Transport Characteristics of Two Carcinostatic Compounds, Hydroxyurea and Hadacidin, with Rat Small Intestine

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Hydroxyurea has numerous biochemical and biological effects, including the inhibition of DNA synthesis (Brachet, 1967), and also has anti-tumour properties (Stearns *et al.*, 1963). Hadacidin (*N*-formylhydroxyaminoacetic acid) suppresses growth of human adenocarcinoma *in vitro* (Gitterman *et al.*, 1962). It is an aspartate analogue inhibiting reversibly the enzyme adenylosuccinate synthetase (Shigeura & Gordon, 1962).

We investigated the transport of these potentially therapeutic compounds across the mucosa of the rat small intestine *in vitro*. Either everted sacs (Randall & Evered, 1964) or everted segments (Wass & Evered, 1970) were used.

Neither hydroxyurea (assay method: Nery, 1966) nor hadacidin (assay method: Iqbal & Ottaway, 1970) was transferred across everted sacs against a concentration gradient. The concentration ratio (serosal concn./mucosal concn.) was, for 5mm-hydroxyurea, 0.97 ± 0.02 S.E.M. (16 sacs), and, for 5mm hadacidin, 0.87 ± 0.09 S.E.M. (16 sacs). Transport of hydroxyurea with the concentration gradient was linear over the range 2–15 mm external concentration. This transfer was not diminished by the presence of 1 mM-KCN.

Similarly, hadacidin transport down the gradient with everted sacs gave linear kinetics over the range 3–20 mm. Uptake of hadacidin (1 mM) by intestinal sacs and segments was not inhibited by 1 mM- or 10 mM-KCN, methionine, L-aspartate, glycine, betaine, L-proline or L-hydroxyproline. The result with hydroxyurea is not unexpected, since urea (Jervis & Smyth, 1959) and thiourea (Esposito *et al.*, 1969) both show simple diffusion across the intestine.

At 5 mM hadacidin did not show active transport with everted sacs (Spencer & Brody, 1964), but the lack of inhibition by structurally related analogues or a metabolic inhibitor has not previously been demonstrated.

Our results all suggest that hydroxyurea and hadacidin pass across the mucosa of the rat small intestine by simple diffusion.

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The Site of Action of Antimicrobial Cationic Proteins on Staphylococci

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Cationic proteins may constitute an important biochemical determinant in the natural defence of a host against invading micro-organisms. These proteins have been isolated from the teat canal, milk leucocytes and the cervical mucus of the cow (Brownlie & Hibbitt, 1972; Hibbitt *et al.*, 1969, 1971), and experiments *in vivo* and *in vitro* have shown that they produce considerable morphological changes in the fine structure, involving the plasma membrane, of staphylococci (MacMillan & Hibbitt, 1969).

In an extension of this work the region of the staphylococcal cell directly affected by cationic proteins has been studied by incubating the bacteria with calf thymus histone conjugated with Lissamine Rhodamine B (RB200) and examining the distribution of fluorescence in the cell-wall, membrane and protoplasm fractions. In another series of experiments designed to provide a comparison with an anionic protein, the staphylococci were incubated with a bovine serum albumin-Rhodamine B conjugate.

The staphylococci were fractionated into their component parts by lysing the cell wall with lyso-staphin (Schwarz/Mann) in 2.0M-sucrose containing 0.145M-NaCl. The protoplasts prepared in this manner were ruptured osmotically in double-glass-distilled water and the membrane fraction was sedimented at $60000g_{av}$ for 30 min. Electron-microscopical examinations were employed to monitor the purity of the cell fractions and to provide further evidence for the site of action of the cationic protein.

The incubation of staphylococci with the calf thymus histone conjugate resulted in an incomplete lysis of the cell wall by lysostaphin. A complete