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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Awdeh, Z. L., Williamson, A. R. & Askonas, B. A. (1968). *Nature, Lond.*, **219**, 66.

Dale, G. & Latner, A. L. (1968). *Lancet*, **i**, 81.

Fawcett, J. S. (1968). *FEBS Lett.* **1**, 81.

Leaback, D. H. & Rutter, A. C. (1968). *Biochem. biophys. Res. Commun.* **32**, 447.

Lewin, S. (1969a). *Abstr. Eur. Ass. Study Diabetes, Montpellier*, nos. 102 and 104.

Lewin, S. (1969b). *Post-grad. med. J.* **45**, 729.

Wrigley, C. (1968). *Sci. Tools*, **15**, 17.

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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- Chan, D. K. O., Jones, I. C., Henderson, I. W. & Rankin, J. C. (1967). *J. Endocr.* **37**, 297.
- Florkin, M. & Schoffeniels, E. (1965). In *Studies in Comparative Biochemistry*, p. 6. Ed. by Munday, K. A. Oxford: Pergamon Press Ltd.
- Florkin, M. & Schoffeniels, E. (1969). *Molecular Ecology*, pp. 89-111. New York: Academic Press Inc.
- Potts, W. T. W. & Parry, G. P. (1964). *Osmotic and Ionic Regulation in Animals*. Oxford: Pergamon Press.
- Sharratt, B. M., Jones, I. C. & Bellamy, D. (1964). *Comp. Biochem. Physiol.* **11**, 9.

The Metabolism of Trehalose during Morphogenesis of the Brine Shrimp *Artemia salina*

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High concentrations of trehalose are characteristic of many systems that are capable of undergoing cryptobiosis, e.g. plant seeds, fungal spores, diapausing insects and the brine shrimp *Artemia salina*.

Earlier work (Clegg, 1964, 1965) indicated that a rapid conversion of trehalose into glycogen and glycerol occurs on rehydration of dormant *Artemia* embryos. It was suggested that trehalose provides the necessary oxidizable substrate for the maintenance of the metabolic requirements during morphogenesis, and that the activation of trehalase might be important as a means of mobilizing this disaccharide.

In an investigation to study the metabolic changes underlying the initiation of morphogenesis in this species, we have attempted to correlate the changes in the concentrations of a number of carbohydrates with the enzymic profiles at different stages of development. Our findings show that trehalase activity (expressed as μmol of glucose formed/min per g dry wt.) does not alter significantly between dry cysts (0.16), cysts hydrated for 6h (0.18), 12h (0.17) and 24h (0.19), or adult shrimps (0.18); although the activities of maltase and sucrase were higher at the adult stage compared with dormant or hydrated cysts (maltase, 0.18 and 0.05; sucrase, 3.53 and 1.15 respectively). Thus an alteration in the total detectable trehalase activity does not appear to be a significant factor in initiating the metabolism of trehalose by developing *Artemia* embryos.

Further, although our results confirm those of Clegg in that there is a very much lower content of trehalose in hatched nauplii and adult shrimps compared with the encysted stage, we find that very considerable quantities (approx. 75%) of the trehalose initially present remains in the cyst remnants after hatching. Thus the low concentration of trehalose in hatched nauplii does not necessarily indicate that it has actually been degraded during morphogenesis. The functional significance of this residual carbohydrate is far from clear. It does not

appear to provide a long-term energy store, since the concentration of trehalose in dormant cysts remains essentially unchanged for over 20 years (Clegg, 1962), and though it could conceivably provide an additional source of external nutrient after hatching this seems unlikely in view of the non-utilization by *Artemia* of various externally added substrates (Emerson, 1967; Huggins, 1969).

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Clegg, J. S. (1962). *Biol. Bull. mar. biol. Lab., Woods Hole*, **123**, 295.

Clegg, J. S. (1964). *J. exp. Biol.* **41**, 879.

Clegg, J. S. (1965). *Comp. Biochem. Physiol.* **14**, 135.

Emerson, D. N. (1967). *Comp. Biochem. Physiol.* **20**, 245.

Huggins, A. K. (1969). *Comp. Biochem. Physiol.* **29**, 439.

Fractionation of the β -Glucanases in a *Cytophaga johnsonii* Culture Filtrate Lysing Yeast Cell Walls

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When grown in the presence of autoclaved yeast cell walls *Cytophaga johnsonii* produces extracellular enzymes that will lyse these cell walls *in vitro* under suitable conditions (Bacon, Milne, Taylor, & Webley, 1965). There is no evidence that mannan is degraded during the lytic process, and so attention has been concentrated on other cell-wall components, especially glucan.

We have fractionated the culture fluid, examining particularly its action on insoluble laminarin from *Laminaria cloustonii*, a β -(1 $\rightarrow$ 3)-glucan, and lutean, a β -(1 $\rightarrow$ 6)-glucan. An Auto-Diluter (Mk II; Hook and Tucker Ltd., London S.W.9, U.K.) was used to sample each chromatographic fraction, add buffered substrate and deliver the mixture into an Auto-Analyzer cup. Ribonuclease was added, a cap fitted and the cup incubated at 30°C, usually overnight, before being placed on the sampler module of the Auto-Analyzer for measurement of reducing sugar (Hoffman, 1937).

After concentration by freeze-drying and precipitation with ammonium sulphate the enzymes were applied to a Sephadex G-100 column and developed with 5mM- Na_2HPO_4 . A variable amount of laminarinase emerged at the void volume, but the greater part was eluted later as a rather broad peak. A peak of luteanase followed closely on the laminarinase, and two further luteanase peaks were eluted with and after the ammonium sulphate; in this region there was also a small laminarinase peak.

When the fractions containing it were applied to