Clinical principles

Cell therapy as biomedical fundamental concept

From the preceding chapters on the principles and experimental studies on cell therapy it appears that this therapeutic trend is far better founded both theoretically and experimentally than many other forms of therapy, and that the assertion that it «lacks scientific significance» can be explained only with the absence of corresponding experience and knowledge of literature.

The question whether clinic and practice have been equally verified and substantiated cannot be affirmed and substantiated unrestrictedly in spite of 4–5 million patients treated already with cell therapy. This is due chiefly to the insufficient documentation of the results obtained mainly in the practice so that knowledge transmitted by word of mouth and by individual experience rather than a systematology suited to serve for science has been developed. Questions like the dose of the implanted quantities, number of the tissues to choose, intervals of implantations can not be answered free from contradiction – which no doubt is true for many biological methods.

The following chapters try to systematize the clinical questions; owing to the situation described above they must be a side by side of «hard», clinically significant data and facts and casuistic individual results (individual experience).

First, a general introduction on technique, indications, basic tests and side-effects is given, then the clinic is subdivided after the 4 indications crystallizing from the variety of applications:

I innate dysfunctions of organs and organic systems;

II pathological dysfunctions of organs and organic systems;

III impaired functions of organs, organic systems and of the whole organism as a result of ageing processes;

IV concomitant therapy of tumours.

«Cell therapy» is a biological form of therapy. It is defined as an injection-implantation of fetal or juvenile cells and tissular particles in physiological solutions.

As a matter of principle, it must be pointed out that cell therapy like any other medical trend should be part of a comprehensive wholistic concept. Than more this therapy with biological components is embedded into a number of necessary concomitant measures, than more convincing the effect will be.

The opinion often expressed formerly that «if cell therapy then nothing else beside it» has never been founded and cannot be justified in the apodictic postulate. Of course, supporting procedures ought to be used – not such of contrary action. It is of minor value to apply some hundreds of mg (100–400) of fetal tissue for reconstruction of defective tissues, and to administer at the same time antagonistic tissue-destroying drugs by grams or kilogramms. It must be emphasized that cytostatics, anticonvulsives, a few antibiotics and electromagnetic short wave radiations counteract the constructive principle of cell therapy as they destroy structures. Except these groups and narcotics, however, there are hardly any other medicaments that, if necessary, could not be used as concomitant remedies.

Cell therapy should in all cases be the constructive part of a medico-biological therapeutic general concept.

Under this aspect, the doctor using cell therapy must proceed on a broader base than usually. The purpose of therapy is not to remove a conspicuous symptom but the elimination of the cause of the symptom. In other words, not only the affected organ, the entire complex of regulations of the organ should be taken into account also.

Choosing the tissues indicated in an individual case is an art in the strictest sense of the word, which requires a deep insight into the biological general condition of an affected body.

The effect of cell therapy depends not only on the selection of the proper tissue but also on the body's demand for the substrates and enzymes offered. Where there are no defective structures or want of enzymes, biological components can neither be built in nor take effect.

The demand for biological structures and enzymes is a presupposition for the effect.

Preliminary tests

By extensive preliminary tests the doctor should

a) gain a detailed picture of the individual clinical aspect, and
b) find out any possible risks of side-effects.

It is of less importance to establish an exactly defined scheme of laboratory data than to carry out the necessary clinical and biochemical tests relating to age, sex, and disease. These tests differ from each other according to whether a child with infantile cerebral paresis or a potency problem in a middle-aged patient or loss of vitality in old age are in question.

It is essential

a) to exclude acute infections, especially such of the respiratory tract, and
b) to treat chronic infections (otitis, mastoiditis, tonsillitis, sinusitis, cholecystitis, pyelonephritis).

Useful, if possible, is the knowledge of complement factors and of the IgE level as immunological side-effects may occur on this base.

The detailed biological general aspect (state of the illness, vitality status) is necessary because from this assessment primarly cell-type selection and concomitant therapies adjusted to the individual situation of life can be derived. On the other hand, it does not promise an optimal therapy to rely only on a clinical guiding symptom or on striking laboratory findings.

Besides the reasonable therapy, the preliminary test has the purpose to prevent avoidable risks. The injection-implantations are followed within the first, generally 2–3 but also even 7 days by a stress on the body, which is due to the high metabolic function of disintegrating and transporting the implanted tissues. Whereas an organism capable of bearing stress experiences this phase of stress objectively as an agreeable fatigue or lassitude, the stress on an already strained organism may provoke unwelcome side-effects. Three starting conditions must be taken into account:

a) Infectious stress by acute bacterial and viral infections, or stressing chronic infections. In either condition, the implantation may strain the reactivity of the body beyond the expected limit of stress. High blood sedimentation, high complement titres, high IgE or the proof of specific tissular antibodies give valuable contraindications.
b) The organism is stressed (exhausted) by the basic disease to such an extent that its reacting mechanisms – espe-

cially the reactivities of the adrenal glands and of the hypothalamus/diencephalic system– are reduced enough to allow a stronger influence of the «physiological» general effects of the stressing phase. Then the lassitude is frequently felt as an unwelcome side-effect.

c) In patients of advanced age, the conditions of vessels and blood circulation at the site of injection have often been worsened by degenerations of vessels to such an extent that the absorption and removal of the implanted material are impaired and intensive local reactions may occur. The same conditions apply to diabetics advanced in years and patients suffering from chronic hypertension.

In all situations mentioned, it must be found out whether or not a cell therapy is feasible. This question should be analysed critically as the stressing factors are high and

cell therapy remains often the only possible therapeutic alternative.

The decision lies between the extremes «no treatment because risk is high and perhaps not justified» and «the higher risk must be taken in order to try and stop the fatal course of the disease» ; moreover, precautionary measures are indispensable.

Precautionary measures

Cell therapy requires perfectly sterile working as a generally necessary measure. The implant is tissue that, after resuspension, forms a substratum favourable for ingested microorganisms and those already existing in the body. The technical equipment and disinfection of the skin must be perfect, but bacterial or mycotic disseminating focuses cannot be excluded safely.

Technical equipment

The technical equipment (injection syringes, needles) must be sterilized immediately before use unless disposable articles in commercial packings are used. Wing canules offer the advantage of better handling when the syringe is changed i. e. they can be operated without touching the nozzle of the canule; on the other hand, there is the disadvantage that the metal cylinder of the needle is usually thicker.

The disinfection of the skin

at the site of injection ought to be double. Primarily, a sufficient area of the skin should be disinfected with a preparation containing iodine or an iodine substitute. After at least one minute of action the iodine must be cleaned away from the site with alcohol or ether. Besides the double disinfection, this method avoids traces of iodine getting into the body through the prick of injection and causing allergies. Disinfection with alcohol or ether alone – benzene should not be used – is not sufficient as the bactericidal effect of these agents cannot set in before the lapse of several minutes whereas the interval between the disinfection and injection uses to be shorter. Before the injection, the site must be dried with a sterile muslin tampon so as to avoid the penetration of even traces

of alcohol or ether through the puncture channel.

After the injection, the puncture channel must be covered for 3 days with an adhesive tape to bar the invasion of germs. As the injections are preferably applied to the gluteal region, this subsequent measure is necessary specially for patients with insufficient hygiene. Among these persons are disabled children and persons advanced in years, for whom a contact of the site of injection with urine or faeces cannot be excluded. Consequently, the protective tape must be renewed after every bath on the first three days following the injections.

Immunological precautionary measures

The complexity of the immunological processes has for consequence that

a) interrelations with other immunological processes through the non-specific bridging links (complement factors) cannot be excluded
and
b) immunizations against partial components of the used tissues – specially cell membranes, connective tissue – may occur if the implantations are repeated.

Experience with 4–5 million cell implantations carried out so far has shown that the immunological side-effects are extremely rare in contrast to the rate of expectance postulated by certain immunologists. Many patients treated over a long time tolerate completely without reaction even up to 40 injections administered at intervals of 5–6 months.

On the other hand, intensive local or even urticarial reactions may occur already after the 3rd or 4th implantation in patients suffering from acute infections or chronic suppurative focuses (otitis, mastoiditis, tonsillitis, sinusitis). Special caution is indicated in acute infections of the respiratory tract because stenosing laryngitis is possible.

As all these responses provoke the unwelcome hyperergic reactions via virtually non-specific mediators, extensive precautionary measures are necessary.

Since the reactions of the vascular apparatus – irrespective of how they are elicited – are caused mostly by the release of mediators from the basophils and mast-cells, the following measures are advisable though allergic reactions may occur:

1. Single dose, according to patient's age, of a *cortison preparation* (prednisolon, triamcinolon, bedneson) and *antihistamine product* 5–10 minutes before the injection/implantation.
2. Single dose of an *adrenalin derivative* (epinephrin, suprarenin, novadral, norphen, effortil and similar derivates) immediately after the implantation.

A favourable combination of a cortison- and antihistamin preparation is *celestamine* in the form of syrup and tablets.

The cortison derivative covers generally the antiallergic effect, the antihistamin preparation the effect of histamin, the catecholamin derivatives answer the reactions by the IgE-Complement.

«Antiallergic» treatments several days before are not necessary. A single dose of the above groups of drugs is usually enough; in case of need, they may be given several days.

Together with chronic bacteremia – especially Otitis chronica, mastoiditis – retarded urticarial reactions may occur weeks later in extremely rare cases.

Then, one must not rest content with the antiallergic covering, for only the elimination of the focus calms down the relapsing urticaria. From his own experience relying on 3 cases in which urticaria helped to detect otitic complications with chronic osteomyelitic lesions, the author points to the rare sequence of combinations; the implantation plays the part of a non-specific stimulator in a labile-latent immunological structure.

If intense local reactions (fig. 219) or an urticarial general reaction (fig. 220) occur, the alternative of choice is a parenteral (subcutaneous) dose (according to the age) of suprarenin or of a related adrenalin derivate.

Cortison in the form of suppositories, tablets or injection can also be given simultaneously though it must be taken into consideration that its optimal effect occurs 20–30 minutes later and that cortison preparations alone are not a proper remedy to control an acute allergic reaction.

A cup of a *mixture of vitamin-C-calcium* sparkling effervescent tablets: Cal-C-Vita, Ce-Ca-bion) is for children a placatory end of the injection. It is difficult to answer or to refute the question whether this beverage has an essential antiallergic-antiphlogistic effect.

Implantation technique

Although the implantation by injection is an injection, in practice there are more problems than one should expect.

The resuspension

of the tissular lyophilisate extremely poor in water can be effected in the ampoule. As the surface of the lyophilisate forms a film difficult to penetrate when getting in touch with the resuspension liquid, the lyophilisate dissolves slowly. This becomes a time factor if several ampoules must be drawn up for a treatment.

It is therefore recommended to fill the contents of 1–3 ampoules of the lyophilisate from behind into the syringe barrel; for this purpose, the end of the syringe must be provided with a needle to prevent any loss of material. After putting the piston into place, the solution is drawn in and the resuspension effected by shaking the barrel (fig. 210–214).

As many tissues (e.g. cartilage, connective tissue, placenta) swell, it is expedient to let pass 5–10 minutes, not more, between the resuspension and injection; if it takes longer, an injection canule with a wider calibre should be chosen.

Equipment for injection

The syringe should have a capacity of 10–20 ml. Syringes of 10 ml are easier to handle and hold well 3 × 100 mg of lyophilisate for the resuspension.

The injection canule must have a lumen of 1.0–1.2 (needle No. 1 or 20); for connective tissue and placenta, a lumen of 1.2–1.4, for cartilage and fetal, skin one of 1.4–1.8 should be selected.

One-ware equipments for every tissue are avaiable 1983 (fig. 219–226).

Site of injection

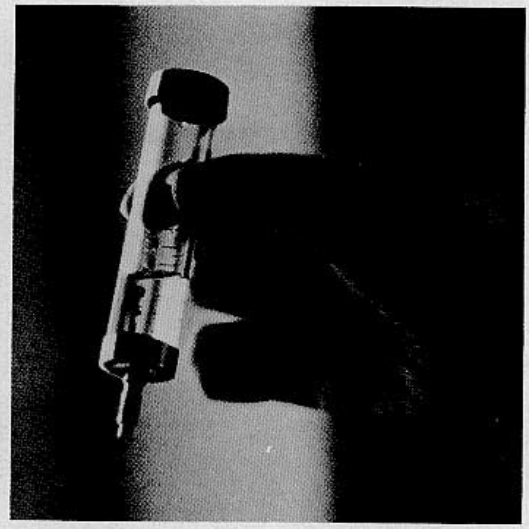
Implantations of cell material can be injected

a) subcutaneously,
b) intramuscularly,
c) intraperitoneally.

(Text continue page 197)

Fig. 210–218:
Implantation technique

Fig. 210:
Owing to the high dryness of the lyophilisates, with a residual humidity of less than 0.4%, it is not easy to resuspend them in the ampoules; the surface of the lyophilisates is moistened with water, which inhibits the penetration of the suspension liquid into the lyophilisate powder. It is therefore advisable to fill the cylinder of a 10–20 ml syringe with 3–5 ml of the suspension liquid after closing the nozzle of the cannula with a sterile small-calibre cannula broken off at a right angle.
Moreover, the lyophilisate can be brought into the cylinder of the syringe with the cannula put on and provided with a protective cap (see fig. 214) direct i. e. without a solvent in the cylinder.



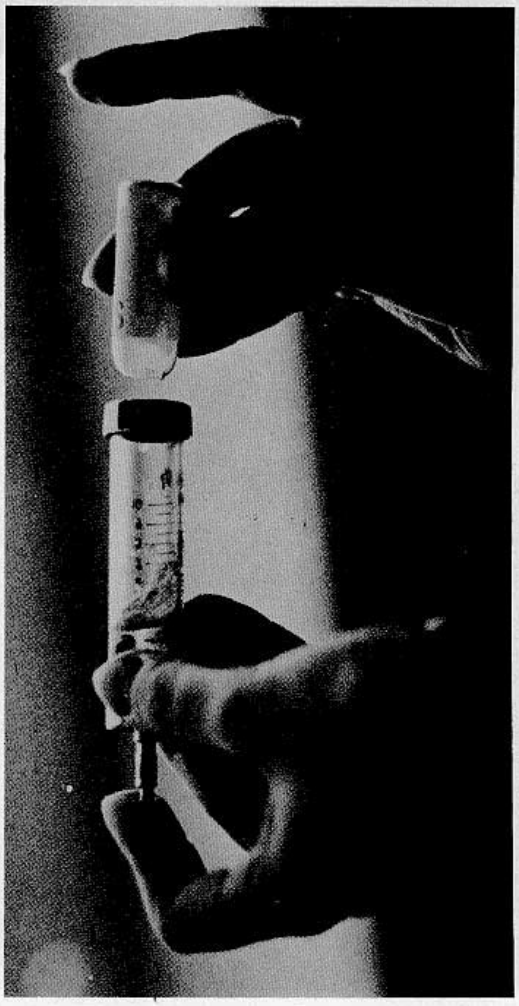


Fig. 211:
After loosening the contents of the ampoule by knocking on a hard bottom, the lyophilisate is strewn into the cylinder of the syringe from above.

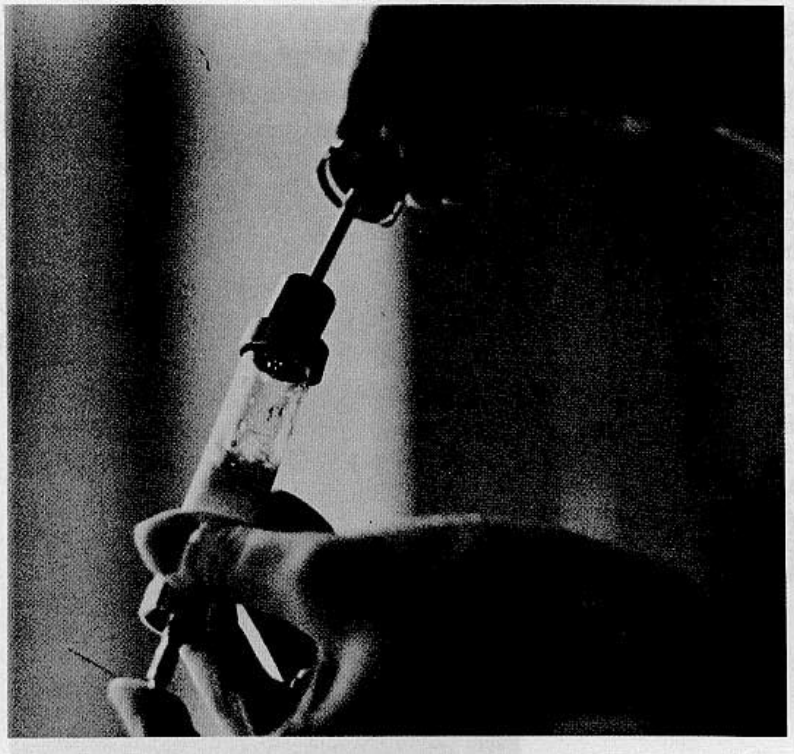


Fig. 212:
After emptying the complete contents of the lyophilisate ampoules into the cylinder, the piston is put on and screwed.

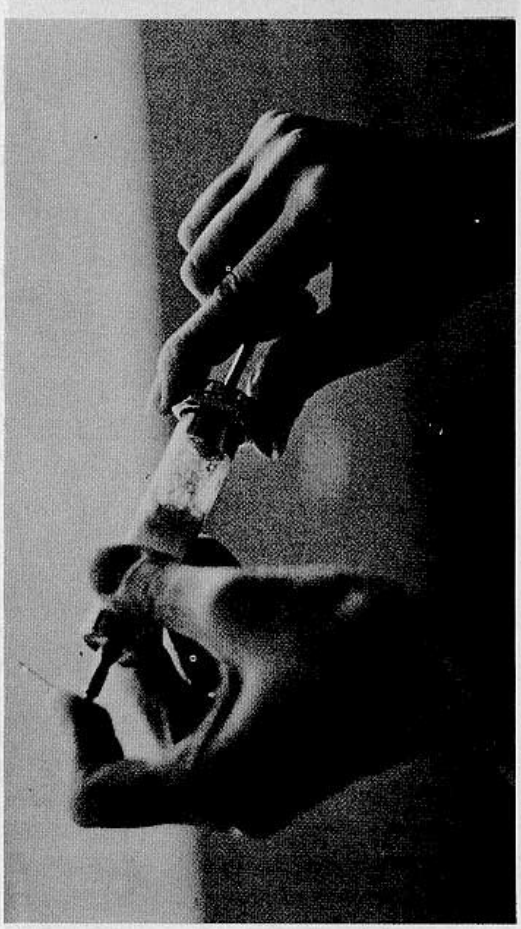


Fig. 213:
The use of sterile disposable syringes spares the screwing. In contrast to this advantage, there is a disadvantage: more tissue remains in the nozzle of the cannula.

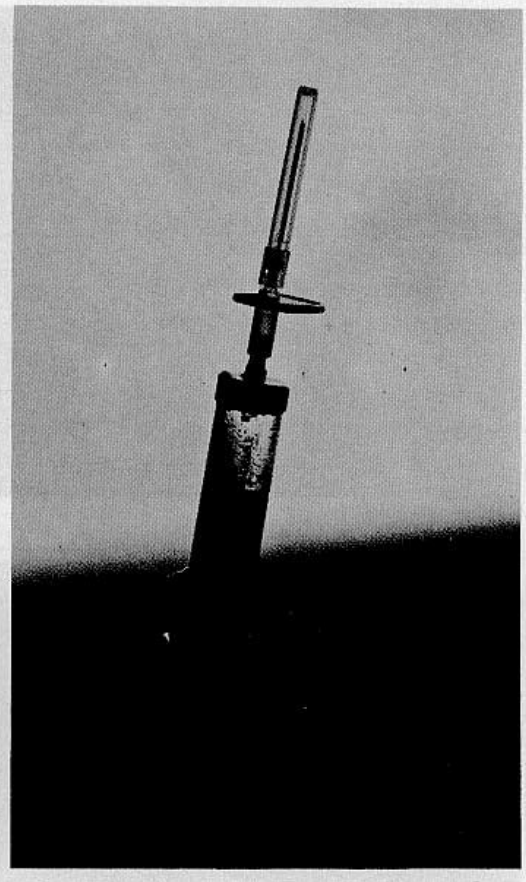


Fig. 214:
The lyophilisate and suspension liquid are mixed in the cylinder by shaking till the dry substance has disappeared completely. The contents of 2–3 ampoules of lyophilisate can be suspended in a cylinder at the proportion of 100 mg of lyophilisate: 4–5 ml of suspension liquid.

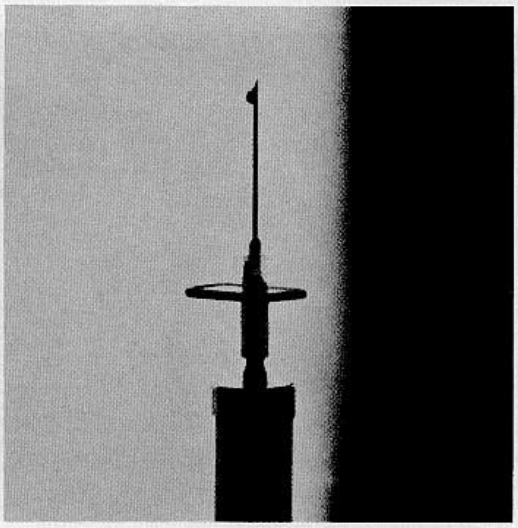


Fig. 215:
With the syringe held in a vertical position, the air needed for mixing is evacuated till the first drop of the suspension appears at the needle top. The filling of the needle with suspension avoids the closing of the orifice with a cylinder of skin when the needle is pricked. For most of the tissues, cannulae with a calibre of 1.0–1.2 will suffice, for placenta and connective tissue 1.4, for cartilage and bone-marrow a diameter of 1.8 mm is recommended. Not more than 10 min should pass between the resuspension and the injection or else the swelling would impede the injection and the preservation of the biochemical substance after the rehydration could no longer be controlled.

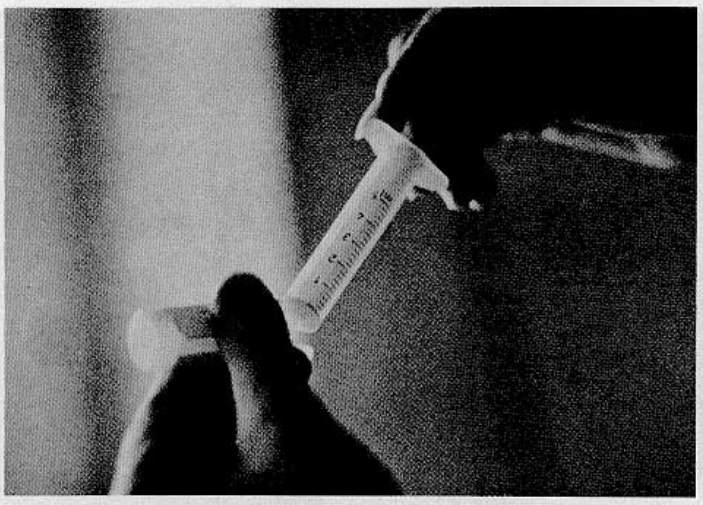


Fig. 216:
To empty the cannula and the puncture channel it is advisable to inject some more suspension liquid. This suspension liquid can be used for the complete cleaning of the lyophilisate ampoules.

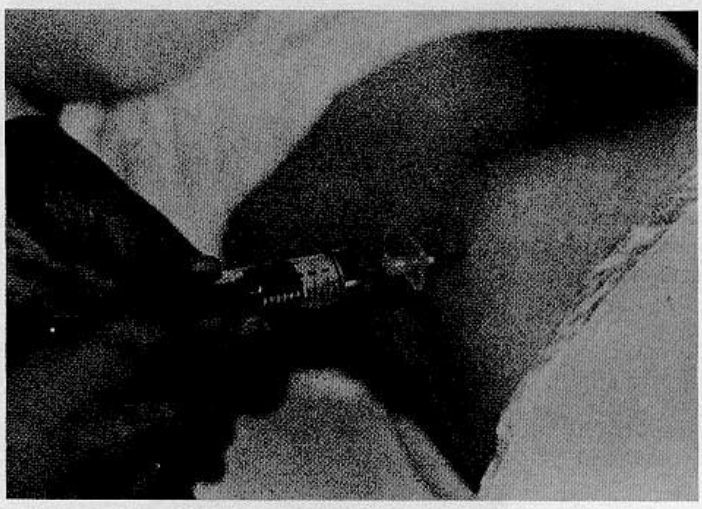


Fig. 217:
The injections (implantations by injection) are applied by deep subcutaneous (epifascial) punctures; there should not be any resistance, which would indicate an improper position of the needle-point. Lumbar regions (i.e. above and outside the gluteal region) and abdominal walls are the most suitable sites for the absorption.

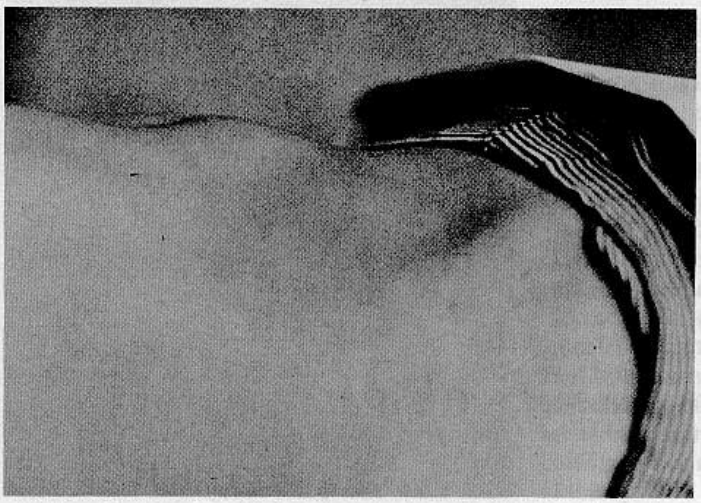


Fig. 218:
The injection lifts off skin and subcutaneous tissue, which causes tenderness on pressure and tension for about 2–3 min. The protuberance will disappear after a professionally applied epifascial injection within 10–15 min. Injuries to blood vessels and bleedings into the area of injection may evoke inflammatory processes (same as with injections of own blood). The puncture-channel should be covered for 2–3 days with an adhesive tape against infections.

Fig. 219–226:
Implantation-technic by using
One-way combination injection systems

Preparation of the syringe

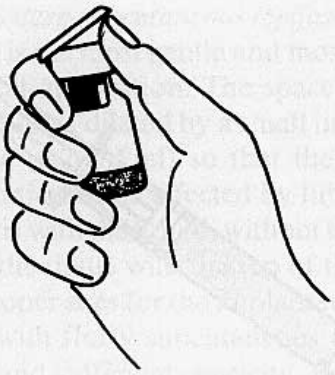


Fig. 219:
Ease open the protective cap on the pierceable vial.

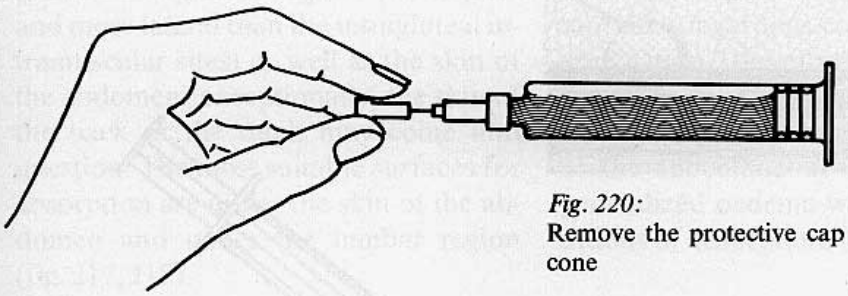


Fig. 220:
Remove the protective cap from the injection cone

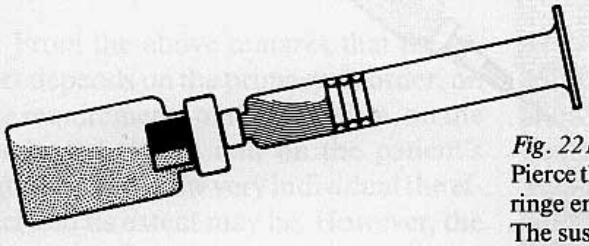


Fig. 221:
Pierce the rubber stopper until the cone of the syringe engages.
The suspension agent is sucked by vacuum into the pierceable vial.

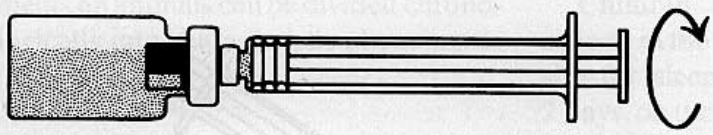


Fig. 222:
Screw in the piston rod

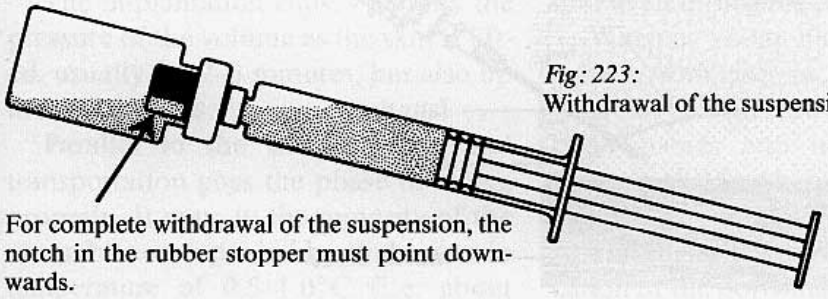


Fig: 223:
Withdrawal of the suspension

For complete withdrawal of the suspension, the notch in the rubber stopper must point downwards.

The implantation must be carried out immediately after withdrawal of the suspension by deep subcutaneous injection.

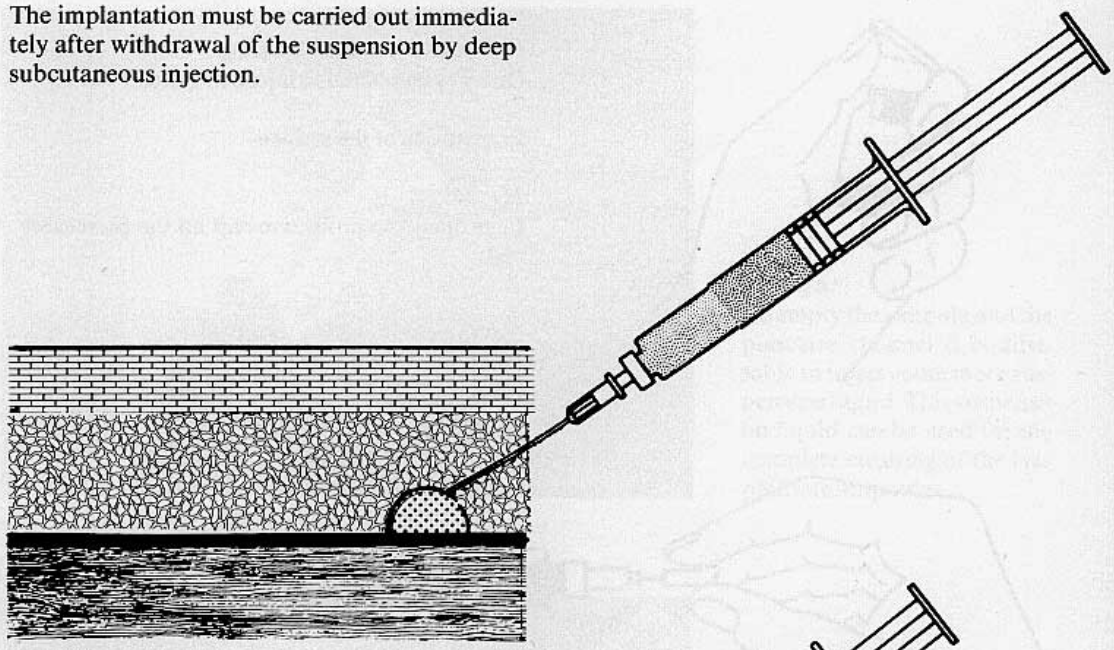


Fig. 224

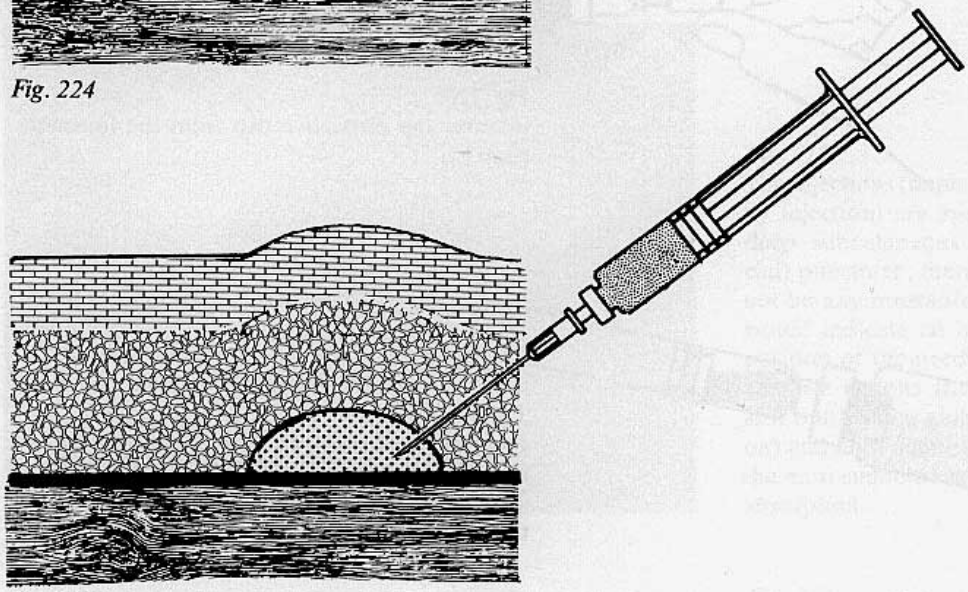
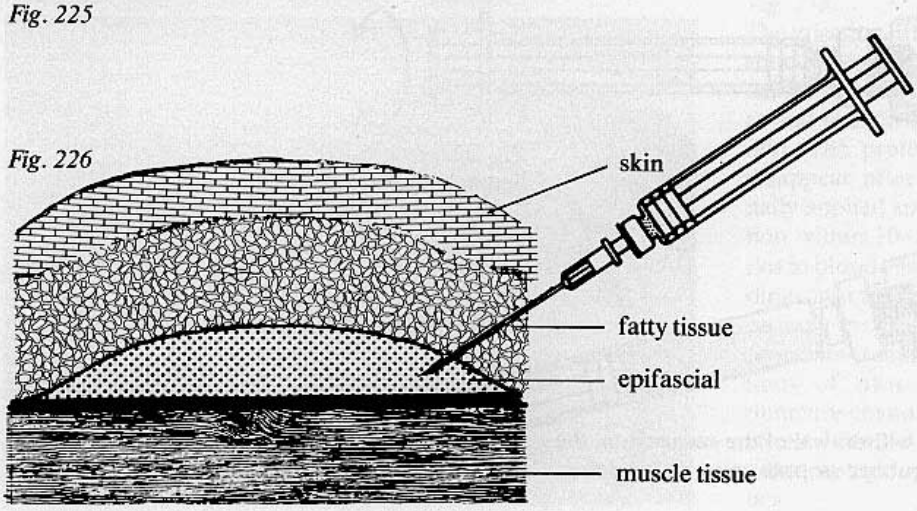


Fig. 225

Fig. 226



The *deep-subcutaneous (epifascial) injection* is the most gentle and most physiological application. The space of implantation is dilated by a small injection volume (0.2–0.5 ml) so that the subsequent injection is effected by lifting the subcutis with the liquid, without traumatizing the tissue with the top of the needle. Proper sites for the implantation are areas with fluffy subcutaneous cellular tissue and sufficient elasticity. Best suited are dorsal-lateral areas between the lumbar and gluteal regions (i.e. higher and more lateral than the intragluteal intramuscular sites) as well as the skin of the abdomen; exceptionally, the skin of the back or the thigh may come into question. The most suitable surfaces for absorption are under the skin of the abdomen and under the lumbar region (fig. 217, 218).

The *intramuscular injection* was preferred formerly; for intramuscular injections it must be considered that the comparatively large volume causes spreads and traumatization of the muscles and, possibly, bleedings. Restricted functions of muscles, pain and hematoma are remarkable consequences if more than 2 injections are administered. Rest in bed for several days may be necessary in such cases.

The *intraperitoneal injection* is optimal with respect to the absorption but too risky regarding complications; this application, therefore, should be reserved to special situations, which may occur in cases of cachexia with atrophy of the subcutaneous tissue, further in generalized oedema with doubtful conditions of absorption.

Concomitant phenomena

From the above remarks that the effect depends on the primary disorder, on the requirements of the organism, on the implanted tissues and on the patient's age, it follows how very individual the effect and its extent may be. However, the variety of clinical treatments, subtile casuistics in certain areas and of experiments on animals can be divided chronologically into characteristic phenomena.

The phase of stress

follows the implantation immediately.

The implantation causes pain by the pressure of the volume as the skin is lifted, usually for 2–3 minutes, but also up to 5–10 minutes.

Parallel to the disintegration and transportation goes the phase of stress, properly. It may, in the minority of the cases, be accompanied by slight rises in temperature of 0.5–1.0°C (i.e. about 37.8–38.5°C of body temperature) for some hours or a day. The blood-count shows a rise in leukocytes while the polynuclear multiply (fight of leukocytes). Adults describe a pleasant desire for rest, some a feeling as after a heavy meal, others speak of lassitude for several days.

Children may show two contrary aspects in the phase of stress, namely desire for sleep for several hours up to 2 days, on the one hand, and hypermotility to restlessness, on the other. Most of the children do not change their behaviour at all during the phase of stress.

Whereas young babies respond only seldom with rises in temperature, such rises can be observed more frequently in older babies and infants, practically never in children between 3–15 years of age.

The slight leukocytosis with multiplication of the polynuclears subsides after

a few days and results in a monocytic-eosinophilous «overcoming phase» though the qualitative changes are not very impressive.

Inconsistent though it may sound: the stronger the symptoms of the phase of stress the better the therapeutic outcome may be anticipated.

Phase of effect

The positive effect of a «cell therapy» appears usually from the 3rd to 4th week after the injection. This has been registered most convincingly by observations of thousands of parents of disabled children, who speak of *«developmental outburst»* or *«developmental leap»*, sometimes of a *«developmental explosion»*, which may receive expression in motor, linguistic and mental criteria or criteria of conduct. This phase lasts about 3 months, then subsides and falls below the threshold of what can be registered, after 5–6 months.

Besides this general *«time-table»*, there are impressive examples of earlier or later effects. From animal tests we know that according to the effective tissues (spleen, liver) the normalizing deviations of the blood-count are fully developed in leukemic mice already after 3 days. A mongoloid baby who owing to complex immaturity of the liver, sepsis and uncontrollable diarrhea was unable to gain in weight in spite of 3 months of parenteral feeding and moribund got peritoneal injections of 150 mg of liver, 150 mg of placenta and 100 mg of cerebrum, improved within an hour though his condition had lasted for months. After a second series of implantations, the improvement was definitive.

A boy of eight suffering from familial congenital nephrosis was carried to the clinic with a hydrops on a hard plank because his body looked like a jelly-fish. All conventional measures (prednisolon, infusions of protein) failed. Diuresis set in after peritoneal injection of 150 mg of kidney and 150 mg of placenta already on the following day and swept out the edemata completely.

A latency-period

of 1–2 weeks follows the phase of stress till the registrable onset of the action. It is the period in which, after disintegration and removal of the implanted material, the dispersion in the body and the incorporation in the homologous organs (structures) take place. Not until the functions of the organ (tissues) have improved at the site of incorporation, the effect can be registered biologically or clinically. In contrast to the above extremely short intervals pending the onset of the effect, latent periods of several months have been reported for older persons. STÜHLINGER (1979) collected 20 impressive examples showing the different onsets and durations of effect.

The repetition

of the implantations depends on the duration of the effect. As a rule, repetitions should not be initiated earlier than after a time-interval of 5 months. In favour of this minimal interval speak immunological considerations and reasons of effect. In adults and especially old persons, the duration of the therapeutic result is decisive for the indication and time of a repeated treatment. Generally, the intervals will come to 1–2 years.

An exception to this recommendation is the concomitant tumour therapy with a fetal mesenchyme; intervals of several weeks are usual and necessary here.

The frequent question how often a cell therapy should or must be repeated, can be answered very simply: as long as concrete success is obtained.

Lyophilised tissues in physiological solutions are well tolerated, there is no toxicity. As however a xenogenous tissue injected subcutaneously by comparatively large volumes (6–15 ml per injection) is in question, physical irritations at the site of injection are caused, on the one hand, and immunological concomitant symptoms may be caused by repeated injections, on the other hand. The long interval between the injections of implantations, from 5–6 months up to 2 years, is medically justified and provides moreover some safety from an anaphylactic reaction after sensitization.

The side-effects are astonishingly slight even under the forced therapeutic conditions of the frequent implantations in the tumour-immunotherapy. The following picture results from an extensive study by H. RENNER (1979):

71 patients got 324 injections of fetal mesenchyme (Resistocell®), several injections (2–22) were administered to 55 patients at intervals of 4–6 weeks. Even those 324 injections intended for the immunization against fetal antigens showed no threatening allergo-anaphylactic reactions; in one case, a patient developed urticaria but tolerated several equal injections without reaction later.

The evaluation by H. RENNER provided the following outcome:

Of those 324 immunizations, 254 were specified under the following parameters: fever, local pain and local reactions at the point of puncture such as erythema, swelling, overwarming. The reactions were rated by an arbitrary scale from 0–4:

0 = no reaction
1 = slight
2 = distinct
3 = intense
4 = very intense.

No reaction whatever was observed in 57.8% of the immunizations, the rest showed: fever in 6.7% (scale 1 and 2), local pain in 29.9% (among them only 3.2% scale 3) and local reactions in 19.3% (among them only 2.3% in scale 3). These side-effects were partly combined.

But once more it must be made plain: most of these reactions were slight to moderate (scale 1 and 2), only very few were distinct (scale 3). Excessive reactions were not observed (scale 4).

Especially it must be emphasized: all side-effects (100%) were entirely regredient within a short time; there were never any lasting ulcerations, fistulae or fever.

The several immunizations were applied to a group of women with metastasising mamma carcinoma as part of a combined treatment with cytostatic interval-polychemotherapy and local palliative radiation during the first chemotherapeutic courses. The immunization was performed at intervals of 4–6 weeks when the therapy had been interrupted. RENNER treated a woman who by now has got already 31 regular immunizations in the therapy-free intervals within about 2¾ years. Also these several immunizations at short intervals were just as well tolerated as single immunizations or such repeated at longer intervals.

N. WOLF (1966, 1969) obtained similar *time-accelerated results* when treating 65 patients suffering from brain-atrophic processes. The implantations were lyophilisates of placenta and fetal frontal brain, sometimes combined with hypothalamus and ovary; 2–6 treatments at intervals of 4 weeks constitute also an unusual, immunologically provocatory accumulation of implantations at short intervals. Even under these conditions

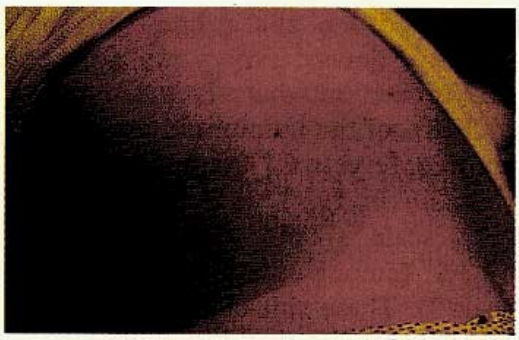


Fig. 227:
Repeated injections at the same site may cause a local reaction round the point of puncture in the form of reddening and swelling. – Intense local reaction in adipositas (striae). Girl of 16.

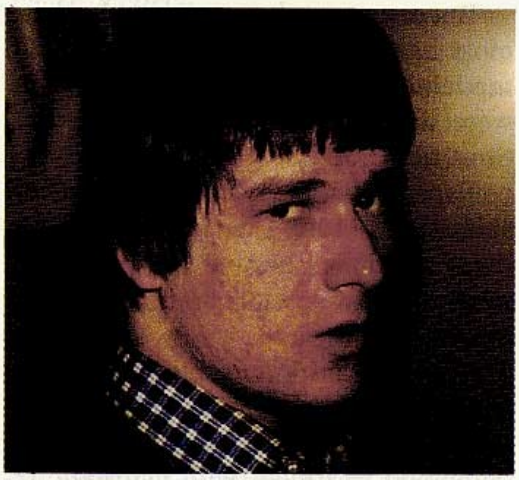


Fig. 228:
High IgE titres may evoke general reactions in the form of a rush or a Quincke edema. Catecholamins (adrenalin derivatives) applied orally or parenterally (specially suprarenin) work faster and more causally than cortison derivatives.

«no complications or remarkable side-effects whatever occurred» .

From his own experience based on 74,000 implantations of lyophilised tissues of various organs applied in the course of 25 years, the author can derive the following conclusions: if intervals between implantations of 5–6 months and the precautionary measures described are observed, the percentage of side-effects and complications is much inferior to the rate anticipated for xenogenous tissue.

In particular, the following circumstances must be taken into account:

1. *Local reactions (erythema, swellings)* on the first 2–3 days after the injection; they depend on the injection technique, injected quantity and number of the preceding implantations applied at the same site. Such local reactions must be expected in about 5–10% of the cases if injections are repeated.
2. Irrespective of this, extended non-allergic erythema is often seen immediately after injections of *placental tissue;* in question is a circulatory reaction, which subsides untreated or with only physical measures (cooling, gel).
3. One *abscess* caused by the injection must be taken into account for every 5,000 implantations, according to all serial observations made so far. It should be realized that the cleanliness at the site of injection cannot be assured especially with disabled and young children. The cases observed so far are without exception pyogenous infections from inside the body or infections of the puncture channel.

4. *Hypersensitivity reactions* of the immediate type must be expected for repeated injections – more than three preceeding injections – in 8‰, especially if no freedom from infection is guaranteed. Immediate reactions usually occur through IgE via the complement system and do not constitute a hypersensitivity for the xenogenous tissues.
5. *Infections* of animal epidemics frequently feared specially in the beginning years have so far not been observed. Substantiated reports are available neither for the original fresh-cell method nor for the dry-cell doses.

The general risks and the problems of the complications are treated in papers written mostly in the fresh-cell era. Most of them analyse the matter generally and theoretically. Here is a survey of the more important authors:

Bösch 1958;
Boms 1956;
Camerer 1961;
Castens 1957;
Dahmen 1953;
Döderlein G. Fanconi G. and Nonnenbruch W. 1955;
Hupfeld 1980;
Griffel A. 1957
Iversen 1956, 1959;
Jores 1955;
Jussek 1970;
Schulten 1957;
Vorländer 1958;
Kanzow 1960;
Klein 1967;
Knüchel 1956;
Pischinger 1955;
Rappold 1959;
Rietschel 1955, 1956;
Rümelin 1969;
Rupp 1955;
Schmid F. 1955, 1978, 1980;
Sprado 1955, 1958;
Schütze 1956.

Much more useful than the above figures is the statement that in only 40 of more than 74,000 implantations, suprarenin or cortison had to be applied parenterally (subcutaneously) owing to the dimensions of the urticarial reaction, which is a rate of less than 1‰. Even these situations could have been avoided for the most part if infections of the respiratory tract and pyogenous focuses (otitis, mastoiditis, suppurative sinusitis) had been respected as strict contraindications and no compromises e. g. for excessive travelling distances and economy of time had been agreed to.

Of all postulated incompatibilities and complications for which a temporal coincidence seemed to indicate a causality with cell therapy, the casuistically reported cases of *encephalomyelitis* and *polyradiculitis* deserve special notice. Most of the published cases (Gsell 1975; Hupfeld and Wenzel 1980; Bennhold 1954; Seitelberger et al. 1957; Brauch 1956) are so-called «fresh-cell treatments», for which the quantity and kind of the used material were not known and there was no safety from the conveyance of microbes. As the declarations of the materials used and of their doses were insufficient, the conditions of these complications extremely rare in proportion to the number of the treatments have not been found out.

The fact that, in contrast thereto, more than 40 series of implantations of lyophilised cerebral tissues were applied to mentally ill children in the course of development without a case of neuroallergic reactions becoming known so far, shows that the possible causalities depend on special conditions.

If any substantial quantities of cells are implanted, it is necessary to avoid any excessive physical overexertion, exposure to intensive sunlight and X-ray radiation, further the use of cytostatic

drugs within the first 10 days following the implantation.

Cell therapy is a highly specific method, which must be learned. Indications, contraindications, methods of application and, above all, the use of standardized, bacteriologically controlled products declared as to quantity and contents, are prerequisites for an adequately riskless and promising therapy.

Forms of the cell therapy

The publicity for various forms of cell therapy has helped to confuse the public. The «fresh-cell therapy» (tissues of freshly slaughtered animals are dissolved and injected) was wrongly believed to use fresh cells, living to continue life in the organism of the recipient. Decisive for the effect, however, is not the «freshness» but the composition and quantity of the biochemical contents (substrates and enzymes). The faster the tissue is implanted and preserved, the more active ingredients remain intact. Lyophilisation (freeze drying) is the method of choice because all chemical disintegrating processes of the donor's tissue are stopped at once when the water is withdrawn. This is the safest method of guaranteeing the native form of the biochemical outfit of fetal tissues.

A therapy largely applied by the doctors must assure that

a) the quantity of the used «pharmacon» can be declared,

b) the contents are generally known,

c) freedom from microorganisms can be anticipated.

The way of meeting these requirements is not equal for the 3 variations usual nowadays (tab.). Applied by well versed experts and if a team collaborates smoothly, the «fresh-cell method» guarantees a great deal of efficiency and safety provided that not more than 30 minutes elapse between the taking of the donor's tissue and the implantation. Roughly, the lyophilisates offer the highest degree of safety and effect. The entire elementary research has been done with lyophilisates because only these provide the prerequisites for experimental work.

Safety conditions	«Fresh-cell method»	Lyophilisates (freeze drying)	Freeze-cell method
Quantity	not determinable, roughly estimable for experienced	declared by mg of liquid-free tissues	roughly determinable
Biochemical composition	undefined	about 20 cellular substances cytochemically demonstrated Content on minerals and trace elements is analysized	undefined
Sterility	sought to obtain by asepsis, cannot be guaranteed	released for use only after bacterial control	freezing and thawing reduce bacteriological safety usually controlled

Instructions for the production of sterile cell-therapeutic preparations and for the health control of the donor animals

The German Federal Health Gazette 13, pages 116–117 (1970) contains the instructions for the production of cell-therapeutic preparations. These instructions comprise 4 chapters:

I the importance of using healthy donor animals;
II provenance of the donor animals;
III slaughtering hygiene and meat inspection;
IV taking of organs and further treatment.

Here is the wording of the instructions for a safe production and application.

Conditions of production
I The importance of using healthy donor animals

a) Necessity of additional examinations and measures

The cellular therapy in the meaning of these directions is a technique by which material of animal tissue or native animal fluids are incorporated into the body of a patient. Under § 1 paragr. 1, in connection with § 2 No. 3 of the Drugs Act of May 16th, 1961 (Fed. Gaz. I p. 533) – version now valid – such substances become medicaments. Their circulation, however, is prohibited under § 6 No. 1 of said Act if their use as prescribed implies a risk of harmful effects exceeding a degree acceptable in the understanding of medical science. The harmful effects include the transmission of the morbific agents of zooanthroponosis, to which the fresh-cell therapy may give rise. To avoid such a risk, certain precautionary measures must be observed when the donor animals are selected and the preparations are extracted. Some of these measures are already part of the provisions on vaccines and sera (ordinance of the Pr. min. of public welfare and agriculture of July 15th, 1929 (LMBL. p. 447) – still valid). As a matter of principle, only animals healthy in every respect can be used. It is the veterinarian's concern to select and to examine them. Irrespective of this, the doctor is responsible for the application and its consequences.

Slaughter cattle is tested alive and after killing for pathologico-anatomical processes under the Act on meat inspection (FLBG). However, the findings relying usually just on a rough examination allow only to decide on the edibility of meat. These findings therefore do not indicate that certain tissues are suited to be used for the cell therapy. A painstaking bacteriological examination is carried out only in suspicious cases and if animals are ill; they do not come into question to serve for cell donors anyway. The examination under the Act of meat inspection, consequently, is not sufficient to detect all diseases transmissible to man by parenteral application because many infectious diseases and stages of diseases proceed without clinical symptoms or pathological changes. If in cell therapy cells are transmitted immediately from the animal to the patient, the time available between the taking of cells and the application is too short for relevant additional examinations.

b) Zoonosis

As chiefly horned cattle, sheep and pig, and the rabbit of the small animals, come into question to serve for donors, especially the diseases of these animals that can be transmitted to man are of interest, namely:

blue-tongue disease (sheep)	brucellosis
enzootic sheep abortion	gas edema
influenza	leptospirosis
looping ill	listeriosis anthrax
lyssa (rabies)	pneumococcosis
foot-and-mouth disease (aphthous fever)	erysipelas
animal small pox	salmonellosis
Rift-Valley fever (sheep)	tuberculosis, psittacosis, piroplasmosis, rickettsiosis, toxoplasmosis
Wesselbron fever (sheep)	tularaemia (e. g. Q-fever)
ornithosis	vibriosis

II Descent of the donor animals

a) Selection

To judge of whether an animal is suited to be used as a donor of cells, a time of observation as long as possible is necessary in order to submit it to thorough repeated clinical and additional tests in the laboratory. Best suitable are SPF animals, in special cases gnotobiotic, at least animals kept for the particular purpose of extracting cells and supervised by a veterinarian. If, exceptionally, this requirement cannot be met, the animals used must have lasting distinctive marks, be of a known descent and have been examined thoroughly by veterinarians at regular intervals.

Obligatory are veterinary certificates of health, from which it appears that repeated examinations of the stock of animals in question have not revealed any symptoms indicating an epidemic (e. g. foot-and-mouth disease, erysipelas, myxomatosis), especially one of the diseases mentioned under Ib), and that an animal shows no clinical signs of a disease. These certificates of health should state moreover that the animals have not been submitted to a therapy likely to exert an unfavourable influence on the results of the examinations (e. g. certain inoculations with destroyed or living cultures – possibility of eliminating morbific agents or influence on the results of serological examinations – or treatments with sulphonamides and antibiotics – possible influence on the results of bacteriological examinations -). Sterility, abnormalities of the cycle, miscarriages or abortions and mastitis must not be there. If possible, artificial insemination ought to be used in the original stock of animals; a natural mating can be accepted if the father animal is submitted to the same supervision as the donors. If there was an epidemic in the immediate environment of the original stock and the donors are free from the epidemic though susceptible to infection, the animals of this stock cannot be used unless passing first a quarantine, the duration of which must be fixed by an official veterinarian according to the kind of the epidemic.

The veterinarian and official veterinarian should collaborate closely when a proper stock of animals is selected.

b) Species of animals

Donors of cells are chiefly horned cattle, calf, pig and sheep. The sheep seems to be preferred as it is easy to keep and supervise. Further, small laboratory animals as guinea-pigs and rabbits are used.

c) Quarantine

If animals are to be included in a supervised stock, they must be put under quarantine. This is indispensable unless the animal comes from a known stock looked after by a veterinarian, which must be certified by a veterinarian. The quarantine should last 3 weeks at the least. The stall must be built in such a way as to keep the animals strictly separated from other animals. It must be easy to clean and disinfect and allow clinical inspection of the animals any time. Contacts with wild animals or rodents must be avoided and stinging insects, ticks and flies be kept away.

d) Examinations

The animals selected for donors of cells must be subjected to repeated clinical, bacteriologico-virological, serological and allergological examinations. If parasitical diseases are suspected, supplementary tests (faeces, skin) must be made. In case, the animal experiment must be included. These tests ought to be made also during the quarantine or time of observation.

III Slaughtering hygiene and meat inspection

a) Conditions expected from the slaughter rooms

The room used for slaughtering cell donors ought to be reserved exclusively for this purpose. All necessary structural requirements for hygienic equipment must be met. If such a room is not available, slaughtering must be done in a slaughter-house, with strict separation from the normal slaughtering. The place where the slaughtering is done and its equipment must meet the minimum requirements for slaughtering under the provisions for meat inspection.

b) Qualities expected from the animals

Animals qualified as malnourished by the veterinarian or very excited before slaughtering, unfed for some time or not sufficiently reposed, do not come into question to be used as cell donors. The animals must be cleaned thoroughly before slaughtering. The hygiene of cutting up is very important. Any contaminations of the meat and organs must be avoided (minimum requirements for slaughter-houses under the provisions of meat inspection). Pigs should be brothed preferably in a hanging position. It is advisable to slaughter only one donor animal at a time. Slaughtering several animals simultaneously may impair the hygienic conditions.

c) Examinations under the meat inspection Act

Whilst the meat inspection Act allows, in certain cases, persons other than veterinarians to examine meat, only a veterinarian is authorized to do so if the extraction of cells comes into question. As a rule, it is advisable to release the cell material only after the examination according to § 21 and the foll. of the regulatory statutes A of the meat inspection Act i. e. as soon as the inspection has proved the perfect quality of the meat for human consumption. The parts of the body (genitals, fetuses, etc.) declared unsuit-

able for human consumption (§ 35 sentence 1 of the regulatory statutes A of the meat inspection Act) can be used for the extraction of cells if the meat and the part of the body in question have been found to be in order and a permission under § 7 of the meat inspection Act has been issued. Animals not subject to the provisions of meat inspection (e.g. rabbits) must be examined in analogy to these instructions. This means that a veterinary examination is obligatory also if an animal is killed only for the production of cells and not used for human consumption.

d) Additional examinations

When tissues are produced for the so-called fresh-cell method (immediate transmission from the donor to the patient), additional laboratory tests on the extracted cell material will usually not be feasible as time is short. The health control ought to be concentrated above all on the living animal. If however parts of tissue are taken for the production of quick-frozen and lyophilised cellular preparations, these additional examinations must be made. They help to reduce still more the risk of infection.

IV The taking of organs and further treatment

a) Qualifications expected from the personnel

The personnel occupied with the extraction and production must, respecting the state of health, come up to the requirements for persons working in the food trade. The provisions of the Federal Epidemic Act apply. The persons entrusted with the taking of tissue must be thoroughly familiar with the special technique of dissection for the various species of animals and, above all, with anatomy.

b) The taking of the organs

The tissue must be taken under aseptic conditions. The unintentional opening of non-sterile organs (oesophagus, trachea, stomach, intestine, etc.) must be avoided.

c) Additional laboratory examinations

For reasons of time, bacteriological examinations can come into question only for tissues that are to be quick-frozen or lyophilised before use (see IIId). If only fetal tissue is taken, these examinations may be restricted to the foeti. Recommended is a bacteriological test more thorough than that provided by annex 1 to § 20 par. 3 of the regulatory statutes A of the meat inspection Act. Quick-frozen and lyophilised tissues require moreover sufficient bacteriological after-controls of the preparations ready for injection.

d) Further treatment up to the processing or injection

The cell material must be put into sterile containers immediately after taking. Fresh cell material intended for the application must be transported and preserved at low temperatures (+3° to +6°C). When cells are quick-frozen, a temperature of about −70°C is necessary. The material for quick-frozen and lyophilised preparations must also be treated in such a way as to avoid an unfavourable influence during the subsequent processing. Gradual bacteriological tests should be made during the various steps of production. The methods of production must guarantee the manufacture of sterile injection preparations. The compatibility ought to be tested on small experimental animals before the injection.