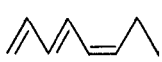
Table 2. The response of spermatozoid-attractants and related compounds

Attractant		Activity* *P. wrightii*	*F. evanescens*
	natural fucoserratene (**1**)	+ + +	+ + +
	synthetic fucoserratene (**1**)	+ + +	+ + +
	synthetic 1, trans-3, trans-5-octatriene** (**2**)	+ + +	+ + +
	synthetic 2-methyl-1,3,5-heptatriene*** (**3**)	±	±

* The assay for spermatozoid-attracting activity was carried out at a concentration of 10^{-2} and 10^{-3}M according to Müller's method[11,12]; + + +, very active; ±, slightly active or inactive. ** Over 95% purity. *** A mixture of geometrical isomers[9].

P. wrightii and *F. evanescens* was only slightly less than that of **1**. Müller et al.[13] have demonstrated that fucoserratene (**1**) attracts the male gametes of dioecious brown algae, *F. serratus* and *F. vesiculosus*. Recently, we have reported that the male-attracting substance of a dioecious brown alga *Sargassum horneri* consists of **1** (15%), **2** (10%), 1, cis-3, trans-5-octatriene (65%) and cis-2, cis-4, trans-6-octatriene (10%) which have been shown to attract the male strongly[7,9]. Experiments to clarify the roles of **2** and **3** in the seriated sex behavior of hermaphrodite brown algae, particularly an examination of the synergistic and inhibitory effects and of the specificity of sexual chemotaxis, are still in progress.

1 Acknowledgments. The authors would like to express their thanks to Mr A. Nishikawa for technical assistance, to Dr M. Abe (Yamagata Univ.) for valuable discussion on biological work and to Prof. Y. Sakai (Hokkaido Univ. Director of the Institute of Algological Research, Muroran) for the facilities during the investigation of *P. wrightii* and *F. evanescens*. Financial support from the Ministry of Education Science, and Culture (Grant-in Aid for Scientific Research No. 556092) is gratefully acknowledged.

2 To whom correspondence should be addressed.
3 D.G. Müller and L. Jaenicke, FEBS Lett. *30*, 137 (1973). The structure of fucoserratene was determined by GC, UV and GC-MS using synthetic specimens: no ^{13}C-NMR and ^{1}H-NMR spectral data of the natural fucoserratene were reported because the isolated amount of the attractant was minute.
4 L. Jaenicke and K. Seferiadis, Chem. Ber. *108*, 225 (1975).
5 M. Abe, Bot. Mag., Tokyo *83*, 254 (1970).
6 M.P. Schneider and M. Goldbach, J. Am. chem. Soc. *102*, 6114 (1980).
7 T. Kajiwara and A. Hatanaka, 24th TEAC meeting 1980, abstracts p. 199. Chem. Soc. Japan, Tokyo.
8 T. Kajiwara, K. Kodama and A. Hatanaka, Bull. Jap. Soc. scient. Fish. *46*, 555 (1980).
9 T. Kajiwara, K. Kodama and A. Hatanaka, Naturwissenschaften *67*, 612 (1980).
10 T. Kajiwara and A. Hatanaka, 23rd TEAC meeting 1979, abstracts p. 180. Chem. Soc. Japan, Tokyo.
11 D.G. Müller, Z. PflPhysiol. *80*, 120 (1976).
12 K. Lüning and D.G. Müller, Z. PflPhysiol. *89*, 333 (1978).
13 D.G. Müller and K. Seferiadis, Z. PflPhysiol. *84*, 85 (1977).

Effects of hypochlorite on thiamine and its derivatives

J. Jaroensanti and B. Panijpan[1]

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 4 (Thailand), 3 October 1980

Summary. Thiamine and its phosphorylated derivatives reacted with hypochlorite with reaction rates following the order: thiamine > thiamine monophosphate > thiamine diphosphate from pH 4.0 to 6.5. At least one unknown transient intermediate was formed and at least one non-thiochrome product was fluorescent. Chemiluminescence was also observed.

Recently Yagi and Itokawa[2] made an important report that chlorinated tap water and hypochlorite solution destroyed thiamine. In the reaction the hypochlorite ion was considered to be the species that cleaved thiamine mainly into its 4-amino-2-methyl-5-hydroxymethylpyrimidine (**I**) and 5-hydroxyethyl-4-methylthiazole (**II**) moieties. Only the reaction with thiamine was reported and there was no indication of any reaction intermediates or other products.

Here we wish to report our comparative studies on the hypochlorite destruction of thiamine (Th) and its 2 commonly-found biological phosphorylated derivatives thiamine monophosphate (ThMP) and thiamine diphosphate (ThDP). In addition, evidence is presented to show that at least one transient intermediate and product other than **I** and **II** above occurred in the hypochlorite reaction with thiamine. Chemiluminescence and fluorescence were also observed.

Materials and methods. Th, ThMP and ThDP were from Sigma. **I** and **II** were gifts kindly provided by Dr Y. Ito of Takeda Chemical Co. The chlorine concentration of sodium hypochlorite from BDH and that present in the tap water was determined by titration with sodium arsenite and the end point indicated by the iodide and starch system[3]. A Beckman Acta V, a Gilford 2000 and a Perkin-Elmer (Coleman 55) spectrophotometers were used for spectral scanning, absorbance vs time plots and absorbance measurements respectively. An Aminco Bowman spectrofluorometer was used for obtaining the fluorescence spectra and quantitating free thiamine in the form of thiochrome (excitation at 375 nm, emission at 430 nm) produced by

CNBr[4]. Products were analyzed by high voltage paper electrophoresis[5] and TLC[6]. The reaction rate was obtained from the initial slope of the percentage thiamine remaining vs time (min) plot.

Results and discussion. We found the destruction of Th, ThMP and ThDP by hypochlorite and Bangkok chlorinated tap water (~ 2–3 ppm chlorine) to be dependent on the hypochlorite concentration, pH and temperature in essentially the same way as reported by Yagi and Itokawa[2]. However, it is interesting to note that the reaction rate followed the order of Th > ThMP > ThDP from pH 4.0 to pH 6.5 and Th > ThDP > ThMP above pH 6.6 (fig. 1). The rate differences may reflect the overall pH-dependent effects of various charged groups on the conformation and electronic distribution of various thiamine species and thus the latter's reactivity with hypochlorite[8–10]. The rate of reaction was significant even at 17 °C. From 30 to 42 °C, the reaction rate for all 3 thiamines was enhanced to a similar extent, ~ 2-fold/10 °C at pH 7.4, indicating similar activation energy. It is to be noted that simple pseudo-first order kinetic plots with respect to thiamine were not possible at the highest hypochlorite/thiamine ratio that did not cause interference with CNBr assay.

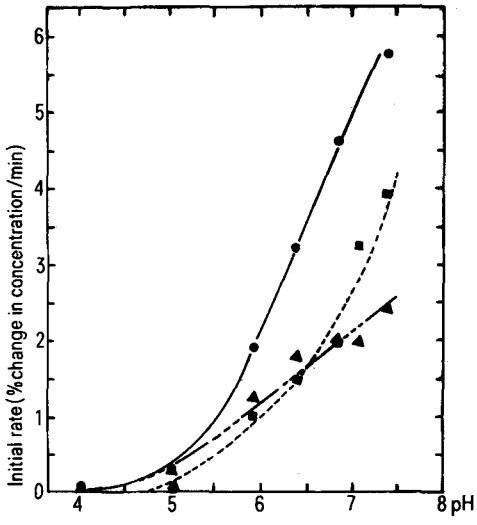


Figure 1. The pH-rate profile of 2.96 μM each of thiamine (—●—), thiamine monophosphate (—▲—), thiamine diphosphate (—■—) and 7.87 ppm chlorine, 35 °C.

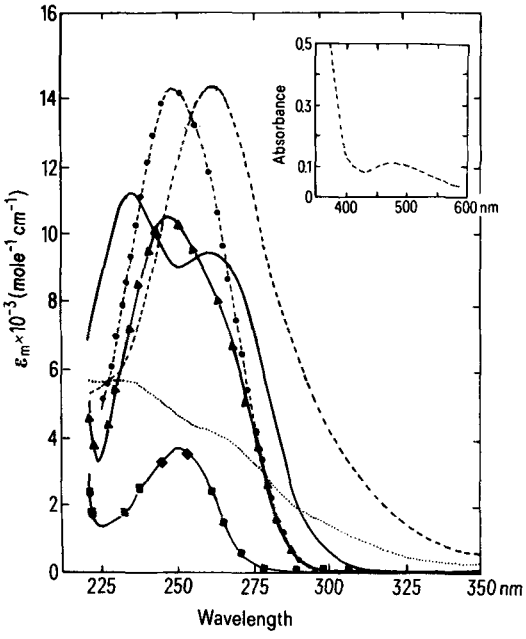


Figure 2. The UV-visible spectra at pH 5.0 of thiamine (——), I (—■—), II (—▲—) mixture of I and II (---●---), and the reaction mixture (initial thiamine concentration: 200 μM and hypochlorite: 56.8 ppm chlorine) at 2 min (- - - - - - -) and 3 h (· · · · · · · ·). Concentration of each component in thiamine, I, II and mixture of I and II solutions was 200 μM, 40 °C. Inset at 5 min, 25 °C, 1.8 mM thiamine and 78 ppm chlorine (- - - - -).

Time-dependent UV-visible spectra of the reaction between hypochlorite and Th, ThMP and ThDP indicated the existence of at least 1 transient intermediate and at least 1 product besides I and II. Figure 2 shows the individual spectra of solutions containing 200 μM thiamine, I and II. The spectrum of a mixture of I+II (each component at 200 μM) gave the same spectrum as that from the combination of individual spectra of I and II at the same concentration as in the mixture. Also shown in figure 2 are the rapidly changing spectra of the thiamine (200 μM) hypochlorite (56.8 ppm chlorine) reaction mixture at 2 min and the more time-invariant spectrum at 3 h. From detailed spectral analyses, it was clear that the spectra at 2 min and 3 h, and indeed those at times in between, could not be represented by any proportion and combination of I, II and thiamine alone (see also Doherty et al. 7). Following the reaction at various wavelengths from 225 to 305 nm, it was found that some of the absorbance vs time curves did not peak, bottom out or flatten at the same time. Besides, during and after the reaction, absorbance at wavelengths tending towards the visible region, e.g. ⩾ 360 nm, where absorbance is not detectable for thiamine or the maximum I and II products formed from it at the concentration used, absorption was clearly observed even at 20 μM thiamine and 1.42 ppm chlorine. At high concentrations of reactants at both pH 5.0 and 7.4, this long-wavelength absorption band gave rise to a pinkish-orange colour which changed intensity and hue rapidly with time at first and became more stabilized after 3 h at 25 °C. As can be seen from figure 2, I and II could not be the main components that gave rise to the final spectrum at time 3 h.

It should be added here that the 3-h thiamine-hypochlorite mixture exhibited an intense fluorescence (a property not exhibited by Th, I nor II) with a maximum excitation and emission wavelengths of 375 and 460 nm respectively at pH 7.4. That this fluorescence was not due to thiochrome alone was also indicated by an intense fluorescent spot on the thin-layer plate and paper electrophoretogram at a position not corresponding with that reached by the authentic thiochrome used. From tests with several compounds similar to or derived from thiamine, it appeared that the 4-aminopyrimidine moiety and not the thiazole moiety was essential for yielding the pinkish-orange solution with hypochlorite.

Another interesting observation is that during the first min of mixing thiamine with sodium hypochlorite intense chemiluminescence (much higher than backgrounds of controls) was observable in a Packard scintillation counter with the coincidence circuit switched off. This reaction-related luminescence was lowered by the presence of added bovine superoxide dismutase indicating the participation of superoxide. Probably, besides simple cleavage of the 2 heterocyclic rings at the methylene bridge as implied by Yagi and Itokawa, oxidation and chlorination also took place[11,12]. Elucidation of the mechanism of hypochlorite destruction of thiamine and derivatives needs further studies involving product identification, kinetics and structural analysis of the thiamines.

The above observations could have vast implications for the thiamine status of foods and feeds kept or prepared in water treated with chlorine. Chlorination of water is widely practised in many parts of the world where the thiamine intake in humans may only be marginal[13,14].

1 We thank the International Foundation for Science, Sweden, and the Thai University Development Council for financial support.
2 N. Yagi and Y. Itokawa, J. nutr. Sci. Vitaminol. *25*, 281 (1979).
3 A.I. Vogel, in: A Textbook of Quantitative Inorganic Analysis, 3rd edn, p.364. Longmans, London 1961.
4 M. Fujiwara and K. Matsui, Analyt. Chem. *25*, 810 (1953).
5 B. Panijpan and P. Detkriangkraikun, Am. J. clin. Nutr. *32*, 723 (1979).
6 P.P. Waring, W.C. Goad and Z.Z. Ziporin, Analyt. Biochem. *24*, 185 (1968).
7 J.J. Doherty, N. Cane and F. Wokes, J. Pharm. Pharmac. *7*, 1053 (1955).
8 A.A. Gallo, I.L. Hansen, H.Z. Sable and T.J. Swift, J. biol. Chem. *247*, 5913 (1972).
9 A.A. Gallo and H.Z. Sable, J. biol. Chem. *249*, 1382 (1974).
10 A.M. Chauvet-Monges, C. Rogeret, C. Brand and A. Crevat, Biochim. biophys. Acta *304*, 748 (1973).
11 S.K. Chakrabartty and H.O. Kretschmer, J. chem. Soc. Perkin *1*, 222 (1974).
12 Y. Ushijima and M. Nakano, Biochem. biophys. Res. Commun. *93*, 1232 (1980).
13 S.L. Vimokesant, S. Nakornchai, S. Dhanamitta and D.M. Hilker, Am. J. clin. Nutr. *28*, 1458 (1975).
14 C.S. Farkas, Can. med. Ass. J. *122*, 1356 (1980).

Plasma lecithin: cholesterol acyltransferase activity in high- and low-responding rhesus monkeys

A.K. Bhattacharyya

Departments of Pathology and Physiology, Louisiana State University Medical Center, New Orleans (LA 70112, USA), 3 February 1981

Summary. The initial rate of esterification of plasma cholesterol by lecithin: cholesterol acyltransferase (LCAT) was measured in high- and low-responding rhesus monkeys fed a moderately high cholesterol (0.15 mg/kcal) diet. The results show that the rate of esterification of cholesterol in the plasma of the high-responders was significantly ($p < 0.025$) higher than that of the low-responding animals. In view of known relationships between LCAT activity and plasma lipoprotein metabolism, it is suggested that the lipoprotein metabolism in the high-responders would differ from that in the low-responders.

Among rhesus monkeys fed cholesterol some develop severe hypercholesterolemia (high-responders), while some show only mild elevation in serum cholesterol level, (low-responders)[2]. Previously, we reported that high-responders absorb significantly higher percentage of intestinal luminal cholesterol than low-responders[2-4]. This difference in the intestinal absorption of cholesterol has been the only difference observed so far in respect to cholesterol metabolism between high- and low-responders that explains the differential response in plasma cholesterol concentration when fed cholesterol[2-4]. Based on free and esterified cholesterol content and specific radioactivity of free and esterified cholesterol in plasma chylomicrons 4 h after feeding a test meal by gavage containing radiolabeled cholesterol, we have suggested that the high absorption of cholesterol in high-responders is probably due to increased uptake and esterification of cholesterol by the intestinal mucosa[3]. Cholesterol, besides being esterified within the intestinal mucosa by the esterifying enzymes, cholesterol esterase[5] and acylcoenzyme A cholesterol acyltransferase (ACAT)[6-7], is also esterified in the systemic circulation by the enzyme present in the plasma, lecithin cholesterol acyltransferase (LCAT)[8]. It was of interest, therefore, to study the rate of esterification of cholesterol by LCAT in the plasma of the 2 highly selected groups of rhesus monkeys.

Materials and methods. 10 adult, male rhesus monkeys, weighing between 8 and 14 kg, were used in the study. The monkeys were selected previously as high- or low-responders from a group of 36 young adult male monkeys on the basis of the response of plasma cholesterol to an atherogenic diet[2]. Of the 10 animals, 6 were high-responders and 4 were low-responders.

The monkeys were fed for more than a year a semisynthetic moderately high cholesterol diet providing fat at 38% of calories and proteins at 15% calories. The cholesterol content of the diet was 0.15 mg/kcal[4]. The animals were fed once daily about 150 g diet or 600 calories a day which was sufficient to maintain body weight.

Blood samples were obtained after an overnight fast in tubes containing disodium EDTA (1 mg/ml) and were immediately cooled in crushed ice. The plasma was separated by centrifugation at 4 °C and was used immediately for determination of LCAT activity.

Plasma cholesterol esterification or LCAT activity was measured by the method of Marcel and Vezina[9] by measuring the decrease in the concentration of plasma free cholesterol before and after incubation. Aliquots of the plasma in triplicate were incubated in stoppered erlenmyer flasks in a shaking incubator at 37 °C for 30 min. The reaction was stopped by addition of 5 ml methanol and lipids were extracted by the method of Folch et al.[10]. The lipid extract was evaporated to dryness under N_2 and the residue dissolved in small volume of hexane was subjected to TLC for free and esterified cholesterol separation using silica gel G plate with light petroleum/diethyl ether/glacial acetic acid (80:20:1, v/v). The free and ester bands were scraped and eluted with diethyl ether. The free sterol was subjected to as trimethylsilyl ether derivative with 5 α-cholestane used as the internal standard as described below. The sterol esters were saponified with alcoholic KOH, extracted with hexane and processed as the free sterol.

GLC was equipped with a hydrogen flame ionization detector and an automatic digital integrator. The glass column (183 cm × 4 mm inner diameter) was packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q (Applied Science Labs, State College, PA). Temperatures of column, detector and flash heater were 235,220 and 250 °C, respectively. Helium was used as carrier gas at 30 ml/min; the inlet pressure was 40 psi.

Results. The plasma total cholesterol in the high-responding rhesus monkeys was significantly higher than the low-responding animals (230 vs 142 mg/dl, $p < 0.025$) (table). Similarly plasma free and esterified cholesterol concentrations in the high-responders were also significantly higher than the low-responding group. However, plasma esterified cholesterol concentration when expressed as percent of