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# NEUROENDOCRINE CONTROL OF PROTEIN AND AMINO ACIDS IN THE TISSUES OF FRESHWATER CRAB, *BARYTELPHUSA GUERINI* (H. MILNE EDWARDS) (DECAPODA, POTAMIDEA)

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STUDIES ON *Hemigrapsus nudus* showed that loss of eyestalk factor through removal of sinus gland led to acceleration of catabolic processes in the tissues<sup>1</sup>.

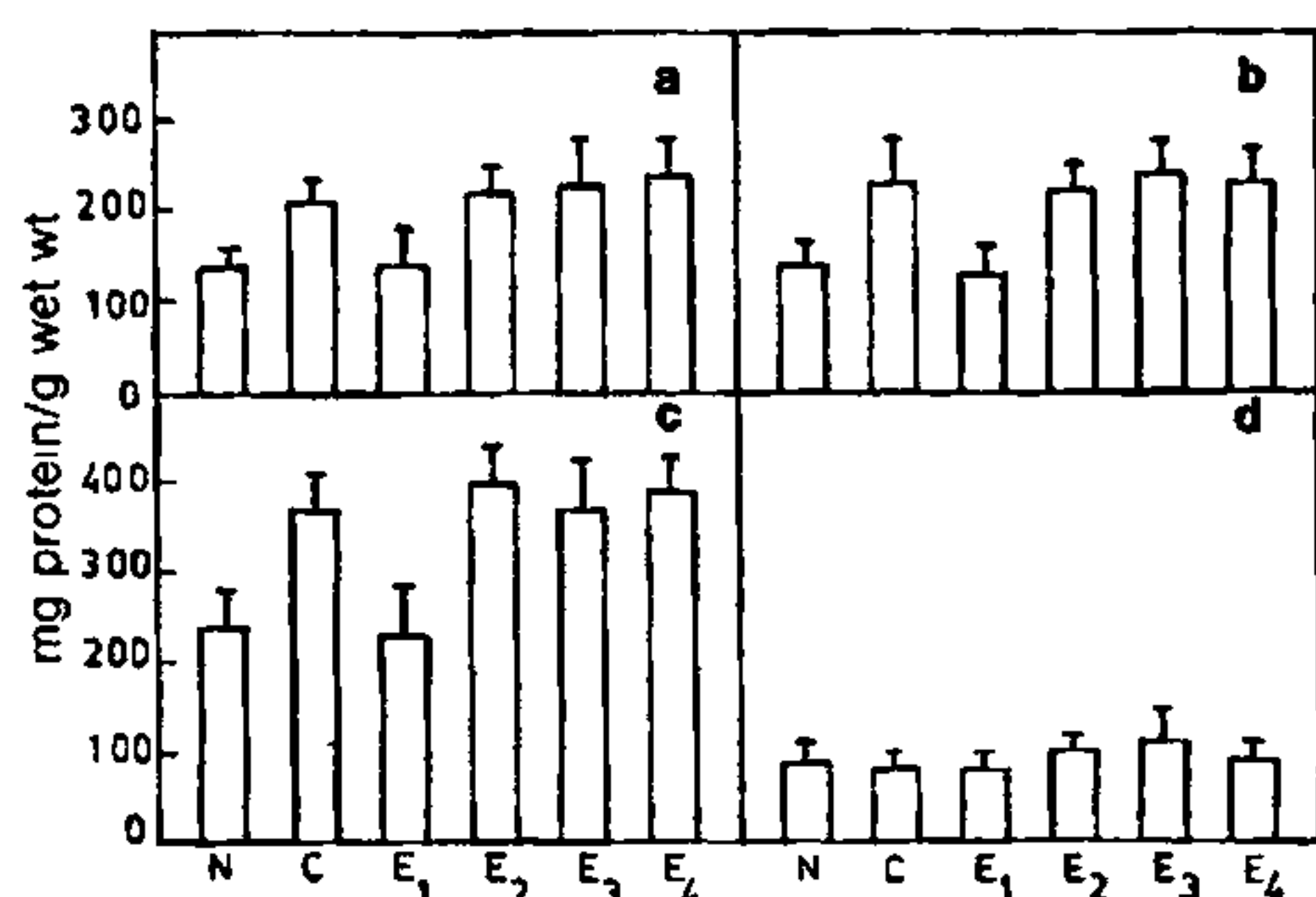
Eyestalk removal led to decrease in total nitrogen content, amylolytic activity and RNA content of *Procambarus clarkii*<sup>2</sup> and *Barytelphusa cunicularis*<sup>3</sup>, while eyestalk injections into eyestalk-ablated animals increased RNA levels. Histochemical studies on the hepatopancreas of *Scylla serrata* showed that glycoproteins and glycolipids were depleted following eyestalk ablation<sup>4</sup>. Variations in free amino acid levels in relation to eyestalk factors were also noticed in a few crustaceans<sup>5-7</sup>. To understand the neuroendocrine control of protein and amino acid levels in the tissues of the freshwater crab *Barytelphusa guerini*, the effects of eyestalk ablation, eyestalk injection into ablated animals, and other neuroendocrine structures were studied.

The collection and maintenance of the freshwater crab *B. guerini* were described earlier<sup>8</sup>. Males weighing between 30 and 50 g were used for the experiments, carried out at room temperature (26–28°C). The total protein and amino acid content of tissues like muscle, gill, heart and hepatopancreas were estimated quantitatively<sup>9,10</sup> in normal animals with intact eyestalks. Another group of laboratory-adapted crabs was employed for eyestalk ablation experiments. Forty-eight hours after eyestalk ablation, the animals were divided into five batches. One batch of eyestalk-ablated animals was used as control ablated animals and total protein and amino acid content were estimated. Animals in the remaining four experimental batches were given injections of extracts of eyestalks, sinus gland, brain and thoracic ganglionic mass respectively. Protein and amino acids were estimated 48 h after extract injection.

Eyestalk ablation and preparation of neuroendocrine extracts were done according to the procedures described earlier<sup>8</sup>. Total protein was determined<sup>9</sup> and the amino acids were quantified<sup>10</sup> by using 1 ml of 1% homogenates of tissues prepared in 0.025 M sucrose solution. At least 6 animals were used for each determination and the results were statistically analysed using Student's *t* test.

Figure 1 shows the results for total protein of muscle, gill, heart and hepatopancreas. Total protein was increased by 44.39% in muscle, 62.28% in gill and 57.32% in heart 48 h after eyestalk ablation. In each case the increase is highly significant ( $P < 0.02$ ). Injection of eyestalk extract into eyestalk-ablated animals brought down protein content to the normal level in about 48 h. The recovery was almost complete, and the difference in protein content between animals that received eyestalk extract and

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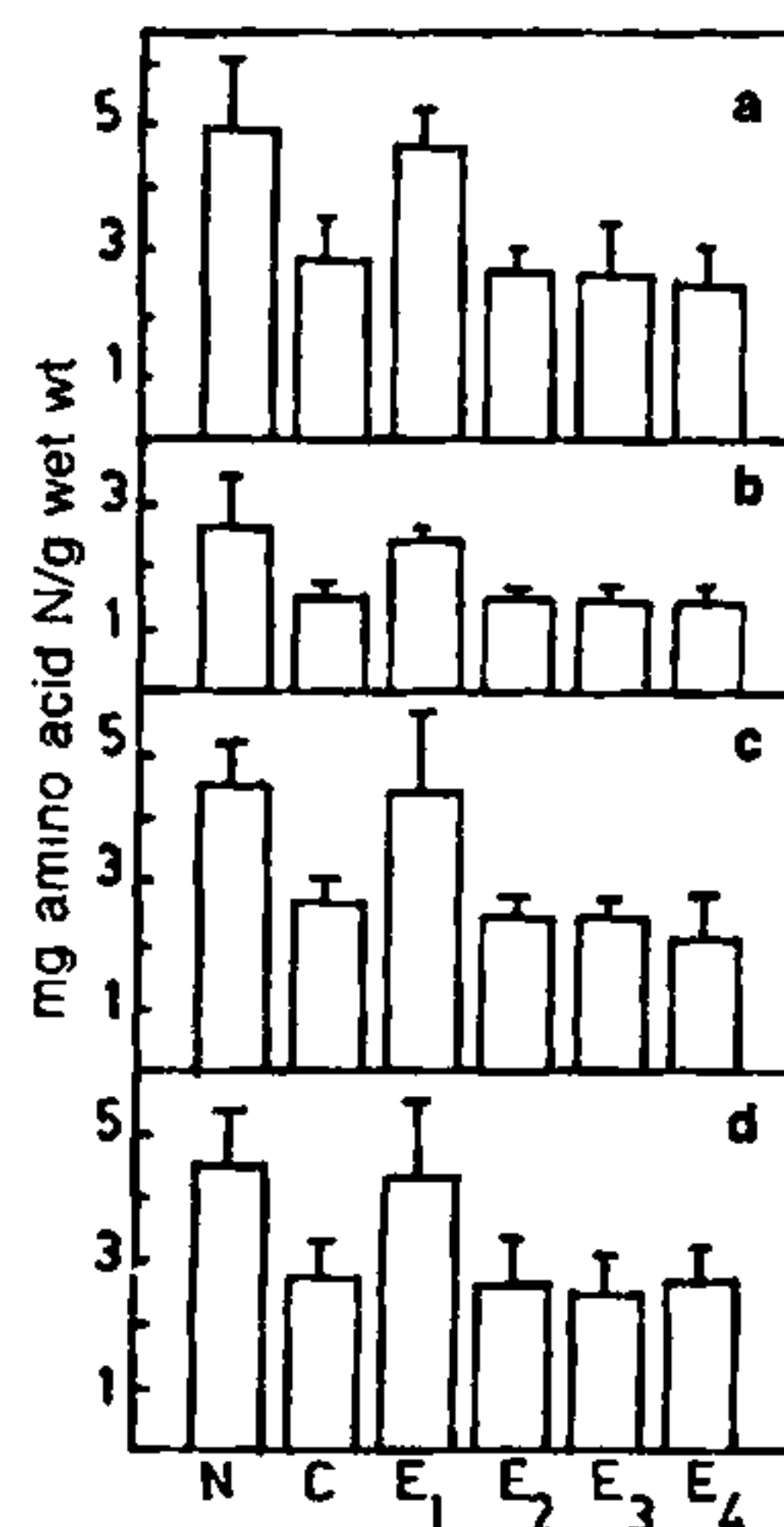
**Figure 1.** Total protein content of muscle (a), gill (b), heart (c) and hepatopancreas (d) of *B. guerini*, in normal, intact animals (N), after eyestalk ablation (C), and in ablated animals after injection of extract of eyestalk (E<sub>1</sub>), sinus gland (E<sub>2</sub>), brain (E<sub>3</sub>) and thoracic ganglionic mass (E<sub>4</sub>). Bars show means of 6 determinations.

normal animals was insignificant ( $P > 0.1$ ). In the hepatopancreas, however, protein content was increased by about 8.25% 48 h after eyestalk ablation. This change is not significant ( $P > 0.1$ ). Injection of eyestalk extract into eyestalk-ablated animals did not produce any significant change in protein content of hepatopancreas.

Amino acid content was decreased by 43.57% in muscle, 44.85% in gill, 41.70% in heart and 39.77% in hepatopancreas 48 h after eyestalk ablation (figure 2). This decrease is also highly significant ( $P < 0.02$ ). Injection of eyestalk extract into eyestalk-ablated animals elevated amino acid content to the normal level in about 48 h. Again, the recovery was almost complete, the difference in amino acid content between extract-injected animals and normal animals being insignificant ( $P > 0.1$ ).

Injection of extracts of sinus gland, brain and thoracic ganglionic mass into eyestalk-ablated animals did not reverse the effects of ablation in any of the four tissues (figures 1 and 2). Eyestalk ablation did not produce significant change in total protein content of hepatopancreas (figure 1), but caused a decrease in amino acid content, as in the other tissues examined (figure 2).

Earlier studies on *Astacus astacus*<sup>5</sup> and *Callinectes sapidus*<sup>6</sup> showed that eyestalk ablation facilitates the incorporation of amino acids into tissue proteins and their utilization for metabolic energy release. It was suggested earlier that, in *B. guerini*, decreased blood sugar level after eyestalk ablation is possibly



**Figure 2.** Amino acid content of muscle (a), gill (b), heart (c) and hepatopancreas (d) of *B. guerini*, in normal, intact animals (N), after eyestalk ablation (C), and in ablated animals after injection of extract of eyestalk (E<sub>1</sub>), sinus gland (E<sub>2</sub>), brain (E<sub>3</sub>) and thoracic ganglionic mass (E<sub>4</sub>). Bars show means of 6 determinations.

due to stimulation of glycogenesis<sup>11</sup>. It has also already been reported that decreased amino acids in the blood after eyestalk ablation may be due to their utilization for energy release in the absence of sugars, because the sugars are utilized in the process of lipid synthesis and glycogenesis<sup>12</sup>. There is also evidence to show that eyestalk ablation leads to increased lipid synthesis and gluconeogenesis<sup>4,13-15</sup>. The utilization of amino acids as an important source of energy-producing compounds has been well demonstrated in crustaceans<sup>16</sup>. The present results suggest the possible role of eyestalk factor in regulating protein and amino acid content of tissues. It appears that the eyestalk contains a factor that inhibits incorporation of amino acids into tissue proteins. Thus the present study provides good evidence for a protein synthesis inhibitory factor in the eyestalk of *B. guerini*. This view is further strengthened by the fact that eyestalk removal in *Orconectes virilis* in premolt stages produced a greater incorporation of amino acid into tissue proteins in all tissues<sup>7</sup>.

Studies on *H. mudus*<sup>1</sup>, *P. clarkii*<sup>2</sup>, *B. cunicularis*<sup>3</sup>, *S. serrata*<sup>4</sup> and *Palaemon vulgaris*<sup>17</sup> showed a



significant decrease in the protein content of hepatopancreas. The absence of any such significant change in the present study may perhaps be due to the fact that RNase and RNA content of hepatopancreas in *B. guerini* are less susceptible to the action of eyestalk hormone and therefore the protein synthetic rate is not adversely affected. Alkaline phosphatase activity in hepatopancreas of *Astacus leptodactylus* did not change significantly after sinus gland removal<sup>18</sup>.

Sinus gland extract did not restore protein and amino acid levels, suggesting that the factor influencing protein metabolism is not present in the sinus gland. It is possible that the factor produced in the X-organ complex of the eyestalk is released directly into the blood stream instead of being stored in the sinus gland<sup>19</sup>. Central nervous system structures also did not affect protein and amino acid content, suggesting the absence of any factors influencing protein metabolism in these structures. The present study thus clearly shows that total protein and amino acid content of tissues in *B. guerini* are under the control of a factor in the eyestalk. It is also evident that the eyestalk factor shows some tissue specificity.

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#### A REPORT ON THE PRESENCE OF VARIOUS PATHOGENIC MICROBES IN A WILD POPULATION OF BIHAR HAIRY CATERPILLAR, *DIACRISIA OBLIQUA*

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BIHAR hairy caterpillar, *Diacrisia obliqua*, is one of the most serious pests on mulberry plantations, and harbours many pathogens<sup>1-3</sup> common to the silkworm *Bombyx mori* and serves as a reservoir host. Cross-infectivity of *Nosema bombycis* and *Borrelina* virus between *Diacrisia obliqua* and *Bombyx mori* has already been reported<sup>4</sup>. The present study was carried out to find out the presence of pathogenic microbes in *D. obliqua*.

Larvae of *D. obliqua* were collected from the mulberry field of this Institute. One thousand larvae each of II, III, IV, V, VI and VII instars were examined for the presence of various pathogenic microbes. Tissues examined were haemolymph and midgut. Per cent incidence of various pathogens is presented in table 1.