Placental Grafts in Rats

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WITH TWO PLATES

INTRODUCTION

We have previously reported that certain parts of the rat placenta are capable of growth and differentiation when transferred from the uterus to the mother's omentum (Payne & Payne, 1960). Yolk-sac membrane, transplanted on the 15th day of pregnancy, produced grafts containing a variety of tissues which included epidermoid cysts, mucus-secreting epithelium, muscle, bone, and cartilage. The purpose of this paper is to describe the work in detail and to report the results of further experiments.

METHODS

Stock female rats (Albino Wistar strain) were used throughout except in 2 experiments where male rats of the same strain or stock albino mice were employed. The rats came from a colony at Compton which was not inbred although the population had been 'closed' for many years and bulk breeding had been practised. All animals were mature when incorporated in the experiments, rats weighing 200–250 g. and mice 20–25 g.

Mating of animals

Males were caged with the female rats and vaginal smears taken daily. When spermatozoa were found this was taken to indicate successful mating and the date of conception was recorded. Mated females were then transferred to cages labelled with this date so that they could be incorporated in experiments when the appropriate stage of pregnancy had been reached.

Procedure for grafting

Each experiment involved the removal of the uterus from a pregnant rat, the dissection of the placentae and the grafting of certain portions into the mother or another host. Surgery was carried out under general anaesthesia with strict aseptic precautions.

Anaesthesia. Various anaesthetics were used in order to find one which would perhaps be safer and more acceptable to the rat than the conventional open

ether method. In most experiments anaesthesia was induced by 'Avertin' (tribromoethanol in amylene hydrate, Bayer Ltd.) or 'Cyclonal Sodium' (hexobarbitone sodium BPC, May & Baker Ltd.) given intraperitoneally and supplemented where necessary by the volatile anaesthetics, 'Vam' (mixture of vinyl and ethyl ether, May & Baker Ltd.) or 'Trilene' (trichloro-ethylene BP, I.C.I. Ltd.). Experiments indicated that the choice of anaesthetic had no demonstrable effect on the results of grafting.

Hysterectomy. The gravid uterus was removed by means of a midline laparotomy, haemorrhage being prevented by ligation of the uterine blood-vessels. The uterus was then transferred for dissection to a sterile Petri dish. Meanwhile, the mother's abdominal wound was left open for a few minutes until placental grafts could be prepared for transplantation to the omentum. In certain experiments indicated in the text the ovaries were also removed.

Dissection of the uterus. The uterine wall was carefully opened with scissors so as to expose the conceptuses undamaged. The disk-shaped chorio-allantois could then be seen attached mesometrially, whilst beneath hung the visceral yolk-sac wall containing fluid and the foetus. The yolk sac was then cut near its point of attachment to the disk and opened so that the foetus and contained fluid flowed out. The attachments of the foetus to the membranes were now severed and the foetus removed, leaving the visceral yolk-sac wall, amnion, and possibly a little umbilical cord. Further dissection to remove the amnion was carried out in Ringer fluid over a dark background so that the yolk sac could be identified as a thick membrane with blood-vessels in its wall as compared with the amnion, which was thin, flimsy, and avascular. Tissue deemed to be pure yolk sac was used for grafting, but histological sections of such material frequently revealed the presence of small pieces of amnion and even umbilical cord. Contamination with tissue from the foetus was not observed. Plate 1, fig. B illustrates the histological appearance of the yolk sac and fragments of attached amnion.

Most grafts were taken from the uteri on the 14th or 15th day of pregnancy but, where indicated in the text, material was also taken on the 11th or 18th day. Accurate dissection was not possible on the 11th day, when all preparations were found to be mixtures of various placental and foetal tissues. On the 18th day dissection was relatively easy, for by then the various membranes were well formed.

Grafting technique. One-half of a yolk sac as prepared above is sufficient for a successful 'take', but as ample material was available one yolk-sac preparation was used for each host. If the host was also the donor mother-rat a fold of omentum was gently lifted out through the same incision as that used for the hysterectomy. For other hosts a small midline incision was made specially for the grafting procedure. The fold of omentum was spread fanwise on a sterile cloth and a yolk-sac preparation laid upon it. The omentum was then wrapped over so as to completely enclose the transplant and the whole structure returned
to the abdominal cavity. Grafts remained where placed and no further anchoring was necessary. All abdominal wounds were closed by two layers of sutures.

Modifications of this basic technique were employed in certain experiments mentioned later. These included transplanting rat yolk-sac preparations to the omentum of mice, and adult skin, foetal skin, or amnion to the omentum of rats.

Necropsy. Animals were killed at intervals of 1, 2, and 3 months after operation. The gross appearance of the graft was recorded before fixation in 10 per cent. formal saline. Histological sections were prepared and stained with Ehrlich's acid haematoxylin and eosin or with special stains to demonstrate particular features.

RESULTS

The macroscopic and microscopic appearance of grafts varied from rat to rat, but a number of well-defined patterns of growth commonly occurred. These will now be described.

Typical macroscopic appearance of grafts of yolk-sac preparations taken on the 14th–15th day of pregnancy

At 1–3 months after transplantation the grafts varied in size and appearance but usually took the form of cysts with nodular bulges or convolutions projecting from the surface. The largest attained a diameter of 5 cm. (Plate 1, fig. A) but ½–1 cm. was a more usual size. The cysts were filled with mucus which was either clear or turbid and yellow. Some grafts were not cystic but consisted of a series of small granular masses which on histological examination were usually found to be epidermoids. In cases where the graft had not grown there was usually no visible remnant of the original transplant or a tiny nodule of granulation tissue might be observed. No differences were observed between grafts in the mother and in other stock rats.

Typical microscopic appearance of grafts of yolk-sac preparations taken on the 14th–15th day of pregnancy

Although a variety of tissues developed in the grafts there were four main structural patterns: (a) the cysts mentioned above, which usually resembled intestinal wall, (b) skin, and skin derivatives, (c) skeletal tissues such as cartilage or bone, and (d) various kinds of connective tissue. These four types of growth could occur separately, or in any combination in the same graft.

Cysts resembling intestinal wall

These were the commonest structures found. They contain mucin, which stains red with mucicarmine and PAS stains, together with a few clumps of degenerating columnar cells which have apparently broken away from the cyst wall. The lining epithelium, in which there are often many dividing cells, is usually of the tall mucus-secreting type with many goblet cells (Plate 1, fig. C).
It is rarely smooth, but thrown into folds closely resembling intestinal villi (Plate 1, fig. D), each covered by columnar epithelium around a core of connective tissue containing histologically normal and functional blood-vessels. At the base of the villi are cells which differ from the rest of the epithelium in that their cytoplasm contains masses of coarse eosinophilic granules (Plate 1, fig. D). They closely resemble the cells of Paneth which occur in the small intestine.

The connective tissue of the cyst wall may contain cells such as lymphocytes, polymorphs, and macrophages which are usually associated with inflammatory reaction. This might well be due to a homograft reaction by the host against graft antigens. It may also be the result of excessive secretion of mucin, which ruptures the cyst wall and escapes into the surrounding tissues. In addition, secondary bacterial infection may have supervened in old, very distended cysts, so that the contents become converted to muco-pus and the lining epithelium severely ulcerated. In both cases the epithelium actively attempts to heal the eroded areas.

Cysts are nearly always surrounded by thick bands of smooth muscle in which the fibres are orientated in transverse and longitudinal layers. In some well-developed cysts there may even be a rudiment of muscularis mucosa, but this is rare. The resemblance to intestinal structure is completed by clusters of neurones between the muscle layers reminiscent of the myenteric plexus of Auerbach (Plate 1, fig. E).

Whilst the above description is that of the structure of a typical cyst, several variations do occur. Some cysts are simple and composed only of mucus-secreting epithelium and thickened strands of hyaline fibrous tissue (Plate 1, figs. F, G). It is convenient to mention here that this type of connective tissue is a common component of yolk-sac grafts; it resembles, and appears to be derived from, Reichert's membrane, which is a normal structure in the yolk sac of rat placentae.

**Skin and skin derivatives**

Simple round epidermoids are the next most common structure found in the grafts. They contain a centre of keratin surrounded by all the structural layers commonly present in mature skin (Plate 1, fig. G; Plate 2, figs. H, I); from the periphery inwards can be traced the strata germinativum, granulosum, lucidum, and corneum. Mitoses are common in the basal layers. In rare cases structures akin to skin derivatives, such as hair follicles with hair and sebaceous glands, are produced (Plate 2, fig. J).

**Skeletal structures**

These are rare as compared with mucus-secreting cysts and epidermoids (see Table). In their simplest form they occur as small round nodules of cartilage (Plate 2, fig. K). Larger pieces of cartilage undergo central degeneration or
endochondrial ossification (Plate 2, fig. L). In different grafts stages in bone formation may be traced from preliminary ossification to the development of structures closely resembling long bones with cartilaginous extremities and ossified shafts. Haemopoietic cells are present in the medulla of one such bone.

**Table**

*All grafts transplanted to omentum unless otherwise stated*

<table>
<thead>
<tr>
<th>Factors investigated</th>
<th>Day of pregnancy when yolk sac taken</th>
<th>Host</th>
<th>No. takes</th>
<th>No. failures</th>
<th>Graft constituents</th>
</tr>
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<tr>
<td></td>
<td>14th day</td>
<td>Mothers</td>
<td>4</td>
<td>4</td>
<td>4M, 3E</td>
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<td></td>
<td>,</td>
<td>Stock females</td>
<td>3</td>
<td>5</td>
<td>2M, 1C</td>
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<tr>
<td></td>
<td>‏</td>
<td>Ovariectomized stock males</td>
<td>3</td>
<td>5</td>
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</tr>
<tr>
<td></td>
<td>‏</td>
<td>Males</td>
<td>6</td>
<td>7</td>
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<tr>
<td>Age of yolk sac</td>
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<td>23</td>
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<td></td>
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<td>‏</td>
<td>15</td>
<td>10</td>
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<tr>
<td></td>
<td>18th day</td>
<td>‏</td>
<td>3</td>
<td>21</td>
<td>3E</td>
</tr>
<tr>
<td>Age of graft</td>
<td>14th day</td>
<td>Stock females killed after 1 month</td>
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<td>5</td>
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<td>8</td>
<td>1</td>
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<tr>
<td></td>
<td>‏</td>
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<td>4</td>
<td>4</td>
<td>5M, 2E</td>
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<tr>
<td>Transplantation site</td>
<td>‏</td>
<td>Stock females subcuta grafts to 6 sites neonos</td>
<td>8</td>
<td>13</td>
<td>7M, 1E, 1C, 1B</td>
</tr>
</tbody>
</table>

M, mucus-secreting cyst; E, epidermoid cyst; c, cartilage; B, bone.

**Connective-tissue stroma of the grafts**

The various components of the graft are usually bound together into a discrete mass by fibrous tissue. Sometimes this is mature and collagenous, staining red in Van Gieson preparations, but at other times there is relatively little collagen and mesenchyme cells predominate. Thick strands of hyaline connective tissue (Plate 1, figs. F, G) resembling Reichert’s membrane occur in most grafts even when no other placental structures have grown.

**Experiments to determine the factors concerned in successful placenta-grafting**

**The importance of the sex and hormone status of the host**

Our initial report (Payne & Payne, 1960) described the grafting of yolk-sac preparations to the omentum of the donor mother. In later experiments it was shown that grafts grow equally well in other hosts, i.e. stock female rats, intact males, and ovariectomized females. The results of these experiments are summarized in the Table.

**The stage of pregnancy at which the yolk sac is removed**

Yolk sacs taken on the 14th or 15th day of pregnancy will grow successfully when transplanted. The Table records the corresponding results for yolk sacs
removed from the rats on the 11th, 14th, and 18th day of pregnancy. Very few ‘takes’ occurred with the 11th- and 18th-day material, and these were all small and poorly differentiated. One of the mucus-secreting cysts in the 11-day group was curious in that its lining consisted of mucus-secreting cells interspersed with areas of stratified squamous epithelium (Plate 2, fig. M).

The site for transplantation
In most of the experiments yolk-sac preparations were transplanted into the omentum. This involved a short but tedious surgical operation which might have been avoided had subcutaneous implantation proved successful. However, the Table records the relatively inferior results obtained when 14-day yolk sacs were implanted into 6 subcutaneous sites in each of 21 stock female rats.

Time allowed for development of graft
Groups of rats which received 14-day yolk-sac preparations were killed at 1, 2, and 3 months after operation. The Table records that the number of successful grafts did not decrease as time progressed to 3 months. Though some of the grafts examined at 1 month showed inflammation, this was not a prominent feature of those taken at 2 and 3 months, when the various structures were better differentiated.

Grafts of rat yolk-sac preparations to mice
Rat yolk-sac preparations were transplanted into the omentum of each of 47 stock mice. When the animals were killed 1 to 2 months later there was no evidence of any transplant growth, whereas yolk-sac preparations taken from the same donor rats and grafted to the omentum of rats grew successfully.

The homogeneity of the rat stock
In this experiment portions of shaved abdominal skin were removed from rats and divided into two parts. One part was transplanted to the omentum of the donor rat as an autograft and the other to the omentum of another stock rat to form a homograft. Ten pairs of such animals were used in the experiment. When the rats were killed 1 month later all the autografts were healthy but only 1 homograft had grown; 5 homografts were represented by remnants of degenerating skin, whilst the other grafts were entirely replaced by granulation tissue resulting from chronic inflammatory reaction.

Grafts of foetal skin
As adult homografts were rarely successful in our stock rats the lack of homograft reaction to placental tissue must be due either to a particular propensity of placental tissue for grafting or to a property of foetal tissue in general. In an attempt to clarify this point portions of foetal rat limbs were taken on the 14th day of pregnancy and grafted into the omentum of stock rats which were kept for 2 months and then autopsied. Of 13 such grafts all grew and produced skin, skin derivatives, and bone, but evidence of skin degeneration was present in four.
Attempts to graft chorio-allantoic placenta

In spite of repeated attempts, we have been unable to produce a successful graft of chorio-allantois. Cells of the developing chorio-allantoic placenta were included in yolk-sac preparations from 11-day pregnant rats (see above) but the trophoblast cells ceased to divide and none survived for more than a few days. Slices of chorio-allantoic disk taken at 14 days' pregnancy and grafted to the omentum failed to grow. Implantation of similar material into the spleen was also without success. Finally, in each of three pregnant animals undergoing partial hysterectomy one placenta proximal to an ovary was left in situ and covered with omentum. By this procedure the placenta had every chance of survival as its blood-supply was intact, but at necropsy 4 weeks later only a necrotic remnant was found.

Grafts of amnion

As a certain amount of amnion was known to be included in yolk-sac preparations, it was decided to determine how it would develop if grafted alone in the same way as the yolk-sac preparation. Thirteen stock female rats received pieces of this membrane which had been carefully dissected from the placentae of mothers on the 15th day of pregnancy. Two months later only one graft had grown and this had produced a small simple epidermoid cyst.

DISCUSSION

There are very few references in the literature to the successful grafting of placental membranes. Peer (1959) quotes the experimental work of Douglas et al. (1954), which suggests that foetal membrane grafts are tolerated for 2 to 3 times the average survival time of skin homografts. Foetal membrane transplants in mice were apparently viable and capable of undergoing cellular division and epithelization when observed by the transparent tissue chamber technique. Willis (1958), in a review of the literature, described his own and other's experiments on the transplantation of embryonic tissues but gave no reference to any work which had resulted in the successful grafting of placental membranes. He states that a wide range of foetal tissues have been transplanted and that homografts of foetal origin survive and differentiate whereas similar adult tissues are rejected.

Our previous communication (Payne & Payne, 1960) recorded that yolk-sac membranes transferred from the uterus to the omentum of the mother rat grew and differentiated into a variety of tissues. The present paper describes further experiments which confirm and extend our knowledge of this original finding.

The first subject for discussion is the origin of the various types of tissue which we have found in the grafts. These have included mucus-secreting cysts which closely resemble intestine, epidermoid cysts, and skin derivatives, together with skeletal elements such as bone and cartilage. The origin of tissues resembling intestine is perhaps the easiest to explain, in that the yolk sac is composed of
endoderm and mesoderm cells embryologically related to the gut. It seems that the yolk sac at the 14th–15th day of pregnancy retains its potentiality for differentiation into intestine, but that by the 18th day of pregnancy it loses this capacity, as grafts taken at this stage rarely grow. Alternatively, it may be that by the 18th day the placenta is capable of inducing a homograft reaction which prevents its growth.

The growth of skin is more difficult to explain. As there is no ectoderm in the yolk sac the possible sources are from tissue other than yolk sac included in the graft, or metaplasia of yolk-sac endoderm. On the basis of present evidence neither source can be said to be entirely confirmed or excluded. The amnion has cells of ectodermal origin, and it has been shown to contaminate yolk-sac preparations used in these experiments; when isolated and grafted without yolk sac it did produce an epidermoid cyst in one case. However, metaplasia of yolk-sac endoderm cannot be ruled out.

The origin of skeletal structures in the grafts is particularly difficult to explain. There are two possibilities: either that cartilage or bone-producing elements are present in the original transplant, or that these tissues are produced from host cells. It seems unlikely that they could have arisen from the transplanted cells, for the mesoderm associated with the placental membranes has little connexion with the skeletal mesoderm of the embryo. In support of the alternative hypothesis, induction of bone in the omentum was described by Huggins (1931), who transplanted pieces of bladder wall to the omentum and found that membrane bone developed in the neighbouring tissues. Later, Huggins & Sammet (1933) showed that gall-bladder epithelium also had osteogenic potentialities. It is just possible that yolk-sac grafts might induce bone formation in the same way, but it must be stressed that the bone which developed in Huggins's experiments was membrane bone, whereas cartilage and endochondrial ossification occurred in the present experiments. Even though yolk-sac mesoderm is not skeletal, in an unusual environment it might produce bone and cartilage like the omentum mesoderm in Huggins's experiments. The discrete nature and morphology of the skeletal derivatives in our yolk-sac grafts suggest origin from the graft itself, but this may be a misleading impression. Until further evidence is available the origin of bone and cartilage in the grafts remains uncertain.

An aspect of the work which must be stressed is the importance of taking the placenta at about the 14th or 15th day of gestation. Very few successful results were obtained with tissue taken on the 11th and 18th day. It may thus be assumed that the period of potentiality for growth and differentiation of tissue from the yolk sac is circumscribed; it is not attained by the 11th day and by the 18th day the yolk sac is apparently incapable of growth in the new environment.

Our previous work (Payne & Payne, 1960) reported the successful grafting of yolk-sac preparations into the omentum of the donor mother. The present paper presents evidence that similar grafts grow equally well in other stock females, whether the ovaries are present or absent, and also in stock male rats. It
therefore seems unlikely that the sex-hormone environment of the host tissue has any effect on the development of yolk-sac grafts.

Placental grafts are homografts even if they are implanted into the donor mother and their successful growth raised problems of tissue tolerance. It was therefore necessary to assess how far our stock rats were capable of accepting tissues transplanted from one to another. Our experiments indicated that homografts of adult skin, when transplanted into the omentum, were rejected or underwent homograft reaction, whereas foetal skin was comparatively well tolerated. These results are similar to those of Toolan (1957), who found that foetal tissue could be successfully transplanted to homologous adults. Thus it is likely that the tolerance of adult rats to placental tissues is part of a wider phenomenon, that of tolerance of adult animals to foetal tissues in general. The tolerance does not extend, however, to heterografts, for rat yolk-sac preparations transplanted to mice failed to grow. Further experiments are in progress to investigate the problem of tolerance, using inbred strains of rats.

SUMMARY

Yolk sacs from rat placental membranes taken on the 14th or 15th day of pregnancy grew and differentiated in a remarkable manner when transplanted into the mother's omentum. A variety of tissues developed within them, including mucus-secreting cysts, epidermoid cysts, cartilage, and bone. The mucus-secreting cysts were surrounded by smooth muscle and closely resembled intestine; they probably arose from the endoderm and mesoderm of the yolk sac. The epidermoids could have originated either from amnion remnants included accidentally in the transplant, or from metaplasia of the yolk-sac endoderm. It is obscure whether the cartilage and bone developed from host or graft. Transplants were rarely successful when removed from rats at the 11th or 18th day of gestation.

Similar results were obtained when the hosts were other stock rats, either normal or ovariectomized females or males.

The grafts were well tolerated by the hosts and homograft reactions did not occur. Members of the rat stock used would not tolerate adult skin homografts in the omentum, but transplants of foetal skin and bone grew and differentiated well. It is concluded that the tolerance of yolk-sac membranes is not a characteristic peculiar to placental tissue, but is part of a wider phenomenon of tolerance to foetal tissues in general.

RÉSUMÉ

Greffes placentaires chez le Rat

La croissance et la différenciation de sacs vitellins de rat, prélevés dans les membranes placentaires le 14ᵉ ou le 15ᵉ jour de la gestation, se sont remarquablement effectuées après transplantation de ces sacs dans l'épiploon de la mère.

Les transplantations ont été rarement réussies quand les prélèvements ont été faits le 11ᵉ ou le 18ᵉ jour de la gestation.

Des résultats semblables ont été obtenus en prenant pour hôtes des rats d’autres souches, femelles normales ou ovariectomisées et mâles.

Les hôtes ont bien toléré les greffes et il n’y a pas eu de réaction d’homogreffe. Les individus de la souche de rats utilisé ne toléraient pas d’homogreffes de peau adulte dans l’épiploon, mais des transplant de peau et d’os fœtaux s’y sont accrus et se sont bien différenciés. On en conclut que la tolérance à l’égard des membranes du sac vitellin n’est pas une caractéristique particulière au tissu placentaire, mais fait partie d’un phénomène plus étendu de tolérance envers les tissus fœtaux en général.

ACKNOWLEDGEMENTS

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REFERENCES


EXPLANATION OF PLATES

PLATE I

FIG. A. Large mucus-secreting cyst removed from the omentum 3 months after transplantation. The wall has been cut so as to allow the mucoid contents to escape. ×4/9.

FIG. B. Yolk sac and amnion membranes taken on the 15th day of pregnancy. The yolk sac is thick and contains blood-vessels, whereas the amnion is thin. Haematoxylin and eosin. ×300.
Fig. C. Mucus-secreting cyst 2 months after transplantation. Goblet cells containing mucin are present in the villi of the lining epithelium. Periodic acid/Schiff stain. × 300.

Fig. D. Wall of mucus-secreting cyst. At the base of the villi are cells closely resembling cells of Paneth. Smooth muscle is visible towards the edge of the photograph. Haematoxylin and eosin. × 300.

Fig. E. Wall of mucus-secreting cyst. A group of neurones which resemble part of the myenteric plexus of Auerbach can be seen. Haematoxylin and eosin. × 300.

Fig. F. A simple mucus-secreting cyst surrounded by thickened strands of hyaline fibrous tissue. Haematoxylin and eosin. × 300.

Fig. G. A simple mucus-secreting cyst and an epidermoid in the same graft separated by thickened strands of hyaline fibrous tissue. Haematoxylin and eosin. × 300.

Plate 2

Fig. H. A simple epidermoid cyst. The cell layers normally associated with mature skin are present. Haematoxylin and eosin. × 300.

Fig. I. An epidermoid cyst the wall of which appears to be developing a hair follicle or gland. A mitotic figure can be seen. Haematoxylin and eosin. × 300.

Fig. J. An epidermoid cyst in which hair and sebaceous glands have been formed. Haematoxylin and eosin. × 300.

Fig. K. Round nodules of cartilage present in a graft. Haematoxylin and eosin. × 300.

Fig. L. A graft containing a piece of cartilage which is undergoing endochondral ossification. Haematoxylin and eosin. × 300.

Fig. M. The wall of a cyst which is lined by mucus-secreting cells and also by stratified squamous epithelium. Haematoxylin and eosin. × 300.

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