however, sympathetic secretion had been prevented by injection of the β-blocking propranolol, a temporary retardation, attributable to myoepithelial relaxation, appeared when stimulation ceased; before propranolol, this phase obviously did not come to light because the secretory rate was augmented for a short period after stimulation. In the first experiment of the Figure, it can be seen that, after the α-receptor-blocking drug dihydroergotamine, some acceleration of the flow persisted after sympathetic stimulation for 2 sec, but scarcely for 1 sec. Such a persisting acceleration, abolishable by propranolol, can be ascribed to augmented secretion, suggesting that part of the acceleration at stimulation for 2 sec before dihydroergotamine was in fact due to secretion, adding its accelerating effect to that of the myoepithelial contraction and possibly reducing the retarding effect of subsequent myoepithelial relaxation even in this experiment.

The investigation shows that sympathetically evoked myoepithelial contraction can be studied in the submandibular gland of the dog if allowed to act on a slow, parasympathetically induced secretion, provided the period of sympathetic stimulation is short and a β-adrenoceptor-blocking drug has been given.

Physiological Studies on the Effects of Nutritional Imbalance on the Central Nervous System. II. Effects of Thiamine Deficiency on Oxidative Enzymes in the Brain of Chicken, *Gallus domesticus*

U. P. NALINI and NAYEEMUNNISA

Department of Zoology, Bangalore University, Bangalore 560001 (South India), 25 June 1975.

Summary. The activity levels of succinate dehydrogenase, glutamate dehydrogenase and pyruvate dehydrogenase in the fore, mid and hind brain regions of the thiamine deficient chicken, *Gallus domesticus* were determined. The activity levels of succinate dehydrogenase and glutamate dehydrogenase in all the 3 regions of brain showed augmentation on inducing thiamine deficiency. In contrast the activity levels of pyruvate dehydrogenase decreased in the brain of thiamine deficient animals. It is suggested that these changes in the oxidative enzymes indicate disturbance caused in the operation of the tricarboxylic acid cycle in thiamine deficiency.

Vitamin deficiency is known to cause metabolic defects in man and other vertebrates[1]. Many clinical signs and biochemical changes induced by vitamin deficiencies in chick have been described[2,3]. It has been reported that absence of thiamine causes cellular anorexia[2] and affects the distribution of thiamine-dependent enzymes that operate in the carbohydrate metabolism in birds and mammals[2,4,5]. Thiamine deficiency is also known to produce a rise in pyruvate content of the tissues in vertebrates, and this is believed to be the key to many deficiency-induced lesions[6]. However, information about the regional distribution of dehydrogenases in the CNS, and changes occurring in their activity during thiamine deficiency, is lacking. Hence it was felt desirable to study the dehydrogenases which play a significant role in the carbohydrate metabolism on inducing thaimine deficiency. The paper presents information about the pyruvate, succinate and glutamate dehydrogenases in the fore, mid and hind brain regions of the thiamine deficient chick, *Gallus domesticus*.

Materials and methods. 3-day-old white Leg-horn chicken, *Gallus domesticus*, ranging in weight from 15–20 g, were purchased from a local dealer and reared in the laboratory in electrically heated cages at 37 $\pm$ 1 °C. The controls were fed on standard chicken feed purchased from 'Mysore Feeds', Bangalore, India. The experimental birds were fed on polished rice for more than 3 weeks to induce thiamine deficiency[3,7]; water was given ad libitum.

The normal and thiamine deficient chicken were sacrificed by decapitation after 30 days. The brain was dissected with sterilized instruments and kept in normal saline on ice at 0 °C, and adhering blood vessels were removed. The fore, mid and hind brain regions were separated with sterilized bent forceps and scalpel; they were weighed quickly in Ringer[8] in an electric balance and were used for analysis.

Assay of dehydrogenase. A 10% (wt/vol) homogenate of brain cortical matter was prepared in 0.25 *M* cold sucrose solution using glass homogenizer and centrifuged at 3000 rpm for 15 min. The supernatant was used for the assay of enzyme activities. The levels of dehydrogenase activities were estimated by modified triphenyl tetrazolium chloride reduction method[9]. The incubation mixture contained 0.5 ml each of 0.09 *M* sodium succinate, and 0.27 *M* sodium glutamate and 0.1 *M* sodium pyruvate as substrates for the estimation of succinate, glutamate and pyruvate dehydrogenases respectively (these concentrations of substrates were found to give optimal activity[10]), 0.5 ml of sodium phosphate buffer of 0.1 *M*, 0.5 ml triphenyl tetrazolium chloride (0.2% solution at neutral pH) and 0.1 ml of the 10% homogenate. The incubation was carried out at 37 °C for 45 min after which 6 ml of glacial acetic acid (BDH) and 6 ml of toluene were added and kept in the refrigerator overnight. The toluene layer was extracted and the optical density was read at 505 nm in Du[2] Beckman's spectrophotometer.

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Activity levels of oxidative enzymes[a] in the brain of normal and thiamine – deficient chicken, *Gallus domesticus*[b]

Name of the enzyme	Cerebrum		Cerebellum		Medulla oblongata	
	Control	Test	Control	Test	Control	Test
Pyruvate dehydrogenase	440 ± 30.0	165 ± 35[d] − 73.4[c]	375 ± 7	140 ± 20[d] − 62.0[c]	387 ± 38	75 ± 15.0[d] − 80.6[c]
Succinate dehydrogenase	592 ± 65.7	740 ± 114[d] + 25.0[c]	478 ± 96	706 ± 98[e] + 47.6[c]	276 ± 36.7	448 ± 90[e] + 61.0[c]
Glutamate dehydrogenase	110 ± 14.2	260 ± 55.0[d] + 81.0[c]	158 ± 30.6	213 ± 20.4[e] + 34.7[c]	120 ± 28	200 ± 41.3[e] + 66.6[c]

[a] Activity expressed as μg of NTC reduced/g wet wt/h. [b] Values are mean ± SD of 5 observations. [c] Percentage change, + and − signs indicate increase and decrease in the activity level with respect to the control. [d] $p > 0.001$; [e] $p > 0.01$.

Proteins from the aqueous extracts were precipitated with 5% TCA (BDH) and the concentration was measured by microbiuret method[11].

Results and discussion. The data after statistical analysis are presented in the Table. Thiamine pyrophosphate is known to act as a co-enzyme in carbohydrate metabolism, and its absence is known to affect the activity and distribution of thiamine-dependent enzymes[2,12]. Pyruvate dehydrogenase acts in the oxidative decarboxylation of pyruvate to acetyl CoA and requires a co-enzyme, thiamine-pyrophosphate for the process[13]. In the present investigation, activity levels of PDH are found to decrease significantly in all the three regions of the thiamine-deficient brain, remarkably so in the brain stem regions. It is obvious that dietary absence of thiamine is the direct cause for such a decrease in the activity levels of pyruvate dehydrogenase in all the three regions of the brain analyzed. Earlier work done on these lines report an impaired utilization of pyruvate in the brain of thiamine-deficient rats[6]. A rise in the pyruvate content in the thiamine-deficient tissues was observed by Peters[7]. In the light of these reports, the decreased activity levels of PDH could be due to accumulation of pyruvate, since it is known that accumulation of substrate beyond a certain level leads to the inhibition of the enzyme activity[14–16]. The magnitude of decrease in the activity levels of PDH is comparatively greater in medulla and least in the cerebellum, and confirms the earlier observation by PINCUS and WELLS[12] that the brain stem region is the most sensitive region to thiamine deficiency and the cerebellum less sensitive.

Succinate dehydrogenase activity was observed to be significantly increased in all the three regions of the thiamine-deficient brain. A constant production of succinate could be one of the reasons for such an increase in the activity levels, since it has been found that the total lipid contents decrease and unsaturation of fatty acids increase in all the three regions of the brain in the same chicken on inducing thiamine deficiency (NAYEEMUNNISA, unpublished observations). This may account for the increased production of acetyl CoA via enhanced β oxidation of fatty acids, ultimately leading to the augmentation in the activity level of succinate dehydrogenase.

The activity levels of glutamate dehydrogenase (GDH) exhibited considerable increase in all the three compartments of the brain studied in thiamine deficient chicken (Table). Since GDH is involved in the deamination reaction pertaining to glutamine acid, as well as amination of α-keto-glutaric acid, the level of activity of the enzyme will have a decisive role in regulating the ammonia toxicity in the tissue[17]. An increase in the level of activity indicated a greater potentiality in decreasing the ammonia toxicity, which is produced in several reactions in the brain of the thiamine-deficient chicken.

It is clear from the data (Table) that the effects of thiamine deficiency varied with the enzyme measured and the regions studied. The greater sensitivity of some regions as compared to others is strikingly evident in the case of pyruvate dehydrogenase (cerebrum and medulla oblongata), glutamate dehydrogenase (cerebrum) and succinate dehydrogenase (medulla oblongata). The significance of these differences in the different enzymes studied is far from clear. However, the differences in the magnitude of response exhibited by the different regions of the brain are related to differences in the functional status of the regions concerned[18].

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