Effect of pH Placing of Sample in Isoelectric Focussing of Proteins

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Reproducibility of the results of isoelectric focussing of proteins on polyacrylamide gels requires strict adherence to specifically laid-down procedures. Several of these have been noted in a number of publications (e.g. Dale & Latner, 1968; Leaback & Rutter, 1968; Wrigley, 1968; Fawcett, 1968; Awdeh, Williamson & Askonas, 1968; Lewin, 1969a,b). There is, however, one aspect, namely that of sample placing at different pH values, that does not appear to have been duly considered.

Present isoelectric-focussing methods involve the establishment of pH gradients by using Ampholines (LKB Produkter, Stockholm, Sweden). The most extensive range available is that of pH 3–10, which can be applied to separation of protein mixtures with isoelectric points within these limits. Several proteins exist that are correspondingly denatured on being subjected to pH values within increasing differences with their respective isoelectric points. Hence placing of a protein with an alkaline isoelectric point at the acid pH end of the gel (or a protein with an acid isoelectric point at the alkaline pH end of the gel) could lead to denaturation and therefore to additional bands. Similar difficulties can arise when protein complexes or protein-nucleic acid complexes are involved that are pH-sensitive. In such cases it would be advantageous to place the protein sample in a pH region as near as possible to its isoelectric point, preferably within ± 1.5 pH units of it. When a mixture of proteins is used several additional bands may be involved, depending on the pH-sensitivities of the individual proteins. This applies to sera (e.g. human and bovine), and mixtures of antibodies or detection of reactions (e.g. the effect of heavy water in protein biosynthesis or antigen-antibody reactions) must be carried out under specified conditions of pH placing of sample so as to avoid artifacts and erroneous conclusions.

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The Non-Protein Nitrogenous Constituents in the Parietal Muscle of the Eel *Anguilla anguilla* and their Possible Role in Adaptation to Changes in Salinity

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The osmoregulatory importance of the non-protein nitrogenous constituents of both intercellular and intracellular fluid is well established in many invertebrate groups (see Florin & Schoffeniels, 1965, 1969). With a few exceptions interest in vertebrates has centred around nitrogenous excretory products, especially ammonia and urea, or the inorganic ion changes that occur during salinity stress (see Potts & Parry, 1964).

With the eel *Anguilla anguilla* it is well known that changes in Na^+ , K^+ , Cl^- etc. in blood and parietal muscle follow the direction of any alteration in the external salinity (Sharratt, Jones & Bellamy, 1964; Chan, Jones, Henderson & Rankin, 1967), but little is known of changes in other body fluid constituents. Using two-dimensional paper chromatography and amino acid analyser techniques we have studied the distribution of the non-protein nitrogenous substances present in the parietal muscle of eels adapted to fresh- and salt-water environments.

A significant increase from 13.88 ± 1.53 to 22.50 ± 1.41 mmol/kg of tissue water in the total free amino acids occurred on adaptation of two groups of seven eels to fresh and salt water (approx. 15 g of Cl^-/l) respectively, norleucine being used as an internal standard for colour development. When the contribution of the individual constituents to this change was examined, the concentration of alanine, glutamine, glutamate, glycine, tyrosine and ornithine was increased in salt water, whereas taurine and the remaining amino acids did not show any appreciable change.

A notable feature of vertebrate muscle is the presence of the dipeptides carnosine and anserine, and we have detected large quantities of carnosine but not anserine in eel muscle. This carnosine must also contribute to the intracellular osmotic pressure, but we find that the quantity present, although variable, does not appear to correlate with the change in the external salinity.

Thus our findings with regard to the changes in the amino acids provide further evidence for a generalized mechanism of operation of these compounds in biochemical adaptation to salinity stress, in vertebrates as well as invertebrates. Carnosine does not appear to participate in the eel, although present in very considerable quantities.

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