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

By R. M. CHESHIRE and M. V. PARK. (*Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX, U.K.*)

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

By J. COCHRANE, J. S. CHAHL and J. H. OTTAWAY. (*Department of Biochemistry, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, U.K.*)

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.