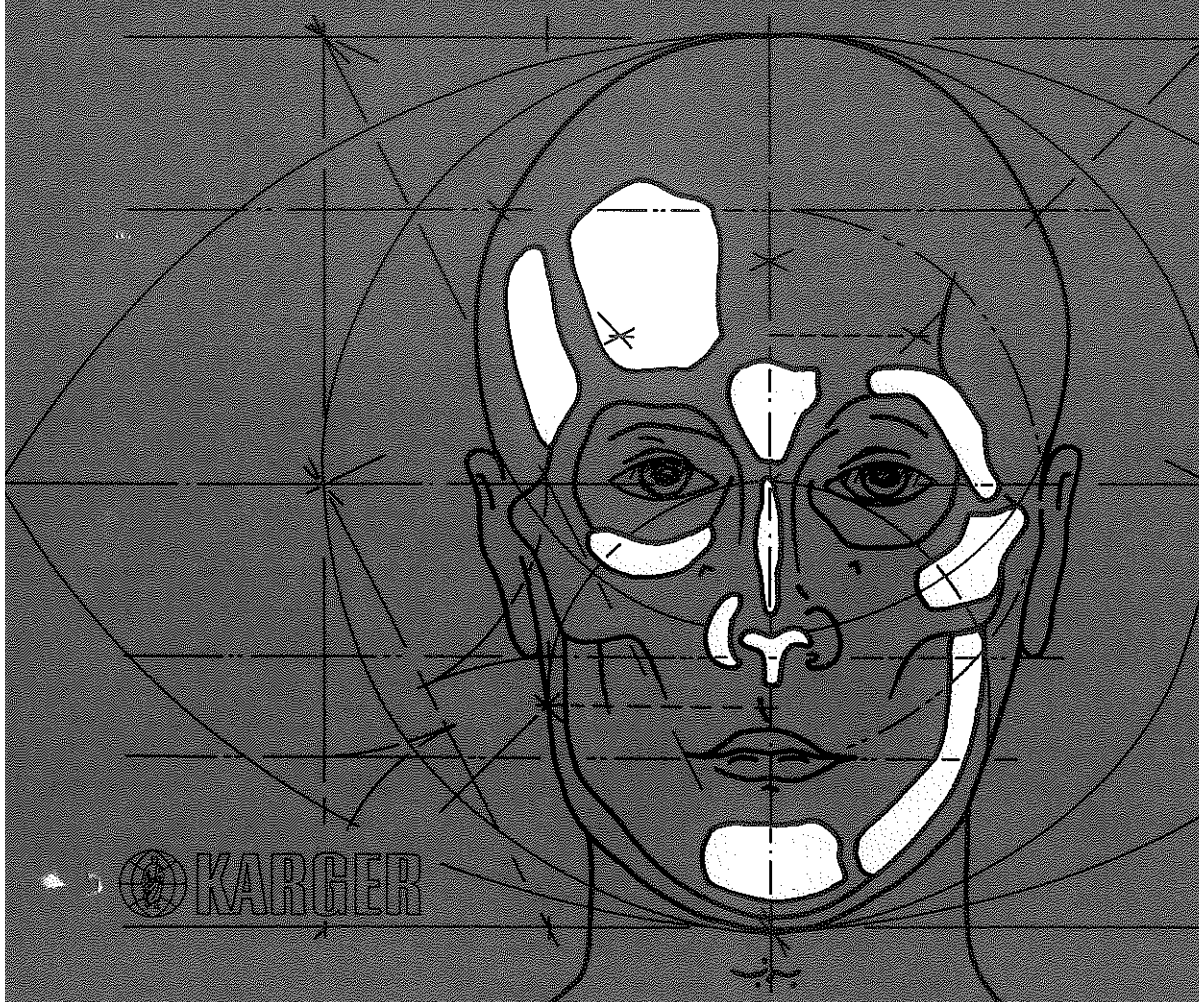
Hermann F. Sailer

Transplantation of Lyophilized Cartilage in Maxillo-Facial Surgery

Experimental Foundations and Clinical Success



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84 figures and 24 tables, 1983

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2. Basic Comments on Lyophilization

Definition and Standardization of Terminology in German, English and French

Lyophilization or freeze-drying is a process in which water is removed from the frozen biological material by sublimation under vacuum, i.e. the liquid state is omitted, to some extent, and the ice in the material is transformed directly into vapour. The term 'lyophilic' (Greek: having an affinity for solution) is attributed by *Flosdorf and Mudd* [1935] to *Reichel,* who applied the term to freeze-dried serum on account of its ready solubility after the addition of distilled water. 'Gefriertrocknung' and 'Lyophilisation' are used in German today practically without differentiation.

In American and British English the method is described as 'freeze-drying' or 'drying by sublimation' or 'lyophilization'. In the French literature on the subject [*Thomas,* 1963], the method is referred to as 'dessiccation en vide' or 'congélation-dessiccation' or 'lyophilisation'. The name of *Altmann* [1890], who was probably the first to use a true freeze-drying process in the field of medicine (fixing of organ fragments for histological purposes), is also used to describe the method. References to the Altmann-Gersh method or simply to the work of *Gersh* [1932] are also found occasionally in the Anglo-American literature.

The German medical literature on the subject reveals that *Neumann* [1952, 1954, 1955a–c, 1956a, b] and *Zurbuchen* et al. [1959] in particular did valuable work to propagate and improve the method and to widen its scope of application. Since, apart from minor authographic differences, the expression 'lyophilization' is current and understood in all the languages of western and eastern Europe, we would plead for the universal use of this standard term.

Practical Principles of Lyophilization

The lyophilization process begins with the freezing of the material. Ideally the drying process should follow immediately, but may be carried out later, the material having been stored in the meantime at the prescribed temperature. After having been dried, the material should be vacuum-packed and stored. Before use, the dried substrate is rehydrated by adding water.

Freezing

The same conditions apply for the freezing of material to be lyophilized as for preservation by deep-freezing (see below).

It is difficult to say categorically whether fast freezing (e.g. shock-freezing at −183 °C by immersion in liquid nitrogen) or slow freezing (e.g. 1 °C/min) is preferable; the choice will depend on the tissue being treated and the purpose for which it is intended. It

seems certain, however, that for histochemical or histological investigations fast freezing is essential [*Neumann,* 1961]. In the case of slow deep-freezing it may be assumed that, as the ice crystals form, they extract water from the environment and consequently bring about an increase in the concentration of all of the substances dissolved in the tissue fluid [*Chambers and Hale,* 1932]. This also results at the same time in a reduction of the freezing-point. If the selected temperature is low enough, i.e. not below the eutectic point of each of the salt components in the tissue, it is possible for a frozen and a liquid phase to exist simultaneously, but macroscopically the tissue appears to be frozen.

Nevertheless, it seems that for certain applications good results can be achieved with slow freezing [*Neumann,* 1955a–c]. This applies particularly to the freeze-drying of particles of skin [*Billingham and Medawar,* 1951], where de-antigenization is of special importance. In contrast, *Luyet and Gehenio* [1940] and *Luyet* [1949] regard the fast freezing of cells as the preferred method. *Luyet and Gonzales* [1950], using fast freezing and defreezing techniques, were even able to observe cardiac contractions in chicken embryos. Less spectacular results obtained with fast freezing have also been described by other authors [*Campbell,* 1950; *Blumenthal and Walsh,* 1950]. *Meryman and Kafig* [1955] also found that erythrocytes subjected to fast freezing with liquid nitrogen showed a high survival rate of 80%. However, if protective solutions are added, slow freezing also seems to yield outstanding results with respect to the maintenance of vitality.

The most commonly used protective solutions are probably glycerine [*Lovelock,* 1953; *Smith,* 1954; *Polge and Soltys,* 1960] and various kinds of sugar. According to *Meryman* [1960a, b] slow freezing at a rate of 1 °C/min is the most favourable method for maintaining cell vitality. After rehydration the protective solution must be eliminated.

In 1936 *Hoerr* observed that sections of tissue of larger dimensions placed in isopentane at −190 °C were frozen only to a depth of 5 mm after 15 s. Similar observations were made by *Simpson* [1941] and *Yokoyama and Stowell* [1951], who attributed the loosening of the tissue to the slow dissipation of heat from its interior. If a section of tissue is not to be destroyed by freezing, its thickness should not exceed 1 mm.

In the transplantation of homeostatic allogeneic and xenogeneic tissue the preservation of vitality is normally not required [*Neumann,* 1955a; *Friedlaender,* 1976]. Moreover, it is not yet technically feasible [*Neumann,* 1961] to use deep-freezing to preserve the vitality of the large portions of homeostatic tissue necessary for many reconstructive operations, nor is it desired, as far as foreign tissue is concerned, in view of the risk of rejection.

Consequently, most preservation processes are designed to either reduce or eliminate the antigenicity of the material in question entirely. This has been shown to be the case for the deep-freezing and the lyophilization of a number of tissues, but not for lyophilized cartilage. We shall revert to this matter in chapter 6.

Drying

For the drying process the following physical properties are of importance:

(1) The property of water below the triple-point, i.e. at a temperature of less than 0 °C and a pressure of less than 4.6 Torr, to pass from the solid to the gaseous condition without having to go through the liquid

phase. This property is known as sublimation. As it is a question here of an endothermic process, heat has to be added, but only to the extent that the frozen water in the tissue does not melt.

(2) Water vapour will continue to issue from the frozen water and the frozen tissue until the partial pressure of the water vapour molecules is so high that no further sublimation of molecules can occur, i.e. a state of equilibrium between the water molecules in the frozen block and its environment has been established. If the formation of this state of equilibrium is prevented by bonding the water vapour chemically or physically, by allowing it to condense or by removing it with a vacuum extraction pump, in order to create a pressure gradient, the ice contained in a piece of frozen tissue is gradually removed. This process is described as primary drying. This phase normally takes place at lower temperatures (approximately −40 °C and below) to eliminate any possibility of melting. The residual moisture still present in the tissue amounts to 2–4% of the dry weight [*Neumann,* 1961]. This residual moisture is bonded to the inner surfaces of the tissue by adsorption [*Neumann,* 1961]. Because of the stronger adsorptive bonding of the residual moisture, the second drying phase, i.e. the reduction of the residual moisture to 1% and below, can take several times as long as primary drying. The removal of this residual moisture, also referred to as secondary drying, is generally carried out at temperatures above freezing-point, as there is no longer any risk of the dry material melting.

Storage

According to *Neumann* [1961], a higher moisture content in the dried material can have an unfavourable effect on chemical reactions and enzymatic processes; bacterial growth occurs only if the moisture content exceeds 20%.

It does seem very important, however, as far as the duration of storage is concerned, to store the dried material in vacuum containers, as described for example by *Naylor and Smith* [1946] for bacterial cultures. The simplest method is to store in a sealed polyethylene bag, in metal or glass containers fitted with a sealing cock, or in glass ampoules sealed with a welding apparatus.

It is also possible to fill the storage containers with an inert gas such as nitrogen. If oxygen is present, oxidation of certain components of the material, e.g. fatty acids or acids containing sulphur, may occur [*Neumann,* 1961].

A major advantage of freeze-drying is that the material is not temperature-sensitive, an important consideration from the point of view of transportation. It can be stored for a practically unlimited period at room temperature and even in tropical conditions [*Klen and Heger,* 1970; *Neumann,* 1961]. Even highly sensitive viruses are known to have retained their infectiousness for many years after freeze-drying and storage at room temperature [*Munce and Reichel,* 1943].

If lyophilized devitalized material is to be used immediately after the drying process, i.e. rehydrated without delay, an extremely low level of residual moisture is unnecessary in our view. However, for long-term preservation during several years the residual moisture must be as low as possible and ideally about 1% of dry weight.

Rehydration

During this process the dried material is placed in distilled water; Ringer's solution or solutions of common salt or glucose can also

be used. If the material is to be used for transplantation, it should preferably be rehydrated in a solution containing an antibiotic, e.g. penicillin and streptomycin. We use 1 million units of penicillin G and 2 g streptomycin per litre. Depending on the nature of the material, it will return to its original condition within a short time, i.e. between several minutes and a few hours.

Lyophilization in Medicine and Research

Hammer [1911] reported on the preservation of bacteria by lyophilization and *Swift* [1921] developed a freeze-drying method for the preservation of bacterial strains which subsequently demonstrated vital functions with characteristic properties after rehydration. Extensive investigations in this field were carried out somewhat later by *Elser* et al. [1935]. In their work on the survival rate of micro-organisms after lyophilization, *Proom and Hemmons* [1949] investigated more than 1,500 strains of bacteria and provided detailed data for the improvement of the vitality rate.

In 1933, convalescent and normal serum and plasma were lyophilized and used for clinical purposes for the first time by *Flosdorf* [1949]. These were the first products of the human body to be preserved by lyophilization. Thanks to this development and to the research effort of the pharmaceutical manufacturers Sharp and Dohme, freeze-dried human blood plasma was available to the allied forces from 1941 onwards during World War II [*Flosdorf,* 1949]. The use of lyophilized blood plasma and the literature on the subject expanded rapidly during the next few years. *Flosdorf* [1949] and *Neumann* [1955b] cite numerous references in this connection.

The lyophilization of solid tissue is a more recent development, starting in the 1950s. In this field, in the USA the tissue bank of the National Naval Medical Centre in Bethesda, Maryland, has become pre-eminent.

The first lyophilized material from this tissue bank to be used clinically was bone processed by a method developed by *Flosdorf and Hyatt* [1952]. *Kreuz* et al. [1951, 1952] used it chiefly in orthopaedic surgery of the extremities. Further reports of favourable results with lyophilized bone in orthopaedic surgery followed [*Carr and Hyatt,* 1955; *Gresham,* 1964a, b; *Brown and Townsend,* 1970]. At the same time the results of numerous experimental studies with animals were being published. These results were almost unanimous in their favourable appraisal of the tested lyophilized material [*Gresham and Thomas,* 1962; *Cooksey,* 1954; *Turner* et al., 1955; *Heiple* et al., 1963; *Lane* et al., 1972].

An important step forward for reconstructive surgery in general and for the maxillofacial region in particular appears to be the use of lyophilized mandibular allotransplants as replacements in defects of the mandible, a method described by *Plotnikov* [1966]. The outstanding clinical results obtained by *Plotnikov* [1966] were confirmed by *Sailer* [1978, 1980a, b] in work under experimental conditions on monkeys. The simultaneous use of autogeneic spongiosa in combination with lyophilized bone transplants was also mentioned by *Plotnikov* [1966]. On the basis of animal experiments conducted by *Burwell* [1966], *De Fries* et al. [1971] reconstructed a large mandibular defect by means of an allogeneic mandible segment transplant, the bone marrow of which was replaced by autogeneic spongiosa. Using the De Fries method, *Cantrell* [1974] reported that grafts had taken

fully in 6 out of 11 patients. In patients having previously received radiation therapy, the treatment was unsuccessful. Lyophilized spongiosa is also frequently used for filling up cystic defects in the jaw [*Marble,* 1968] and in the bones of the extremities [*Mlynek,* 1969; *Spence* et al., 1969; *Wilber and Hyatt,* 1960]. Relatively unfavourable results obtained when using lyophilized bone for the bridging of defects after anterior movement of the middle face are reported by *Epker* et al. [1976]; in 7 out of 18 patients the transplants had to be removed because of infection.

In the field of periodontal surgery lyophilized bone has been used [*Mellonig* et al., 1976; *Sepe* et al., 1978], and *Yukna and Sullivan* [1978] conducted experimental tests on monkeys to establish the possibilities of using lyophilized skin and mucous membrane. According to *Hyatt* [1960], lyophilized allogeneic split skin tissue shows initial adherence of 80% on body surfaces suffering from burns, but is rejected on average after 21 days. According to *Snyderman* [1957], lyophilized fascia appears to give good results in reconstructive thoracic surgery after thoracoplasty. Lyophilized arteries have also been used experimentally [*Marrangoni and Cecchini,* 1951; *Pate* et al., 1952; *Pate and Sawyer,* 1953] and clinically [*Brown* et al., 1953; *Crawford* et al., 1956; *Creech* et al., 1956] with very good results.

Thanks to its outstanding compatibility, lyophilized dura has been used world-wide in almost all forms of surgery, e.g. neurosurgery [*Jacoby and Marguth,* 1968; *Bettag and Kersting,* 1969], thoracic surgery [*Hofmann,* 1969; *Specht,* 1969], maxillo-facial surgery [*Luhr,* 1969; *Schilli,* 1969], ophthalmic surgery [*Niedermeier,* 1969] and in otorhinolaryngology [*Pfaltz,* 1969].

Allogeneic lyophilized material has also been used in the surgical reconstruction of peripheral nerves. *Weiss* [1943] and *Weiss and Taylor* [1943] carried out the first successful animal experiments in this field, transplanting short nerve segments measuring 1–2 cm. Good clinical experience with lyophilized nerve transplants sheathed with lyo-dura was reported by *Jacoby* et al. [1970, 1972]. *Sailer* [in preparation] achieved adequate functional results with lyophilized allogeneic nerve transplants up to 6 cm in length (without lyo-dura sheating) in the reconstruction of the facial nerve in monkeys and in the replacement of the mandibular nerve in humans. Slower neurotization in comparison with autogeneic transplants was observed by *Samii* et al. [1971, 1972]. A more critical view is taken by *Meier and Sollmann* [1972], who observed neurotization of the lyophilized nerve transplants, but drew attention to the appearance of large numbers of eosinophilic leucocytes as evidence of immunoreaction.

The purpose of this work is not to present a complete survey of the many uses of lyophilized products, for that the reader is referred to the outstanding summaries by *Flosdorf* [1949], *Neumann* [1955a–c, 1961], *Harris* [1954], and *Parkes and Smith* [1960] on the techniques and range of applications.

In contrast to the many publications dealing with the use of lyophilized bone, dura, arteries, etc., relatively few investigatory studies appear to have been carried out on the behaviour of lyophilized cartilage. It is also striking that most of the latter publications stem from countries in eastern Europe.

3. Survey of Literature on the Use of Lyophilized Cartilage (Lyo-Cartilage)

Clinical Use of Lyo-Cartilage

The use of lyo-cartilage in the maxillofacial region has not been described either clinically or experimentally to any great extent. Almost half the studies on the subject are to be found in Russian, Czech and Polish literature.

In 1959, *Zurbuchen* reported on a process for the lyophilization of cadaveric cartilage and *Held and Spirgi,* in the same work, discussed its use in oral surgery as a means of filling bone defects due to maxillary cysts or the removal of non-erupted canine teeth. No post-operative complications occurred although all defects were regarded as infected. After only 2–3 months the formation of new bone was observed. In 7 cases with complex parondontitis with vertical bone resorption the authors also used lyo-cartilage for the reconstruction of bone defects and again no complications occurred. In a further 19 patients suffering from mandibular alveolar process atrophy, the process ridge was rebuilt with lyo-cartilage placed in subperiosteal tunnels. 3 weeks after the operation the cartilage was firmly attached to the osseous substrate. No post-operative complications were reported.

In a subsequent article, *Held and Spirgi* [1960] described 10 cases of complex parodontitis treated with lyo-cartilage without any complications. In another study *Held* et al. [1961] report on their experience with lyo-cartilage on a total of 167 patients. They observed increasing calcification of the implants. In 2 cases the authors were able to remove the lyo-cartilage 3 weeks and 11 months, respectively, post-operatively. Histologically the more recent biopsy material showed a chondrocyte area filled with cell debris and containing numerous fibroblasts and few elements of inflammation. Giant cells of the foreign body type were not observed. The cartilage was in some places permeated by very fine granulation tissue. The formation of new bone was visible only in the older biopsy material.

Clark [1969] used xenogeneic lyophilized cartilage produced from calf embryos by Squibb & Sons. The cartilaginous tissue was also treated with unspecified 'surface agents'. This preserved tissue was implanted in only 5 cases in the nasal region (dorsum 2, ala 1 and septum 2) and observed in 2 patients for 6 and 12 months, respectively. In the area of the ala and septum one partial resorption was clinically observed in each case. No post-operative infection occurred.

Mikhelson [1962] mentions summarily that he had carried out over 100 transplantations of deep-frozen and lyophilized cartilage, but does not present any detailed data. The author claims that lyophilized cartilage is brittle when cut.

Gresham [1964a] reports on the use of lyophilized alogeneic tissue produced in the tissue bank of the Naval Medical School in

Bethesda. It appears from a table that in addition to the use of lyophilized bone (3,247 cases), fasciae (341), skin (438), dura (145) and arteries (101), lyophilized cartilage was also transplanted in 183 cases, but – in contrast to the information provided on the other tissue – no reference whatever is made in the report to the behaviour of lyo-cartilage.

Obwegeser and Edlan [1967] and *Obwegeser* [1969, 1970] use allogeneic lyo-cartilage for the correction of facial contours. They consider this material more suitable than alloplastic material, suggesting that in the case of the latter there is a greater risk of a secondary infection emanating from the neighbouring dentition.

Chumakow and Paranova [1969] described an isolated case in which, after resection of an angiofibroma in the cartilaginous area of the nose, a defect measuring 1.0 × 1.0 × 1.5 cm was reconstructed using lyophilized cartilage. A 4-year period of post-operative observation revealed no modification of the implant as far as could be clinically ascertained (it is not clear from the report whether allogeneic or xenogeneic cartilage was used). The authors also emphasize the favourable properties of lyo-cartilage, mentioning specifically its high elasticity, simplicity in use and low susceptibility to infection.

Ganina et al. [1963] reported on their experience with lyo-cartilage, which they had used during the preceding 3 years in more than 100 cases of facial contour correction with good results.

Lazowski and Sarnowska [1970] used xenogeneic lyo-cartilage in the treatment of infected fractures of the mandible. They resected the infected bone in 12 cases and filled the defect with lyo-cartilage. The fractures are reported to have been clinically stable after 6 weeks. In defects up to 0.5 cm in length, complete ossification is said to have taken 12 months, and 18 months in the case of large defects.

After various animal experiments (see p. 11), *Rassumowska* [1970a] treated 30 patients suffering from 'parodontia inflammata profunda' or 'dystrophica cum inflammatione' with a total of 42 periodontal bone pockets (3–12 mm deep) using xenogeneic lyophilized calf cartilage. Post-operative infection did not occur in any of these cases. The period of observation varied from 2 to a maximum of 4 years. Bone regeneration in the pocket areas amount to 5–10% in 14 cases and in 1 case was as high as 35–45%. According to the author, the results can be regarded as distinctly favourable compared with those reported elsewhere in the relevant literature.

Without quoting any exact data, *Moskalewski* [1973] mentions that, clinically, the use of pulverized lyophilized calf cartilage has a generally beneficial effect on the healing of ulcers, and suggests that it can be successfully used in stomatology for the treatment of deep bone pockets and alveolar osteitis (alveolalgia), adding however that lyophilized allogeneic and xenogeneic cartilage inserted into bone cavities does not lead to faster ossous restitution.

Sailer [1976] reported on experience gained in the Zürich clinic for maxillo-facial surgery over a 12-year period (1962–1974) in the use of lyo-cartilage for facial contour correction. During this time 265 implants in various areas of the face were carried out on 122 patients. The degree of resorption observed clinically amounts to about 20%; at 2.6% the infection rate was relatively low. In the light of additional experience gained in a further 173 implant cases (1974–1978) the infection rate was reduced to 1.7% [*Sailer*, 1979]. From the clini-

cal viewpoint the material introduced subperiosteally is very suitable for the reconstruction of facial contours.

Animal Experiments

Lynch et al. [1956a, b] investigated in dogs the behaviour of allogeneic rib cartilage which, having been removed in non-sterile conditions, was bacterially infected under experimental conditions and subsequently subjected to various forms of preservation. In addition to lyophilization and radiation (no information on the lyophilization process), the material was also treated by immersion in Merthiolate, by irradiation alone and in a common salt solution. It was then transplanted into the rectus sheath and under the scalop on the pericranium. Strikingly, one-third of all the implants could not be found later and the remainder showed evidence of severe resorption. The cartilage irradiated in the salt solution performed best. The material transplanted onto the periosteum of the vault of the skull showed more marked resorption than that in soft tissue locations.

Ushkalow [1970] conducted a comparative study on 40 rabbits to acquire data on bone regeneration after the implanting of lyophilized bone, lyophilized cartilage, plaster of Paris, Gelastypt and musculature in circular defects in the mandible measuring 1.1 cm in diameter and only 0.3 cm in depth. It was found that the defects filled with lyophilized bone were completely reconstituted with new bone 120 days after implantation, whereas a defect of 4 mm in diameter was still present in cases where lyo-cartilage had been implanted. The central area of the cartilage was partially destroyed and marked infiltration was observed in the environment.

Stadnicki et al. [1968] implanted lyophilized allogeneic and xenogeneic cartilage into artifical defects in the mandible, the dorsal musculature and the subcutaneous dorsal tissue of a total of 36 rabbits (no data on species). Autogeneic bone was used as the reference material for comparison. In the soft tissue (musculature, subcutaneous tissue) resorption was fastest in the case of xenogeneic cartilage and slowest in the case of autogeneic cartilage. In the osseous environment calcification occurred slightly earlier in the case of xenogeneic cartilage than was the case with the allogeneic and autogeneic material. Nonetheless, with all three forms of implant the bone defects are reported to have shown complete ossification within approximately the same time. Evidence of a foreign body reaction manifested itself in the soft tissues – in those cases in which allogeneic and xenogeneic cartilage was implanted, – in the form of an infiltration of lymphocytes and plasma cells increasing in intensity until the 30th day and then remaining constant, whereas the immunological reaction after the transplantation of autogeneic tissue gradually subsided. In the osseous environment the immunological reaction was much less pronounced in allogeneic than in xenogeneic grafts. The intensity of round cell infiltration did not subside in any of the grafts in the osseous bed during the observation period of 60 days, the immunological reaction however was never severe enough to have resulted in rejection. The authors found that all cartilage implants undergo modification in accordance with their environment, i.e. they transfer into muscular, osseous and subcutaneous tissue. The rate of resorption (neither the rate nor the method of evaluation is specified) is said to be greater in tissue well supplied with blood, since in these circumstances the grafts

were reached more quickly by cells of the lymphatic system. Cartilaginous tissue implanted in the spongiosa of the mandible is alleged to induce bone regeneration. The authors consider that lyophilized allogeneic and xenogeneic cartilage tissue is unsuitable for plastic operations on account of its strong tendency to resorb.

Rassumowska [1970a, b] investigated the suitability of lyophilized allogeneic and xenogeneic cartilage from dogs and calves and lyophilized allogeneic bone from dogs for the reconstruction of alveolar walls in the treatment of experimentally induced vertical bone defects in 5 dogs. The defects were surrounded by bone and tooth substance. The lyophilization method and procedure are not described. The total observation period varied between 14 and a maximum of 100 days. In the light of these experiments the grafts described are said to be suitable for reconstruction work being replaced by trabecular bone after resorption. However, after implantation of xenogeneic lyophilized cartilage, the commencement of osteogenesis is said to be, comparatively speaking, somewhat retarded: with this material an inflammatory reaction was also observed in the initial phase. The material was also used clinically (see p. 9).

Krajnik et al. [1970] grafted lyophilized cartilage (the method of lyophilization is not described) from cattle into the pre-auricular tissue of 14 male rabbits in order to test the immunological reaction of the host tissue. All the transplants showed signs of resorption. In view of the cellular reaction the authors diagnosed a retarded immune reaction.

Sauer et al. [1971] observed for a maximum of 70 days discs of ear cartilage implanted under the skin of the abdomen and back of 14 rabbits. Compared with fresh autogeneic and allogeneic cartilage, preserved grafts (lyophilized and preserved in Palacos) showed a degree of resorption increasing in proportion to the duration of the observation period. Resorption invariably proceeded from the incision surface or from areas of interrupted perichondrium. Lyo-cartilage is said to show signs of swelling, rupture and compression, while the material preserved in Palacos shows severe deformation. The authors consider the preserved cartilage as unsuitable for grafting into areas of the body subject to mechanical stress.

Störig [1972] mentions that cartilage grafts from joints, having been deep-frozen, lyophilized, preserved in Cialite and synovial fluid, produced rather unfavourable results compared with fresh allografts in experiments on dogs. The author does not go into further detail.

In his work on the reconstruction of infrastructures of the nose and face, *Hellmich* [1974] carried out experimental grafts on 22 rabbits, using allogeneic (rabbit) and xenogeneic (human) rib tissue prepared in different ways. All the grafts were implanted under the skin of the back and the maximum period of implantation was 18 months. 3 animals died and in 4 other cases, in which xenogeneic cartilage preserved in Ringer-Locke solution and subjected to irradiation of 4 Mrad (^{60}Co) had been grafted, infection occurred. Nine different methods of preserving allogeneic and xenogeneic cartilage, respectively, were investigated on these 22 test animals. As the grafts were removed from 1 rabbit every month (from 2 animals after 6 and 12 months), one implant for each method of preservation was available each month for examination. In the case of allogeneic cartilage the degree of substance loss was almost uniformly lost and hardly measurable for all

forms of preservation (including vital, deep-freezing, irradiation, Merthiolate, and lyophilization). Among the xenogeneic chips on the other hand those preserved in Merthiolate and lyophilized did best. It must be mentioned that the various grafts, including those subjected to vital preservation, could well have had an effect on one another in one and the same animal, specially from the immunological point of view.

Using xenogeneic lyo-cartilage, *Krajnik* et al. [1978] closed artificially induced palatal defects measuring 1.5 × 1 cm in dogs. The mucosa defect was not closed nasally, so that the graft was covered only orally with soft tissue. Over a period of up to 6 weeks no rejection of the cartilage was observed. On the nasal side the lyo-cartilage was covered with a thick layer of fibrinous exudate after 6 days and exhibited, after 14 days, a layer of connective tissue rich in cells.

Conclusions and Evaluation

Study of the literature reveals several points:

(1) The process of lyophilization applied to the implanted cartilage is described in detail only in the work of *Zurbuchen* et al. [1959]. The other studies provide either no information at all or insufficient data, although the temperature range, the duration of the drying stage and the time needed for rehydration, for example, could all have a significant influence on the properties of the material.

(2) Lyo-cartilage is chiefly used clinically in periodontal surgery [*Held and Spirgi,* 1960; *Rassumowska,* 1970a], for the filling of cystoid cavities [*Zurbuchen* et al., 1959; *Moskalewski,* 1973], in the treatment of infected fractures of the mandible [*Lazowski and Sarnowska,* 1970] and in nasal surgery [*Clark,* 1969; *Chumakow and Paranova,* 1969]. In the Zürich clinic for maxillo-facial surgery, lyo-cartilage, prepared according to *Zurbuchen* et al. [1959], is also used for reconstruction of the floor of the orbit [*Obwegeser and Chausse,* 1975; *Sailer* 1977a] and the modelling of facial contours [*Obwegeser and Edlan,* 1967; *Obwegeser,* 1969, 1970, 1974; *Sailer,* 1976; 1979].

(3) Clinical and experimental results vary greatly in certain respects. While the material seems to be clinically very suitable for reconstruction work in the facial area [*Obwegeser and Edlan,* 1967; *Obwegeser,* 1970; *Sailer,* 1976, 1979], *Stadnicki* et al. [1968] conclude from the results of their experiments in rabbits that it is unsuitable for plastic operations.

(4) Contradictory results have also been obtained in purely experimental studies. Whereas *Rassumowska* [1970a, b] observed retarded calcification of xenogeneic lyophilized cartilage transplanted in alveolar bone in experiments on dogs, *Stadnicki* et al. [1968] noted in rabbits earlier calcification of the xenogeneic than of the allogeneic lyo-cartilage. The species of animal used may play a role here (see p. 86).

(5) Some investigators have limited their post-operative observation of experimental grafts to 40 days [*Stadnicki* et al., 1968], 6 weeks [*Krajnik* et al., 1970], 70 days [*Sauer* et al., 1971] and 120 days [*Ushkalow,* 1970]. These periods seem relatively short. The experiments conducted by *Hellmich* [1974] on rabbits suggest that taking the initial behaviour of a cartilage graft as a basis for extrapolation as to its subsequent behaviour after the experiment has been concluded may not be entirely permissible, since modifications do

still occur in such transplant cases after more than 120 days.

(6) Rabbits have been used almost exclusively for experiments [*Ushkalow,* 1970; *Stadnicki* et al., 1968; *Sauer et al.,* 1971; *Hellmich,* 1974]. Only *Rassumowska* [1970a, b] used dogs (5 test animals). The number of test rabbits used varies between 14 and 40 and may not always be adequate [*Hellmich,* 1974] considering the number of different grafts used.

It is questionable whether, following transplantation of lyo-cartilage or cartilage preserved by other means under the dorsal or abdominal skin of rabbits, valid conclusions can be drawn as to the behaviour of the graft material in the facial region of the human being. Even facial grafts carried out on rabbits or dogs, considering the fundamentally different functions and anatomic forms involved, probably allow only limited extrapolation of the results obtained to the human species. Monkeys, in which the form, function and metabolic factors can certainly be described as being similar to those of the human being, have not been used so far for experimental reconstruction work with lyo-cartilage in the cranio-facial area.

were observed. However, as highly concentrated beta-propiolactone applied continuously for several months to the skin of hamsters and guinea pigs has been shown to cause skin cancer [*Parish and Searle,* 1966a, b], appropriate safety precautions (rubber gloves, good ventilation, etc.) are advisable.

Countries in which the application of beta-propiolactone is not allowed, gaseous sterilization following lyophilization may serve as an alternative method, both simplifying and accelerating the technical process.

Lyophilization: Method and Procedure

Freezing

For freezing the tissue the LC-80 Low Temperature Liquid Cooler manufactured by FTS Systems Inc., Stone Ridge, N.Y., USA was used, a thermos vessel being half filled with acetone and the flexible refrigerating tube of the equipment being inserted into it. Thin-walled sterile glass tubes measuring 15 mm in diameter and closed with a plug of cotton wool are then immersed in the acetone and attached to the rim of the thermos vessel with wire. To save time, this preparatory work is begun when the cartilage material is entering the second rinsing stage after sterilization. After 2 h, when the temperature in the thin-walled glass tubes is −70 °C, the fragments of cartilage are transferred to these tubes under aseptic conditions and left for 30 min.

Drying

The pieces of cartilage still in the glass tubes plugged with cotton wool are then transferred within a few seconds to the dryer (model 30P2 manufactured by Edwards High Vacuum Ltd.), which must be showing an initial temperature of −40 °C, and left there for 36 h. The ambient air inflow after completion of drying is purified in a Millipore filter (pore size 0.22 mm). The residual moisture in the cartilage material amounted to approximately 5%. There are currently on the market various types of fully tested freeze-drying equipment which could probably be adequate for the lyophilization of homeostatic tissue. *Neumann* [1955a] has described the different systems.

Storage

The dried tissue was transferred immediately to airtight metal containers, which were then placed under a vacuum of 10^{-2} Torr. We had no facilities for sterile vacuum packing in plastic envelopes.

Rehydration

After being removed from the storage container under sterile conditions, the lyophilized cartilage material was placed for 2 h in distilled water containing 1 million units penicillin G and 2 g streptomycin, respectively, per litre. For unusually thick human rib cartilage 2 h rehydration time could well be the lower limit; after that period it is still hard in the centre and difficult to cut.

In vitro Experiments

Vitality of the Lyophilized Cartilage

Definition of the Problem

No difference can be ascertained histologically between lyophilized rehydrated cartilage and vital cartilage in HE, AB-PAS and other staining solutions [*Sailer,* 1976]. On the otherhand, opto-electronic studies conducted by Dr. *Spycher* (Pathological Institute of the University of Zürich) clearly reveal the destruction of the nuclear membranes of the chondrocytes after lyophilization.

The vitality of cartilaginous tissue can however be established under the light microscope by resorting to vital staining with neutral red. The method dates back to 1935 when *Kredel and Roberts* used neutral red in a Ringer solution (1 part in 20,000) for the vital staining of cartilage fragments. The cytoplasmic granules turn red, indicating vital cell structures. The method was subsequently modified by *Hagerty* et al. [1960a], who used Tyrode's instead of Ringer's solution, the proportion of neutral red toTyrode's solution being 1:10,000. According to *Krüger* [1964] this modified method results in less intensive

staining of the granules. In the same year he described a fast-test method capable of providing qualitative information on the vitality of cartilaginous tissue.

In our preliminary experiments we used the method described by *Hagerty* et al. [1960a] and *Krüger's* [1964] fast test to determine the influence of sterilization and lyophilization on the vitality of human and simian rib cartilage.

Procedure

Composition of Tyrode's solution

NaCl	9.0 g
KCl	0.2 g
$CaCl_2$	0.2 g
$MgCl_2 \cdot 6H_2O$	0.1 g
$NaH_2PO_4 \cdot H_2O$	0.05 g
$NaHCO_3$	1.0 g
glucose	1.0 g
aqua dest. ad	1,000 ml

Method of *Hagerty* et al. [1960a]

Three different forms of 3-mm thick rib cartilage from humans and *M. irus* monkeys, namely (a) fresh and untreated, (b) treated with beta-propiolactone alone and (c) treated by lyophilization alone, were immersed for 60 h at room temperature in a fresh solution of neutral red and Tyrode (1:10,000). The cartilage was then sectioned with microtome without being embedded. This is relatively difficult on account of the small diameter of the simian cartilage (approximately 5–6 mm), with the result that the small pieces can hardly be fixed on the carriage – a disadvantage which does not arise in the method developed by *Krüger* [1964], in which a razor-blade suffices for cutting the cartilaginous tissue.

Fast-Test Method of *Krüger* [1964]

Again using the same three forms of cartilage, extremely fine sections are produced with a razor-blade and stained for 40 min at room temperature in a solution of neutral red and Tyrode (1:10,000), then placed in a drop of Tyrode's solution on a baseboard and placed under cover-slips. The sections must be subjected to microscopic examination immediately, since in our experience the red stain of the granules is no longer visible after 20–40 min.

Results

(a) With both methods fresh human and simian cartilage (fig. 1) show staining of the cytoplasm granules. Both methods seem to be suitable for testing the vitality of simian cartilage *(M. irus).*

(b) After sterilization alone (in a 1% solution of beta-propiolactone) none of the sections showed any sign of staining of the cytoplasm granules.

(c) After lyophilization alone – without previous sterilization – there was again no sign in any of the cells of red staining of these cytoplasmic granules.

(d) Under the experimental conditions stated, the *Krüger* [1964] fast test is superior to the method developed by *Hagerty* et al. [1960a] as far as speed and simplicity are concerned, since with the latter method the staining time of 48–60 h is considerably longer and does not yield any additional qualitative data.

Susceptibility of Lyo-Cartilage to Infection

Kiseleva [1952] and *Watkins* [1957] calculated the infection susceptibility rates of autogeneic cartilage of 9.4% and more than 10%, respectively. The figures for preserved allogeneic cartilage are much lower, as many studies have shown.

Over the last 40 years, storage in Merthiolate was probably the most widely used method of preserving allogeneic cartilage. Examination of the relevant studies covering a large number of cases reveals infection rates between 1 and 6.2%, with an average of

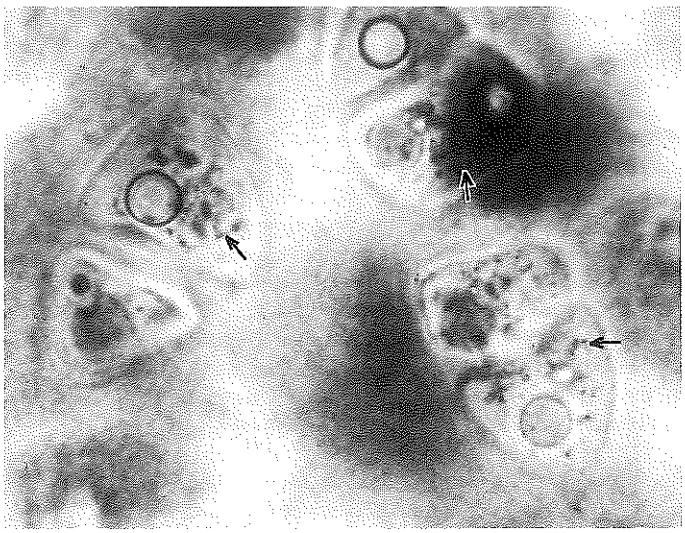

Fig. 1. Vital staining of a fragment of costal cartilage from *M. irus* using neutral red in Tyrode's solution. Fast test [*Krüger,* 1964]. The cytoplasm granules visible in the picture (arrows) do not accept the stain after sterilization (beta-propiolactone) or lyophilization. Fat vacuoles are present in some cells.

approximately 3.1% *O'Connor* [1939, 1940] reported on a total of 400 cases, *Rasi* [1959] on 398 cases and *Masing and Hellmich* [1968] on 113 cases. Allogeneic cartilage preserved by refrigeration was also transplanted relatively frequently: *Limberg* [1961a, b] 368 cases; *Köle* [1962] 92 cases. The rates of infection were between 1.1 and 4.3%, i.e. an average of 2.7%.

Rates of infection as high as those for autogeneic cartilage have also been calculated for preserved xenogeneic material. For example, *North* [1953] reports 19 cases of infection in 205 transplants of bovine cartilage preserved in Merthiolate.

Unfortunately, the literature contains no statistical data on the susceptibility to infection of lyophilized allogeneic and xenogeneic cartilage, so that we have no figures to compare with the infection rates published by us. In an initial series of 265 implants we recorded an infection rate of 2.6% [*Sailer,* 1976], but after further extensive experience with this material at the Zürich clinic we had an infection rate of only 1.7% [*Sailer,* 1979] in a second series of 173 implants of lyophilized allogeneic cartilage. Only deep-frozen cartilage yielded a comparably low average, while the rate for material preserved in Merthiolate was twice as high on average (see above).

Definition of the Problem

An explanation of the low susceptibility of lyophilized cartilage to infection might be that, during the rehydration process in an aqueous antibiotic solution, it is capable of absorbing over a short period larger quantities of antibiotics than other cartilage grafts reconstituted in antibiotic solutions; for it is with respect to the rehydration progress in particular that lyophilized cartilage differs from deep-frozen and all other forms of preserved material. The purpose of our experiments was to examine this question and to verify at the same time whether cartilage preserved by freezing, i.e. relying, in common with lyophilized material, on refrigeration, differed in any way from the latter in our experimental environment.

Procedure

(1) Agar plates, all containing 0.2 ml of the penicillin-sensitive *Staphylococcus aureus* (Oxford strain), were prepared. Four holes measuring 7.5 mm in diameter were cut in the agar at intervals of 3.5 cm.

(2) In order to establish for purposes of later comparison the growth-inhibiting effect of penicillin G on the 'Oxford' Staphylococcus, the cavities were filled up with 0.05 ml of various concentrations of penicillin (10, 7.5, 5, 2.5 and 1 U/ml of penicillin G) and the Petri dishes were incubated for 24 h at 37 °C. The areas of growth inhibition were determined by placing the Petri dishes on illuminated templates and reading off three diameters (60° apart) of the inhibition zone in millimeters.

(3) Lyophilized and deep-frozen (stored for 4 weeks at −20 °C) rib cartilage from *M. irus* was placed for 2 h in penicillin G solutions of the same concentrations (10 million U/l, 1 million U/l), weighed, adjusted to the same weights (50 mg cartilage substance/ml) by the addition of broth and homogenized (MSE tissue homogenizer).

(4) Equal quantities of the homogenized substance (0.05 ml) were then introduced into the cavities described under (1) and incubated for 24 h at 37 °C (a total of 10 Petri dishes, each with four cavities, one cavity in each case containing 0.5 ml of a 5 U/ml solution of penicillin G).

Results

As expected, the inhibition zones became larger as the concentration of the antibiotic increased. The growth inhibition zones are shown in table I; plotting against the base-10 logarithms of concentration produces a use-

Table I. Inhibition of growth of staphylococci as a function of penicillin concentration

Standard penicillin solutions (concentration) U/ml	10_{log} (concentration)	Absolute quantity of penicillin per cavity units	Diameter of inhibition zones, mm	Average mm
10	1	0.50	28, 27, 28, 30 28, 28, 28, 29 27, 28, 27, 29	28
7.5	0.875	0.375	26, 26, 28, 25 26, 26, 27, 25 26, 27, 28, 25	26
5	0.699	0.25	23, 25, 26, 23, 23, 26, 24, 23, 23, 24, 24 24, 25, 25, 24, 23, 26, 25, 24, 23, 24, 24 23, 24, 25, 23, 23, 25, 24, 24, 23, 24, 22	24
2.5	0.398	0.125	20, 20, 21, 20 20, 20, 21, 20 20, 20, 21, 21	20
1	0	0.05	12, 14, 14, 13 12, 15, 14, 14 12, 15, 13, 13	13.5

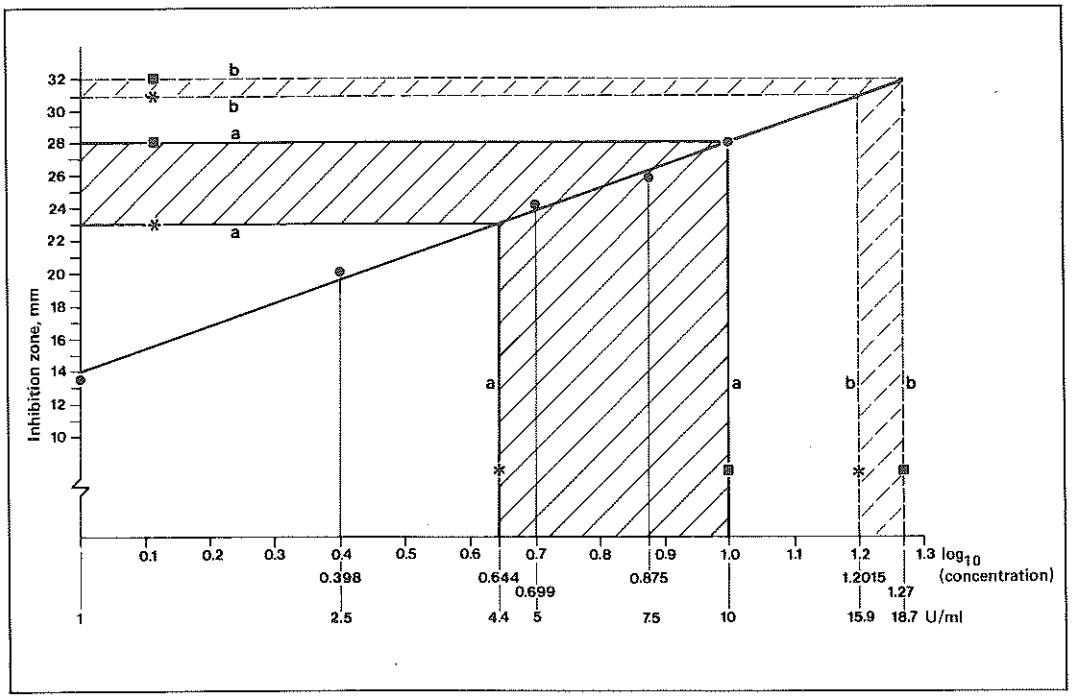

Fig. 2. Lyophilized cartilage (–■^{a}–), rehydrated in 1,000 U/ml penicillin solution (a), causes growth inhibition (28 mm) equivalent to 10 U/ml of the pure standard penicillin solution. In the case of deep-frozen cartilage (–★$_{a}$–) growth inhibition (23 mm) is equivalent to only 4.4 U/ml penicillin ($2\alpha = 0.01$). If rehydration is effected in 10,000 U/ml penicillin (b), the calculated concentrations of penicillin for lyo-cartilage (--■^{b}--) at 18.7 U/ml and for deep-frozen cartilage (--★$_{b}$--) at 15.9 U/ml show no significant differences.

ful linear relationship for comparison purposes (fig. 2). Data on the inhibition of the growth of 'Oxford' staphylococci caused by lyophilized and deep-frozen cartilage treated in a 1,000 U/ml solution of penicillin are presented in table II. Here the following observations are of interest:

(a) In the case of lyophilized cartilage rehydrated in the 1,000 U/ml (or 1 million U/l) solution of penicillin G, the inhibition of growth corresponds to that of a pure standard solution of 10 U/ml, namely 9.8 U/ml. In contrast, in the case of the deep-frozen cartilage, identically treated and also reconstituted in a 1,000 U/ml solution, the inhibition of growth recorded is smaller than that of the pure standard solution of 5 U/ml, namely 4.4 U/ml (fig. 2). Statistical evaluation (ranking test) reveals that the difference in growth inhibition is significant ($2\alpha = 0.01$). We conclude from the above that during rehydration in a penicillin G solution of 1 million U/l lyophilized cartilage is capable of absorbing more than twice as much antibiotic as deep-frozen bank cartilage treated in exactly the same way. It may well be that deep-frozen cartilage must be reconstituted in an antibiotic solution for considerably longer than 2 h for it to absorb the same quantities of antibiotic as lyophilized cartilage.

Table II. Inhibition of growth of staphylococci due to diffusion of penicillin from cartilage preserved by various means

	Diameter of inhibition zones mm	Average mm	Penicillin concentration U/ml
A Deep-frozen material immersed in 1,000 U/ml	24, 24, 19, 23, 23, 23, 25, 21 24, 24, 23, 24, 24, 24, 24, 23 24, 24, 24, 22, 22, 22, 24, 21	23	4.4
B Deep-frozen material immersed in 10,000 U/ml	33, 30, 32, 30, 32, 31, 30, 31 32, 30, 33, 30, 32, 32, 31, 30 32, 31, 32, 30, 31, 31, 30, 31	31	15.9
C Lyophilized material rehydrated in 1,000 U/ml	28, 30, 26, 27, 28, 29, 26, 29 28, 29, 26, 27, 28, 29, 27, 28 28, 30, 26, 28, 27, 27, 27, 28	28	9.8
D Lyophilized material rehydrated in 10,000 U/ml	30, 35, 31, 32, 33, 33, 33, 32 32, 35, 32, 30, 32, 31, 31, 30 30, 35, 32, 30, 33, 33, 33, 32	32	18.7

(b) The inhibition of growth caused by the lyophilized cartilage rehydrated in a 10,000 U/ml (or 10 million U/l) solution of penicillin G is admittedly greater than that produced by the deep-frozen material, but is, marginally, no longer significant (table II). For lyo-cartilage the inhibition of growth corresponds to a solution of 18.7 U/ml compared with 15.9 U/ml for deep-frozen cartilage (fig. 2).

These experiments show that, apart from the method of preservation, the concentration of the penicillin solution used is also of significance. For the concentrations of penicillin quoted, lyo-cartilage is superior to deep-frozen material in practice due to the fact that the faster and more intensive absorption of antibiotic by the former represents a time gain and may in certain circumstances also lead to a reduction in susceptibility to infection as a result of a higher local concentration of penicillin. We wish to point out that the cartilage material used by us was homogenized to ensure that the conditions for the experiments were as uniform as possible.

Experimental Grafting of Lyophilized Allogeneic and Xenogeneic Cartilage

Maxillo-Facial Region

It should be mentioned at the outset that, in contrast to other investigators working with fresh or preserved material, we implanted both intact rib cartilage and material trimmed to size. However, the grafting of totally intact rib cartilage into the facial areas in human beings must be regarded as excep-

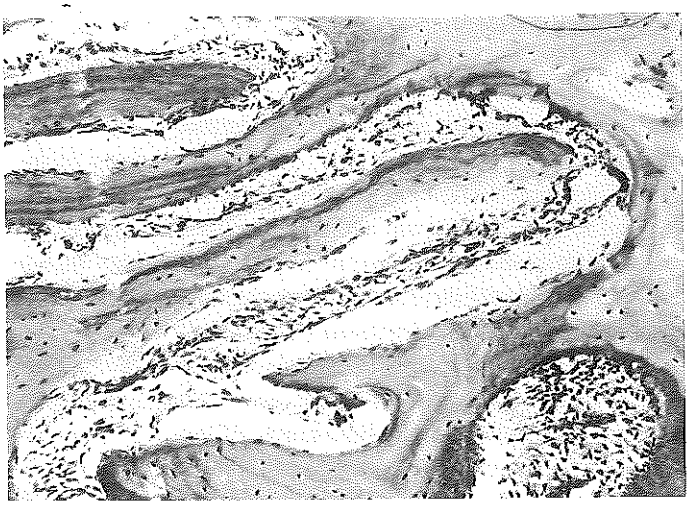

Fig. 53. 240 days after being grafted into a palatal defect, this graft of lyophilized allogeneic costal bone is completely transformed and incorporated. HE. × 130.

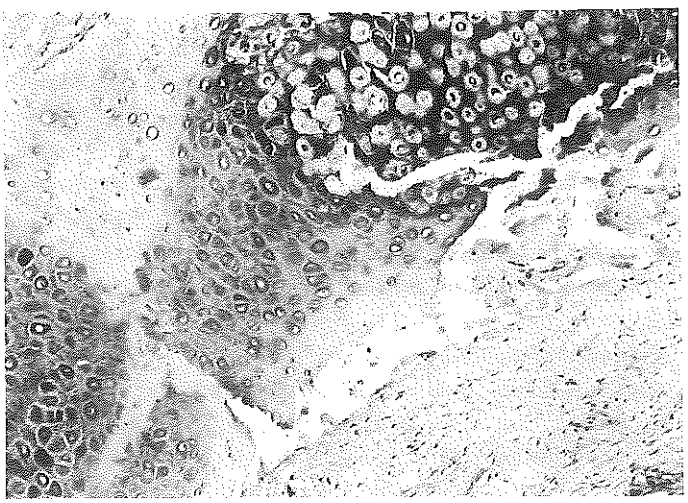

Fig. 54. Lyophilized elastic allogeneic cartilage from the ear 240 days after subcutaneous grafting: degenerative alterations and the decomposition of the graft can be seen in all areas. Toluidine blue. × 100.

cally, total destruction of the preparation can be observed (fig. 54).

Conclusions

(1) No graft was lost because of infection. As far as the clinical behaviour of graft material is concerned, the extra-oral and trans-oral operative routes were equally suitable.

(2) With one exception it was possible to identify all the grafts throughout the entire period of the experiment. For periods of observation up to 260 days no cases of resorption were identifiable by palpation, although resorption in the form of a reduction in surface smoothness was occasionally revealed by macroscopic examination after grafts had been removed. Reliable appraisal was possible only after histological examination.

(3) The behaviour of lyo-cartilage was not dependent on the location of the grafts, provided the applications consisted of subperiosteal implantation for contour correction.

(4) The grafts showed differences in behaviour after subperiosteal and subcutaneous implantation (see below).

(5) After 23–50 days subperiosteal grafts have acquired osseous fixation, but can still be removed with an elevator. After periods of observation of 240 days or more, however, this was possible with only 24 out of 64 grafts, the remaining 40 being fixed to their substrates by bone regeneration. Only 3 non-fixed grafts were still movable.

(6) All complete and bisected blocks of cartilage grafted subcutaneously and also the upper sections of the bisected blocks implanted subperiosteally were movable.

(7) Entire and bisected blocks of cartilage grafted subcutaneously give rise to macroscopically and microscopically visible resorption of the subjacent bone covered by soft tissue (fig. 42a, b).

(8) Histologically, subperiosteally grafted lyo-cartilage undergoes gradual osseous substitution starting at the point of contact with the bone, but only after the narrow gap between the bone and the surface of the cartilage has been bridged by bone regeneration, with the graft material even being slightly 'lifted' (fig. 22). After 240–260 days, about 1/10 to 1/5 of the cartilage substance has been ossified from the base upwards.

(9) Between 30 and 50 days after subperiosteal grafting microscopic examination reveals massive bone regeneration in the region of 'dead' space covered by periosteum adjacent to the vertical walls of the cartilage grafts (fig. 16–18).

(10) In no case did subcutaneously grafted lyo-cartilage lead to bone regeneration in the surrounding regions.

(11) Minimal symptoms of resorption can be identified histologically on the cut surfaces of the cartilage even after short periods of observation of up to 50 days; after 240–260 days this resorption shows an appreciable increase, especially with xenogeneic grafts.

(12) After 240 days lyo-cartilage grafted subcutaneously also shows signs of incipient ossification.

(13) Two forms of ossification can be distinguished: ossification on the basis of connective tissue via osteoid tissue in the peripheral areas of subperiosteally grafted cartilage (fig. 16–18), and ossification on the basis of secondary calcification in both subperiosteal *and* subcutaneous grafts (fig. 38–40).

(14) No resorption of areas of cartilage showing secondary ossification was observed at any time during the entire course of the experiment, irrespective of whether the ossification had developed via calcification or via an intermediate connective tissue stage after resorption.

(15) Bisected segments of cartilage show greater total resorption than single blocks of the same weight, as resorption also occurs between the segments.

(16) Even after long periods of observation, cartilage surfaces with or without an integument of perichondrium – but free of lesions – show either no resorption at all or at any rate much less than the cut surfaces of the cartilage.

(17) After 50 days of observation, perichondrium implanted with the graft material reveals initial signs of resorption on the side facing the bone surface, but reveals no such signs on the side covered with periosteum (fig. 20).

(18) The central sections of costal cartilage, which contain fewer chondrocytes and are the first to show signs of regressive alteration, are resorbed earlier than the areas of cartilage close to the surface.

(19) Calcified cartilage areas undergo 'turbulent' disorderly resorption (fig. 19).

(20) Two different types of resorption can be differentiated: superficial 'quiet' resorption where cartilage is covered by periosteum or muscle fibres running parallel to the surface (fig. 31, 32), and 'deep' resorption where these conditions do not exist (fig. 33).

(21) Macroscopically, allogeneic and xenogeneic lyo-cartilage demonstrated identical behaviour during the entire observation phase.

(22) Microscopically, xenogeneic cartilage showed much greater resorption than allogeneic material after 240–260 days.

(23) During the first observation period of 50 days, minor round cell infiltration was observed in both allogeneic and xenogeneic cartilage. After 240–260 days the latter showed round cell infiltration arranged in the manner of lymph follicles (fig. 40).

(24) Lyophilized bone grafted in the form of a costochondral junction for contour correction is fully resorbed shortly after grafting (fig. 30).

(25) Lyophilized cartilage implanted in palatal defects with osseous perimeters showed incipient osseous fixation and substitution after only 29 days. The substitution process continued throughout the entire period of observation (fig. 48). Xenogeneic lyo-cartilage appeared to ossify more quickly. In certain circumstances both types of cartilage could replace autogeneic bone in cases of palatal reconstruction.

(26) Non-crafted palatal defects for comparison purposes do not fill up spontaneously with regenerated bone (fig. 45).

(27) Allogeneic lyophilized costal bone grafted into palatal defects ossifies more quickly than allogeneic and xenogenic cartilage under the experimental conditions described, i.e. its behaviour is contrary to that of lyophilized bone grafted subperiosteally for contour correction. In certain circumstances, therefore, allogeneic lyophilized bone could be a suitable substitute for autogeneic bone.

(28) Lyophilized elastic cartilage from the ear shows signs of severe decomposition after 240 days (fig. 13, 54).

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9. Clinical Use of Lyophilized Allogeneic Cartilage

In chapter 3 we presented a literature report on the use, so far, of lyophilized cartilage in the cranial-maxillo-facial region. As a result of the different methods of lyophilization and sterilization adopted and also due to the fact that not only allogeneic but also xenogeneic lyo-cartilage has been used in treating patients, the results, which are often presented summarily without any detailed assessment of the individual cases, are not comparable. Only the results obtained by *Zurbuchen* et al. [1959], *Held* et al. [1961] and *Sailer* [1976, 1979] are comparable, as in all cases the cartilage material used was prepared in accordance with the method described in 1959 by *Zurbuchen* et al. The authors come to similar, positive conclusions regarding implantation of this material into the various maxillo-facial and oral regions. In the clinic for maxillo-facial surgery at the University Hospital Zürich, more than 1,300 lyo-cartilage grafts have been carried out since 1960. This chapter discusses various possibilities of reconstructive surgery using lyo-cartilage and presents a number of case studies.

As described in chapter 4, the cartilage material must be rehydrated before being grafted. Rehydration, which should last for at least 2 h, is carried out in a solution containing any recognized water-soluble antibiotic. As a rule we place the cartilaginous tissue in the solution on the evening before the operation to ensure that even thick pieces of cartilage have optimum cutting properties. Any surplus material can be deep-frozen at approximately −20 °C and stored for subsequent use. The genesis of a defect is of practically no importance in reconstructive surgery with lyo-cartilage. The totally inert biological material, which does not rely on any form of nutrition by the surrounding soft tissues, also heals satisfactorily in areas treated by irradiation. As the experimental results in chapter 4 show, the susceptibility of lyo-cartilage to infection is lower than that of cartilage preserved by other means, due to the fact that during rehydration in the antibiotic solution the lyo-cartilage absorbs large quantities of the antibiotic and acts post-operatively as an antibiotic depot. Lyo-cartilage can of course also be rehydrated without any antibiotic additive.

Reconstruction in the Forehead Area

Lyophilized cartilage is ideal for the reconstruction of defects and irregularities in the area of the forehead (fig. 63a, b), for example after defect fractures, craniotomies leaving visible burr holes, transcranial surgery for hypertelorism with subsequent unevenness of contour, in hemi-facial atrophy, after tumour removal, and in cases of congenital and developmental asymmetries etc.

Technical procedure: If osseous regeneration of a forehead defect is to be attained, the osseous perimeter of the defect (for it is from these perimetric areas that calcification and

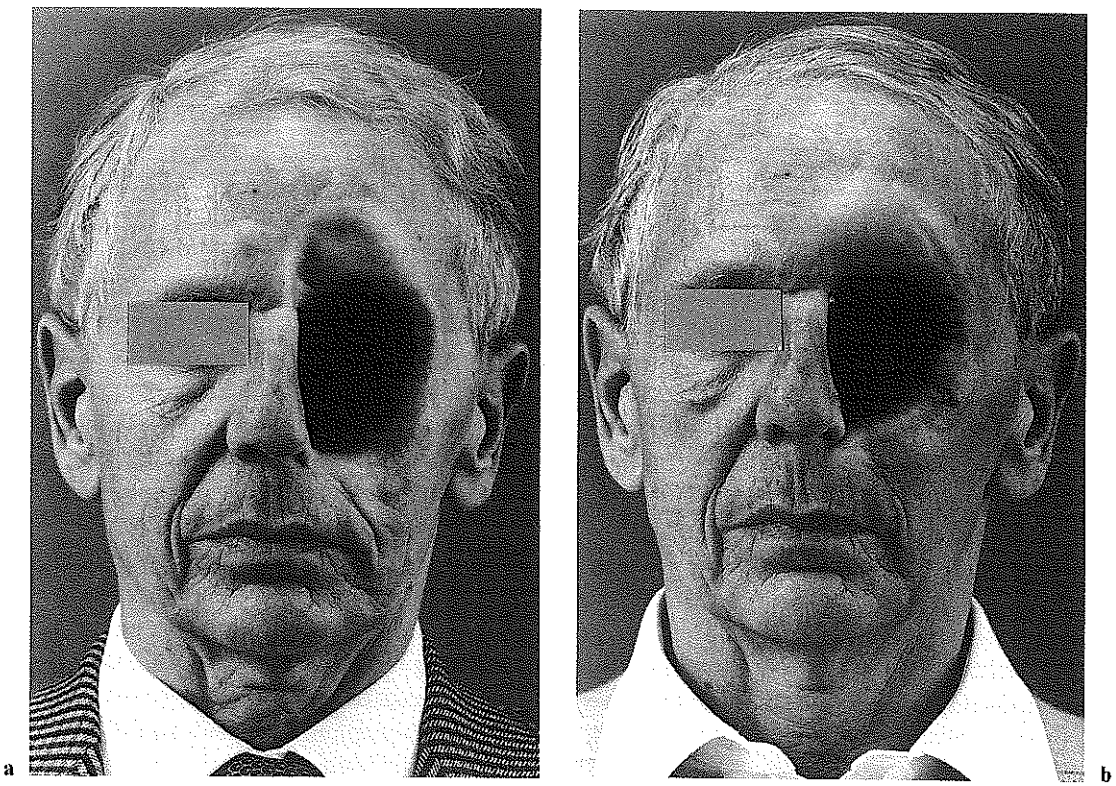
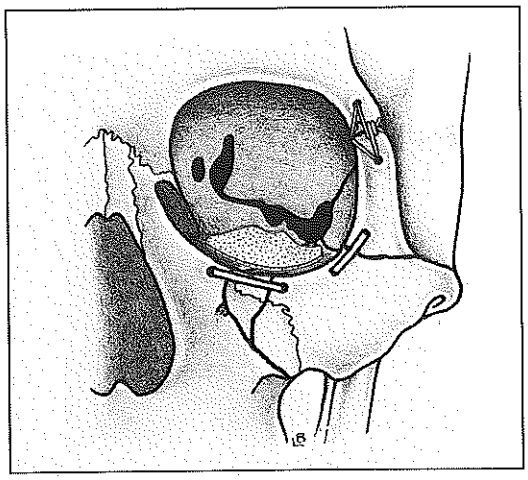


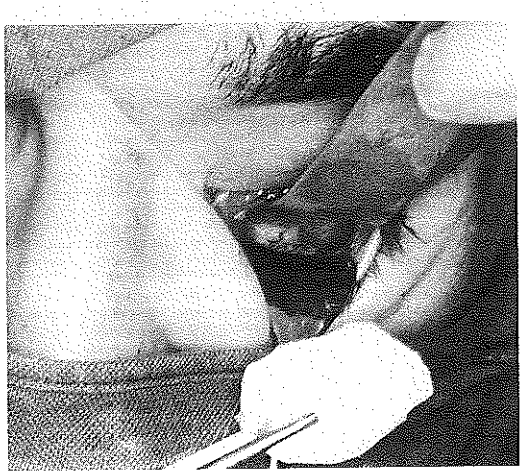
Fig. 63. Reconstruction of a forehead defect and the periphery of the orbita. Condition after orbital exenteration, including removal of neighbouring bone structures (alio loco) and after irradiation treatment **(a)**. The defect in the forehead area was corrected under local anaesthesia by subcutaneous preparation and implantation of several slices of lyo-cartilage. For the defect in the zygoma/maxilla region a single cartilage implant 8 cm in length was used in the same session: caudally it rests on the maxillary bone under the nasal alae; cranially it is supported by the zygomatic bone, where it is fixed with a wire suture. The soft tissues were tunnelled between the contact points. This method does not hinder post-operative control of the resection cavity. The surface to be covered with a dressing has been reduced by almost 50% **(b)**.

subsequent ossification of the implanted lyo-cartilage must proceed) must be exposed and freed of connective tissue by means of subperiosteal preparation. Naturally, this must be done with great care, avoiding any damage to the dura. The defect can now be filled up with one or several blocks of cartilage, which should be in close contact with the bone. Fixation with resorbable suture material (chrome catgut, Dexon) through burr holes is recommended.

Another technique consists of the subcutaneous preparation over the defect of a pocket into which slices of lyo-cartilage cut very finely (fig. 69a) with the Dermatome, as suggested by *Obwegeser,* are then inserted. Access in the surgical reconstruction of forehead defects is invariably via existing scars, in the region of the eyebrows or hair line, via a temporal approach [*Obwegeser,* 1978] or by means of a bitemporal curved incision [*Unterberger,* 1959] in order to camouflage scars.



64a



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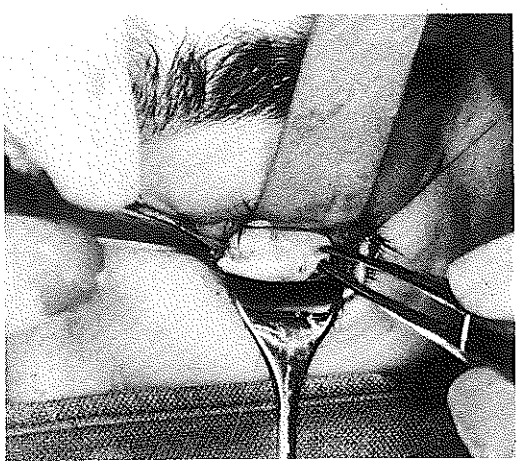

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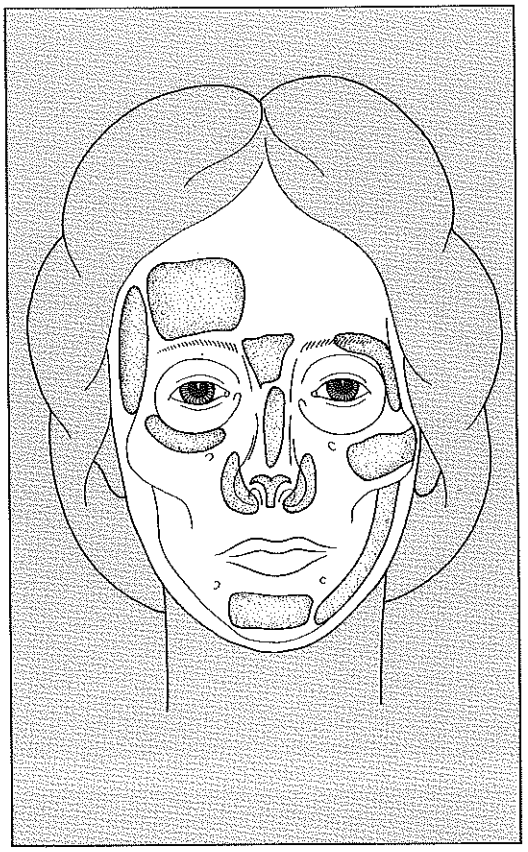
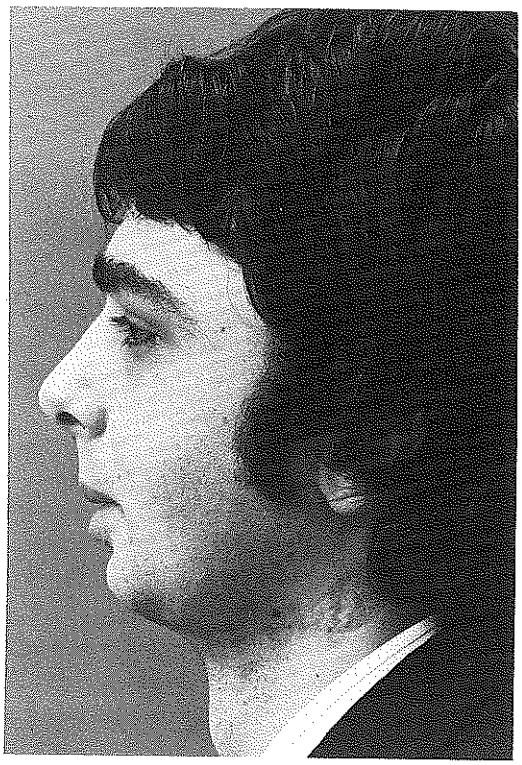


Fig. 65. Regions in which common congenital, developmental or acquired defects (flattened contours, asymmetry) were corrected with lyo-cartilage.

Fig. 64. Reconstruction of the orbital floor after zygomatic fracture. The diagram **(a)** shows the position of a lyo-cartilage implant bridging a defect in the orbital floor; the cartilage graft is usually inserted transconjunctivally, as are the osteosynthesis staples in the infra-orbital border [*Sailer*, 1977b], in order to avoid an infra-orbital incision. Using the same method, it is also possible to implant very large pieces of cartilage for bridging a defect or for raising the bulbus **(b, c)**.



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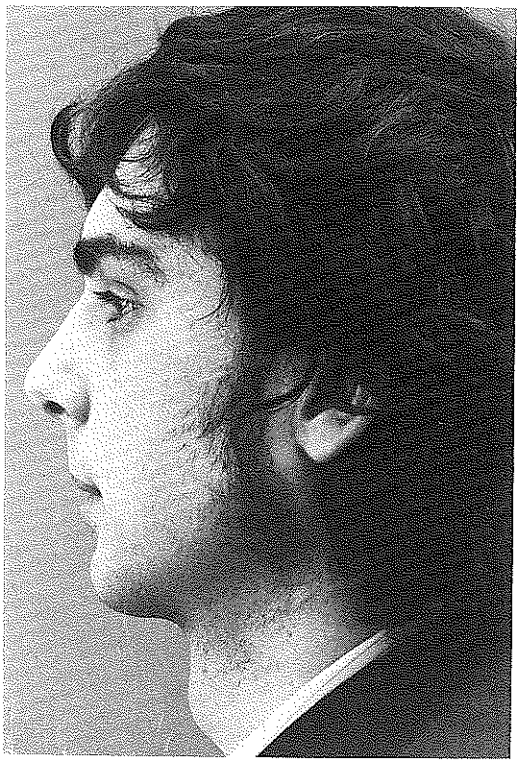

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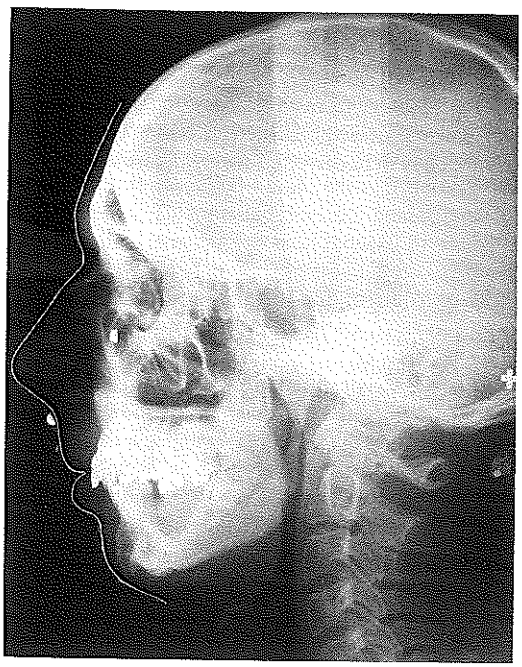

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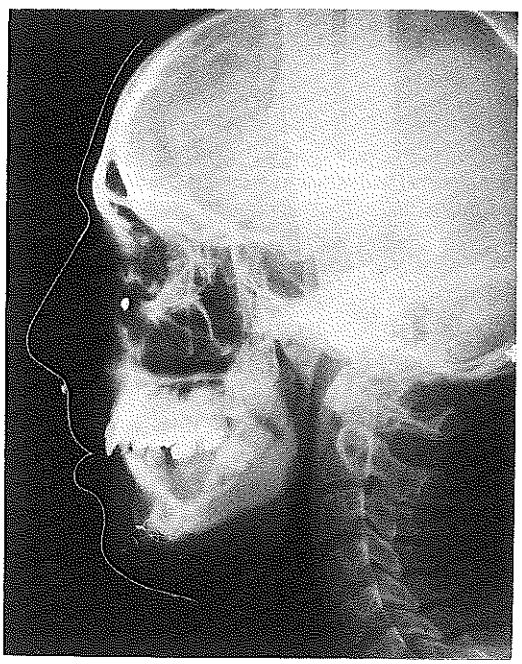

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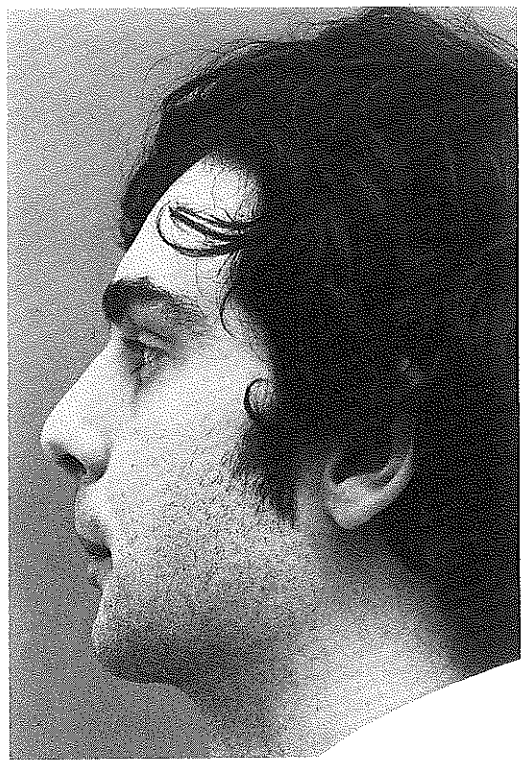

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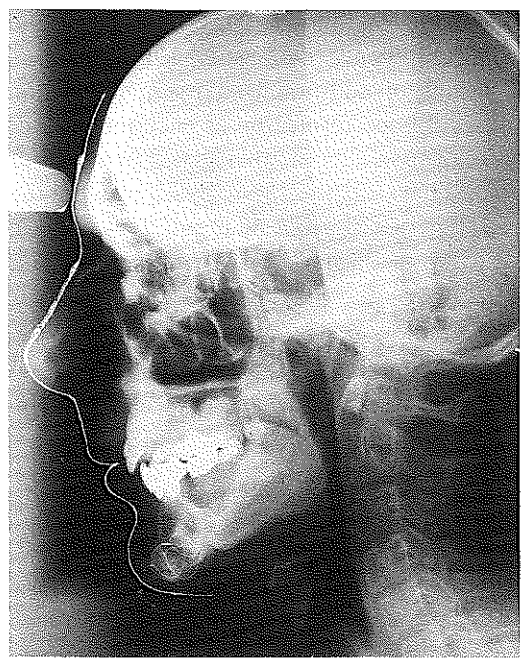
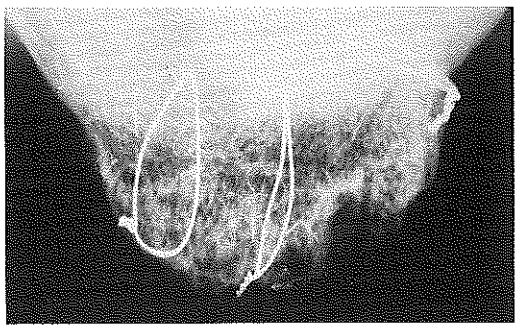


f

Reconstruction in the Orbital Region

Defects of the upper, lateral, medial and lower orbital borders can be reconstructed with pieces of cartilage cut to the appropriate shape. They are normally fixed to the residual bone with 0.4 mm wire. In certain conditions a tunnelling preparation through very small incisions is also a possibility, for example in areas treated by radiation (fig. 63a, b), to avoid compromising blood supply and lymph drainage.



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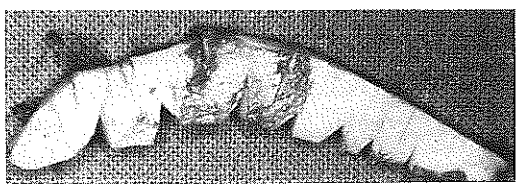


h

Fig. 66. Enlargement of the chin prominence using lyo-cartilage [*Sailer*, 1979]. The series of illustrations show the pre-operative situation **(a, d)**, the lyo-cartilage graft trimmed to size **(h)**, the situation 2 months post-operatively **(b, e)** and 6 years later **(c, f)**. Chronologically the cephalometric X-rays **(d–f)** correspond closely to the profile photographs, whereas in the immediate post-operative stage the cartilage material is identified merely by the fixation wires **(e)**, mineralization of the lyo-cartilage is clearly evident 6 years later **(f)**. The chin contact X-rays **(g)** reveal an almost regular structure of bone trabeculae within the cartilage material.

The surgical access routes will again depend on the localization and extent of the defect. For the supra-orbital and also for the lateral and medial regions of the orbital we use an eyebrow incision, while a transconjunctival incision (fig. 64b) is chosen for the reconstruction of defects of the infra-orbital margin. For reconstructions of the lateral orbital margin and in the region of the zygomatic bone and arch, e.g. in cases of Treacher Collins syndrome, the temporal approach [*Obwegeser,* 1978] is the most suitable.

In fractures of the middle face and zygoma and in cases of blow-out fractures we prefer to use slices of lyo-cartilage for the reconstruction of the orbital floor (fig. 64a–c) and side-walls, and also where reconstruction of the orbital floor is necessary after excision of tumour. The transconjunctival access route is preferred, via which osteosynthesis of the infra-orbital margin by means of staples [*Sailer,* 1977 a,b] is also possible (fig. 64a). In not one single case has either infection or abscess formation been observed after reconstruction of the orbital floor using lyo-cartilage. This material is indicated if reliable contact positioning is posible on at least two sides of the defect; special fixation of the transplant is not normally carried out. If reliable positioning is not possible, deep-frozen bisected costal material from the tissue bank is normally used [*Obwegeser and Chausse,* 1975], and is wedged in and remains in position due to its inherent elasticity. We close small cleft-like defects with lyo-dura.

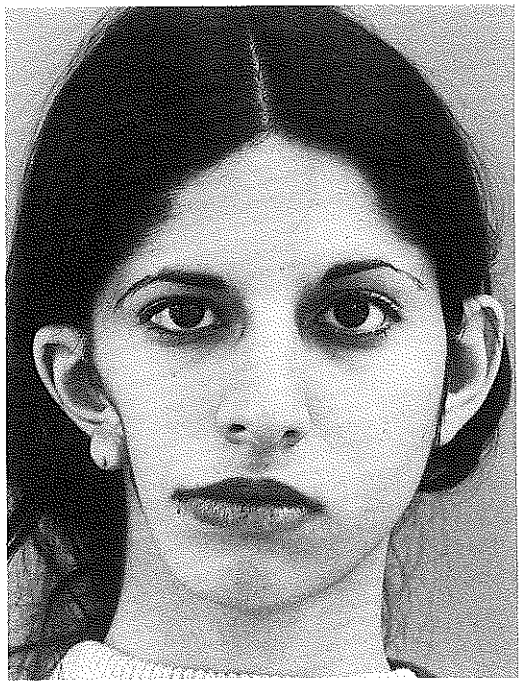
Correction of Contours in Various Facial Regions

In the Zürich clinic for maxillo-facial surgery lyo-cartilage is most frequently used for the correction of developmental hypoplasia and in the treatment of acquired or congenital facial asymmetries (fig. 65).

Very common defects are a flattening of the paranasal region and retro-displacement of the maxillary base in normal occlusion. Following a vestibular vertical incision in the region of the canine and preparation of a subperiosteal pocket, L-shaped blocks of cartilage (fig. 65) of the required size are inserted alongside and below the piriform aperture. If this is done correctly, fixation is unnecessary. Impervious closure of the vestibular incision is then carried out with a non-resorbent material (Supramid).

Lyo-cartilage has proved outstandingly successful for enlargement of the chin (fig. 66a–f) either as a primary measure in microgenia or, following anterior sliding of the chin prominence [*Obwegeser,* 1957, 1958], as a means of additional enlargement. In the event of bone resorption as encountered not infrequently after double-step advancement of the chin [*Neuner,* 1965] or as a result of the rejection of alloplastic materials, lyo-cartilage is strongly indicated for use in subsequent chin enlargement as it is also incorporated in scar tissue. We have also grafted lyo-cartilage repeatedly in tissue treated by radiation without any negative effects. The surgical access route

Fig. 67. Treatment of a moderately severe case of dysostosis otomandibularis exclusively by lyo-cartilage onlays (a case treated by *Obwegeser*). It was possible to achieve almost total correction of the three-dimensional hypoplasia of the right side of the face **(a)** by peroral implantation of lyo-cartilage onto the body and ramus of the mandible **(b)**. The lyo-cartilage and the fixation wires are clearly visible in the xeroradiogram in lateral **(c)** and posterior-anterior **(d)** views. From *Sailer* [1976]. In subsequent surgery the macrostoma was corrected and mentoplasty carried out.



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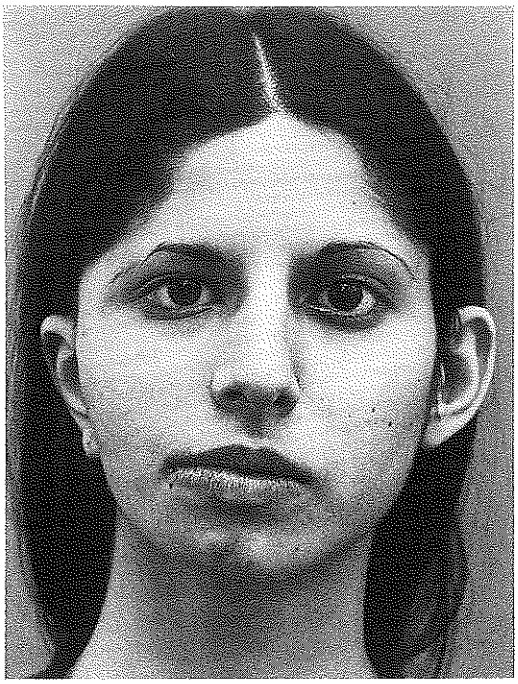

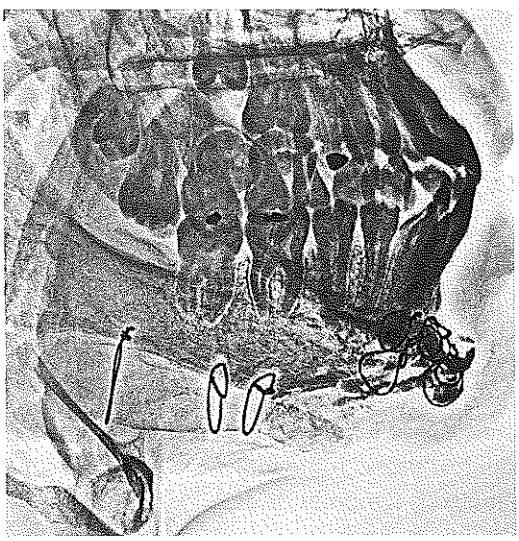

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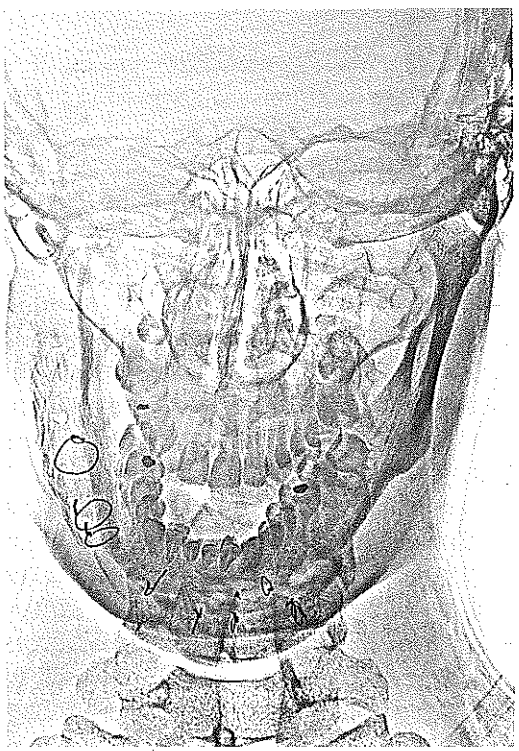

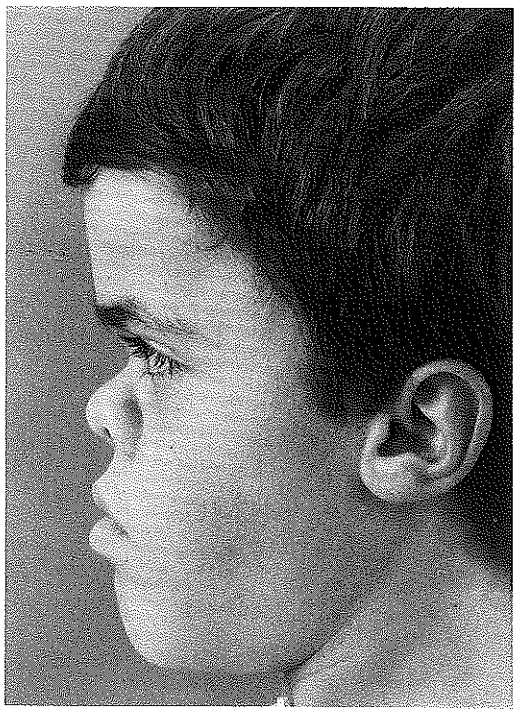

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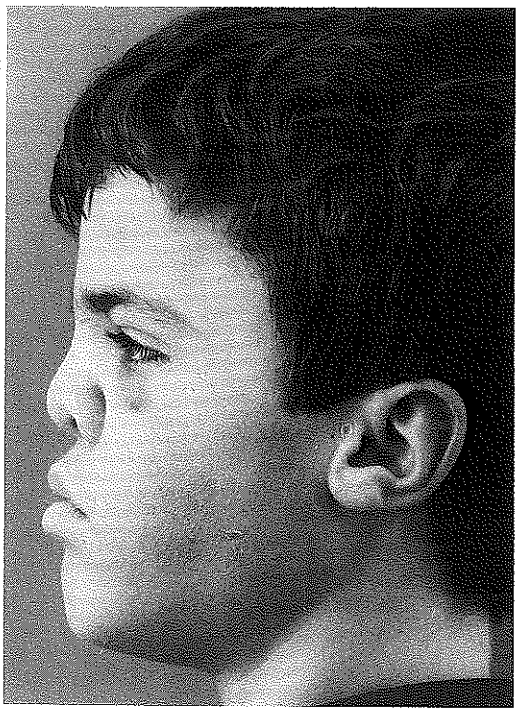


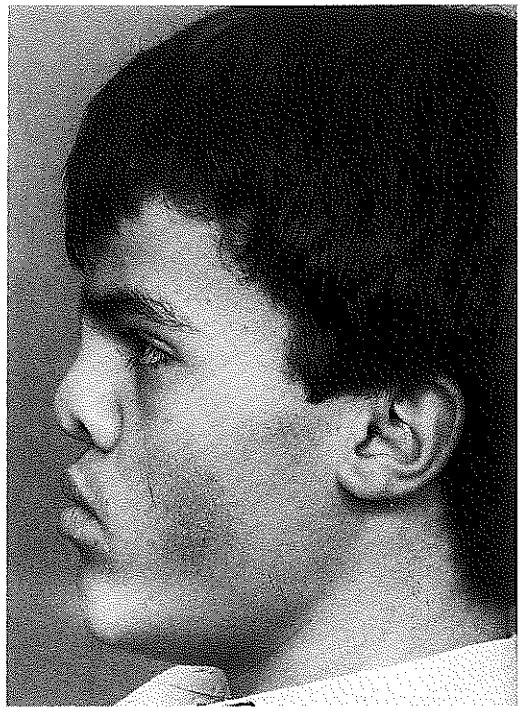
b

is invariably trans-oral via an incision in the labial vestibule.

After reconstruction of the chin region it is simple to demonstrate radiologically the conversion of the allogeneic lyo-cartilage material into autogeneic bone (fig. 66f, g). The same applies with respect to reconstruction of the contours in the lateral mandibular region (fig. 65) as an isolated or supplementary measure in the treatment of developmental malformation accompanied by asymmetry [*Obwegeser and Edlan,* 1967].

Special mention must be made of malformations within the category of otomandibular dysostosis (fig. 67a–d), since even after rotation of the lower half of the face [*Obwegeser,* 1970, 1973] additional correction is still necessary [*Sailer* 1981]. The need to remove more autogeneic bone and cartilage would be very unsatisfactory in these patients, from whom costal and pelvic crest material had previously been removed. In order to avoid visible scars and possible damage to the facial nerve, lyo-cartilage is positioned via the oral route subperiosteally adjacent to the mandible and generally fixed with wire sutures.

Where the problem consists of flattened areas in the region of the zygomatic bone, for example after zygomatic fractures or in the case of hemi-facial hypoplasia or dysostosis manidublofacialis, or for the bridging of clefts in the zygomatic bone, or for purely cosmetic reasons, the reconstruction of contours with lyo-cartilage in the form of blocks or slices is a possibility. In these cases the surgical access route may be either transconjunctival, temporal or oral.



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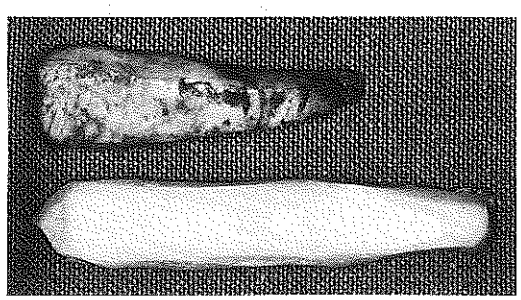

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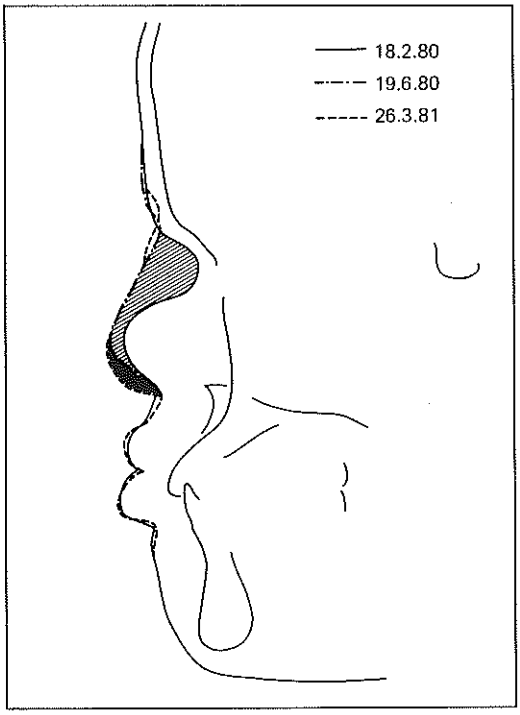


e

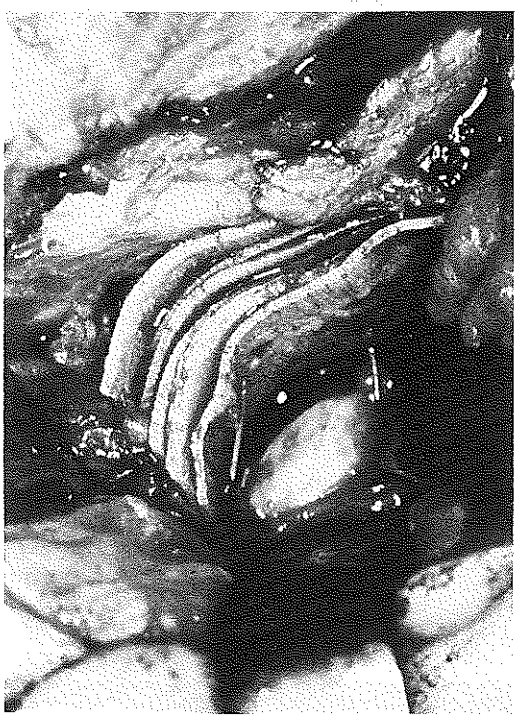
Fig. 68. Correction of a saddle nose with chondrodystrophy by two-stage implantation of lyo-cartilage. As the 21-year-old patient would not permit visible scars, the correction of the saddle nose **(a)** was achieved exclusively by extension of the soft tissues by means of a strip of lyo-cartilage 3 cm long **(d**, top) inserted via an intercartilaginous incision. The result is shown in figure **b**. 5 months later a second strip 5 cm long **(d**, bottom) was implanted, and the result is shown in figure **c**. Tracing of the cephalometric X-rays **(e)** shows the results after the first (– · – · –) and second (– – –) graft and the additional length gained by the second graft (black area). Correction of the bimaxillary protrusion by the patient.

From our experimental studies we know that lyo-cartilage gives first-class results when implanted subcutaneously, although the ossification processes take place more slowly in comparison to subperiosteal transplantation. Apart from the forehead region, subcutaneous implants are most common in the nasal area, either as cosmetic treatment or in the reconstruction of facial malformations. The technical details given on pages 114–118 relate to nasal corrections carried out on patients with cleft lip and palate.

The use of lyo-cartilage is particularly indicated in the case of the short saddle nose (fig. 68a–e), as it is possible by successive grafting of consistently larger pieces of lyo-cartilage (fig. 68d) to extend the soft tissue and lengthen the nose without external surgery resulting in visible scars [*Jackson and Reid,* 1978] caused by cutaneous flaps.

Cartilage Slices in the Treatment of Ankylosis or after Resection of the Condylar Process

The interpositioning of cartilage slices between the base of the skull and the stump of the mandible was first described by *Escher and Schilli* [1967] who used autogeneic material. In order to avoid a second operation with all its concomitant complications, *Obwegeser* has been using cartilage slices systematically in operations for ankylosis since 1962 (fig. 69 a–c). Extremely thin slices are cut with the Dermatome and 5–10 of them are tied loosely together with Dexon (fig. 69a, b). Our experimental studies with animals have shown that connective tissue develops between the slices, making them mobile in relation to one another. These layers of connective tissue prevent bonding of the gradually ossifying slices and (fig. 69c), consequently, re-ankylosis.



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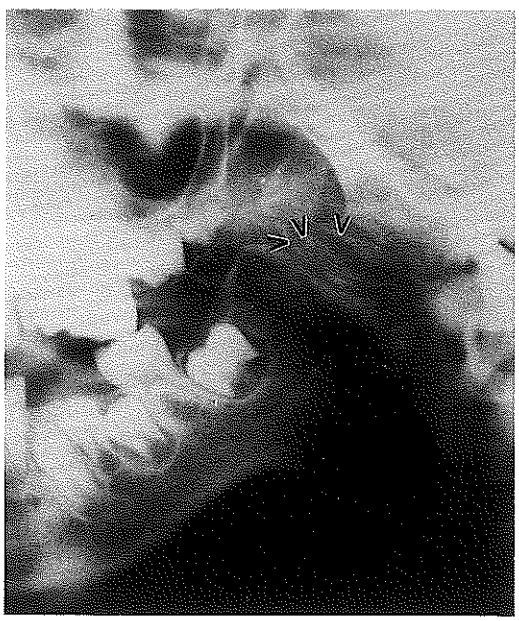


c

Fig. 69. Treatment of ankylosis of the temporomandibular joint using lyo-cartilage. After wide excision of the osseous ankylosis, slices of cartilage **(a)**, loosely tied together with Dexon, are inserted between the stump of the mandible and the base of the skull **(b)**. The calcification and ossification of the slices **(c)** visible in the X-ray does not lead to renewed ankylosis since the connective tissue ensures permanent separation of the pieces.

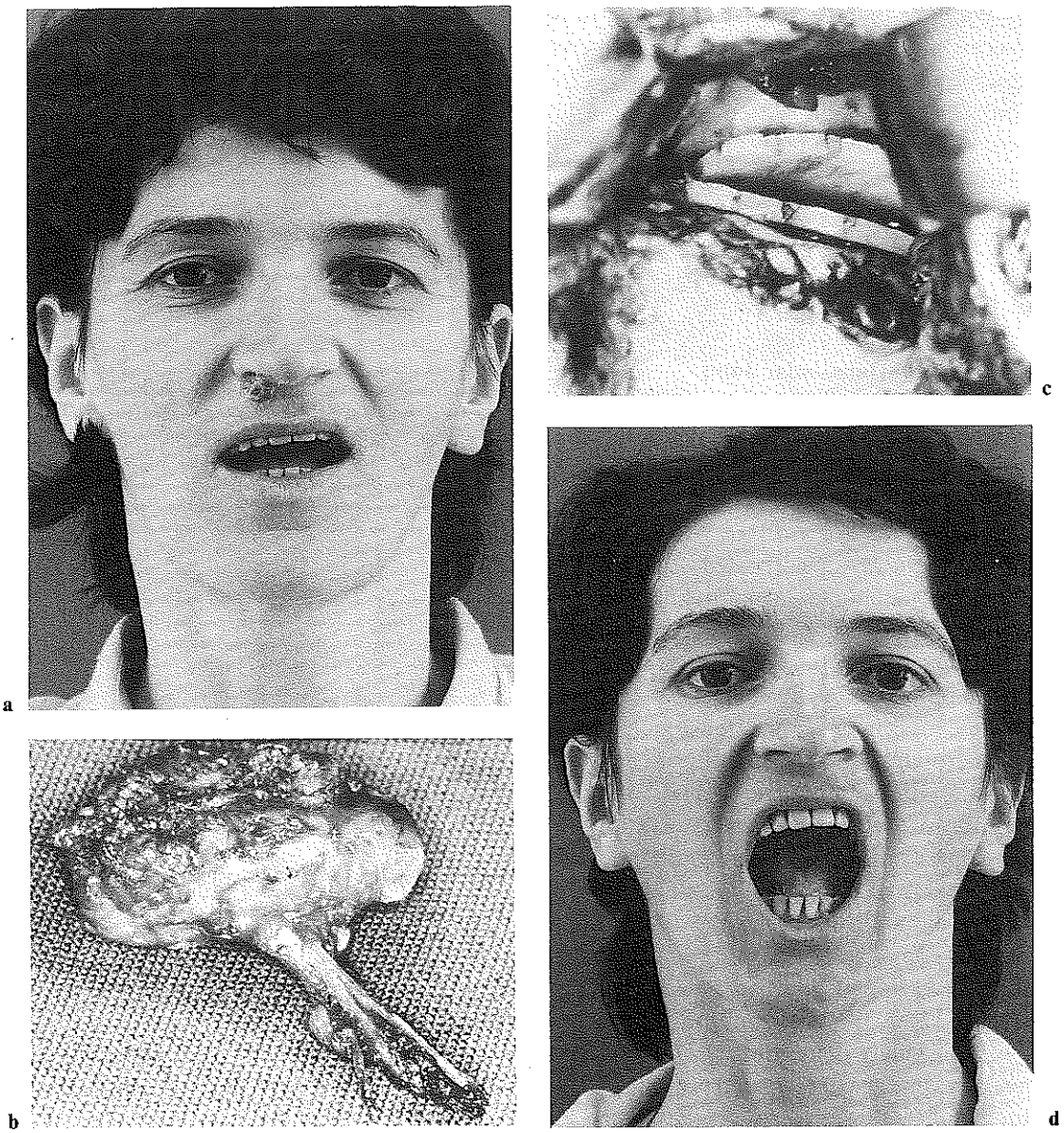
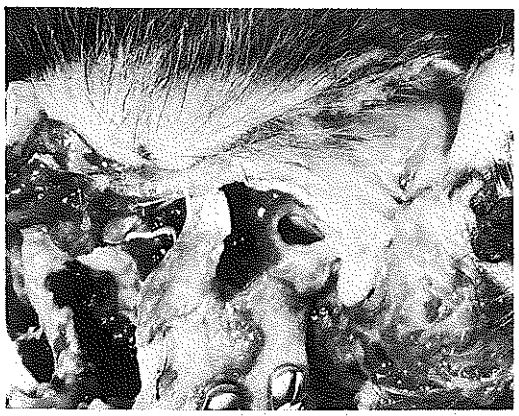


Fig. 70. Treatment of severe arthrosis deformans using lyo-cartilage. Female patient (32) with history extending over a number of years of arthrosis deformans of the right temporo-mandibular joint and painful inhibition of movement **(a)**. After resection of the altered condylar process **(b)**, lyo-cartilage is implanted within the joint to support the mandibular ramus **(c)**. The absence of anterior movement of the right side of the mandible as a result of the surgical intervention is acceptable considering that the patient has now been free of pain for 3 years and normal mouth opening has been restored **(d)**.

We have found a similar procedure to be successful in cases of resection of the altered condylar process on account of arthrosis deformans (fig. 70a–d). With this technique it is possible not only to prevent displacement of the mandible to the operated side, but also to avoid an open bite.



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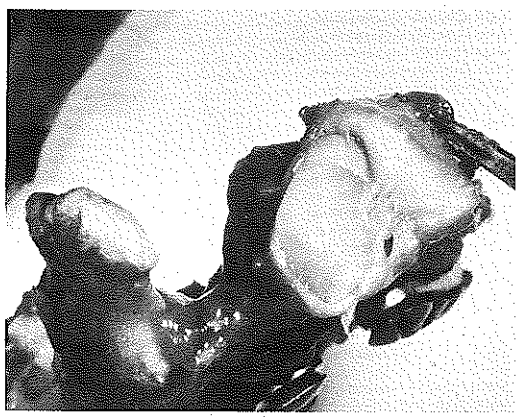
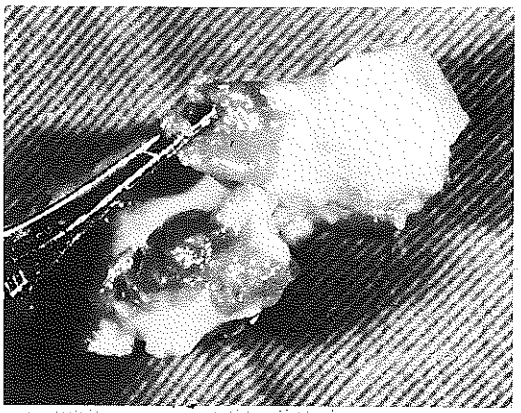


b

Cartilage-Bone Grafts in the Reconstruction of the Temporo-Mandibular Joint

Previous experimental studies with monkeys *(Macaca irus)* have shown [*Sailer,* 1978, 1980a] that lyophilized allogeneic mandibular rami including a cartilage-bearing condyle are aesthetically unsurpassed, can achieve complete functional rehabilitation and can also be subjected immediately to post-operative stresses (fig. 71a–e). With the aid of polychrome sequence labelling it was possible in these experiments to demonstrate the total transformation of the lyophilized cadaveric implant into autogeneic bone within 6 months. After this period of observation microscopic examination revealed that the articular cartilage with its typical layers was largely unmodified. Using the LMI test, it was also possible at the same time to show that the reconstruction had a relatively high level of residual antigenicity. In view of the low-antigenicity determinants of lyophilized hyaline cartilage (see chapter 6), the chief source of this residual antigenicity is the lyophilized bone transplanted with the graft material. It is thought that this residual antigenicity is necessary to initiate the osteoclastic and osteoblastic processes and thus to transform the allogeneic bone transplants into autogeneic tissue. This is consistent with the observation that immunologically inert bone does not show any sign of degradation or transformation, as is the case with alloplastic material.

On the strength of the positive results of our experiments and in the light of *Plotnikov's* [1966] clinical experience, we also carried out articular reconstructions on patients using lyophilized allogeneic cartilage-bone grafts (fig. 72, 73). Since lyophilized bone does not have any osteogenetic properties, the presence of residual periosteum is essential for the transformation processes [*Sailer,* 1980b]. In the absence of periosteum, i.e.



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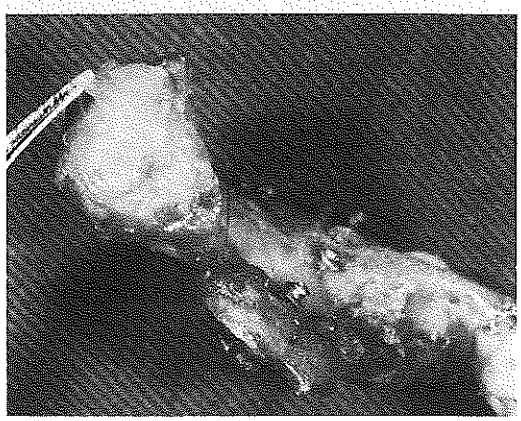

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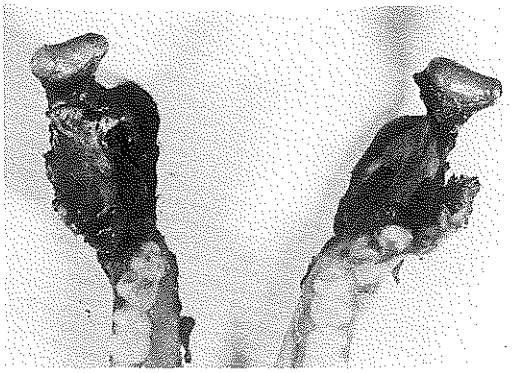


e

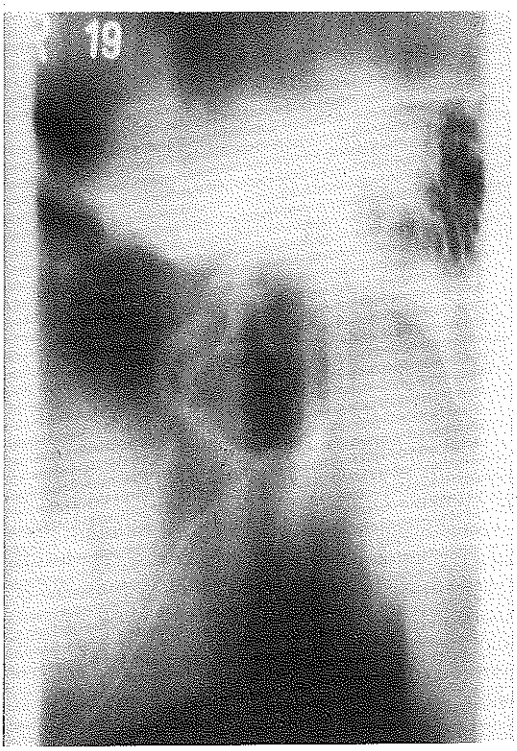
Fig. 71. Articular reconstruction by means of similar grafts of lyophilized rami including condyles in experiments on monkeys. From *Sailer* [1980a]. The resected mandibular ramus was replaced immediately by a similar ramus graft of lyophilized allogeneic material. On re-opening the intact joint capsule 39 days after operation a normal upper and lower articular joint space was found **(a)**. The disc was in the correct position and the articular surface was intact **(b)**. The lateral pterygoid muscle was attached to the graft **(c)** and the articular surface was covered with a fine membrane of connective tissue **(d)**. Similar symmetrical conditions **(e**, reconstruction on the right) are unlikely to have been achieved with autogeneic bone (rib, pelvic crest).

after epiperiosteal resections, the additional transplantation of osteogenetic medullary bone from the iliac crest is necessary.

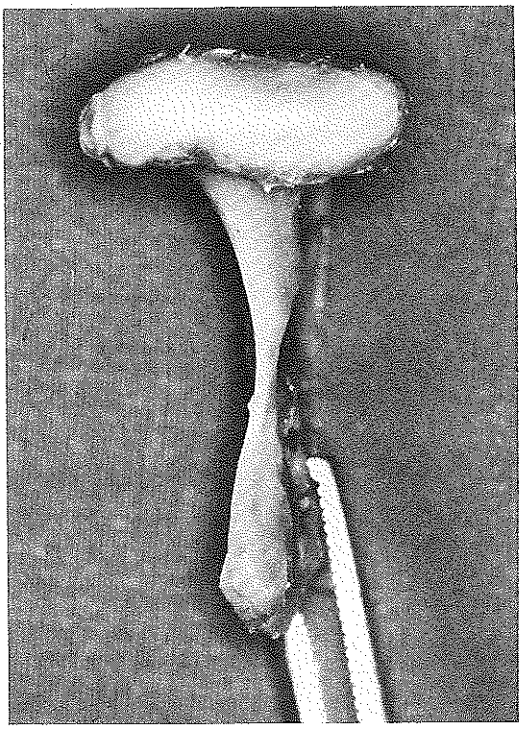
Oral, Parodontal and Pre-Prosthetic Surgery

Lyo-cartilage was first used in stomatological surgery for the filling of residual cavities [*Zurbuchen* et al., 1959] following mandibular cystectomies. This is regarded as a feasible application; it seems likely that a cavity filled with lyo-cartilage would be less susceptible to infection that one filled with blood. The disadvantage of using lyo-cartilage for this purpose is the subsequent slow rate of calcification and ossification. It is therefore indicated only if there is no danger of spontaneous fracture.

A further application is in parodontal surgery, namely in the treatment of deep pockets exhibiting inflammatory alterations. In such cases the lyo-cartilage can be rehydrated with any preferred antibiotic, which then acts in situ as an inert antibiotic carrier, which, because of its non-vascular structure, does not



72a



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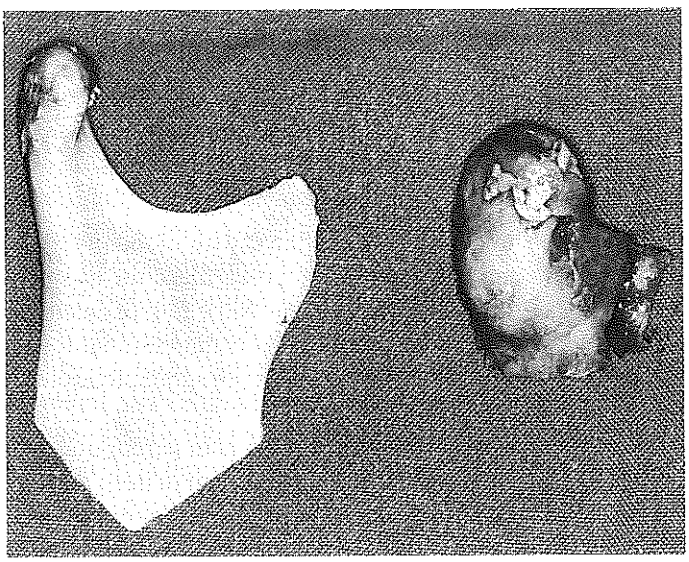
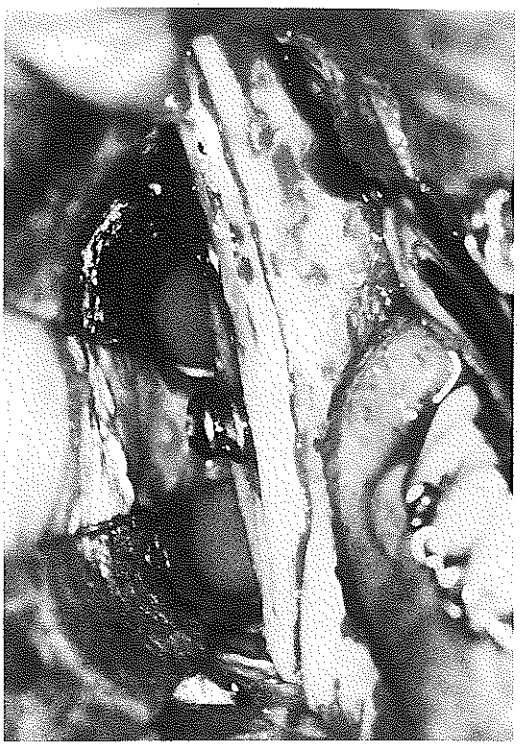


72b

Fig. 72. Articular reconstruction by means of a lyophilized cadaveric cartilage-bone graft of the mandible. Condylar resection due to a cystoid tumorous lesion (**a; b** right) and replacement by a ramus graft of lyophilized allogeneic material (**b** left). The intact cartilage surface (**c**) is placed in loose contact with the non-resected articular disc. Fixation of the graft, inserted trans-orally, is achieved by means of three compression screws inserted trans-buccally through a 5-mm cutaneous incision (**d, e**). No intermaxillary fixation was performed in this case.

(For legend to fig. 73, see page 111.)



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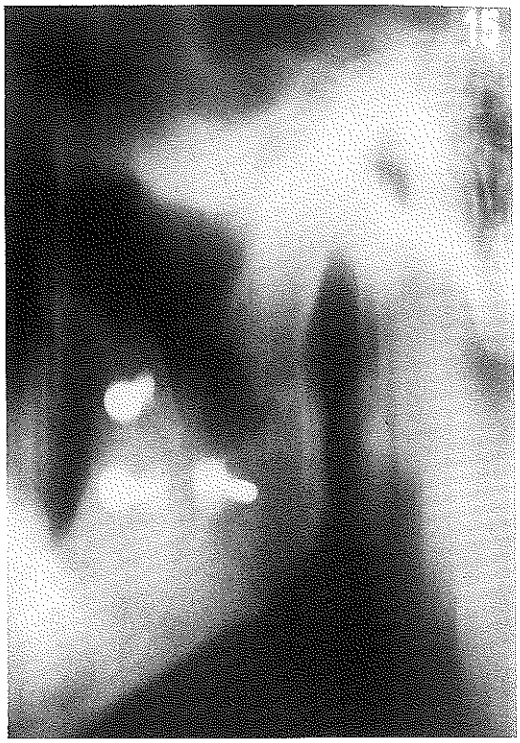

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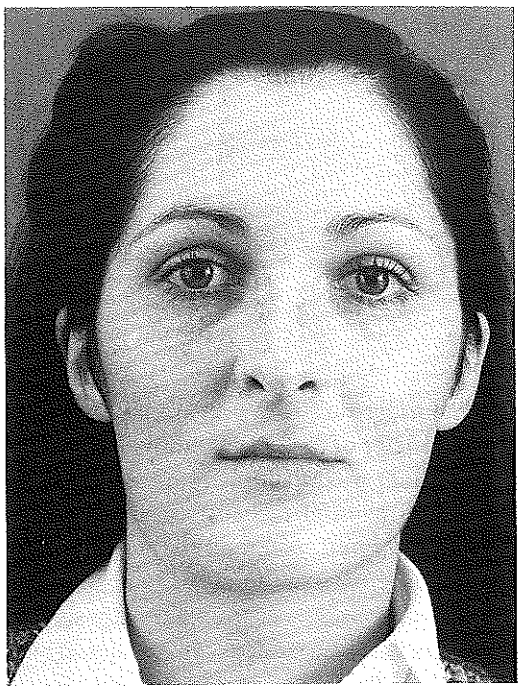
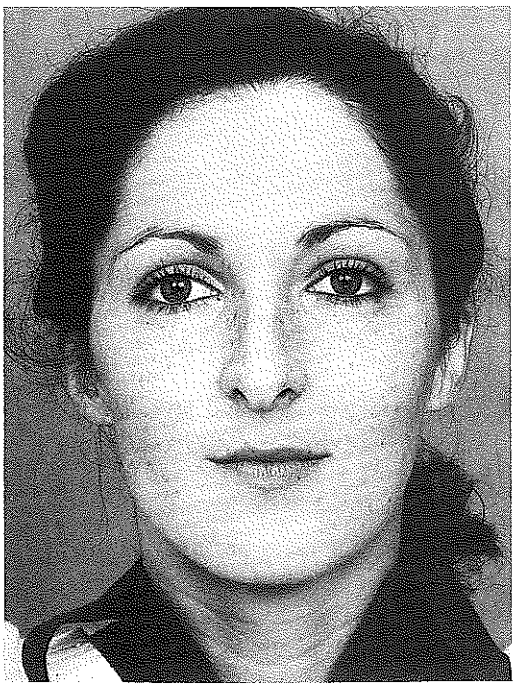
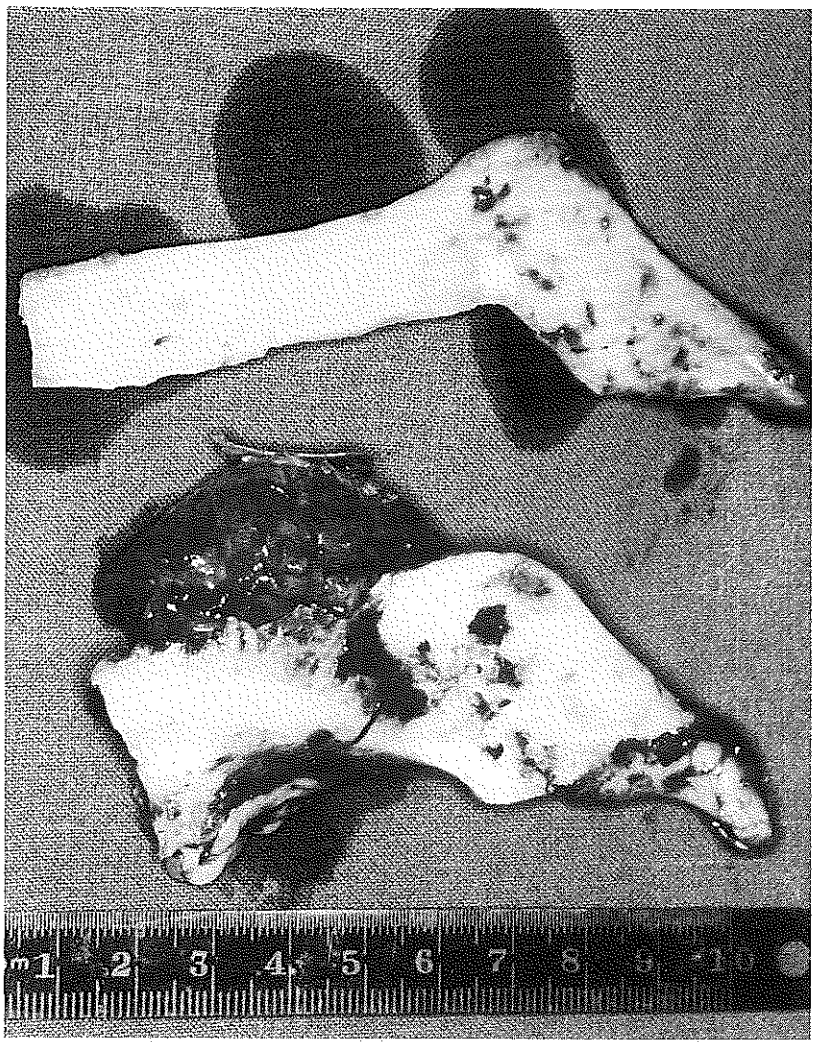


73a



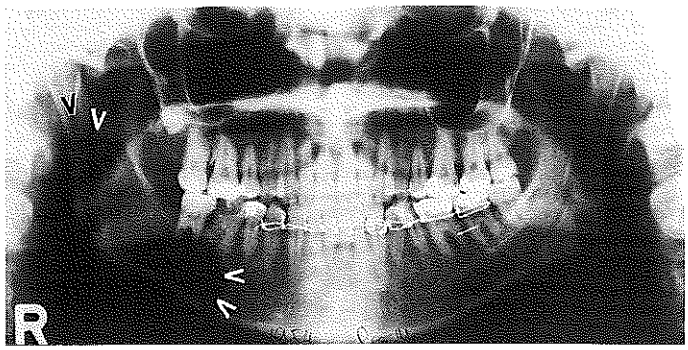
73b



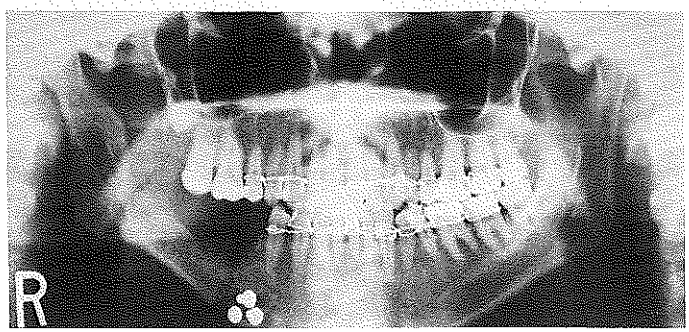
73c

become infected itself, but still calcifies and ossifies following elimination of the infection (fig. 74a–c).

The possibilities of using lyo-cartilage in pre-prosthetic surgery are many and varied. Of note is the reconstruction of alveolar process defects following fractures (fig. 75a) and the resection of tumours. In such cases the normal procedure is to prepare holes around the perimeter of the defect and then to fix the lyo-cartilage grafts with wire or slow-resorbing suture material such as Dexon. Long needles are required to thread the wire through the cartilage material in order to ensure that in the event of pressure from a prosthesis perforations will be avoided.



73d



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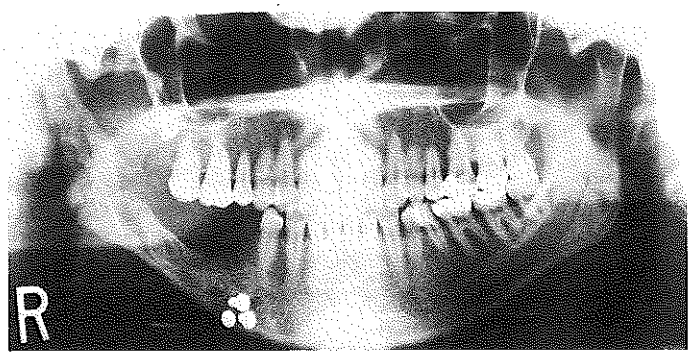
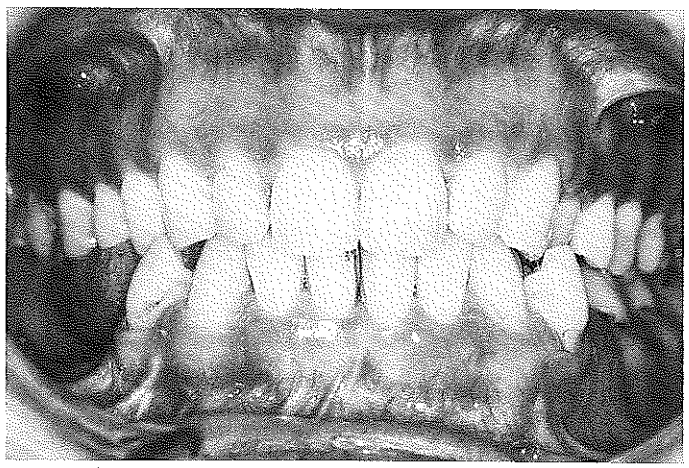
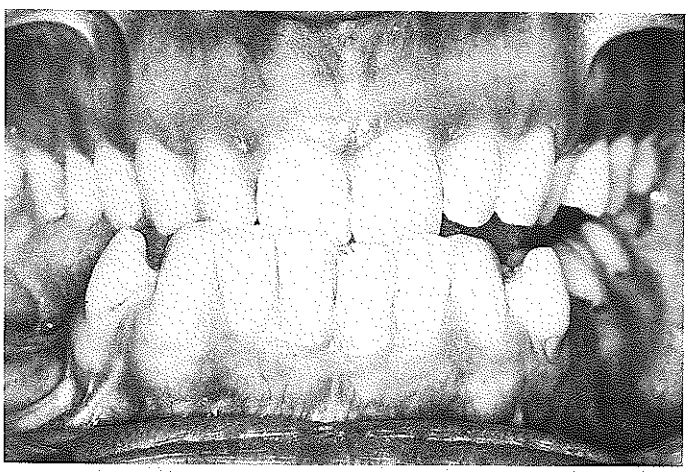


73f

Fig. 73. Reconstruction of the right mandible including condyle by means of a lyophilized cadaveric mandible. From *Sailer* [1980b]. Female patient (25) with distention in the region of the right mandible **(a)** due to an odontogenic fibroma extending into the body and ramus region (**d**, arrows). After resection of the right mandible (**c**, below), reconstruction was performed by grafting lyophilized allogeneic mandible (**c**, top). Fixation was accomplished by means of compression screws **(e)**. Surgery was effected exclusively trans-orally. After 1 year the graft is largely integrated and revitalized **(f)**. Perfect articular function, including anterior translation in the right joint **(g, h)** was achieved. Additionally an excellent aesthetic result 3 years post-operatively is evident **(b)**.



73g



73h

No fixation is necessary if lyo-cartilage slices can be inserted under minimally mobilized periosteum for filling of alveolar process undercuts.

Lyo-cartilage is also ideal in cases of scaphoid atrophy of the mandible in the molar area with the remaining anterior dentition still present (fig. 75b). Here and with circular augmentation (fig. 75c, d) Dexon is preferred to fix the graft as closely as possible to the bone, i.e. with a minimum of intervening dead-space. The closer the contact between cartilage and bone, the sooner clacification and ossification will begin, starting from the surface in contact with the bone. A prosthesis can be fitted 3–4 weeks post-operatively; frequent check-ups during the first 3 months and, if necessary, relining of the prosthesis to avoid pressure at specific points are recommended.

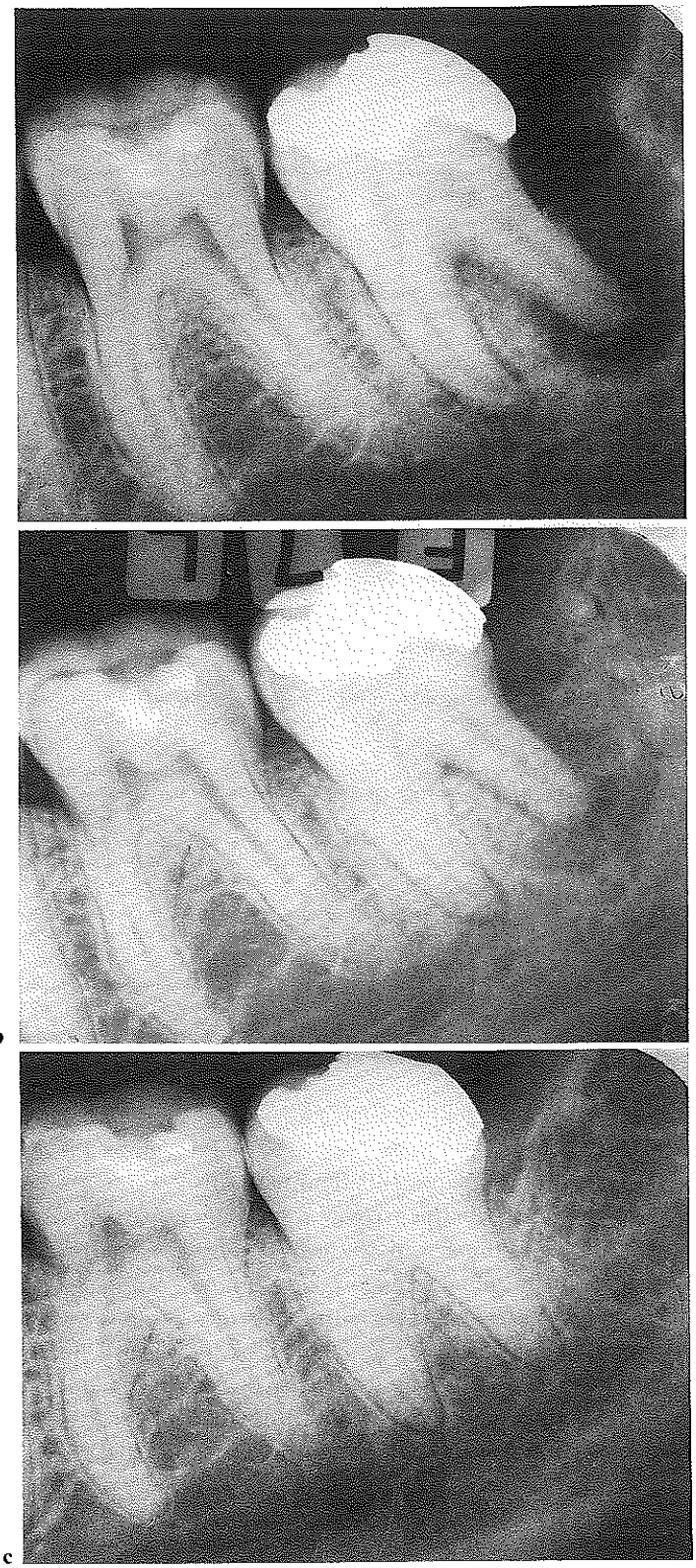
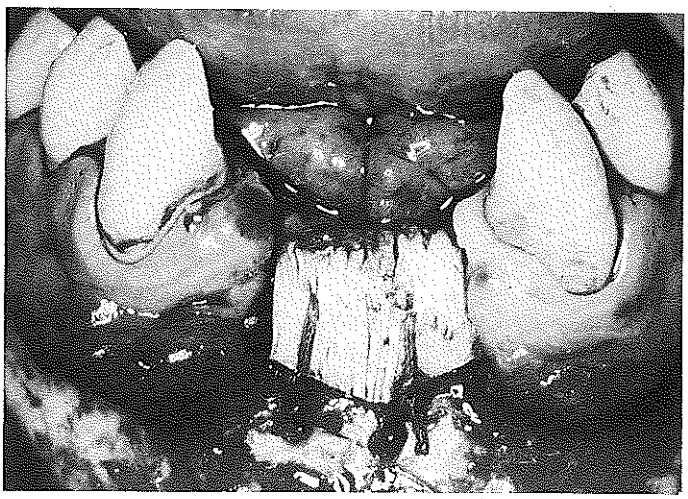
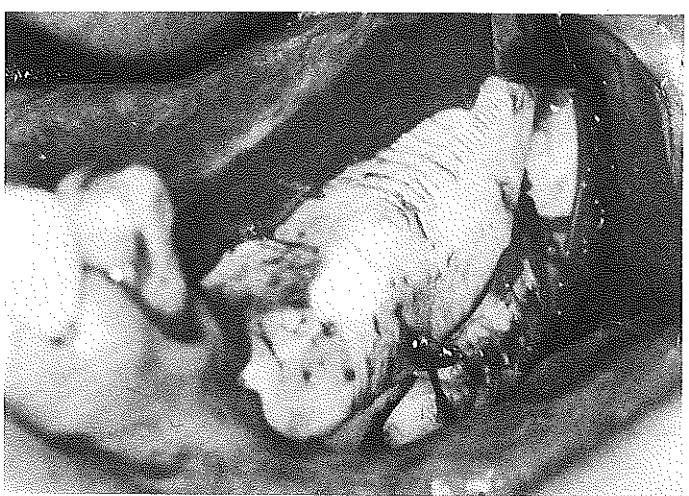


Fig. 74. Transplantation of lyo-cartilage into an infected area. Osteolysis, suppuration and fistula formation in the region of the lower second molar's distal root, with a 4-year history **(a)**. After carefully curetting the lesion, chips of lyo-cartilage were inserted into the defect. These show partial ossification after 2 years **(b)** and complete ossification after 8 years **(c)** with restitution of the periodontal space. The molar in question is vital.



75a

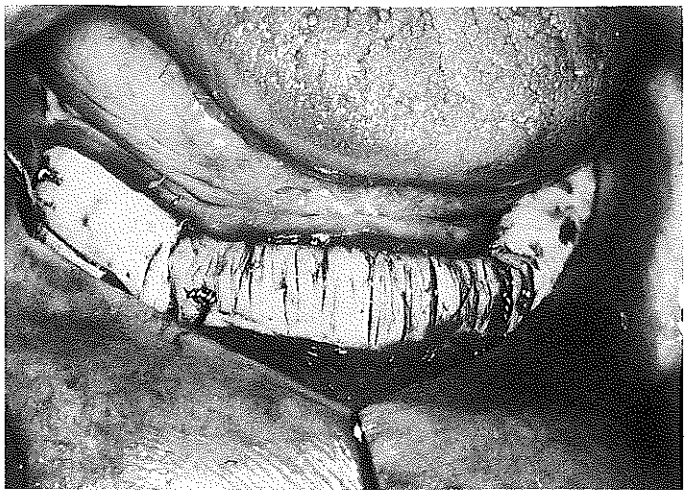


75b

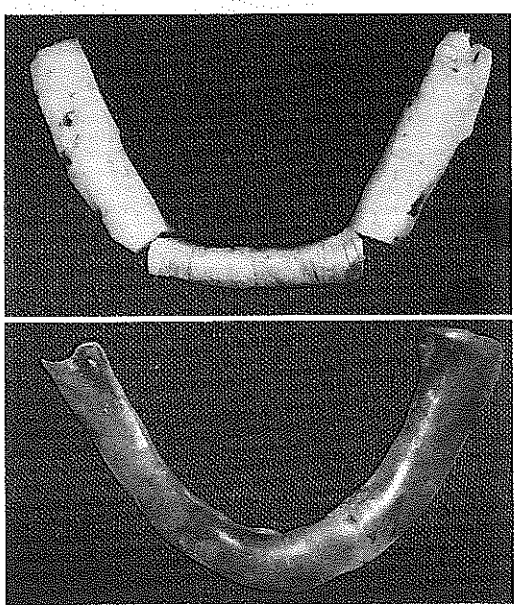
Fig. 75. Reconstructions in the region of the alveolar process. The illustrations show the possibilities of reconstruction in the frontal area after trauma **(a)**, in the area of the molar teeth following atrophy caused by a prosthesis **(b)** and in the case of circular atrophy of the alveolar process **(c)**. With the aid of a plastic template **(d**, bottom) prepared prior to surgery, the size and the position of the pieces of lyo-cartilage **(d**, top) can be determined extraorally in order to simplify the operative procedure. The fixation material used may be either long-term resorbable suture material (e.g. Dexon) **(a, b)** or wire **(c)**. To prevent the development of perforating pressure points in the covering mucosa, the fixation material is never taken across the surface of the cartilage in areas serving as a base for the denture. It is always taken through the cartilage away from the pressure zone **(a–c)**.

Correction of Secondary Cleft Deformities

Cleft lip and palate patients require multifaceted specialist treatment over a long period of time. By the age of 20 such patients have frequently undergone 5–8 operations. The secondary surgical interventions for the purpose of acquiring autogeneic bone or cartilage, for example in cases of primary osteoplasty, Le Fort I osteotomy of the maxilla, the closure of residual palatal and alveolar clefts, and the occasionally multiple corrections of the typically deformed asymmetrical nose, are particularly uncomfortable for the patient. Allogeneic sustentacular tissue in the form of deep-frozen and lyophilized bone and cartilage have therefore been in use for many years in the Zürich maxillo-facial surgery clinic for the correction of secondary cleft deformities [*Obwegeser,* 1969, 1970; *Obwegeser and Chausse,* 1975; *Obwegeser and Edlan,* 1967; *Sailer,* 1976, 1979].



75c



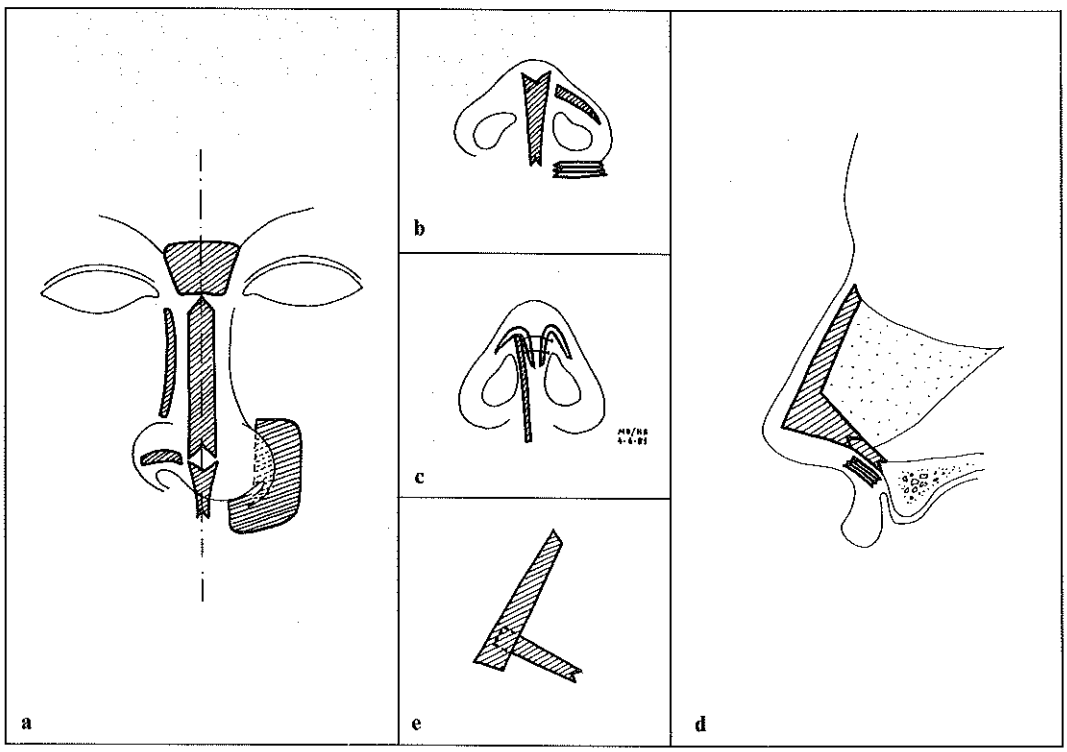
75d

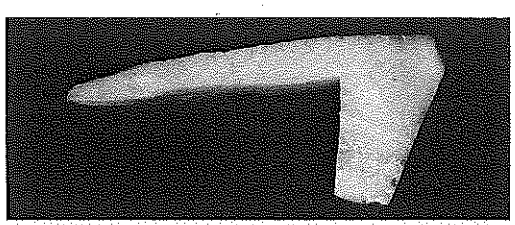
Nose Correction in Cleft Patients

Between 1966 and 1980, 105 lyo-cartilage transplants were carried out on 77 patients in various regions of the nose (fig. 76a–e). In 50% of them, lip and nose corrections were performed simultaneously. The shape of the transplants depended on the deformation to be corrected (fig. 76a–c). The most frequent location (62 implants or 59%) was the columella, followed by the ridge of the nose (21 implants or 21%), the base of the alae (14 implants or 13.3%) and the alae (8 implants or 7.6%). Check-ups were carried out by palpation, by reference to standardized photographic projections (e.g. full face, profile from both sides, face from below), and cephalometric X-rays. Palpation of the transplant was only possible in the region of the columella and the dorsum of the nose. The grafts in the region of the dorsum were all mobile since they had not been implanted subperiosteally on the nasal bones, but epiperiosteally. This procedure is preferred in order to ensure mobility in the caudal region of the nose.

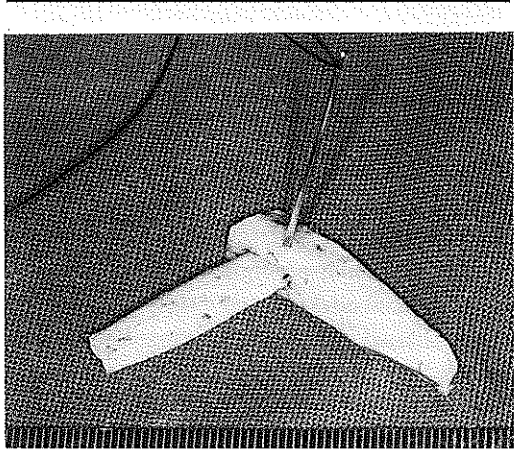
Implants to the ridge of the nose should always be L-shaped (fig. 76d, f). If the access route described by *Rethi* [1929] is selected, the L-shaped implant can be inserted caudally; straight pieces combined to L-shaped grafts (fig. 76e, g), equally stable, can be inserted via a transfixion incision.

76





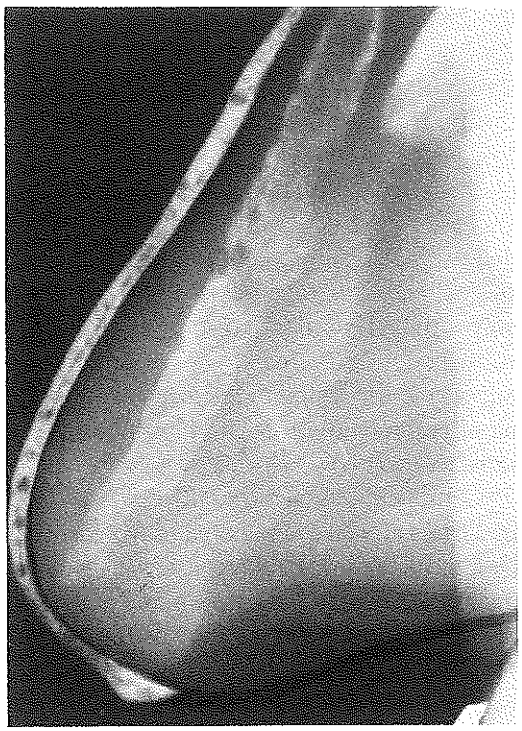
f



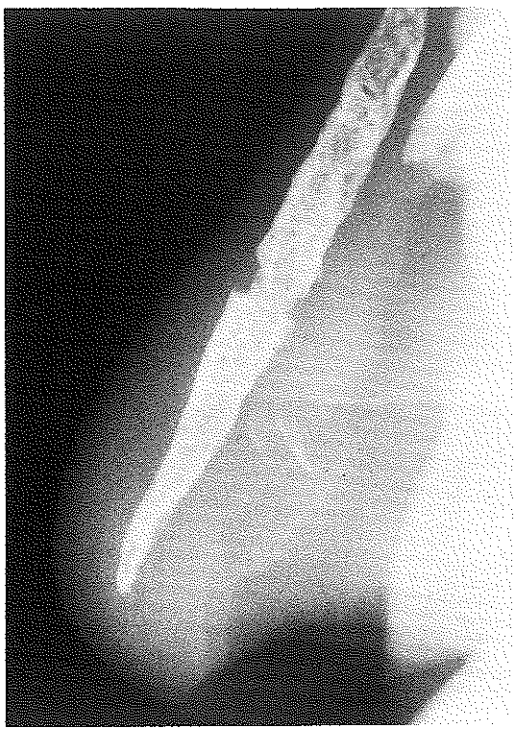
g

Fig. 76. Localization and potential application of lyo-cartilage in the correction of asymmetrical noses in patients with cleft lip and palate. Apart from cosmetic nasal surgery, lyo-cartilage implants in the region of the alae **(a, b)**, the columella **(a–c)** and the ridge of the nose in the form of a straight **(a)** or L-shaped **(d–g)** piece are often necessary in such cases due to the severe asymmetry and shortened columella typically encountered. In open-nose surgery L-shaped pieces trimmed to size **(f)** can be used, while combinations of pieces **(e, g)** are more suitable for implanting when the transfixion incision is used.

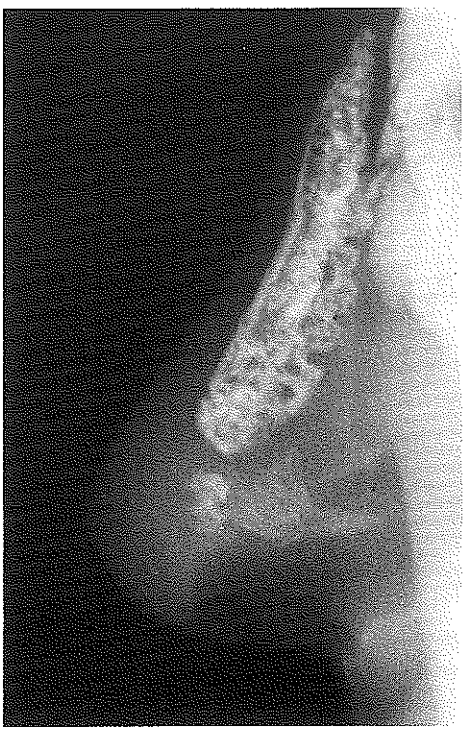
Fig. 77. Calcification and ossification in the subcutaneous bed. 2 years after operation **(a)** there is already clear evidence of mineralization, which 2 years later has practically been replaced by ossification with incipient corticalization **(b)**. The L-shaped piece of cartilage is located epiperiosteally and thus guarantees mobility. In the case of the combined pieces **(c)** both ends calcify but are still slightly mobile in relation to one another in the region of the tip of the nose.



77a



77b



77c

No clinical signs of resorption of these implants were observed; after approximately 2 years all these implants showed signs of calcification on the X-rays (fig. 77a–c).

A slight deformation tendency in the caudal region of straight, non-L-shaped implants was observed in those cases in which the anterior nasal spine was not used for support, despite the fact that considerable tension of the soft tissue in the region of the upper lip indicated that this support was necessary. In one case the implant was removed after 5 months because of this planning and technical error and replaced by an L-shaped graft from the pelvic crest, which in turn underwent severe resorption probably on account of the tension within the soft tissues. It was

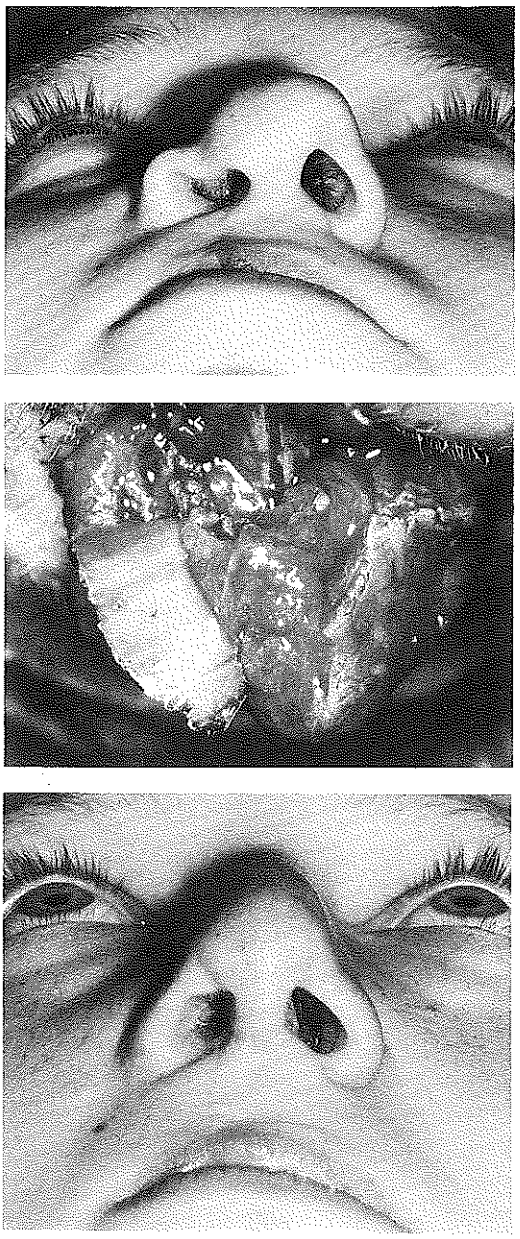


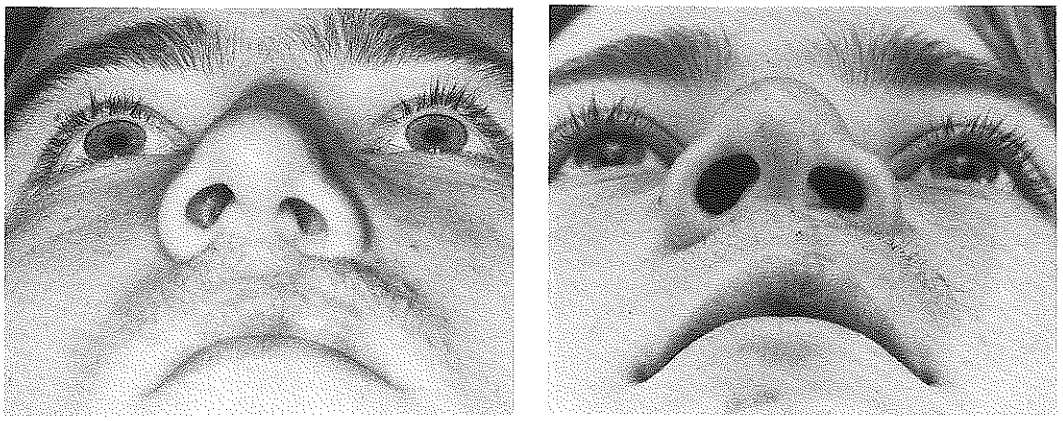
Fig. 78. Transplantation of lyo-cartilage in the region of the nasal alae. The asymmetry typical of the cleft nose **(a)** cannot always be corrected in a one stage procedure. Following total septorhinoplasty, the alae region was corrected **(c)** during a subsequent intervention using lyo-cartilage **(b)**.

not possible to palpate delicate implants in the region of the alae (fig. 78a–c), however, the contours corresponded predominantly to the immediate post-operative result as late as 3½ years post-operatively. In the case of implants in the columella it was found that the very fine fleur-de-lis constructions used for delicate tip-defining points were not entirely successful, we therefore changed to considerably stronger swallow-tail-type lyo-cartilage constructions (fig. 76a, b) for this purpose. Support in the region of the alae base and the nasal entrance (fig. 76a, b, 79a, b) was very satisfactory.

Complications occurred in very few cases: in 2 cases (1.9%) a small area of lyo-cartilage (4–6 mm) became exposed a few days post-operatively, but in both cases – one in the site of transfixion and one in the nostril dome, – subsequent healing occurred spontaneously. In 1 case (0.95%) an abscess developed in the nasal tip, but healed following neomycin irrigation on three separate occasions. The abscess was apparently unrelated to the lyo-cartilage implant in the nasal ridge. Loss of lyo-cartilage implant, as a result of infection did not occur in any case of nose correction in cleft lip and palate patients.

Reconstruction of Palate and Alveolus

Only *Stadnicki* et al. [1965] and *Krajnik* et al. [1978] have reported on the use of lyophilized cartilage for the closure of palatal defects. Our experimental studies with monkeys (chapter 4) show that not only allogeneic but also xenogeneic cartilage is ideally suited for palatal reconstruction. Under experimental conditions there was clear evidence of exceptionally rapid bony transformation of the cartilage, as this was located between two



a

Fig. 79. Transplantation of lyo-cartilage for correction of asymmetry of the floor of the nasal entrance. The posterior displacement of the base of the alae **(a)** can frequently be rendered symmetrical by implanting lyo-cartilage pieces **(b)**.

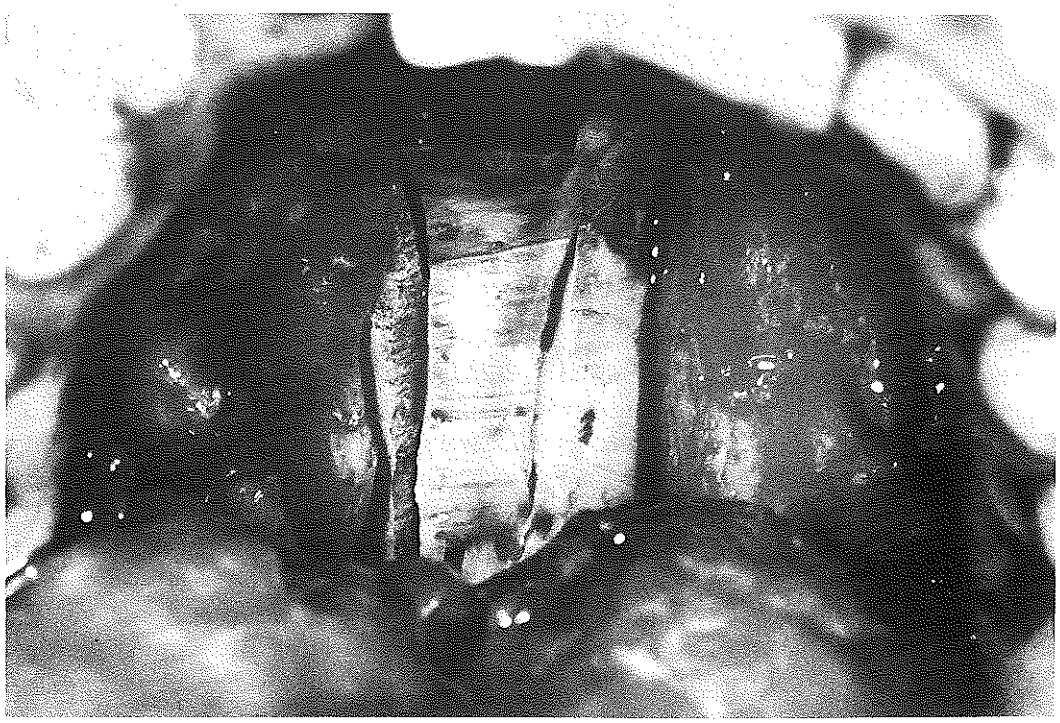


Fig. 80. Closure of the hard palate in adults using lyo-cartilage. The nasal mucosa is already closed, the pieces of lyo-cartilage are in immediate contact with the palatal bone.

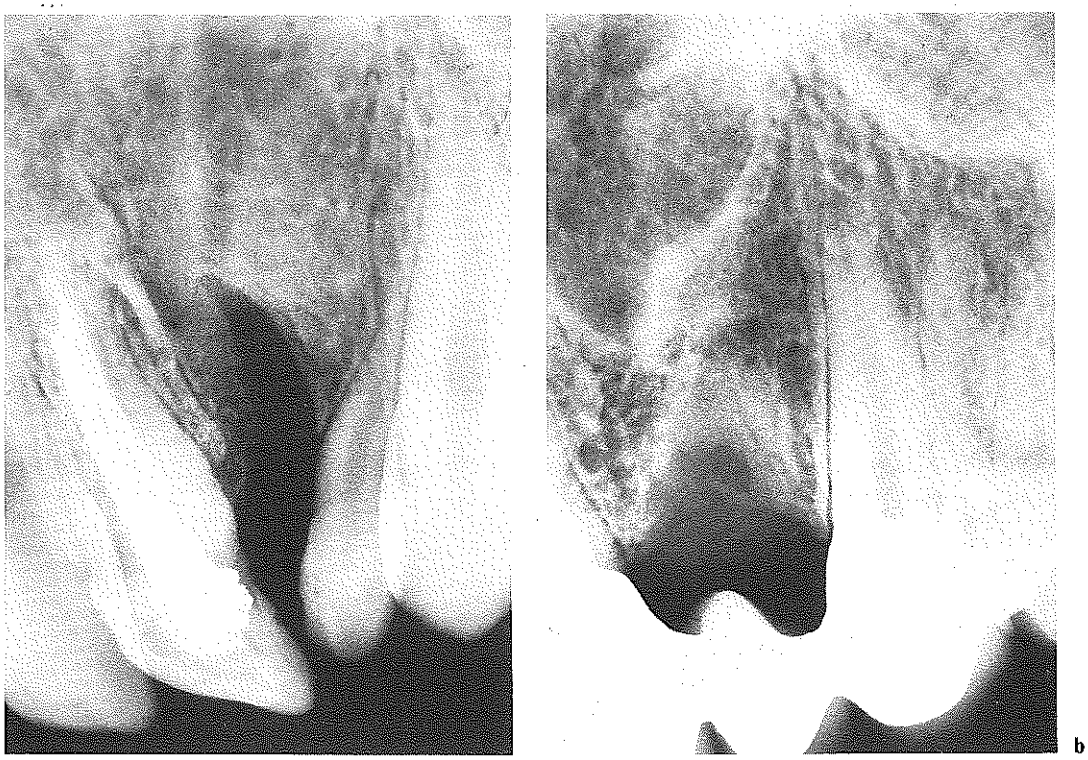
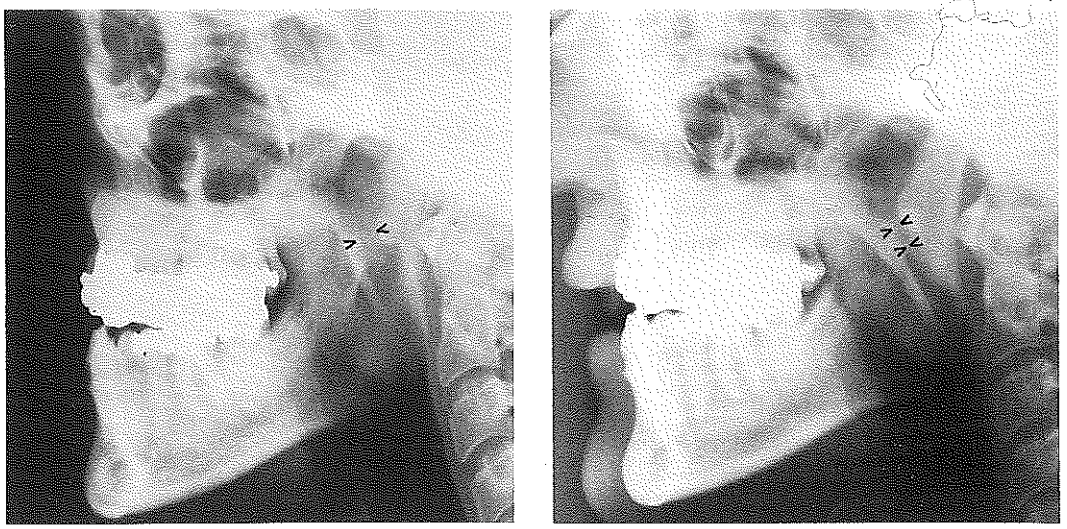


Fig. 81. Reconstruction of the alveolar process in the cleft region with lyo-cartilage to ensure favourable conditions subsequent prosthetic work. The peg-shaped tooth besides the defect **(a)** was extracted; 2 years after reconstruction the lyo-cartilage graft is partially calcified and ossified **(b)**. The bridge work was carried out 5 months after operation.

layers of periosteum. To date, no oro-nasal resiudal fistula has occurred following palatal or alveolar fissure correction with allogneic lyo-cartilage. The material is generally inserted in the form of one or several blocks in close contact with the bone and to each other (fig. 80), the surface of the bone having been lightly decorticated beforehand.

Lyo-cartilage has been used hitherto only on adult cleft patients for the reconstruction of the hard palate. With certain defects, e.g. gunshot or explosion wounds, closure of the nasal mucoperiosteal layer is occasionally not possible due to loss of tissue and subsequent cicatrization. In 2 cases the lyo-cartilage 'took' successfully despite being completely exposed on its nasal surface with coverage on its oral surface being effected by palatal mucosa. The cartilage was fixed to the palatal bone with Dexon.

Lyo-cartilage also appears to be suitable for the bridging of alveolar clefts (fig. 81a, b) but good bone contact is again essential. To ensure rapid ossification, light decortication of the bone in the areas of cartilage contact is recommended. If teeth close to the cleft have to be moved orthodontically into the alveolar cleft at a later date, the use of autogeneic



a b

Fig. 82. Retropharyngeal lyo-cartilage implants in cases of velopharyngeal incompetence. Where the soft palate is short or insufficiently mobile **(a)** it is sometimes possible to reduce the velopharyngeal distance **(b)** and consequently the nasality of speech by implanting a block of cartilage under the retropharyngeal mucosa. The arrows indicate the velopharyngeal space before surgery **(a)** and 1 year post-operatively **(b)**. In this case speech was substantially improved.

bone rather than lyo-cartilage is indicated. Lyo-cartilage is indicated if the deficiency in dentition in the region of the cleft is to be remedied by crown and bridge work (fig. 81b).

Grafts for Speech Improvement

Retraction of or irregular relief in the hard palate as a result of primary operative palatal closure leads occasionally to speech defects. In such cases palatal relief levelling can be accomplished by subperiosteal implantation of lyo-cartilage in conjunction with a bridging of the defect in the cleft areas (see p. 118).

In the event of velopharyngeal incompetence as a result of a short soft palate, nasality can be mitigated or eliminated by means of the retropharyngeal submucous implantation of a block of lyo-cartilage, which is best positioned on the vertebral fascia (fig. 82). This method is not indicated, however, in cases with a large velopharyngeal distance and an immobile soft palate. A vertical incision in the posterior pharyngeal wall is used in order to prepare a pocket above the vertebral fascia; generous lateral mobilization is neces-

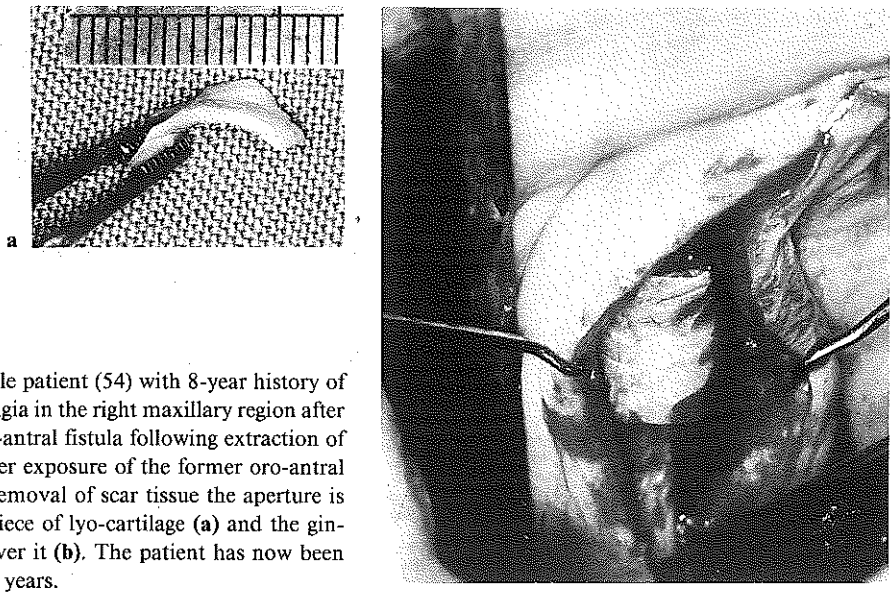


Fig. 83. Female patient (54) with 8-year history of an atypical neuralgia in the right maxillary region after closure of an oro-antral fistula following extraction of a first molar. After exposure of the former oro-antral connection and removal of scar tissue the aperture is covered with a piece of lyo-cartilage **(a)** and the gingiva is sutured over it **(b)**. The patient has now been free of pain for 2 years.

sary. The lyo-cartilage implant (approximately 3 cm long) is then inserted perpendicular to the incision. If the edges of the incision tend to gape and direct closure of the wound is possible only under considerable tension, a Z-plasty is necessary. Dehiscence of a retropharyngeal wound generally remains undetected, but can lead to mild infection of the implant bed and possibly to gradual resorption of the cartilage.

Prevention and Treatment of Neuralgiform Conditions

Cases of neuralgiform symptoms in the maxillary region are occasionally observed emanating from the area of a former oro-antral fistula, which sometimes occurs after the extraction of molars. The cause of this condition may be due to cicatricial contractions located within the bone defect linking the oral and sinus mucosa. It is possible that this tissue contains a neuroma or nerve fibres which have been strangulated or compressed by the cicatricial tissue. The closure of such bone defects with lyo-cartilage after removal of the cicatricial tissue can help to eliminate the neuralgiform condition (fig. 83a, b).

Similar neuralgiform conditions may also develop following radical surgery to the maxillary sinus. *Pfeifer* [1973] therefore removes the cicatricial tissue in the region of the bone fenestration in the canine fossa and closes the defect with autogeneic bone. We in fact close the fenestration with lyo-cartilage not only from the therapeutic point of view but also for prophylactic reasons (fig. 84a, b).

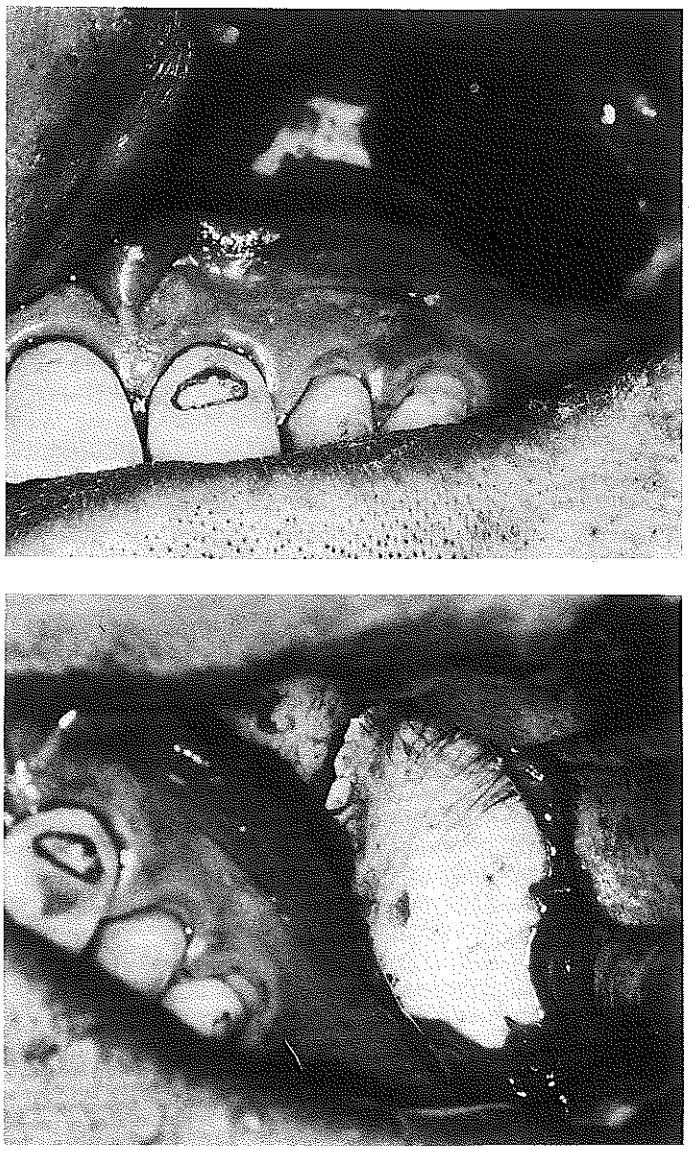


Fig. 84. Closure of a defect in the facial wall of the maxillary sinus after removal of a dislocated tooth. Fenestration in the canine fossa **(a)** which is closed with a piece of lyocartilage **(b)** to prevent transfenestral cicatrization and neuralgic complications. Fixation is carried out with Dexon sutures (through two drill holes).