decreased adherence of cells, reduced rate of DNA synthesis and increased generation time. It thus seems that in normal cells attachment to the substratum is linked to cellular proliferation.

There is evidence that in epidermal cells such fixation to the substratum is provided by hemidesmosomes. Our observation of a greatly increased number of HD in rapidly multiplying cells supports the concept that these membrane specialisations are positively correlated with cell proliferation. Under *in vivo* conditions strong adherence to the basal lamina would thus be provided for cells engaged in reduplication.

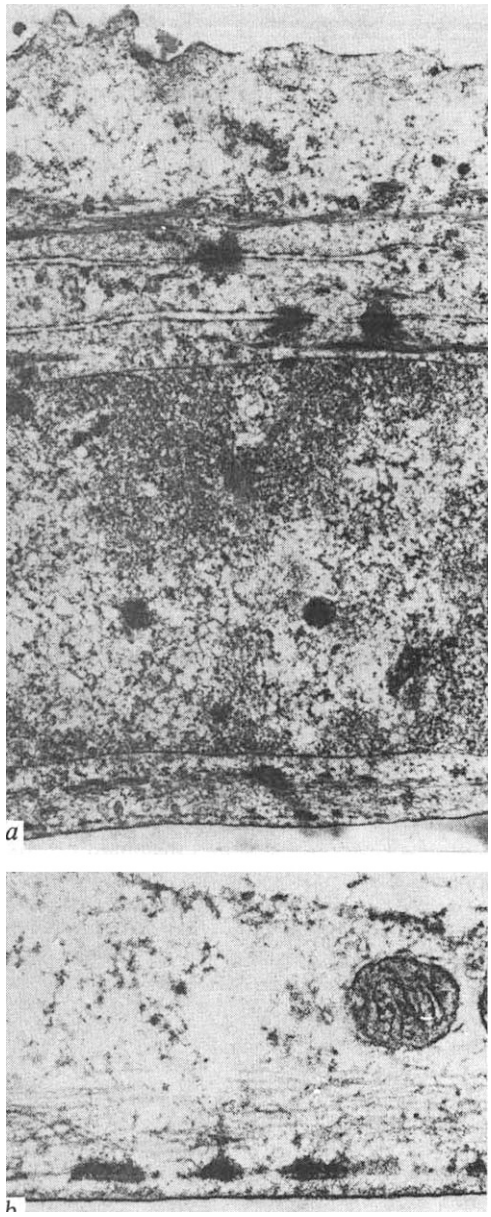

Fig. 1 High density epidermal cell culture grown *in vitro* for 3 d (control). Two types of membrane specialisations are formed: D, located at sites in contact with adjacent cells (*a*), and HD, exclusively present at the undersurface of cells contacting the substratum (*b*). Cultures were prepared[5] by gentle treatment with trypsin of adult guinea pig ear skin fragments. Cell suspensions consisting of single cells were seeded at a density of $1.8–2.2 \times 10^6$ ml^{-1} in 35 mm Falcon culture dishes and maintained in 5% CO_2–air at 37 °C. *trans*-Retinoic acid (Nordmark-Werke, Uetersen) was dissolved in DMSO and added to the culture medium (McCoy 5a, supplemented with 10% foetal calf serum, penicillin and streptomycin) at a final concentration of 10 μg ml^{-1}. The concentration of DMSO in all cultures, including controls, was 0.1%. *a*, ×27,540; *b*, ×60,000.

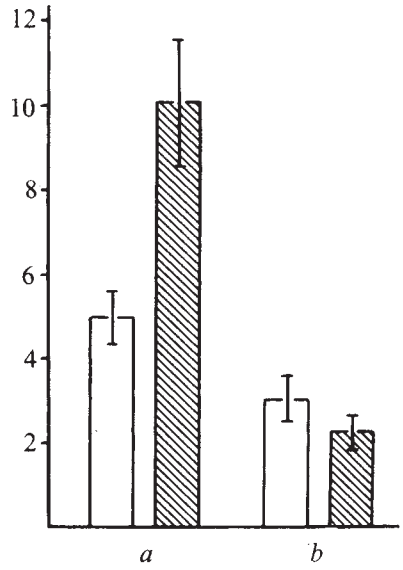

Fig. 2 Number of HD (*a*) and D (*b*) in 3-d-old cultures of PEC treated with 10 μg ml^{-1} RA (hatched bars) and untreated controls (open bars). Epon-embedded specimens were obtained after *in situ* fixation of entire culture plates. Selected areas were excised and cut with a diamond knife. Vertical sections were stained with uranyl acetate and lead citrate, and viewed with a Philips EM300 microscope. Photomicrographs were taken for HD and D counts at magnifications between 8,000 and 26,000. Length of plasma membrane on both sides of the cells was measured using a map roller. HD and D were counted and the results calculated as HD or D per 10 μm surface length + s.e.

On the other hand, upward movement and tissue-specific differentiation of epidermal cells coincides with the disappearance of hemidesmosomes and increased formation of desmosomes. The basic functional difference between the two attachment devices of the epidermal cell membrane thus seems to be closely linked to the mechanism of epidermal cell renewal.

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Demonstration of intermolecular forces in cell adhesion using a new electrochemical technique

The problem of cell adhesion has been approached from both chemical[1–3] and physical[4,5] viewpoints. We present here new evidence that a purely physical attraction acts between cells and other surfaces and that electrostatic forces can overcome this attraction. Previous attempts to demonstrate

and interpret such forces[6-8] have largely been frustrated by inadequately defined test surfaces, biological media including complex macromolecular components, and inappropriate assays for cell adhesion.

The unique features of our experimental approach are that the interfacial electrostatic charge of a well defined clean metallic surface can be varied continuously from positive to high negative values, and measured accurately. We have consequently been able to determine the critical electrostatic condition for cell adhesion. Another significant feature of our system is that the very low contamination rate of the test surface can be monitored continually during the course of the experiment.

We have used the relatively new development of solid polarisable electrodes: the test surface was a lead electrode[9] whose high polarisability in contact with electrolyte enables the interface to be charged positively or negatively by means of a battery and potentiometer: the magnitude of the charge being determined accurately from differential capacitance measurements. Since a current density of only a few $\mu A\,cm^{-2}$ flowed across the electrode surface, the experiment was virtually electrostatic. The lead surface was prepared by chemical polishing, after which it is probably molecularly smooth over large areas. Contamination of the electrode by oxygen, the adsorption of ions or organic molecules, could be detected by capacitance changes, providing a criterion for the rejection of poorly prepared electrodes. In practice, high stability over a 6-h period was frequently achieved following exhaustive cleaning and deoxygenation of the entire system.

We examined the adhesion of glutaraldehyde-fixed human red blood cells to the electrode in dilute sodium fluoride solutions of electrochemical purity. Aldehyde fixation, which has little or no effect on cell surface charge[10], was necessary to render cells stable at low ionic strength and to avoid loss of cellular proteins which might otherwise contaminate the electrode. Adhesion was assessed by allowing cells to settle for twenty minutes (settling for a period of 1 h does not affect the results) on to the polarised electrode and then inverting it while under continuous microscopic observation: cells which fell off were scored as non-adhesive. Cell falling was usually completed in 10 min.

In 10 mM NaF all cells stuck to the electrode, even at high negative surface charge. As the concentration was reduced to 1.1 mM, however, a most interesting pattern of behaviour was seen. When the electrode was positively charged ($+1.2\times10^3$ e.s.u. cm^{-2}) the negatively charged cells adhered irreversibly—our criterion for irreversibility being that the cells continued to adhere when the negative polarisation was subsequently increased to the maximum value of -4.1×10^4 e.s.u. cm^{-2}, approximately ten times the cell surface charge density. But when cells were allowed to settle on the negatively polarised electrode, within the range -5×10^3 to -3×10^4 e.s.u. cm^{-2}, reversible adhesion occurred. The cells failed to fall off when the electrode was inverted even after 20 min, but within a few minutes of the negative polarisation being increased beyond -3×10^4 e.s.u. cm^{-2} most of the cells were forced off electrostatically and were seen to sediment away from the electrode under the effect of gravity. Cells which adhered to the electrode at different charge densities within the range -5×10^3 to near -3×10^4 e.s.u. cm^{-2} fell off close to the critical charge density of -3×10^4 e.s.u. cm^{-2}.

The explanation for these entirely novel observations can be provided in terms of electrostatic and electrodynamic forces. The physical force theory[5] predicts strong, probably irreversible, adhesion at the limit of molecular approach (primary potential energy minimum) and a weaker reversible adhesion where cell and surface are separated by a finite gap and the forces of attraction and repulsion are equal (secondary potential energy minimum), the two energy minima being separated by an energy barrier. Preliminary calculations suggest that the distance of closest approach between a red cell and the metal surface may be several hundred Å in the secondary minimum position. We therefore feel that the essential criteria for the duplex adhesive behaviour predicted by the physical force theory have been demonstrated, and it is hard to think of an alternative explanation for the experimental facts. It is anticipated that when the distance of separation between cell and electrode has been measured directly it will be possible to estimate the size of the attractive force from the repulsion and make a quantitative comparison with the theory.

Whether secondary minimum adhesion occurs in physiological conditions remains uncertain. In our experimental system it has been found only at very low ionic strength where electrostatic repulsion can be maximised. But the calculated electrodynamic attraction to metal is large[5], so that lower repulsion coupled with lower attraction may give reversible secondary minimum adhesions in physiological media. Further experiments on cell adhesion at charged oil–water interfaces[11] may answer this question.

Our experiment has demonstrated that attractive forces which presumably include both image forces and electrodynamic (van der Waals') forces, and repulsive electrostatic forces, all of which can act over distances greatly in excess of chemical bond lengths, are responsible for the adhesive behaviour of red blood cells to a defined surface in the most stringently controlled conditions yet used in such work. The very nature of these rigorous controls makes the experiment unavoidably non-physiological; this, however, by no means detracts from the fact that long range interactions have been shown to occur between the complex architecture of protein, lipids and glycoprotein comprising the cell periphery, and the atoms of a solid surface. The conclusion that these forces also operate between cells would seem to be unavoidable.

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Increased spontaneous transmitter release from presynaptic nerve terminal by methylmercuric chloride

In recent years, several incidences of methylmercury pollution have been documented[1,2], one of the most striking alterations induced by the compound being extensive damage to the nervous system. Experimental mercury poisoning has been produced in animals[3,5], detailed morphological studies of which have shown that acute changes occur initially in the peripheral nerve fibres and thereafter in the central nerve cells[3,4]. Little