induce formation of pseudopods in the nearby area of the surface; in this way the active zone will gradually increase. In contrast, the zones of the edge in which pseudopodial attachment is not efficient will be gradually inactivated. Possibly, this inactivation is achieved first by the microtubule-independent mechanism and later strengthened and extended by the microtubule-dependent process. In such a way the cell may 'choose' the most adhesive substrate, it may orient itself with regard to the curvatures of the substrate etc. Thus, we are now beginning to distinguish the main groups of functionally different reactions which form the basis of complex cell morphogenetic behaviour. Our knowledge of the phenomenology of these reactions is still far from complete and our ignorance of the molecular mechanisms of these reactions is still profound. Another fundamental unsolved problem is that of the role of morphogenetic reactions in the regulation of cell metabolism, proliferation and differentiation. To take only 1 example, normal fibroblasts proliferate only when they are attached to the substrate but not in suspended state[32-34]. We know nothing about the mechanisms of this 'anchorage dependence of growth'.

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Concluding remarks

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All in vitro cell cultures are artificial systems which allow investigators to analyse the responses to cells in their immediate environment and hopefully to relate these responses to normal and malignant states in vivo. Pioneers in this field were confronted by the inherent difficulties of working with structured tissues and they decided to establish viable in vitro methods and define the basic terminology for cellular activities such as contact guidance and inhibition. Their common language has allowed comparisons to be made between differing cell types to establish both the similarities and differences.

Since this initial work, numerous factors affecting in vitro cell movement have been reported and analyzed in detail. The composition of the media, the physical conformation and chemical composition of the substrates, the cell type, age and state of differentiation all play an integral role. Joan Heaysman describes how, until recently, the majority of in vitro studies were devoted either to cell locomotion or growth and

division. To further complicate analysis of events studies were frequently undertaken independently by morphological workers and biochemists.

The morphological studies revealed the patterns of cell movement allowing the investigator to analyse them in a step-by-step manner and the biochemical investigations played an important role in establishing cell membrane configurations and correlating internal cell morphology with chemical composition. However, the strength of combined morphological and biochemical analysis is apparent from the recent surge of information and as in the puzzle-review. Cells are no longer described solely in terms of their shape and all of the contributors consider the combination of morphological and biochemical information. For example, Vasiliev considers pseudopodia not only in their relation to their cytoskeletal elements, but also to their position in the culture system, and the clustering of membrane receptors. Grinnell discusses in vitro and in vivo cell-substratum interactions as a biochemical problem. With this multidisciplinary approach it has become apparent that substrata morphology and biochemistry are of even greater importance than previously anticipated. However, as more information is correlated from 3-dimensional studies with collagen lattices and Sterispon, the inherent weakness of the 2-dimensional substrate system becomes apparent. The magnitude of the 3-dimensional problem is also apparent in the difficulties which Mareel found when assessing in vitro penetration of malignant cells into normal ones by 2-dimensional methods of assessment. As a possible solution to 3-dimensional analysis Löfberg and Ebendal have suggested using embryonic tissues as models; Trinkaus and his co-workers have used such tissues to great advantage. The suggestion is an exciting one! Perhaps more workers should now return to the transparent in vivo environment of the embryo to study the movement of normal cells and their encounters with malignant ones.

SPECIALIA

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Microbiological transformations of β-sitosterol and stigmasterol by a soil pseudomonad[1]

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Summary. Fermentation of β-sitosterol by a soil pseudomonad resulted in the formation of 4-stigmasten-3-one, 4-stigmasten-3-one-6β-ol and 5-stigmasten-3β,7α-diol. In case of stigmasterol the metabolites isolated and characterized were 4,22-stigmastadien-3-one, 4,22-stigmastadien-3-one-6β-ol and 5,22-stigmastadien-3β,7α-diol.

β-Sitosterol is widely distributed in the plant kingdom. Enormous quantities of sitosterols accumulate during the isolation of stigmasterol from plant sources[2], but remain unutilized due to the absence of a suitable process for degradation of the saturated C-17 side chain. A few microbiological processes[3-5] for oxidizing and cleaving the side chain have been reported, but until now none is in use on a production basis. During our work on the isolation of microorganismus capable of cleaving the side chain of β-sitosterol we came across a soil pseudomonad capable of using β-sitosterol as a sole source of carbon. This bacterium can also convert stigmasterol in the same way as it does β-sitosterol.

The soil pseudomonad was isolated by enrichment culture on β-sitosterol. The microorganisms were found to grow on β-sitosterol and stigmasterol and produce metabolites under various aerobic conditions, but the best yields were obtained at 30 °C in mineral salt medium (substrate concentration, 500 µg/ml). Fermentation of β-sitosterol (**I**) by the bacterium yielded 3 compounds.

Compound 1 (yield 15%), m.p. 97–98 °C, $[\alpha]_D^{25}$ +79° (c, 1.56 in $CHCl_3$), was found to be identical with 4-stigmasten-3-one (**II**) by comparison with an authentic sample.

Compound 2 (yield 2%), $C_{29}H_{48}O_2$ (M^+ 428), m.p. 207–209 °C, $[\alpha]_D^{25}$ +18° (c, 0.56) was characterized as 4-stigmasten-3-one. 6β-ol (**III**) by comparison with an authentic specimen prepared from β-sitosterol[6].

Compound 3 (yield 2%), $C_{29}H_{50}O_2$ (M^+ 430), m.p. 210–212 °C, $[\alpha]_D^{25}$ −70.5° (c, 0.55) gave a deep blue colour with $SbCl_3$ (in $CHCl_3$) indicating it to be a 7-hydroxy derivative of β-sitosterol[7-8]. This compound was finally characterized as 5-stigmasten-3β,7α-diol (**IV**) by direct comparison with an authentic specimen[9].

Fermentation of stigmasterol (**V**) with the bacterium also yielded three metabolites.