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

separation of the bacterial cell wall from the membrane was impossible under these conditions, so the comparison between the action of the two conjugates was restricted to the combined wall and membrane fractions. The bovine serum albumin conjugate was not bound to the walls and membranes, whereas there was a highly significant increase in the fluorescence of these cell components after incubation with the calf thymus histone conjugate. It is probable, nevertheless, that the major part of the fluorescence is bound to the membrane, since the incubation of staphylococcal protoplasts with the calf thymus histone conjugate led to a ninefold increase in the membrane fluorescence.

No bovine serum albumin conjugate passed through the membrane of whole staphylococci into the protoplasm, whereas the calf thymus histone conjugate entered the protoplasm and produced a sixfold increase in its fluorescence. The results of these experiments suggest that the staphylococcal plasma membrane may be the target site of action for the cationic proteins.

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Induction of Adenylate Cyclase Activity in Cultured Human Fibroblasts during Increasing Cell Population Density

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As diploid mammalian cell cultures become confluent on the surface of the culture vessel there is a decrease in the rate of cell division and of migratory movements (Stoker & Rubin, 1967). Such characteristics of 'contact inhibition' are considerably decreased in some tumour or transformed cell lines, but may be restored by the addition of cyclic AMP (adenosine 3':5'-cyclic monophosphate) (Ryan & Heidrick, 1968; Heidrick & Ryan, 1970; Johnson *et al.*, 1971). These data, together with the observation that polyoma-transformed cells are deficient in adenylate cyclase (Burck, 1968), are compatible with the model that, as the population density of diploid cells increases, there is a rise in the concentration of cyclic AMP, which acts as a mediator of contact

inhibition. This concept is compatible with the result of our studies.

Human fibroblasts were cultured in Eagle's minimum essential medium containing 10% of foetal calf serum and subcultured at 1-week intervals. Adenylate cyclase activity was assayed on successive days after subculture of testicular fibroblasts (F. Zaccello & P. F. Benson, unpublished work). Specific activities were as follows: day 1, 21.1 and 21.8 units (nmol of cyclic AMP formed/m per g of fibroblast protein); day 2, 55.1 and 55.4 units; day 3, mean 71.9 units (four assays, range 64.5–78.3 units); day 4, mean 151.6 units (four assays, range 117.3–165.3 units).

In fibroblasts derived from skin, the mean specific activity in non-confluent cultures 4 days after subculture was 60.1 units (three assays, range 52.0–69.1 units) and in confluent cultures 10 days after subculture was 156.8 units (four assays, range 144.2–167.3 units).

Our results indicate that adenylate cyclase activity in fibroblasts (derived from both human testis and skin) becomes enhanced during monolayer culture *in vitro* as the cell population density increases. When considered with the data cited above, that cyclic AMP restores some characteristics of contact inhibition to transformed cells that lack adenylate cyclase, our findings are consistent with an 'emitter-acceptor' mechanism for contact inhibition (Stoker & Rubin, 1967; Johnson *et al.*, 1971) in which cyclic AMP acts as mediator.

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The Use of Protonated Alumina for Purifying Anionic Substances for Subsequent Spectroscopy

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Anionic substances such as taurine and glycine conjugates and organic sulphates can be separated by t.l.c. on silica gel (e.g. Kieselgel G from E. Merck A.-G., Darmstadt, Germany, or MN-Kieselgel G-HR from Macherey Nagel und Co., Düren, Germany), and the material, located with iodine vapour, eluted with methanol and purified from silica-gel extractives on alumina protonated with HCl ($\text{Al}_2\text{O}_3/\text{HCl}$).

Alumina (500g of grade H, Peter Spence and Sons,