

Figure 1. Choline (a) and glycinebetaine (b) accumulation in shoots and roots of wheat at different salinity levels of growth medium.

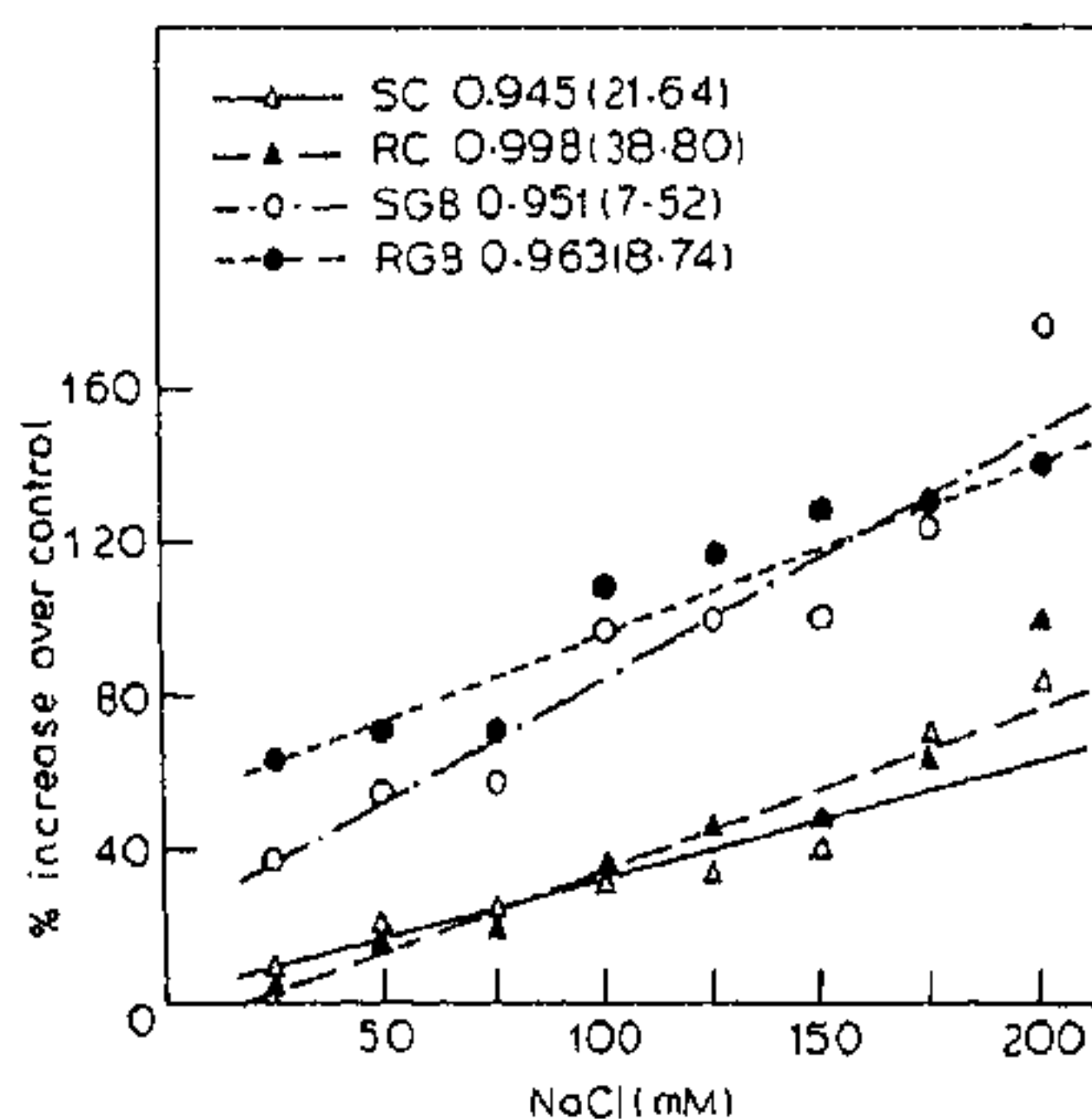


Figure 2. Linear relationship between accumulation of choline and glycinebetaine in shoots and roots of wheat seedlings and salinity of growth medium. SC, Shoot choline; RC, root choline; SGB, shoot glycinebetaine; RGB, root glycinebetaine. Figures in parentheses are  $r$  values.

It has been suggested that ethanolamine gets methylated thrice to produce choline<sup>11</sup>. The choline is

further oxidized to glycinebetaine. There is a view that, once formed, glycinebetaine constitutes an inert end-product that is not further metabolized by the plant<sup>11</sup>. Although a precise physiological or adaptive role has not been assigned to choline and glycinebetaine, it is believed that these quaternary ammonium compounds may function as an organic nontoxic cytoplasmic osmoticum<sup>4</sup>, associated with salt resistance<sup>5,6</sup>, protection of enzymes against electrolyte inhibition<sup>12</sup>, and increased membrane stability<sup>13</sup>, thus representing an important adaptation of plants to water shortage<sup>14</sup>.

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## Increased acetylcholinesterase activity in aestivating snail *Trachia vittata* (Muller)

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Acetylcholinesterase activity was significantly higher in nervous tissue, mantle fold and foot muscle of aestivating snails than in tissues of normal, active snails. Protein content of these tissues was decreased in aestivating snails. Increased acetylcholinesterase activity in some tissues may be a requirement in aestivation physiology.

ACETYLCHOLINESTERASE (EC 3.1.1.7) hydrolyses acetylcholine and is found in all classes of animals<sup>1-3</sup>. Acetylcholine, a well-known neurotransmitter, brings about diverse physiological functions in different tissues<sup>4,5</sup>. When it acts through a muscarinic cholinergic receptor, the phosphoinositide messenger system is stimulated<sup>5-7</sup>. Muscarinic cholinergic stimulation, depending on the tissue involved, regulates diverse

functions such as neurotransmission, muscle contraction, glycogen metabolism, secretion and permeability changes<sup>5-8</sup>. Neuromuscular activity is altered during aestivation in snails. I determined acetylcholinesterase activity in nervous tissue, foot muscle and mantle fold of aestivating and normal snail to get some information about aestivation physiology.

Terrestrial snails, *Trachia vittata*, were collected from their habitats in Madurai and divided into 2 groups. One group was maintained in a special device<sup>9</sup> designed to maintain them active under laboratory conditions and the other was allowed to aestivate inside an aestivation chamber for one year<sup>9</sup>. The snails were removed carefully from the shell using a bone cutter, and nervous tissue, mantle fold and foot muscle were quickly excised and weighed. Each tissue was homogenized at 0-4°C in 0.1 M phosphate buffer, pH 8.4. Acetylcholinesterase activity was assayed in the crude homogenate as described by Ellman *et al.*<sup>10</sup> with the following optimum conditions: 0.075 M acetylcholine iodide (substrate), 0.1 M phosphate buffer, pH 8, and 7 min incubation at 37°C. Absorbance at 415 nm was recorded in a Perkin-Elmer 402 spectrophotometer. Protein was estimated according to the method of Lowry *et al.*<sup>11</sup>

Acetylcholinesterase activity was higher by 112, 101 and 45% in nervous tissue, mantle fold and foot muscle respectively in one-year-aestivated snails compared to that in normal, active snails (Table 1). In contrast, protein content was decreased in all three tissues in aestivating snails (by 24, 23 and 10%).

A decrease in acetylcholinesterase activity was reported in the nervous system of *Pila globosa* during aestivation<sup>12</sup>. However, enzyme activity was shown to be increased in non-nervous tissues<sup>13</sup>. In the present study, acetylcholinesterase activity was increased in both nervous and non-nervous tissues during aestivation. Furthermore, in the body wall tissue of aestivating earthworm, appearance of an additional isozyme of acetylcholinesterase has been observed<sup>14</sup>. Therefore

increase in activity of this enzyme may be a requirement in aestivation physiology.

The increased acetylcholinesterase activity is likely to lower acetylcholine concentration. However, this likelihood remains to be investigated.

In aestivation, neuronal activities of snails are, in general, reduced<sup>15</sup>. Activity of osmoregulator neurons in the CNS is low during aestivation owing to osmotic stress<sup>16</sup>. Increased acetylcholinesterase activity in aestivating snails suggests an alteration in acetylcholine-mediated neuronal activity. Stimulation of the acetylcholine receptor has a crucial role in the control of ion transport across cell membranes, particularly in nerve and muscle cells<sup>17,18</sup>. Under hyperosmotic conditions, probably, a change in the translocation of ions across plasma membranes is a likely adaptation.

Acetylcholine is known to induce secretion by activating muscarinic cholinergic receptor in rat parotid glands<sup>4</sup>, avian salt glands<sup>7</sup>, etc. Acetylcholine probably has a role in the secretory process in the mantle in molluscs. This remains to be studied. However, the increase of enzyme activity in foot muscle and mantle of aestivating snails suggests a role for the enzyme in foot muscle and mantle in aestivation physiology.

**Table 1.** Acetylcholinesterase activity and protein content of tissues of active and aestivating snails.

	Nerve	Mantle	Foot muscle
	Acetylcholinesterase activity (nmol min <sup>-1</sup> mg <sup>-1</sup> protein)		
Normal snail	39.7 ± 7	18.7 ± 6	25.5 ± 6
Aestivating snail	84.1 ± 11	36.6 ± 5	37.0 ± 4
	P < 0.001 (112)	P < 0.001 (101)	P < 0.01 (45)
	Protein (mg g <sup>-1</sup> wet tissue)		
Normal snail	46.2 ± 4.54	94.9 ± 8	71.6 ± 5.86
Aestivating snail	31.45 ± 6.05	72.9 ± 1.06	64.43 ± 2.5
	(24)	(23)	(10)
		P < 0.001	P < 0.05

Values are mean ± SD (n=6). Figures in parentheses are per cent increase or decrease over control.

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