# Thiamine triphosphate metabolism and its turnover in the rat liver

H. Sanemori and T. Kawasaki

*Department of Biochemistry, Hiroshima University School of Medicine, Hiroshima 734 (Japan), 22 September 1981*

*Summary.* When rats were injected with a thiamine disulfide derivative, the content of thiamine triphosphate (TTP) in the liver doubled within 3 h after a preceeding rise in thiamine pyrophosphate; it then returned to the basal level within the next 3 h, indicating a net increase of TTP in vivo and its rapid turnover.

The role of thiamine pyrophosphate (TPP) as coenzyme is well documented; and possible coenzyme-independent functions of thiamine, especially of thiamine triphosphate (TTP), have been reviewed from the point of view of its neurophysiological effects[1-4]. It had been estimated that most animal tissues contain TTP as 5-9% of the total thiamine[5], but this percentage was more recently shown to be 0.7-1.6% when analyzed by high performance liquid chromatography (HPLC)[6-8].

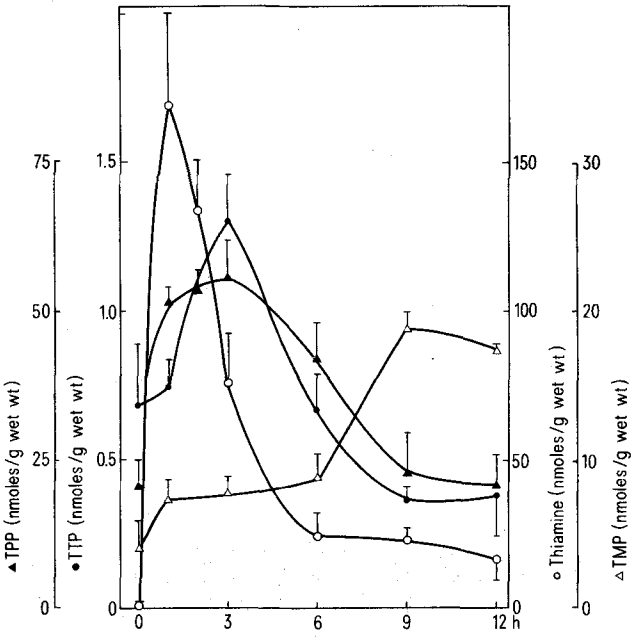
TTP synthesis from TPP was reported to be catalyzed by TPP phosphotransferase (EC 2.7.4.15) in rat brain[9,10]. However, Schrijver et al.[11] could not confirm a net synthesis of TTP by rat brain preparations either by direct measurement of TTP formation or by following the ATP consumption. We also tried to determine directly by HPLC the enzymatic formation of TTP from TPP under the conditions described by Itokawa[12], but failed to detect any enzyme activity, even though the sensitivity of the HPLC method used was enough to detect 0.1 pmoles of TTP[8]. In addition, metabolism of TTP in vivo has not yet been reported. Although a preliminary paper[13] described the turnover of thiamine derivatives in rat organs, no evidence has been obtained for a net increase in TTP content in animal tissues so far as we are aware. Recent development in analysis of thiamine phosphates by HPLC at subpicomole level[6-8], however, has allowed us to study the metabolism and turnover of thiamine and its phosphates, especially of TTP in rat liver, which is described in this paper.

*Materials and methods.* Male Wistar rats (250-300 g) were fasted overnight and injected i.p. with approximately 0.1 ml of a thiamine disulfide derivative solution at a dose of 25 mg/kg b.wt. The disulfide used was thiamine tetrahydrofurfuryldisulfide (TTFD) hydrochloride obtained from Takeda Chemical Industries, Osaka. TTFD was used in the experiments because of its property of being readily transferable to tissues[14]. The animals were sacrificed at the indicated times after the injection, and the liver and brain were immediately removed and frozen in liquid $N_2$. Procedures for preparation of the tissue extract and conversion of thiamine phosphates in the extract to corresponding thiochrome compounds were identical to those described in previous papers[7,8]. Straight phase HPLC[6] equipped with the same fluorometer as for reverse phase HPLC[8] was used to determine thiamine and its phosphates in the identical manner as described[6]. The content of thiamine and its derivatives in the liver was expressed as an average value for 5 different animals with SD's in the figure. TTP was a gift from Dr M. Yamazaki, Research Laboratory of Sankyo Co., Tokyo, and the other reagents were all of analytical grade.

*Results and discussion.* Changes in the level of thiamine and its phosphates in the liver after injection of TTFD are shown in the figure. Thiamine, at first, was markedly and rapidly increased within 1 h to a maximum level of 170 nmoles/g wet wt and then evenly reduced to a level of 34 nmoles/g wet wt during the following 3 h. The level of TPP, which is formed from thiamine by thiamine pyrophosphokinase (EC 2.7.6.2), was also increased to 57 nmoles/g wet wt (2.6-fold higher than the basal level) within 1 h after injection, and remained at this level for about 2 h. Thereafter, there was a gradual decrease to the basal level during the following 6 h. Thiamine monophosphate (TMP) content gradually increased for 6 h after injection and then doubled in the next 3 h as the level of TPP decreased.

The concentration of TTP started to rise only after the increase in TPP level, reached a maximum (1.3 nmoles/g wet wt), 2-fold higher than the basal content 3 h after TTFD injection, and then decreased fairly rapidly to the basal level within the following 3 h. The TTP level was further reduced thereafter, showing a minimum of 0.37 nmoles/g wet wt 9 h after the injection, followed by a tendency to return to the basal level. The balance among thiamine metabolites changed between the 1st and 3rd h after TTFD injection; thiamine decreased by 113 nmoles/g wet wt, while TMP, TPP and TTP increased by 0.40, 4.3 and 0.47 nmoles/g wet wt, respectively.

The time taken for the content to fall to half its maximum level was calculated to be approximately 2, 5, and 3 h, for thiamine, TPP, and TTP, respectively. The turnover of thiamine and its phosphate esters in rat organs was investigated[13] by the pulse labeling method with [$^{35}$S]thiamine, and thiamine compounds were shown to be turning over in the brain, heart, and liver at approximately same half-life of 33-35 h. Our results indicate that the turnover of TTP synthesized in vivo in the rat liver is much more rapid, and after administration of TTFD the synthesis of TTP in vivo follows TPP synthesis, which is consistent with the formation of TTP from TPP by TPP phosphotransferase.




Profile of the change in the content of thiamine and its phosphates in the livers of rats injected i.p. with TTFD (25 mg/kg b.wt). Experimental procedures are given in the text and each point represents mean ± SD (n = 5). ○, Thiamine; △, TMP; ▲, TPP; ●, TTP.

The change in the content of TPP and TTP in the brain after TTFD injection was analyzed by the same procedure as that in the liver, and a slightly increased level of both TPP and TTP was observed at 3 h after the injection (data not shown). However, this increase was not sufficiently distinct to get exact kinetics of these changes. This may be due to a slow penetration of thiamine through the blood-brain barrier when injected in large doses[15,16].


1 P.M. Dreyfus, in: Thiamine, p.229. Eds C.L. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
2 R.L. Barch, in: Thiamine, p.283. Eds C.L. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
3 Y. Itokawa, Vitamins (Japanese) *49*, 415 (1975).
4 H. Iwata, Vitamins (Japanese) *50*, 1 (1976).
5 G. Rindi and L. de Guiseppe, Biochem. J. *78*, 602 (1961).
6 K. Ishii, K. Sarai, H. Sanemori and T. Kawasaki, Analyt. Biochem. *97*, 191 (1979).
7 K. Ishii, K. Sarai, H. Sanemori and T. Kawasaki, J. nutr. Sci. Vitaminol. *25*, 517 (1979).
8 H. Sanemori, H. Ueki and T. Kawasaki, Analyt. Biochem. *107*, 451 (1980).
9 Y. Itokawa and J.R. Cooper, Biochim. biophys. Acta *158*, 180 (1968).
10 J.H. Pincus, Y. Itokawa and J.R. Cooper, Neurology *19*, 841 (1969).
11 J. Schrijver, T. Dias and F.A. Hommes, Neurochem. Res. *3*, 699 (1978).
12 Y. Itokawa, in: Thiamine, p.361. Eds C.L. Gubler, M. Fujiwara and P.M. Dreyfus. Wiley, New York 1976.
13 I. Sen and J.R. Cooper, Neurochem. Res. *1*, 65 (1976).
14 M. Fujiwara, J. nutr. Sci. Vitaminol. *22*, suppl. 57 (1976).
15 A. Inoue, S. Shim and H. Iwata, J. Neurochem. *17*, 1373 (1970).
16 R. Spector, Am. J. Physiol. *230*, 1101 (1976).


## Relationship between the molecular weights of pesticides and their bioconcentration factors by fish


J. Kanazawa

*National Institute of Agricultural Sciences, Kannondai, Yatabe, Ibaraki, 305 (Japan), 12 October 1981*



*Summary.* A significant positve correlation was found between the molecular weights (187–412) of 19 pesticides and their bioconcentration factors (4–37,800) in 2 fresh-water fishes; topmouth gudgeon and fathead minnows.


For many non-ionic organic compounds, including pesticides, it has already been shown that the bioconcentration factor (BCF) from water by fish increases as the solubility in water decreases, or as the 1-octanol-water partition coefficient (PC) increases[1–5]. These 2 physico-chemical properties of chemicals were used to predict their bioconcentration potential in living organisms[6]. Zitko and Hutzinaer[7] reported that for both chlorinated and brominated biphenyls, in Atlantic salmon, the accumulation coefficients from water decrease with increasing molecular weights. But, on the contrary, Southworth et al.[8] found that for 3 azaarene homologues, isoquinoline, acridine and benz (a) acridine, the BCFs in *Daphnia pulex* increased dramatically with increase in molecular weight. It is not clear whether this difference is due to the structure of the compounds or the physiological characteristics of the test organisms. The present study is directed towards obtaining a basic understanding of the relationship between the BCFs of 15 pesticides in fresh-water fish; adding to the results for topmouth gudgeon, already reported by the author[5], the BCFs of 4 reference pesticides and their molecular weights.

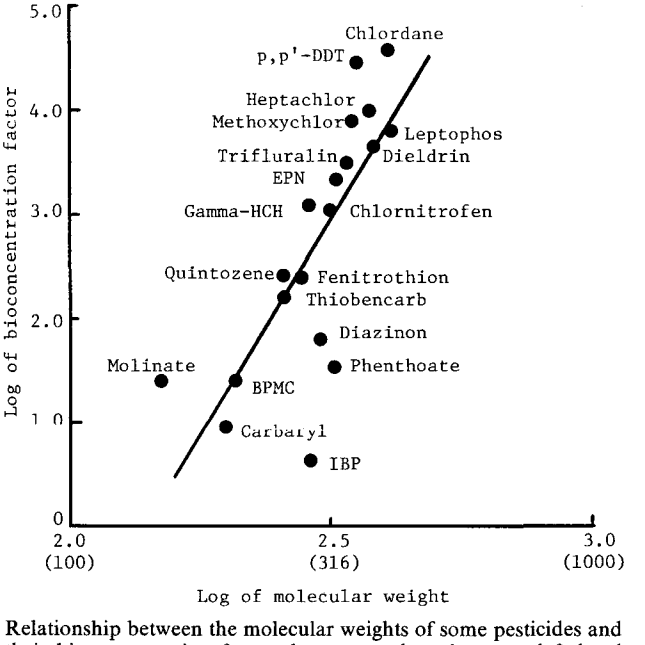
*Material and methods.* For the BCFs for topmouth gudgeon *(Pseudorasbora parva)* from water using a flow-through test at equilibrium, and the PCs of 15 pesticides, the values already reported by the author[5] were used. The BCFs for methoxychlor, p,p'-DDT, heptachlor and chlordane were the values in fathead minnows *(Pimephales promelas)* reported by Veith et al.[4].

*Results and discussion.* The molecular weights, BCFs and PCs of 19 pesticides including 6 organochlorine insecticides, 5 organophosphorus insecticides, 2 carbamate insecticides, 2 fungicides and 4 herbicides are shown in the table. A plot of log (BCF) vs log (molecular weight) is shown in the figure. As shown in the figure, for the 19 pesticides tested, which had a wide range of structures, the BCF increased as the molecular weights increased from 187 to 412. A satisfactory linear relationship is observed between the log of BCFs and the log of molecular weights. The regression equation shows:

$$\text{Log } Y = 10.9 \log X - 24.2$$

Where Y is the BCF by fish and X is the molecular weight of the pesticides. The correlation coefficient was 0.846 which was significant at the 1% probability level. Therefore, it is possible to predict the bioconcentration potential of a pesticide by the molecular weight.

It is already well known that a significant correlation is present between the PC values of many non-ionic organic compounds and their BCFs from water by fish[1,2,4,5]. Therefore, it can be presumed that the partition of chemicals in water through the gills into the blood of fish, and the rate




Relationship between the molecular weights of some pesticides and their bioconcentration factors by topmouth gudgeon and fathead minnows. Parenthesis shows an integral number.