Incorporation and distribution of injected foreign tissues

Actually, an organism has three possibilities of coping with foreign tissues:

1. Use (enzymes) or incorporation (substrates) in the own tissues.
2. Disintegration with selective utilization and selective elimination.
3. Elimination in toto.

What becomes of a tissue in a foreign organism depends on the phylogenetic and ontogenetic affinity. The less differentiated the tissues and organisms, the better the mutual tolerance. It is therefore much easier to obtain an incorporation in lower species of animals than in man. Good chances for an effective in-

corporation in the human organism are offered chiefly by endogenic (transplantations of skin and bones) and homogeneous tissues (transplantations and implantations of bones, bone-marrow, vessel and cornea). Blood transfusions provide at least a functional incorporation. The incorporation of foreign tissues is seldom obtained. But even this is possible with juvenile tissues and good conditions of contact. An effective incorporation will always depend on an intimate contact between corresponding tissues.

If a direct contact between corresponding tissues is not feasible (as in most of the parenterally supplied suspensions of cells and tissues) the chances for a direct incorporation will dwindle. Generally, the other two ways namely disintegration with selective utilization and total elimination will have to be taken. A classical example is the implantation of calf hypophysis; the implant is disintegrated in foreign tissue (skin of the abdomen) and can develop a selective effect or is eliminated as foreign matter in the form of a sterile abscess.

The following tests are to show the principles of dispersing injected tissues. Studied were homogeneous fresh tissues and foreign dry tissues (lyophilisates).

Material and methods

To follow the interrelations between the donor and recipient tissues:

1. single cells ought to be estimable,
2. longitudinal studies must be conducted, without
3. disturbing the physiological regulations of recipient's whole organism.

The studies on the contents of irritable blisters conducted first could not be continued because the hairy animals selected for the test do not develop sufficiently provoked blisters. Consequently, cells of abdominal exudates from white rats and guinea pigs were used as standard objects. The exudates were concentrated by intraperitoneal applications of 5–10cm^3 of paraffin oil (equal quantities in each series). Suspensions of heterogeneous cells were brought into this exudate rich in cells also by intraperitoneal injection, namely in the

1st test series organspecific, homologeous foreign «fresh cells», in the

2ndtest series organdifferent, heterogeneous dry tissues.

To distinguish the cells of the recipient animals from the donor cells or tissues, vital stainings were effected with congo-red, janus-green or trypan blue; dry tissues histologically well differentiable remained unstained. The staining with janus-green had soon to be given up as the mitochondria were injured.

The distribution of the donor and recipient cells in the abdominal exudate was registered by regular punctures within 4–6 days. The cells showing a colour of neither the donors nor recipients were recorded by auramin counter-staining. By this vital labelling, the quotient of distribution was found at any time in the cells obtained by puncture. In addition, tampons of spleen, liver, thymus as well as net-mesenterium preparations from selected animals were made.

The intraperitoneal technique ranges between the intramuscular and intravenous applications, and can therefore be regarded as a model of the administrations customary for man.

Tested were 61 albino rats weighing 180g on an average. The tests were conducted together with P. ROHRBACH and FLÖRSCHINGER.

1. Homologeous, organspecific cells

Although by rinsing the entire abdominal cavity of a donor animal all cells were transmitted so that, theoretically, equal quantities of donor and own cells must be in the abdominal cavity of the recipient, the first punctures made after 10 to 30 minutes showed many more own cells than donor cells. The relation of vitally stained own cells to vitally stained donor cells was about 3:1 to 6:1, but also proportions of 11:1 were registered. The percentage of donor cells came to 1–14% of all cells in the first puncture (including those not stored). These different capacities of storing cells indicate that practically every animal must be valued individually; still, the following rules can be deduced:

1. Removal of the heterogeneous cells from the site of injection sets in at once;
2. heterogeneous cells diminish in an exponentially declining curve (fig. 110);
3. even after 4–6 days, sporadic cells with the heterogeneous vital substance can be traced.

The foreign, homologeous organoid cells begin to diminish at once and dwindle rapidly, though not in a constantly declining curve.

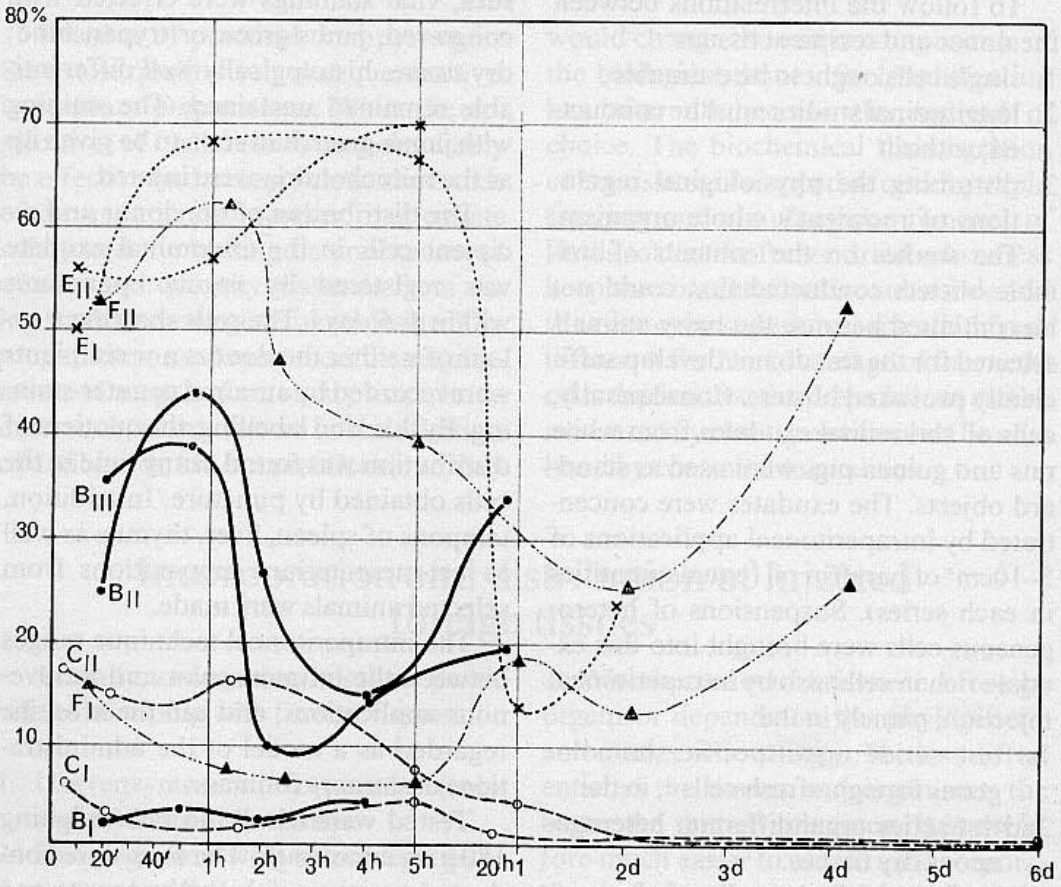

Fig. 109:
Decrease of the own cells marked by vital storage in the peritoneal exudate (guinea-pig).

In contrast thereto, the vitally stained own cells diminish much slower and stay considerably longer in the organism (fig. 109, 112).

The tests did not disclose anything about a fermentative disintegration. It can hardly be supposed that an extracellular fermentative disintegration occurs in the short time during which virtually the donor cells disappear.

2. Heterogeneous (xenogenic) organdifferent cells

The tests described above related to homologeous, organspecific cells. Of practical interest were the studies on the situation when tissues of other organs from other bodies were injected under the same conditions. For this purpose, unstained tissues easily identifiable by their morphological properties (siccacell preparations) such as cartilaginous, renal and placenta tissues were used. The tissues were suspended in Ringer solution and injected intraperitoneally in a volume of 2.5 cc (50 mg of dry substance).

Heterogeneous cells disappear very quickly from the site of application. This may occur theoretically

1. by *dissolution*
2. by *phagocytosis*
3. by *transportation-removal.*

The reparations provided safe criteria for a phagocytosis (fig. 114, 119–128) of the heterogeneous cells and certain criteria for an increasing dissolution (fig. 117). Both processes seem to coincide because accumulations of phagocytes around fragments of cells and stain particles of donor cells were largely detected in the autogenous cells. These

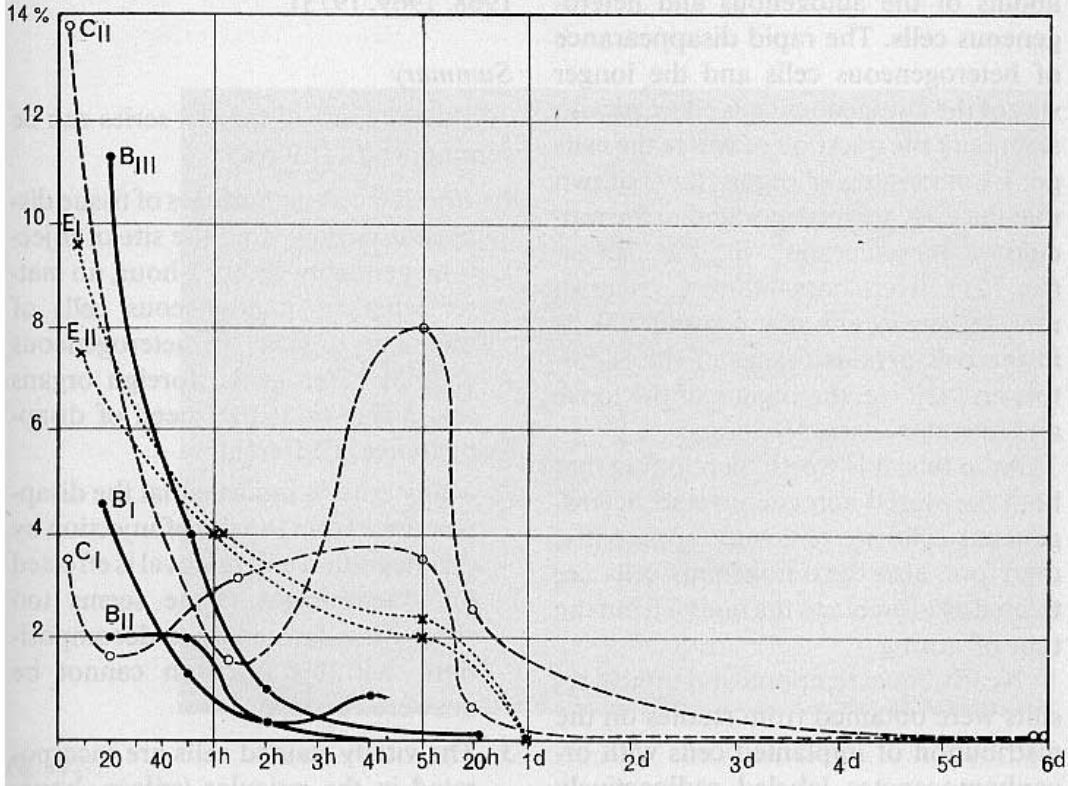

Fig. 110:
Decrease of the homologous donor cells marked by vital storages in the peritoneal exudate. Most of the foreign cells disappear within the first hour after injection.

«mixed colour» cells with much autogenous stain and little heterogeneous stain were found chiefly during the first five hours by 2%–4%, max. 6%.

The disappearance of these cell complexes from the injection area seems to be subject to the same laws as found for the autogenous cells. As complexes of fetal tissue are in question, the disintegration seems to take longer. Many individual pictures indicated that the autogenous cells gather round the heterogeneous tissues (fig. 114, 121–128, 160–189), push branches of protoplasma (fig. 119) to or into the heterogeneous tissue and «eat» the tissue complexes from the periphery. In coping with heterogeneous tissues, the body seems to rely mainly on the dissolution, the parenteral disintegration and the removal by autogenous phagocytes. It has been observed repeatedly that autogenous macrophages can take up in toto and disintegrate other cells (fig. 121, 125, 126).

The lyophilised fetal dry tissues have apparently a less «heterogeneous» effect in the recipient organism as they hardly provoke any changes of cell pictures worth mentioning. The antigenic stimulus is small, the formation of large basophile cells (plasma cells) does not take place or remains abortive. Several days (proved up to 6 days post injectionem) tissue complexes can stay at the site of injection where they are slowly destroyed by phagocytosis.

The removal

Vital labelling has revealed the whereabouts of the autogenous and heterogeneous cells. The rapid disappearance of heterogeneous cells and the longer stay of the autogenous cells charged with stain raise the question of where the cells go. Examinations of organs have shown that the cells are incorporated in the reticulum, mesenterium, in the spleen (fig. 111), liver, bone-marrow, thymus, muscle fasciae, articular coats as well as in the collagenous tissues of the snout, feet and tail i. e. the organs of the loose and reticular connective tissue.

As to this, it is worth mentioning that both the stored autogenous and heterogeneous cells are removed; apparently, therefore, also the autogenous cells are treated as «foreign to the body» from the time of storing.

Nearly equal temporal and topical results were obtained from studies on the distribution of implanted cells with organhomogenates labeled radioactively with L-histidin-2,5-tritium and L-lysin-4,5-tritium (KMENT, ZABAKAS, BINDER, HOFECKER, NIEDERMÜLLER and DREIER, 1968, 1969, 1973).

Summary

The outcome of the test series can be summarized as follows:

1. Injected cells or particles of tissue disappear rapidly from the site of injection, generally within 1 hour, no matter whether homogeneous cells of identical organs or heterogeneous cells of heterogenic, foreign organs are in question; the speed of disappearance is different.
2. Many criteria indicate that the disappearance from the site of injection by disintegration and removal is effected by macrophages. Time seems too short for a fermentative decomposition; but this question cannot be answered by tests.
3. The vitally stained cells are incorporated in the reticular (spleen, bone-marrow, thymus, liver) and loose connective tissue (collagen areas, articu-

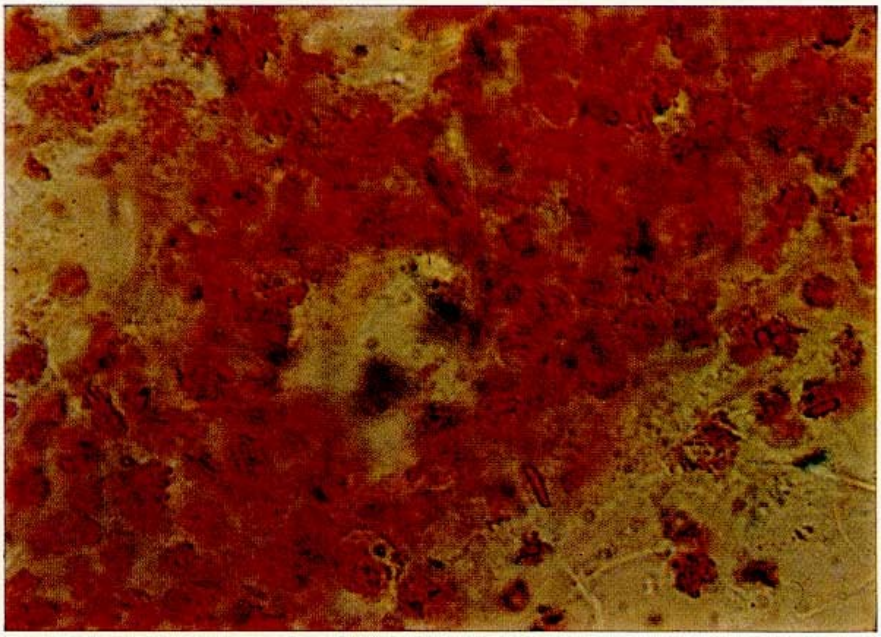

Fig. 111:
Storage of Congo red in the cells of the omentum.

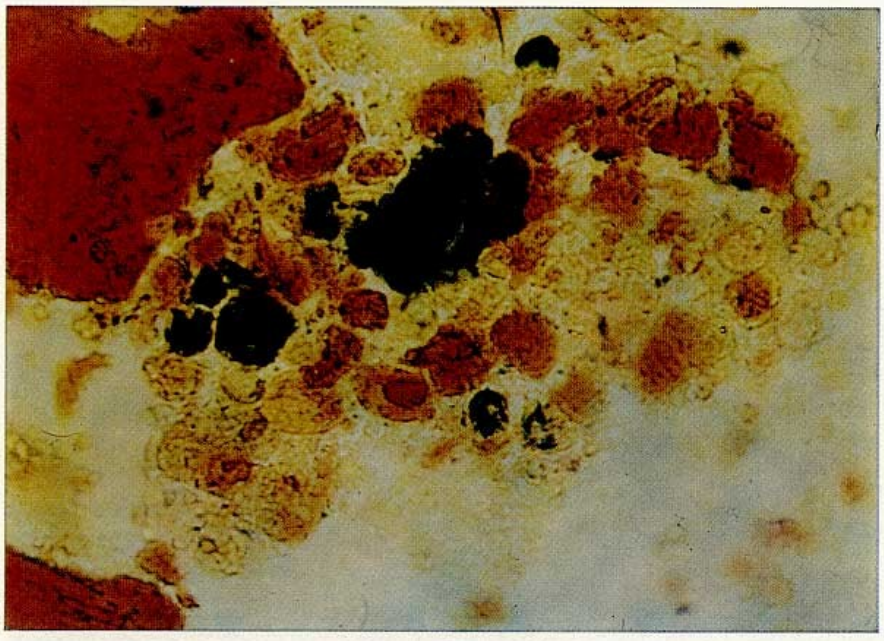

Fig. 112:
Pattern showing the own cells (storage of Congo red) and homologous heterogeneous cells (storage of trypan blue) 30 minutes after injection of the heterogeneous cells. Auramin countercolouration for the identification of the cells without storage.

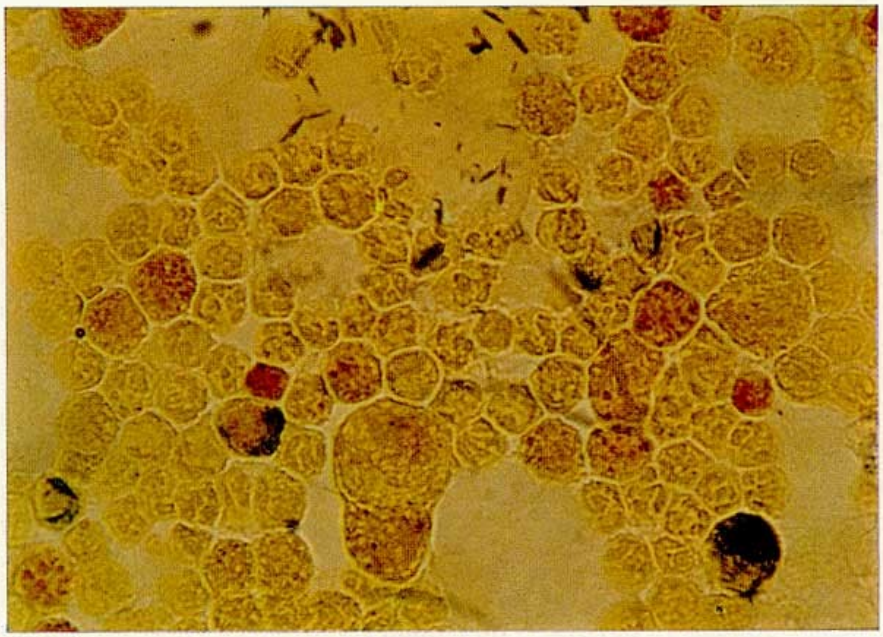

Fig. 113:
Pattern showing vital-stored own cells (Congo red) and homologous foreign cells (trypan blue) 5 hours after the latter's injection.

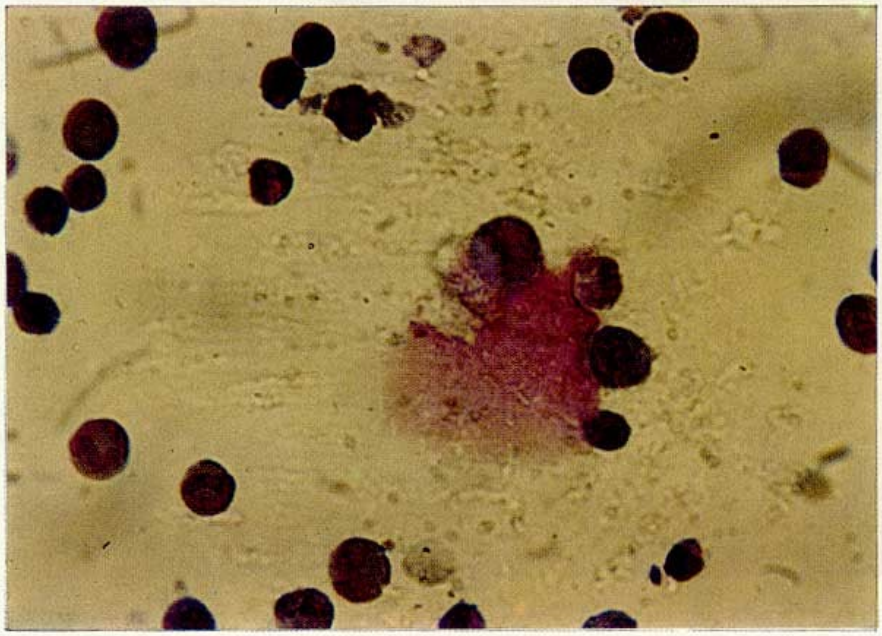

Fig. 114:
Body-produced cells with basophil plasma have «corroded» the remaining tissue of a fetal renal particle.

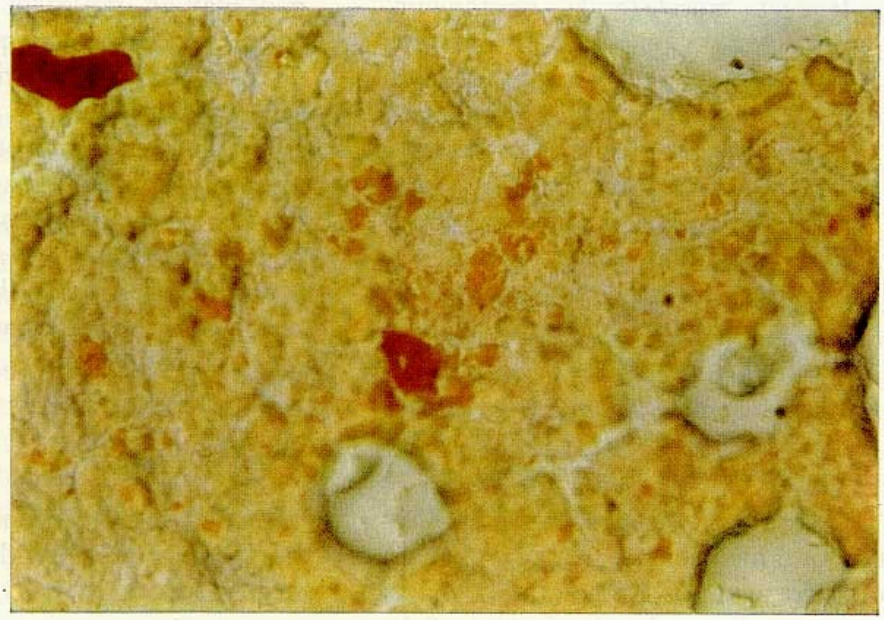

Fig. 115:
Storage of cells carrying vital dyestuffs in the thymus.

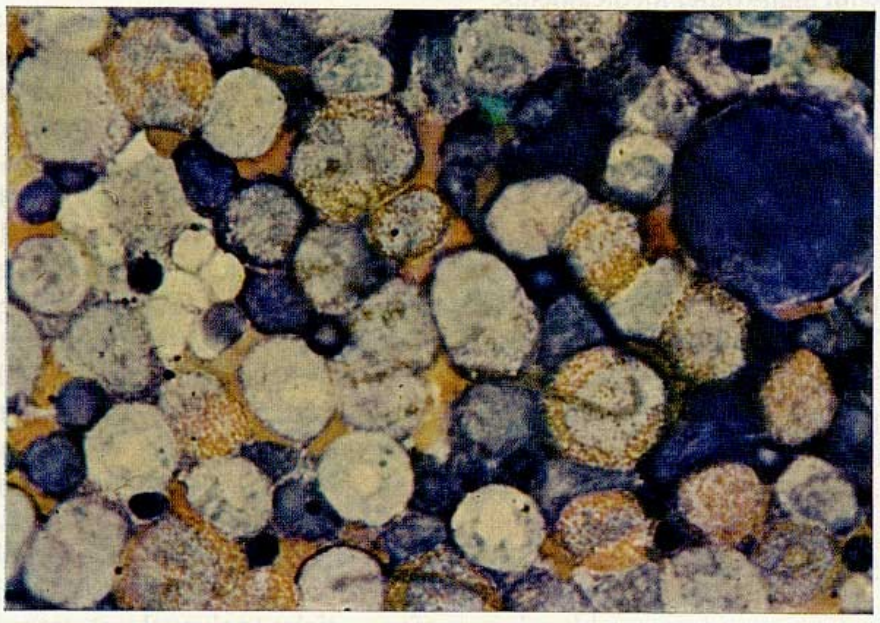

Fig. 116:
Dispersion of own and foreign cells in the spleen. Blue: homogeneous storage with trypan blue, red: heterogeneous cells stored with Congo red.

lar coats, muscle fasciae, peritoneum, mesenterium, omentum). From these tests, however, it can only be concluded that the cells are incorporated in related tissues. As the intraperitoneal application has an intermediate position between the intravenous and intramuscular administrations, the foregoing statement must be restricted. In the intramuscular application, fermentative disintegration may play an important part, but on principle the same mechanisms of disintegration and distribution must be supposed.

4. All partial results indicate that the heterogeneous cells cannot act in toto but as building elements in the size of oligopeptides, enzymes and substructures of biochemical substrates serve as material for the reconstruction of defective cell structures.