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cyclase activity was assayed on successive days after subculture of testicular fibroblasts (F. Zaccello & P. F. Benson, unpublished work).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,

Widnes, Lancs., U.K.) was digested on a boiling-water bath with 1 M-HCl (1 litre) for 1 h, collected on a filter and washed with water (8 litres), triturated with methanol (600 ml), refiltered and 'dried' at room temperature. It was reactivated by heating for 1 h at about 255°C at 2 mmHg pressure (cf. Evans & Shoppee, 1953). The product (0.1 g) was packed into columns (internal diam. 0.25 cm) in methanol and the anions (0.5–2 mg of sodium or ammonium salts) put on in methanol. Methanol (about 5 ml) eluted non-anionic impurities and the anions were then obtained by elution with a mixture of methanol (5 ml) and aqueous ammonia (0.1 ml, analytical quality, 'sp.gr. 0.88'). The total amount of NH_4Cl obtained by thus eluting a 'blank' column with ammoniacal methanol was determined (for one batch of $\text{Al}_2\text{O}_3/\text{HCl}$ it was 17.32 mg of $\text{NH}_4\text{Cl/g}$), and for i.r. spectroscopy of anionic organic compounds in 'test' runs Na_2CO_3 was added in amount sufficient to destroy no more than

about 90% of the NH_4^+ ion. This ion, in KBr, gives absorption bands at (maxima) about 3.1 and 7.1 μm , which do not interfere with bands important for the identification of steroid conjugates. Bands due to excess of carbonate did so interfere and were avoided.

The use of protonated alumina as an anion-exchanger has been previously noted (e.g. Lewis, 1956); it cannot contribute organic contaminants. An equivalent of Cl^- is displaced from $\text{Al}_2\text{O}_3/\text{HCl}$ for each anionic equivalent and can be used for analysis.

Satisfactory i.r. spectra have thus been obtained in the identification of bile salt mixtures from various animals, chloroform–methanol–aq. ammonia systems being used for t.l.c. (Subbiah & Kuksis, 1968).

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DEMONSTRATION

The Analysis of Serum Lipoproteins by 'Crossed Electrophoresis'

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A method has been developed for the analysis of serum lipoproteins by means of a modification of the Laurell (1965) technique of electrophoresis, with

antisera specific for the human apolipoproteins. By this means it is possible to distinguish the superimposed compounds that form the profile of β -lipoproteins and to improve the characterization of the hyperlipoproteinaemias. The analyses also provide support for the theory of lipoprotein families (Alaupovic, 1971).

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