presence of sugars: sucrose > fructose > glucose. As there is no cell division, increases in length and fresh weight are due to growth of existing cells.

During extension growth the invertase (β-fructofuranosidase) activity follows the growth rate (Heyes & Vaughan, 1967). Since Brown (1963) postulated that extension growth is an expression of protein synthesis, the effect on growth and changes in invertase activity of several amino acids and some of their analogues was studied over 18h in the presence of 2% (w/v) sucrose.

Methionine, phenylalanine and proline did not affect extension growth compared with controls. Two analogues (ethionine and *p*-fluorophenylalanine) inhibited extension growth some 60% at 3mM; inhibition was measurable after 6h. The inhibitions could be prevented by adding 3mM-methionine and -phenylalanine to the culture medium.

In sucrose media, with or without amino acids, changes in invertase activity closely follow the growth rate, being maximal after 10–12h and thereafter declining. Ethionine and *p*-fluorophenylalanine prevented increases in invertase activity, an effect abolished by adding the natural amino acid. The increase of acid phosphatase activity was similarly affected, but there were no changes in the total protein content of segments cultured under any of the conditions described.

The cell walls contain about 20% of the invertase activity and 90–95% of the 4-hydroxy-L-proline of the cell. The hydroxyproline content of the wall trebles during incubation in sucrose. Hence segments were cultured in the *cis* or *trans* isomers of this imino acid. Extension growth was enhanced by 15–20%, but invertase activity was depressed by 20–25% compared with the sucrose controls. The effects are abolished by adding proline.

The results support the idea that protein synthesis is required for cell growth. But results with hydroxyproline raise the question as to whether this imino acid is an analogue of proline or exerts its effect on growth because of its special position in cell-wall proteins.

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Transfer of Adsorbed Bilirubin to Specific Binding Proteins

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In a screening study of the interaction of alkaline bilirubin solutions with water-insoluble compounds in bead form, it was observed that cross-linked dextran behaves as a relatively strong adsorbent (more than 95% adsorbed on Sephadex G-10 at pH 9.0). Alkaline destruction of bilirubin was drastically decreased by the presence of Sephadex in the suspension. A saturation limit of the solid matrix for the pigment could be demonstrated and measured quantitatively.

After stepwise neutralization of the suspension with phosphate buffers (final pH 7.4) combined with a washing at each step, followed by a final washing with water to remove ions, a bilirubin–Sephadex powder was prepared by freeze-drying and stored *in vacuo* over P_2O_5.

In agitated suspension of the bilirubin–Sephadex complex (in buffers pH 4.0–10.0), the pigment remained adsorbed below pH 8.0. This is probably due to the extremely low solubility of bilirubin at these pH values (Brodersen & Theilgaard, 1969). In contrast, when the suspension contained specific bilirubin-binding proteins, rapid solubilization of the pigment took place. The adsorbed bilirubin seems to be present in a kind of activated state as, in otherwise identical conditions, solubilization of crystalline bilirubin did not occur.

The fast desorption of the pigment from the bilirubin–Sephadex complex was exploited to construct a sensitive assay of bilirubin-binding capacity. Bilirubin–Sephadex complex and either bovine serum albumin, lysozyme, β-lactoglobulin, rat serum, human serum or rat liver cytosol were incubated with continuous agitation. Lysozyme and β-lactoglobulin did not desorb the pigment. In the other preparations the amount of solubilized bilirubin was related hyperbolically to protein concentration. Equilibrium was attained after 16h at 4°C or after 4h at 25°C. On the basis of total protein concentration the amount of bilirubin solubilized by rat liver cytosol was about one-tenth that found with rat serum.

If during the preparation of the bilirubin–Sephadex complex the saturation limit of the solid matrix was exceeded, very high bilirubin/protein ratios were found in the subsequent binding assays (up to 33mol of bilirubin/mol of albumin). This is probably due to the presence on the solid matrix of loosely bound bilirubin. It is likely that the desorbed pigment in this case is largely in colloidal form (R. Brodersen, personal communication), as indicated by spectrophotometry and restoration of the original bilirubin spectrum by alkalinization (Brodersen & Theilgaard, 1969).

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