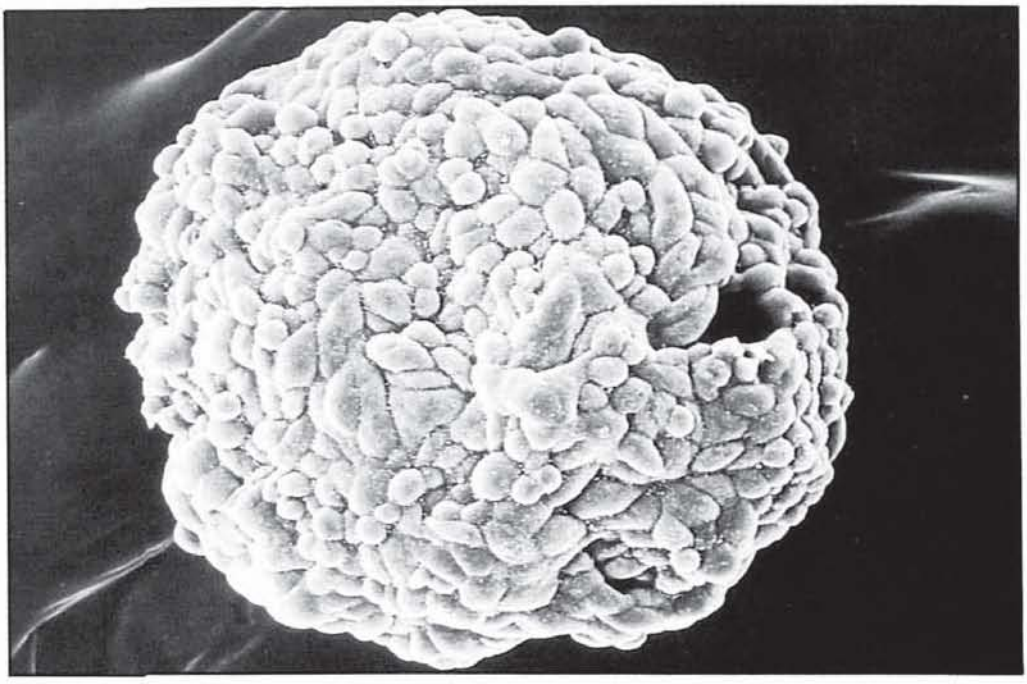
A Million Cells in Search for Contact

Multicell Spheroids not only for Cancer Research

Three-dimensional, spherical aggregates of cells – so-called multicell spheroids – have many practical applications. In cancer research, for example, they contribute to a drastic reduction in the number of experiments with animals. The authors of the following article were awarded a special DM 10,000 prize under the Felix - Wankel - Tierschutz - Forschungspreis (Felix Wankel Animal Protection Research Prize) in November 1986 for their work on intercellular communication in multicell spheroids.

Much of the information now available on the origin and behaviour of malignant cells has been gained as a result of experiments with cell cultures. This sounds strange considering the fact that traditional methods of cell culture only permit the cells to grow in one plane, namely on the bottom of glass or plastic dishes where they become two-dimensionally arranged. This so-called monolayer culture is a form of growth that in no way corresponds to the three-dimensional organisation to which the majority of cells in the organism grows. Hence, the questions which have been answered to date using these monolayer cultures have related to events or circumstances which occur independently of the spatial order of the cells, such as changes in their growth rate under the influence of toxic substances, or the appearance of tumorspecific components in the cell membrane after carcinogen-induced changes in the genetic programme of the cells. However, concerning questions of regulation and characteristic organisation of the intact organism, monolayer cell cultures can only be of limited assistance. Nevertheless, this kind of question can also be answered with cells in culture, since special techniques now enable cultivation of three-dimensional spherical cell aggregates. These multicell spheroids were introduced into cancer research in the 1960's as a nodular tumor model by Anneliese Schleich, a scientist from Heidelberg. However, this ability to grow into three-dimensional cultures is not a characteristic property of tumor cells, as cells from normal tissues or organs can also be induced to do this. Just as multicell spheroids from tumor cells display the



Transmission electron micrograph of a section through a multicell spheroid of chicken embryo liver cells. Adjacent cells form small biliary channels (above). Right: Scanning electron micrograph of a three-day-old multicell spheroid of rat mammary tumor cells.

typical characteristics of solid tumor growth in the organism, so liver cells form multicell spheroids with structures known from the intact organ and with the organotypical synthetic capacity. In multicell spheroids from heart cells, regular contractions can be observed. Changes in their pulse-rate can easily be determined and this simple method has been used to measure the reaction of the cells to the administration of drugs to these cultures. Isolated brain cells in suspension reaggregate to multicell spheroids which facilitate investigation of the interaction between the individual cell types. This cell culture technique provides a highly complex cellular organisation which permits significantly closer approximation to the conditions found in animals than is possible with the two-dimensional growth of monolayer cultures.

But how are multicell spheroids cultivated? Some million single cells collected either from permanently growing cell lines or from biopsies of normal or malignant tissues are transferred into petri dishes. Unless their surface is specifically treated these plastic dishes provide no substrate for adhesion of animal cells which, therefore, arrange themselves in close mutual contact.

Monolayers and Multicell Spheroids

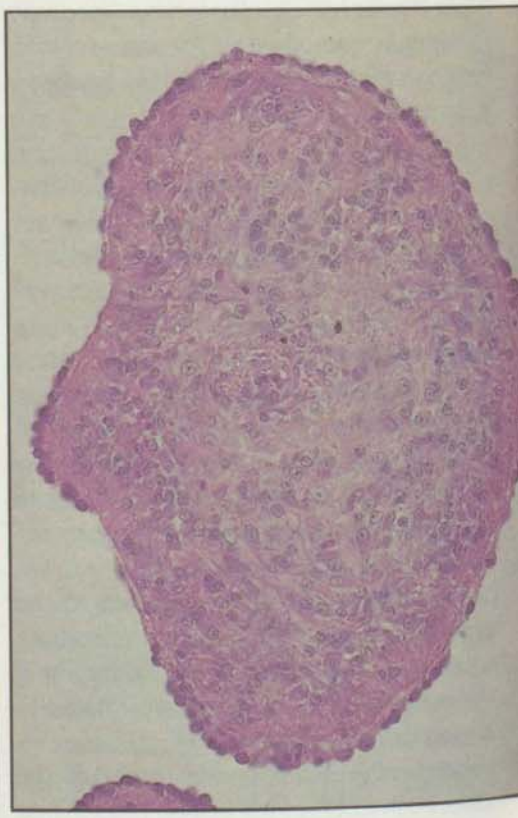
After only a few days, depending on the growth rate of the cells, aggregation and cell division will have led to the formation of numerous clumps of cells. These are then transferred into bottles where cells and medium are kept in constant motion to ensure optimum supply of nutrients for the cells. Under normal culture conditions, i. e. a steady temperature controlled at 37° C and buffering at pH 7,4, spherical cell aggregates of various sizes will have formed after a few days. These are then separated with fine-mesh sieves to cultures of uniformly sized multicell spheroids which continue to grow for a further 20 days or more under controlled laboratory conditions.

For extended series of comprehensive tests hundreds of multicell spheroids can

Multicell spheroid of rat mammary tumor cells. After injection of the fluorescent dye Lucifer yellow into a cell the dye spreads via channels into adjacent cells (above). Right: Section through a multicell spheroid of chicken embryo brain cells.

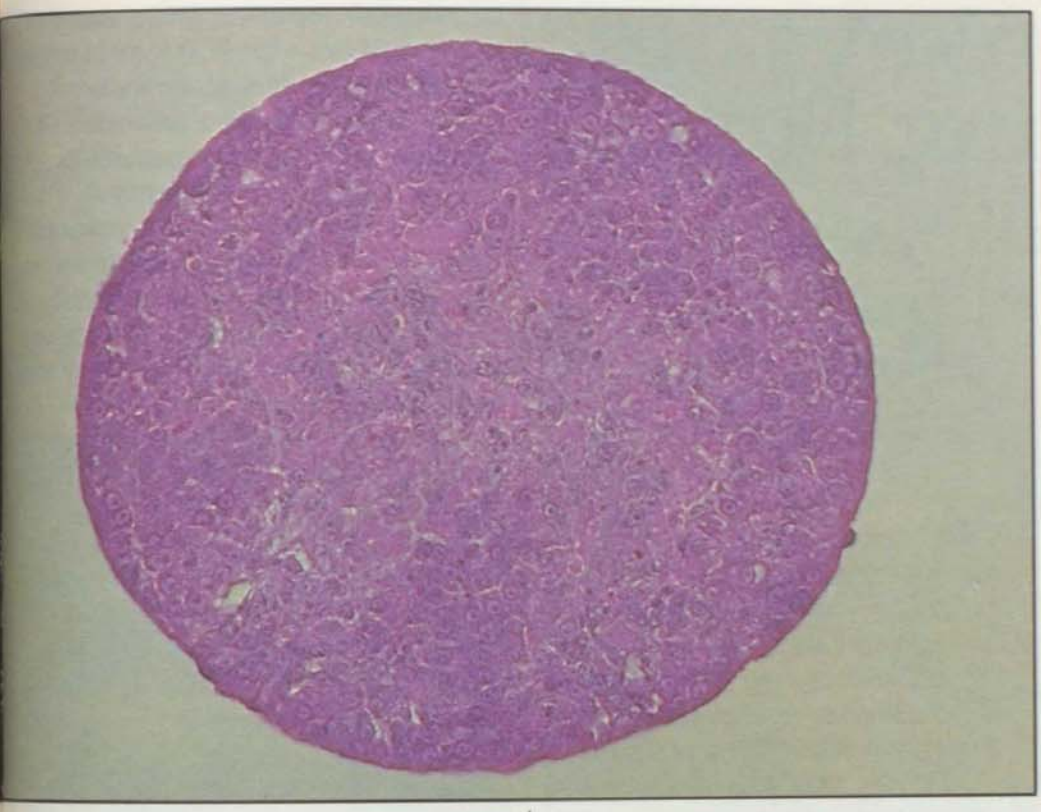
be obtained from the various organs and tissues of an embryo isolated from a chicken egg after nine days of incubation. Even if cells have been cultured for years as monolayers, in the multicell spheroid they will always revert to the original tissue- or organospecific three-dimensional structure. Thus human HeLa cells, transferred more than thirty years ago from a cervical cancer to a monolayer culture, arranged themselves to a multicell spheroid with a densely packed external layer of epithelial morphology. As a result, the cells inside the spheroid are deprived of their optimum supply of nutrients from the medium. Their fading leads to central necroses which characterise the growth of many tumors. The thickness of the external layer of fully viable cells varies, depending on the nature of the tumor.

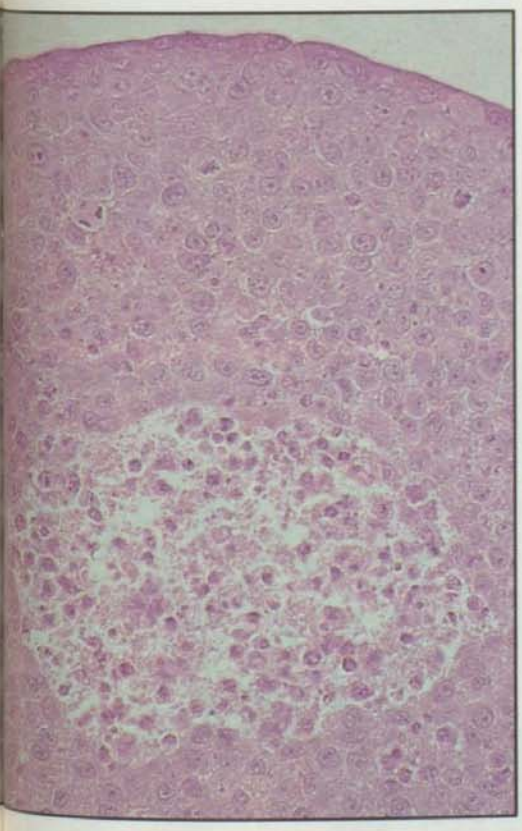
Tumors can be treated either by surgical removal or by destroying the tumor cells in the body by ionising radiation. However, they react with varying sensitivity to radiation, and thus the aim of treating the patient



with the minimal radiation dose compatible with effective treatment cannot always be achieved.

Bob Sutherland's group in Rochester, New York, provided experimental evidence that, for comparable damage to the cells after radiation treatment, tumor cells of the Chinese hamster in multicell sphe-





Section through a multicell spheroid of chicken embryo liver cells (above) and of a human tumor (right) with central necrosis (cervical cancer, HeLa cells).

roids require a higher dose than the same cells grown in monolayer cultures.

This finding – known as contact effect – has been attributed to the fact that due to the three-dimensional arrangement a cell in a multicell spheroid has considerably more neighbours than it would in a monolayer, and thus it can expect more support in case of damage. Using electrophysiological techniques, our laboratory has succeeded in further elucidating this contact effect. Co-operating with Hermann Dertinger from the Nuclear Research Centre in Karlsruhe, where the radiation experiments were performed, we examined a number of different cell lines and discovered that radiation-resistant cells were linked by fine channels (gap junctions). It became apparent that this contact effect was the more evident the better the cells in the monolayer were capable of intercellular communication via these gap junctions.

In a preliminary study, scientists in France used these findings for a clinical application. They determined the degree of intercellular communication in tumor biopsies from patients, and subsequently took this into account in their attempt to find a radiation dose small enough to protect the patient and yet effective enough to destroy the tumor cells.

As we continued our research on intercellular communication in multicell spheroids, results accrued which took us far beyond any applications for tumor therapy. Cells in monolayer cultures linked via gap junction channels are not able to regulate the flow of information from one cell to the other. This can only be achieved if the cells are cultured three-dimensionally as multicell spheroids. We proved this by forcing extrinsic signal molecules of varying sizes into the cells. As we are equally ignorant of both the intrinsic cell signal molecules for most cells and the messages which are exchanged between them, we used easily traceable substances injected into the cells by means of glass microelectrodes. Such a substance is the fluorescent dye Lucifer yellow, whose molecules are over ten times the size of the potassium, sodium or chloride ions which carry the electric signals for synchronous pulsation in heart cells.

In two-day-old multicell spheroids from coupled cells both Lucifer yellow and potassium ions spread from one cell to another. In four-day-old multicell spheroids the dye molecules in the injected cell are retained and only the potassium current flows through the gap junction channels. Two days later, however, these channels are closed and the potassium current is also retained. These results indicate that the actual state of intercellular coupling cannot be made responsible for the contact effect described above, but that the reason for the reduced sensitivity to radiation found in many tumors must be sought in the earlier coupling period.

Multicell spheroids can thus have many applications: in cancer research they are contributing to a drastic reduction in the number of experiments with animals but, nevertheless, enable the investigation of the origin of cancers and their therapy, or the behaviour of cancer cells attacking normal tissue. In cell biology they provide insights into cellular control mechanisms which cease to function in monolayer cultures, but which reappear in three-dimensional growth. In this way they have brought cell culture one step closer to the conditions prevailing in animals.

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