when stained for peroxidase activity. There were 2 extra faint bands on the corresponding gel stained with Coomassie Blue which did not have peroxidase activity (not shown). Their approximate mol. wts were 100,000 and 150,000. However, 2-dimensional immunoelectrophoresis of the purified haptoglobin fraction with rabbit anti whole human serum antibodies demonstrated a single peak. A similar result was obtained with rabbit anti human haptoglobin antibodies. Although it is uncertain whether these 2 bands are protein contaminants they appear to co-precipitate with haptoglobin on immunoelectrophoresis. The purified haptoglobin preparation did not cross react with antiserum to α_2M, IgG, IgA or IgM the only other proteins which have been suggested to have some inhibitory action against cathepsin B. The immunoglobulins are only inhibitory in very high concentration[6].

The purified fraction inhibited the activity of cathepsin B and this effect could be reversed by the use of monospecific antiserum to haptoglobin up to a maximum mean of 85% of its original activity. The inhibition was partial as in the presence of excess haptoglobin approximately 40% of the activity was still retained. The reversal of inhibition by antibodies to haptoglobin may be due to the greater affinity of the antibodies for haptoglobin.

This effect is clearly not due to α_2M and suggests that haptoglobin is an inhibitor of cathepsin B and it remains to be determined if it is associated with the general thiol proteinase inhibitor isolated from human plasma[13,14].

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Effect of spike disease on the 5′- and 3′-nucleotidase activities in sandal plants

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Summary. In the sandal plant affected by spike disease, caused by mycoplasma-like organisms, the changes occurring in the 5′- and 3′-nucleotidase activities were studied and their possible significance discussed.

Study of 5′-nucleotidase activity has received attention in virus-infected animal tissues[1,2]. Information on changes occurring in the activity of this enzyme in infected plant tissue is scanty. In the sandal plant (*Santalum album* L.) affected by spike disease, caused by mycoplasma-like organisms, a disturbance in the nucleic acid metabolism has been reported[3]. It was therefore of interest to examine the changes occurring in the 5′-nucleotidase (5′-Ntdase) and 3′-nucleotidase (3′-Ntdase) activities in the spiked sandal plant.

Materials and methods. Leaf samples were taken from 6 healthy and 6 spiked sandal plants as described earlier[4]. Preparation of the enzyme extract and assay of the nucleotidase activity were done adopting the method described by Yee Foong Lai et al.[5]. The leaves (2 g) were homogenized with pre-cooled $NaHCO_3$ buffer (0.05 M, pH 7.4), and the homogenate filtered through a cloth, made up to 25 ml with the same buffer and centrifuged (3500 rpm, 10 min). The supernatant solution formed the enzyme extract. The reaction mixture (2 ml), among the other components referred to in the procedure described by Yee Foong Lai et al.[5], contained 0.5 ml enzyme extract and 2.5 mM nucleotide substrate (5′- or 3′-monophosphate of adenosine/guanosine/cytidine/uridine as needed) and was incubated for 2 h at 37 °C. In the control, addition of trichloroacetic acid preceded the addition of the enzyme extract. The liberated Pi was estimated in an aliquot of the reaction mixture. The enzyme activity was expressed as µg Pi liberated/40 mg tissue/2 h under the conditions of the experiment.

Results and discussion. Results obtained are presented in the table. From the table it can be seen that, during infection by spike disease, characteristic changes occurred in the 5′- and 3′-Ntdase activities. Compared to the healthy, in the diseased plant the 5′-Ntdase activity in respect of 5′-GMP, 5′-CMP and 5′-UMP increased in the young leaves and decreased in the mature ones; similar variations occurred in the enzyme activity in respect of 5′-AMP also, but to a less extent. While the activity of 5′-Ntdase increased from young to mature stage of the leaves in the healthy plant, it was the opposite in the spiked one. In contrast, the 3′-Ntdase activity declined from the young to the mature stage of the leaves in both healthy and spiked plants, the activity in the latter tending to remain at a relatively higher level throughout, more so in respect of 3′-AMP.

It has been suggested that 5′-Ntdase regulates the level of ribonucleotides for ribonucleic acid (RNA) synthesis in the tissue[6,7]. However, 5′-Ntdase activity in sandal did not show a determinant effect on the actual RNA level in the tissue, since despite the opposite trends of variations observed in the 5′-Ntdase activity in the healthy and spiked plants, the RNA level in both the plants was at a maximum level in the young leaves[3]. Nevertheless, it is possible that the increased activity of 5′-Ntdase in the young leaves of the diseased plant, where it has been indicated that multiplication of the

Levels of 5′-nucleotidase and 3′-nucleotidase activities in healthy (H) and spiked (S) sandal leaves

Description of the leaves	Nucleotidase activity* (μg Pi liberated/40 mg tissue/2 h)							
	5′ Nucleotidase activity							
	5′-AMP		5′-GMP		5′-CMP		5′-UMP	
	H	S	H	S	H	S	H	S
Young	33.5	35.0	55.6	70.0	42.5	54.5	43.3	60.4
	(1.0)	(1.4)	(2.0)	(2.3)	(1.8)	(2.2)	(1.1)	(2.4)
Mature	37.0	31.3	68.5	55.4	63.0	44.0	65.2	48.5
	(1.7)	(1.4)	(2.5)	(2.0)	(2.1)	(2.1)	(2.5)	(1.6)
	3′-Nucleotidase activity							
	3′-AMP		3′-GMP		3′-CMP		3′-UMP	
	H	S	H	S	H	S	H	S
Young	62.3	86.5	32.5	35.0	46.4	50.2	15.0	15.2
	(1.5)	(1.4)	(1.2)	(1.7)	(1.5)	(2.2)	(1.0)	(0.8)
Mature	42.5	70.2	30.8	32.0	41.5	45.6	10.7	9.5
	(1.8)	(2.3)	(1.4)	(1.2)	(1.4)	(1.4)	(0.9)	(0.8)

* Average of 6 replications. Figures within brackets show SD.

pathogen occurs[3], may adversely affect the level of ribonucleoside triphosphates serving in the energy transfer mechanism of the cell.

Compared with the mature leaves, the relatively higher level of 3′-Ntdase activity in the young leaves of both healthy and diseased plants could possibly be correlated with the high level of ribonuclease activity noticed in them[3], since the 3′-ribonucleotides released from RNA by the plant ribonuclease are hydrolyzed to ribonucleosides, which, either directly or after conversion to the bases, are retrieved for recycling for RNA synthesis[8].

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Enhancement of lipid peroxidation in rat brain mitochondria by polyamines

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Summary. Ascorbic acid-induced lipid peroxidation in rat brain mitochondria is enhanced by the addition of spermine and spermidine.

Ascorbic acid at low concentrations induces nonenzymatic lipid peroxidation of membrane lipids in mitochondria of rat liver[2] and brain[3]. Mitochondria are membraneous organelles rich in polyunsaturated fatty acids (PUFA) and the normal functions of mitochondria depend on highly integrated membrane structures. In vitro peroxidation of PUFA in cell membranes leads to the formation of a wide variety of toxic products, destruction of lipid-requiring functions and deterioration of the membrane structure[4]. The peroxidation of lipids is inhibited in various tissues by ascorbic acid at high concentrations, but the physiological significance has not been adequately stressed[5]. Since polyamines are present in relatively high concentrations in mammalian nervous tissues[6], the effects of spermine and spermidine on ascorbic acid-induced lipid peroxidation in rat brain mitochondria were examined.

Materials and methods. Spermine tetrahydrochloride, spermidine phosphate and 2-thiobarbituric acid were purchased from Sigma Chemical Co., USA. Other reagents were of analytical grade. Male Wistar rats, weighing 150–200 g and fed on stock laboratory diet, were used in this study. The animals were killed by decapitation and the brains excised, homogenized in 10 vol. of chilled 0.25 M sucrose, and centrifuged at $800 \times g$ for 10 min. The supernatant was centrifuged at $18{,}000 \times g$ for 20 min. The mitochondrial pellet was washed with 0.15 M KCl and suspended in 25 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl to a final protein concentration of 3.0 mg/ml. Mitochondria were incubated with 0–5 mM ascorbic acid at 37 °C for 75 min and aliquots removed at different time intervals for estimations. In some experiments, spermine and spermidine (1–40 mM) were added to the incubation mixture. The reaction was stopped by the addition of 1 ml 10% TCA which contained 0.02% by volume of an ethanolic solution of butylated hydroxytoluene (BHT), and centrifuged. The resultant supernatant was mixed with 1.0 ml. 0.67% thiobarbituric acid (TBA) and placed in a boiling water bath for 10 min. The absorbance at 535 nm was read and the amount of malonyldialdehyde (MDA) formed[7] calculated by using the extinction coefficient $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Protein was determined by the procedure of Miller[8]. Values are expressed as means ± SE.