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Occurrence of Histones in the Trypanosomatid Flagellate *Crithidia oncopelti*

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Although histones are known to be present in the nuclei of several species of protozoa, Stewart & Beck (1967) could not demonstrate their presence in 40 parasitic species by an immunofluorescence staining technique that employed antibody to DNA-histone. However, the isolation of histones from the parasitic flagellate *Crithidia oncopelti* is now reported. Because of difficulties encountered in purifying nuclei from this organism, the histones were isolated by acid extraction of DNA-protein preparations.

Harvested cells were washed once with 0.10M-NaCl-0.50M-trisodium citrate, adjusted to pH 7.0 with HCl. A cell homogenate was obtained by rapidly freezing and thawing the wet pellet three times and then resuspending it in the NaCl-citrate solution. The material that could be sedimented from the homogenate by centrifugation at 10000g for 15 min was washed several times with more of the NaCl-citrate solution. DNA-protein was then isolated by extraction with 1M-sodium chloride followed by precipitation on dilution to 0.15M (Jordan, 1960). After one reprecipitation the DNA-protein was dried by washing with ethanol and ether. DNA-protein preparations obtained in this way normally contained 20-30% DNA, 5-10% RNA and 15-20% histone.

Histones were extracted from the DNA-protein with 0.2M-hydrochloric acid and precipitated by the addition of 7 vol. of acetone. They were freed from any contaminating non-basic proteins by chromatography on CM-cellulose (Leaver & Cruft, 1966).

The amino acid analysis of the histone isolated from *C. oncopelti* was very similar to that for unfractionated calf thymus histone. However, polyacrylamide-gel electrophoresis (Panyim & Chalkley, 1969) indicated the presence of more components in the *C. oncopelti* histone. Several of the *C. oncopelti* components had electrophoretic mobilities similar

to calf thymus histones, but notably there were two components with much higher mobilities. Amino acid analysis of these two components isolated together by preparative polyacrylamide-gel electrophoresis indicated that they are extremely rich in lysine (30 mol/100 mol of residues) and alanine (29 mol/100 mol of residues).

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Normalization Factors in the Fitting of Exponential Curves to Results Obtained in Isotopic-Tracer Studies

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When computing optimum values for the constants of equations, for example those connected with isotopic-tracer studies, by minimizing the sums of squares of the differences between computed and experimental values of a variable, it is essential that the squares of the differences shall be 'normalized' by dividing each by a suitable factor. Otherwise the largest differences, which are normally associated with the numerically largest observations, have a disproportionate weight in the sums of squares. If a single decay curve were being fitted, this would have the effect of always providing the best fit to the early observations; if observations on two or more variables were available, the fit to the numerically small observations would be neglected.

If replicate observations are available, it can be shown that the best normalizing factor for each set of points is the variance, so that the contribution to the sum of squares of deviations should be:

$$n\sum(x_{\text{calc.}} - x_{\text{exp.}})^2 / \sum(\bar{x} - x_{\text{exp.}})^2$$

Occasionally, however, it is desired to fit a curve to a single set of observations, whether of one or several variables. In this case the variance is not available. The obvious expedient is to divide the deviation at each point either by $x_{\text{calc.}}$ or $x_{\text{exp.}}$, i.e. to use a term such as $[(x_{\text{calc.}} - x_{\text{exp.}})/x_{\text{calc.}}]^2$ or $[(x_{\text{calc.}} - x_{\text{exp.}})/x_{\text{exp.}}]^2$. It can readily be shown, however, that the use of such terms will lead to a systematic bias towards large values of $x_{\text{calc.}}$ if this is the normalizing factor, or to small values if $x_{\text{exp.}}$

is the factor. This is simply because the larger the numerical value of the denominator the smaller is the value of the fraction, especially when squared.

There is no entirely satisfactory solution to this problem, other than the highly recommended procedure of never using single sets of observations, but it has been found that the mean of experimental and calculated values is a normalizing factor that considerably decreases the bias and provides a fairly satisfactory neutral weighting between large and small experimental variables. The form of each deviation term is thus:

$$4[(x_{\text{calc.}} - x_{\text{exp.}})/(x_{\text{calc.}} + x_{\text{exp.}})]^2$$

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Inhibition by Salicylate of Porcine Lactate Dehydrogenase Isoenzyme 5

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Salicylate has been shown to inhibit a number of dehydrogenase enzymes by competing with the NAD molecule for its binding site at the active centre (Hines & Smith, 1964). The work now reported is part of an investigation to determine with which part of the NAD site on lactate dehydrogenase the salicylate molecule is competing. During this study evidence was obtained that the pattern of inhibition of porcine lactate dehydrogenase isoenzyme 5 by salicylate is more complex than previously (Dawkins, Gould, Sturman & Smith, 1967) reported.

Lactate dehydrogenase activity was measured during L-lactate (17–22mM) oxidation at pH 10 at 25°C by following extinction changes at 340nm. The reversibility of salicylate inhibition of lactate dehydrogenase, as reported by Dawkins *et al.* (1967), was confirmed for isoenzyme 5 by using the method of Ackermann & Potter (1949).

The inhibition by salicylate at concentrations up to 18mM with varied NAD⁺ concentrations was predominantly competitive, but some effect on the intercept of the reciprocal plot was noted, indicating that, although the major inhibitory effect arose from the combination of the inhibitor with the free form of the enzyme, some combination with the enzyme-NAD⁺ form did occur. This assumes an ordered mechanism for lactate dehydrogenase, in which NAD⁺ is the leading substrate (Novoa & Schwert, 1961; Zewe & Fromm, 1962).

The intercept replot against salicylate concentration shows considerable variance, but a definite

deviation of the gradient from zero was noted, indicating combination of salicylate with the enzyme-NAD⁺ form. This intercept effect was noted at L-lactate concentrations as high as 44mM, a result not expected were combination of inhibitor occurring at the lactate-binding site.

The slope replot against inhibitor concentration consistently produced a parabola even under conditions of constant ionic strength. The parameters of these parabolas were determined by using a weighted least-squares analysis (Cleland, 1963). This observation can be explained on the assumption that up to 2 molecules of salicylate/catalytic centre were combining with the same form of the enzyme as did NAD⁺.

The weighted mean values of K_{i1} and K_{i2} , the stepwise dissociation constants of the enzyme-inhibitor complexes EI and EI₂, were (means ± s.e.m.) 7 ± 1 and 7 ± 1mM respectively. It can be shown that the relative magnitudes of these two values may indicate that the two molecules of salicylate bind by a sequential mechanism, the binding of the first causing unmasking of the second binding site.

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Liver Perfusion with a Closed Gas Circuit

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An apparatus for the perfusion of isolated rat liver has been constructed in which the gas and the perfusate are both recycled in closed circuits. Temperature control is achieved by circulating water from a thermostatically controlled bath through the water jackets of the component parts of the apparatus. The medium, 50ml of Krebs-Ringer bicarbonate buffer containing 4% (w/v) of bovine serum albumin, is equilibrated with the O₂+CO₂ gas phase by passing it over a series of rotating discs on which the medium spreads in a thin film. This system gives efficient gas exchange with negligible frothing. The apparatus is fitted with oxygen electrode, flow meters and a gas-flow radioactivity counter.