FMDV neutralizing antibodies which attain maximum titers in serum at about 3 weeks p.i.

Our results show that pretreatment of mice 4 days before infection with a low dose of Cy prevents the appearance of pancreatic lesions and decreases the virus yield. This was associated with an enhanced synthesis of anti-FMDV neutralizing antibodies, which not only reached titers in serum higher than those present in control mice, but also persisted for a longer time. This effect could be tentatively ascribed to the immunomodulating activity of Cy[4]. Given at a large dose Cy is a potent immunosuppressor agent[9-11]. However, it is known that Cy can also display an immunostimulating activity, probably by its action on the suppressor cascade[12]. Several reports claim that Cy, administered before antigenic challenge and in a low non-immunosuppressive dose, can enhance antibody formation[13].

In other systems, such as in implanted experimental tumors, a low dose of Cy can induce rejection and eventual cures, even when administered at a time where the tumors grow to a considerable size[4]. This phenomenon has also been associated to the activity of the drug on the T-suppressor cells[14]. Our results show that in Cy-treated mice the synthesis of anti-viral antibodies was markedly enhanced. This phenomenon could be responsible for the decrease in virus production in the pancreas and mildness of pathological alterations. Although the lower titer of neutralizing antibody in the serum of infected mice not treated with Cy could be ascribed to the presence of virus-antivirus complexes, this possibility seems unlikely since the level of circulating immune-complexes was similar both in the Cy-treated and non-treated mice.

The possibility that the drug can display a direct anti-viral effect seems also unlikely. The drug was administered 4 days before infection and according to its short half-life[15], a very small amount of Cy would be present at the time when virus replicates in the pancreas. Moreover, the fact that the first cycle of virus replication was not affected (at 12 h p.i. virus yields from pancreas of Cy-treated and non-treated mice were similar) supports the opinion that Cy, in the dose and schedule of administration we used, has no direct effect on virus replication.

Another alternative possibility for explaining the lack of pancreatic alterations is the well-known cytocidal effect of Cy on polymorphonuclear leukocytes[16]. Although leukopenia could be responsible for the absence of inflammatory infiltrates in the pancreas, other phenomena such as the acinar necrosis, which appears earlier than the polymorphonuclear infiltrate and the diminished virus replication, are unlikely to be related to the decrease in the leukocyte number.

1 Gershwin, M. E., Goetzl, E. J., and Steimberger, A. D., Am. Int. Med. *80* (1974) 531.
2 Scott, D. G. I., and Bacon, P. A., Am. J. Med. *76* (1984) 377.
3 Natanson, N., Monjan, A., Panitch, S. H., Johnson, E., Petursson, G., and Cole, G. A., in: Viral Immunology and Immunopathology, p. 357. Ed. A. L. Notkins. Academic Press, New York 1975.
4 Dray, S., and Mokyr, M. B., in: Immunomodulation. New Frontiers and Advances, p. 349. Eds H. H. Fundemberg, H. D. Whitten and F. Ambrogi. Plenum Press, New York 1984.
5 Creighton, W. D., Lambert, P. H., and Miescher, P. A., J. Immun. *111* (1973) 1219.
6 Snedecor, G. W., in: Métodos estadísticos aplicados a la investigación agrícola y biológica, p. 91. Compañía Editorial Continental, México 1964.
7 Brown, F., Ann. Inst. Pasteur/Virol. *136 E* (1985) 547.
8 Platt, H., Virology *9* (1959) 484.
9 Fass, L., and Fefer, A., J. Immun. *109* (1972) 749.
10 Lubet, R. A., and Carlson, D. E., J. natl Cancer. Inst. *61* (1978) 897.
11 Turk, J. L., Parker, D., and Poulter, L. W., Immunology *23* (1972) 493.
12 Glaser, M., J. exp. Med. *1499* (1979) 774.
13 Kaufman, S. H. E., Hahn, H., and Diamanstein, T., J. Immun. *125* (1980) 1104.
14 Bollinghoff, M., Starzinski-Powitz, A., Pfizenmaier, K., and Wagne, H., J. exp. Med. *145* (1977) 455.
15 Graul, E. M., Schaumloffel, E., Hundeshagen, H., Williams, H., and Simon, G., Cancer *20* (1967) 896.
16 Van Bekkum, D., Sem. Hemat. *21* (1984) 81.

0014-4754/89/010110-03$1.50 + 0.20/0

Effect of consumption of green and black tea on the level of various enzymes in rats

M. Ali*, L. H. J. Hayat, J. Al Saleh and C. J. Gubler +

Department of Biochemistry, Faculty of Science, Kuwait University, P.O. Box 5969, 13060 Safat (Kuwait)
Received 16 June 1988; accepted 13 September 1988

Summary. Drinking of both green and black tea as the only liquid ingested resulted in significant decreases in the activity of transketolase in whole blood of rats both before and after the in vitro addition of thiamin diphosphate. Liver transketolase activity was decreased only by green tea. Mucosal transketolase activity was not affected by either type of tea. The activity of lactate dehydrogenase (LDH) was not affected by either type of tea, while whole blood LDH was decreased by both green and black tea. Neither tea had any affect on mucosal alkaline phosphatase, but thiamin diphosphatase activity was decreased by both teas. An increase in liver total thiamin resulted from the drinking of both types of tea.
Key words. Green tea; black tea; transketolase; lactate dehydrogenase.

Tea, both green (unprocessed) and black (processed), has been consumed as a beverage from time immemorial and in some societies fermented leaves are chewed as a stimulant. In some cultures, tea is the common drink in place of water. Many plants of nutritional value, including tea, have been shown to possess antithiamin activity[1-4]. Somogyi and coworkers[5,6] showed that the antithiamin activity was chiefly due to polyhydroxy phenols in the products. Tea is rich in such compounds, notably tannic acid, and has been shown to possess considerable antithiamin activity[3,7]. Hence studies have been made on the effects of tea consumption on thiamin nutrition and status in humans[8-10]. Heavy consumption of tea was shown to lead to a decrease in thiamin excretion in the urine and to an increase in the percent increase in the red cell transketolase activity after the in vitro addition of thiamin diphosphate (%TPP effect), which is considered diagnostic of thiamin deficiency if it is above 20%.

In vitro tests using everted sacs of rat intestines indicated that some of these antithiamin compounds may interfere with the active transport of thiamin [11,12]. On the other hand, studies involving antithiamin compounds in animal feeding tests have been inconsistent. Hayakawa and Murata [13] found no significant effect on body weight, urine thiamin, erythrocyte transketolase or the %TPP effect after feeding caffeic acid or tannin to rats. Schaller et al.[14] also found no changes in the thiamin status parameters in rats fed caffeic acid. Yoo and Hilker [15] also found no effect of Korean teas on weight gain and urine thiamin in rats.

Ruenwongsa and Pattanavibag [16], however, showed that the administration of tea to rats for 14–20 weeks resulted in significant decreases in total thiamin and α-ketoglutarate and pyruvate dehydrogenase activities in the brain of rats.

In view of these inconsistencies, it seemed worthwhile to make a study with rats on the effects of tea as the only source of drinking water on their status with respect to thiamin. Also, since Matsuda et al.[17] showed that thiamin deficiency caused a marked decrease in intestinal alkaline phosphatase activity, it seemed worthwhile to see if tea might have an effect on this enzyme.

Materials and methods. Sprague-Dawley female rats of 200–250 g weight were supplied to us by the Department of Zoology of Kuwait University. In a second experiment, weanling female rats of 90–110 g were used, but since no significant difference was observed in the various parameters studied between the older and the younger rats, the data were combined for statistical treatment using Student's t-test for significance of the means for the groups. The rats were divided into three groups, a control group, which received water ad libitum; a group which received a green tea extract ad libitum; and a group which received black tea extract ad libitum. All groups received a purified synthetic thiamin deficient diet [18]. Tea was purchased on the commercial market. The extracts were prepared by boiling 20 g of green (unprocessed) or black (processed) tea in 1 l of water for 10 min. After cooling, the tea was filtered through cheese cloth and dispensed in regular drinking bottles.

After 8 weeks on this regimen the rats were sacrificed by ether anesthesia followed by collection of the blood through the abdominal aorta into citrated tubes. The liver and the heart were removed immediately and placed on ice. The first 15 cm of the intestine was removed and washed with saline and the mucosa collected and prepared as described by Bai et al.[18]. Mucosal alkaline phosphatase (Alk-P) activity was assayed essentially by the method described by Matsuda et al.[17], but using assay kits provided by Wako Pure Chemicals Inc., Ltd, Japan. Transketolase (TK) activity was determined was described by Bai et al.[18]. In line with the findings of Ali et al.[19], whole blood was used to measure the red cell TK activity; for the liver and heart, 1–10 homogenates were prepared in the buffer used for assay. TK activity was determined according to Al Hassan et al.[20] and lactate dehydrogenase (LDH) activity as described by Bergmeyer and Bernt [21]. For the assay of total thiamin in the heart, 1 g of heart was homogenized in 10 ml of cold 0.3 M $HClO_4$ for 3 min, then centrifuged at 3000 × g for 15 min. The pellet was rehomogenized in 5 ml of the 0.3 M $HClO_4$ and centrifuged as above. The two supernates were combined, and the pH was adjusted to 5–6 with 30% KOH, followed by centrifugation again at 3000 × g to remove precipitated KClO.

The supernatant was then adjusted to pH 4–5 with 4 M acetate buffer, pH 4.5, and made up to 25 ml with distilled H_2O; thiamin was measured according to the official method of the Association of Vitamin Chemists [22]. Alkaline phosphatase and thiamin diphosphatase activities in intestinal mucosa were assayed by methods described by Iwata et al.[23]. Calcium in the heart was assayed by the method of Hulanicki and Trojanowicz using an Orion calcium electrode model 93–20.

Results and discussion. As shown in table 1, both green and black tea consumption resulted in significant reductions in whole blood TK activities both with and without the addition of thiamin diphosphate (ThDP). The finding of a significant decrease in total TK after ThDP addition (in vitro) and the lack of a significant increase in the percent increase in TK activity after ThDP shows an inability of the blood to respond in vitro to ThDP. This has been observed repeatedly after chronic deficiency of thiamin. It would suggest an actual decrease in the apoenzyme level as well as the coenzyme. Consumption of green tea also led to a significant decrease in liver TK activity after ThDP. The activity without ThDP was decreased but the decrease was just on the border of significance, i.e. p = just slightly over 0.05. Black tea had no significant effect. Mucosal TK activity was not significantly affected by consumption of either green or black tea. There is no obvious reason why these values did not decrease as was reported in an earlier study [18] with thiamin-deficient rats without tea.

As shown in table 2, tea consumption had no effect on liver LDH activity. Black tea led to a significant decrease in the LDH activity in the heart, and both green and black tea resulted in a significant decrease in whole blood LDH activity.

Table 3 indicates that neither green nor black tea consumption had any effect on the alkaline phosphatase activity of the mucosa while both resulted in a significant decrease in ThDPase activity. Iwata and coworkers [17,22] reported a decrease in both Alk-P and ThDPase in rats with simple thiamin deficiency.

Total thiamin levels were only determined in the liver. The results are presented in table 4. In this case the values for old and young rats differed significantly and are therefore treated separately. In both groups, the tea-treated rats showed a

Table 1. Effects of tea on transketolase activity in rat tissues following eight weeks of drinking tea extract.

Group	No. of animals	−ThDP	+ThDP	Increase (%)
Whole blood (U/g Hb)				
Control	11	0.70 ± 0.058	0.82 ± 0.071	14.3 ± 1.91
Green tea	10	0.50 ± 0.061***	0.63 ± 0.068***	15.6 ± 2.14
Black tea	12	0.51 ± 0.039*	0.60 ± 0.051	13.6 ± 1.86
Liver (U/g liver)				
Control	11	3.21 ± 0.126	3.39 ± 0.148	4.0 ± 0.70
Green tea	11	2.78 ± 0.084**	3.02 ± 0.103	7.1 ± 1.49
Black tea	12	2.81 ± 0.183	3.04 ± 0.162	8.2 ± 1.93
Mucosa (U/g protein)				
Control	11	0.205 ± 0.0122	0.226 ± 0.0785	11.2 ± 2.98
Green tea	11	0.178 ± 0.0186	0.200 ± 0.0195	11.6 ± 3.76
Black tea	12	0.207 ± 0.0227	0.232 ± 0.0227	10.8 ± 2.27

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Values are given as the mean ± standard error.

Table 2. Effects of tea on lactate dehydrogenase activity in rat tissues following eight weeks of drinking tea extract.

Group	Control	Green tea	Black tea
No. of animals	11	10	12
Liver (U/g liver ±SE)	189 ± 10.3	186 ± 14.0	190 ± 13.0
No. of animals	6	6	7
Heart (U/g heart ±SE)	15.4 ± 2.11	14.7 ± 1.45	9.41 ± 1.61**
No. of animals	11	10	12
Whole blood (U/g Hb)	132 ± 6.4	99 ± 15.3**	112 ± 6.2**

**$p < 0.05$. Values are expressed as units/g fresh liver ± the standard error.

Table 3. Effects of tea on enzyme activities in the intestinal mucosa of rats following eight weeks of drinking tea extract.

Group	Control	Green tea	Black tea
No. of animals	11	11	12
Alkaline phosphatase	113 ± 22.6	113 ± 13.6	122 ± 13.3
No. of animals	6	6	7
Thiamin diphosphatase	3.29 ± 0.040	1.97 ± 0.258**	2.06 ± 0.396**

**$p < 0.05$. Values are expressed as units of activity/mg protein ± the standard error.

Table 4. Effects of tea on liver total thiamin (μg/g liver) levels of rats following eight weeks of drinking tea extract.

Group	No. of animals	Adult rats	No. of animals	Young rats
Control	8	1.40 ± 0.25	3	2.38 ± 0.23
Green tea	8	1.87 ± 0.12*	3	3.76 ± 0.05*
Black tea	8	2.32 ± 0.33**	4	3.81 ± 0.09***

*$p = 0.05$; **$p < 0.05$; ***$p < 0.005$.

significant increase in the concentration of total thiamin in the liver. This was unexpected and no explanation is evident. One would have expected the values to decrease if tea consumption interferes with thiamin absorption [3, 8–10]. It is interesting that young rats had higher thiamin levels than the older rats.

Tea consumption had no effect on the total calcium in the heart.

Acknowledgments. We acknowledge with thanks the support supplied by Kuwait University grant No. SB015.

* To whom all correspondence should be addressed.
+ Visiting Professor from Brigham Young University, Provo, Utah, USA.

1 Wesig, I. H., Green, A. M., and Haag, J. R., J. biol. Chem. *165* (1946) 737.
2 Kundig, H., and Somogyi, J. C., Int. J. Vit. Res. *34* (1964) 135.
3 Hilker, D. M., Chan, V. C., Chan, R., and Smith, R. L., Nutr. Rep. int. *4* (1971) 223.
4 Hilker, D. M., and Somogyi, J. C., Ann. N.Y. Acad. Sci. *378* (1982) 137.
5 Somogyi, J.C., and Bonicke, R., Int. J. Vit. Res. *39* (1969) 65.
6 Davis, J. S., and Somogyi, J. C., Int. J. Vit. Res. *39* (1969) 401.
7 Rungruansak, K., Tosukhowong, P., Panijpan, B., and Vimokesant, S. L., Am. J. clin. Nutr. *30* (1977) 1680.
8 Vimokesant, S., Nakornchai, S., Dhanamitta, S., and Hilker, D. M., Nutr. Rep. int. *9* (1974) 371.
9 Bukr, S., and Hilker, D. M., Fedn Proc. *36* (1976) 443.
10 Vimokesant, S., Kunjara, S., Rungruansak, K., Nakornchai, S., and Panijpan, B., Ann. N.Y. Acad. Sci. *378* (1982) 123.
11 Schaller, K., and Holler, H., Int. J. Vit. Nutr. Res. *43* (1976) 143.
12 Sarkar, L., De, B. K., and Chaudhuri, D. K., Int. J. Vit. Nutr. Res. *46* (1976) 412.
13 Hayakawa, F., and Murata, K., Vitamins (Japan) *2* (1979) 55.
14 Schaller, V. K., Brammertz-Buchen, C., Brammertz, K. H., and Holler, H., Tierphysiol. Tierernähr. Futtermitt. *38* (1977) 65.
15 Yoo, Y., and Hilker, D. M., Korean J. Nutr. *12* (1979) 33.
16 Ruenwongsa, P., and Pattanvibag, S., Experientia *38* (1982) 33.
17 Matsuda, T., Baba, A., and Iwata, H., Experientia *34* (1978) 18.
18 Bai, P., Bennion, M., and Gubler, C. J., J. Nutr. *101* (1971) 731.
19 Ali, M., Gubler, C. J., Al Saleh J., and Abu Farsak, F., Comp. Biochem. Physiol. *87B* (1987) 833.
20 Al-Hassan, J. M., Ali, M., Thomson, M., Fatima, T., and Gubler, C. J., Toxicon *23* (1985) 532.
21 Bergmeyer, H. U., and Bernt, E., in: Methods of Enzymatic Analysis, 2nd edn, vol. 2, p. 590. Ed. H. U. Bergmeyer. Academic Press, New York 1974.
22 Methods of Vitamins Assay, 2nd edn, p. 107. The Association of Vitamin Chemists, Inter Science Publishers, Inc. New York 1975.
23 Iwata, H., Matsuda, T., and Baba, A., Experientia *32* (1976) 1252.
24 Hulanicki, A., and Trojanowicz, W., Analyt. chim. Acta *68* (1974) 155.

0014-4754/89/010112-03$1.50 + 0.20/0

Instructions to Authors

Experientia is a monthly journal for life sciences devoted to publishing articles which are interdisciplinary in character and which are of general scientific interest. Considered for publication will be hitherto unpublished papers that fall within the following categories:

Reviews (one-man and multi-author reviews)
Mini-reviews (1–2 printed pages)
Full Papers
Short Communications (1–2 printed pages, published within 3 months of acceptance)

Papers reporting on work that is preliminary in nature, or wherein animal experiments have been conducted without the appropriate anesthesia, will not be accepted.

Manuscripts (including all tables and figures) must be submitted in *triplicate* and must be in *English*. *Title pages* should bear the author's name and address (placed directly below the title), a brief *abstract* (of approximately 50 words for short communications) mentioning new results only, and a listing of *key words*. *Footnotes* must be avoided. *Tables*, and then *figures*, are to follow the body of the text and should be marked with self-explanatory captions and be identified with the author's name. All *data* should be expressed in units conforming to the Système International (SI). *Drawings* are to be on heavy bond paper and marked clearly in black. *Photographs* should be supplied as glossy positive prints. Please note that we use two different systems for citing references. 1. For *Review Articles*, references should be arranged alphabetically and be numbered. Within the text, literature should be referred to by number and, where indicated, by author. The references should contain full journal article titles and the first as well as the last page of the article cited. 2. For *Short Communications, Full Papers* and *Mini-reviews*, an abbreviated bibliography is requested and references should be listed chronologically. Please consult a current issue of Experientia or inquire at the editorial office for details on form.

Authors are requested to specify into which discipline their papers fall:

1. Anatomy, Physiology
2. Biochemistry and Biophysics
   Metabolism
   Neurobiology
   Pharmacology
3. Endocrinology
4. Cellular Biology
   Molecular Biology
   Immunology
5. Genetics, Developmental Biology
6. Ethology, Ecology
   Natural Product Chemistry

All incoming manuscripts are acknowledged immediately. Authors will be notified of the editorial board's publishing decision once their manuscripts have been evaluated by a minimum of two field experts. Fifty reprints of papers accepted for publication will be sent to authors free of charge; additional reprints may be ordered.

Manuscripts and all communications to the editors should be addressed to:

Experientia
Birkhäuser Verlag
P.O. Box 133
CH-4010 Basel/Switzerland
Tel. 061 73 53 00