EXPERIMENTAL STUDIES ON VIABILITY, ANTIGENICITY AND PERSISTENCE OF CARTILAGE TRANSPLANTS IN RABBITS

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INTRODUCTION

Paul Bert, a Frenchman, is generally credited with having initially reported the outcome of transplanting cartilage when, in 1865, he published the results of his experiments in which he implanted skinned rat tails subcutaneously in the same and in different rats. He concluded that the cartilage present in the grafts retained its structure as both autograft and homograft throughout the five months over which his observations extended. His pioneer animal experiments were followed by those of Ollier (1867), Tizzoni (1878), Prudden (1881), Leopold (1881), Fischer (1882) and Zahn (1884) but the conclusions reached by these various authors as to the fate of transplanted cartilage were not wholly in accord with those of Bert. Ollier, Tizzoni, Fischer and Zahn each described degenerative changes as occurring in the grafts, which were ultimately absorbed whether transplanted elsewhere in the donor, into another animal of the same species, or into an animal of different species. Ollier claimed, in addition, that

a cartilage graft is absorbed more rapidly if the perichondrium is removed prior to implantation, and this contention was later reiterated by Fischer. (Both Ollier and Fischer, however carried out their experiments with chickens as hosts and, as will be seen later, foreign cartilage grafts are rapidly destroyed in the fowl). Prudden, in distinct contrast, was of the opinion that cartilage autografts and homografts in rabbits may persist largely unchanged for 399 days, whether implanted with or without perichondrium. His findings were confirmed by Leopold, who reported, further, that rabbit foetal cartilage continues to grow after transplantation; both found, however, that cartilage homografts sometimes became rapidly absorbed.

On the strength of what would seem, therefore, to be rather slim evidence, Koenig, in 1896 introduced the use of cartilage autografts to plastic surgery when he employed such material to effect cosmetic repair of laryngeal wall defects. This use of cartilage autografts in man was extended, shortly afterwards (von Mangoldt, 1899;

Nélaton and Ombredanne, 1904) to the correction of nasal defects.

The first record of the use, in plastic surgery, of homografts of cartilage alone was by Morestin (1915) although both Lexer (1908) and Tuffier (1911) had earlier implanted composite long bone grafts including articular cartilage, taken from amputated limbs, to patients under their care. A few years later Imbert, L'Heureux and Rouslacroix (1916) reported their pioneer experiments using bovine scapular cartilage to repair human cranial defects. They met with only moderate success, as they found that the cartilage became progressively absorbed.

The utilisation of cartilage grafts by the plastic surgeons stimulated anew the investigation of the fate of transplanted cartilage. Davis (1917) found cartilage autografts in dogs to be histologically intact after 505 days whether implanted with or without perichondrium. Gillies (1920) reported a three-year follow-up study of clinical cases of cartilage autografts and homografts, both types of graft being found

to give satisfactory results. He also examined one autograft and one homograft histologically after 18 months, and found the cartilage apparently normal in both instances, although the cells of the homograft were vacuolated and degenerate in places. The first reviews of the field of tissue transplantation appeared soon afterwards (Neuhof, 1923; Lexer, 1924). Writing in the latter publication, Rehn and Ruef substantiated Gillies' contention that cartilage autografts and homografts exhibit great powers of persistence: a human cartilage homograft removed after 9 months in situ was found to be histologically normal, but these authors found that cartilage heterografts were unable to survive more than a few months. Further histological proof of the ability of cartilage autografts to persist for a year in rabbits (1926) and for 30 months in dogs (1927) was provided by Mannheim and Zipkin.

This immunity to destruction in foreign hosts exhibited by cartilage grafts was not shared by most other tissues however. In 1912 Schoene, largely influenced probably by his teacher Ehrlich, had

advanced the view that the rejection of foreign tissue grafts by the host was akin to an active immune response. This was partially borne out by the results of an extensive series of experiments (Loeb and Harter, 1926; Loeb, 1926a,b, 1927a, 1927b) in which tissues, including cartilage, were exchanged between donors and hosts possessing graded degrees of genetic dissimilarity. Loeb's conclusions were (1930) (1) that the greater the variance of genetic constitution between host and donor, the greater was the host reaction to the graft and (2) that the host reaction to cartilage is considerably less than the reaction to other tissues from the same donor. Here, then was the first advocation of a reason for the immunity to destruction enjoyed by a foreign cartilage graft. Loeb and Siebert (1935) later discovered that cartilage homografts in the fowl are rapidly destroyed, in sharp contrast to the conditions obtaining in all mammals studied.

In recent years, many instances of proved histological survival of cartilage auto- and homografts have been provided. In man, normal

cartilage has been found in autografts removed after six (Peer, 1939), ten (Kirkham, 1940), twelve (Young, 1941), thirteen (Padgett and Stephenson, 1948; Peer, 1941), twenty (Peer, 1945) and twenty-one, twenty-three and thirty-seven (Davis and Gibson, 1956) years. Cartilage homografts of normal histological appearance have been found after two years in rabbits (Rollo, 1930), eighteen months in dogs (Young, 1945) and four years in man (Peer, 1954a). In agreement with the findings of the earlier writers, survival of cartilage heterografts beyond a few months has not been demonstrated.

During the last 20 years, plastic surgeons have reverted to the use of preserved homogenous cartilage, as first advocated as long ago as 1881 by Prudden, in order to preclude the necessity of performing mutilating and debilitating operative procedures to obtain autogenous cartilage. Homogenous cartilage, obtained at post-mortem and preserved in a variety of ways, gives fairly satisfactory results clinically (O'Connor and Pierce, 1938; Brown, 1940; Kirkham, 1940;

Straith and Slaughter, 1941; Lamont, 1944; Vidaurre, 1952; Mir y Mir, 1952; Eisenstodt, 1953; Schofield, 1953a; Rasi, 1959).

A common and distressing sequel to reparative cartilage grafting is post-operative warping of the graft. Of 35 cartilage grafts implanted by Mowlem (1938) 17 underwent twisting and a further nine became warped; all 26 reparative procedures had to be repeated. New and Erich (1941) advocated immersion of cartilage in boiling water prior to grafting as rendering it proof against this complication. Wardill and Swinney subsequently (1947) reported that heterogenous cartilage so treated elicited practically no host reaction whatever, and that such material gave satisfactory results clinically. This latter claim was also made by Marino and Niklison (1949) and Gillies and Kristensen (1951) but long-term follow up studies have shown that the results fall short of expectations, since the majority of the grafts show some degree of absorption within two years (Gibson and Davis, 1953; North, 1953). As a result of these reports, the use of boiled heterogenous cartilage

has declined.

This brief review of the literature of cartilage grafting leads one to the conclusion that cartilage retains its histological structure for very long periods after auto-transplantation, and for at least several years after homo-transplantation, but that hetero-grafts of cartilage are, by comparison, short-lived. Substantiation of the claim for continued viability of cartilage autografts and homografts in rabbits and man was provided by Dupertuis' observations (1941, 1950) that increase in size of such grafts occurs if the cartilage be taken from a young donor. Moreover, cartilage autografts and homografts in rabbits possess a distinct and measureable carbohydrate metabolism for at least 150 days (Laskin, Sarnat and Bain, 1952). Finally, Peer (1954a) demonstrated that the cells of a human cartilage homograft removed after 4 years in situ retained their ability to take up supravital dyes. In face of this weight of accumulated evidence, it is rather surprising to find modern writers expressing

the view that the cells in all cartilage grafts die within a short time of implantation of the cartilage (Bossi, 1949; Schofield, 1953b; Longmire, Cannon and Weber, 1953; Maumenee, 1953; Billingham, 1954). In a leading article in the British Medical Journal of November 1953 it was stated - "As with bone grafts, the effectiveness of cartilage transplants does not depend on the survival of the cells of the transplanted tissue, and it is because of this that grafts from other individuals or even from other species can be of great use in reconstructive surgery. It is known from the work of Peer that cartilage cells may survive for many years in auto-transplants. But the survival of cartilage cells from other individuals of the same species (homotransplants) and from individuals of other species (heterotransplants) is transient and unimportant". This represents a concession to cell survival in autografts, but the belief that the cells in any cartilage graft die shortly after implantation is held by many present-day plastic surgeons. Part I of this thesis will therefore be devoted to an attempt to clarify the situation regarding

the viability of cartilage implants. The problem will be approached by a histochemical study of cartilage implants in rabbits, and by examining the uptake of radioactive sulphur by such grafts. Parts II, III and IV will deal with experiments designed to elucidate the reasons underlying the ability of foreign cartilage grafts to survive, and to determine the status of such grafts in relation to modern views of tissue transplantation immunity. Finally, part V will deal with transplantation experiments using cartilage treated so that it may become permanently acceptable to a homogenous host.

PART I

THE FATE OF THE CELLS IN TRANSPLANTED CARTILAGE

INTRODUCTION

It is nearly 100 years since it was first reported (Ranvier, 1872; Neumann, 1877) that glycogen is a normal cytoplasmic constituent of chondrocytes; and that fat is also a normal inclusion has been known for half a century (Hammar, 1894; Sacerdotti, 1898). Recent studies of these substances in cartilage cells have been made by Sheehan (1948), Montagna (1949) and Follis and Berthrong (1949). Hyaline cartilage matrix is composed of mucoprotein - an acid mucopolysaccharide (chondroitin sulphate) in combination with various proteins, notably collagen (Eichelberger, Brower and Roma, 1951; Kellgren, 1952; Loeven, 1955). The acid mucopolysaccharide can be selectively demonstrated by a number of histochemical methods, including the Hale technique, the PAS reaction, alcian blue and the metachromatic reaction (Pearse, 1953). It was felt, therefore, that a histochemical study of cartilage grafts would

provide much valuable information regarding their structural integrity.

Radioactive sulphur administered as sulphate is rapidly incorporated into cartilage in vivo (Dziewiatkowski, Benesch and Benesch, 1949; Odeblad and Boström, 1952; Davies and Young, 1954; Pelc and Glücksmann, 1955; Curran and Kennedy, 1955). The ion is taken up in the first instance by the cells (Pelc and Glücksmann) and occurs finally as the ester sulphate of the chondroitin sulphuric acid of the matrix. Cartilage will also incorporate $^{35}SO_4$ in vitro: if, however, the chondrocytes be killed by heating or by chemical poisons no incorporation of the ion will take place (Layton, 1950; Boström and Mansson, 1952, 1953; Curran and Gibson, 1956). Purified chondroitin sulphate alone will not take up $^{35}SO_4$ in vitro (Boström, 1952). It is manifest, therefore, that $^{35}SO_4$ uptake provides a reliable means of determining chondrocyte viability. Bacsich and Wyburn (1955a) have shown that the cells in guinea-pig cartilage homografts take up $^{35}SO_4$ in vivo three weeks after implantation. The extension of this

investigation to long-term cartilage grafts was considered to be indicated.

Three series of experiments were performed. In the first of these, histochemical studies were carried out on cartilage autografts and homografts recovered from intramuscular beds in rabbits at intervals up to 22 months. The second series consisted of histochemical studies of rabbit cartilage auto- and homografts recovered from the anterior chamber of the eye after varying periods up to 18 months. The third series constituted a study of the uptake of $^{35}SO_4$ *in vivo* by cartilage auto-, homo- and heterografts which had been in situ for periods ranging between 659 and 757 days.

SERIES ONE

A HISTOCHEMICAL STUDY OF INTRAMUSCULAR CARTILAGE AUTO- AND HOMOGRAFTS

MATERIAL & METHODS

Each of 28 rabbits was given an implant intramuscularly of freshly-removed autogenous cartilage in one buttock and a freshly-removed

costal cartilage homograft in the other. The cartilage, in each instance, was implanted with perichondrium intact. The rabbits were killed after 3, 5, 7, 10, 14, 21 and 28 days, and at monthly intervals thereafter to 22 months.

All the recovered grafts were cut into three pieces which were treated as follows:-

(1) Fixed in formol-calcium, sectioned with the freezing microtome and stained with a saturated solution of Fettrot 7B in absolute propylene glycol for the demonstration of lipids.

(2) Fixed in Rossman's fluid at 0°C for 24 hours and paraffin sections were stained with the periodic acid-Schiff technique. Both the glycogen of the chondrocytes and the acid mucopolysaccharides of the matrix are demonstrated by this reaction. Control sections were incubated in ½% diastase for half an hour in order to digest the glycogen, and were subsequently subjected to the PAS reaction.

(3) Fixed in 4% neutral formaldehyde, embedded in paraffin, sectioned,

and stained with Rinehart and Abu'l-Haj's (1951) modification of the Hale (1946) method for mucopolysaccharides.

Sections of each portion of each graft were also stained with Haematoxylin and Eosin.

RESULTS

Intramuscular cartilage autografts. Every graft implanted was recovered and appeared to be outwardly unchanged: each was of normal histological appearance and no evidence of invasion was seen (Fig. 1). Some of the

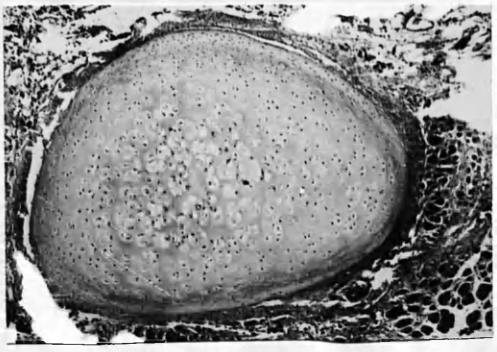

Fig. 1. Autograft after 22 months intramuscularly, to show normal cartilage. H & E x 25.

grafts recovered during the first 21 days occasionally presented regions in which many of the chondrocytes had lost their lipid or glycogen deposits, but were otherwise normal. Since these compounds were not

studied on adjacent sections but on different portions of each graft, it was not ascertained whether both were lost from any given cell at the same time. No histochemical evidence was found of any disruption of the composition of the matrix during this same period. All other grafts in the series gave normal histochemical reactions for lipid (Fig. 2), glycogen (Fig. 3) and mucopolysaccharide: in a

Fig. 2. Intramuscular autograft after four weeks. The chondrocytes possess normal lipid deposits in their cytoplasm. Fettrot 7B & Haemalum. x 370.

few, degeneration of the cells in the central zone of the graft had occurred, and the effete cells in this region had lost their lipid

and glycogen deposits. The surrounding matrix nevertheless gave

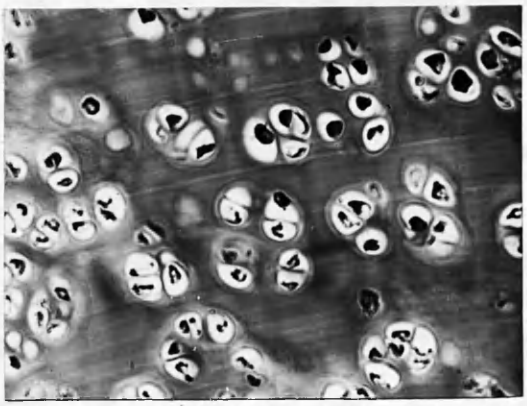

Fig. 3. Autograft after 28 days intramuscularly. Large glycogen deposits are seen within the cytoplasm of the chondrocytes, and the matrix reacts normally. P.A.S. x 33.

normal histochemical reactions to the techniques employed.

Intramuscular cartilage homografts. Every graft was again recovered

Fig. 4. Intramuscular homograft after 22 months, showing normal cartilage. H & E x 25.

and appeared macroscopically unchanged (Fig. 4). As with the auto-grafts, there was evidence of localised loss of lipid and glycogen content of the cells in some regions of the grafts removed during the first 21 days. All these grafts were otherwise normal and there was no evidence of any histochemically-detectable alteration of their matrix. Host mononuclear leucocytes were rarely seen in the vicinity of grafts removed after 7 or 10 days: by the fourteenth day such cells were found in moderate numbers, and during the third week they were seen in greatest concentration.

Most of the later homografts, also, were histologically and histochemically normal (Figs. 4,5), with certain exceptions. A few

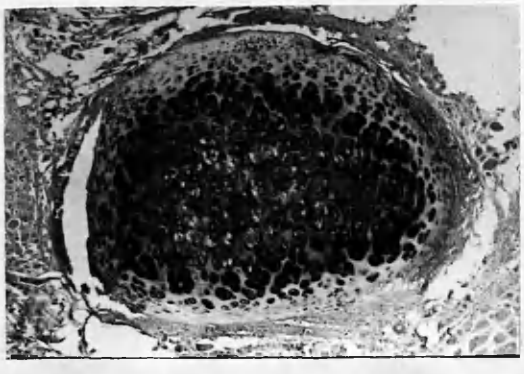

Fig. 5. Homograft after 3 months intramuscularly. The mucopolysaccharide content of the matrix is demonstrated, and is within normal limits. Hale method. x 25.

exhibited degeneration of the cells in the central regions: this was associated with absence of histochemically-demonstrable lipids and glycogen in the cells of the same zone. A further three homografts

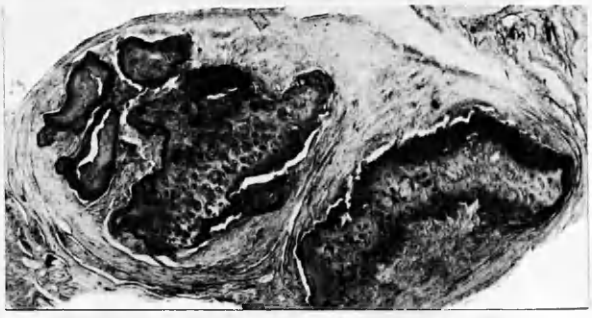

Fig. 6. Extremity of an intramuscular homograft after 16 months. The cartilage has become invaded and broken up into several pieces.
H & E. x 25.

(those removed after 10, 16 and 22 months) had suffered a degree of invasion from the raw surface at one extremity (Figs. 6,7). In these

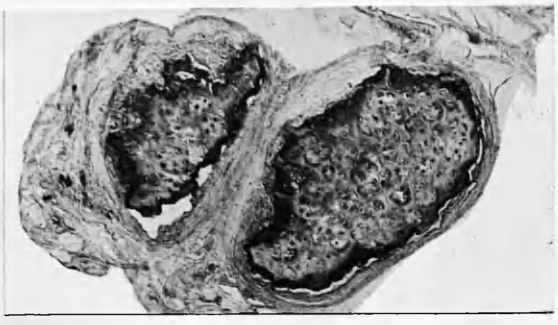

Fig. 7. Extremity of homograft after 10 months intramuscularly. The cartilage has again been fragmented.
Fettrot 7B & Haemalum x 25.

areas the matrix was eosinophilic, in contrast to the normal mild basophilia, and gave negative Hale and PAS reactions: the cells

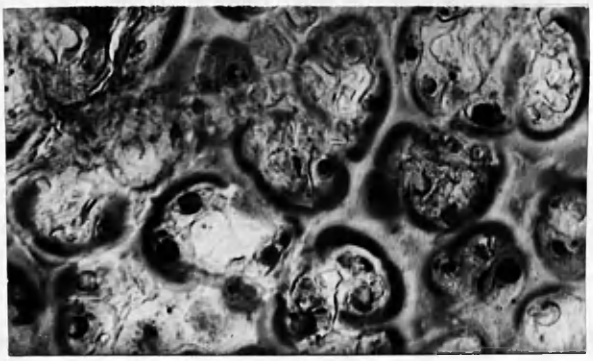

Fig. 8. High power view of a portion of Fig. 7 to show fragmentation of the lipid globule in the cytoplasm of many of the chondrocytes. Fettrot & Haemalum x 370.

possessed pyknotic nuclei and the lipid globules in their cytoplasm were fragmented (Fig. 8), an appearance which Sheehan (1948) found to be typical of degenerating chondrocytes. The great bulk of even these three grafts was normal cartilage, however (Fig. 9).

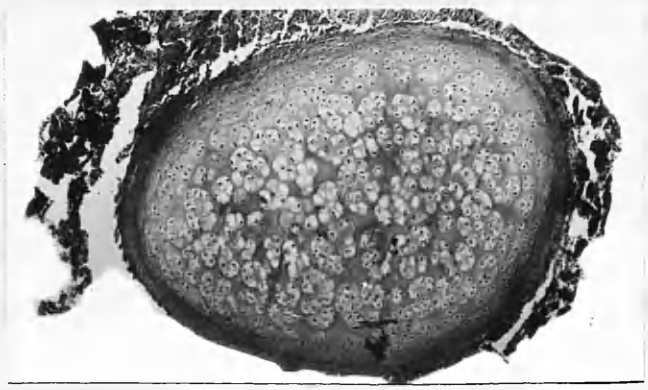

Fig. 9. Middle portion of homograft shown in Figs. 7 and 8 to show normal cartilage. H & E. x 25.

SERIES TWO

A HISTOCHEMICAL STUDY OF CARTILAGE AUTO- AND HOMOGRAFTS IMPLANTED IN THE ANTERIOR CHAMBER OF THE EYE

MATERIAL & METHODS

In this series, 24 rabbits received intraocular autografts, and a further 24 received intraocular homografts. Two $\frac{1}{25}$ mm. thick slices of freshly-removed costal cartilage were placed in the anterior chamber of one eye of the rabbit through a small incision at the margin of the cornea just in front of the limbus. The animals were killed in pairs so that autografts and homografts were obtained after 3, 5, 7, 10, 14, 21 and 28 days, and subsequently at monthly intervals to 18 months.

The two grafts recovered from each rabbit were treated as follows:-

(1) One implant was fixed in formol-calcium, sectioned with the freezing microtome and stained with :(a) a saturated solution of Fettrot 7B in absolute propylene glycol for the demonstration of lipids (b) the Rinehart and Abu'l-Haj (1951) modification of the

Hale (1946) method for mucopolysaccharides.

(2) The other implant was fixed in Rossman's fluid for 24 hours at 0°C and stained with the periodic acid-Schiff technique. Both glycogen of the chondrocytes and acid mucopolysaccharides of the matrix are demonstrated by this reaction. Control sections were incubated in ½% diastase for ½ hour in order to digest the glycogen, and were subsequently subjected to the PAS reaction. Sections of each graft were also stained with Haematoxylin and Eosin.

RESULTS

Intraocular cartilage autografts. All the grafts were recovered and were found, for the most part, to be adherent to the back of the cornea, although some had become attached to the iris. Every graft was found to be of normal histological structure (Fig. 10) and gave normal reactions to the histochemical tests applied.

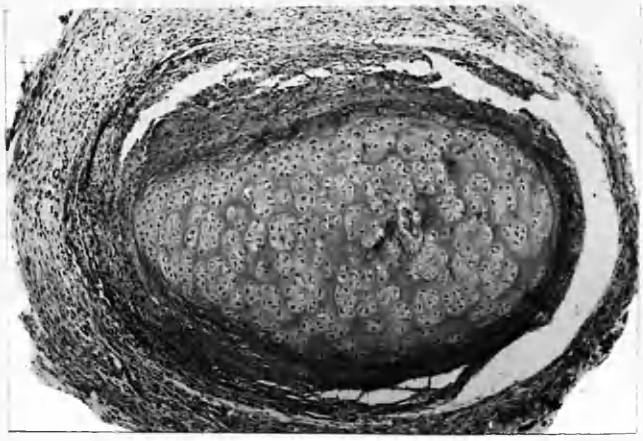

Fig. 10. Autograft after 14 months in anterior chamber of eye. H & E. x 25.

Intraocular cartilage homografts. In two of the rabbits, the grafts were found to have been completely destroyed. All the remaining grafts were recovered and appeared to be unchanged to the naked eye:

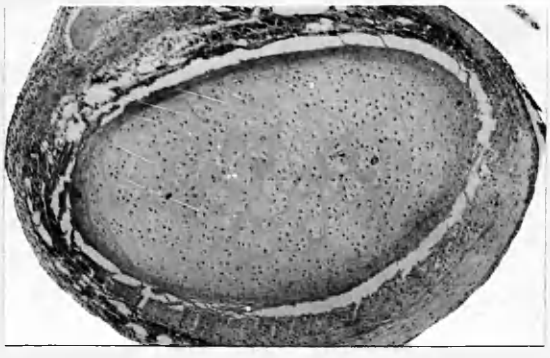

Fig. 11. Intraocular homograft after 18 months. Normal cartilage. H & E. x 25.

each was of normal histological appearance and no evidence of invasion

was seen (Fig. 11). All gave normal histochemical reactions.

SERIES THREE

THE UPTAKE OF $^{35}SO_4$ BY LONG-TERM CARTILAGE AUTO-, HOMO- AND HETEROGRAFTS

MATERIAL & METHODS

Seven rabbits, each of which bore long-term cartilage grafts, as detailed in Table 1, were given a subcutaneous injection of 1 ml. of $Na_2\ ^{35}SO_4$

TABLE 1

Rabbit No.	Type(s) of Graft	Site of Graft	Duration in situ
1	Autografts and homografts	Intramuscular	659 days
2	Autografts and homografts	Intramuscular	671 "
3	Homografts	Subcutaneous	667 "
4	Homografts	Subcutaneous	757 "
5	Heterografts+	Intramuscular	679 "
6	Heterografts+	Intramuscular	684 "
7	Heterografts+	Subcutaneous	731 "

+Obtained from freshly-killed guinea-pigs.

containing 5 mc. of ^{35}S twenty-four hours before attempted recovery of the grafts. All grafts recovered, and control pieces of costal cartilage

removed from two of the rabbits, were fixed in 4% neutral formaldehyde and embedded in paraffin. As a further control, a piece of rabbit costal cartilage was incubated in vitro for 24 hours at 37°C in 2 ml. of Ringer solution containing 5 mc. of ^{35}S. Using the stripping film method, autoradiographs were performed on 7 μ sections of the grafts and of the control pieces of cartilage. The exposure time was 90 days. In addition, sections of the grafts and the control pieces of cartilage were stained by:-

(1) Haematoxylin and Chromotrope

(2) 0.5% aqueous alcian blue

(3) 0.01% aqueous toluidin blue

(4) the periodic-acid-Schiff reaction.

RESULTS

In this group, each autograft and homograft implanted was recovered and appeared to have undergone no macroscopic change. Of all the heterografts implanted however, only a small fragment of one graft was

recovered from animal 5 (see Table 1) after 679 days: in rabbits 6 and 7 all the guinea-pig cartilage had disappeared without trace.

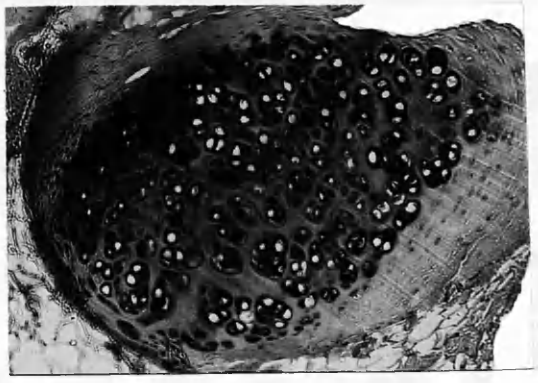

Fig. 12. Subcutaneous homograft after 757 days. The graft is composed of normal cartilage.
H & E x 80.

Every autograft and homograft, and the heterograft fragment was seen to consist of histologically-normal cartilage (Figs. 12,13) although

Fig. 13. Intramuscular heterograft after 679 days, to show normal structure.
H & E x 140.

degeneration of the cells in the central regions had occurred in several of the implants.

All grafts and control pieces of cartilage gave a positive autoradiograph(Figs. 14-17). Aggregations of silver granules were found, at 24 hours after injection of the isotope, to be confined to areas localised over or near adult chondrocytes: the subperichondral chondroblasts and immature chondrocytes do not exhibit uptake of the isotope (Fig. 14) and the matrix in general gives a faintly positive result, but

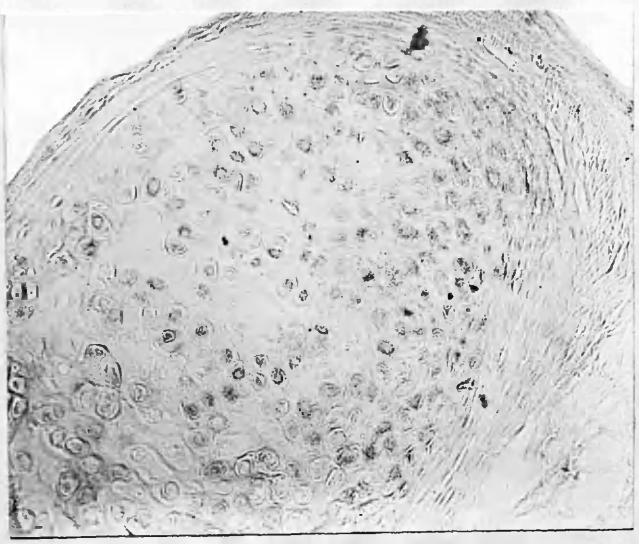

Fig. 14. 757 day homograft 24 hours after administration of ^{35}S. Autoradiograph. Aggregations of granules can be seen over the mature cartilage cells, whilst the matrix and the cells of the perichondrium are negative. x 27.

is strongly positive in the immediate vicinity of mature chondrocytes.

The greatest activity was found in the young adult cells in a zone

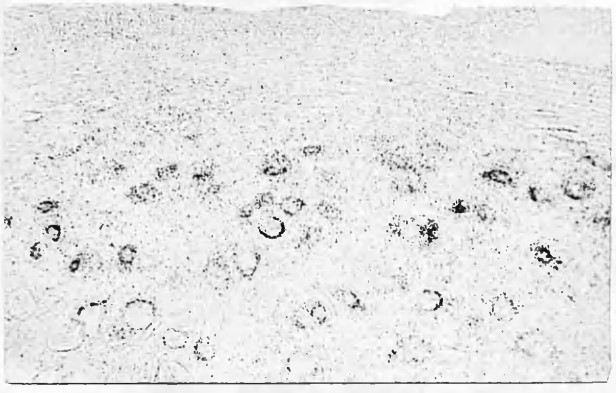

Fig. 15. 671 day homograft 24 hours after a subcutaneous injection of ^{35}S. Autoradiograph. Uptake of ^{35}S is confined to the young adult chondrocytes in a zone below the perichondrium. x 240.

some distance below the perichondrium (Figs. 15, 16), and in those

Fig. 16. 679 day heterograft 24 hours after administration of ^{35}S. Autoradiograph. Uptake of ^{35}S is exhibited by the graft cells. x 470.

grafts in which cellular death was observed histologically in the central regions, activity was confined to the aforementioned zone (Fig. 15). The control piece of cartilage incubated <u>in vitro</u> in a medium containing a high concentration of the isotope presented a very

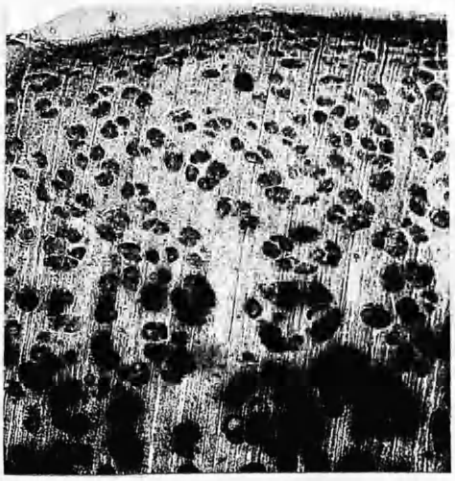

Fig. 17. Costal cartilage of young adult rabbit incubated in a medium containing high concentration of ^{35}S. Heavy incorporation of the ion is shown by the adult chondrocytes. Autoradiograph. x 105.

strong measure of incorporation of ^{35}S (Fig. 17).

The ground substance of the grafts gave a normal or only somewhat reduced staining response to alcian blue and toluidin blue, and a normal or even slightly increased PAS reaction by comparison with the controls.

DISCUSSION

The evidence presented from the three experimental series establishes that cartilage auto- and homografts in rabbits can persist wholly or largely unchanged macroscopically and histologically for very long periods of time under widely differing environmental conditions. Further, the experiments adduce that many of the cells in both types of graft possess normal lipid and glycogen deposits, and the the ground substance of the grafts has maintained its histochemical integrity. The third group of experiments establishes, further, that the cells did not lose their capacity to metabolize sulphate. From all this evidence one is forced to conclude that the grafts have remained viable. During the first three weeks, when the implants are undergoing a phase of readjustment to their new environment, temporary and reversible cellular depletion of lipid and glycogen may occur.

In long-standing implants the oldest cells in the centre of the graft and furthest removed from the nutritive tissue fluid bathing the

cartilage commonly degenerate. Hass (1943) in a study of ageing cartilage writes: "The number of cells in the matrix diminishes, and those which remain display morphologic changes which are ordinarily accepted as evidence of diminished activity, if not of decreased viability." This finding, in conjunction with the well-known fact that central degeneration is a particularly common pre-ossification finding in ungrafted costal cartilage allows one to attach little significance to the degeneration seen in the centre of several long-term autografts and homografts.

The slight degree of invasion seen at one extremity in three of the homografts loses most of its significance in face of Davis and Gibson's (1956) findings. They discovered that, during cutting of cartilage, localised areas of damage occur at the cut surface: if the cartilage be grafted subsequently, and even if it be implanted as an autograft, these damaged areas will rapidly liquefy and disintegrate and the cavity so formed will become occupied by fibrous tissue from the host. This occurs during the few weeks immediately following grafting and does not

increase in extent thereafter.

The presence of living cells in long-term foreign cartilage grafts is open to the interpretation that these are not donor cells but replacement cells from the host which have become incorporated in the graft. This has been shown to be an invalid criticism in the case of human cartilage homografts which had been in situ for 8 and 19 months (Peer, 1958, 1959). The chondroblasts and chondrocytes in septal cartilage homografts from a female donor implanted in male patients for such periods contained the female sex chromatin. There was no indication therefore that even the peripherally-placed youngest cells were host cells.

Cartilage heterografts do not share with cartilage homografts the distinction of being able to persist unchanged in foreign hosts. They are progressively destroyed in a period during which the homograft undergoes no alteration. Nevertheless, as long as any histologically-normal and fully-stainable cartilage remains, the heterogenous chondrocytes therein remain viable.

Davis and Gibson (1957) and Gibson, Davis and Curran (1958) have concurrently reported that human cartilage autografts and homografts take up $^{35}SO_4$ when they have been in situ for up to 37 and 2 years respectively.

PART II

THE ANTIGENICITY OF FOREIGN CARTILAGE GRAFTS

INTRODUCTION

Homografts of most tissues are rejected, usually within a few days or weeks, by virtue of the active immune response which they elicit in the host animal: this response is mediated through activated lymphoid cells (Medawar, 1958). Homografts of certain tissues do, however, persist for periods in excess of a few weeks. Thus the cortical tissue of adrenal homografts can survive for up to seven months (Darcy, 1952a)and splenic homografts for nearly a year (Knake, 1953). Ovarian homografts continue to function for at least 153 days (Smith and Parkes, 1954) and survival is surmised. Corneal homografts, too, can remain for several months if implanted subcutaneously (Bacsich and Wyburn, 1957). Homografts of all other tissues except cartilage rapidly succumb to the host homograft reaction: for this response to become manifest, three conditions must obtain (Medawar, 1948). First, there

must be continuity of lymphatics between the graft bed and the host antibody-forming cells, allowing free access of graft antigens to the latter. Secondly, the host antibody-forming mechanism must be intact. Finally, the graft must become vascularised to render possible the close contact between the activated host lymphoid cells and the graft cells which is imperative for destruction of the latter. Hence homograft survival can be achieved by blocking the machinery of the homograft response at any one of three levels: (1) by interrupting the "afferent" flow of antigen (2) by inhibiting the "central" antibody-forming mechanism or (3) by interfering with the "efferent" antibody outflow (Billingham, Brent and Medawar, 1956a).

It has been seen that, by virtue of their ability to remain unaltered in foreign hosts for quite exceptional periods, cartilage homografts hold a unique position in the field of tissue transplantation immunity. Three explanations have been suggested for the ability of the cartilage homograft to survive (and presumably the explanation

would extend with equal force to the heterograft). Bacsich and Wyburn (1955b) believe that the continuous production, by the homogenous chondrocytes, of a protective shield of mucopolysaccharide renders them safe from inimical host agents. Medawar (1959) suggests that the avascular nature of cartilage suffices to explain the survival of the cartilage homograft, since a close approximation between the activated host lymphoid cells and the graft cells is not possible. In both postulates, inhibition of the homograft response by interruption of the "efferent" antibody outflow is implicit. The third explanation is that cartilage lacks antigen (Allbrook, 1954; Wyburn, 1959) or possesses but a poor complement of it (Loeb, 1930): this would involve an inhibition of the homograft response at an "afferent" level.

Immersion of cartilage in boiling water as a prophylactic measure to prevent post-operative warping was, as mentioned previously, introduced by New and Erich (1941). Wardill and Swinney later (1947) claimed

that such pretreatment caused cartilage to lose its "biological personality" since boiled cartilage evokes practically no local leucocytic reaction in heterogenous hosts. Boiled bovine cartilage, however, does produce a local host leucocytic reaction in man, and successive implants undergo increasingly rapid absorption (Gibson and Davis, 1953). The antigenic status of heterografts of boiled cartilage is thus controversial. On the other hand there seems little doubt that boiling would destroy the ability of a tissue to elicit any immune response in a homogenous host (Siebert, 1928, 1931; Billingham, Brent and Medawar, 1956b).

These considerations and observations led of necessity to the question; is it possible to demonstrate antigenicity of foreign cartilage grafts?

One method of proving that a foreign tissue graft elicits a state of transplantation immunity in the host is to show that survival periods

are less for second-set grafts than for first-set grafts from the same donor in a single recipient. This approach is impractical in the case of cartilage homografts in view of the powers of persistence of the first set-graft. However, since skin homografts produce a distinct and typical regional lymph node response (Gallone, Radici and Riquier, 1952; Scothorne and McGregor, 1955; Scothorne, 1957), the regional lymph node response to fresh cartilage homografts and to both fresh and boiled cartilage heterografts has been studied in order to determine whether the response is also elicited by cartilage. In addition, since (1) a non-fitted skin homograft of 0.36-0.44 gm. weight will fully immunise an adult rabbit (Medawar, 1944), and (2) antigens are shared in common by every tissue in any given animal (Medawar, 1958) the effect of implantation of an aliquot of homogenous cartilage on the behaviour of a skin homograft subsequently carried over from the cartilage donor to the same recipient, has been studied.

SERIES ONE

REGIONAL LYMPH NODE CHANGES INDUCED BY CARTILAGE AUTO-, HOMO- AND HETEROGRAFTS IN RABBITS

MATERIAL & METHODS

Eighty rabbits were used in this investigation. Each received a subcutaneous implant on the dorsal aspect of one ear, near the base, of approximately 0.4 gm. of cartilage, the source and nature of which is indicated in Table 2. In 70 rabbits the cartilage was implanted

TABLE 2.

SINGLE SET IMPLANTS

Type	No. of experiments	Form of implant	See Table
Autografts	9	Diced	3
Homografts	21	7 diced) 7 whole) 7 ground)	4
Heterografts from guinea-pigs	13	3 whole) 10 diced)	6
Heterografts from cattle	9	Diced	7
Boiled (1 minute)hetero-grafts from cattle	9	Diced	9
Boiled (10 minutes)hetero-grafts from cattle	9	Diced	10

TABLE 2 (cont.)

DOUBLE SET IMPLANTS

Type	No. of experiments	Form of implant	See Table
Homografts	6	Diced (on both occasions)	5
Heterografts (guinea-pig)	4	Diced (on both occasions)	8

in a single dose, but in 10 it was implanted in two half-amounts with an interval of four days between each operation. Nine rabbits received autografts, 27 received homografts, 26 received heterografts and 18 received heterogenous cartilage which had been immersed in boiling water for either one[+] or ten[++] minutes. In the majority of cases (63 of the 80 rabbits) the cartilage was implanted in the form of small dices of $\frac{1}{2}$ mm. face; 7 homografts and 3 heterografts were implanted as pieces, and 7 homografts in the form of a paste after having been ground with mortar and pestle.

[+]The preparation of bovine cartilage marketed by Messrs. Armour Laboratories has been so treated.

[++]Bovine cartilage boiled for such length of time has been recommended by New and Erich as being exempt from post-operative warping.

All 80 rabbits were sacrificed between the fourth and fourteenth day after grafting (Tables 3-10). Half an hour before each rabbit was killed, 0.5 ml. of a 2% aqueous solution of pontamine sky blue was injected subcutaneously into the graft site and into a corresponding position on the other ear. By this means the regional lymph node of both sides was clearly outlined: the lymphatic drainage from the dorsum of the ear in rabbits is ipsilateral (Scothorne and McGregor, 1955). The lymph nodes were secured, fixed in 4% neutral formaldehyde, weighed and embedded in paraffin. 7 μ sections of the nodes were stained by (1) haematoxylin and chromotrope and (2) pyronin and methyl green according to the method of Kurnick (1955).

RESULTS

Weight changes. A cartilage autograft did not produce any appreciable increase in weight of (Table 3), nor any cytological changes within, the regional lymph node of the operated side by the fourth day after

grafting, and this finding precluded the necessity of performing sham

TABLE 3.

CARTILAGE AUTOGRAFTS

Animal No.	Days after Grafting	Weights of Regional Nodes (mg.) non operated side	operated side	% enlargement of Nodes on operated side
R486	4	180	180	0
R470	4	140	150	+7
L480	4	170	190	+12
L488	4	90	100	+11
R475	4	100	100	0
R497	4	90	110	+22
R495	4	100	120	+20
L489	4	120	100	-20
R466	4	160	180	+12.5

operations on the control side in the remaining experiments.

In 20 of 21 rabbits, a single cartilage homograft did not produce any significant increase in weight of the regional lymph node during the fourteen days after grafting, regardless of whether the cartilage was diced, ground, or in a piece (Table 4). A single skin homograft

TABLE 4.

FIRST-SET CARTILAGE HOMOGRAFTS

Animal No.	Days after Grafting	Form of Implant	Weights of Regional Nodes (Mgm) Non-operated Side	Operated Side	% enlargement of node on Operated Side
L355	4	Ground	60	60	0
L354	4	Diced	50	50	0
R373	4	Whole	100	100	0
R339	6	Ground	120	130	+8.5
R302	6	Diced	70	50	-29
L305	6	Whole	110	130	+18
L352	7	Ground	50	50	0
R411	7	Diced	50	40	-20
L353	7	Whole	50	50	0
L449	8	Ground	100	110	+10
R374	8	Diced	60	80	+33
L314	8	Whole	110	110	0
L340	9	Ground	70	70	0
L299	9	Diced	160	160	0
L349	9	Whole	100	100	0
L303	12	Ground	60	80	+33
R34	12	Diced	90	150	+67
L308	12	Whole	90	110	+22
L456	14	Ground	80	90	+12
R145	14	Diced	60	50	-16
L315	14	Whole	70	70	0

produces an approximately two-fold weight increase in the regional lymph node by the fourth day after grafting (Scothorne and McGregor, 1955). However, implantation of a similar quantity of homogenous cartilage in two half-doses produced a moderate (33-100%) degree of weight increase of the node on the operated side by the fourth day after the second implantation (Table 5).

TABLE 5.

SECOND-SET CARTILAGE HOMOGRAFTS

Animal No.	Interval between First & Second Implants (days)	Interval between Second Implant & Removal of Node (days)	Weight of Regional Nodes (Mg.) non-operated Side	Operated Side	% Enlargement of Node on Operated Side
L411	4	4	70	140	+100
L412	4	4	60	80	+33
R413	4	4	130	190	+46
R414	4	4	160	290	+81
L421	4	4	90	130	+44
L423	4	4	80	120	+50

A heterograft of either guinea-pig (Table 6) or bovine (Table 7) cartilage produced a marked (80-400%) degree of weight increase of the

TABLE 6.

GUINEA-PIG CARTILAGE HETEROGRAFTS

Animal No.	Days after Grafting	Form of Implant	Weights of Regional Nodes (Mg.) Non-operated Side	Operated Side	% Enlargement of Node on Operated Side
L343	4	Diced	100	100	0
R467	4	"	110	250	+127
L339	6	"	50	110	+120
R464	6	"	120	290	+142
L472	6	Whole	120	300	+150
L350	8	Diced	100	300	+200
L430	8	"	130	650	+400
L471	8	Whole	110	440	+300
R427	10	Diced	140	640	+357
L444	10	"	60	190	+217
L474	10	Whole	110	350	+218
R465	12	Diced	200	360	+80
R468	12	"	120	300	+150

regional lymph node in 21 of 22 experiments: in one animal (L343, Table 6) no weight increase occurred, and no underlying reason for the discrepancy

could be ascertained. There again seemed to be no correlation between

TABLE 7.

FRESH BOVINE HETEROGRAFTS

Animal No.	Days after Grafting	Weights of Regional Nodes (mg.) Non-operated Side	Operated Side	% enlargement of Node on operated Side
R473	4	90	450	+400
L477	4	60	230	+283
R474	4	60	180	+200
L475	4	60	180	+200
L478	4	50	190	+280
L473	4	90	270	+200
R479	4	100	290	+190
R476	4	150	440	+193
L482	4	80	320	+300

the surface area of the implant and the degree of weight increase of the node (Table 6). Implantation of two successive heterografts of cartilage from a single guinea-pig, without increasing the dosage, also induced a marked (100-230%) weight increase of the regional lymph node

on the operated side by the fourth day after the second implantation (Table 8).

TABLE 8.

SECOND-SET GUINEA-PIG HETEROGRAFTS

Animal No.	Interval between First & Second Implants (days)	Interval between Second Implant & Removal of Node (days)	Weight of Regional Nodes (mg.) Non-operated Side	Operated Side	% Enlargement of Node on Operated Side
L419	4	4	110	280	+155
L420	4	4	150	300	+100
R452	4	4	160	480	+200
L466	4	4	100	330	+230

Implantation of boiled bovine cartilage produced a moderate (11-133%) weight increase of the regional lymph node in 17 of 18 rabbits (Tables 9, 10) but in one rabbit (R502, Table 10) the control lymph node, for reasons which did not emerge, greatly outweighed the node on the operated side.

Histological changes. The weight increase in the node from the operated side was seen to be due to cortical hyperplasia (Figs. 18, 19).

TABLE 9.

BOVINE HETEROGRAFTS BOILED 1 MINUTE

Animal No.	Days after Grafting	Weights of Regional Nodes (mg.) Non-operated Side	Operated Side	% Enlargement of Node on operated Side
R484	4	190	260	+37
L479	4	90	210	+133
L485	4	120	190	+58
L486	4	170	340	+100
R472	4	110	220	+100
R488	4	90	170	+90
R487	4	180	280	+55
R481	4	90	140	+55
R485	4	130	190	+46

TABLE 10.

BOVINE HETEROGRAFTS BOILED 10 MINUTES

L483	4	70	150	+114
L493	4	90	100	+11
L496	4	130	160	+23
R502	4	250	130	-92
R500	4	40	60	+50
R501	4	100	140	+40
R506	4	130	190	+46
R503	4	100	170	+70
R504	4	120	220	+83

The hyperplasia often led to fusion of the lymphatic nodules so that their outlines became indistinct (Fig. 19), and this was true especially

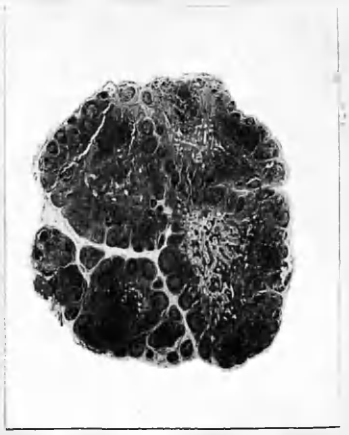

Fig. 18. Control node from unoperated side of rabbit L419 (Table 8). The cortical lymphatic nodules are sharply delineated. H & E x 10.

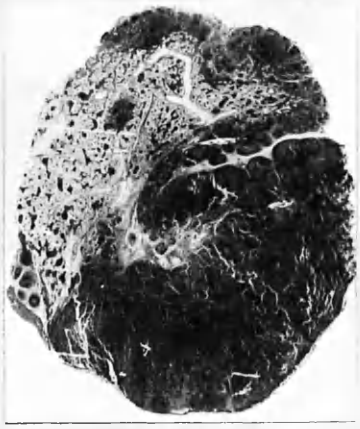

Fig. 19. Node from operated side of rabbit L419 four days after a second-set cartilage heterograft. Massive cortical hyperplasia, in which the nodules have become largely indistinct, can be seen. H & E x 10.

of the fresh heterograft experiments. Owing to the well-recognised difficulty in identification of cell types in lymphoid tissue, and also to the wide variations with which these cell populations occur in the several parts of any given node, differential cell counts were not attempted. The hyperplasia was accompanied by signs of great proliferative activity amongst large and medium-sized "lymphoblastic" cells

whose cytoplasm stained conspicuously with pyronin (Fig. 20): a

Fig. 20. Rabbit R476 (Table 7). Cortex of lymph node on fourth day after implantation of fresh bovine cartilage. Many large and medium-sized "lymphoblasts" with conspicuously pyroninophilic cytoplasm are seen. Methyl green & pyronin. x 715.

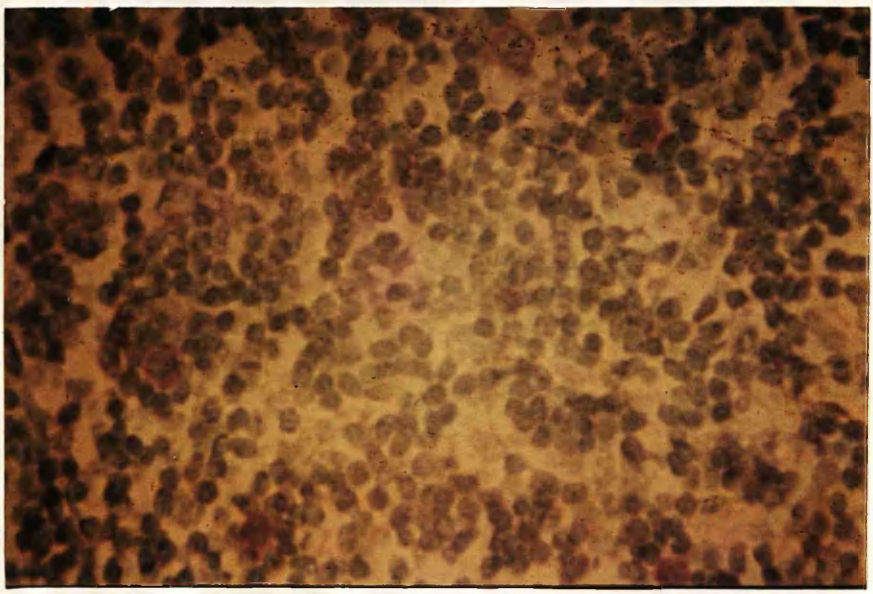

Fig. 21. Cortex of control node from unoperated side of rabbit R476. The great majority of the cells are lymphocytes. Methyl green & pyronin. x 715.

decidedly greater number of these pyroninophilic cells was found both within the germinal centres of, and in the cortex surrounding the lymphatic nodules in the hyperplastic nodes from the operated side by comparison with the contralateral control node (Figs. 20, 21). The large type in particular rarely occurred in control nodes removed before the eighth day. This cell (Fig. 22), it is thought, conforms

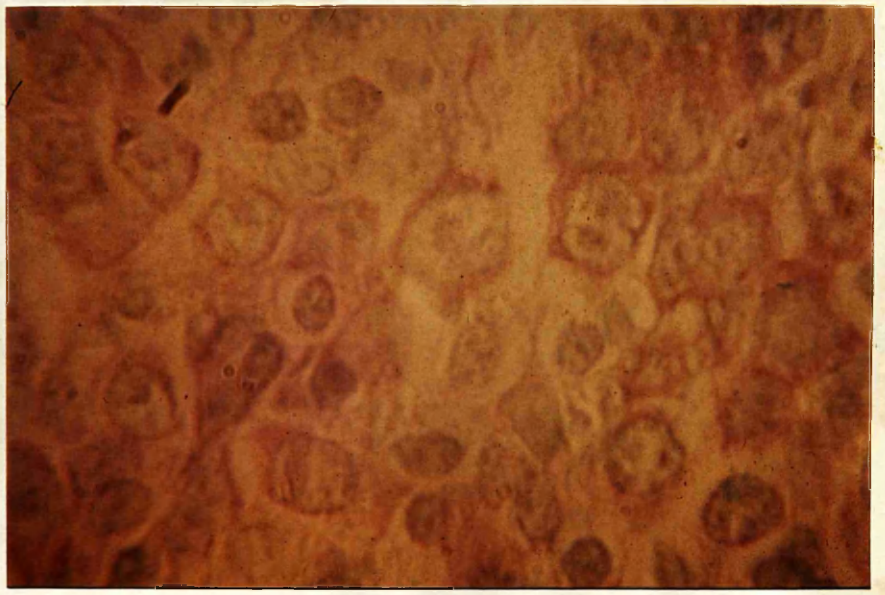

Fig. 22. High power view of a field from Fig. 20 showing several large "lymphoblastic" cells. Methyl green & pyronin. x 1690.

in its morphological characteristics to the "transitional cell" of Fagraeus (1948) and the "large lymphoid cell" of Scothorne and McGregor (1955); it is of larger size (14-17 μ) than the reticulum cell from

which it is thought to be derived, and possesses a large pale round nucleus with one or more distinct nucleoli and a narrow rim of rather vacuolated pyroninophilic cytoplasm. Since many of the nodes were removed on or after the 8th day of grafting, by which time the immune response has become systemic (Medawar, 1945), some of the control nodes in these experiments therefore showed signs of having been stimulated. However, since the experimental node was, in the second-set homograft and heterograft experiments larger than the control node it follows that the absolute number of pyroninophilic lymphoblasts in the experimental nodes exceeds that in the control nodes. Moreover, as mentioned above, these cells appeared also to be relatively more numerous in the hyperplastic nodes.

High concentrations, which often amounted to massive accumulations, of typical mature plasma cells were common in the medullary cords of control nodes (Fig. 23). These cells were, if anything, less frequently seen in the medullary cords of the enlarged

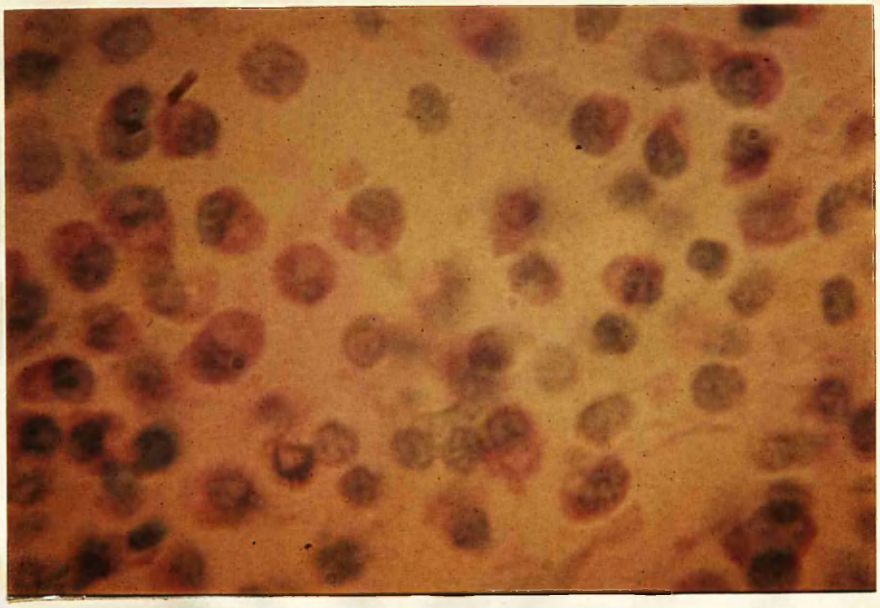

Fig. 23. Medullary cord of control node of Rabbit R495 (Table 3). Large numbers of lymphocytes and plasma cells are present.
Methyl green & pyronin. x 1690

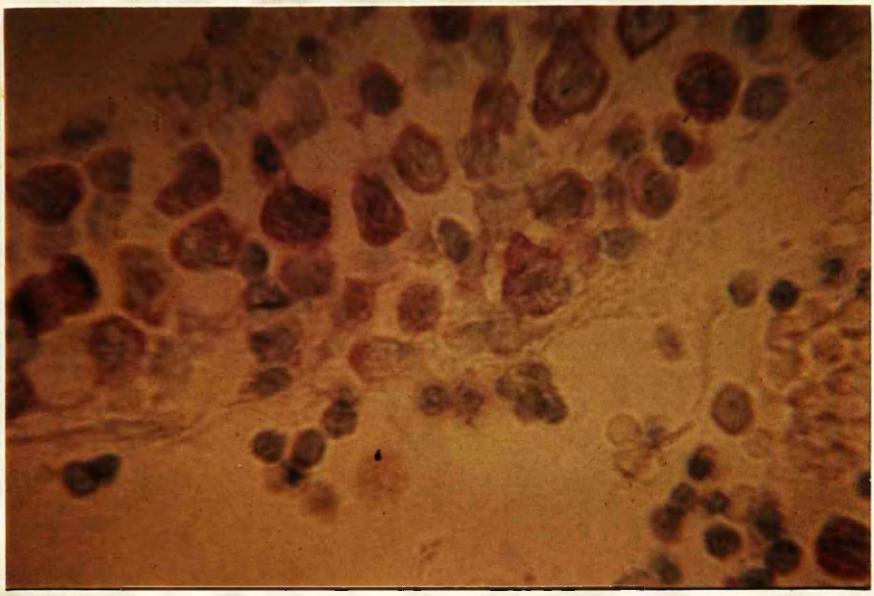

Fig. 24. Medullary cord of lymph node from operated side of rabbit R479 (Table 7), four days after implantation of boiled bovine cartilage. Several lymphobastic cells can be seen among the lymphocytes and plasma cells.
Methyl green & pyronin. x 1690

experimental nodes; instead, more medium-sized pyroninophilic "lymphoblastic" cells were often present in this situation (Fig. 24).

SERIES TWO

THE ABILITY OF CARTILAGE HOMOGRAFTS TO SENSITISE THE RECIPIENT IN RESPECT OF A SKIN HOMOGRAFT FROM SAME DONOR

MATERIAL & METHODS

A skin homograft behaves in different fashion when implanted on non-immune and immune hosts. The median survival time[+] of first-set skin homografts in high graft dosage (0.36-0.44 gm.) in rabbits is 10.4 ± 1.1 days, whilst the median survival time of skin homografts in immune rabbits is 6.0 ± 0.6 days (Medawar, 1944). In the non-immune recipient the graft epithelium exhibits proliferation (Figs. 26-29) and migration prior to breakdown, but in immune hosts these phenomena are not seen (Figs. 30-32). This difference is conditioned by the fact that skin homografts in intact hosts become revascularised

[+]The Median Survival Time represents the time at which the last surviving remnants of the epithelium of the skin homografts carried by 50% of the experimental subjects have just broken down.

by vessels from the graft bed, and although this occurs to some extent in immune hosts, the vessels undergo precocious stagnation and breakdown (Medawar, 1944). Hence, the epithelium of most first-set skin homografts will be intact and proliferating on the sixth day after grafting, but the epithelium of most skin homografts in immune hosts will have been destroyed by this time, or at least will not show signs of proliferation. The following scheme of experiments was therefore designed:-

Eighteen "control" rabbits received a high dosage homograft of ear skin (Fig. 25). A further 18 rabbits were given 0.2 gm. of very finely diced homologous cartilage subcutaneously on each of two occasions four days apart, followed by a homograft of ear skin from the cartilage donor on the eighteenth day after the initial cartilage implant. All 36 skin homografts were removed on the sixth day after grafting, fixed in alcoholic Bouin (Duboscq-Brasil) and embedded in paraffin. Sections of the grafts were stained by Ehrlich's haematoxylin and chromotrope.

RESULTS

The histological condition of the 36 skin homografts was assessed using the following classification:-

(A) Survival, with proliferation, of the entire graft epithelium. (See figs. 26-28).

(B) Breakdown of proliferated epithelium in progress. (See fig. 29).

(C) Breakdown of non-proliferated epithelium in progress or complete-"immune" type of breakdown (Medawar, 1944, 1945). (See figs. 30-32).

The results are presented in Table 11. They indicate that implantation of 0.2 gm. of homologous cartilage from the same donor on each of two occasions elicited a state of transplantation immunity in only 5 (28%) of the 18 rabbits.

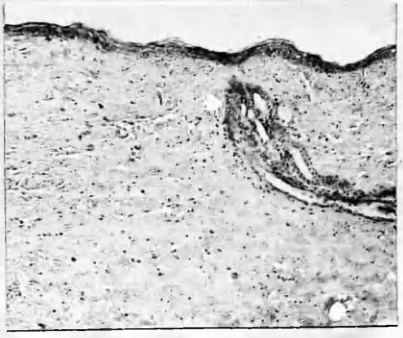

Fig. 25. Ear skin as used for homografting. H & E x 70.

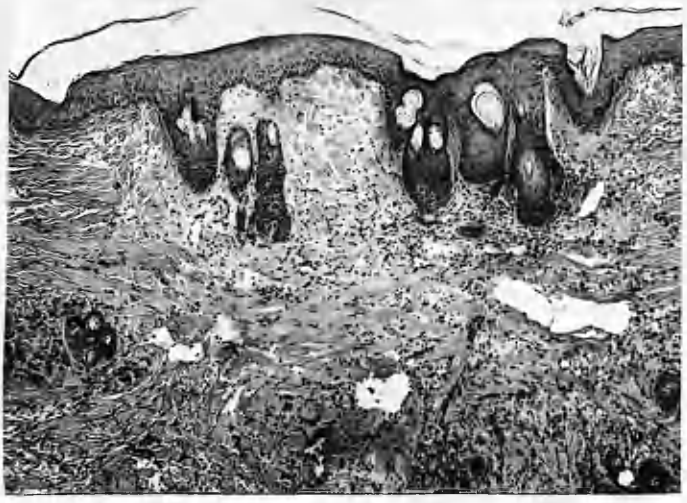

Fig. 26. Homograft of skin after 6 days in a non-immune host. cf Fig. 25. Proliferation has occurred in the epidermis, which remains intact. Grade A condition (see text). H & E. x 70.

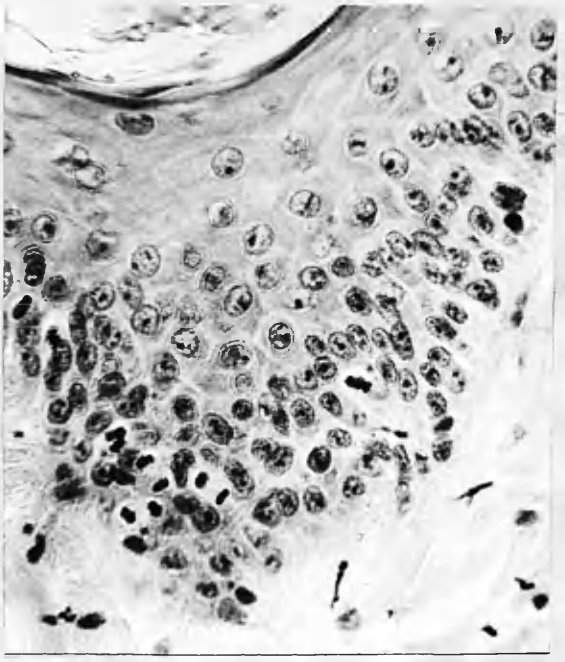

Fig. 27. Homograft of skin after 6 days in a non-immune host. Mitotic activity is to be seen in the epidermis. H & E. x 530.

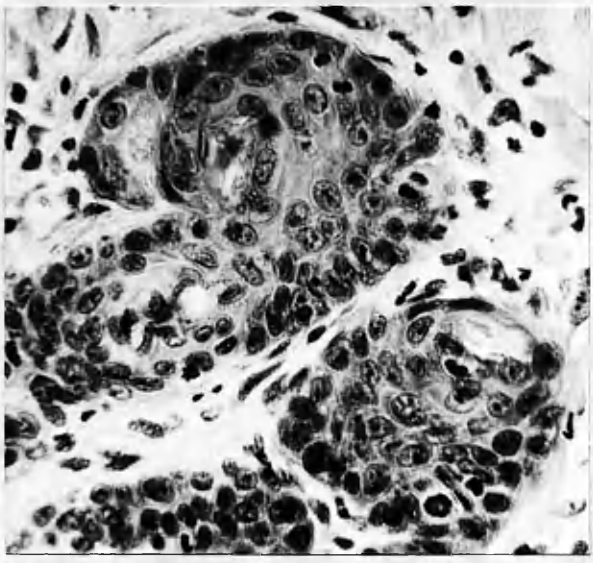

Fig. 28. Homograft of skin after 6 days in a non-immune host. Mitotic activity is present in the epithelium of the hair follicles.
H & E. x 530.

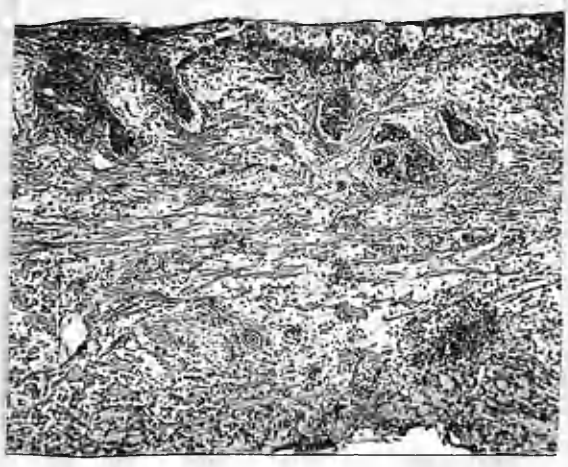

Fig. 29. Homograft of skin after 6 days in a non-immune host. The epidermis has proliferated but exhibits blistering indicative of incipient breakdown. Grade B condition (see text). H & E. x 70.

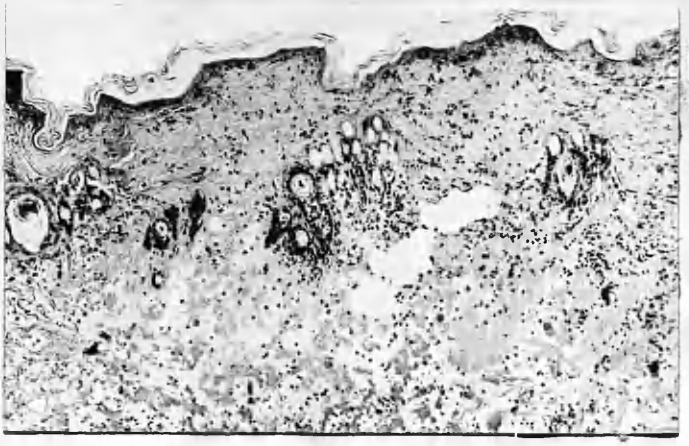

Fig. 30. Skin homograft after 6 days in a rabbit immunised by implantation of cartilage from the skin donor. Proliferation has not occurred in the graft epithelium, which has been destroyed. Grade C condition (see text). H & E. x 70.

Fig. 31. High power view of epidermis from Fig.30 to show nuclear pycnosis and complete epithelial disintegration. H & E. x 530.

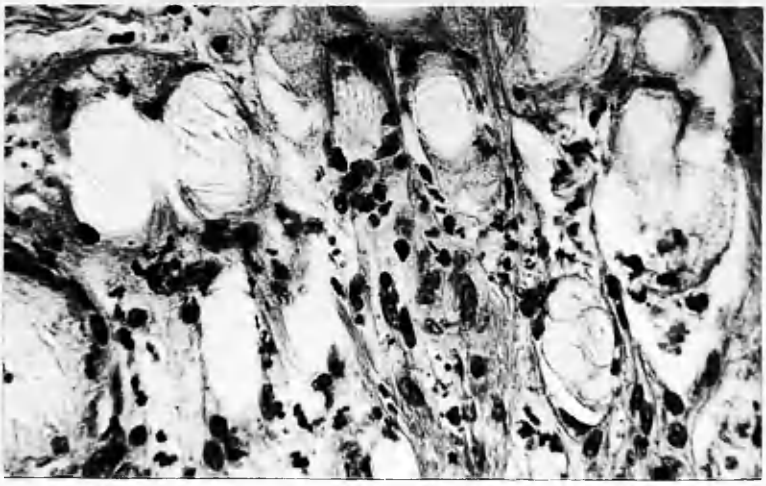

Fig. 32. High power view of a group of hair follicles from Fig. 30 to show epithelial breakdown. H & E. x 530.

TABLE 11.

Graft condition	Control homografts	Experimental homografts
A	8	4
B	10	9
C	0	5

DISCUSSION

Lymph nodes have long been known to be sources of antibody (McMaster and Hudack, 1935; McMaster and Kidd, 1937), and that there is concomitant enlargement of the node seems well established (Habel, Endicott, Bell and Spear, 1949; Erich, Drabkin & Forman, 1949; Pinkus, Albert and Johnson, 1954; Andreini, Drasher and Mitchison, 1955). The demonstration that lymph node cells from homografted animals can confer a state of sensitivity if introduced into normal animals (Mitchison, 1954; Billingham, Brent and Medawar, 1954, 1956c) establishes the complicity of the lymph node in antibody production to homografts. Tissue transplantation immunity differs from orthodox immune responses, which are mediated by humoral antibodies, in that it is mediated through both activated lymphoid cells and humoral antibodies (although circulating antibodies need not participate in the reaction by which skin homografts are destroyed) and thus belongs to the same class of immunological reaction as do the drug and bacterial allergies (Medawar, 1958). Hence the histological changes at the seat of antibody production may not

necessarily be the same for both types of immune response. There seems little doubt that, in orthodox immune responses, there is transformation of undifferentiated mesenchymal cells into "lymphoblastic" or "immature lymphoid" cells (Rich, Lewis and Wintrobe, 1939; Dougherty and White, 1947; Harris and Harris, 1948, 1949; Parsons, 1943; Bjorneboe and Gormsen, 1943; Bjorneboe, Gormsen and Lundquist, 1947; McNeil, 1948, 1950; Fagraeus, 1948; Marshall and White, 1950; Ringertz and Adamson, 1950; Keuning and van der Slikke, 1950; Leduc, Coons and Connolly, 1953) but the subsequent development of this cell is widely disputed. The majority opinion is that it becomes a plasma cell, but there is a group of writers who favour the view that it becomes a lymphocyte. In view of the fact that it has been suggested that transformations between lymphocytes and plasma cells can occur (Darcy, 1952b; Jordan, 1954; Sundberg, 1955) the distinction between the two cell types may be much less rigid than has hitherto been drawn.

The changes in the lymph node regional to the site of a skin

homograft are basically similar to those occurring in orthodox immune responses: there is increase in weight of the node accompanied by hyperplasia of the cortex and production of large "lymphoid" cells (Scothorne and McGregor, 1955; Scothorne, 1956), appearances which are manifest by the fourth day after grafting. A single cartilage homograft did not produce enlargement of the regional lymph node during the 14 days consequent upon implantation. However, implantation of a similar amount of cartilage in two successive half-doses sufficed to produce some enlargement of the regional node. Enlargement of a considerably greater degree was produced by a single implantation of fresh bovine or guinea-pig cartilage, or by two successive implants of the latter: the degree of enlargement was approximately equal in all three instances. Implantation of heterogenous (bovine) cartilage which had been boiled for either one or ten minutes produced a moderate degree of enlargement of the node on the operated side. Every enlarged node from the operated side possessed

great numbers of large- and medium-sized pyroninophilic lymphoblast-like cells in the cortex. The large type was rarely to be seen in control nodes, especially in those removed before the eighth day: after this time, the medium-sized pyroninophilic lymphoblastic cell was often seen in control nodes, and the large type was not uncommon. This finding can be explained by the fact (Medawar, 1945) that the immune response has sometimes become systemic by the eighth day. Plasma cells were found, if anything, to be present less frequently in the enlarged hyperplastic nodes from the operated side than in the control nodes.

In view of these changes induced in the regional lymph node by double-set cartilage homografts, and by both single and double-set heterografts of cartilage, it would appear that foreign cartilage grafts are antigenic. The investigation leaves unanswered the question of to what extent the regional lymph node response is elicited on the one hand by intranuclear antigens responsible for calling forth tissue

transplantation immunity in the host, and on the other hand by antigens present in the cytoplasm of the graft cells provoking orthodox immunity in the host (for discussion see Billingham, Brent and Medawar, 1956a), or present even in the matrix. Heterografts must indeed have added antigenicity in respect of their ground substance since heterologous collagen is antigenic (Watson, Rothbard and Vanamee, 1954; Rothbard and Watson, 1956) and heterologous chondroitin sulphate acts as a haptene (Glynn and Holborow, 1952). The experiments do nevertheless indicate that the overall antigenicity of a cartilage homograft is much more feeble than that of a fitted skin homograft of comparable weight (see Scothorne and McGregor, 1955; Scothorne, 1956), since the latter produces a two-fold increase in weight of the regional lymph node by the fourth day after grafting.

Cartilage boiled for up to ten minutes continues to elicit a moderate regional lymph node response after heterografting. Gibson and Davis (1953) showed that implants of boiled (for 10-15 minutes) bovine cartilage in man call forth an intense local cellular reaction and that successive implants underwent increasingly rapid absorption

even when the cartilage was taken from different donors. Medawar (1944) noted that the immune response generated by skin homografts "does not necessarily extend with equal vigour to a graft of second planting derived from a donor other than that which provided the graft of first planting". These various findings, taken in conjunction, would suggest that a heterograft of cartilage boiled for up to 10 minutes is antigenic.

The results of the second series of experiments indicate that implantation of 0.2 gm. of finely diced homologous cartilage on each of two occasions will render only 28% of the recipient animals capable of exhibiting the "immune" type of destruction of a skin homograft from the cartilage donor. Homologous skin weighing 0.36-0.44 gm. openly transplanted will render 100% of rabbits immune to a second skin homograft from the same donor (Medawar, 1944). These findings show that cartilage and skin from the same donor possess histocompatability antigens in common, but that cartilage, by comparison with skin, is poorly endowed with such antigens. As mentioned previously, an openly implanted skin homograft undergoes epithelial proliferation and

migration after implantation, so adding greatly to its effectiveness in sensitising the recipient. These phenomena are not seen in transplanted cartilage, but it was felt that implantation of the cartilage in two amounts would have tended to offset this in some measure.

Histocompatibility antigens reside wholly within the nuclei of the graft cells (Billingham, Brent and Medawar, 1956b). The ability of a homograft to elicit transplantation immunity in the host will therefore be related to nuclear density within the graft, and to the capacity of the graft parenchyma to proliferate after implantation. Cartilage has a sparse cell population and the chondrocytes do not exhibit post-implantation hyperplasia. These considerations would seem sufficient to account for the poor ability of a cartilage homograft to sensitise the host, although the antigen liberated from the graft into the surrounding host tissues may have originated only from cells exposed on the surface of the cartilage dices, and may not have represented the gross complement of antigen

present in the graft. The lymph node experiments did suggest, however, that no relationship existed between the surface area of the cartilage implants and their ability to elicit the lymph node response.

PART III

THE FATE OF CARTILAGE HOMOGRAFTS IN RABBITS SENSITISED BY A SKIN HOMOGRAFT FROM THE CARTILAGE DONOR

INTRODUCTION

It has just been demonstrated that cartilage, by comparison with weights-equivalent of skin, is poorly endowed with the ability either to evoke a regional lymph node response or to elicit a state of transplantation immunity in a homologous host. Complement fixation tests have shown (Khvorostukhin, 1958) that articular cartilage possesses distinct antigenic properties but that, weight for weight with skin it is much less antigenic. It has been suggested that the paucity of the cells in cartilage, and their lack of proliferation after transplantation, in sharp contrast to a skin homograft, both contribute to its poor capacity to elicit a state of transplantation immunity in the host as the antigens responsible for calling forth such immunity are believed to reside within the nuclei of the graft cells.

Absorption of cartilage homografts in man is generally believed to occur as a late sequel to implantation. The questions which now presented were these. Are cartilage homografts enabled to survive on account of their poor complement of histocompatibility antigens? Does the late absorption of a cartilage homograft, since it is not seen with autografts, represent a delayed immunisation of the host by the cartilage? Answers to these questions, it was felt, might be provided by studying the behaviour of cartilage homografts in hosts sensitised by a skin homograft from the cartilage donor since the two tissues possess iso-antigens in common (Part II).

MATERIAL & METHODS

Fifteen rabbits were used in the investigation, 5 albinos as donors and 10 brown rabbits as hosts. Each of the latter received, under Nembutal anaesthesia, homologous ear skin weighing approximately 0.4 gm. on a large open bed prepared on the dorsal thoracic wall, a procedure which will render adult rabbits immune (Medawar, 1944).

After two weeks each host had rejected the homologous skin, and then received a subcutaneous implant of 3 to 5 pieces of freshly removed costal cartilage from the skin donor. Every cartilage homograft was at least half an inch long, and was accurately measured in length before implantation. In all, 38 pieces of cartilage were implanted in the 10 hosts.

One recipient, bearing four cartilage homografts died after two weeks and was excluded from the series. The 34 grafts in the remaining nine host rabbits were left in situ for 400 days, and then recovered, fixed in Bouin's fluid, decalcified in nitric acid and embedded in paraffin. Subsequently, 7 μ paraffin sections were stained by:

(1) Haematoxylin and chromotrope
(2) Best's carmine stain
(3) The PAS reaction
(4) 0.01% aqueous toluidin blue
(5) 0.5% aqueous alcian blue

Sections of each graft incubated in saliva for half an hour at 37° to

digest any glycogen present were also subjected to the PAS reaction and to Best's carmine stain.

RESULTS

The 34 cartilage homografts were all recovered after 400 days from the nine hosts, and in none had any reduction in length occurred. In 7 of the nine recipients (rabbits 1,2,3,4,5,6 and 9 in Table 12), bearing collectively 26 pieces of cartilage, all the grafts were found to consist of normal cartilage histologically (Fig. 33). The chondro-

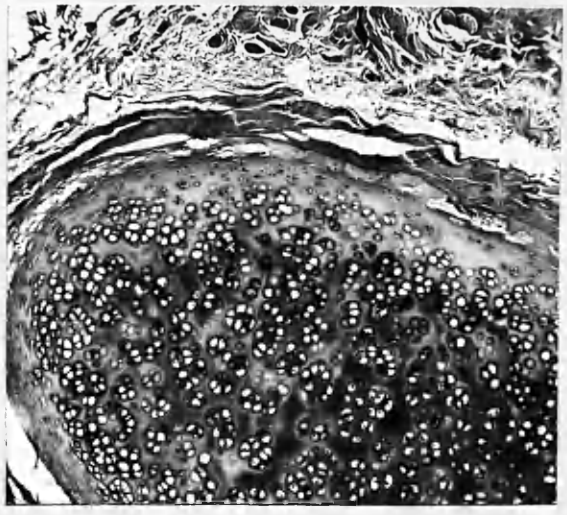

Fig. 33. Cartilage homograft after 400 days in a sensitised host. Normal cartilage. H & E. x 54.

cytes possessed a normal complement of glycogen as demonstrable by Best's

carmine stain and the PAS reaction (Fig. 34) and the matrix gave normal

TABLE 12

Donor Rabbit	Recipient Rabbit	Number of Implants	Condition of Cartilage Homograft Viable	Non Viable
A	1	3	3	0
	2	3	3	0
B	3	4	4	0
	4	5	5	0
C	5	4	4	0
	6	4	4	0
D	7	4	3	1
	8	4	0	4
E	9	3	3	0
	10	Excluded from series.	-	-
	TOTALS	34	29	5

histochemical reactions to alcian blue, toluidin blue and the PAS reaction. Five of these 26 cartilage homografts showed some measure of degeneration of the centrally-situated cells, which possessed neither nuclei nor cytoplasmic glycogen: there seemed to be no correlation between the presence of this degeneration and the cross-sectional area of the implant, however. The remaining two rabbits (Nos.7 and 8 in

Table 12) each bore four cartilage homografts, all eight having been

Fig. 34. Chondrocytes, with normal glycogen deposits, in a 400 day cartilage homograft from a sensitised recipient.
PAS. x 280.

contributed by a common donor. In the first of these hosts three implants consisted of normal cartilage with extensive central degeneration and the fourth implant contained only dead cells (Fig. 35), since

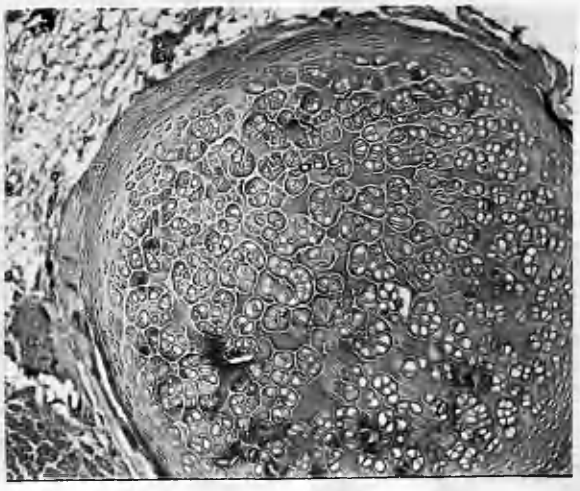

Fig. 35. One of five completely non-viable cartilage homografts recovered after 400 days from a sensitised host. The matrix is eosinophilic (cf. Fig. 33) and there is absence of nuclear staining in the chondrocytes.
H & E. x 54.

there was absence of nuclear staining in, and loss of glycogen from the cytoplasm of (Fig. 36), every chondrocyte; in the second host,

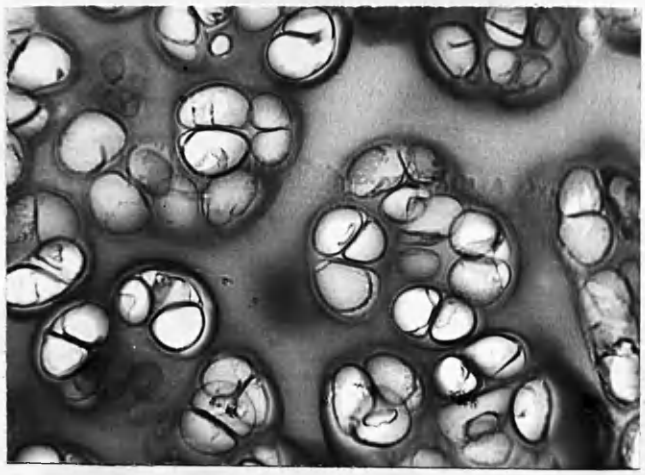

Fig. 36. Cartilage homograft shown in Fig. 35. PAS reaction. Absence of glycogen deposits in every chondrocyte. x 280.

none of the four cartilage homografts was found to contain any living cells. Little evidence of any host cellular reaction was found in

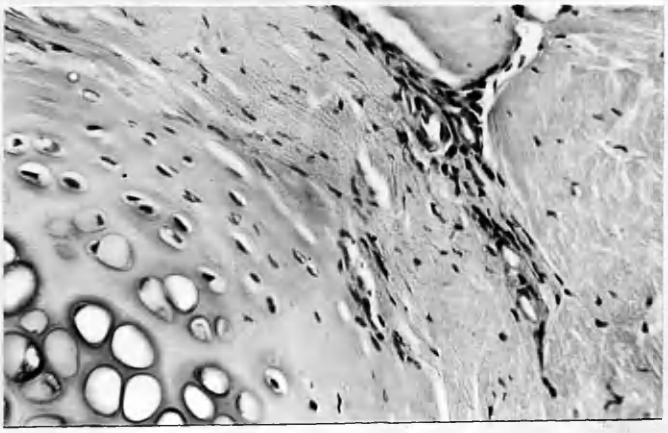

Fig. 37. Normal cartilage homograft after 400 days in a sensitised host. A perivascular collection of host leucocytes is seen near the graft perichondrium. H & E. x 270.

the vicinity of all the implants, whether viable or non-viable (Figs. 33 and 35) although small localised accumulations of host cells were occasionally seen (Fig. 37). In one or two of the grafts a slight degree of invasion had occurred through the perichondrium

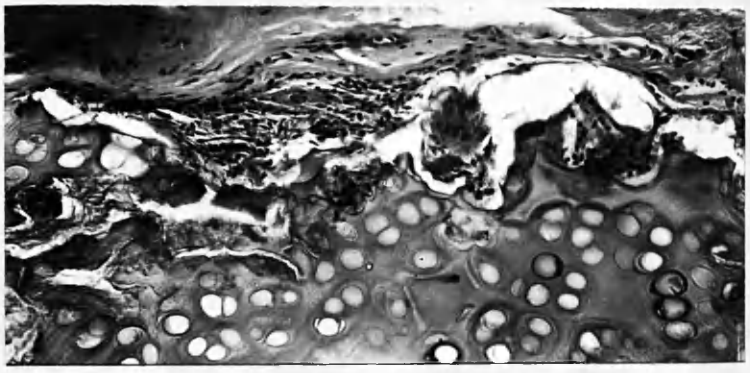

Fig. 38. Edge of a normal viable cartilage homograft removed from a sensitised host after 400 days. Some invasion has occurred through the perichondrium, and the cartilage in the immediate vicinity is non-viable. H & E. x 165.

(Fig. 38), but this finding was not encountered exclusively in either the viable or the non-viable implants.

DISCUSSION

This investigation shows that cartilage homografts underwent no gross change during 400 days in rabbits which had been sensitised -

if not rendered immune - by an openly-transplanted high dosage homograft of skin from the cartilage donor. Microscopically, it was seen that no greater degree of invasion or absorption had occurred than in comparable cartilage homografts implanted in non-immune rabbits for a similar period (Part I). From this it may be concluded that the degree of transplantation immunity of the host is of minor import as far as persistence of the graft is concerned, and by corollary, that cartilage homografts do not persist because of their poor ability to elicit the homograft response. Further, the late absorption of cartilage homografts which is reputed to occur is possibly not attributable to a delayed appearance of the homograft response in the host. Yet there seems to be a correlation between the capacity of a graft of foreign cartilage to elicit a response in the regional lymph node - indicative of the overall antigenicity of the implant - and its ability to persist. Thus, fresh cartilage heterografts evoke a strong regional lymph

response and such grafts have a short life span; heterografts of boiled cartilage call forth but a moderate reaction in the regional node and are longer-lived than fresh heterografts. Homografts of fresh cartilage elicit a very weak response in the regional lymph node and exhibit longevity of a high order. Add to this that the best clinical results using cartilage homografts have been obtained with refrigerated cartilage (O'Connor & Pierce, 1938; Straith & Slaughter, 1941; Eisenstodt, 1953; Rasi, 1959). Khvorostukhin (1958) has shown that such cartilage is much less antigenic than fresh cartilage. This he determined by complement fixation tests which take cognisance of circulating antibodies and so will indicate overall antigenicity since both orthodox and transplantation immunity are mediated by this means. The immune response generated by intranuclear histocompatibility antigens will be directed against the graft cells, which are however out of reach of the antibody-bearing host lymphoid cells; it will not be directed against the

matrix. Persistence of a cartilage homograft is an attribute of the matrix, as was suggested by Bacsich and Wyburn (1947), since it occurs even if the graft cells are not viable. If the graft ground substance were antigenic (in the orthodox sense) or if the antibodies called forth by cytoplasmic antigens level themselves against the ground substance then the association between overall antigenicity and ability to persist can be explained. The matrix is composed of a collagen-chondroitin sulphate complex, and recently, after many failures, collagen (Watson, Rothbard and Vanamee, 1954; Rothbard and Watson, 1956) has been shown to be antigenic and chondroitin sulphate (Glynn and Holborow, 1952) haptenic in another species. Hence the ground substance of cartilage heterografts must be considered as antigenic but whether the matrix of cartilage homografts is similarly endowed must await further investigation by the immunologists: at most, however, the ground substance of homografts can be but poorly antigenic in view of the mild lymph node response generated by such grafts. The difference in ability to persist between cartilage homografts and hetero-

grafts may lie with the difference in antigenicity of matrix between the two types of implant.

In the present series of experiments, a few implants showed evidence of some invasion having occurred through the perichondrium, a finding which was never seen in cartilage homografts in non-immune recipients (Part I) and hence the skin homograft may have procured a slight degree of sensitisation, in the orthodox sense, in respect of the subsequent cartilage homograft: it has to be admitted, however, that the evidence is, to say the least, frail. Gibson and Davis (1955, 1959) have recently observed that heterografts of boiled bovine cartilage in man persisted largely unaltered for periods up to 7 years if they came to be surrounded by a bursa-like sac. This finding could be interpreted to mean that such a development will serve either to reduce release of orthodox antigens from the graft, or to prevent host agents directed against the ground substance from gaining contact with it to bring about its dissolution.

Microscopic examination of the 34 cartilage homografts in the present series revealed that 29 were viable, and 5 were non-viable. Loss of viability in a cartilage implant is not infrequently seen in clinical practice, and does not appear to occur with any greater frequency in grafts of foreign cartilage than in autografts: it is thought to be due to haematomata or dead spaces in the immediate vicinity of the graft interrupting the diffusion of nutritive substances from the graft bed (Gibson, Davis and Curran, 1958). The distribution of the five non-viable implants within the recipients in the present series suggests that some mishap at the time of implantation may have been the underlying cause of the death of the chondrocytes, but the possibility that it represented a manifestation of the host homograft response cannot be completely ruled out. It may be that sometimes a host possessing a state of transplantation immunity furnishes conditions which are incompatible with survival of the grafted cartilage cells, e.g. by producing ischaemia of the

graft bed. It would seem, however, that generally the degree of host transplantation immunity has little influence on the fate of foreign chondrocytes. Survival of the cells in cartilage homografts is almost certainly attributable to the peculiar physicochemical nature of the ground substance, which behaves in a manner comparable to the diffusion chamber devised by Algire, Weaver and Prehn (1954). This chamber allows homologous cells to survive in hosts possessing a state of transplantation immunity by preventing activated host lymphoid cells from establishing contact with the homologous cells to effect their destruction. Whether the matrix achieves this by virtue of its avascularity (Medawar, 1959) or by its being continually replenished from within by the chondrocytes (Bacsich and Wyburn, 1955) has not been established.

In view of the importance of the matrix in promoting both persistence and cellular survival of cartilage homografts, a study of the fate of such grafts after enzymatic degradation of their ground substance will now be described.

PART IV

CARTILAGE HOMOGRAFTS IN PAPAIN-TREATED RABBITS

INTRODUCTION

Crude papain administered intravenously to young rabbits brings about ear collapse within 24 hours (Thomas, 1956; Spicer and Bryant, 1957, 1958; McLuskey and Thomas, 1958, 1959) and there is a concomitant loss of metachromasia of the ear cartilage (Spicer and Bryant, 1957). A return to normal after a single dose occurs within a few days, but can be delayed for about 28 days by the administration of cortisone (Spicer and Bryant, 1957; McLuskey and Thomas, 1959). Biochemical studies have shown that there is release of chondromucoprotein from the ear cartilage and a reduction of chondroitin sulphate content in the chondromucoprotein that remains in the cartilage (Tsaltas, 1958): there is also liberation into the blood and urine of a mucopolysaccharide resembling, in chemical and physical properties, chondroitin sulphates A and C

(Bryant, Leder and Stetten, 1958). In view of this and of the importance which attaches to the matrix in promoting cellular survival and persistence of cartilage homografts, it was decided to investigate the fate of such homografts whose matrix had been degraded by papain and maintained in that condition for some time after implantation by administration of cortisone to the host. Cortisone, however, has the property of prolonging survival of homografts of skin (Billingham, Brent and Medawar, 1951a, 1951b) ovary (Ingram and Krohn, 1954) and thyroid (Woodruff, 1953). Since a study of the regional lymph node response to skin homografts in cortisone-injected rabbits showed that cortisone achieves its effect, probably, by reducing the effective overall antigenicity of the graft (Scothorne, 1956), it was thought to be advisable to attempt to counteract this property of cortisone by using as hosts, rabbits sensitised by skin homografts from the prospective cartilage donors. This precaution would ensure that the hosts be sensitised

to the donor in respect of transplantation immunity and perhaps, also in the orthodox sense (Part III).

MATERIAL & METHODS

The papain sample used[+] was found to produce ear collapse within 24 hours in each of three rabbits given 3 ml. of a 2% aqueous solution intravenously. The ear cartilage from all three rabbits continued to exhibit the property of metachromasia. The metachromasia was, however, more alcohol-labile than that of normal cartilage.

Twenty rabbits of under 1 Kg. body weight were used, ten as donors and ten as recipients: the latter group comprised five control and five experimental animals. All ten recipients were given a skin homograft of 0.4 gm. average weight on an open bed prepared on the

[+] The papain used in these experiments was kindly donated by Messrs. Chas. Zimmermann & Co. Ltd., Perivale, Middlesex.

dorsal thoracic wall: fourteen days later, at which time all had rejected the skin homograft, each received a subcutaneous implant of a large piece of ear cartilage from the skin donor. For 28 days thereafter, all ten recipients received an intramuscular injection of 5 mg. of cortisone daily. The five experimental recipients differed from the five control animals in two respects: the cartilage implant was taken from the donor after the latter had had ear collapse brought about by papain, and secondly the experimental recipients were given an ear-collapse-inducing dose of papain after implantation of the cartilage homograft; they showed ear collapse throughout the 28 days during which cortisone was administered; the matrix of the homografts was presumably simultaneously prevented from being restored to normal during this same time. One control and one experimental recipient died during the period of cortisone administration. The cartilage homografts in the eight remaining rabbits were left in situ for 3 months, then recovered, fixed in Bouin's fluid, decalcified in nitric acid and

embedded in paraffin.

Sections of each graft were stained by:-

(1) Haematoxylin and chromotrop

(2) The PAS reaction

(3) 0.01% aqueous toluidin blue.

RESULTS

Seven of the eight cartilage homografts were found to have become grossly warped and coiled as opposed to the form of a flat sheet at the time of implantation (Fig. 39): they were otherwise unchanged

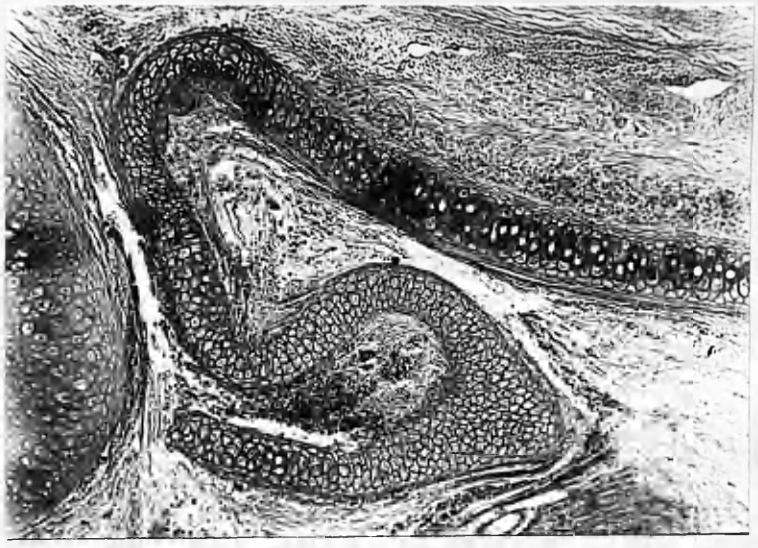

Fig. 39. "Control" homograft of ear cartilage after 3 months. The graft has become coiled and is non-viable in places. The intensity of the surrounding host mononuclear leucocyte reaction varies from place to place. H. & E. x 55.

macroscopically. In the eighth recipient, which was an experimental rabbit, the graft site was very swollen after operation and a secondary infection was suspected. In this rabbit the cartilage homograft had become very much reduced in size and the implantation site resembled a fibrous nodule containing central cartilage fragments. The seven other cartilage homografts (i.e. four control and three experimental homografts) were more or less surrounded by host mononuclear cells, which in places were present in very large numbers (Figs. 39, 41), but penetration of the graft tissue by these cells was rarely seen. The grafts consisted for the most part of normal cartilage histologically and histochemically (Figs. 39-42) but each presented small localised areas where the cells were effete and possessed neither nuclei (Fig. 39) nor cytoplasmic glycogen, and the matrix in their vicinity was non-metachromatic (Fig. 40) and gave a weak PAS reaction. There was no correlation between the presence of this degeneration and the intensity of the surrounding host mononuclear reaction.

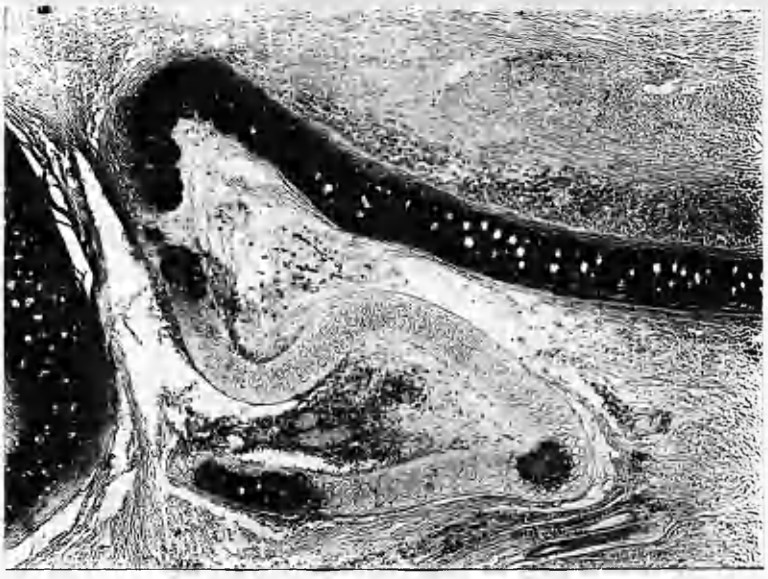

Fig. 40. Adjacent section to that shown in Fig. 40. Toluidin blue. The graft is metachromatic in those regions with viable cells, but is non-metachromatic elsewhere. x 55.

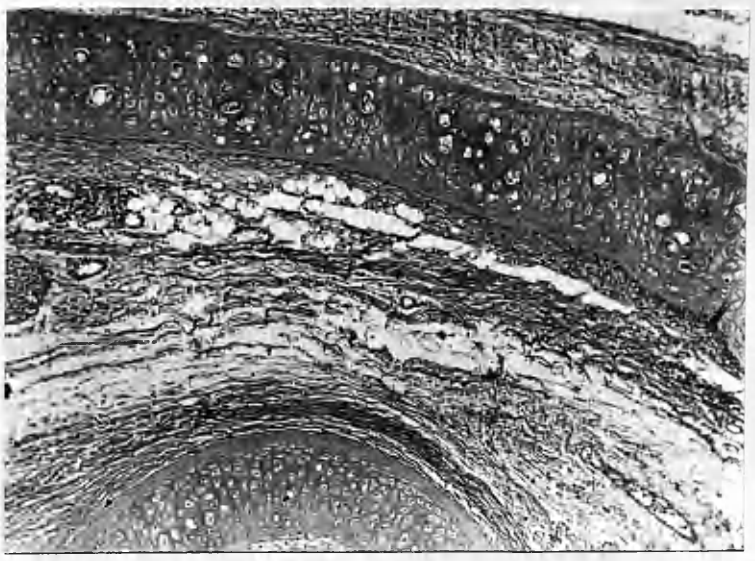

Fig. 41. "Experimental" homograft of ear cartilage after 3 months in a papain-injected recipient. Two viable portions of the coiled graft are seen, with surrounding host mononuclear cells.
H & E. x 55.

Fig. 42. Adjacent section to that shown in Fig. 41. Toluidin blue. Both portions of the graft exhibit metachromasia of the matrix. x 55.

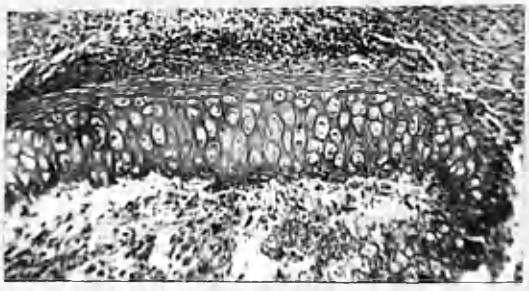

Fig. 43. A portion of normal viable cartilage found in the midst of a fibrous nodule in one papain-injected host. H & E. x 85.

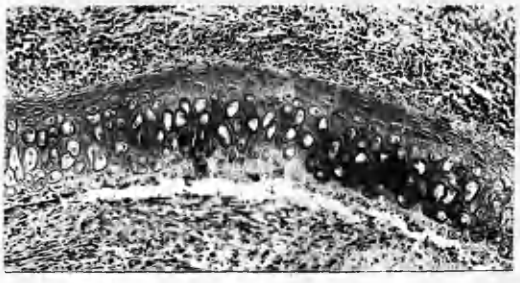

Fig. 44. Fragment of cartilage shown in Fig. 43. Toluidin blue. The matrix is faintly metachromatic. x 85.

No histological or histochemical difference could be detected between the three experimental and the four control homografts, with the exception that the intensity of metachromasia of the ground substance was less in the grafts of the experimental group. Histologically the fibrous nodule, consisted of a few islands of viable cartilage (Fig. 43) giving normal histological and histochemical (Fig. 44) reactions to the techniques employed, but a considerable bulk of the graft had been invaded and replaced by host cells.

DISCUSSION

Cartilage matrix is so degraded chemically by papain as to alter the physical properties of the cartilage. The results of these experiments indicate that cartilage homografts whose matrix has been degraded by papain and maintained in this condition for about a month after implantation are capable of remaining grossly unchanged for three months. One homograft had been largely destroyed but

due, as mentioned, to a secondary infection as it is well known from clinical practice that transplanted cartilage rapidly disintegrates in the presence of pus. The remaining experimental and control homografts were composed, substantially, of normal viable cartilage although each presented localised areas where the cartilage cells were patently non-viable. The explanation for this is to be sought in the rolling-up of the transplanted cartilage which in places would inevitably produce detachment of the cartilage from contact with the graft bed, with consequent interruption of the diffusion of nutritive tissue fluid to the chondrocytes. However, an alternative interpretation that the death of the cells represented a manifestation of the state of transplantation immunity in the host cannot be entirely ruled out. Yet it would seem that the protection from destruction in immune hosts conferred on homologous chondrocytes by their surrounding ground substance is of a high order since matrix containing only residual amounts of chondroitin

sulphate continued to be effective.

Furthermore, the cartilage homografts with papain-induced degradation of their ground substance were found to have undergone no invasion or absorption after three months. This finding would appear to indicate that the powers of persistence exhibited by the matrix are unaffected by its being subjected to partial enzymatic breakdown.

PART V

A STUDY OF HOMOGRAFTS OF BOILED CARTILAGE

INTRODUCTION

At present the ultimate fate of cartilage homografts in man is uncertain. Peer (1954a) reported that homografts of fresh human cartilage after 1, 2, 2½ and 3 years were viable although "all showed definite invasion and partial absorption of the matrix"; a further fresh homograft which had been measured before implantation had lost approximately one third of its bulk after four years. Gibson, Davis and Curran (1958), on the other hand, found that 21 fresh human cartilage homografts recovered at intervals up to two years had undergone only very superficial absorption on raw surfaces, a process which also occurs with autografts (Davis and Gibson, 1956), which is due to localised damage of the cartilage during cutting, and which is completed within a few weeks after implantation; of the 21 homografts, 19 were viable as determined by their ability to take up radioactive sulphur.

Cartilage homografts preserved in merthosaline and stored in refrigerators (at temperatures around 0°C) have also been used extensively in plastic surgery since their introduction by O'Connor and Pierce in 1938; these authors claimed that no absorption occurred within five years in 375 instances. Brown (1940), on the contrary, thought that such implants gave good clinical results for up to three years but that "absorption takes place to some extent in all instances and complete loss may occur by a gradual process without visible reaction over a period of several months. This process is not always uniform." Kirkham (1940) reported that merthosaline-preserved cartilage homografts could persist for up to four years. Straith and Slaughter (1941) using similar implants claimed satisfactory results in 94% of their cases followed-up from 18 months to four years. Eisenstodt (1953) found that no reduction in size had occurred in 14 preserved cartilage homografts after periods varying between 4 and 11 years. Schofield (1953a) also

reported that 86% of his cases were satisfactory for up to two years. Finally Rasi (1959) in a long-term follow-up study of 49 of Straith and Slaughter's cases found that, after between 2 and 18 years, total loss had occurred in 11.9%; 32.2% had undergone no loss of bulk and the remaining 55.9% showed some loss of bulk; of the latter group a quarter were poor, a third were fair and the rest good as regards contour.

Many of these authors have reported distortion and early total absorption as complications but the frequencies with which these occur is probably no greater than with cartilage autografts. Late absorption, however, is unique to homografts and it very likely represents some host response to the foreign nature of the cartilage. Brown (1940) thought fresh cartilage homografts were to be preferred to merthosaline-preserved homografts whilst Peer (1954b) wrote: "clinically, preserved homogenous grafts retain their structure about as well as fresh homogenous cartilage grafts."

Cartilage grafts possess the supreme advantage over grafts of most other tissues that the parenchymatous cells are not required to be viable for the graft to be effective; there is indeed, some evidence that preserved non-viable cartilage homografts have greater powers of persistence than fresh cartilage homografts. Consequently, it is rational to attempt to modify cartilage so that it may become more, or even completely acceptable to homologous hosts even if this would involve destruction of the graft cells, a sequel which would render the majority of tissue homografts, other than cartilage, useless. The ideally-modified cartilage homograft would be required to fulfil four conditions:

a) that it would not warp
b) that it would be non-antigenic and therefore exempt from late absorption
c) that its qualities as a prosthesis would not have been altered during modification
d) that its ability to persist would not be influenced by alteration of the matrix during modification.

One method of modification of cartilage consists of immersing it in boiling water for 10 minutes prior to implantation. New and Erich (1941) found that this militated against post-operative warping and suggested that even autografts be so treated. Hence modification by boiling will render cartilage capable of fulfilling condition a), and there is strongly presumptive evidence that cartilage after boiling would also meet condition b). Siebert (1928; 1931) noted that cartilage homografts no longer attracted host leucocytes after they had been heated to 47°C for 30 minutes. Billingham, Brent and Medawar (1956b) moreover showed that transplantation immunity antigens are inactivated by heating cells to 48.5°C for 20 minutes. As regards condition c), clinical experiences with <u>heterografts</u> of boiled cartilage indicates that boiling does not unduly interfere with the physical property of the cartilage. A question mark, however, lies opposite the ability of boiled cartilage to fulfil condition d). The surgeons, in view of their disappointing experiences with <u>heterografts</u> of boiled

cartilage now look with suspicion on any implant of boiled cartilage without realising that, as has already been discussed, the heterologous nature of the implants was almost certainly the reason for their rapid destruction, and not the boiling. Homografts of boiled cartilage however have never been used, as far as is known, in reparative surgery. Consequently, it was decided to investigate the histochemical nature and fate of long-term homografts of boiled cartilage in rabbits.

MATERIAL & METHODS

Seven rabbits were used in the investigation. Costal cartilage, taken from newly-killed donor rabbits unrelated to the prospective recipient, was immersed in distilled water in a test tube suspended in a beaker of boiling water for exactly one (4 experiments) or ten (3 experiments) minutes. One piece boiled for each time period was fixed in Bouin's fluid and processed for histological examination. Several pieces, after being measured in length, were implanted

subcutaneously into each of the seven rabbits. Two rabbits, one bearing cartilage homografts boiled for one minute and the other bearing cartilage boiled for ten minutes, were sacrificed after 24 days and the grafts recovered. The 20 grafts of the remaining five rabbits were removed after 400 days, measured, fixed in Bouin's fluid, decalcified in 5% nitric acid and embedded in paraffin. 7 μ sections of every graft and of the control pieces of boiled cartilage, were stained by:-

(1) Haematoxylin and chromotrope
(2) The PAS reaction
(3) 0.01% aqueous toludin blue
(4) 0.5% aqueous alcian blue.

RESULTS

The control pieces of cartilage boiled for one or ten minutes were found to possess nuclei of normal appearance and a matrix which was metachromatic, alcian blue- and PAS-positive.

The homografts of boiled cartilage recovered after 24 days

were unchanged macroscopically. Histologically it was seen that occasional groups of chondroblasts had retained their nuclear staining (Fig. 45). Little evidence of any host mononuclear reaction

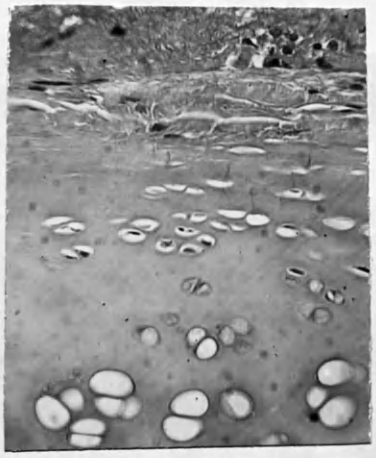

Fig. 45. Portion of the edge of a homograft of cartilage(boiled 1 minute) removed after 24 days to show absence of a host leucocytic reaction in the vicinity of the graft. H & E. x 295.

was seen in the vicinity of these implants (Fig. 45). The matrix of the implants possessed normal histochemical responses to toludin blue, alcian blue and the PAS reaction.

All 20 homografts recovered from the remaining five rabbits after 400 days appeared macroscopically unchanged; no reduction in

length had occurred. Microscopically the cartilage was found to have retained its structure and little evidence of invasion or destruction was seen in any of the implants (Fig. 46). Few host mononuclear cells

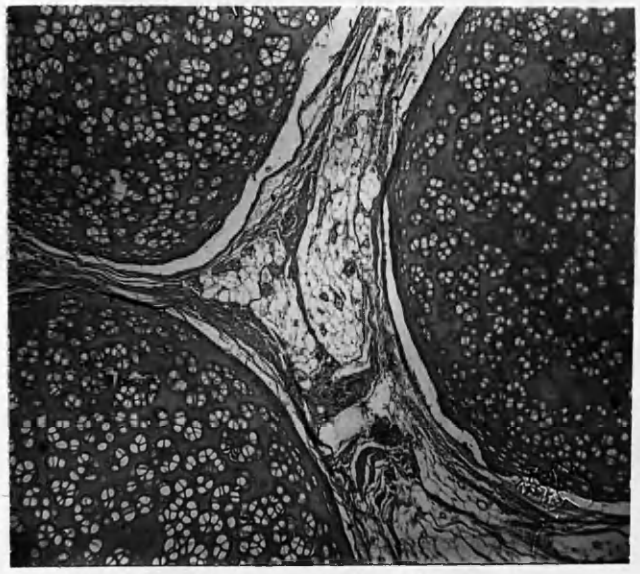

Fig. 46. Portions of three 10 minute-boiled homografts of cartilage after 400 days, to show that the grafts have remained unaltered.
H & E. x 50.

were found in the vicinity of the implants, but occasional foreign body giant cells were seen in eroded regions of the grafts (Figs. 47, 48). In several of the 20 implants small groups of cartilage cells

Fig. 47. Portion of the edge of a boiled (1 minute) homograft after 400 days to show an attenuated multinucleated giant cell lying in a recess in the perichondrium.
H & E. x 460.

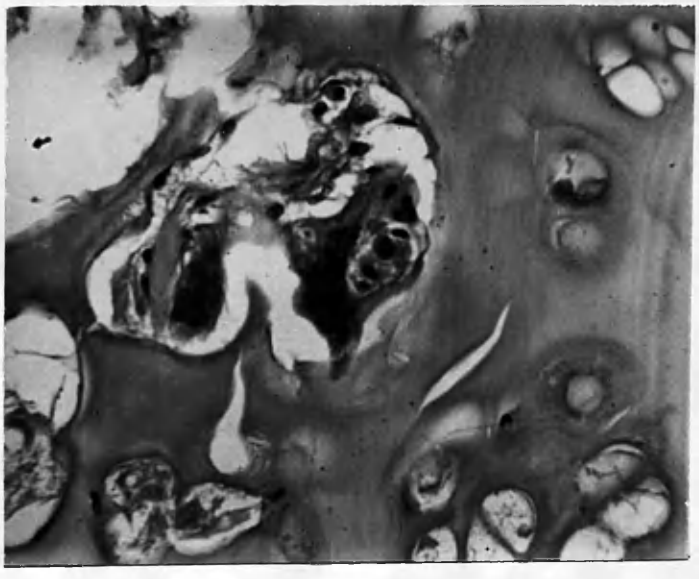

Fig. 48. Portion of a one-minute-boiled homograft after 400 days showing two multinucleated giant cells in an erosion in the graft.
H & E. x 410.

with normally-staining nuclei were seen in or just under the perichondrium (Figs. 49, 50) and even occasionally in the central

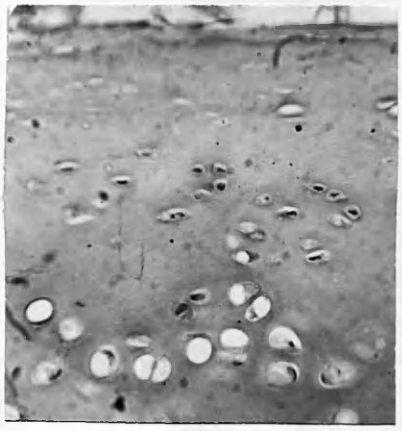

Fig. 49. Part of a one-minute boiled cartilage homograft after 400 days showing a group of cells under the perichondrium with normally staining nuclei.
H & E. x 295.

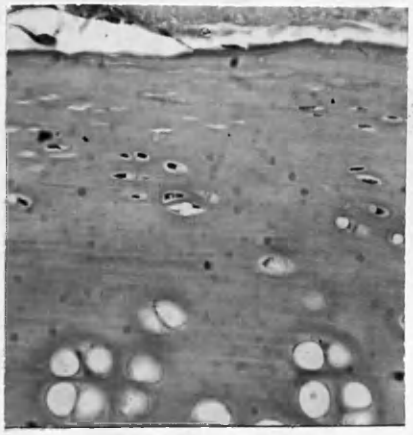

Fig. 50. Edge of a one-minute boiled cartilage homograft after 400 days showing a group of normally staining nuclei in and under the perichondrium.
H & E. x 295.

regions of the implants, but the bulk of the cartilage cell cavities did not show a nuclear stain, especially in those implants which had been boiled for 10 minutes (Fig. 46). The matrices of all 20 homografts were metachromatic (Fig. 51) and alcian blue- and PAS-positive (Fig. 52).

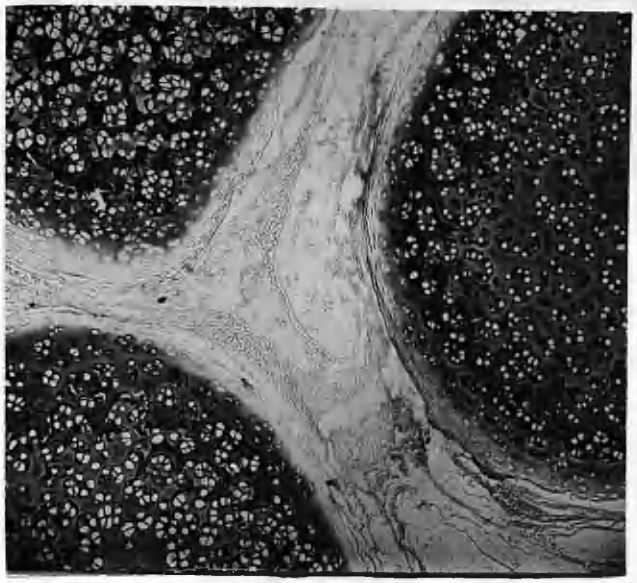

Fig. 51. Three 10-minute-boiled homografts shown in Fig. 46. All possess a metachromatic matrix.
Toluidin blue. x 50.

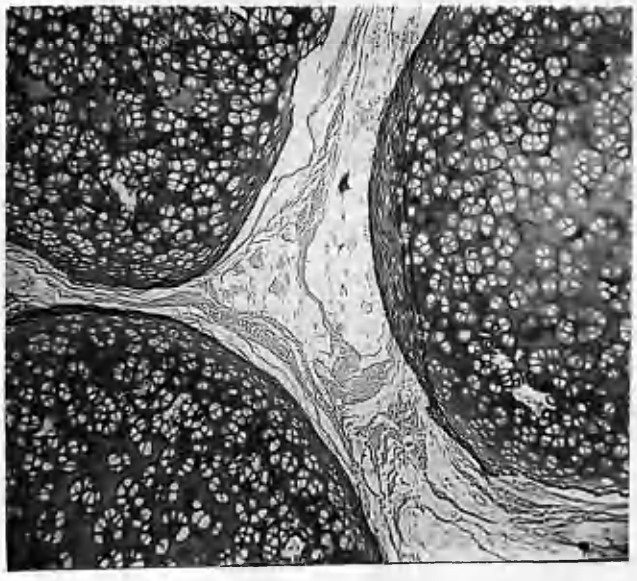

Fig. 52. Three 10-minute-boiled homografts after 400 days. The matrix of each graft is PAS positive. x 50.

DISCUSSION

These experiments supplement the observations of Siebert (1928, 1931) (see p.97) in that they include cartilage homografts heated to 100°C for one minute. The only host reaction seen to occur in the vicinity of homografts of boiled cartilage was the presence of an occasional foreign body giant cell. Moreover, the grafts, on microscopic examination, had not been subjected to invasion to any great extent.

The survival of a few cartilage cells in some of the implants is of interest. It could mean that in these grafts some antigens must remain in both nucleus and cytoplasm of the surviving cells, but there can be little doubt that homografts of boiled cartilage are less antigenic than fresh cartilage homografts.

The capacity of the homografts of boiled cartilage to persist was equal to that of fresh autografts or fresh homografts over the period studied (see Part I). Moreover the histochemical reactions

of the matrix of the boiled implants had remained unaltered during this period, suggesting that the ability of the graft to persist even longer is unlikely to be impaired.

Collectively, the findings of the present experiments would indicate that homografts of cartilage boiled for up to 10 minutes might constitute a very satisfactory implant in reparative surgery. They do not warp; they can be only very mildly, if at all antigenic; and the preservation of the properties of the matrix may well assure persistence of the implant. Since this study was undertaken, Gibson and Davis (1958) have shown,in an ingenious series of experiments, that post-operative warping of a cartilage implant can be avoided by the simple expedient of trimming the graft so that it has a 'balanced' cross section. Hence it is not now necessary to submit cartilage to such an extreme of temperature as 100^{o} during modification. If the implant can be rendered non-antigenic and thus exempt from late absorption, the case will be met. Tissue homografts heated to $47^{o}C$

for 30 minutes (Siebert, 1928, 1931) or frozen to -70°C and thawed to 37°C on five occasions (Darcy, 1955) do not attract host leucocytes and may be devoid of antigens or may be at best mildly antigenic. Cartilage modified by either of these methods may constitute an even more satisfactory implant after homografting than boiled cartilage, since the matrix may suffer less degradation. Steps will accordingly be taken to determine whether this is so.

GENERAL DISCUSSION

The readiness with which cartilage can be shaped with a scalpel renders it the material of first choice in reparative procedures involving restoration of contour, and it has been widely used by cosmetic surgeons since the beginning of the present century. The cartilage utilised has been obtained from a variety of sites not only from the patient himself, but also from other human beings or even other animals; and it has been implanted fresh, after refrigeration or boiling, or after having been stored in a variety of preservative fluids for varying lengths of time, or even after having been subjected to various combinations of these treatments. Not surprisingly, the fate of transplanted cartilage has been the subject of much controversy, because many of the above variants have not been tested under controlled experimental conditions, and outcome often has been assessed purely on clinical results. This has led to condemnation of guilty and guiltless alike, and, in modern times, to a considerable decline in the use of cartilage as a prosthesis

in favour of other bland substances. This is unfortunate, for the autograft of fresh cartilage resected in such fashion that the internal stresses of the implant are evenly-distributed so that it will not warp post-operatively (Gibson and Davis, 1958) constitutes the perfect cosmetic graft - pliant, durable, non-irritant and easily shaped as it is. One weakness of character alone detracts from the make-up of the heroine of the piece - to obtain the implant is tedious and necessitates submitting the patient to disfiguring and debilitating operative interference. On account of this shortcoming, the leading lady has been dismissed by the reparative surgeon and all understudies so far tested have been found to possess other and equally serious failings.

The obvious second choice was fresh cartilage from a relative or an unrelated donor, but in both instances the same defect prevailed as with autografts. Copious supplies of homologous cartilage are obtainable however, at post-mortem but the material was rarely

available fresh when required and so the preserved cartilage bank came into existence (O'Connor, 1939). Many, but not necessarily all, of the methods of preservation brought about the death of the cartilage cells but since it was generally believed that the cells in any graft of fresh cartilage died soon after implantation in any case, this seemed of little consequence. The investigations described in the first part of this thesis indicate that the cells in cartilage auto- and homografts in rabbits retain both their histochemical characteristics and their ability to metabolise $^{35}SO_4$ for at least 22 months, and it is reasonably certain that the cells remain viable. Cartilage heterografts however are largely destroyed during a similar period, but the cells in any persisting fragments also contain normal histochemical deposits and are able to metabolise $^{35}SO_4$.

The cosmetic surgeons, in their quest of a suitable alternative to autogenous cartilage readily accepted, at its face value, the

claim of Wardill and Swinney (1947) that, after boiling cartilage loses its "biological personality" and that after introduction into heterologous hosts it elicits "practically no reaction whatever". Early results of the use of boiled bovine cartilage heterografts in man were encouraging (Marino and Niklison, 1949; Gillies and Kristensen, 1951) but later it was reported that the majority of the implants showed some degree of absorption within two years (North, 1953; Gibson and Davis, 1953). The latter authors found that successive implants of such material called forth an increasingly intense local reaction and underwent increasingly rapid absorption. It has been shown in this thesis (Part II) that a heterograft of boiled cartilage retains its ability to elicit a response in the regional lymph node. All these findings suggest that boiled cartilage is "antigenic" in heterologous hosts. Collagen (Watson, Rothbard and Vanamee, 1954; Rothbard and Watson, 1956) has been shown to be antigenic and chondroitin sulphate

(Glynn and Holborow, 1952) haptenic for a member of another species, and it is suggested (see p.78) that the difference in fate between cartilage homografts and cartilage heterografts may be related to this fact.

Clinical experience has shown that homografts of preserved cartilage are tolerated by the host as well as, if not better than, homografts of fresh viable cartilage, and so the presence of living cells is not a prerequisite for persistence of a cartilage homograft. It should be stressed that survival (in its strict biological sense) and persistence are not synonymous terms when applied to cartilage homografts. Tissue homografts, other than cartilage, fail to survive on account of the host homograft response which is called forth by intranuclear antigens present in the graft cells and is mediated through activated host lymphoid cells (Medawar, 1958). Three reasons have been advocated for the ability of cartilage homografts to survive. Firstly, it was suggested that cartilage is mildly antigenic (Loeb, 1930) or non-antigenic (Allbrook, 1954;

Wyburn, 1959). Secondly that the continuous production, by the homologous chondrocytes, of acid mucopolysaccharide renders them safe from destructive agents of the host (Bacsich and Wyburn, 1955). Finally it is held that the avascularity of cartilage prevents activated host lymphoid cells from gaining access to the chondrocytes (Medawar, 1959). In part two of this thesis it has been shown that double-set cartilage homografts induce changes in the regional lymph node indicating some degree of antigenicity (in a general sense) which is, however, poor compared with that of a skin homograft of comparable weight (see Scothorne and McGregor, 1955); it was also shown that cartilage is endowed with histocompatibility antigens since it can confer a state of sensitivity in respect of a skin homograft from the same donor. Again, however, its ability to do so is less definite than that of an aliquot of homologous skin (see Medawar, 1944). Sparseness of the cells in cartilage and their lack of proliferation after implantation could well be sufficient reason to account for the poor complement of histocompatibility

antigens present. Other factors however enter into the survival of homologous chondrocytes for it was found that the cells remained viable after cartilage homografts had been implanted in hosts sensitised by a skin homograft from the cartilage donor (Part III); moreover, they remain alive in sensitised hosts even after their surrounding matrix has been degraded by papain and maintained in this condition for the first month after implantation (Part IV). The matrix, even if partially degraded, thus appears to protect the homologous chondrocytes by behaving in a manner comparable to that of the diffusion chamber devised by Algire, Weaver and Prehn (1954) which allows homologous cells to survive in immune hosts; and the matrix may be assisted in doing so by the fact that it is being continually replenished from within (cf. Bacsich and Wyburn, 1955).

Persistence of a cartilage homograft is obviously an attribute of the ground substance since it is independent of the viability of

the graft cells. The experiments described in the third part of this thesis indicate that the power of persistence is not materially affected by the degree of transplantation immunity of the host, as cartilage homografts remained unaltered after 400 days in hosts sensitised by a skin homograft from the cartilage donor. Gibson, Davis and Curran (1958) are of the belief that cartilage matrix is non-antigenic in homologous hosts. No compound present in the ground substance has ever been shown to be antigenic within a species. However, the clinical results using merthosaline-preserved refrigerated cartilage homografts suggest that such implants may have a greater ability to persist than homografts of fresh cartilage. Whilst the critical factor here may be the preservative ("Merthiolate") Khvorostukhin (1958) has shown by complement fixation tests that refrigerated cartilage is less antigenic than fresh cartilage. This suggests that a measure of orthodox immunity may be called forth in the host by the homologous cartilage and that persistence is indirectly proportional to the strength of this immune response. The conclusion

that an immune response underlies the rejection of homologous cartilage is substantiated by the fact that autogenous cartilage is not absorbed. Hence in homotransplantations cartilage ground substance may be antigenic in the orthodox sense, but further immunological elucidation of this point is required.

In view of the fact that the presence of living chondrocytes is not essential for the clinical success of cartilage homografts, it seemed rational to attempt to modify cartilage before implantation into homologous hosts so that it might become permanently acceptable, even if the cells were killed furing the process of modification. If, at the same time the implant could be rendered proof against post-operative distortion, then a very satisfactory implant would ensue. Boiled cartilage does not warp (New and Erich, 1941) and does not attract host leucocytes after homografting (Siebert, 1928, 1931) so is possibly non-antigenic. Such implants however have

never been used by cosmetic surgeons because they attributed their lack of success with boiled cartilage heterografts at least in part to the act of boiling. In part V of this thesis it has been shown that homografts of boiled cartilage persist unchanged, and retain histochemical integrity of matrix, for 400 days in rabbits. It is suggested that such implants may constitute a very satisfactory prosthesis in plastic surgery. Gibson and Davis (1958) have recently shown that post-operative distortion can be avoided by trimming the cartilage so that it has a 'balanced' cross-section. Hence boiling is not necessary and implants of cartilage heated to 47°C for 30 minutes or frozen to -70°C and thawed to 37°C on five occasions, procedures which will render the cartilage unable to attract host leucocytes after homografting, may also comprise satisfactory implants. Long-term studies of cartilage homografts thus modified are at present being undertaken.

SUMMARY

1. The history of experimental and clinical transplantation of cartilage is reviewed.

2. Autografts and homografts of fresh costal cartilage in rabbits remain virtually unchanged in various implantation sites for up to two years whereas heterografts of fresh cartilage are almost totally destroyed during a similar period.

3. Histochemical and autoradiographic (using ^{35}S) studies indicate that the cells have remained viable in the autografts and homografts and in a heterograft fragment that had persisted and appeared normal histologically.

4. Cartilage homografts induce a very poor regional lymph node response by comparison with that which has been described for skin homografts of comparable weight.

5. A heterograft of fresh cartilage provokes a much greater regional lymph node response than that produced by a fresh

homograft in similar dosage. The capacity of heterografts of boiled cartilage to call forth the lymph node response is weaker than that of fresh heterologous cartilage.

6. Implantation of 0.2 gm. of finely diced homologous cartilage on two occasions will produce a state of transplantation immunity (as determined by the fate of a subsequent skin homograft taken from the cartilage donor) in only 28% of hosts. It is known that homologous skin in comparable dosage will render 100% of adult rabbits immune.

7. Cartilage homografts survive and persist in homologous hosts possessing a state of heightened homograft sensitivity induced by a previous skin homograft in high graft dosage (0.4 gm.) from the cartilage donor.

8. Cartilage homografts, whose matrix has been degraded by papain and maintained in that condition for about four weeks after grafting, nevertheless survive and persist for at least 3 months

in hosts previously sensitised by a skin homograft in high graft dosage from the cartilage donor.

9. Cartilage boiled for up to 10 minutes does not attract host leucocytes after homografting, and persists unaltered for at least 400 days.

CONCLUSIONS

1. It is stressed that a distinction should be drawn between the ability of a cartilage graft to survive and its ability to persist. Persistence is a property of the matrix, since it is exhibited by non-viable implants.

2. Fresh cartilage auto- and homografts survive and persist for up to two years in rabbits. Fresh cartilage heterografts are largely destroyed within the same period.

3. Cartilage homografts have a low overall antigenicity and a poor capacity to elicit transplantation immunity in the host. It is suggested that the latter phenomenon is in large measure due to the sparseness of the cells in cartilage and their lack of proliferation after implantation in view of the fact that transplantation immunity antigens are nuclear compounds.

4. Neither persistence, nor survival of the cells, of cartilage homografts appear to be related to the poor complement of "histocompatibility" antigens, since both properties are

retained by cartilage homografts implanted in hosts possessing a state of transplantation immunity. Survival of homologous chondrocytes appears to be a measure of the protection afforded them by the matrix against the activated host lymphoid cells which are the effector agents of the homograft response. Matrix degraded by papain continues to ensure survival and persistence of cartilage homografts.

5. It is suggested that the ground substance of foreign cartilage grafts is antigenic in the "orthodox" sense and that the period of persistence is inversely proportional to the strength of the host orthodox immune response.

6. Cartilage may be subjected to pretreatment, before being introduced into foreign hosts, to render it non-antigenic in the orthodox sense, and thus proof against late absorption. Choice of pretreatment is not restricted to methods which will allow the cells to survive. Any given pretreatment may not render cartilage non-antigenic for both homologous and hetero-

logous hosts: thus boiled cartilage is antigenic in hetero-logous hosts and probably non-antigenic in homologous hosts.

7. Three methods render cartilage unable to attract host leucocytes (and hence probably non-antigenic) after homografting: boiling for one minute; heating to 47°C for 30 minutes and repeated freezing (to -70°C) and thawing. Cartilage homografts modified by the first of these methods persist grossly and histochemically unaltered for at least 400 days in rabbits.

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