- Delaney, M. E., Owen, W. J. & Rogers, L. J. (1971). *Biochem. J.* **124**, 24P.
- Izawa, S., Connolly, T. N., Winget, G. D. & Good, N. E. (1966). *Brookhaven Symp. Biol.* **19**, 169.
- Lawler, P. D. & Rogers, L. J. (1968). *Biochem. J.* **110**, 381.
- Owen, W. J., Rogers, L. J. & Hayes, J. D. (1970). *Biochem. J.* **121**, 6P.

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 15min 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 7vol. 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 (30mol/100mol of residues) and alanine (29mol/100mol of residues).

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Jordan, D. O. (1960). *The Chemistry of Nucleic Acids*, pp. 10-12. London: Butterworth and Co. Ltd.

Leaver, J. L. & Cruft, H. J. (1966). *Biochem. J.* **101**, 665.

Panyim, S. & Chalkley, R. (1969). *Archs Biochem. Biophys.* **130**, 337.

Stewart, J. M. & Beck, J. S. (1967). *J. Protozool.* **14**, 225.

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 $x_{\text{exp.}}$