

### Biotin uptake in cultured human fibroblasts

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The uptake of the vitamin biotin by the intestine has been studied in a number of animal species using the inverted sac technique, intact enterocytes, and the *in vivo* intestinal loop technique (Spencer & Brody, 1964; Goré *et al.*, 1986; Bowman *et al.*, 1986). There has also been some work on isolated membrane vesicles from kidney tubules of the rat (Podevin & Barbarat, 1986), and the passage of biotin across the blood-brain barrier of the rat (Spector & Mock, 1987), however, little is known about uptake in humans.

Holocarboxylase synthetase (Ghneim & Bartlett, 1982) catalyses the covalent binding of biotin to acetyl-CoA, pyruvate, propionyl-CoA and 3-methylcrotonyl-CoA apocarboxylases which generates the active holocarboxylases (EC 6.4.1.2; EC 6.4.1.1; EC 6.4.1.3; EC 6.4.1.4). These carboxylases are involved in fatty acid oxidation, gluconeogenesis, isoleucine metabolism, and leucine metabolism, respectively. Acetyl-CoA carboxylase is cytosolic, the others are mitochondrial. Biotinidase (EC 3.5.1.12) catalyses the production of biotin and lysine from biocytin which is the final biotin-containing breakdown product of the carboxylases.

A number of inborn errors of metabolism occur in humans due to carboxylase deficiencies. Low or undetectable levels of these enzymes often results in death in infancy. Deficiencies of holocarboxylase synthetase and biotinidase also result in reduced carboxylase activities, because of impaired biotinylation of apocarboxylases. These defects can be corrected by the administration of large doses of biotin, up to 10 mg bd (Bartlett, 1982). This is 1000 times the normal daily intake.

It has been suggested that biotinidase is required for normal biotin uptake. We report studies of biotin uptake in fibroblasts derived from normal subjects and patients with biotinidase deficiency.

The fibroblasts were made biotin deficient by culture in a medium (MEM, Ghneim *et al.*, 1980, and Ultrosor G, LKB, Croydon U.K.) containing a low biotin concentration (0.07 nmol/l). Ultrosor G was used instead of fetal calf serum because this contains high biotin concentrations (10–200 nmol/l). Biotin deficiency was assessed by measurement of mitochondrial carboxylase activity (Ghneim *et al.*, 1980); after four passages the activities were about 10% of normal. The  $K_m$  and  $V_{max}$  for biotin uptake were determined by incubating the cells in small wells (9.6 cm<sup>2</sup>), with various concentrations of D-[8, 9(n)-<sup>3</sup>H]biotin (specific activity

Table 1.  $K_m$  and  $V_{max}$  values for biotin uptake in biotin deficient fibroblasts

Cell line	$K_m$ values (nmol/l)	$V_{max}$ (nmol/mg protein per h)
Control 1	65	9.7
Control 2	86	13.0
Control 3	80	12.1
Biotinidase deficient 1	64	4.5
Biotinidase deficient 2	210	4.5
Biotinidase deficient 3	105	176
Biotinidase deficient 4	255	19.2

30  $\mu$ Ci/nmol<sup>-1</sup>; ranging from 1.67 nmol/l to 166.6 nmol/l) at 37°C for different lengths of time up to 20 min. The incubation medium was then removed and the cells washed four times with ice-cold Hanks balanced salt solution (Hanks & Wallace, 1949) containing sodium bicarbonate (4.4 mmol/l) and biotin (140 nmol/l). The cells were harvested using 0.005% (w/v) trypsin/0.002% (w/v) EDTA (1:250) washed and sonicated at 0°C. A proportion of the homogenate was counted and protein measured. Biotin uptake was linear with respect to time with all concentrations used.

The results are shown in Table 1. The  $K_m$  values for biotin uptake by normal fibroblasts and biotinidase-deficient fibroblasts are not significantly different, neither are the  $V_{max}$  values. These results suggest that biotinidase does not function in biotin uptake.

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### High precision automated off-line analysis of sedimentation equilibrium data

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Low-speed sedimentation equilibrium procedures in the analytical ultracentrifuge provide one of the most powerful methods of characterizing the behavior of biological macromolecules in solution, particularly difficult, non-ideal heterogeneous systems. Information that is potentially available includes molecular weight distributions of polydis-

perse macromolecular systems (e.g. proteoglycans, mucus glycoproteins, polysaccharides) and association constants of interacting systems (e.g. self assembly phenomena).

The Rayleigh Interference optical system provides the most accurate way of recording the equilibrium solute distributions. Manual data capture procedures (using for example 'microcomparators') currently in use, are both tedious and of a relatively low precision (normally to between 1/50th and 1/100th of a fringe).

A relatively simple adaptation of a commercially available laser densitometer (the LKB Ultrosan 2022) permits automatic data capture. Scans of the solution fringes are

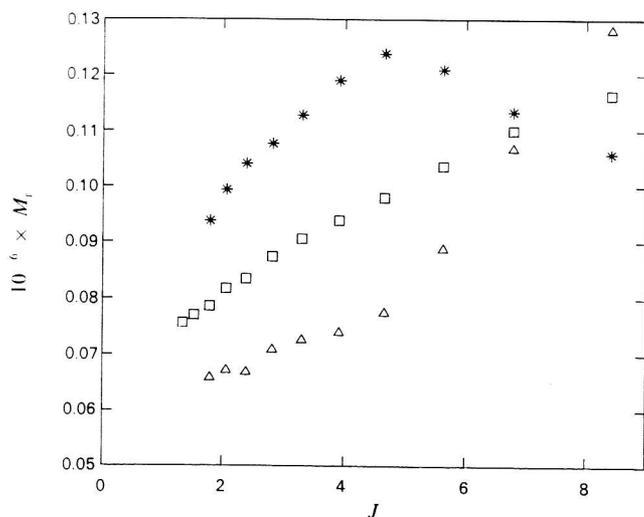


Fig. 1. Plot of point average  $M_w$  (□),  $M_z$  (\*) and  $M_{y_2}$  (Δ) data versus (absolute) fringe concentration,  $J$  for a low speed sedimentation equilibrium experiment on a polyuronide from ripe tomato fruit (Seymour & Harding, 1987)

Rotor speed 9341 rev./min, temperature = 20.0°C, loading concentration  $\approx 0.4$  mg/ml. Fringe data were captured off-line by the Ultrascan, and fringe displacement as a function of radial position determined using the routine 'ANALYSER'.  $M_w$  and  $M_z$  values were obtained using sliding strip quadratic fit procedures to the fringe data.

made at up to 50 radial positions in the pattern, using the UCSD PASCAL program GELSCAN for the Apple II. The data is then analysed for fringe shifts in the vertical direction (as a function of radial position). This is done by using an algorithm 'ANALYSER' we have written (in UCSD PASCAL) for the iterative maximization of the amplitude of the Fourier cosine coefficient, of order  $Q$ , where  $Q$  is the number of fringes within the data set analysed, by translation of a reading frame within the total data set (Harding & Rowe, 1987, 1988). The precise degree of translation required to maximize  $A(Q)$  is thus a measure of the phase (or fringe) shift. The air or reference fringes are routinely measured at a series of radial positions, and by a simple

linear regression analysis of the measured fringe increment the optical baseline can be obtained to correct the data obtained from the region of the solution column.

An accurate record of the fringe displacement data for the range of solution radial positions can be obtained within minutes. More significantly, the improvement in precision is remarkable: a final precision of 1/300–1/500th of a fringe is readily obtained from typical real experimental data (Harding & Rowe, 1988). The extra accuracy in the fringe data results both in improved point weight average molecular weight data,  $M_w$  and also the ability to realistically evaluate point  $z$ -average ( $M_z$ ) and 'ideal'  $y_2$ -point average molecular weight ( $M_{y_2}$ ) data (Fig. 1), where  $M_{y_2} = M_w^2/M_z$  (Roark & Yphantis, 1969).  $M_{y_2}$  is independent of first-order thermodynamic non-ideality effects and first-order effects of self-association and polydispersity.

The algorithm would therefore appear to offer the possibility that, even with fringe data generated by a conventional mercury arc light source (and not a laser), a series of most interesting methods derived during the past 20 years (e.g. Roark & Yphantis, 1969; Teller, 1973; Kim *et al.*, 1977; Johnson *et al.*, 1981; Tindall & Aune, 1982; Harding, 1985), which have much potential for protein and glycoconjugate biochemistry, but which have proved difficult to implement because of the severe demands placed upon the smoothness of data, may turn out to be readily usable, using a device commonly found in laboratories in the life sciences.

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