cin. The minimal detectable amount of AVP was 0.5 fmoles/tube at which the enzyme activity was lower than the value of $\bar{x}-2$ s calculated from that of the blank. This indicates that the enzyme immunoassay for AVP is sufficiently sensitive for the determination of physiological levels of AVP in plasma. Furthermore, the inhibition-curve with the samples which were prepared with a plasma extract by serial dilution or addition of known amount of AVP, was parallel to the standard curve of AVP. The mean values of AVP determined at 3 separate periods were 3.2, 3.6 and 3.5 fmoles/tube in the dehydrated state and were 1.6, 1.4 and 1.3 fmoles/tube after the hydration. This indicates that the enzyme immunoassay for AVP is highly reproducible. The AVP-β-D-galactosidase complex was stable for at least 1 year with respect to the enzyme activity and to the binding activity to the anti-AVP-antibody. Therefore, the enzyme immunoassay for AVP described here could be applicable in clinical chemistry.

1 G.L. Robertson, E.A. Mahr, S. Athar and T. Sinha, J. clin. Invest. *52*, 2340 (1973).
2 K. Shimamoto, T. Murase and T. Yamaji, J. Lab. clin. Med. *87*, 338 (1976).
3 P.H. Baylis and D.A. Heath, Clin. Endocr. *7*, 91 (1977).
4 E. Ishikawa, Y. Yamada, Y. Hamaguchi, S. Yoshitake, K. Shiomi, T. Ota, Y. Yamamoto and K. Tanaka, in: Enzyme labeled immunoassay of hormones and drugs, p.45. Ed. S. B. Pal. Walter de Gruyter, Berlin 1978.

Effect of tea consumption on the levels of α-ketoglutarate and pyruvate dehydrogenase in rat brain[1]

P. Ruenwongsa and S. Pattanavibag

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 4 (Thailand), 13 November 1981

Summary. Administration of tea to rats fed on a normal diet results in a marked drop in brain levels of total thiamine as well as of α-ketoglutarate and pyruvate dehydrogenase activities. The patterns of decrease in both enzyme activities are similar to that of total thiamine content; they drop to about 65% of the control at 14–20 weeks after continuous consumption of tea.

Tannin, the main antithiamine component in tea has been reported to interact with thiamine, resulting in a decrease in thiamine absorption[2,3]. A thiamine deficiency status has been shown among people who have the habit of chewing fermented tea leaves[4]. Since thiamine deficiency can lead to a dysfunction of the nervous system, it is of considerable interest to investigate the possible effects of prolonged tea consumption on the thiamine status of the brain. Moreover, the thiamine pyrophosphate dependent enzymes, α-ketoglutarate and pyruvate dehydrogenase are involved in the synthesis of γ-aminobutyric acid and acetylcholine respectively. Impairment of these enzyme activities could affect neurotransmitter synthesis and might contribute to disturbances in neurological function[5,6]. Therefore, in addition to brain thiamine content, the brain levels of α-ketoglutarate and pyruvate dehydrogenase were also determined in the rat at various times after tea administration.

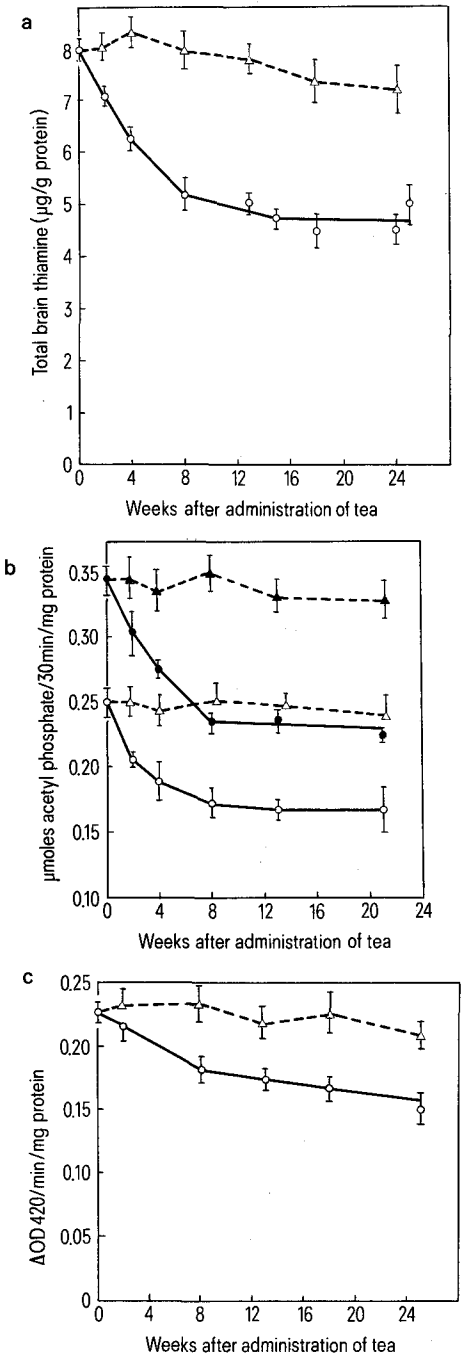
Materials and methods. Male albino weanling rats (aged 3 weeks, weight 30–40 g), fed ad libitum on a normal basal diet were divided into 2 groups of 60 animals, namely tea-treated and control. The experimental group was given black tea (The Raming Tea, Thailand), 1:50 w/v while drinking water was given to the control group. At different times after tea administration, 8 animals from the tea-treated group and 8 animals of the same ages from the control group were decapitated, and their brains were removed and separately processed immediately. 4 brains from each group were used for the determination of thiamine content in brain while another 4 brains were used for the assay of α-ketoglutarate and pyruvate dehydrogenase activity. For determination of total thiamine content, the whole brain was homogenized in 8 vol. of cold 0.3 M $HClO_4$ and then centrifuged at $6000\times g$ for 30 min. The supernatant obtained was digested with Takadiastase[7] and the total thiamine content was assayed by the thiochrome method[7].

The activities of brain α-ketoglutarate and pyruvate dehydrogenase were assayed in the mitochondrial fraction which was prepared from freshly removed brain. Each brain was homogenized in 2 vol. of Krebs-Ringer phosphate buffer, pH 7.4 and centrifuged at $1000\times g$ for 10 min. The pellet was discarded and the resulting supernatant was further centrifuged at $12{,}000\times g$ for 20 min to isolate the mitochondrial fraction. The mitochondrial pellet obtained after washing was suspended in Krebs-Ringer phosphate buffer, pH 7.4 and used while fresh for assaying the enzyme activity. The α-ketoglutarate dehydrogenase activity was measured by using $Fe(CN)_6^{3-}$ as an electron acceptor[8,9]. The rate of reduction to $Fe(CN)_6^{4-}$ was followed spectrophotometrically at 420 nm and the enzyme activity was expressed as changes in OD_{420} per min per mg mitochondrial protein. For pyruvate dehydrogenase activity, the assay system as described by Wieland et al.[10] involved the oxidative decarboxylation of pyruvate, resulting in the formation of acetyl phosphate. Pyruvate dehydrogenase exists as an active nonphosphorylated and an inactive phosphorylated form[11]. To determine the total pyruvate dehydrogenase activity, the mitochondrial fraction was preincubated at 25 °C for 1 h with 10 mM $MgCl_2$, prior to the assay[10].

Results and discussion. The fluid intake and body weights of rats in the 2 groups, consuming either drinking water or tea (1:50 w/v) were not significantly different. However, in the tea-treated group, as early as 2 weeks after tea consumption, the brain total thiamine content had already decreased. When compared to the nontreated control, a progressive decline was observed in the tea-treated group from 2 to 14 weeks, where about 60% of the control value remained (fig.a). After 14 weeks, there was no further decrease in brain thiamine content even though the rats were still maintained on tea drinking for 24 weeks. The results suggest that prolonged consumption of tea can lead to a status of thiamine deprivation in the brain although the rats still do not yet show neurological symptoms. It has been reported that when the brain thiamine declines to less than 25% of the normal value, neurological signs are observed[12,13]. The decrease in brain thiamine content following tea consumption could possibly result from interfer-

ence with thiamine absorption by complex formation between thiamine and tannin, the polyphenolic compound in tea[2,3]. However, coffee and its orthophenolic constituents, which have been reported to interact with thiamine[14], have been shown to have no effect on the thiamine status of experimental rats, as determined by blood thiamine level and erythrocyte transketolase activity[15]. In our experiment, blood transketolase activity, which has been widely used as an index of thiamine deficiency status[16], also dropped below the normal value in the tea-treated rats (results are not shown).

There were no significant changes in brain thiamine content or in the activities of α-ketoglutarate and pyruvate dehydrogenase in the control group during the course of the experiment, therefore, the reduction in these parameters were not related to the maturation process of the animals. Figure b shows the activities of total and active pyruvate dehydrogenase at 0–24 weeks after administration of tea. In a similar way to the pattern of decrease in brain thiamine content, at 8 weeks after tea treatment the enzyme activities decreased to 75% and 65% of the control for total and active pyruvate dehydrogenase respectively. From 8 to 24 weeks on tea treatment, no further drop in the enzyme activity was found. The oxidative decarboxylation of α-ketoglutarate was also disturbed in thiamine deprivation by tea consumption, as shown in the result in figure c. A marked drop in α-ketoglutarate dehydrogenase activity occurred from 2 to 8 weeks after tea administration, followed by a slight decline afterwards. About 65% of the control value was observed after 16–24 weeks. The results from this study indicate that the oxidative decarboxylation of α-ketoglutarate and pyruvate can be affected by prolonged tea consumption, and they also suggest that both activities are more or less equally susceptible to a status of thiamine deficiency. The extent of decrease in both enzyme activities seems to be compatible with that of brain thiamine content, although the decrease in thiamine is slightly greater. The similar reduction in both α-ketoglutarate and pyruvate dehydrogenase has also been demonstrated in thiamine-deficient rats produced by a thiamine deficient diet[9]. The decrease in the enzyme activities is partly due to lack of thiamine pyrophosphate, the coenzyme needed for their activities; however, the synthesis of apoenzyme may be reduced as well[9]. This finding suggests that prolonged consumption of tea results in a decrease in brain thiamine content which further affects the function of α-ketoglutarate and pyruvate dehydrogenase, and may thus lead to an impairment in neurotransmitter metabolism.



Brain levels of total thiamine (a), pyruvate dehydrogenase (b) and α-ketoglutarate dehydrogenase (c) in rats at different times after tea administration: —○—, the tea-treated rats; ---○---, the control rats of the same ages. The results are presented as means ± SEM (n=4). The total pyruvate dehydrogenase (●, ▲) and active pyruvate dehydrogenase (○, △) are expressed as µmole acetyl phosphate formed per 30 min per mg mitochondrial protein. The activity of α-ketoglutarate dehydrogenase is expressed as changes in OD at 420 nm per min per mg mitochondrial protein.

1 Supported by a grant from the International Foundation for Science (IFS), Sweden.

2 K. Rungruangsak, P. Tosukhowong, B. Panijpan and S. Vimokesant, Am. J. clin. Nutr. *30,* 1680 (1977).
3 D.M. Hilker, V.C. Chan, R. Chen and R.L. Smith, Nutr. Rept. int. *4,* 223 (1971).
4 S.L. Vimokesant, S. Nakornchai, S. Dhanamitta and D.M. Hilker, Nutr. Rept. int. *9,* 37 (1974).
5 C.J. Gubler in: Thiamine, p.121. Eds C.J. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
6 C.P. Heinrich, H. Stadler and H. Weiser, J. Neurochem. *21,* 1273 (1973).
7 D.S. Murdock and C.J. Gubler, J. Nutr. Sci. Vitam. *19,* 43 (1973).
8 V. Massey, Biochim. biophys. Acta *38,* 447 (1960).
9 K. Takahashi, A. Nakamura and Y. Nose, J. Vitam. *17,* 207 (1971).
10 O. Wieland, E. Siess, F.H. Schulze-Wethmar, H.G. Von Funcke and B. Winton, Archs Biochem. Biophys. *143,* 593 (1971).
11 T.C. Linn, F.H. Pettit, and L.J. Reed, Proc. natl Acad. Sci. USA *62,* 234 (1969).
12 D.W. McCandless and S. Schenker, J. clin. Invest. *47,* 2268 (1968).
13 D.S. Murdock and C.J. Gubler, J. Nutr. Sci. Vitam. *19,* 237 (1973).
14 J.C. Somogyi, Wld Rev. Nutr. Diet. *29,* 42 (1968).
15 R. Stalder and M. Marchesini, Experientia *37,* 633 (1981).
16 P.M. Dreyfus and G. Hauser, Biochim. biophys. Acta *104,* 78 (1965).