Transport-routes and effects

A basic requirement for the application of a medicament or a combination of active agents – as found in biological preparations – is the clarification of the principles governing the absorption and distribution of a remedy in the body. Since cell implants are of a size that can be detected under the microscope, the passage route from the stage of absorption to that of decomposition in submicroscopic particles is much better clarified than is possible and actually the case with many other medicaments.

This has been substantiated by the following tests:

a) Radioactive tagging of the injected tissular suspensions with P^{32} and measuring the radioactive concentration in the organs of the recipient (LETTRÉ, 1955; HARBERS, 1955).
b) Studies on the distribution of implanted cells by radioactive tagging of the organhomogenates with L-histidin-2,5-tritium and L-lysin-4,5-tritium (KMENT, ZABAKAS, BINDER, HOFECKER, NIEDERMÜLLER, DREIER).
c) Longitudinal studies on cellular suspensions tagged with vital stains (congo red, trypan blue, janus green, auramin) in guinea-pigs treated intraperitoneally (SCHMID, 1963).
d) Intravital studies on phagocytosis in guinea-pigs treated with intraperitoneal injections (SCHMID).

These basic experimental tests give the following uniform picture of the conditions of absorption and the fate of the implanted cell lyophilisates:

The heterological fetal tissue particles are loosened up immediately after the injection in net-like fashion, the chromosomes are «despiralized» . This process is achieved virtually after 20 minutes. During that time, microphages move into the loosened cell structures and attach – microscopically clearly visible – particles of the implanted cells to their cytomembranes. The process is completed after about two hours to the extent that the entire heterological cell material degrades into small particles and is absorbed by the macrophages i. e. on their cell surface (fig. 117–124).

The second phase of the incorporation of the implanted cell material starts with the phagocytosis of the microphages (loaded with foreign material), which is effected by large mononuclear macrophages. The microphages proper, which are loaded with foreign tissue particles (polynuclear), are evidently felt by the body as being «foreign» and phagocytised by monocytary macrophages.

After 48 hours, this process has continued to an extent that under an optical microscope no implanted fetal cell material is left to be identified (fig. 112–126).

The extent to which the decomposition of the implanted cell material continues, is probably controlled by the recipient organism. On the one hand, there is evidence that the decomposition may take place up to the short-chain peptides (KMENT); on the other hand, there is substantiated proof that the recipient organism incorporates proteins of high molecular weight in specific functions. Cellresident immunoglobines M of a molecular weight of 5 × 160,000 are taken over in specific functions; the recipient shows a positive tuberculin reaction three days after the cell transfer and ob tains the transferred property of the life term of the implanted immunoglobines for 3–4 months, without developing tu berculosis. It is irrelevant here whether the donor material is homologous (after exchange transfusions from animal to animal) or heterologic (fluid cells of humans on guinea-pigs, pleura cells, lymphocyte concentrates, spleen-pulp); (M. CHASE, 1945, STAVITSKY, 1948; SCHMID F., 1949–1952; LAWRENCE, 1952; SCHLANGE, H.).

Principles of distribution

Whereas the absorption of implanted cell material, up to the stage of magnitudes identifiable by optical and electronic miscroscopy, has been clarified almost completely, the principles of distribution have been ascertained only in an incomplete manner.

The «Hallstedt principle» advanced by some representatives, according to which planted cells migrate to the «place of need», is difficult to prove or refute by way of experiment. The following stud ies on this partial question are available:

a) Increased growth of corresponding organs (ANDRES, 1953, 1959; MURPHY, 1916; DANCHAKOFF, 1916);
b) Specific effect of cell inoculates in the embryonic and growing organism (ANDRES, 1963); fig. 135a–c.
c) Induction of the growth of organs by implantation of homologous tissues (NEUMANN, 1963).
d) Principles of distribution of injected foreign tissues (SCHMID, 1963).
e) Measuring of radioactive concentration in the organs after application of radioactive tissues (LETTRÉ, HEMPRICH and SPIRIG, 1953; LETTRÉ, 1954, 1955).
f) Rates of absorption of tagged tissues after splenectomy and experimental renal lesion (HARBERS, 1954).
g) The specific effect of implanted endometrium of rabbits on the uterus of castrated rabbits (BERNHARD and KRAMPITZ, 1960).
h) Studies on the distribution of implanted cells by tritiating organhomogenates (L-histidin-2,5-tritium; L-lysin-4,5-tritium; KMENT, ZABAKAS, BINDER, HOFECKER, NIEDERMÜLLER, DREIER, 1966–1973).

These experimental data can answer satisfactorily two important questions:

1. The injected (implanted) suspended cells and their stages of decomposition are rapidly distributed over the body in an exponentially declining curve, with the main activity taking place within the first hour after application; after five hours, the greater part of the distribution process has been completed. The taggings with

radioactive substances and vital staining widely agree in terms of the principle of distribution and in terms of time.

2. The degraded implantation material is removed authentically by microphages (polynuclear) and macrophages (monocytes, histiocytes); it has not been clarified but is probable that submicroscopic fragments are carried along with the flow of fluids.

Regardless of the type of tagging used, high concentrations of the implanted materials can be identified in various organs of the body already one hour after implantation. The tagged cells and their components are mainly, but not exclusively, identifiable in the implanted tissues of corresponding organs.

A few experiments suggest that besides the tissue relationship the «need» of an organ in the recipient organism plays a part. HARBERS (1954) found the rate of absorption of injected liver cells to rise from ordinarily 5–7% a day to 15–20% a day if the liver of the recipient animal was damaged by CCl_4-injections before testing; among these predamaged animals, the nucleic acid fraction in the liver was about double that in healthy animals. LETTRÉ (1955) did not find any specific concentration in the corresponding organs after injection of heart, liver and kidney cells tagged with P^{32}, but after injection of tagged brain cells the increase in activity in the brain of the recipient animal was double the level expected for uniform distribution.

The distribution studies by KMENT et al. (1968–1972) undoubtedly have an indicative value in biostatistics; they show that the highest concentrations of the implanted material are obtained in the corresponding organ. However, this can also be established for other organs and systems.

Increase in growth and action of corresponding organs of the recipient were repeatedly proved statistically (MURPHY, 1916; DANCHAKOFF, 1916; ANDRES, 1953, 1959, 1963; NEUMANN, 1963).

Fig. 117–124:
Fate of the implanted tissues in the recipient's organism.

Fig. 117:
Hypothalamic tissue immediately after implantation in the peritoneal cavity of the guinea-pig.

Fig. 118:
Thymic tissue is loosened net-like 20–40 min. after the implantation (DNA disspiralled).

Fig. 119:
Already in the first hour, endogenic microphages penetrate into the loosened fetal foreign tissues. Microphage: dark colour.

Fig. 120:
The degraded heterogeneous tissue is attached to the membranes of the endogenic microphages (dark colour) within 2 hours; here: cerebral tissue.

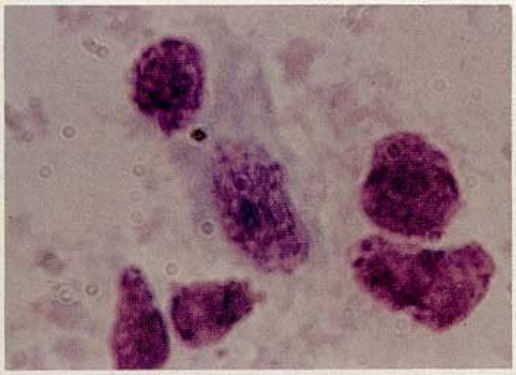
Fig. 121:
After 2 hours, fetal heterogeneous tissues in the peritoneal cavity of the guinea-pig have been attached nearly completely to the membranes of the microphages.

Fig. 122:
Within the following 48 hours, the complexes of microphages plus foreign particles are taken for heterogeneous and phagocyted by body-produced macrophages. «Battle of microphages».

Fig. 123:
Contact of a microphage marked with alkaline phosphatase (dark brick-red), with a monocytary macrophage.

Fig. 124:
Destruction of the segment-nuclear microphage (phosphatase colouration = dark red) by a monocyte at the site of injection (peritoneal cavity).

The donor material is degraded in the first hours after the injection of autogenous microphages and, later, macrophages to such an extent that it can no longer be traced optically and is dispersed all over the body. The material is incorporated specifically where the structures are of use and where they are needed. Whereas the cellular contents can rather quickly be disintegrated,



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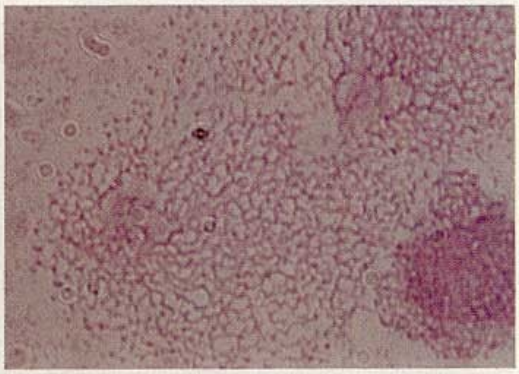

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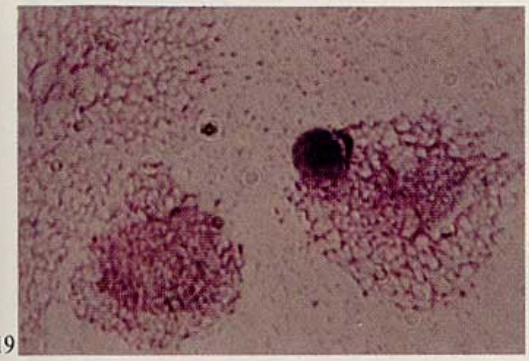

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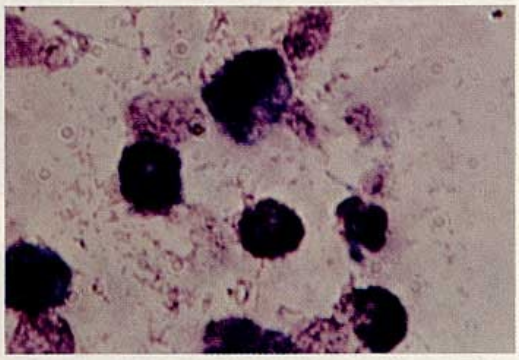

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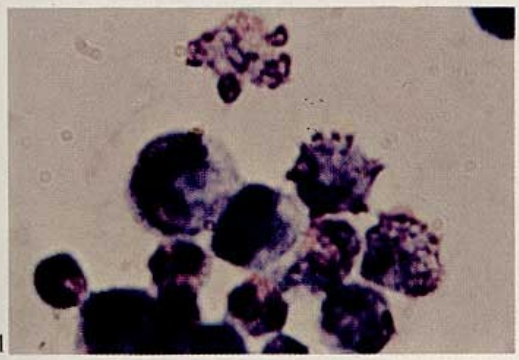

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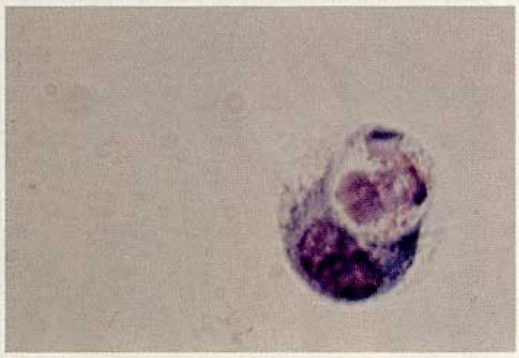

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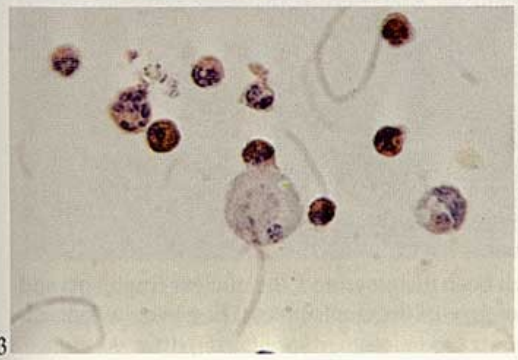

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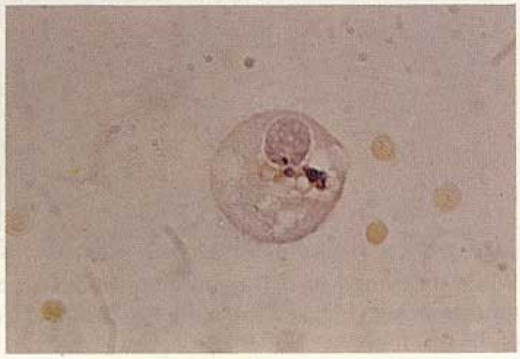

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Fig. 125–128:
Disintegration of the microphages in the phagocyting macrophages in electron-optical dimensions. The process takes place in the peritoneal cavity, 2–48 hours after the injection.

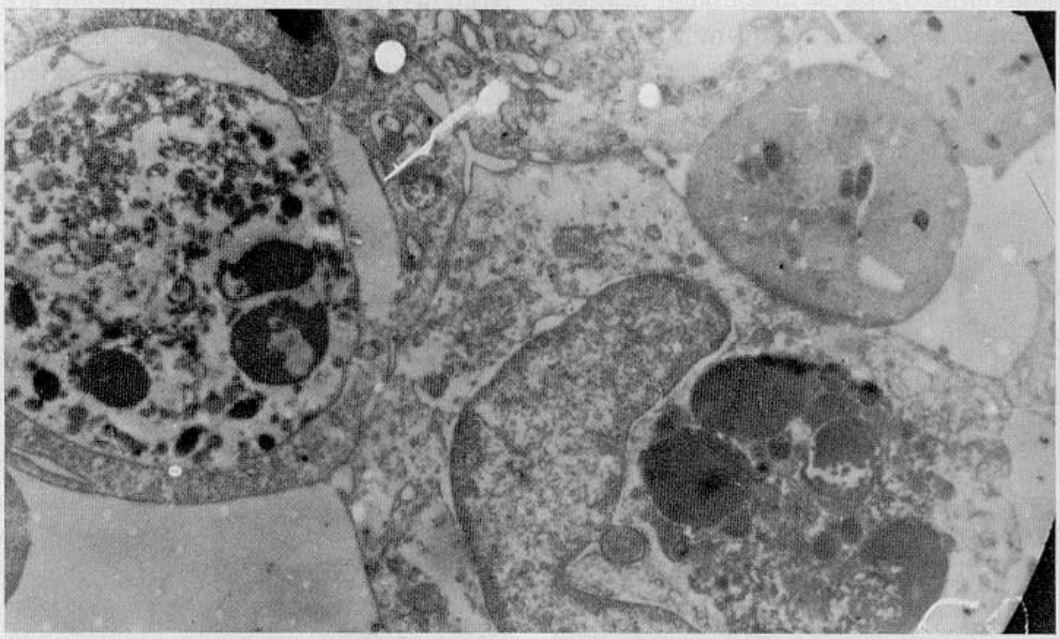

Fig. 125:
2 segment-nuclear microphages in various phases of disintegration (dark) within the cytoplasm of macrophages (1:11,000).

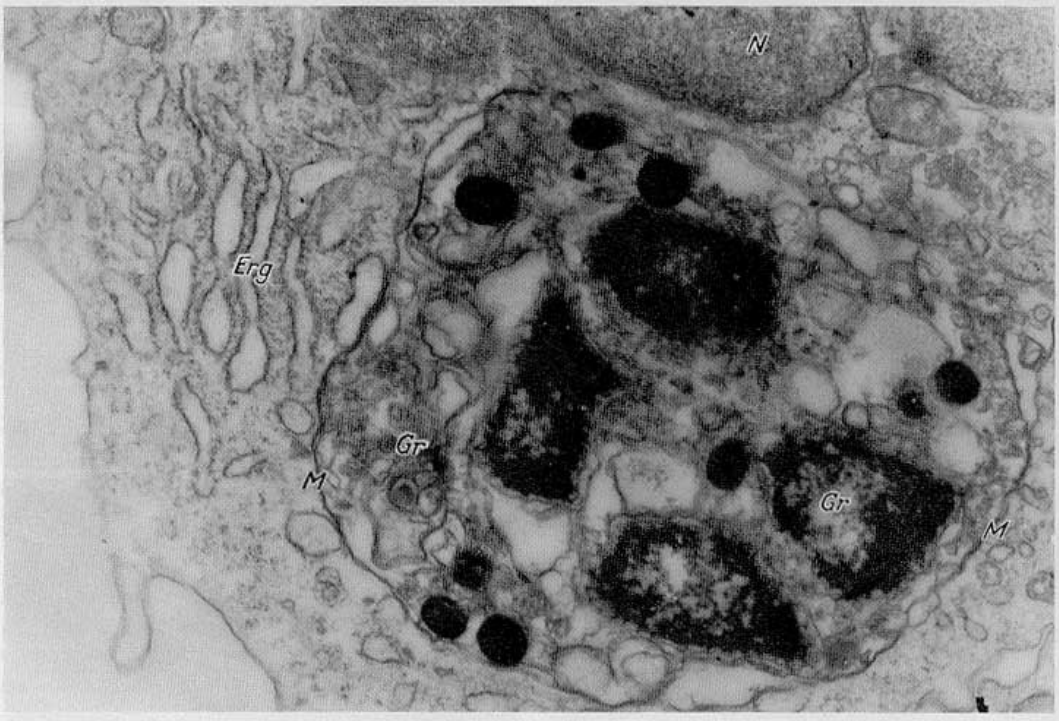

Fig. 126:
Whilst the cytoplasm of the granulocyte (Gr) has much been disintegrated, the nuclear fragments and the membrane (M) are still clearly perceptible. N = nucleus of the macrophage; Erg = ergastoplasm of the macrophage (1:30,000).

transported and incorporated by the recipient organism, the degradation of the membranes charged with heterogeneous particles of the autogenous microphages offers greater difficulties and may provoke immunizing processes.

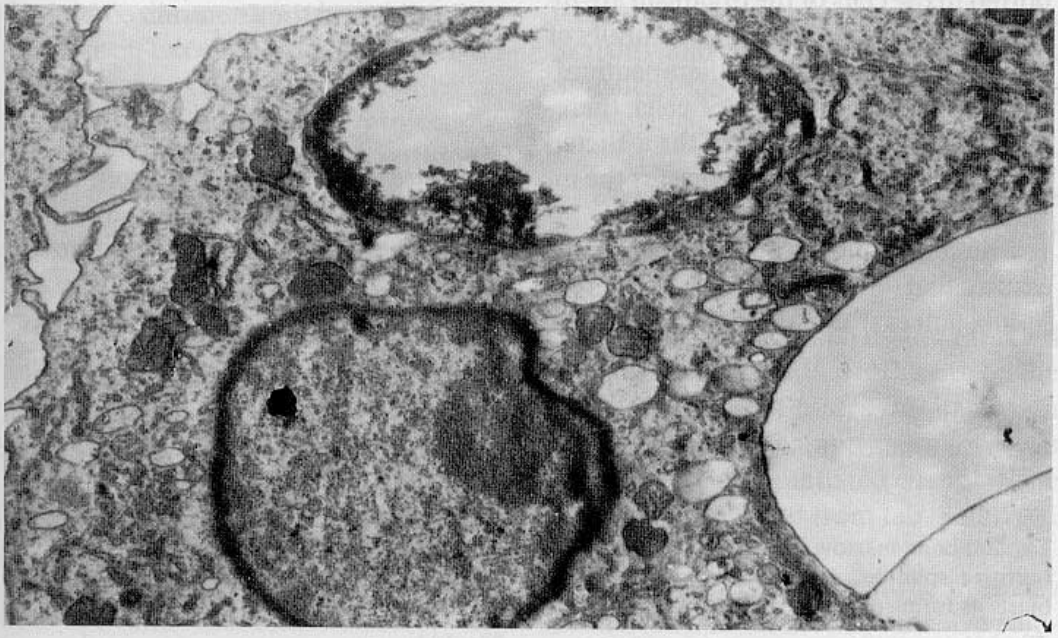

Fig. 127:
The structure of the granulocyte is no longer perceptible, thickened cellular membrane and nuclear particles form a digestive cistern (1:15,000).

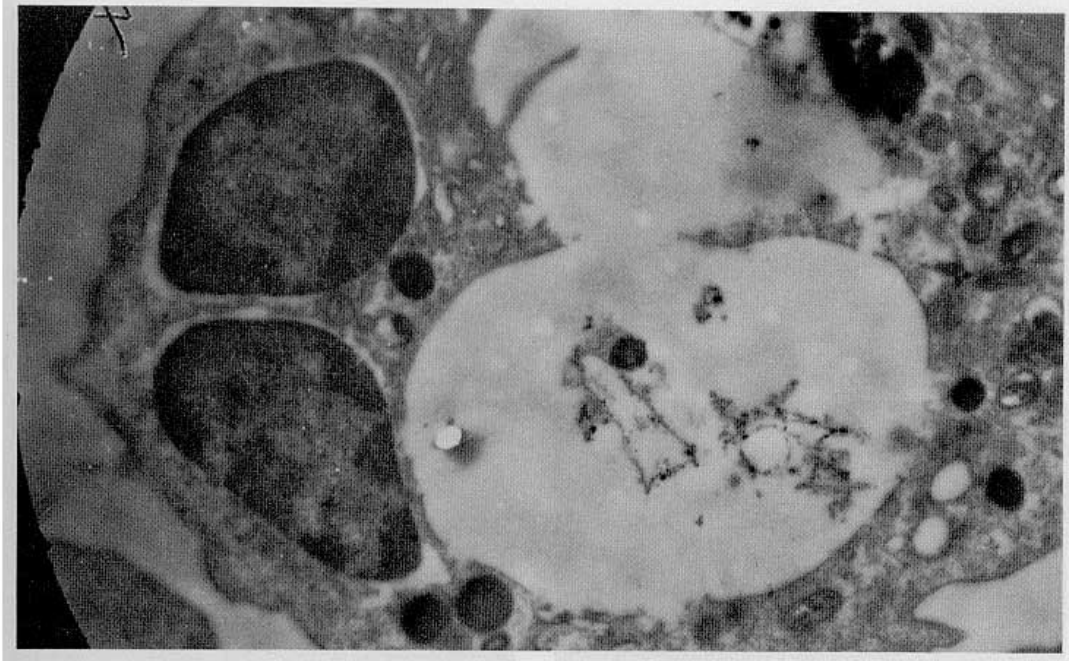

Fig. 128:
The last optical traces in the digestive cisterns of the macrophages are remainders of the granulocyte membrane with particles of heterogeneous tissue and nuclei (1:15,000).

Principle of self-distribution

Under a principle of self-distribution, the macromolecular structures transported in the cells of the organism are incorporated where they are structurally of use. A prerequisite for the therapeutic efficiency of implantations of cells, therefore, is a structural defect of the afflicted tissues i. e. an ecological space for the incorporation of structures.

The metabolic autonomy of the cells assures that

substances that are needed can be built in where their molecular structure permits so;

substances that cannot be disintegrated or infiltrated are wrapped up by antibodies and thus neutralized biologically.

Latency period

A latency period results from the regularities of disintegration and infiltration of the implanted tissues into the paths of autogenous metabolism and structures of the body, between the im-

Fig. 129–132:
In tissular cultures, growth can be stimulated by adding fetal tissues and the redifferentiation can be avoided.

Fig. 129:
Explant-bone-marrow culture of white rats in nutritive solution. After a short while, fibroblasts as forms of redifferentiation will prevail.

Fig. 130:
Addition of fetal cartilage to the nutritive solution multiplies derivatives of bone-marrow and extends the zone of migration.

Fig. 131:
Fibroblasts in the suspended culture with pure nutritive solution.

Fig. 132:
Multiplication of fibroblasts in the suspended culture by adding placenta-lyophilisate (LANGER v. LANGENDORFF).

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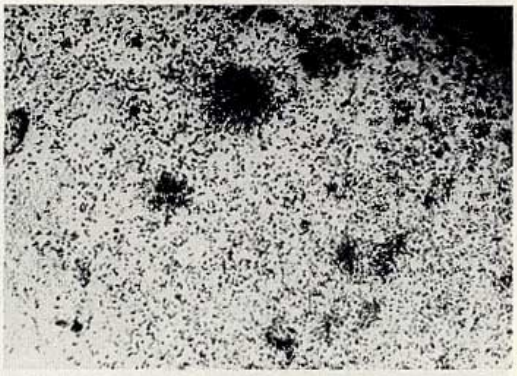

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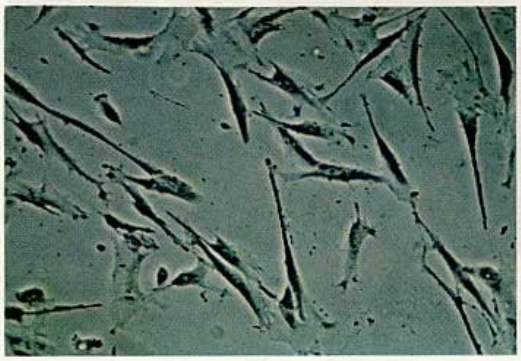

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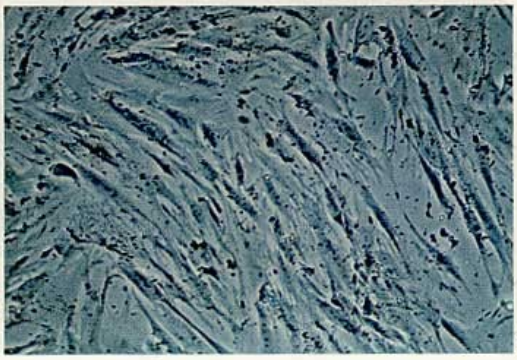

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plantation and the experimentally or clinically seizable effect. The latency period lasts at least 3 days in mesenchymal organs and at most 2 to 3 weeks in specific organic tissues such as cerebral or renal tissues. Exceptions are various endocrine organs as placenta in that after implantations of these tissues by injection a remarkable influence on the peripheral blood circulation, on the general state of health in the form of general revitalisation can be observed already within a day or a few hours. In question are probably hormonal effects and influences of other cellular contents, which by release from the implanted cells can take effect at once.

STÜHLINGER (1979) has presented a number of impressive casuistics on the latency periods to the onset of the clinical (biological) action; they depend on the age and the basic disease, may amount to days or months. For most of the indications, the effect sets in subjectively and under objective parameters in the 3rd or 4th week after the implantation.

Tissue cultures of rat bone-marrow served for models so as to test the influence of heterologous, chiefly fetal, tissues on the growth of the tissue cultures in the absence of immunological defence. Eighteen tissues indicated various influences on extension, density and cytologic picture of the emigration zones. Most of the tissues exerted a distinct growth impulse but also inhibitions were seen. The kind of the added tissue influences the prevailing cellular form. Many circumstances seem to indicate that the heterologous tissular particles are dissolved in the nutritive medium and selected as additional nutritive substances. A detailed representation of the tests and results can be found in F. SCHMID and H. LEWALD (see also F. SCHMID, 1963).

Density and extension of the emigration zone in bone-marrow cultures after addition of dry tissues

Many of the 18 tested tissues revealed very distinctly a gradual influence on the emigration zone. Besides this extension of surface, the compactness of cells showed very differentiated changes. The results can be noted from tab. 8 and fig. 129–134.

A comparison between the averages of the control cultures in nutritive solution shows considerable growth impulses in certain tissues. The index of extension and compactness of the emigration zones e. g. of the hypothalamus exceeded the control figures by 66 %. These absolute maximum ciphers are followed by a group of about 40 % growth increase (liver 45 %, placenta 40 %, cartilage 37 %). The majority of the tissues, above all the lymphoreticular and cerebral tissues, registered a growth increase of 20 %–30 %. Striking was the absence of influence by fetal bone-marrow, which showed even a slightly negative tendency (−2 %); the inactive osteoid fragments constitute a mechanical obstacle and dissolve very sparingly. There is no theory to explain the growth-retarding effect of testicles (−13 %).