The absorption maximum of fluorescence spectrum (515 nm at neutral pH) was also coincident with that of isosepiapterin. Furthermore, on alkaline permanganate oxidation the compound gave 2-amino-4-hydroxypteridine-6-carboxylic acid (Rf = 0.03, solvent system 1 in the Table). Hydrogen peroxide oxidation gave both xanthopterin (Rf = 0.10, solvent system 1) and 2-amino-4-hydroxypteridine-6-carboxylic acid. In alkaline solution, the compound was readily oxidized with bromine to xanthopterin. Labile hydrogen was determined by iodine titration[8,9]. Iodine equivalent to 0.81 μmoles of hydrogen was consumed per μmole of the compound. The carbonyl group of the compound was determined by the 2,4-dinitro-phenylhydrazine method[10] and gave 1.07 μmoles of carbonyl group per μmole. These chemical characteristics are consistent with those of isosepiapterin described by FORREST et al.[11,12]. From the above results it can be concluded that the yellow compound is identical with isosepiapterin. Besides acid treatment, adsorption of dihydrobiopterin at neutral pH on Filtrol Grade 58 followed by elution with 20% aqueous acetone also leads to the formation of isosepiapterin. Biopterin gives no oxidized form of isosepiapterin by similar treatment. Studies on the biological significance of the reaction are being undertaken.

Résumé. La dihydrobioptérine obtenue par le mélange réactionel de sépiaptérine et de sa réductase se transforme en substance jaune par traitement à l'acide sulfurique. Employant des techniques chromatographiques, l'analyse du spectre d'absorption UV et l'analyse chimique, on a pu mettre en évidence que cette substance est identique de l'isosépiaptérine.

S. KATOH and M. AKINO

Department of Biology, Faculty of Science, Tokyo Metropolitan University, Tokyo (Japan), July 4, 1966.

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Changes in Alanine Transaminase Activity in the Liver of Riboflavin-Deficient Rats

NICHOL et al.[1] and EISENSTEIN[2] observed a direct correlation between alanine transaminase activity and glycogen deposition in liver through gluconeogenesis. They have studied this association in various ways, including the use of pyridoxine-deficient rats. It has also been reported that alanine transaminase activity varies according to the size of the free amino acid pool in the liver[1].

In riboflavin deficiency MORGAN et al.[3,4] observed an increased gluconeogenesis in the liver at the beginning of riboflavin deficiency. After 5 weeks on riboflavin-deficient diet, rats showed depressed gluconeogenetic activity. These workers subjected the normal and deficient rats to physiological stress and observed an impaired gluconeogenetic response to stress in riboflavin deficiency, which was attributed to the failure of pituitary adrenal axis or 'trigger mechanism' to release ACTH by the pituitary.

When normal rats were injected with deoxycorticosterone, hepatic alanine transaminase activity was lowered. This inhibitory effect of deoxycorticosterone on alanine transaminase activity was suggested to be due to the suppression of ACTH release by the pituitary[1]. This situation is similar to that observed in riboflavin-deficient rats by MORGAN et al.[4].

If such a defect exists under riboflavin deficiency, it is natural to expect low activity of alanine transaminase in the liver of riboflavin-deficient rats. On the contrary, it has been reported that there is increased activity of this enzyme in the liver of riboflavin-deficient rats[5].

In the present investigation, changes in alanine transaminase activity, glycogen and pyridoxal phosphate concentration of liver have been studied in riboflavin deficiency.

Young male albino rats of 80–100 g were divided into 2 groups of equal average body weights. Group A consisted of normal animals, 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[6]. Water-soluble vitamins were supplied daily by subcutaneous injection.

After the experimental period was over, the rats were kept fasting for 24 h and then decapitated. The livers were removed, cleaned of adherent blood, weighed, and chilled in ice. A weighed quantity of liver was digested in 30% KOH and used for the determination of glycogen[7]. Another part of the liver was homogenized in phosphate buffer of pH 7.4 and used for the determination of alanine transaminase activity[8] and pyridoxal phosphate concentration[9]. It can be seen from the accompanying Table that alanine transaminase activity and the concentration of pyridoxal phosphate and glycogen in liver are increased in riboflavin deficiency.

In riboflavin deficiency, the oxidative enzyme system is affected by decreased concentration of flavin enzymes[10]. Therefore the oxidation of amino acid is ex-

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Changes in alanine transaminase activity, pyridoxal phosphate and glycogen concentration in the liver in riboflavin deficiency, averages ± S.D.

Nutritional state	No. of animals	Liver						
		Weight, g/100 g body wt.	Alanine transaminase activity, U[a]		Pyridoxal phosphate in µg		Glycogen in mg	
			Per mg	Per 100 g body wt.[b]	Per g	Per 100 g body wt.	Per g	Per 100 g body wt.
Group A (normal pair-fed)	7	2.78 ± 0.092	1.2 ± 0.02	3.336 ± 0.27	5.14 ± 0.126	18.07 ± 1.126	0.156 ± 0.035	0.433 ± 0.087
Group B (riboflavin deficient)	8	3.848 ± 0.06	3.07 ± 0.67	12.88 ± 1.5	7.10 ± 0.17	26.65 ± 1.71	0.186 ± 0.021	0.713 ± 0.10

[a] U = µmoles of keto acid produced per hour. [b] Expressed in thousands.

pected to be decreased. This may result in an increased free amino acid pool and consequently an elevation of alanine transaminase activity in the liver of riboflavin-deficient rats. Pyridoxal phosphate, a cofactor for alanine transaminase, is increased under this condition. This may also be the reason for increased alanine transaminase activity in the liver of riboflavin-deficient rats. NICHOL et al.[1] and EISENSTEIN[2] observed a direct correlation between alanine transaminase activity and glycogen deposition. In the present investigation also an increase in alanine transaminase activity is associated with more deposition of glycogen in the liver of riboflavin-deficient rats.

It is noted in the introduction that suppression of ACTH release by the pituitary presumably lowers the alanine transaminase activity in the liver[1]. Further treatment of intact rats with cortisol[11–13] or cortisone[11,12,14,15], or adrenalectomized rats with cortisol[12,13], increased the alanine transaminase activity of the liver. It has also been observed that s.c. injection of ACTH produced a twofold increase in the alanine transaminase activity of the liver[12], and adrenalectomy lowered it[13]. In the present investigation alanine transaminase activity is increased in the liver of riboflavin-deficient rats. This suggests rather more release of ACTH by the pituitary and consequent stimulation of adrenal cortical function, and is in agreement with the earlier reports[6]. Recently the authors[16] observed an increased plasma ascorbic acid level in riboflavin deficiency, which is also suggestive of increased adrenal cortical secretion as plasma ascorbic acid level increases on administration of cortisone to normal rats[17]. These studies might therefore provide indirect evidence of increased adrenal cortical activity in riboflavin deficiency.

Zusammenfassung. 45tägiger Riboflavinmangel bewirkte bei männlichen Albinoratten in der Leber eine Erhöhung der Alanin-Transaminase-Aktivität sowie des Pyridoxal- und Glykogengehaltes. Diese Befunde weisen darauf hin, dass die Aktivität der Nebennierenrinde beim Riboflavinmangel erhöht ist.

A. K. CHATTERJEE, S. C. JAMDAR, and B. B. GHOSH[18]

Department of Physiology, University College of Science, Calcutta (India), July 14, 1966.

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[18] Acknowledgment: The authors wish to express their sincere gratitude to Prof. S. R. MAITRA, Head of the Department of Physiology, Calcutta University, for constant encouragement and for providing the laboratory facilities. The authors are also thankful to Dr. D. SEN of the Department of Applied Chemistry, Calcutta University, for translating the English summary into German.

Versuche zum Calciphylaxie-Phänomen an HeLa-Zellkulturen[1]

Als Calciphylaxie definierte SELYE[2] eine durch einen doppelten Mechanismus erzeugte Gewebsreaktion, die zu einer Calciumablagerung führt. Sie wird vermittels Vorbehandlung mit einer calciummobilisierenden Substanz (Sensibilisierung), der nach Ablauf einer bestimmten Zeitspanne («critical period») die Nachbehandlung mit gewissen chemischen oder mechanischen Agenzien (Provokatoren, Beizstoffe, «challengers») folgt, ausgelöst. Ablagerung von Calciumsalzen erfolgt dort, wo der Provokator mit sensibilisiertem Gewebe in Berührung kommt. Demgegenüber bedeutet die Calcergie eine Ausfällung von

[1] Durchgeführt mit Unterstützung der Deutschen Forschungsgemeinschaft.
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