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EFFECT OF PROTEIN DEPLETION AND REPLETION ON SERUM PROTEINS IN RIBOFLAVIN DEFICIENCY

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The effects of riboflavin deficiency on serum proteins have been studied in rats following protein depletion and repletion with high-protein diet. Riboflavin deficiency causes a reduced serum total protein concentration, reflecting changes in all fractions except β-globulin. The decrease in serum total protein level with changes in albumin and γ-globulin fractions following protein depletion is less pronounced in riboflavin deficiency. Although these changes are reversed upon repletion with 40% protein diet, the values of serum total protein and its different fractions with the exception of α_2- and β-globulins in riboflavin-deficient rats are still lower than those of control rats undergoing the same dietary treatment. It has been suggested that lowered serum protein level in riboflavin deficiency on 16% protein diet or on repletion with 40% protein diet following protein depletion may result from reduced synthesis by the liver as well as increased utilization by the peripheral tissues.

Riboflavin deficiency does not alter the nitrogen concentration in liver (*1*). There is, however, a decrease in the nitrogen concentration of the carcass, especially the muscle. MOOKERJEA and HAWKINS (*2*) also observed that riboflavin deprivation does not seem to impair the ability of the rat to synthesize important protein constituents of liver and blood.

Liver is considered as the site for synthesis of plasma proteins, albumin, fibrinogen and globulins. This has been confirmed by MILLER *et al.* (*3*) by means of the perfused rat liver and C^{14}-lysine. In contrast, the non-hepatic tissues when perfused are able to produce only small amounts of globulin but no fibrinogen and albumin (*4*, *5*). It is, therefore, worthwhile to study the effect of riboflavin deficiency on serum proteins and their partition, and to see how the serum proteins and their partition respond to protein depletion and sub-

sequent repletion with protein-rich diet in riboflavin deficiency.

MATERIALS AND METHODS

Animals and diets. Male albino rats weighing 80–100 g were allocated to control and riboflavin-deficient groups, A and B, respectively. Control rats were pair-fed with the riboflavin-deficient rats. Three diets, varying in protein content were used. The other compositions of the respective diets were the same as used earlier (*6*). Water-soluble vitamins were supplied daily by subcutaneous injections.

The group of control rats was subdivided into three subgroups. One subgroup (A_1) was fed 16% protein for 45 days only; subgroup (A_2) was fed 16% protein for 30 days followed by 0% protein for 15 days; subgroup (A_3) was fed 16% protein for 30 days followed by 0% protein for 15 days and then fed 40% protein for another 7 days. The riboflavin-deficient rats (group B) were similarly subdivided.

Method. After the experimental period for each dietary regimen was over, the rats were killed and blood was drawn out from the hepatic vein. Serum was separated by centrifugation. The proteins of serum were determined by Biuret method (*7*). Electrophoretic analysis of serum proteins was performed by the method as described by LEDERER (*8*). The run was carried out with 0.01 ml of serum in Whatman No. 1 chromatographic paper under a voltage of 180–200 for 16 hours. Veronal buffer (pH 8.6, ionic strength 0.05) was used. The paper strips were dyed with bromophenol blue solution. With each paper strip a graph was plotted with a densitometer (Photovolt Corporation, Model No. 510A) and the area under each peak was measured by an Allbrit planimeter. The percentages of different fractions of serum proteins were calculated from the measured area under each peak. The absolute amount of each fraction was then calculated by referring the percentage composition values to serum total protein level.

Statistical analysis. The significance in serum protein changes in riboflavin deficiency was determined by the *t*-test (*9*).

RESULTS

The results are summarised in the Table 1.

Riboflavin deficiency caused a decreased serum total protein concentration. All the fractions except β-globulin exhibited significant declines in riboflavin deficiency.

Giving a protein-free diet to the pair-fed control animals caused a fall in serum total protein concentration reflecting changes in albumin and γ-globulin fractions. The pattern of response, although differing in magnitude, was similar in riboflavin-deficient rats on giving protein-free diet. Repletion with 40%

Table 1. Effect of protein depletion and repletion on serum proteins in riboflavin deficiency
(Data are represented as means ± SEM of 6 animals)

Dietary regimen	Groups of animals	Total protein g/100 ml	Albumin g/100 ml	Globulins g/100 ml			
				α_1	α_2	β	γ
16% protein for 45 days	Pair-fed control (A_1)	7.63±0.18	2.17±0.13	1.21±0.06	0.89±0.07	1.75±0.15	1.61±0.12
	Riboflavin-deficient (B_1)	5.99±0.36	1.68±0.12	0.94±0.06	0.67±0.05	1.51±0.11	1.18±0.06
16% protein for 30 days followed by 0% protein for 15 days	Pair-fed control (A_2)	4.80±0.17	0.74±0.08	1.10±0.06	0.78±0.03	1.48±0.04	0.69±0.06
	Riboflavin-deficient (B_2)	4.72±0.26	0.74±0.11	0.96±0.04	0.79±0.05	1.42±0.06	0.80±0.09
16% protein for 30 days followed by 0% protein for 15 days plus 40% protein for 7 days	Pair-fed control (A_3)	7.70±0.08	2.35±0.04	1.17±0.05	0.77±0.04	1.81±0.06	1.58±0.04
	Riboflavin-deficient (B_3)	5.76±0.31	1.61±0.22	0.82±0.06	0.64±0.07	1.57±0.12	1.11±0.06
P values Groups	A_1 *vs* B_1	<0.01	<0.05	<0.02	<0.05	>0.05	<0.02
	A_1 *vs* A_2	<0.001	<0.001	>0.05	>0.05	>0.05	<0.001
	B_1 *vs* B_2	<0.02	<0.001	—	>0.05	—	<0.02
	A_2 *vs* B_2	—	—	>0.05	—	—	—
	A_2 *vs* A_3	<0.001	<0.001	—	—	<0.01	<0.001
	B_2 *vs* B_3	<0.05	<0.01	>0.05	>0.05	>0.05	<0.05
	A_3 *vs* B_3	<0.001	<0.01	<0.01	>0.05	>0.05	<0.001

protein in both control and riboflavin-deficient rats restored the values of serum total protein and its different fractions to the corresponding levels obtained with 16% protein feeding. But even on repletion with 40% protein the values of serum total protein and its different fractions, with the exception of α_2- and β-globulins, of riboflavin-deficient rats are lower than those of the control rats undergoing the same dietary treatment.

DISCUSSION

FOY *et al.* (*10*) noted a reduction in serum protein concentration in riboflavin-deficient baboons and the level improved gradually when riboflavin was added. They suggested that absence of riboflavin was directly or indirectly responsible for the serum protein changes, which was confirmed by the rise in the content of proteins that occurred whenever riboflavin was added to the diet. In the present investigation also, riboflavin deficiency produced a decreased serum protein level in rats with significant declines in albumin, α_1-, α_2- and γ-globulin fractions. JACQUOT-ARMAND *et al.* (*11*), however, reported an increase in serum γ-globulin content in rats on a riboflavin-free diet.

Although the riboflavin-deficient rats did not appear to show impairment in the synthesis and concentration of liver proteins (*1*, *12–14*), the data on overall protein synthesis in liver (*2*, *12*, *13*), even if normal, would not exclude the possibility that the synthesis of a rapidly turning over fraction, such as albumin, is reduced. Another factor contributing to the fall in serum protein level in riboflavin deficiency may be the increased utilization of serum proteins by the peripheral tissues. It is accepted that plasma proteins can contribute to the store of intracellular proteins when need arises—that plasma proteins can meet the body's new proteins and energy needs (*15–17*). It is possible that in riboflavin deficiency the animal uses a good deal of plasma proteins for the maintenance of the energy needs of the body and serum protein falls in consequence. The alteration in the serum protein concentrations in riboflavin deficiency may also be brought about by the changes in the plasma volume. But this appears unlikely as riboflavin deficiency does not produce an appreciable effect on plasma volume (*18*).

A number of investigations concerned with the effects of nutritional states on serum proteins of several species have been reported (*17*, *19–24*). In the present investigation feeding a protein-free diet was found to have a similar effect on serum proteins in control and riboflavin-deficient rats, but the effect was less marked in riboflavin-deficient rats. It is noteworthy that there was no apparent difference in the values of serum total protein and its different fractions between control and riboflavin-deficient rats on protein-free diet. It has been suggested that a protein-free diet causes reduced synthesis and a comparatively less breakdown of liver proteins in riboflavin deficiency (*25*). The synthesis of a rapidly

turning over protein fraction, such as albumin, might already be reduced considerably in riboflavin-deficient rats, and therefore a protein-free diet seemed not to have much of a depressing effect on liver albumin synthesis in riboflavin-deficient rats as it had in control rats. Accordingly it would appear that effect of riboflavin deficiency on serum albumin level is eliminated in a protein-free diet. The apparent lack of difference in serum globulin level between the groups may likewise be explained.

Repletion with 40% protein restored the serum total protein level and its different fractions in both pair-fed control and riboflavin-deficient rats to the corresponding levels obtained in the 16% protein diet. This suggests that riboflavin deficiency does not affect the regeneration of serum proteins. Although there is repletion with 40% protein regenerated serum total proteins and its different fractions following protein depletion, the values of serum total protein, albumin, α_1- and γ-globulins are still lower than those of pair-fed control rats receiving 40% protein following protein depletion. Riboflavin deficiency was found to have no effect on the regeneration of liver proteins on repletion with 40% protein diet following protein depletion (*26*). This was based upon the fact that the restoration in the nitrogen or protein concentration in liver after protein repletion following depletion was not affected by riboflavin deficiency. But this may result from reduced synthesis as well as diminished breakdown in liver protein as was evidenced by the observations of CHATTERJEE (*25*), who suggested reduced synthesis as well as greater reduction in liver protein breakdown in riboflavin-deficient rats receiving 40% protein following protein depletion. So the reduced serum protein level in riboflavin-deficient rats receiving 40% protein following protein depletion may result from reduced synthesis by liver as well as increased utilization by the peripheral tissues.

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