C H A P T E R VI.

SOME ADDITIONAL FACTORS CONTROLLING CARBOHYDRATE METABOLISM IN MANGOES

Regulation of metabolism leads to the maximum efficiency of a cellular process by controlling the direction of a particular reaction sequence. Present views on metabolic regulation are based predominantly on interactions between enzymes, in the cell free preparations and the metabolities.

Metabolic regulation due to lipid material has received attention recently.[258-266,270-273] CoA esters of fatty acids have been implicated in the production of more glucose in perfused mammalian tissue.[258-263] Taketa and Pogel[270] have shown the inhibition of enzymes of glycolysis and HMP shunt by esters of fatty acids.

Several studies describing the inhibition of various enzymic activities by free fatty acids emphasising its significance in metabolic control have appeared.[264,265]

Webber et al,[271] Lee et al[272] and Lee and Webber[273] have reported that free fatty acids specifically inhibit the key glycolytic enzymes without affecting the gluconeogenic enzymes. Pande and Mead[274] found that enzymes from varied sources were inhibited by free fatty acids and suggested that the inhibitory effects are due to the detergent properties of these inhibitors. They also found that the inhibition was of the competitive type. Such inhibition was also found for the enzymes from Arthrobacter crystallopoietes.[265]

During ripening in mango a seven fold increase in carotene[275] and a four fold increase in fatty acids[153] have been reported earlier. The increase in carotene and fatty acid content was found to be accompanied by enhancement in the activity of enzymes generating reduced NADP viz the HMP shunt enzyme and malic enzyme.[65] Later it was suggested that carotenogenesis was regulated by phosphatase which deposphorylated the intermediates; β-carotene (constituting 60% of the total carotene in mango) was found to stimulate the enzyme.[267]

Fatty acids have been found to activate the process of citrate breakdown to liberate acetyl-CoA and oxaloacetate for synthetic reactions in mango fruit.[266] The availability of sugars for high respiration and synthetic reactions is limited by the fact that sugars accumulate in the ripening mangoes,[65] thereby, suggesting the involvement of a regulatory mechanism for sugar accumulation. Results in this chapter indicate the role of β-carotene and fatty acids in controlling the activities of some sugar catabolising enzymes.

The results summarised in Table XVII show that glucose 6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase the initial enzymes of HMP shunt as well as malic enzyme were inhibited by fatty acids and β-carotene whereas invertase, amylase from mango and other sources, isocitrate dehydrogenase (NADP dependent) and fructose diphosphatase from

TABLE XVII

Effect of 0.10 µM Oleic acid 0.27 µM Caprylic acid and 0.08 µM β-Carotene on some of the enzymes related to Carbohydrate Metabolism

Enzymes tested	Specific activity (units/mg.protein)			
	Control	Enzyme activity in presence of		
		Oleic* acid	Caprylic acid	β-Carotene
Glucose-6-phosphate dehydrogenase	15.0	-	5.0(66)	5.0(66)
6-Phosphogluconic dehydrogenase	13.0	5(61)	6.6(49)	3.3(77)
Malic enzyme	60.0	-	21.0(65)	42.0(30)
Isocitrate dehydrogenase (NADP)	5.0	-	5.0(0)	5.0(0)
FDPase	7.3	-	7.2(0)	7.0(0)
Invertase:				
a) Mango	0.162	0.162(0)	0.162(0)	0.162(0)
b) Yeast**	2.66	2.66(0)	2.66(0)	2.66(0)
Amylase:				
a) Mango	1.00	1.05(0)	1.00(0)	1.00(0)
b) Banana	1.40	1.42(0)	1.40(0)	1.40(0)
c) Aspergillus oryzae**	1.22	1.22(0)	1.20(0)	1.22(0)

* Oleic acid was omitted in inhibition studies where Mg or Mn ions were used.

** Purified enzyme from Sigma Chemical Co., U.S.A. were used.

Values are mean of 6 experiments.

Values in parenthesis indicate percentage inhibition.

β-Carotene and fatty acids were dissolved in acetone and acetone blanks were run.

mango were not inhibited. The inhibition of the above enzymes was not reversed by the addition of bovine serum albumin (1 mg./ml.). Thus suggesting that the increase in protein concentration did not have any effect on the inhibition of these enzymes and the inhibition was not a nonspecific one.

Crude fatty acid extracts from mango exert the same effect (Table XVIII) on these enzymes demonstrating that a naturally occurring lipid component from the fruit could be of some physiological significance in the control of the activity of these enzymes.

TABLE XVIII

Effect of crude Fatty acid from Mango on Glucose-6-phosphate dehydrogenase, 6-Phospho gluconic dehydrogenase and Malic Enzyme

	Specific Activity (units/mg. protein)		
	Glucose-6-phosphate dehydrogenase	6-phospho gluconic dehydrogenase	Malic enzyme
Control	15.0	13.0	60.0
Control + fatty acid mixture	9.0(40)	6.0(49)	30.0(50)

Figures in parenthesis are % inhibition.

The inhibition of these enzymic activities was progressive with the increasing concentrations of inhibitor (Table XIX). It was also found that the inhibition was of the competitive type with respect to the substrates of these enzymes (Fig. 17A & B, 18A & B, 19A & B). These results suggested that the inhibitor affects that form of the enzyme which combines with the substrate. The Km values with respect to substrate were found to be 1.5×10^{-5} 1.1×10^{-5} and 5×10^{-4} (M) for glucose-6-phosphate dehydrogenase, 6-phospho gluconic dehydrogenase and malic enzyme respectively.

TABLE XIX

Effect of increasing concentrations of Fatty acid and β-Carotene

Concentration in µM	Specific Activity (units/mg. protein) Enzymes tested		
	Glucose-6-phosphate dehydrogenase	6-phospho gluconic dehydrogenase	Malic enzyme
Control	15	13	60
Oleic acid:			
0.025	-	8.3(30)	-
0.05	-	6.6(49)	-
0.1	-	5.0(61)	-
Caprylic acid:			
0.060	10.0(35)	10.0(25)	48.0(20)
0.130	7.0(56)	8.3(30)	36.0(40)
0.210	5.0(66)	6.6(49)	21.0(65)
β-Carotene:			
0.020	8.0(49)	10.0(25)	58.0(3)
0.040	5.3(37)	6.6(49)	53.0(12)
0.080	3.0(84)	3.3(77)	42.0(30)

Figures in parenthesis are % inhibition.

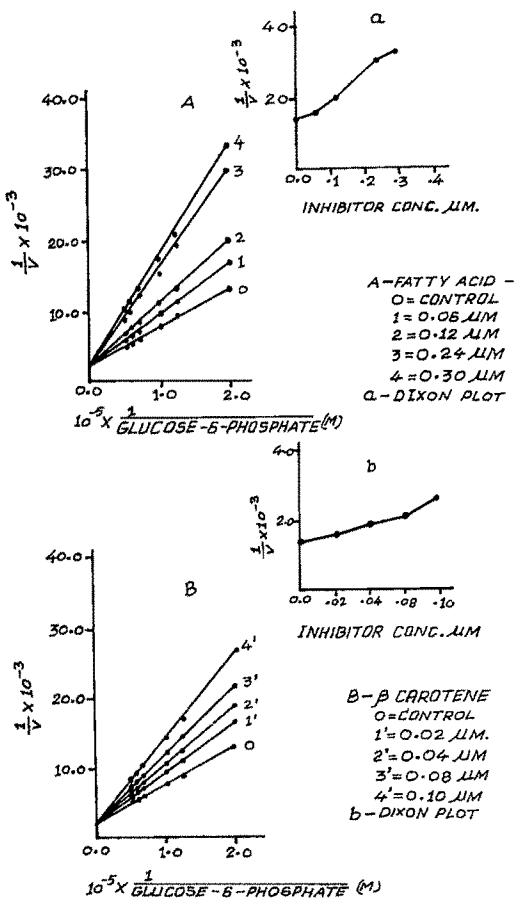

FIG. 17- LINE-WEAVER BURK PLOT FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE

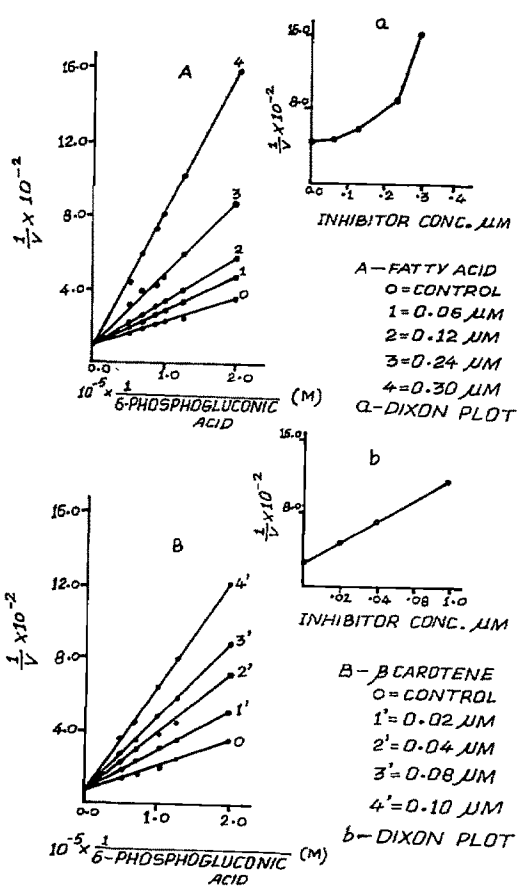

FIG. 18—LINE-WEAVER BURK PLOT FOR 6-PHOSPHOGLUCONIC DEHYDROGENASE

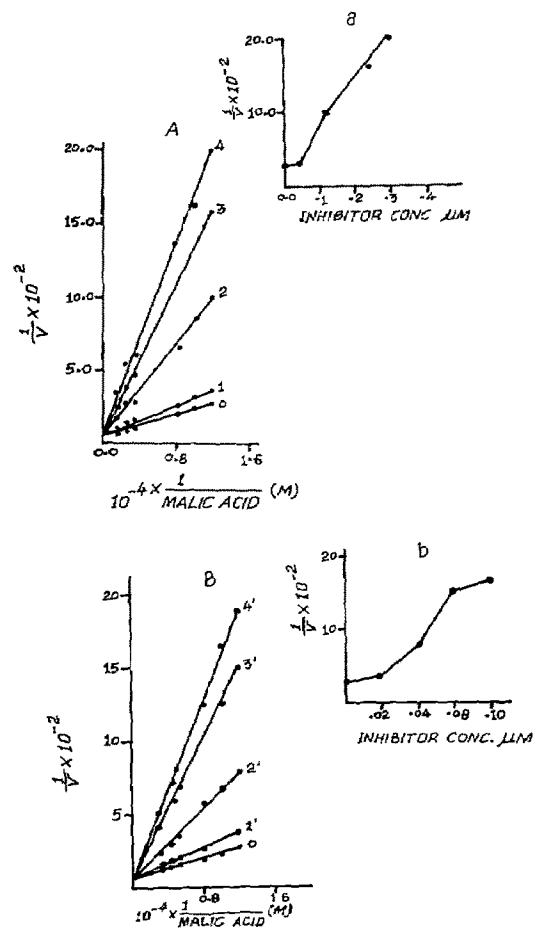

FIG. 13 LINE-WEAVER BURK PLOT FOR MALIC ENZYME
A – FATTY ACID : O = CONTROL, 1 = 0.06 µM
2 = 0.12 µM, 3 = 0.24 µM, 4 = 0.30 µM
B – β CAROTENE : O = CONTROL, 1' = 0.02 µM
2' = 0.04 µM, 3' = 0.08 µM, 4 = 0.1 µM

The values are similar to those reported from yeast,[295] liver[296] and wheat germ.[297] In presence of β-Carotene the Km values changed for the above three enzymes to 1.7×10^{-5} 1.8×10^{-5} and 7.8×10^{-4} (M) and in presence of fatty acid the change was 2.2×10^{-5} 2.5×10^{-5} and 7.1×10^{-4} (M).

Dixon plot of these enzymes revealed that glucose-6-phosphate dehydrogenase had a linear relationship with respect to both β-Carotene and fatty acid (Fig. 17a & b), whereas 6-phospho gluconic dehydrogenase showed a linear plot with β-Carotene but a non-linear plot with fatty acid (Fig. 18a & b). Malic enzyme however showed a non-linear plot with β-Carotene and a linear one (Fig. 19a & b) with fatty acid. These results suggest the possibility that fatty acid and β-Carotene may have multiple binding sites for 6-phospho gluconic acid dehydrogenase and malic enzyme respectively.

To get a comparative data, studies were extended with enzymes from papaya fruit and yeast. β-Carotene and fatty acid disposed a similar effect on both the enzymes of HMP shunt from papaya and yeast glucose-6-phosphate dehydrogenase; 6-phospho gluconic dehydrogenase from yeast was not inhibited (Table XX).

TABLE XX

Effect of Fatty acid and β-Carotene on Papaya and Yeast* Enzymes

	Units/mg. protein				
	Control	Enzyme activity in presence of			
		Caprylic acid		β-Carotene	
		0.05 µM	0.10 µM	0.02 µM	0.04 µM
Glucose-6-phosphate dehydrogenase	5.4(250)	3.3(180)	2.2(110)	4.0(165)	3.3(130)
6-phospho gluconic dehydrogenase	78.0(1300)	68.0(1300)	60.0(1300)	72.0(1300)	64.0(1298)

* Obtained from Sigma Chemical Co., U.S.A.
Figures in parenthesis are values for yeast.

Studies were also carried out to investigate whether the inhibition due to β-Carotene was due to its terpene nature. It was found that vitamin A and geraniol has no effect on the enzymes tested from mango (Table XXI).

TABLE XXI

Effect of Vitamin A and Geraniol on the Mango Enzymes

	Units/mg. protein	
	Glucose-6-phosphate dehydrogenase	6-phosphogluconic dehydrogenase
Control (A)	7.6	9.5
A + geraniol:		
50 Ug	8.0	9.5
100 Ug	8.1	9.0
A + vitamin A:		
50 Ug	7.6	10.0
100 Ug	7.8	*

* Not determined.

These results suggest that β-Carotene and fatty acids play an important role by controlling the utilisation of sugars via HMP shunt. This might be one of the factors for accumulation of sugars in the ripe fruit.