OBSERVATIONS ON TISSUE GRAFTS ESTABLISHED IN RABBIT EAR CHAMBERS

A COMBINED LIGHT AND ELECTRON MICROSCOPIC STUDY

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Early experiments using in vivo microscopy on mammalian tissues were reviewed by Clark et al. (1). The introduction of the transparent rabbit ear chamber, first described by Sandison (2, 3) has allowed microscopic observations to be made of the structure and function of a thin layer of living tissue in the mammal for periods up to several years (4).

A modification of the Sandison-Clark rabbit ear chamber which allowed the mica cover slip to be removed, exposing the surface of the stable vascular membrane and then replaced, was adapted by Williams (4) to study graft tissue fragments implanted on the membrane. He found that autografts of a wide variety of tissues in fragments less than 1 mm diameter became vascularized by the anastomosis of graft vessels to the chamber vessels immediately beneath, and slowly became incorporated into the membrane. These grafts retained their structure and visible function (5). On the other hand, allograft tissues, with the exceptions of choroid plexus, ciliary body, V2 carcinoma, and to some extent adrenal, did not survive (5).

Recently Greenblatt and others (6) established renal allografts in the hamster cheek pouch chamber and described the glomerular blood flow occurring during their brief survival. However, only fetal graft tissues could be used successfully and the glomerular blood flow always followed a preferential path in a relatively direct stream from the afferent to the efferent arterioles. Subsequently he has described fetal and neonatal heart grafts functioning in these chambers (7).

With the eventual aim of studying the process of allograft rejection in vivo, we implanted tissues into the rabbit ear chamber by a method adapted by Cliff et al. (8). Tissues successfully grafted include liver, kidney, myometrium, and thyroid.

**Materials and Methods**

*Animal.*—Rabbits used as recipients were obtained from a closed colony of half-lop rabbits maintained at the Australian National University. They were hardy animals with durable half-lop ears 16 cm in length, very suitable for transparent chamber insertion. Donor rabbits were either of a similar strain, though unrelated, or of an albino strain. Animals were fed on

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Tissue Grafts in Rabbit Ear Chambers

Preparation of Recipient Site.—Graft-type observation chambers were inserted in both ears of half-lop rabbits. 3 wk later the chambers were generally ready for grafting when the table was covered by a fibrous vascular membrane ~80 μ thick. The vascular membrane was covered by a resilient mica cover slip, which allowed clear examination of the full depth at magnifications up to × 500.

Preparation of Graft.—
Donor Selection.—As our initial object was to observe early rejection, unrelated rabbits whose saline-washed red cells were best agglutinated by the recipient serum at room temperature, in some instances were used as donors.

Graft Collection.—The donor was anesthetized with intravenous sodium pentobarbital (Nembutal; Abbott Laboratories, Chicago, Ill.) (0.5 ml/kg weight). Under sterile conditions, the desired organ to be transplanted was exposed and then incised to remove a small portion which was immediately transferred to a cold bath of Hanks' solution, on the stage of a dissecting microscope. Gelfoam (The Upjohn Company, Kalamazoo, Mich.) applied to the cut surface of the organ and held firmly with the finger for 1 min stopped all hemorrhage. The wound was then closed with sutures. Meanwhile another operator very carefully dissected small grafts with maximum dimensions < 1.0 mm from the excised piece of tissue. As each graft was prepared, it was immediately transferred to the recipient site as described below. The remaining pieces were fixed as controls for examination by light and electron microscopy (see below).

Graft Insertion.—While the grafts were being prepared, the conscious recipient rabbit was immobilized supine on a restraining bed. The right and left ear chambers were mounted on two adjacent dissecting microscopes to allow simultaneous observation and manipulation of both chambers. Under aseptic conditions each cover slip was carefully raised and the exposed membrane was kept irrigated with Hank's solution throughout the subsequent procedures. Four to six fragments of graft tissue were carefully positioned on each membrane close to arterioles to facilitate arterial anastomosis. A fresh sterile cover slip was lowered into place and the retaining frame (Fig. 1) and nuts cautiously replaced while the chamber vasculature was watched microscopically for any diminution in blood flow. Each following day during examination, judicious compression of the cover slip reduced the chamber depth and increased optical clarity. However, this was done with great care as good vascular flow was of major importance, as discussed below.

Observation Methods.—Daily observations in vivo were recorded photographically (×10–500). Selected areas were filmed with a 16 mm cine-camera (Vinten Ltd., London, England) with time lapse (60–4 frames/min; normal speed, 16 frames/sec; and high speeds up to 200 frames/sec).

Fixation.—Ear chambers were fixed in formal saline for light microscopy while for electron microscopy 4% glutaraldehyde in cacodylate buffer, pH 7.2, was used (9). After fixation, the tissue was washed in cacodylate at pH 7.3 with sucrose added to a final concentration 0.045 g/ml for at least 2 hr at 4°C. The tissues were then transferred to buffered osmium fixative (10) for 1 hr at 4°C then to uranyl acetate (1% in H2O) for 1 hr at bench temperature. The tissues were embedded in Durcupan (Fluka AG, Basel, Switzerland) after dehydration with acetones. Subsequent staining of sections was performed with an alkaline lead solution (11).

Noradrenaline Solution.—2.5 μg of Levophed (Winthrop Laboratories, New York) in 0.9% saline was administered intravenously in one experiment to produce smooth muscle contraction.
RESULTS

Host Chamber Membrane.—Blood vessels and connective tissue formed in the freshly inserted ear chamber in the manner previously described (1, 4, 12–16).

Lymphatic Vessels.—Lymphatic vessels consistently grew into the recipient

TABLE I
Grafts Performed in the Rabbit Ear Chamber, Showing the Timing of Observations in Days after Grafts Insertion*

| Graft Tissue Grafted | No. of animals | Donor age | Reestab. graft circulation | First evidence of rejection | Termination | Rejection (final episode) |
|----------------------|----------------|-----------|--------------------------|---------------------------|-------------|-------------------------|
| Autograft Myometrium | 2 Adult        | 4 (3-5)   | 133,465                  |                           |             |                         |
| Kidney               | 2 Adult        | 3 (3-3)   | 135,154                  |                           |             |                         |
| Liver                | 1 Adult        | 6         | 7                        |                           |             |                         |
| Lymph node           | 1 Adult        | 5         | 43                       |                           |             |                         |
| Allograft Thyroid    | 1 Adult        | 5 (1-2)   | 42 (22-26)               | One graft                 | One graft‡ |                         |
| Liver                | 2 Fetal        | 1.5 (5-5) | 24 (37-45)               | 71.5 (71-72)              |            |                         |
| Kidney               | 4 Fetal        | 2.7 (2-3) | 16 (7-26)                | One graff§                | 64         |                         |
|                    | 3 Neonatal     | 15 (3-8)  | 37 (36-37)               | 83 (72-93)                | 3 neonatal |                         |
|                    | 2 Adult        | 5 (3-8)   | 41 (36-37)               | 71.5 (71-72)              | 3 neonatal |                         |
|                    | 8 Adult        | 5 (2-14)  | 42 (17-65)               | 72 (43-150)               | 3 neonatal |                         |

Total No. of rabbits 27

*The first figure is the average, and the range is expressed in parentheses. Reestablishment of graft circulation refers to the day that blood flow within the graft vessels became brisk. The first evidence of rejection indicates the first day any microscopic criteria of rejection were noted. Usually, this was transient. Where it was not, and resulted in destruction of the graft, the figure was entered in the rejection column. Termination indicates the day on which, without final rejection having occurred, the ear chamber was fixed for examination.

‡ One animal had fetal grafts of both kidney and liver in the same chamber, both of which rejected at 33rd day.

§ One graft still partially survived as groups of collecting ducts with patchy graft vascularization at time of preparation of this paper, at 86 days.

rabbits' ear chambers and usually were recognized as broad, pale, thin walled channels which generally coursed beside blood vessels. By 3 wk, active lymph flow was seen and filmed. Some channels had semilunar valves (see Fig. 2) which beat at rates up to once per second and cells could be observed moving along
these channels at rates up to 60 μ/sec. After grafts had been placed on the
membrane, the lymphatics could be observed filled with a slowly moving heterogeneous population of cells.

Graft Implantation and Reestablishment of Circulation.—The tissues successfully grafted and their survival are summarized in Table I. In general, primary grafts of all tissues had similar schedules for reestablishment of circulation. On the day of implantation and the following day (day 0-1), grafts had empty vessels and their parenchyma remained clearly visible. On day 2–3 the vessels throughout the whole graft became filled, within a matter of hours, with immobile columns of red cells. Once blood had filled the graft vessels it was usually only a matter of 24 hr before they all had brisk flow. Graft blood flow improved over the following week. For example, in renal grafts the numbers of flowing glomeruli progressively increased and each became more completely perfused as the anastomosis allowed greater flow. Often in this period, a major arteriole in the ear chamber would be remodelled so as to supply exclusively a kidney graft with several glomeruli (Fig. 3), thereby completely diverting its entire flow initially through the glomeruli via the afferent arterioles and then via efferent arterioles to peritubular capillaries which drained into anastomosed ear chamber venules.

As the grafts initially lay on the surface of the vascular membrane, their vessels were in a higher focal plane than those of the ear chamber which could be seen coursing beneath (Fig. 5). Graft vessels traced to the edge of an implant could be seen to join the ear chamber vessels below. After several weeks the grafts sank into the tissue of the membrane so that structures like glomeruli, tubules, and thyroid follicles occupied regions in the middle of the fibrous membrane. Grafts, which for some reason, such as being too large, had become necro-

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Fig. 1. A rabbit ear chamber at time of insertion, showing removable cover slip retention ring, being placed in position. × 1.8.
Fig. 2. Lymphatic channel in vivo showing bicuspid valve (arrows). × 1000.
Fig. 3. Renal adult allograft (G) at day 8 in vivo after vessel anastomosis has diverted the entire flow of the major ear chamber arteriole (A) to supply several glomeruli which lie in a more superficial plane. × 300.
Fig. 4. Thyroid adult allograft at day 14 in vivo showing intimate relationship of reticular capillary blood flow to the follicles, the largest of which contains three granular cells in the colloid. × 1000.
Fig. 5. Hepatic autograft at day 7 in vivo showing fine sinusoidal pattern of graft vessels extending centrifugally from the graft edge. Note large ear chamber vessels lie in a deeper focal plane. × 320.
Fig. 6. Fetal renal allograft at day 25 in vivo, with well-established glomerular flow in renal tissue on upper right, and a fine reticular blood flow pattern in the smooth muscle on the lower left. × 350.
tic, showed a "granulation type" of revascularisation (1, 4, 12-16) in which blood vessels grew in broad loops from which capillary buds advanced into the graft tissue, preceded by a cap of hemorrhage.

When blood flow had been reestablished, tissues whether allografted or autografted had a characteristic vascular pattern quite distinct from that of the ear chamber membrane. These characteristic patterns were best seen soon after reestablishment of graft circulation, when all graft vessels were widely dilated. Indeed it was possible to identify grafted tissues by their blood flow pattern (Figs. 4-6). In one fetal renal graft, a large area of smooth muscle (Fig. 5) was first identified by this pattern and when stimulated by mechanical means or intravenous injections of 2.5 μg of noradrenaline, the muscle graft showed strong contraction. Later some remodelling of the blood flow pattern occurred through adaptation to local circumstances as, for example, the sites of major vascular anastomoses. Hepatic grafts had a sinusoidal vascular pattern (Fig. 5). Thyroid and smooth-muscle grafts had an open capillary network (Figs. 4, 6); renal grafts had interlobular arteries, afferent and efferent arterioles, glomerular tufts, and peritubular capillaries (Fig. 7).

Arteries which had unmistakable graft characteristics were seen best in the renal grafts. Several times a single graft artery gave rise to a number of afferent arterioles supplying glomeruli (Fig. 7). These were interlobular arteries and were often Anastomosed to a major arteriole of the ear chamber and frequently had a prominent refractile elastic lamina which was rather irregularly collapsed (Fig. 8), and this, together with the relative thickness of the vessel wall compared to its lumen diameter, suggested that it was being perfused at a lower pressure than in its original site (17, 18).

**Fig. 7.** Renal fetal allograft at day 21 in vivo. Groups of flowing glomeruli lie along graft interlobular arteries. Tubules are well preserved. × 300.

**Fig. 8.** Renal adult allograft at day 10 in vivo. Graft artery with irregular internal elastic lamina (arrows), giving rise to afferent arteriole (A) supplying glomerulus at lower margin of figure. × 1400.

**Fig. 9.** Renal adult allograft at day 16 in vivo. Adult glomerulus showing delicate epithelium lining Bowman's space (arrow). Note small vessel (V) connecting afferent and efferent arterioles on right side of glomerulus. × 1400.

**Fig. 10.** Same glomerulus as Fig. 9, with focus on the primary division of the afferent arteriole. Note comparative diameter of afferent (A) and efferent (E) arterioles. × 1400.

**Fig. 11.** Renal adult allograft fixed at 17 days. Electron micrograph of the same glomerulus as seen in Figs. 9 and 10. B is Bowman's space. The capillaries, podocytes, and basement membrane are all well preserved. The inset shows characteristic glomerular endothelial fenestrations. × 1900. Inset × 40,000.

**Fig. 12.** Renal adult autograft fixed at 154 days. Electron micrograph of a glomerulus which had poor perfusion in vivo, showing fusion of foot processes and extensive folding of basement membrane, within which vesicular debris and typical collagen is observed. Note well-preserved fenestrated endothelium (E). × 26,000.
Each glomerulus was supplied by an afferent arteriole which had a greater diameter and a thicker wall than the efferent (Figs. 9, 10) and sometimes the two were joined by a fine anastomosing vessel coursing just outside Bowman's capsule (Fig. 9). Occasionally two efferent arterioles were identified leaving a single glomerulus. The afferent arteriole divided into six to eight primary branches, each of which supplied a distinct lobule of the glomerulus. There was no anastomosis between the lobules. Within each lobule, however, the supplying branch divided to form a number of irregularly anastomosing, permanent capillary channels (Fig. 10). The glomeruli in fetal grafts, on the other hand, commenced with a very simple four- to six-channel blood flow which simply arced from afferent to efferent arterioles. Over the succeeding 2 wk these became more convoluted. Light and electron microscopy of glomeruli after fixation of the ear chamber showed normal glomerular structure, with well-preserved fenestrated endothelium, basement membrane, and epithelial cells with distinct foot processes (Fig. 11). Bowman's space was lined by delicate visceral and parietal epithelium (Fig. 9) which was continuous with the epithelium of the proximal convoluted tubules. Autografted glomeruli, which had been observed over periods up to 5½ months to have incomplete perfusion on subsequent electron microscopy, showed collapse of the unperfused capillaries and development of collagen in the folded and thickened basement membrane (Fig. 12). However, in well-perfused capillaries of such glomeruli, the fenestrated endothelium and podocytes were well preserved (Fig. 13). In hepatic grafts, the sinusoidal blood vessels on electron microscopy had definite gaps present between plump endothelial cells (Fig. 14), which showed evidence of phagocytic activity.

Graft Parenchyma Preservation.—Thyroid grafts possessed delicate follicles filled with colloid, at the margin of which numerous prominent vacuoles were seen (Fig. 15). Time-lapse studies at speeds up to 4 frames/min failed to show movement of these vacuoles, but observation from day to day suggested some

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Fig. 13. Same glomerulus as illustrated in Fig. 12, showing a region of normal perfusion with well-preserved foot processes, a thin but folded basement membrane, and a well-preserved endothelium with fenestrae (arrow). Bowman's space (B) contains floccular material. × 21,000.

Fig. 14. Hepatic fetal allograft fixed at 21 days. Electron micrograph shows sinusoidal vessel with plump endothelium and a large gap between endothelial cells. × 13,000.

Fig. 15. Thyroid adult allograft on day 30 in vivo, showing well-preserved follicles lined by well-defined glandular epithelium, with prominent marginal vacuolation of the colloid. × 1750.

Figs. 16 and 17. Serial exposures, at approximately 30 sec interval, of a single peripheral follicle in the same graft as seen in Fig. 15 at day 45, showing intense activity of cytoplasmic veins of a macrophage within the colloid. × 1750.
change in position. Some of the peripheral follicles contained up to three to four highly motile macrophages (Figs. 16, 17). On electron microscopy the follicles were lined by a continuous epithelium with the characteristic appearance of glandular secretory cells (Fig. 18).

Autogenous myometrium was transferred to the ear chamber in one adult rabbit. After reestablishment of the circulation, the smooth muscle was only faintly visible in vivo. The rabbit was then mated and before parturition, intravenous oxytocin produced marked periodic contraction of the muscle in the ear chamber grafts as previously described (19). After parturition, the graft involuted and became virtually indistinguishable from the surrounding ear chamber tissue. However, 1 yr later, the rabbit was again mated and intravenous oxytocin produced precisely the same effect on the graft that had lain dormant since the first demonstration of its contractility. Electron microscopy of the ear chamber which was fixed postpartum revealed numerous large smooth muscle cells with plentiful, centrally located organelles, peripheral dense myofilaments, and large areas of cytoplasmic rarefaction (Fig. 19).

The hepatic graft parenchyma was composed of long cords of large cells which ultrastructurally were comparable to those seen in freshly fixed liver. The hepatocytes formed biliary canaliculi in the usual manner (Fig. 20), and in addition, their margins were at times thrown into a complex series of branches and folds (Fig. 21). When these grafts had been present a few weeks, they developed broad bands of fibrosis separating irregular cellular arcades which resembled the pattern seen in cirrhosis (Fig. 22).

In renal grafts after the circulation was reestablished, the tubular epithelial cells became progressively more granular. Then a fine rim of regenerating epithelium appeared beneath the now loose granular material which drifted into the tubular lumen to aggregate into a refractile granular cast (Figs. 23, 24). By day 3 epithelial regeneration was complete and usually one or more large active macrophages could be seen within the tubular lumen. Most of these casts disintegrated and gradually disappeared.

Occasionally an intact lumen could be traced in vivo from Bowman's space through the proximal convoluted tubule to the descending and ascending limbs.

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Fig. 18. Thyroid adult allograft fixed at 46 days. Electron micrograph shows colloid (C) bounded by characteristic follicular epithelial cells with dark granules. × 8000.

Fig. 19. Myometrial adult allograft fixed 15 months after grafting and 6 wk postpartum. Electron micrograph shows smooth muscle cells with evidence of involution such as cytoplasmic rarefaction (R). × 16,000.

Fig. 20. Hepatic adult allograft fixed at 21 days. Electron micrograph of hepatocytes forming bile canaliculus (arrow). × 14,600.

Fig. 21. Hepatic adult allograft fixed at 21 days. Electron micrograph showing remarkable border on some of the hepatocytes as seen in the "cirrhotic" pattern in Fig. 22. × 11,500.
of the loop of Henle and into the distal convoluted tubule. In each of these regions the in vivo appearance of the epithelium was consistent with their appearance in sections from whole kidneys.

Sometimes a few tubules became cystic and expanded to produce an appearance similar to the "thyroidization" of chronic pyelonephritis. On occasions the epithelial cells lining such cysts developed numerous cilia. These could be seen actively beating in vivo, producing a violent tumbling motion of the cystic contents. Ultrastructurally, epithelial cells with only a few cilia appeared relatively normal but there were quite substantial changes in those which had developed hundreds of typical cilia (Fig. 25).

DISCUSSION

The organ fragments grafted as described above, allowed continuous in vivo observations to be made on parenchymal structure and function, as well as on the graft vasculature which plays such an active role during rejection. In each of the grafts indicated in Table I as successful, the parenchyma was well preserved throughout the in vivo observations before rejection. Fixed at this stage, they had essentially normal structures when examined by both light and electron microscopy.

In contrast to previous transparent chamber work by Williams (4), we found no difficulty in allografting tissues and we had no difficulty with renal grafts from donors at all stages of development including adults, in contrast to Greenblatt and others (6, 7).

An unexpected observation was the prolonged survival of the allograft tissues particularly when obtained from adult donors (Table I). Since it was our eventual aim to study the rejection process, we deliberately chose dissimilar rabbits to act as donors. Despite this, it took nearly 3 months for primary adult and neonatal renal allografts to reject spontaneously. Most of the experience with allografted tissues would suggest that rejection should have occurred within several weeks. This raises the question whether the rabbit ear chamber, as sug-

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Fig. 22. Hepatic neonatal allograft at day 113 in vivo. Note irregular cords of hepatocytes surrounded by whorled fibrous tissue, resembling cirrhosis. × 600.

Fig. 23. Renal adult allograft at day 3 in vivo. Note granular material (G) from tubular necrosis within lumen, and regenerated epithelium (E), at its margin. × 1400.

Fig. 24. Renal adult allograft fixed at 8 days. Electron micrograph shows osmiophilic debris in tubular lumen, lined by regenerated epithelial cells. Note basement membrane near right margin. × 4000.

Fig. 25. Renal adult allograft fixed at 8 days. Electron micrograph of portion of a cystic tubule in which ciliary movement had been observed in vivo. A normal epithelial cell is seen on the lower left while on the right, there is a large pale cell with numerous cilia protruding through the microvillous border. × 13,500. Inset × 46,600.
gested for the anterior chamber of the eye (20), the brain (21), and the hamster cheek pouch (22), is a preferred site (23).

Relatively modest lymphatic function has been described in the rabbit ear chamber (24, 25), but the well-developed lymphatics of these ear chambers and their obvious function during the life of the graft make the usual explanation of preferred site, namely poor or absent lymphatic drainage, unlikely. However, some doubt has been thrown on this general view by the recent demonstration of more effective lymphatic function in the hamster cheek pouch (26). Other possible causes for prolonged allograft survival may be the small quantity of tissues implanted (27) and the repopulation of the vessels of these small grafts by host endothelium. With regard to the former, it was in the fetal grafts that rejection generally occurred in a short period, about 5 wk (Table I). This was despite the usual evidence that fetal tissues are less prone to provoke rejection (23, 28). A possible factor is the spectacular increase in size in the fetal grafts that occurred up to the time of rejection, and perhaps this exceeds some critical level of stimulation. Consideration of the latter is a reflection of the increased interest shown by many in the role of the graft vessels in allograft rejection (29-33). There is some disagreement over the origin of vessels which provide the reestablished circulation. This has been most marked in skin grafts and implantation of organ fragments such as those described above, and the importance of this question was raised by Medawar (34) and Billingham et al. (35). Recently however, it has been claimed that the endothelium of whole transplanted kidney was of recipient origin (36). Consequently, it must be clearly established whether the circulating recipient blood in the graft is in contact with vessels of donor or recipient origin.

Scothorne and McGregor (37) also Converse and Rapaport (38) believe that when graft circulation is reestablished in skin grafts it is entirely by ingrowth from the surrounding recipient tissues, and it is quite independent of the transferred vessels. Others said that it is a combination of these two (39). Wolff and Schellander believe that the transferred vessels deteriorate (40) and Zarem et al. suggest these act as conduits along which the recipient vessels grow to provide circulation (41, 42). Another view, suggested by Trentin (43) as analogous to the rapid development of an endothelial covering on dacron arterial prostheses (44, 45), is that recipient endothelium replaces that of the donor by colonization from the blood particularly in grafts with prolonged survival. He further suggests that this process could account for the concentric intimal proliferation seen in these grafts (43). However most evidence does not support the concept of colonization (46).

Our observations support the opinion expressed by Williams (47, 48) and others (49-54) that the graft vessels are the same vessels that were transferred from the donor and have acquired circulation by anastomosis with surrounding
recipient vessels. Our conclusion is based on three pieces of evidence: the speed with which the circulation is reestablished, the pattern of the established flow, and the structure of the graft vessels.

The reestablishment of graft circulation was rapid and abrupt. Often within 24 hr graft vessels were clearly seen as patent channels and the graft tissues freshly filled with recipient red cells. The brisk flow that followed within a further 24 hr coursed throughout the entire grafts, and though there was progressive increase in the rate of blood flow, the areas perfused did not increase in size, as would have been the case if the recipient vessels were growing in. Such recipient vessel ingrowths were seen in several cases when the graft tissues became necrotic through faulty technique, and here the pattern and rate of revascularization was similar to that seen in granulation tissue (16). Even if they were to use the transplanted vessels as conduits (39), this would still be observed as a progressive process and be quite incompatible with our observations on revascularization of successful grafts.

The pattern of the reestablished graft blood flow was immediately characteristic of the tissue grafted and could be used to identify tissues in vivo. This excludes the possibility that vessels grow in from recipient tissues as in granulation (1, 4, 12-16). Further structural evidence of the donor origin of graft vessels was the recognition in renal grafts of large arteries with irregular, refractile, elastic laminae which were easily seen in vivo. These are not seen in ear chamber vessels. Additional evidence of donor vessel survival is provided by the glomeruli in both allografts and autografts which showed characteristic ultrastructure (55) and their capillaries were lined by a form of fenestrated endothelium which is never seen in ear chamber vessels.

The functional anatomy of the mammalian glomerulus has long been a source of controversy. McGregor (56) has reviewed the early descriptions of glomerular structure and further reviews have been presented by Boyer (57) and Lewis (58). Fig. 26 gives a diagrammatic summary of these structural theories. Our in vivo studies of adult renal allografts allowed direct study of the functioning glomerulus. Other in vivo studies are scarce and limited generally to amphibians (59) with the exception of Greenblatt and others (6) and Baringer (60). The majority have based their opinions on injection techniques (57, 61) and more indirect studies (62, 63).

In the adult glomeruli which we observed flowing, the afferent arteriole arborized into six to eight branches just as Bowman described initially (64) and has been confirmed by others (65, 66). Each of these primary branches supplied a distinct lobule composed of anastomosing capillaries as suggested by Johnston (61), Boyer (57), and Lewis (58). However, we agree with Hall's reservation that these anastomosing channels are confined to the lobules (67), as we did not observe blood flowing between adjacent lobules. This was in contrast to Vimtrup
(65) and Willmer (66) who claimed there is no anastomosis between capillaries as reported by Elias et al. (68, 69) and Borst (70) nor was the tuft produced by a convoluted extension of the arteriolar wall (71).

Our observation of a simplified glomerular flow pattern in fetal tissues is of interest in view of the lower glomerular filtration rate in infants. Edelman (72) suggests this is due to high renal vascular resistance, as well as small glomerular size and low basement membrane permeability, while Webber and Blackbourn (73) claim this is really due to a difference in the degree of endothelial fenestration. Lewis (58) observed large peripheral channels in the injected glomeruli of fetal rabbits, guinea pigs, and sheep and speculated that these could have some functional significance, perhaps providing a more direct channel from afferent to efferent arterioles.

![Diagram of glomerular capillary blood flow pattern](image)

**Fig. 26.** Diagramatic representation of the various theories of glomerular capillary blood flow pattern. Numbers indicate the references.

The effect on the glomerular blood flow produced by varying renal perfusion is poorly understood (74). Berliner (75), summarizing glomerular perfusion studies, felt that normal perfusion of the glomerulus is such that all are perfused and none kept in reserve. Thus in low renal blood flow there should be a uniform and proportionate reduction in the perfusion of each glomerulus. This is supported by indirect studies of glomerular flow in dogs (76) and humans (77) which suggest that during increased and decreased renal blood flow, changes in the filtration rate were the result of alterations in the quantity of filtrate formed in each glomerulus and not by alteration of the number of active glomeruli, as suggested by Richards and Schmidt (78). Previously the only direct evidence of glomerular flow variation came from work on transilluminated frog kidneys. Bieter (59) described shunts when the splanchnic nerves were stimulated during glomerular observation, similar to the preferential path seen by Greenblatt (6) and in our grafts when the afferent flow was reduced by an unfavorable anastomosis. Baringer (60) noted the rates of blood flow in the entire transilluminated frog glomerulus were directly proportional to the degree of afferent arteriolar dilata-
tion, but in addition, flow in single capillary loops could be modified independ-
ently of general glomerular flow. Our findings indicate that when available
afferent flow is reduced, all glomeruli continue to flow but the bulk of the flow
may follow a preferential path through the glomerular tuft, despite patency of
the other capillaries. When afferent flow increases, the glomerular perfusion
becomes more evenly spread through the whole capillary bed.

The fine anastomosing channel connecting the two arterioles of some glo-
meruli suggests that they originated from the juxtamedullary region and that
this is "Ludwig's vessel" (79), about which there has been disagreement.

Generally, graft parenchyma was well preserved in each tissue but it was best
demonstrated in the ultrastructure of the thyroid epithelium and the function-
ing of the myometrial autografts. The marginal vesiculation of the colloid ob-
erved in vivo within thyroid follicles (Fig. 15) was strikingly similar to those
forming the scalloped margin familiar in tissue fixed from hyperthyroid subjects
(80). Although of some diagnostic value in such cases, it is generally regarded as
an artefact of fixation. Williams (81, 82) strongly supported this view as he was
unable to detect such colloid vesicles in vivo by transillumination of rat and
mouse thyroid isthmus, but found they were quite obvious in the sections he
prepared from these areas after fixation. Possibly quartz rod illumination is un-
suitable for visualizing these vacuoles, but he was also unsatisfied by the vac-
uoles in rabbit thyroid autografted into transparent ear chambers (83). The liver
parenchyma we grafted also showed ultrastructural correlation with normal
liver, although it is interesting to note that after many weeks, its histological
appearance became altered to resemble cirrhosis (Fig. 22).

The cystic dilatation of some grafted renal tubules presumably was due to
local environmental factors simulating the circumstances in scarred kidneys
leading to the thyroidization seen in such conditions as chronic pyelonephritis.
The identification of typical cilia projecting from the remnants of a brush
border, together with the other cytological changes observed, indicate that
metaplasia of renal epithelial tissue, whether fetal, neonatal, or adult, may pro-
duce ciliated cells (84), (Fig. 25). This is usually regarded as a characteristic of
immature "rests" where they occur in adult tissue (85). Indeed, the presence of
ciliated cells has been a principal criterion for separating renal dysplasia from
cyctic disease of the kidney (86-88). However, single cilia have been reported in
most parts of the normal rat nephron (89) and also occur in tumors of the ham-
ster, resembling the human Wilms' tumor (90).

This model of transplantation lends itself to a very close observation of the
process of rejection in vivo and this will be the subject of a subsequent report.

SUMMARY

An in vivo microscopic and ultrastructural study of tissues transferred to the
transparent rabbit ear chamber is presented. Fragments of liver, kidney, thy-
roid, and myometrium were successfully auto- or allografted into the chamber
from donors of all ages and allowed continuous in vivo observation of parenchymal structure and function, as well as of the graft vasculature which plays such an active role during rejection.

Circulation was quickly reestablished by anastomosis of graft vessels to those of the ear chamber membrane and only minor reversible parenchymal changes occurred. Both vessels and parenchyma retained the characteristics of the organs of origin on both light and electron microscopy and were observed functioning in vivo for periods up to 1 yr in the case of autografts, and until rejection occurred in allografts. In the latter case, rejection did not occur in tissues obtained from adult and neonatal donors for nearly 3 months, while tissues of fetal origin were generally rejected much earlier.

The kidney grafts provide a unique opportunity for a close comparative study of mammalian fetal and adult glomerular blood flow under varying rates of perfusion, and the tubular epithelium could be observed regenerating after initial acute tubular necrosis. Renal tubules from fetal, neonatal, and adult donors were all capable of metaplastic change to form a highly ciliated epithelium.

Grafts of these tissues will allow the fine detail of the processes of rejection to be studied continuously in vivo.

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