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Influence of the mode of preparation on the long-term efficacy of homologous costal cartilage implants

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Abstract

Devitalized homologous costal cartilage is widely employed as an implant in the management of the saddle nose. The tissue response induced by the implant is a combination of enveloping fibrosis and implant resorption, which will probably, ultimately, be complete. We have studied the balance between resorption and fibrosis, following different modes of cartilage preparation, in the mouse. Homologous costal cartilage was devitalized by four common methods—irradiation, formalin, glutaraldehyde and alochol. Segments of this cartilage were inserted at separate sites in the subcutaneous plain of the tail. These implants were harvested after one year for histology. Variations in the mode of cartilage devitalization, while inducing variations in the degree of the tissue response, did not influence the balance between fibrosis and resorption. Thus the long term maintenance of tissue bulk following homologous cartilage implantation is not influenced by the mode of preparation. Evidence suggests that the ultimate cosmetic results of autologous and homologous costal cartilage implantation would be much the same, and the use of homologous cartilage must be justified on other grounds.

Introduction

Banked homologous cartilage has proved a popular tissue for nasal implantation. during the 1970s and 1980s the view was widely expressed that this method excited a minimal immune reaction with the consequences that implant resorption was relatively slight (Dingman and Grabb, 1961; Mikhelson, 1962; Alichniewicz *et al.*, 1964; Marquit, 1967; Schuller *et al.*, 1977; McGlynn and Sharpe, 1981). More recently, however, the reservations expressed by earlier workers (Brown, 1940; Gibson *et al.*, 1958) have been restated, with the expectation that all such cartilage implants will eventually totally resorb (Welling *et al.*, 1988). But total resorption does not necessarily mean cosmetic failure provided sufficient fibrous deposition occurs at the implant site to compensate for the loss of bulk (Rasi, 1959; Farrior, 1966; Reck, 1978; Stocksted, 1986; Welling *et al.*, 1988).

Animal studies on the fate of implants of devitalized costal cartilage have shown histological features of a foreign body reaction and have confirmed the trend towards resorption and fibrous encapsulation. There was considerable variation in the intensity of the host response both in relation to the species and to the mode of preparation of the implant (Dingman and Grabb, 1961; Hellmich, 1974; Reck, 1978; Babin *et al.*, 1982; Donald, 1986).

It has been stated that variations in the mode of preservation of homologous cartilage implants will influence its absorption potential (Schuller *et al.*, 1977; Donald, 1986; Lefkovits, 1990). For instance, the results of a national survey on cartilage implantation in head and neck surgery (Donald and Col, 1982) indicated that irradiated cartilage exhibited the least absorption of all preservation methods and even less than autologous cartilage. But, as already stated, the long term cosmetic success of a homologous implant depends not only on the degree of resorption but also on the incidence of fibrous disposition. Ideally one is seeking a process of devitalization by which implant resorption is minimized while fibrous deposition is encouraged.

Our present study considers whether variations in the mode of preparation might influence the balance between implant resorption and fibrous deposition at the implant site. The 'host response' to the devitalized implants will be contrasted to the response to viable autologous cartilage grafts.

Materials and method

Five adult mice were sacrificed and the sternum and all costal cartilage up to the costo-chondral junction were removed in one piece. All muscle was cleaned from the cartilages as thoroughly as possible but it was not practical to remove the peri-chondrium. The individual cartilaginous segments were then separated from the sternum and divided into four approximately equal groups to be prepared in the following manner:

Group 1 was immersed in Ringer's solution and irradiated to 50,000 Gy.

Group 2 was immersed in glutaraldehyde solution 5 per cent.

Group 3 was immersed in formalin solution 5 per cent.

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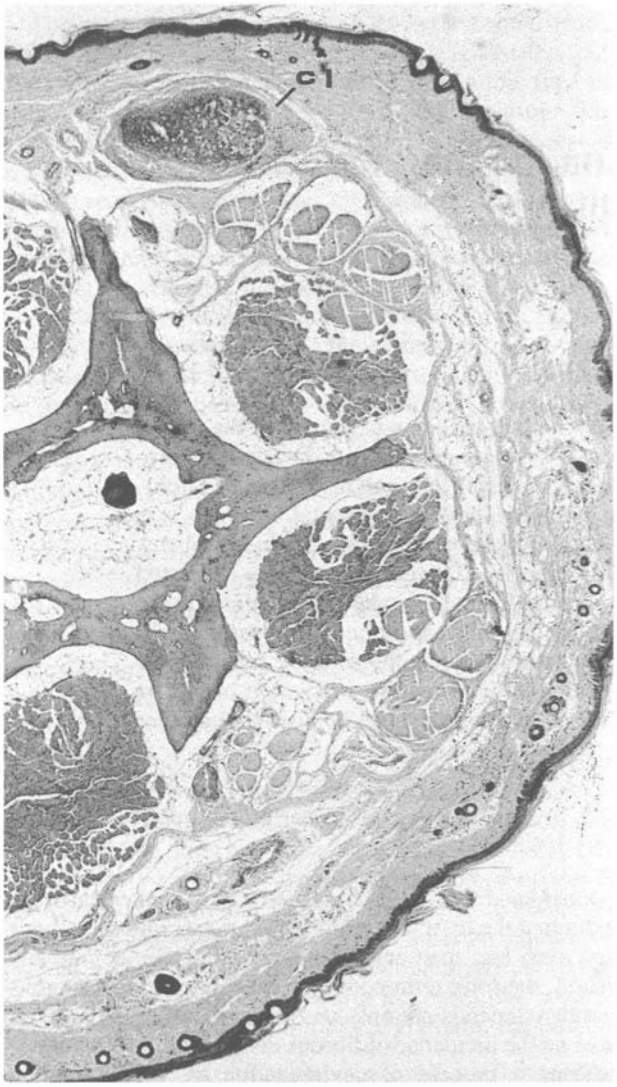

Fig. 1

Portion of a cross section of a mouse tail showing the placement of the cartilage implant (C1) in the subcutaneous plane. Haematoxylin and eosin ×25.

Group 4 was immersed in ethyl alcohol solution 5 per cent.

After one month all cartilage segments were thoroughly washed and transferred to Ringer's solution containing gentamycin 50 micrograms per ml and amphotericin 50 micrograms per ml to prevent secondary contamination.

Segments of costal cartilage of approximately 1 mm in diameter were then identified. From these cross-sectional discs of approximately 1 mm thickness were obtained. Cartilage implants of approximately 1 cubic mm were thus produced.

Thirty adult mice were utilized in this study.

The mouse was anaesthetized and five separate stab incisions were made at intervals along the dorsal mid line of the tail. A subcutaneous plane was readily established and a single cartilage implant was inserted through each incision. The implant was moved away from the site of the incision to lie deep to intact skin. The incision was then sutured. Apart from the cartilage implants a fifth implant of silastic of similar dimensions was also inserted through a separate incision. The site of each individual cartilage implant was then identified accurately by a mark made with a needle tipped with indian ink. The implant sites

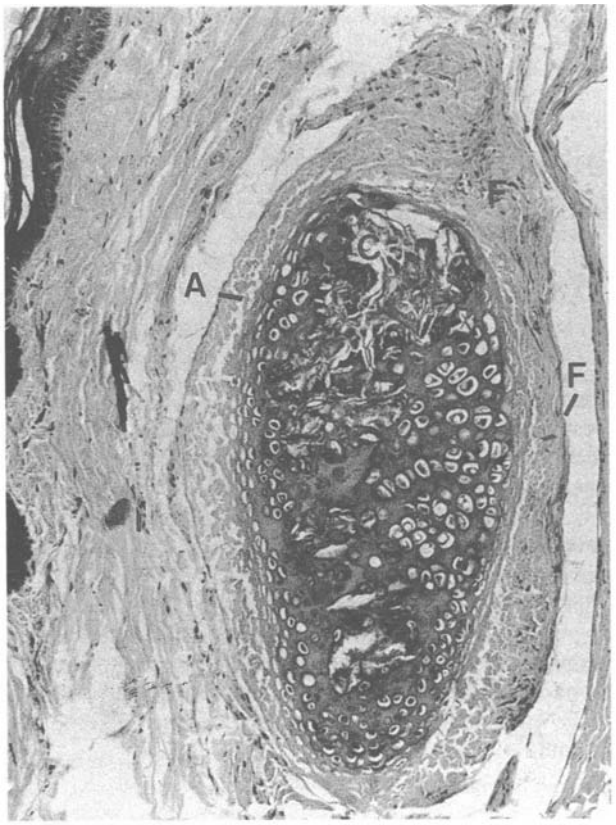

Fig. 2

Cross section of cartilage implant (C) showing minimal fibrous capsule formation (F) and minimal peripheral absorption (A). Haematoxylin and eosin ×100.

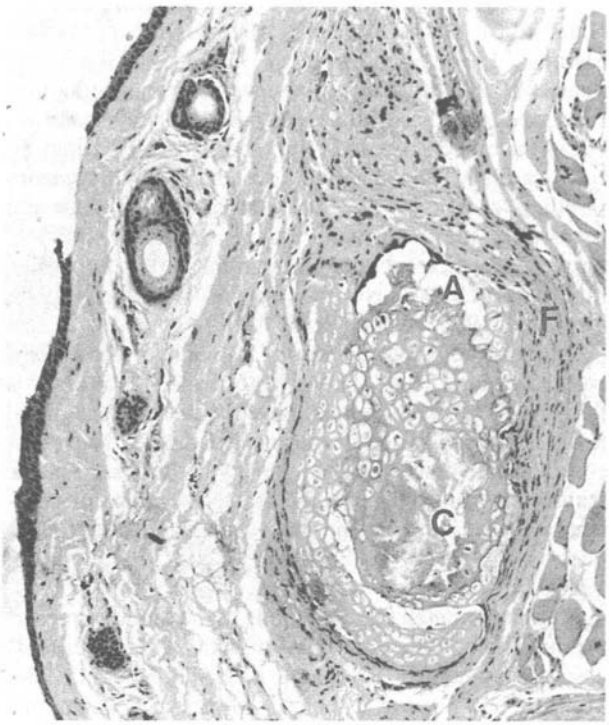

Fig. 3

Cross section of cartilage implant (C) with fibrosis (F) and absorption (A) categorized as moderate. Haematoxylin and eosin ×100.

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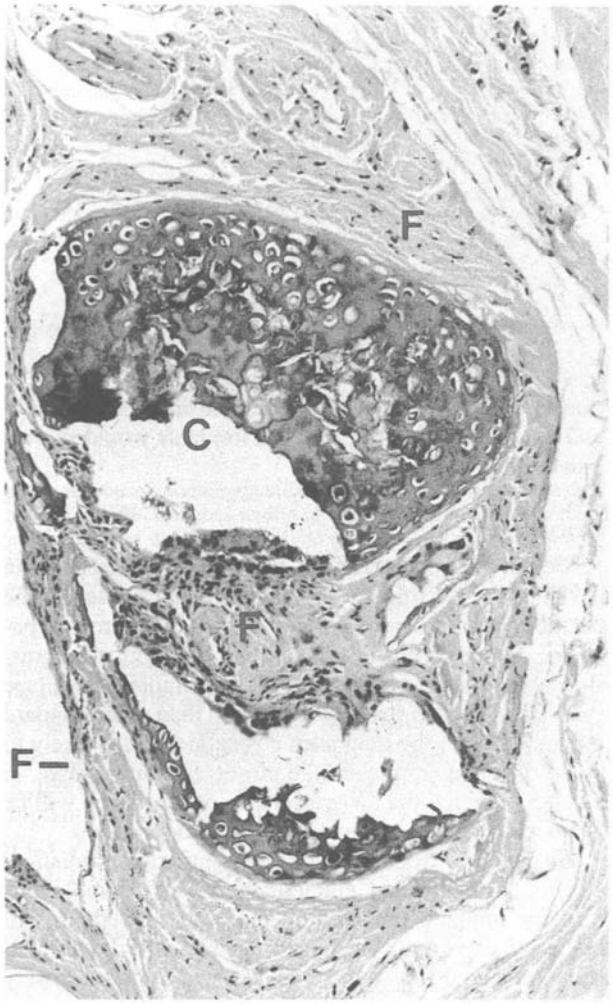

Fig. 4

Cross section of cartilage implant (C) with peripheal fibrosis (F) categorized as mild, but with further fibrous deposition in the centre of the implant where moderate resorption has occurred. The gradation of the fibrosis relates to the thickness of the fibrous layer between the implant and the central vertebral column. Haematoxylin and eosin ×100.

were inspected at regular intervals post operatively to note any evidence of extrusion and the indian ink markings were freshened up if necessary.

A graft of autologous cartilage was obtained from the xyphisternum and was inserted into the subcutaneous plane of the dorsal surface of the pinna. All mice were sacrificed after one year and the segments of tail containing the readily identifiable implant sites were removed for serial section and staining by haemotoxylin and eosin (Fig. 1). Two aspects of the tail cross sections were considered:

1. The thickness of the fibrous capsule laid down around the cartilage implant: The capsule and implant formed a discrete focus in the subcutaneous tissues and it was decided that the thickness of the capsule could be best judged in the area between the implant and the central vertebral column. On account of variations in the configuration of the fibrous capsule in implant shape and size and the somewhat focal nature of implant resorption evaluation of the histological results was inevitably based on careful visual impression rather than accurate measurement.

Fibrous deposition was in general slight and in consequence graded into three categories:

Minimal up to 10 per cent of the diameter of the implant focus (Fig. 2).

Mild up to 30 per cent of the diameter of the implant focus.

Moderate up to 50 per cent of the diameter of the implant focus (Fig. 3).

2. The degree of resorption of the implant that had taken place. This was again graded into three categories but on a score basis as follows:

Minimal implant resorption	+
Mild implant resportion	++
Moderate implant resorption	+++
Total implant resorption	+++++ (Fig. 4)

Autogenous cartilage grafts in the pinna

Serial sections of the graft sites were cut and stained with haemotoxylin and eosin. The histological structure of the cartilage implants were compared with normal cartilage of the pinna (Fig. 5).

Results

Homologous implants.

Histological picture

All implant foci show a similar appearance. There is a fibrous capsule around each implant and a generally mild and somewhat focal inflammatory cell response which is composed of lymphocytes and occasional giant cells. This inflammatory response is of a 'foreign body' type.

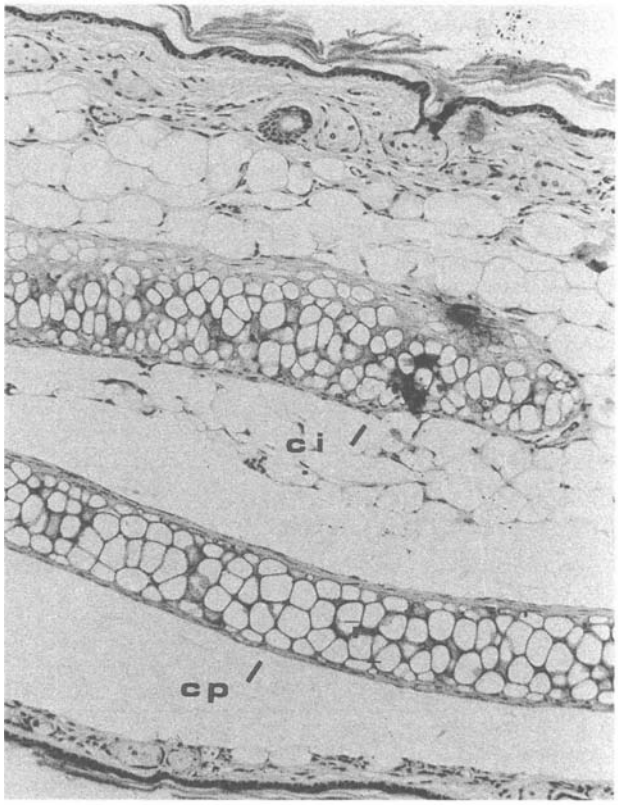

Fig. 5

Portion of cross section of mouse pinna showing the normal pinna cartilage (cp) and the autogenous viable cartilage implant taken from the xyphisternum. Minimal inflammatory reaction is evident. Haematoxylin and eosin ×100.

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TABLE I
EXTENT OF RESORPTION OF IMPLANT

		Irradiation		Glutaraldehyde		Formalin		Alcohol	
Minimal	Total (50%)								
+		16	(54%)	20	(67%)	17	(57%)	7	(23%)
Mild	Total (20%)								
++		7	(23%)	6	(20%)	7	(23%)	4	(13%)
Moderate	Total (30%)								
+++		7	(23%)	4	(13%)	6	(20%)	19	(64%)
Total Resportion									
+++++									

CH1—SQR = 22.60000; P = 0.0009.

There is a mild degree of focal resorption in the periphery of some of the implants, but nowhere is this marked. The peripheral layers of the implants are rarely breached except where they have been cut. In these zones there is often ingress of granulation tissue into the central medullary region with obvious local resorption.

Ten (8 per cent) of the 120 homologous implants had extruded.

One implant had become calcified.

Extent of resorption of implants (Table I)

Fifty per cent of all implants showed only a minimal degree of resorption and none showed more than moderate resorption, 64 per cent of these being prepared in alcohol.

Extent of fibrous deposition (Table II)

Forty per cent of all implants showed only a minimal degree of capsular formation, the thickness of the capsule never exceeding more than 30 per cent of the cross sectional area of the focus, 53 per cent of these being prepared in alcohol.

While the overall impression after one year is of a weak 'foreign body' reaction around all implants, a greater time interval is required to confirm the expectation that total implant resorption will ultimately occur.

It would appear that the 'host response' is least active against those implants devitalized by irradiation and most active against those prepared in alcohol. These results are statistically significant.

From the clinical point of view, however, the outcome is much the same, with the degree of implant resorption being counterbalanced by the extent of the fibrous deposition.

Autogenous cartilage implants

There is no evidence whatsoever of a foreign body reaction around autogenous implants in the pinna of the mouse and the indications are that these implants would survive indefinitely.

Discussion

We have been unable to demonstrate that variations in the mode of cartilage preparation can influence the balance between resorption and surrounding fibrous deposition. Although our results support the clinical impression from Donald's survey (1983) that irradiated cartilage shows a lesser incidence of resorption than other standard methods tested, the long term cosmetic result is likely to be no different from cartilage preserved, for example, in alcohol in which the far greater rate of resorption will be balanced by greater degrees of fibrous deposition.

We conclude from our two studies, utilizing irradiation, alcohol, formalin and glutaraldehyde, that homologous cartilage implants prepared by any of these methods will be easy to carve and, when introduced into the tissues, will show little tendency to distort. They will initiate a closely similar pathological response of varying intensity and the ultimate cosmetic result is likely to be the same. Although cialit was excluded from the *in vivo* studies because cartilage prepared in this solution was less easy to handle (McGlynn and Sharpe, 1981; Ironside, 1982; Adlington *et al.*, 1989) the histological results of previous work on cialit treated cartilage implants inserted into the pinna of the mouse suggested that the host response was much the same (Fig. 6). Hellmich, in fact, who has extensive experience of cartilage implantation in the nose, considers that chemical pre-treatment with cialit should be the first choice (Hellmich, 1974). In contrast to the distinct host response to devitalized cartilage, we find the response to vital autologous cartilage virtually nil.

Lefkovits (1990) recently published results on 27 irradiated cartilage implants followed up to 27 months showing no evidence of resorption. Since 1977 we have inserted 50 irradiated implants into the nasal dorsum. Five

TABLE II
EXTENT OF FIBROUS DEPOSITION AROUND IMPLANT

		Irradiation		Glutaraldehyde		Formalin		Alcohol	
Minimal	Total 42%								
Up to 10% of focus		23	(77%)	16	(54%)	8	(26%)	3	(10%)
Mild	Total 38%								
Up to 20% of focus		6	(20%)	10	(33%)	18	(60%)	11	(37%)
Moderate	Total 20%								
Up to 30% of focus		1	(3%)	4	(13%)	4	(13%)	16	(53%)

CH1—SQR = 46.52444; P = 0.0001.
Incidence of homograft extrusion 10 out of 120 (8 per cent).

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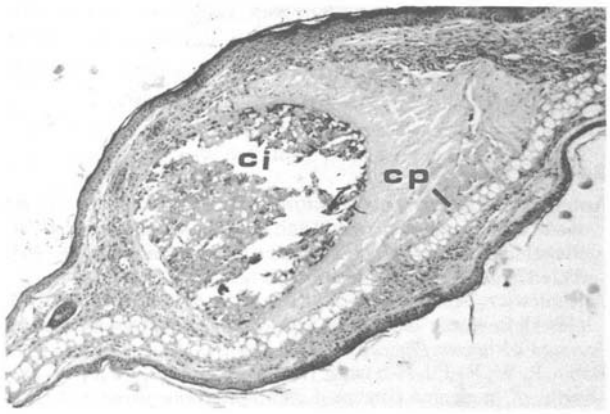

Fig. 6

Cross section of homologous cartilage implant (ci) preserved in cialit in the subcutaneous region of the mouse pinna. The implant has fragmented during histological preparation but the outline is well preserved and indicates minimal absorption after six months. A well established fibrous capsule is evident. Cartilage of pinna (cp) haematoxylin and eosin ×100.

have been replaced on account of excessive resorption. Two patients showed total resorption within two years and in one of these patients a replacement implant was resorbed with equal speed. In other implants the process of resorption is much slower, as demonstrated in an implant removed after five years on account of warping (Figs. 7, 8, & 9), but is probably inexorable. Six additional implants were replaced on account of marked distortion. This produces a total of 22 per cent cosmetic unacceptability which matches 22 per cent recorded by Rasi (1959) and 19 per cent by McGlynn and Sharpe (1981) for homologous implants and 25 per cent and 31 per cent respectively for previous autologous series (Mowlem, 1941; Gibson *et al.*, 1958) in which replacement was required solely on account of distortion. This would indicate, as suggested by Hellmich (1979), that the long term cosmetic results for viable autologous and devitalized homologous costal cartilage would be much the same. While banked homologous cartilage remains a most convenient material for nasal implantation avoiding the need for a 'donor site' operation, concern over HIV and Jacob Creuzfeld disease (Spire *et al.*, 1985; Wilmes *et al.*, 1987; Glasscock, 1988) has forced a re-appraisal of implant policy.

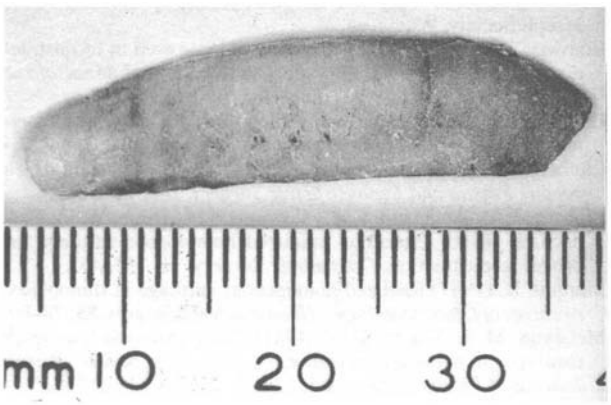

Fig. 7

Homologous costal cartilage nasal implant removed from a patient after five years on account of distortion.

By what other method might the host response to the implant be influenced? Gibson (1959) pointed out that occasionally a devitalized implant becomes surrounded by an adventitial bursa in which it may remain unaltered for many years. He referred to the experimental condition in which prolonged survival of a homograft occurred when cellular contact between the host and the implant is prevented (Medawar, 1948; Woodruff, 1957). The basis of this principle could be applied by wrapping the implant in a layer of autogenous fascia, such as temporalis or fascia lata. The same procedure might also prove beneficial in the use of silastic implants where the extreme inertness of the silicone rubber fails to induce the deposition of a reasonable fibrous capsule which might serve to fix the implant (Brown *et al.*, 1979) and protect the overlying skin from erosion (Braley, 1973) and other undesirable changes (Ham, 1983; Bull, 1987).

Conclusion

We have studied the tissue response induced by costal cartilage implants prepared by four modes of devitalization—irradiation, alcohol, glutaraldehyde and formalin, and conclude that, while irradiation induced least response and alcohol the most, the ultimate long term cosmetic results are the same. Therefore, considering together the conclusions of our previous *in vitro* and present *in vivo* studies, we suggest that, if a bank of devitalized costal cartilage is required, any of the four modes of preparation evaluated will produce an implant which is readily carved with a low incidence of distortion and a comparable long term cosmetic result.

Fig. 8

Cross section of homologous cartilage implant shown in Fig. 7. The irregular deposition of fibrous tissue (f) is evident in the softer medullary zone of the costal cartilage where resorption more readily occurs. Haematoxylin and eosin ×200.

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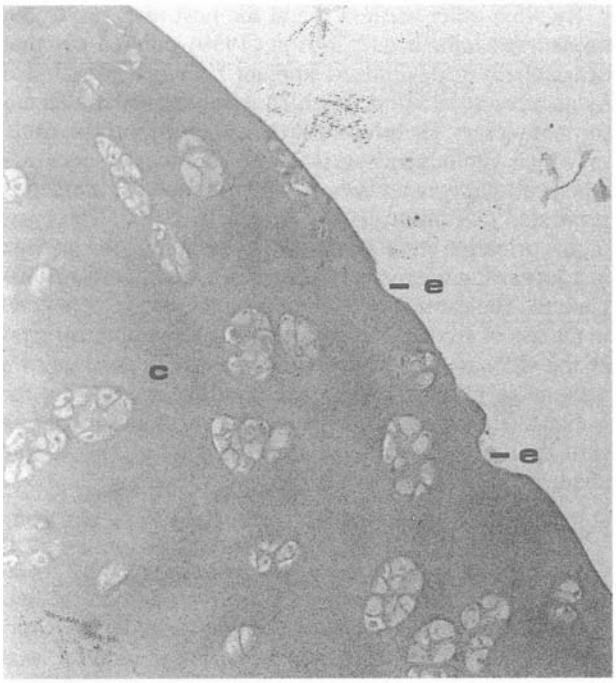

Fig. 9

Magnified view of part of the cortical surface of the cartilaginous nasal implant (c) shown in Fig. 7. Evidence of erosion (e) is minimal. Haematoxylin and eosin ×400.

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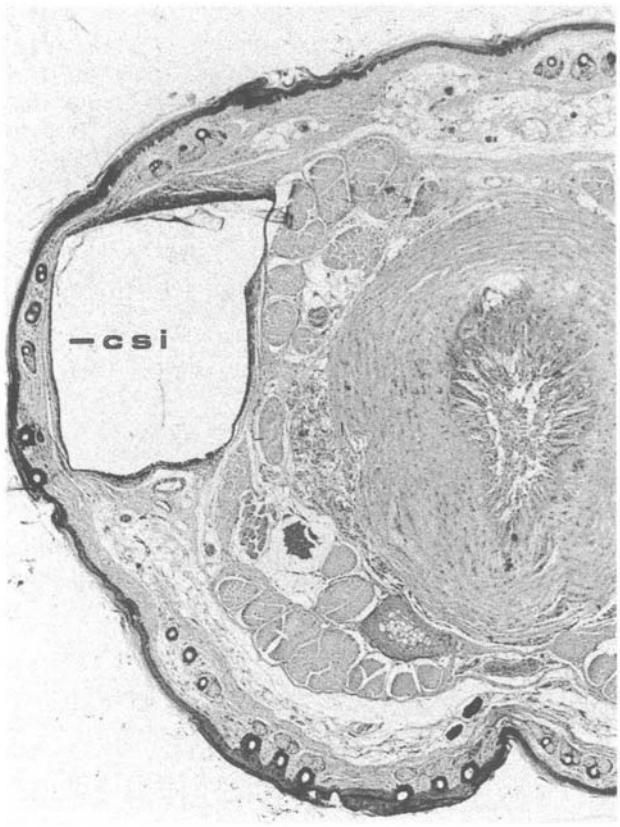

Fig. 10

Cross section of the tail of a mouse showing the site of silastic implantation. The implant has been lost during sectioning, but the minimal fibrotic encapsulation (CSL) is evident. Haematoxylin and eosin ×100.

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