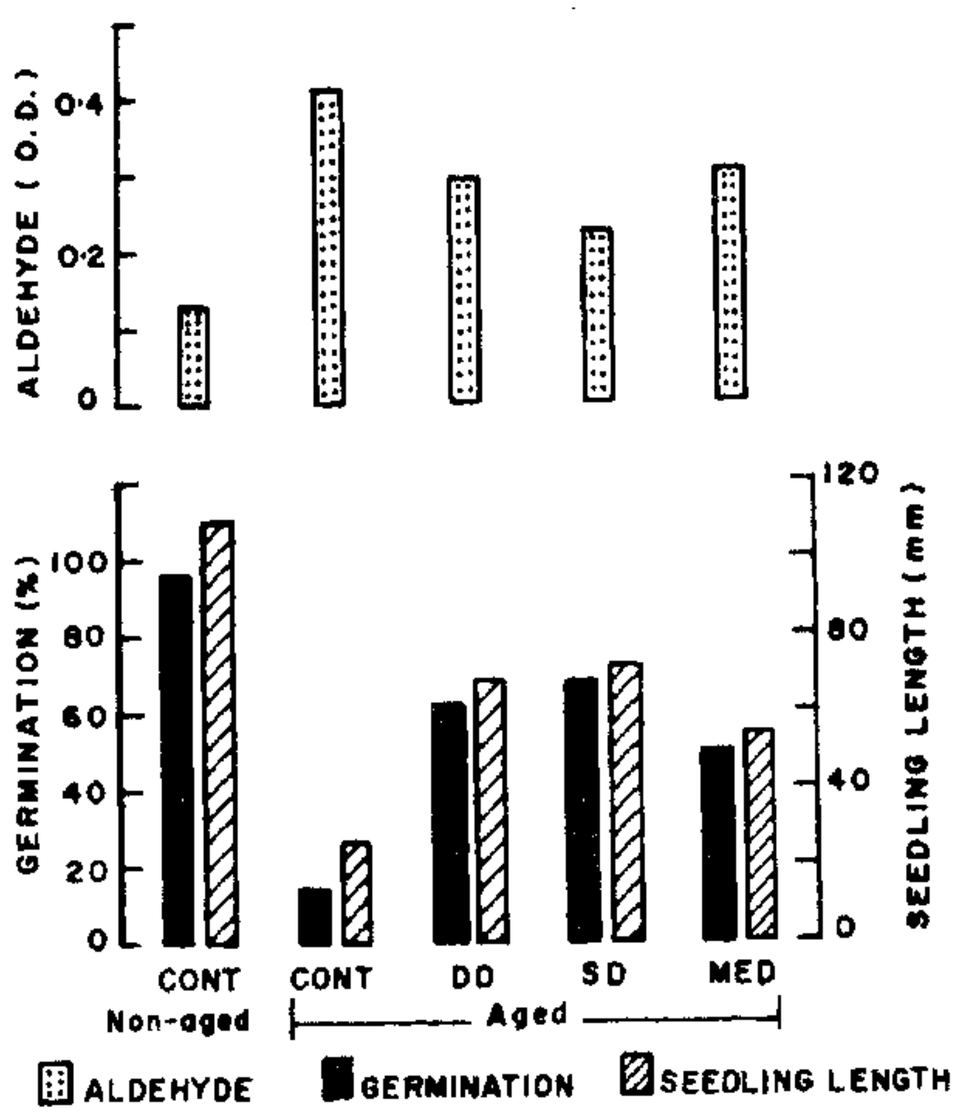

Figure 1. Germinability and volatile aldehyde production by wheat seeds after different hydration–dehydration treatments. The treatments, are CONT (non-aged), seeds stored at – 10°C; CONT (aged), accelerated ageing at 95% RH and 40°C for 7 days; DD, diping-drying; SD, soaking–drying; MED, moisture equilibration–drying. Seeds of DD SD and MED were also subjected to accelerated ageing at 95% RH and 40°C for 7 days.

moisture equilibration–drying treatments.

Immediately after hydration–dehydration treatment (before ageing), volatile aldehyde production from germinated wheat seeds was only slightly lower than in the control and the lowest production of volatile aldehyde was observed in germinating refrigerator-stored (nonaged control) seeds.

Ageing considerably increased the production of volatile aldehydes in wheat seed. Hydration–dehydration treatments effectively reduced the production of such volatile aldehydes and soaking–drying was better than the other treatments (Figure 1). Highly significant negative correlations were noted between germination percentage of wheat and volatile aldehyde production ($r = -0.9291$) and between seedling growth and aldehyde release ($r = -0.9910$).

The present study clearly shows that the hydration–dehydration treatments effectively reduced the post-ageing production of volatile aldehydes by wheat seeds. This would imply that lipid peroxidation, either through autoxidation in dry storage or through the mediation of lipoxygenase during germination, or both, is involved in wheat seed deterioration. This lends support to our earlier reports[10] that the hydration–dehyration seed invigoration treatments maintain greater germinability by reducing lipid peroxide formation in stored seeds.

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Ascorbic acid 2-sulphate, storage form of ascorbic acid in rats

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Ascorbic acid (AA) and ascorbic acid 2-sulphate (AAS) concentrations in rat tissues increased with increase in dietary level of AA. On feeding a high dose of AA to rats for 15 days, AAS levels and ascorbic acid sulphotransferase activity were significantly increased, whereas ascorbic acid-2-sulphate sulphohydrolase activity was reduced. On withdrawing AA, AAS levels and AA sulphotransferase activity were drastically reduced, but AAS sulphohydrolase activity was increased. AAS may be a storage form of vitamin C even in antiscorbutic animals.

ASCORBIC acid sulphate was first isolated and purified from the undeveloped cyst of brine shrimp by Mead and

Finamore[1], who speculated that it may be a storage form of vitamin C. Subsequently, it was shown[2] to be ascorbic acid 2 sulphate (AAS). Later it was concluded[3] that AAS is a major storage form of ascorbic acid (AA) in fish and is converted to AA as and when needed to maintain AA levels. But fish lack the ability to synthesize AA owing to the absence of L-gulonolactone oxidase[3] and so are dependent upon a dietary source of vitamin C. We tried to investigate whether AAS functions as a storage form of AA in vertebrates that can synthesize AA.

Male Sprage–Dawley albino rats (120–180 gm) were divided into two groups of 24 rats each: Group I, given low dose of AA (1 mg per 100 mg body weight); group II, given high dose of AA (200 mg per 100 gm body weight). The diet used had the following composition (in g per 100 g): corn starch, 68; casein, 16; groundnut oil, 8; shark liver oil, 2; yeast, 2; salt mixture[4], 4. AA dissolved in water was given orally by tube for 10 days.

The rats were housed individually in polypropylene cages in a room with temperature maintained at 25 ± 1°C and a 12-h light 12-h dark cycle. Water was available *ad libitum*. Diet consumption in the two groups was similar.

On day 16 half the number of rats from each group were deprived of food overnight and killed by decapitation. Tissues were collected separately in ice-cold containers for various estimations. The remaining rats of each group were fed the same diet but were deprived of AA for the next 15 days, after which they were killed as before and the tissues collected in ice-cold containers for various estimations. AA and AAS were estimated by the method of Terada *et al.*[5]

Tissue homogenates were prepared (1:3w/v) in Tris buffer (0.05 M, pH 7.2) and centrifuged at 4°C at 3000 *g* for 15 min. To assay ascorbic acid sulphotransferase, an aliquot of the supernatant was incubated with 2 μCi of $^{35}SO_4$ for 30 min at 37°C. Reaction was stopped by precipitation of inorganic sulphate with barium chloride. The AAS formed was separated by paper chromatography[6]; solvent used was phenol : water (100 : 40 w/w). AAS was located by spraying 1% ferric chloride in methanol. The spotted region was cut and transferred to PPO-based scintillation fluid and radioactivity was counted in an LKB Wallac 1211 Rackbeta liquid scintillation counter.

Ascorbic acid sulphate sulfohydrolase was assayed by the method of Stevens *et al.*[7], with the modification that the AA formed was measured by the method of Terada *et al*[5].

Protein was estimated after TCA precipitation by the method of Lowry *et al*[8]. Statistical analysis was carried out by Student's *t* test[9].

AA and AAS contents were significantly higher in all tissues analysed in rats fed high dose of AA for 15 days (Table 1). The increase in AAS was much more significant (100–200%) than that in AA (20–50%). When AA feeding was discontinued, there was significant reduction of AA and AAS in all the tissues in both low-dose and high-dose groups. At the end of the deprivation period AA levels were significantly lower in spleen, brain, pancreas and kidney in rats fed low dose of AA compared to those fed high dose, but there was no significant difference in the other tissues. But in the case of AAS, rats fed low dose of AA showed significantly lower levels in all the tissues, both during the AA feeding phase and after deprivation. After AA deprivation AAS content was reduced by 70–90% in both low-dose and high-dose groups but AA content was reduced only to a much lower extent (10–40%).

Ascorbic acid sulphotransferase activity was significantly higher in all tissues in the high-dose group; on withdrawing AA feeding, activity was reduced significantly in both groups (Table 2). On the other hand, activity of the sulphohydrolase was signficantly lower in the high-dose group except in the lungs; on withdrawing AA feeding it was increased significantly in both groups, the extent of increase being more in the high-dose group (300–600% in the high-dose group, 50–150% in the low-dose group). At the end of the deprivation period activities of both enzymes were significantly higher in the high-dose group.

The results indicate that tissue levels of AAS increase with increase in dietary concentration of AA; the increase is more when the dietary intake of the vitamin is also high. This increase in AAS levels is due to increased activity of the sulphotransferase, which transfers sulphate to AA, and decreased activity of the sulphohydrolase, which removes sulphate from AAS. The marked decrease in the activity of the sulphohydrolase is in agreement with an earlier report of Russel *et al.*[10], who purified sulphohydrolase from bovine liver and found that it was inhibited by AAS *in vitro*. Similar results were obtained by Benitez *et al*[11] in fish. The increased concentration of AAS now observed in rats on feeding AA may inhibit the activity of sulphohydrolase. But on withdrawing dietary AA, AAS levels in the tissues were decreased drastically, whereas the decrease in AA was only marginal. The decrease in AAS may be due to the increased activity of the sulphohydrolase on withdrawing AA feeding. This marked decrease in the concentration of AAS in the tissues on withdrawing AA and the marginal decrease observed in the concentration of AA may suggest that the rat tries to maintain tissue concentration of AA at the expense of AAS. This may be in addition to endogenous synthesis of the vitamin.

AAS, which is a more stable form compared to AA[3], may be stored in the tissues and it may be hydrolysed by the sulphohydrolase as and when AA is required for tissue function. Thus the sulphohydrolase and the sulphotransferase may play an important role in providing AA to the tissues as and when needed.

Table 1. Ascorbic acid and ascorbic acid 2-sulphate levels in rat tissues.

Group	Adrenals	Spleen	Brain	Lungs	Liver	Kidney	Pancreas
Ascorbic acid (mg/100 g tissue)							
a. Ascorbic acid-fed							
1. Low dose	400 ± 12.23^{a}	50.54 ± 1.61^{a}	35.80 ± 1.61^{a}	28.70 ± 0.93^{b}	30.29 ± 1.31^{a}	18.7 ± 0.95^{b}	10.2 ± 0.74^{a}
2. High dose	496 ± 14.88^{Aa}	66.20 ± 1.92^{Aa}	44.94 ± 1.84^{Aa}	39.05 ± 1.81^{Aa}	35.97 ± 1.45^{Ba}	24.5 ± 1.12^{Ab}	13.3 ± 0.813^{Ba}
b. Ascorbic acid-deprived							
3. Low dose	340 ± 10.34	36.7 ± 1.28	24.52 ± 0.94	24.20 ± 0.93	20.2 ± 0.95	14.9 ± 0.63	6.9 ± 0.46
4. High dose	375 ± 11.23	44.5 ± 1.31^{A}	28.48 ± 0.93^{B}	26.13 ± 1.21	22.4 ± 1.21	19.4 ± 0.84^{B}	8.7 ± 0.53^{B}
Ascorbic acid 2-sulphate (mg/100 g tissue)							
a. Ascorbic acid-fed							
1. Low dose	10.8 ± 0.45	8.16 ± 0.33	7.4 ± 0.32	6.6 ± 0.25	7.02 ± 0.32	6.33 ± 0.26	7.10 ± 0.31
2. High dose	30.2 ± 1.2^{Aa}	16.12 ± 0.74^{Aa}	17.2 ± 0.75^{Aa}	13.4 ± 0.61^{Aa}	15.50 ± 0.62^{Ba}	12.98 ± 0.51^{Aa}	15.33 ± 0.61^{Aa}
b. Ascorbic acid-deprived							
3. Low dose	3.46 ± 0.13	2.86 ± 0.12	1.88 ± 0.11	2.07 ± 0.11	2.66 ± 0.12	2.54 ± 0.12	3.80 ± 0.16
4. High dose	6.20 ± 0.25^{A}	4.94 ± 0.21^{A}	4.40 ± 0.21^{A}	2.84 ± 0.14^{A}	3.37 ± 0.13^{A}	3.40 ± 0.12^{A}	7.86 ± 0.32^{A}

Each value is the average ($\pm$ SE) of six animals.

Significance of difference:

(i) Group 1 vs group 2, group 3 vs group 4. $^{A}P > 0.01$, $^{B}0.01 < P < 0.05$

(ii) Group 1 vs group 3, group 2 vs group 4. $^{a}P > 0.01$, $^{b}0.01 < P < 0.05$

Table 2. Ascorbic acid sulphotransferase and ascorbic acid 2- sulphate sulphohydrolase activities in rat tissues

Group	Adrenals	Spleen	Brain	Lungs	Liver	Kidney	Pancreas
Ascorbic and sulphotransferase activity (cpm/mg protein/min)							
a. Ascorbic acid-fed							
1. Low dose	294 ± 13.24^{a}	310 ± 13.95^{a}	266 ± 11.97^{a}	214 ± 9.14^{b}	234 ± 10.53^{a}	222 ± 9.99^{a}	158 ± 7.26^{a}
2. High dose	708 ± 31.80^{Aa}	623 ± 26.78^{Aa}	544 ± 22.80^{Aa}	350 ± 16.45^{Ab}	398 ± 16.7^{Aa}	492 ± 22.14^{Aa}	214 ± 9.63^{Aa}
b. Ascorbic acid-deprived							
3. Low dose	158 ± 7.42	200 ± 9.82	186 ± 7.62	172 ± 8.08	176 ± 7.56	136 ± 5.84	90 ± 4.32
4. High dose	318 ± 15.26^{A}	388 ± 17.46^{A}	304 ± 17.46^{A}	276 ± 11.86^{A}	252 ± 11.59^{A}	266 ± 12.23^{A}	128 ± 6.14^{A}
Ascorbic acid 2-sulphate sulphohydrolase activity (µg A A/mg protein/h)							
a. Ascorbic acid-fed							
1. Low dose	9.8 ± 0.41^{a}	4.40 ± 0.21^{a}	9.6 ± 0.45^{a}	7.56 ± 0.32^{a}	4.35 ± 0.21^{a}	8.87 ± 0.42^{a}	3.06 ± 0.15^{a}
2. High dose	6.9 ± 0.31^{Aa}	2.39 ± 0.12^{Aa}	6.8 ± 0.34^{Aa}	6.67 ± 0.31^{a}	3.30 ± 0.15^{Aa}	3.50 ± 0.16^{Aa}	2.43 ± 0.13^{Ba}
b. Ascorbic acid-deprived							
3. Low dose	18.80 ± 0.86	7.80 ± 0.35	15.12 ± 0.72	20.52 ± 0.94	9.08 ± 0.39	11.80 ± 0.55	7.23 ± 0.34
4. High dose	44.30 ± 1.93^{A}	14.30 ± 0.67^{A}	42.20 ± 1.97^{A}	34.00 ± 1.53^{A}	18.24 ± 0.83^{A}	24.30 ± 1.11^{A}	10.56 ± 0.52^{A}

Each value is the average (± SE) of six animals.

Significance of difference:

(i) Group 1 vs group 2, group 3 vs group 4. $^{A}P > 0.01$, $^{B}0.01 < P < 0.05$

(ii) Group 1 vs group 3, group 2 vs group 4. $^{a}P > 0.01$, $^{b}0.01 < P < 0.05$

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Inhibition of thermotolerance by phenothiazines in *Escherichia coli* B/r

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Chlorpromazine and trifluoperazine, which are known potentiators of the hyperthermic effect both *in vitro* and *in vivo* inhibited development of thermotolerance in *E. coli* B/r. In addition, the presence of these drugs during hyperthermia resulted in thermosensitization of normal as well as thermotolerant cells.

THE use of hyperthermia in cancer therapy either alone or in combination with other treatments is currently gaining importance. However, development of thermotolerance in tissues during fractionated hyperthermic treatments is a serious drawback. This temporary resistance towards heat is induced by heat shock and is an adaptive process triggered during initial thermal stress and developing over a period of time[1,2] when the cells are held at 37°C, indicating the necessity of cellulr metabolism for its expression[3]. In fact, the synthesis of heat shock proteins (HSP) has been generally correlated with the appearance of thermotolerance, though there are several exceptions[4]. Hence developent of thermotolerance could be overcome by inhibiting protein synthesis in general[5] and HSP synthesis in particular.

Phenothiazine derivatives chlorpromazine (CPZ) and trifluoperazine (TFP) have been demonstrated to potentiate the effects of hyperthermia both *in vitro*[6] and *in vivo*[7]. Since they are also inhibitors of protein synthesis, their role in the development of thermotolerance was investigated.

E. coli B/r cells were grown overnight at 37°C in nutrient broth (Difco). The washed cells, resuspended in phosphate-buffered saline (PBS, pH 7.0) at concentration of 10^8 ml^{-1}, were used in all the studies. Heat treatment was given in a water-bath shaker with a temperature accuracy of 0.1°C. CPZ and TFP were used as supplied by Sigma, St Louis, USA.

Preliminary investigations were carried out to observe the effect of initial thermal shock at temperatures varying from 40 to 45°C. The resultant cellular lethality in control cells as well as in the presence of either CPZ or TFP at concentrations varying from 2 to 20 μM for a fixed period of 60 min was ascertained. A temperature which was nonlethal to the cells in the presence and absence of a particular concentration of CPZ or TFP was thus selected.

Incubation at 37°C following the initial thermal shock was varied from 30 to 180 min to determine the development of maximum thermotolerance. The temperature for subsequent heat treatment (evaluation of tolerance) was the one at which there was about 10% survival in control cells after a 90-min exposure.

Based on these preliminary investigations, the following protocol was adhered to in all the subsequent investigations: Initial heat shock at 42°C for 60 min with or without either CPZ (10 μM) or TFP (5 μM) + incubation at 37°C for 120 min + re-exposure at 50°C for up to 90 min. At the end of the protocol, the cells were suitably diluted in PBS, plated on nutrient agar and incubated at 37°C for 18 h, and the colonies counted. All the experiments were carried out at least thrice with replicate plates at two plating dilutions. Mean percentage survival was plotted against duration of re-exposure.

Heating the cells at 42°C was nonlethal to both control cells and cells treated with CPZ (10 μM) or TFP (5 μM). Control cells heat-shocked for 60 min at 42°C and subsequently incubated for 120 min at 37°C demonstrated maximum thermotolerance to the challenge at 50°C. As shown in Table 1, the time required for 50% lethality at 50°C (TD_{50}) was 32 min, as against 68 min for cells with fully developed thermotolerance. There was no further development of thermotolerance in cells when the time of incubation was extended to 180 min (data not

Table 1. Effect of phenothiazine derivatives on thermal response in *E. coli* B/r.

Treatment	Time required (min) for 50% lethality when exposed to 50°C		
	Control	CPZ	TFP
Control (heat shock, no incubation, no drug)	32	–	–
Control, with drug	–	15	16
Heat shock, 37°C incubation, no drug	68	–	–
Heat shock, 37°C incubation, with drug	–	20	22
Drug washed off before incubation	–	68	68
Drug washed off after incubation	–	31	33
Drug added before incubation	–	31	33
Drug added after incubation	–	31	33