysectomized rats when marked increase in weight was induced by growth hormone, there was an accompanying small decrease in the size of the liver, which occurred under conditions of paired feeding. They suggested that the liver proteins were supplying material for the enlargement of other parts of the body. Our values, and those of Guggenheim & Diamant (1959), present an opposite set of metabolic circumstances, and could be interpreted to indicate that in riboflavin deficiency, although protein synthesis may not be impaired, some transfer from liver to peripheral tissues may be. If it should be so, the function of the anterior pituitary might be involved. This possibility of impaired transfer of proteins from liver to muscle offers another explanation of our observations than that which postulates increased synthesis in liver and probably decreased synthesis in muscle.

A decreased capacity for glyconeogenesis (Reade & Morgan, 1949; Forker & Morgan, 1954, 1955) and an increased sensitivity to insulin (Ershoff, 1954) have been found in riboflavin-deficient rats, with evidence in each instance that they are the result of impaired adrenal cortical function, and some evidence that the function of the pituitary is involved (Forker & Morgan, 1954, 1955).

There is evidence that cortical hormones are importantly involved in the metabolic production of carbohydrates from amino acids (Evans, 1935–6; Long, Katzin & Fry, 1940; Long, 1942; Lewis, Thorn, Koepf & Dorrance, 1942); therefore reduced cortical function is consistent with conservation of amino acids. On the other hand, the
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administration of cortical extract to rats has been shown to decrease the capacity of the
carcass to increase its protein content (Clarke, 1953) but to increase that of the liver
(Clark, 1953; Roberts, 1953). Guggenheim & Diamant (1959) also observed that
when rats were treated with cortisone the distribution of body proteins was similar to
that in riboflavin deficiency.

The effects of riboflavin deficiency upon protein metabolism in the rat therefore
cannot clearly be ascribed to impairment of pituitary or adrenal function.

**SUMMARY**

1. In four experiments young albino rats were divided into three groups with the
same mean body-weight. They were given a diet containing 16% protein. Those of
one group were deprived of riboflavin. Those of another were given the same amount
of food, and those of the third group were given less food to keep their body-weights
within the same range as for those deprived of riboflavin. Depending upon the experi-
ment, the animals were maintained on the regimen for 45–76 days, at which time the
body-weights of those deprived of riboflavin were 10–15% lower than of those that
had received the same amount of food, with riboflavin.

2. At this stage the concentrations of blood haemoglobin, plasma protein, and liver
nitrogen were essentially the same in all the animals. When dietary protein depletion,
with or without moderate haemorrhage, was imposed, followed by repletion on a diet
containing 40% protein, the concentrations of these three body-protein fractions
behaved in the same way regardless of the state of riboflavin nutrition or of body-
weight. They fell and were restored approximately to the same extent and at the same
rate.

3. The livers of the riboflavin-deficient rats tended to assume a greater proportion
of the body-weight, even during protein repletion, consequently the ratio of liver
nitrogen content to body-weight tended to be greater than in animals with adequate
riboflavin.

4. It was thus demonstrated that in the rat a deficiency of riboflavin does not
impair the synthesis of some of the body’s most metabolically important proteins, and
that at least in one instance it may be enhanced.

We are indebted to Miss Verna G. Leonard, Mrs R. C. Dicks and Mr M. W.
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help with the handling of the animals, a most important function in experiments
such as these.

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has shown an interest in this work, and we have discussed it with him. We are grateful
to him for comments which have been helpful in attempts to interpret the results and
to relate them to phenomena of protein metabolism generally.
REFERENCES