RIBOFLAVIN AND ADRENAL CORTEX

Studies in rats and monkeys show that riboflavin deficiency leads to an initial increase and then a decrease in the activity of adrenal cortex.

Key Words: riboflavin, adrenal cortex, monkeys, erythroid aplasia

Several years ago, H. Foy and co-workers reported the appearance of erythroid aplasia in hospitalized children who were recovering from protein-calorie malnutrition (PCM) in Kenya. The disease appeared at a time when most of the clinical features of PCM had improved and the children were receiving a good-quality diet. It responded to treatment with either cortisone or riboflavin but not to antibiotics, and was not associated with infection.[1]

The same group later demonstrated erythroid aplasia in baboons fed a riboflavin-deficient diet. Unlike the children, monkeys developed aplasia at the peak of deficiency, but like the children, their condition responded to riboflavin or prednisolone. Other symptoms of riboflavin deficiency, such as swollen edematous gums, skin changes, and intermittent diarrhea, responded only to treatment with riboflavin. Features reminiscent of Addison's disease, such as decreased blood volume, oliguria, extreme asthenia, and response to glucose-saline were also present.

Recently, Foy, A. Kondi, and Z. H. M. Verjee[2] have further investigated the relation of riboflavin deficiency to corticosteroid metabolism and erythroid aplasia by looking at the structural and functional changes in the adrenal cortex of riboflavin-deprived monkeys and correlating these changes with aplasia.

All the animals on a riboflavin-deficient diet developed "relative hypoplasia," with a marked fall in marrow red cell precursors and reticulocyte count. Changes in hemoglobin levels were vitiated by changes in plasma volume. The marrow lymphocyte count increased. Total serum proteins and globulin levels tended to increase because of food restriction per se, but this increase was more marked in the riboflavin-deficient animals.

The level of plasma cortisol and the output of urinary steroids showed a tendency to fall from the thirtieth to the sixtieth day of deprivation and to rise again from day 60 to day 120. Urinary steroids fell steeply after that, but plasma steroid levels continued to be high. The initial fall in corticosteroids might be due to adrenal cortex hypofunction, which was followed by a transient rise due to pituitary stimulation in an attempt to restore steroid production. The ultimate fall in urinary steroids would indicate exhaustion or atrophy of the cortex. The serum levels continued to be high, probably because of a rise in transcortin levels indicated by elevated serum globulin levels. The response of the adrenal cortex to ACTH was poor throughout the study.

Histological examination of the adrenal cortex revealed hemorrhage and fibrotic changes in the hypoplastic animals.

Thus this study in monkeys clearly indicated that riboflavin deficiency leads to adrenal cortex dysfunction, which eventually precipitates erythroid hypoplasia and leads to aplasia in some animals. Strangely however, erythroid aplasia has not been reported in other forms of adrenal cortex hypofunction, though anemia is an associated feature of Addison's syndrome.

The relationship between adrenal cortex and riboflavin deficiency is also revealed through several studies in rats. A. F. Morgan and co-workers had observed, in rats fed a riboflavin-deficient diet, that stimulation of gluconeogenesis in response to hypoxic stress was initially high but later (after five weeks) was markedly depressed.[3] Administration of riboflavin could correct the defect in intact animals but not in adrenalectomized animals. The authors speculated that an enzyme containing riboflavin may be a necessary link in the trigger mechanism of the pituitary adrenal system.

In Morgan's experiments, pair feeding was not done, and her conclusions were later contradicted by A. C. Chatterjee, S. C. Jamdar, and B. B. Ghosh.[4] These workers observed that riboflavin-deficient rats after

45 days of deficiency had higher stores of liver glycogen, showed increased incorporation of labeled carbon of alanine into liver glycogen, and had elevated levels of alanine aminotransferase activity, which indicated a stimulation of gluconeogenesis.

The free amino acid pool in plasma and tissues such as liver and muscle was found to go up in riboflavin deficiency.[5] Administration of cortisone could not raise it further, and adrenalectomy prevented the rise resulting from riboflavin deficiency. The authors felt that the increase in the free amino acid pool in riboflavin-deficient rats was due to stimulation of adrenal cortex rather than to an accumulation of unoxidized amino acids.

However, in a subsequent paper[6] Chatterjee and Ghosh reported that ascorbic acid levels in riboflavin-deficient rats were significantly higher than in control animals after 45 days of deficiency, but fell after a period of 70 days on the deficient diet. These changes could not be seen in adrenalectomized animals. Thus, these authors now also conclude that prolonged deficiency of riboflavin does lead to hypofunction of adrenal cortex.

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3. See *Nutrition Reviews* 18: 221, 1960
4. A. C. Chatterjee, S. C. Jamdar, and B. B. Ghosh, *Experentia* 22: 794, 1966
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DIETARY EFFECT ON LIVER FATTY ACID SYNTHETASE

Changes in the level of fatty acid synthetase in rat liver are diet-induced and result primarily from changes in the rate of synthesis rather than degradation of this enzyme.

Key Words: liver, fatty acid synthetase, diet

The nutritional status of mammals has a marked influence upon the level of fatty acid synthetase in their livers. Activity of the synthetase decreases during fasting[1-4] and increases upon refeeding, especially if fat-free diets are ingested.[3-5] Porter and his colleagues have reported that the amount of synthetase that could be isolated from the livers of rats varied according to the nutritional state.[4] That these changes in the level of enzymatic activity are at least in part due to the amount of enzyme protein synthesized was indicated by the prevention of rise of synthetase by administration of puromycin or actinomycin D at the beginning of a refeeding period.[6]

As the net amount of enzyme present must reflect both synthetic and degradative actions, a more detailed appraisal of these parameters has been made for the fatty acid synthetase in rat liver by M. C. Craig, C. M. Nepokroeff, M. R. Lakshmanan, and G. W. Porter.[7] Male, 200 g. Holtzman rats were fed either a fat-free diet or a normal laboratory diet. Fatty acid synthetase was purified from a high-speed supernatant of liver homogenates by chromatography on DEAE-cellulose and centrifugation in a sucrose density gradient. Enzyme labeled with L-[$U^{14}C$] leucine was obtained by injecting animals with a single intraperitoneal dose at the beginning of nutritional steady states wherein the level of hepatic synthetase was essentially constant after a week of normal diet followed by one of three regimens: continuation of the normal diet fed ad libitum, a 48-hour fast, or a 48-hour fast followed by refeeding the fat-free diet for 72 hours. Antiserum to the synthetase was prepared by injecting the purified enzyme together with Freund's adjuvant at ten-day intervals into rabbits who were bled after two further weeks, and the gammaglobulin fraction isolated from the sera. Immunological reactions were assayed by Ouchterlony equivalence point determinations and quantitative precipitin analyses.

Synthetase from livers of normal, fasted, and refed rats reacted with antibody prepared against purified enzyme to give a single precipitin band by the Ouchterlony technique. Hence, the enzyme is immuno-