Effect of Riboflavin Deficiency on Free Amino Acid Nitrogen Concentrations of Liver, Muscle and Plasma

MORGAN et al.[1,2] observed an increased gluconeogenesis in liver at the beginning of riboflavin deficiency and a depressed gluconeogenesis in the later part of riboflavin deficiency. MOOKERJEA et al.[3] demonstrated increased transaminase activity in the liver of riboflavin-deficient rats. Further, increase in alanine transaminase activity of the liver of riboflavin-deficient rats was found to be associated with a greater deposition of glycogen in liver[4]. NICHOL et al.[5] and EISENSTEIN[6] observed a direct correlation between alanine transaminase activity and glycogen deposition in liver through gluconeogenesis. Recently, it was shown that riboflavin deficiency caused an increased in vivo incorporation of C^{14} from labelled alanine into liver glycogen[7]. It has been reported that transaminase activity varies according to the size of the free amino acid pool[5]. All these studies indicate that riboflavin deficiency may have some influence on free amino acid levels of tissues, particularly of liver. The present investigation was therefore undertaken on free amino acid nitrogen concentrations of liver, muscle and plasma in riboflavin deficiency.

Young male albino rats of 80–100 g were divided into groups A and B of equal average body weights. Group A consisted of control rats, and group B of riboflavin-deficient rats. The animals were pair-fed on 16% protein for 45 days. Particulars regarding the diet have been reported elsewhere[8]. Water-soluble vitamins were supplied daily by s.c. injection.

After the experimental period was over, the rats were kept fasting overnight. Then they were killed by a blow on the head. Blood was collected from the hepatic vein. Plasma was separated by centrifugation. Equal volumes of plasma from each of every 4–6 rats were pooled together to give the pooled samples. Protein-free filtrate of this plasma was used for the determination of amino acid nitrogen content by the colorimetric method of ROSEN[9]. Excised livers and muscles (gastrocnemius) were chilled in ice, blotted dry and weighed. A 10% homogenate of each of tissues was prepared in $0.25M$ sucrose solution. The tissue homogenates of each of every 4–6 rats were pooled together to give the pooled samples. Protein-free extracts of these pooled samples were then used for the determination of amino acid nitrogen content by the colorimetric method of ROSEN[9]. The Table shows that riboflavin deficiency increases the free amino acid nitrogen concentrations of liver, muscle and plasma.

In riboflavin deficiency, the oxidative enzyme system is affected by the decreased concentration of flavin enzymes[10]. Therefore, the oxidation of amino acid is expected to be decreased. This may result in an increased free amino acid pool, particularly in liver. As a result of this increased free amino acid nitrogen concentrations in liver, the hepatic gluconeogenesis may be enhanced in riboflavin deficiency as noted earlier[7]. In our earlier studies[7] it was suggested that adrenal cortical activity may be in some way related with increased hepatic gluconeogenesis observed in riboflavin deficiency. The possible role of adrenal cortex on the mobilization of amino acids has often been discussed[11–17]. It was claimed that administration of adrenal cortical extracts to eviscerated rats with intact adrenals increased the rate of rise of plasma amino acids[13]. Further, administration of adrenal cortical extracts[11] or cortisone[11,17], or adrenocorticotropic hormone[14,15] increased the concentrations of amino acids in plasma. It has been demonstrated by a number of workers[12,13,16], using adrenalectomized and eviscerated rats, that increased concentrations of plasma amino acids following administration of adrenal steroids result from the breakdown of peripheral tissue proteins. This was further confirmed by SMITH et al.[18], who studied the effects of adrenalectomy and of cortisol on plasma amino acids of eviscerated animals using alloxan-diabetic rats. They

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Nutritional state	No. of experiments[a]	Free amino acid nitrogen content		
		Liver mg/100 g	Muscle mg/100 g	Plasma mg/100 ml
Group A (control)	7	28.28 ± 0.74	21.54 ± 0.62	5.68 ± 0.11
Group B (riboflavin deficient)	8	36.45 ± 0.78 $P < 0.001$	28.70 ± 0.66 $P < 0.001$	6.88 ± 0.08 $P < 0.001$

Mean values ± S.E. [a] Each experiment involves investigation on pooled samples of 4–6 rats.

noted that the rise in plasma amino acid nitrogen of diabetic rats was markedly reduced by adrenalectomy. Cortisol restored and increased the rate of rise of plasma amino acids of adrenalectomized-diabetic rats. It was also noted that injection of adrenal steroids increases the free amino acid concentrations of liver[19] and muscle[17,20]. In the present investigation, riboflavin deficiency has been found to elevate the free amino acid nitrogen concentrations in liver as well as in plasma and muscle. It is therefore possible that, apart from accumulation of free amino acids in liver due to reduced oxidation of amino acids, there occurs mobilization of amino acids from the breakdown of peripheral tissue proteins, resulting in elevation of free amino acid nitrogen concentrations of liver, muscle and plasma in riboflavin deficiency. This is probably effected by the increased adrenal cortical secretions in riboflavin deficiency[21].

Zusammenfassung. Männliche Albino-Ratten, 45 Tage bei riboflavinarmer Nahrung, zeigten erhöhten Gehalt freien Aminosäure-Stickstoffs in Leber, Muskel und Plasma. Die Erhöhung scheint durch eine gesteigerte Aktivität der Nebennierenrinde beim Riboflavinmangel herbeigeführt zu sein.

A. K. Chatterjee and B. B. Ghosh

Department of Physiology, University College of Science, Calcutta-9 (India),
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The Kidney and Fibrinolysis in Man

It is commonly known that oxygen consumption by the kidney is very high. Both histochemical studies and determinations in renal homogenates revealed the highest known activity of various enzymes in renal tissue. The presence of a potent trypsine inhibitor and a plasminogen activator (urokinase) was demonstrated in urine, both being supposedly produced by the kidney. All these circumstances justified an idea of a possible role of the kidney in maintenance and regulation of blood fibrinolytic activity. When Buluk et al.[1,2] demonstrated in rabbits the potent effect of the ureter obstruction or of renal ischaemia on the fibrinolytic activity in venous blood from the corresponding kidney, we decided to study this problem in man.

It was done in 2 different ways: (a) the blood plasma fibrinolytic activity and urinary plasminogen activator were determined in a large group of patients with various renal diseases and compared to those in healthy subjects. (b) Direct determinations of fibrinolytic activity in the blood plasma from renal veins.

The urokinase was determined according to the procedure described in another paper[3] while the plasma fibrinolytic activity in the euglobulin fraction according to Kowalski et al.[4]. In the first series it was found[3] that no distinct difference in blood plasma activity occurs between the healthy subjects and most of those with renal disease. In uraemia and nephrosis the fibrinolytic activity was decreased ($p < 0.01$). The urokinase elimination, however, decreased distinctly ($p < 0.01$) in chronic glomerulonephritis, nephrosis and uraemia (Figure 1). A similar phenomenon seemed to appear in renovascular obstruction but the small number of patients did not allow a convincing evaluation.

In the second series the blood was drawn by veinpuncture in patients operated on because of a unilateral renal disease (hydronephrosis, TB, nephrocirrhosis). The plasma fibrinolytic activity in cubital and renal veins (the latter on the side of the lesion) was determined. The urine from the sick kidney was taken by the pelvic puncture while from the other it was collected through a catheter inserted after the opposite ureter had been ligated. We were not able to demonstrate any difference in the fibrinolytic activity between the renal and the cubital veins while the urine from the damaged kidney showed much lower content of the urokinase than that from the healthy one (Figure 2).

After some pilot experiments we abandoned further study and these data have not yet been published, because – facing convincing and opposite results of Buluk research – we had many doubts as to the technique of our experiments; it is well understandable that during surgical procedure the kidney and its vessels were maltreated, our approach was difficult and all was done in a hurry in order not to disturb the surgeons.

Now, after many years we have returned[5] to the problem when a modern technique of selective catheterization of various vessels has become available. We took the opportunity of catheterization performed in order to assess the renin activity in 17 patients suspected to have reno-vascular hypertension. The renal artery obstruction was previously demonstrated by selective arteriography and the % of artery narrowing was calculated. Two catheters were inserted in both renal veins, the third in the hepatic vein and the fourth in the vena cava just above its bifurcation. The details of the procedure are reported in the paper by Kokot et al.[6]. They have found the typical behaviour of renin activity in horizontal and

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