ulate the enzyme activity more drastically in a narrow range of their concentration. Both types of hormonal control may cooperate with each other to modulate an appropriate enzyme activity. This hypothesis is compatible with the results of Joshi and Aranda who showed that insulin is obligatory and thyroid hormone is secondary[7].

Our preliminary data also showed that starvation reduced serum insulin levels, thyroxine levels and thyroxine 5'-deiodinase activity in liver, but refeeding with glucose restored the hormonal levels to normal and simultanously elevated the enzyme activity (unpublished). The response in deiodinase activity to starvation and refeeding resembles that for stearoyl CoA desaturase[18]. Therefore, it seems likely that this 'multi-type' regulatory mechanism by hormones is of universal importance.

Since several biological factors[2], in addition to hormones, are involved in regulating the desaturase in vivo, it is difficult to determine the ranking of factors contributing to the enzyme activity. However, it is possible that each biological regulator functions to control the enzyme activity in its own characteristic way.

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Hepatic phosphatidylcholine synthesis in deficiency of lysine and threonine: effect of malathion

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Summary. Rats fed on a rice diet deficient in lysine and threonine showed increased activities of CDP-Choline pathway enzymes and incorporation of (methyl-^{3}H)-choline into hepatic microsomal phosphatidylcholine, compared to rats fed on the same diet supplemented with lysine and threonine. However, the amount of microsomal phosphatidylcholine was significantly decreased in rats fed a deficient rice diet. These results suggest an enhanced phosphatidylcholine catabolism in rats fed on a rice diet deficient in lysine and threonine. Malathion administration reduced the amount of phosphatidylcholine in all the groups.

Phospholipids are important structural and functional components of biological membranes. Reduced hepatic phospholipids after feeding low quality protein or protein-deficient diets have been reported by several investigators[2–4]. The active role of phosphatidylcholine (PC) in microsomal drug metabolism has been well established[5]; PC probably helps in electron transfer processes and also in accumulating nonpolar substrates. Conney et al.[6] have reported that malathion, chlorothion and parathion inhibit the in vitro microsomal metabolism of testosterone.

In the liver, PC is synthesized by more than one pathway; however, the major pathway is the CDP-choline pathway[7]. The rate-limiting steps of PC synthesis by this pathway are catalyzed by choline kinase and cholinephosphate cytidylyltransferase[8,9]. While studying the effect of malathion in various diets in rats, we found that the pesticide reduces metabolism of foreign compounds and inhibits hepatic PC synthesis. We reported earlier that malathion inhibits hepatic PC synthesis in rats by inhibiting CDP-choline pathway enzymes[10]. In the present study, we investigated whether the reduced microsomal PC in rats fed a diet deficient in lysine and threonine is due to reduced synthesis by the CDP-choline pathway and whether it is further reduced by malathion treatment.

Materials and methods. Adenosine triphosphate, choline chloride, cytidine triphosphate, activated charcoal, Dowex 1 × 8 (200–400 mesh), dithiothreitol, and 1-amino, 2,4 naphthol sulphonic acid were obtained from Sigma Chemical Co., St. Louis, MO. All organic solvents were purchased from British Drug House, Ltd., India. Malathion was a gift from M/S Excel Industries, India. (methyl-^{3}H)-choline chloride (sp. act. 6.4 mCi/m mole), phosphoryl-(methyl^{14}C)-choline (sp. act. 52 mCi/m mole) and CDP-(methyl-^{14}C)-choline (sp. act. 51 mCi/m mole) were obtained from Radiochemical Center, Amersham, England.

Male Wistar rats (40–50 g) obtained from the V.P. Chest Institute were fed a rice diet with and without lysine and threonine for fifteen days. The diets were prepared as described by Viviani et al.[11]. Malathion (100 mg/kg b.wt/day dissolved in ground nut oil) was administered orally each day. The controls received the vehicle only. After 15 days the rats were killed, livers were removed, microsomes and soluble fractions were separated. The yield and protein content of microsomes/g liver were not significantly different in all the groups. Choline kinase activity was determined by a modified method of Weinhold and Rethy[12] as described by Ishidate et al.[13]. Cholinephosphate cytidylyltransferase activity was assayed by the modified method of Ansell and

Effect of lysine and threonine deficiency and malathion treatment on microsomal PC synthesis. Rats were fed a rice diet with (supplemented group) and without supplementation (deficient group) of lysine and threonine for 15 days. Malathion (100 mg/kg b.wt/day) was administered orally for 15 days. In a separate experiment (methyl ^{3}H)-choline (10 µCi/100 g b.wt) was injected 60 min before killing. Values are mean ± SE of six animals in each group.

Treatment	Supplemented		Deficient	
	(Methyl-^{3}H)-choline incorporation[a]			
None	617 ± 34		941 ± 51*	
Malathion	561 ± 39		687 ± 31	
	Phosphatidylcholine[b]			
None	71.4 ± 1.5		58.9 ± 3.1*	
Malathion	51.8 ± 3.7		40.5 ± 2.9	
Activity per	mg protein	g liver	mg protein	g liver
	Choline kinase[c]			
None	1.91 ± 0.07	216.1 ± 7.9	3.51 ± 0.18*	330.4 ± 16.9*
Malathion	1.82 ± 0.08	150.7 ± 6.6	1.69 ± 0.06	109.1 ± 3.90*
	Cholinephosphate cytidylyltransferase[c]			
None	0.74 ± 0.06	83.7 ± 2.8	1.14 ± 0.10*	107.3 ± 4.4*
Malathion	0.59 ± 0.02	48.9 ± 1.7	0.81 ± 0.06*	52.3 ± 3.9
	Cholinephosphotransferase[c]			
None	28.77 ± 1.20	448.8 ± 20.1	20.94 ± 1.39*	284.8 ± 13.6*
Malathion	26.96 ± 1.29	415.2 ± 17.9	16.07 ± 1.16*	213.7 ± 9.80*

[a] DPM/µg phosphatidylcholine phosphorus; [b] µg PC phosphorus/g liver; [c] Activity expressed as n mole of product formed /min; * Significantly different from supplemented group ($p < 0.05$).

Chojnacki[14]. Cholinephosphotransferase was assayed by the method of Ishidate et al.[13].

In a second experiment, rats were injected i.p. with (methyl ^{3}H)-choline (10 µCi/100 g b.wt) and were killed 60 min later. Hepatic microsomal lipids were isolated by the method of Folch et al.[15]. Phospholipids were separated by thin layer chromatography, as described by Abramson and Blecher[16]. Phospholipid fractions were visualized in an iodine chamber. Phosphorus was estimated in the PC fraction by the method of Bartlett[17]. Radioactivity was determined in a liquid scintillation counter.

Results and discussion. The activities of choline kinase and cholinephosphate cytidylyltransferase in the supplemented groups of rats were significantly reduced compared to those in the deficient groups. Administration of malathion reduced the activities of both enzymes in supplemented and deficient groups. The effect of malathion was more profound in the deficient group. However, the activity of cholinephosphotransferase was increased in the supplemented group in comparison to the deficient group, and it was not affected significantly by malathion. Vance and Choy[9] suggested that cholinephosphotransferase does not seem to influence the rate of PC synthesis, and one would expect an increased synthesis of PC in rats fed a lysine-threonine deficient diet. Our results did show a significant increase in incorporation of (methyl ^{3}H)-choline into hepatic microsomal PC in rats fed a lysine and threonine deficient diet. In spite of an increased synthesis of PC in deficient rats, the amount of hepatic microsomal PC was significantly decreased in this group suggesting the possibility of an increased catabolism of PC. The results indicate that malathion, in general, inhibits PC synthesis by an effect on CDP-choline pathway enzymes. It appears that malathion also inhibited the catabolism of PC in the deficient group, because the reduction of PC in both groups was the same. The reduced availability of PC in rats fed a diet deficient in lysine and threonine and treated with malathion may alter the structure and function of biological membranes.

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