The influence of fetal mesenchyme cells on a Hodgkin-like lymphoma cell strain

(L. v. LANGENDORFF)

Lymphoma cells modified in a culture containing, «siccacell mesenchyme» not only lose the capability of producing tumours but also exert a protective effect against subsequent inoculations of viable tumours from the cell culture or fresh tumours from SJL/J mice bearing tumours. It has turned out that the «immunity» lasts at least 4 months and resists even repeated inoculations of the same tumour.

Spontaneous course

The effect of fetal mesenchyme cells from sheep (siccacell) on Hodgkin-like lymphoma has been tested. This tumour develops spontaneously after nine months (but not earlier) in mice of an inbreeding strain. This model was chosen because it corresponds much to human cancer and, unlike other tumours in animals, does not involve virous or chemical induction. The tumour is easy to transplant, without impairment by histocompatibility barriers, and grows rapidly in a foreseeable way. It was reproduced over several generations in three to six weeks old female SJL/J mice and leads to death within two to three weeks in 80% of the animals.

Fig. 323 shows a tumour-bearing SJL/J mouse and fig. 324 the excised tumour.

Cultivation in the tissue culture

The tumour grows also in the tissue culture where its cultivation can be continued. The fig. 325–327 show the mixed culture, which was obtained from the tumour shown in fig. 324, after one, two and three weeks in RPMI 1640 culture medium. A uniform population of individual cells was cultivated from this mixed culture. Fig. 328 shows the cell strain after the lapse of one month. Fig. 329 and 330 demonstrate the increased density of the same strain after one- and two-week growth in the RPMI medium; the beginning formation of a lump appears from fig. 330. Fig. 331 shows the cell lump after another two weeks. Although the tumour in situ consists of a population of multiple cell types, its cultivation can be continued in the culture beyond this individual cell strain; it can stand an unlimited number of divisions and retain its full potency as a tumour-producing agent after each cell division. Injecting the cultivated cells intraperitoneally or subcutaneously into

the axilla of three to six weeks old SJL/J mice will produce tumours not differing from the original tumour as shown in fig. 332 and 333. But the tumours originating from the cultivated cells develop more rapidly and cause the deaths of 100% of the test animals within one to two weeks.

Modification by adding fetal mesenchyme cells

This culture of cultivated malign cells was modified by the addition of siccacell-mesenchyme to the nutritive substratum i. e. 15 mg/100 ml. Such a modified culture as appearing one month after the transplantation is seen in fig. 336. During that time it was supplied twice a week with fresh medium, which contained siccacell. The change of the form is evident. Two weeks later, as seen in fig. 335, most of the cells have undergone necrosis, and the surviving cells are oblong in contrast to those of the original strain of cells. Whereas the cells grown in the standard medium RPMI 1640 retained their full tumour-producing effect even after 3 months and 15 transplantations, 70% of the cells cultivated in parallel cultures in the presence of siccacell had undergone necrosis after an equal time. The surviving 30% showed morphological changes. They were longer, became pyknic and the dendrite-like appendages were smaller.

Fig. 336 shows the modified culture in this stage. The cultivation of the surviving cells was continued, and while they propagated themselves, cell counts were used to adjust the quantities of the cells

Fig. 323:
A tumour-bearing SJL/J mouse.

Fig. 324:
The excised tumour.

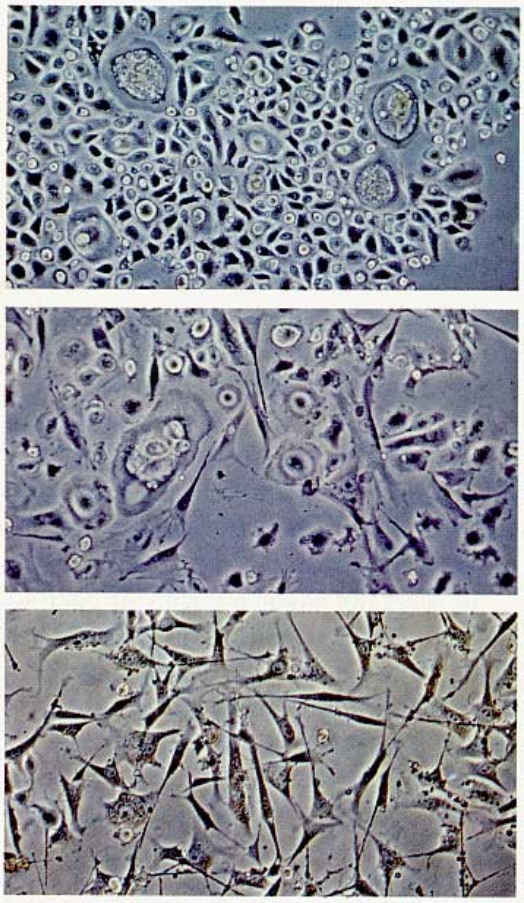

Fig. 325–327:
Mixed culture of tumour in fig. 324.

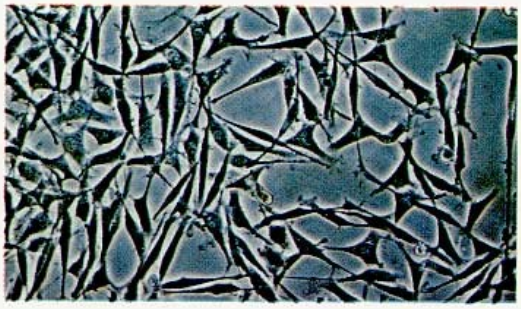

Fig. 328:
Cellular strain after 1 month.

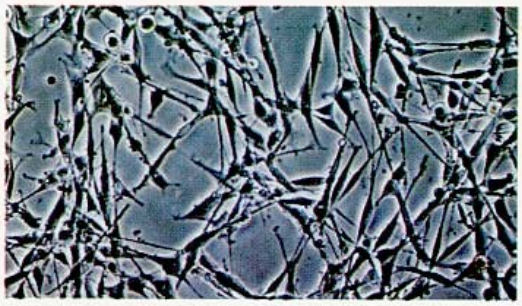

Fig. 329:
Cellular strain (fig. 328) after another week of growth.

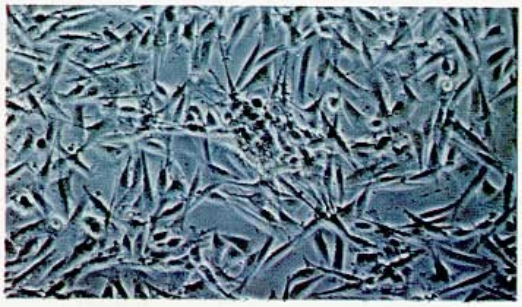

Fig. 330:
Cellular strain (fig. 328) after another 2 weeks of growth.

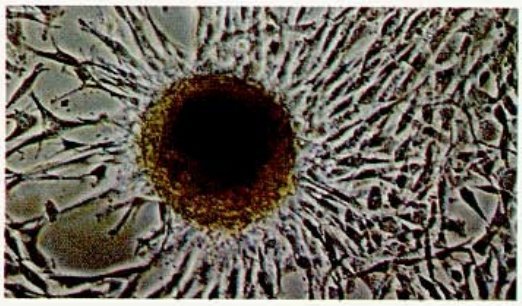

Fig. 331:
Cellular strain tumour tissue after 2 months.

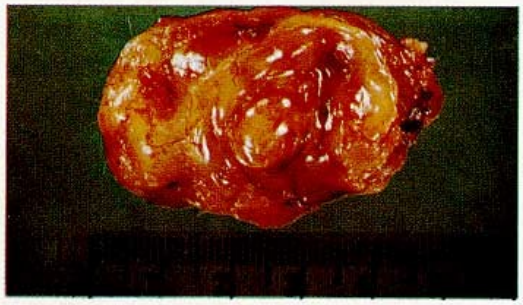

Fig. 332, 333:
Implantation tumour in SJL/J mice 3–6 weeks of age.

to be injected to the numbers of cells of the untreated cell strains, which were injected into the mice of control groups. Fig. 337 shows such a culture. After a month in a siccacell-containing medium, the cells were no longer capable of producing tumours. All mice that got injections of these pretreated cells were free from tumours after 3 months. A typical animal of this group is shown in fig. 338.

Protective effect

Further, the mice that had got modified cells, were protected from the effect of more inoculations of transplants of the original tumour or of the original cell strain of the tumour of the culture. No tumours developed if the mice were inoculated 4, 6, 8, 10 weeks after they had got an injection of cells cultivated in a nutritive medium containing siccacell. This appears from the diagram in fig. 340. On the left, once more the essential items of the test without siccacell-mesenchyme are shown: the tumour-

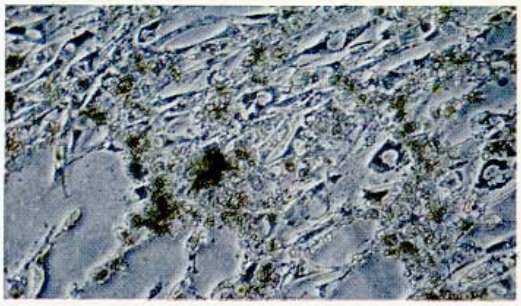

Fig. 334:
Addition of fetal mesenchyme cells to the nutritive medium, after 1 month.

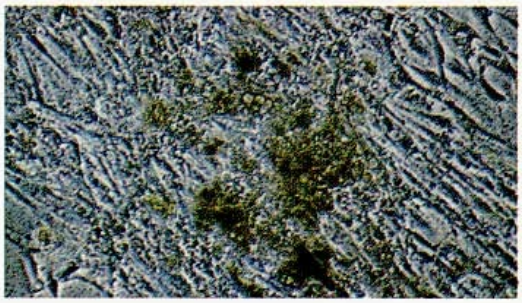

Fig. 335:
Culture 2 weeks later (2 × per week addition of siccacell).

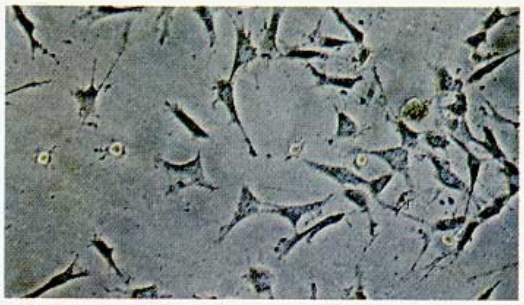

Fig. 336:
Death of tumour cells in the culture modified by addition of mesenchyme.

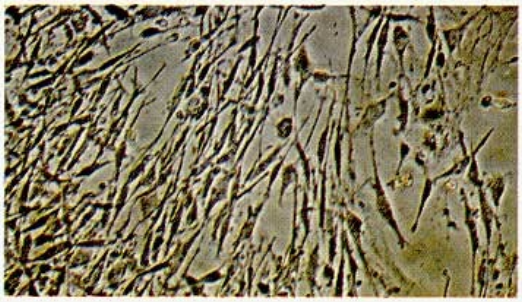

Fig. 337:
Modified culture in continued cultivation.

Fig. 338:
All mice that got injections of cells pretreated with mesenchyme are free from tumours after 3 months.

bearing mouse; the cell strain from the tumour culture and the rapid development of a fatal tumour after the injection of the cell strain. The upper line represents the changed morphology and the reduced number of cells, which results from the addition of siccacell mesenchyme to the nutritive medium, as well as the subsequent increase of the density growing to correspond to that of the unmodified cell strain. If these modified cells are injected into SJL/J mice, no tumour will develop. As seen on the right, the mice are free from tumours even after 10 weeks. If these mice get new injections 2–10 weeks after the implantation with siccacell-modified cells (either tumour-producing culture cell-strains / second line / or excised tumour from a tumour-bearing mouse / third line /), no tumours will develop. All pretreated animals remain free from tumours whereas all control animals succumbed to the tumour. Consequently, cells modified in a siccacell-mesenchyme-containing culture not only lose the capacity of producing tumours but also exert a protective effect against subsequent inoculations with viable tumours from the cell-culture or fresh tumours from tumour-bearing SJL/J mice. So far it has turned out that the «immunity» continues at least 4

months and resists even repeated inoculations of the same tumour.

LANGENDORFF, v. W. L. and his coworkers conducted similar tests to find out the influence of fetal lyophilised mesenchyme cells on the morphology and function of *Wilms-tumour tissue* cultures. The Columbia-Fürth-Wilms-tumour of the rat is a model corresponding much to the Wilms-tumour in children; it spreads in the same way and responds just so to therapeutic measures like the Wilms-tumour in man. This tumour is transplantable and can be transplanted over generations of Fürth-Wistar rats, which are predisposed to it. After the transplantation, a massive tumour develops within a week; it consists of hardly differentiated polygonal and fusiform cells, which are connected with each other in a loose texture. The tumour cell has a large central nucleus, which is surrounded by a small quantity of cytoplasm. The latter contains an endoplasmatic reticulum with much flocculent material, which indicates an active protein synthesis.

If lyophilisate of fetal mesenchyme from the umbilical cord is added to a growing tissue-culture of a Wilms-tumour tissue, part of the tumour-cells will die. The surviving cells show remarkable morphological changes. In comparison with the cells of the original culture, they become larger and flatter. The capacity of forming clusters of cells is lost. The retransplantation into an original nutritive medium did not provoke a tumour, which suggested that after the addition of mesenchyme the transformation into a not tumour-forming kind of the cells is final (LANGENDORFF, v. W. L., 1979).

Tests of many years have proved beyound doubt the effect of fetal mesenchyme on various tumours. The prophylactic and protective action i. e. the prevention of metastases seems to be more important than the reduction of tumours. The explanation of this sequel is far more complicated than the experimental facts of the outcome. RENNER, H., supposes a cross immunity between the onco-fetal antigens and the fetal antigens of the mesenchyme. An immunostimulation against fetal antigens causes, thanks to the affinity to the tumour antigens, an immunization, which covers also the tumour cells. This explanations is substantiated by results obtained in the lymphocyte transformation test (fig. 341).

FUENTE PERUCCHIO et al. (1979) have drawn from the most extensive immunological tests ever conducted in this field the following conclusions: The influences of fetal mesenchyme (resistocell) on the lymphoblastic transformation by means of laboratory cultures of leukocytes from peripheral blood of 22 patients with swellings of the gastrointestinal tract and a carcinomatous mammal affection in certain phases of development showed clearly immunological effects. Ten patients (7 with cancer of the colon and 3 with gastric cancer) were given a dose of Resistocell® 30 days after the excision of the tumour, 5 got the second dose 160 days later. Phytohaemagglutinin (PHA) as a non-specific mitogen was added in 4 experimental models used for 4 in-vitro leukocyte cultures, one of them received moreover 0,5 ml of Resistocell. In the comparative study of the models with and without Resistocell, the results showed a positive effect of the fetal mesenchyme on the blastic transformation of the lymphocytes in the laboratory during the entire course of studies. The differences were statistically significant 55 days after the operation. As however the tests were not sufficient to draw valid conclusions on the therapeutic efficiency of the fetal mesenchyme, these statements are restricted to the immunimodu-

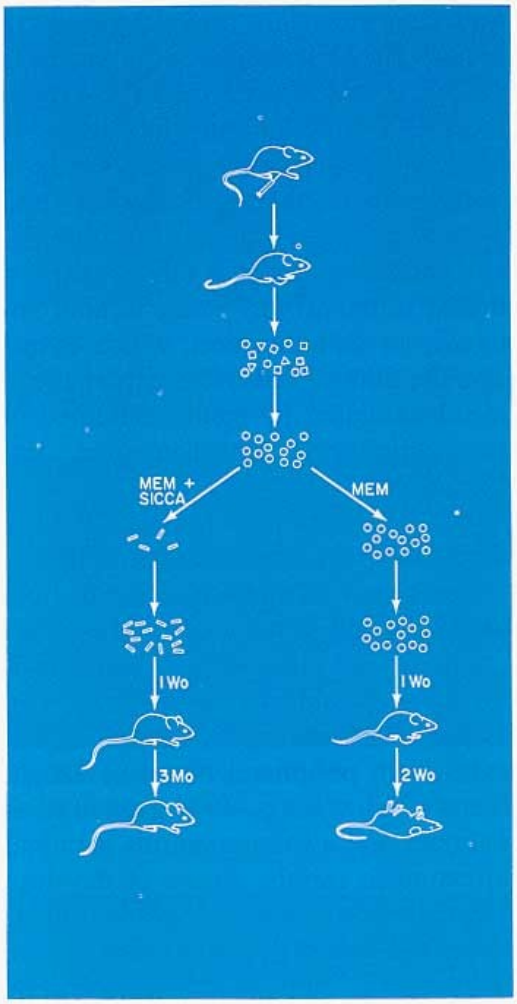

Fig. 339:
is a diagram of these observations. An SJL/J mouse got an axillary injection of the Hodgkin-like lymphoma from another animal and develops the typical tumour. The tumour is excised and cultivated, a cellular strain is taken from the mixed culture. On the right, the processes after cultivation of the cellular strain in standard MEM or RPMI 1640 are represented. A week after the injection, a tumour has formed, and the animals are dead after another two weeks. The left side of the diagram shows, in contrast thereto, the outcome resulting from the addition of siccacell mesenchyme to the standard nutritive medium. Only few cells survive and have been changed morphologically. Their cultivation is continud till their density equals that of the tumour-producing controls, then they are injected into SJL/J mice. No tumour develops, the animals remain safe and sound for 3 months and longer. These mice remain permanently free from tumours.

Fig. 339

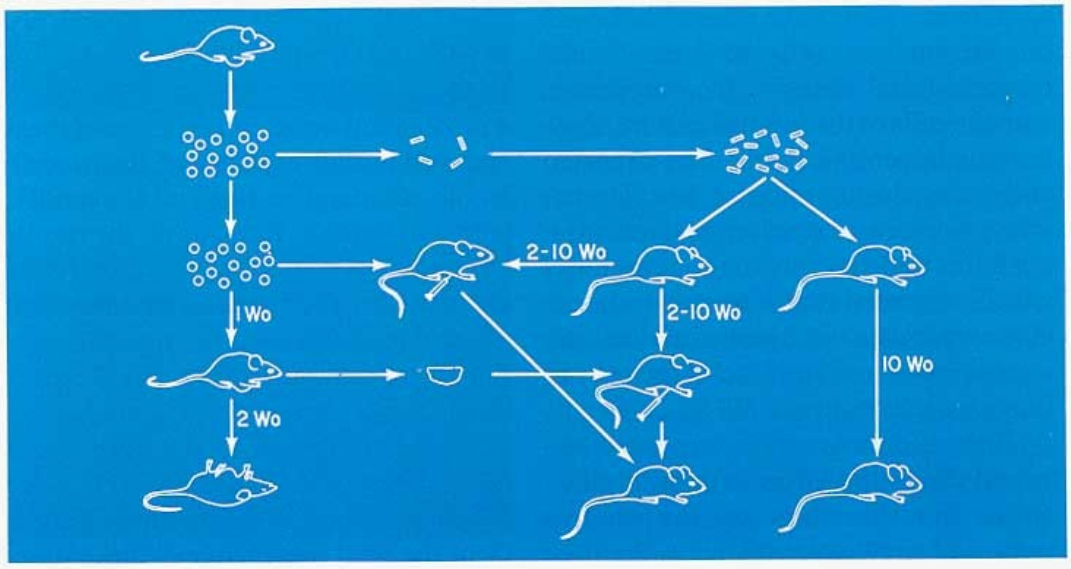

Fig. 340

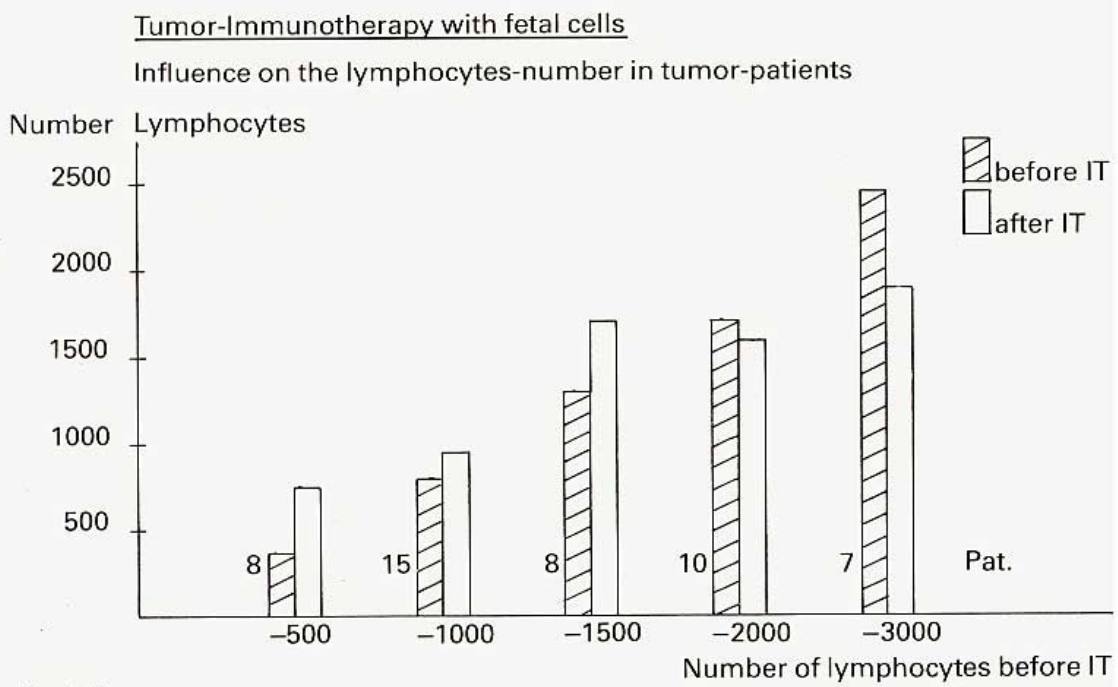

Fig. 341:
Tumour-immunotherapy with fetal cells. Influence on number of lymphocytes in tumour patients (after H. RENNER).

lation using fetal mesenchyme in the cancer therapy.

Not until these last three years (1979–1982), methodical studies by A. LANDSBERGER et al., HAGER, D. 1981, WACKER 1982, provided another very essential point of view.

Fetal mesenchyme in the form of the Resistocell from the umbilical cord may be capable of increasing the *interferon* level to an extent impossible so far with other methods. As interferon is an essential component of the tumour defence, this component of the resistocell effect seems to be of considerable importance.

For all, partly interesting partly convincing, experimental and clinical detailed findings, the clinical reality of cancer prevention and cancer therapy is unsatisfactory. New ideas and reconsideration seem necessary to open promising ways. Moreover, the basic conception of « malignity» will have to be abandoned. As a rule, malignities result in biology from inhibitions of structures and functions. Consequently, it would be more expedient for the therapy to promote the ripening in order to eliminate the inhibition of ripening characteristic for all tumours. This basic conception ought to supersede the present conventional view that tumorous tissue, as a matter of principle, must be removed or destroyed. The deviations of cellular metabolism will become more important and the cell therapy will have to be put on a broader basis than hitherto.