A STUDY OF SKIN ALLOGRAFT BED CELLS BY TRANSPLANTATION BENEATH THE KIDNEY CAPSULE

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
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A STUDY OF SKIN ALLOGRAFT BED CELLS BY TRANSPLANTATION BENEATH THE KIDNEY CAPSULE

By

Dale Corwin Peerbolte

A THESIS

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INTRODUCTION

The recent increase in the importance of tissue and organ transplantation to the practice of medicine has led to an increased awareness of the phenomenon known as allograft rejection. Although many studies have been performed, the exact mechanism by which a host destroys genetically dissimilar tissue still remains obscure. A more complete understanding of the complex processes involved is essential for greater success in transplantation as well as the treatment of other delayed type hypersensitivities.

The orthotopic skin graft has been used frequently as a model in order to study the rejection process. A prominent feature of the reaction is the extravascular accumulation of mononucleated cells from the blood in the graft and graft bed. The fact that these cells are prominent in the grafts being rejected seems to indicate that they may be directly involved in the rejection process.

The present experiment was designed to study the cells of the graft bed and expand knowledge about their properties. Skin allograft bed cells transplanted beneath the kidney capsule of F_1 recipients were studied in regard to transplantation survival, structural characteristics, and influence upon their immediate environment.

REVIEW OF THE LITERATURE

Local cellular reactions in certain immune responses have been observed by many investigators. Waksman (1960), in comparing various experiments dealing with delayed hypersensitivities including graft rejection, noted that a feature common to these states, but not prominent in isografts, is the formation of perivascular islands of lymphatic cells in the immediate area of the reaction.

Other workers have specifically studied the cells underlying allografts and isografts. Medawar (1944) noted a massive invasion of lymphocytes and monocytes in rabbit skin allografts. He described some of these cells as having large amounts of basophilic cytoplasm and eccentrically placed nuclei. Darcy (1954) postulated that the large pyroninophilic cells seen infiltrating subcutaneously grafted tissue in rabbits were proliferating lymphocytes and possibly precursors of plasma cells. Walker and Goldman (1963) labeled the hemopoietic tissues of mice with tritiated thymidine prior to skin grafting and studied the appearance of leukocytes in the graft beds. In both allografts and isografts, labeled lymphocytes, monocytes, and neutrophils were seen to infiltrate the graft area. In four to eight days, large cells with elongated, labeled nuclei and large amounts of basophilic cytoplasm were seen in allograft beds, but not in

isograft beds. Their origin was traced to the large lymphocyte of the blood on the basis of similarity in percentage of labeled nuclei in these two populations.

Electron microscope studies also have been applied to the cells of graft beds. Walker, Yates, and Duncan (1964) studied mouse skin allografts and found four distinguishable cell types. Neutrophils were easily identified by their characteristic cytoplasm. Fibroblasts and hypertrophied lymphocytes were separable in that the former had a prominent granular endoplasmic reticulum whereas the latter was typified by numerous free ribosomes. The fourth cell type was identified as the macrophage. Wiener, Spiro, and Russell (1964) also described cells present in allograft beds with ultrastructures similar to those of lymphocytes or monocytes.

Lymphocytes have been implicated by various methods in playing a prominent role in allograft rejection. Billingham, Brent, Medawar, and Sparrow (1954) demonstrated that lymphocytes from an animal which had previously rejected a skin allograft could accelerate the reaction when transferred to an isologous host. André, Schwartz, Mitus, and Dameshek (1962) reported that the prior destruction of lymphatic cells resulted in an increase of the survival time for allografts to the treated animal. Nevertheless, the exact mechanism by which the cells of the graft bed destroy the grafted tissue is not well understood.

The presence of numerous free ribosomes in the cytoplasm of the hypertrophied lymphocytes of the allograft bed

indicates that they are actively producing some sort of material. Lawrence (1959) called the extract of sensitized human lymphocytes "transfer factor" and found that it was capable of producing a positive skin test reaction in normal hosts. Najarian and Feldman (1962) reported acceleration of allograft rejection when sensitized lymphocytes were transferred in millipore chambers to the peritoneal cavity of isologous hosts. They also successfully performed passive transfer of allograft immunity with a supernatant of mouse lymphocyte extract (1963). Ruddle and Waksman (1968) in correlating skin reactivity with the in vitro response of lymphocytes, postulated that upon contact with antigen, specific lymphocytes release a "mediator" which damages cells in the immediate area. This substance may affect the vascular endothelium as well as causing destruction of the graft parenchyma. More study is needed in order to determine the exact relationship of the lymphatic cells in the allograft bed to the rejection process mechanism.

One method for studying properties of cells is transplantation of these specific cells to other animals. If the host is not genetically similar and is immunologically mature, the transplanted cells will be recognized and rejected, leaving the recipient sensitized to subsequent transplants from the same donor. But if the animal is immunologically immature or incompetent, the transplanted cells will remain at least long enough to induce tolerance so that the host will accept transplants from donors

genetically similar to the original transplant donor (Billingham, Brent, and Medawar, 1955, and Billingham and Brent, 1957a). Repeated injections of donor lymphatic cells to adult immunocompetent animals can also induce tolerance (Shapiro, Martinez, Smith, and Good, 1961, and McKhann, 1964). Brent and Gowland (1962) studied the lymphatic organs after inducing tolerance by repeated injection of spleen cells, and found that the transplanted cells survived in the tolerant animals producing cellular chimeras. Transplantation of large numbers of immunologically competent lymphatic cells into immature or incompetent animals (e.g. parental cells to F_1 hybrid hosts) results in the graft-versus-host (GVH) reaction known as runt disease (Billingham and Brent, 1957b, Martinez, Smith, and Good, 1961, and Simonsen, 1962). In such a situation the grafted cells react against the host without themselves being rejected. Gowans, Gesner, and McGregor (1961) demonstrated that some of the large pyroninophilic cells found in the lymphatic tissues at the height of the GVH reaction are transformed cells of donor origin. Thus it appears that lymphatic cells can survive transplantation and can continue to recognize and react against foreign tissue. Furthermore these reacting cells seem to be the same as those appearing in allograft beds at the time of rejection.

Transplantations of lymphatic cells by methods other than intravenous or intraperitoneal injections and in dosages smaller than those causing fatal runt disease have

also been studied. Elkins (1964) injected F_1 rats beneath the kidney capsule with cells from various lymphatic organs of parental strain donors. He noted proliferation of pyroninophilic blast cells, invasion of the kidney cortex, and subsequent destruction of those tubules which were completely surrounded. Ford (1967) injected lymph node cells and thoracic duct lymphocytes from parental rats intradermally to F_1 hybrid recipients. The lymphocytes were found to change into large pyroninophilic cells and possibly into plasma cells, but no systemic or local tissue destruction was evident. These investigations of local GVH reactions are examples of experiments which support the validity of studying cellular properties by transplantation to a new and tolerant environment.

The kidney capsule has been used extensively as an implantation site, chiefly because of the rich vascularity of the underlying renal parenchyma. Russell (1961) mentioned it as a satisfactory site for endocrine organ implantation. Larkin (1960) found kidney capsule implantation useful for studying first and second set rejection of rat testis grafts. Rolston (1967) studied skeletal muscle implanted beneath the kidney capsule of mice. Many other experiments have involved transplantations beneath kidney capsules, however, a complete list is not relevant to the present study.

MATERIALS AND METHODS

I. Animals

Parental animals used in this experiment were inbred A/J (H-2a) and C3H (H-2k) adult mice from Jackson Memorial Laboratories, Bar Harbor, Maine. Females weighed from 21-27 gm and males from 24-30 gm. All F_1 animals were from breedings within our laboratory between these two strains. F_1 animals were used as donors of allogeneic skin grafts to parental strain hosts. They also served as hosts for implantations beneath the kidney capsule. All F_1 animals were at least eight weeks old before receiving implantations or grafts. Males weighed from 25-35 gm and females weighed from 22-26 gm. All animals were housed in air conditioned rooms and given food* and water ad libitum. Ether was used for anesthesia in all operations and for sacrificing the animals.

II. Skin grafting

Hair was clipped from the dorsal thorax of anesthetized animals and areas larger than the anticipated graft were shaved and cleansed with 70% alcohol. Surgical steps were

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*Mouse diet containing approximately 19% protein, 6% fat, and wheat germ, as prepared for Jackson Laboratories by Emory Morse Company, Guilford, Connecticut.

carried out alternately on donor and host in order to minimize the time between removal of the graft from the donor and placement on the host. Cleaned instruments were soaked in 70% alcohol before operating and care was taken not to touch the graft or graft bed except with cleaned instruments during the grafting procedure. The skin in a 1 cm^2 area was held gently with a forceps and a cut made on three borders of the graft with a small scissors to the depth of the panniculus adiposus. One corner of the resulting flap was lifted slowly with the forceps while the panniculus adiposus was split beneath the graft with a sharp scalpel. Thus the panniculus carnosus with its relatively rich vascularity was left intact in the graft bed. When the flap was completely separated, the fourth border was cut and the graft removed and immediately placed on the host whose graft bed had been prepared in the same way. The graft was then sutured to the surrounding skin and covered with a layer of vaseline. A small pad of gauze slightly larger than the graft was placed over the area and held in place with a plastic strip bandage around the entire thorax. This applied pressure to and protected the grafted tissue. This constituted a slight modification of the method recommended by Billingham (1961). Allografts consisted of F_1 skin transplanted to A/J hosts. A/J skin grafts transferred to F_1 hosts, although not completely isogeneic, were considered to be isografts because of the inability of the host lymphocytes to react against the transplant (the laws of

transplantation, Snell and Stimpfling, 1966). After seven or eight days, which is the time required for hypertrophied lymphocytes to be established in the graft bed (Walker and Goldman, 1963), the dressings were removed and the graft beds transplanted. Each graft bed provided material for three to five implants.

III. Implantation beneath the kidney capsule

Material to be implanted to F_1 kidneys included three nonradioactive allograft beds, two radioactive allograft beds (prepared as in V., below), two isograft beds, one radioactive spleen, one sensitized spleen, and Gelfoam*. F_1 animals receiving implantations were grouped according to the type of implanted material as in Table 1.

Table 1. Numbers of mice receiving implants from various sources.

Type of implanted material	Number of animals
Allogeneic skin graft bed	13
Allogeneic sensitized spleen	2
Radioactive allogeneic skin graft bed	5
Radioactive allogeneic spleen	3
Isogeneic skin graft bed	6
Gelfoam	4
No implant	1

*Absorbable, non-antigenic gelatin sponge, The Upjohn Company, Kalamazoo, Michigan.

Graft beds to be implanted were exposed as in grafting (above) and cut into approximately 1 mm^3 pieces. Each cube was minced with a fine scissors before implantation. The A/J spleen donor was sensitized to F_1 antigens by a skin allograft and intraperitoneal injection of F_1 kidney homogenate seven days before implantation. Implants consisted of minced pieces of splenic tissue measuring about 1 mm^3 each. Gelfoam implants also consisted of approximately 1 mm^3 pieces. All implants were kept moist in Medium 199* diluent which contained 1 unit of penicillin and 1 mg of streptomycin per ml. At no time were tissue implants separated from a living environment for more than ten minutes.

Implant recipients were anesthetized and the mid-dorsal region was clipped, shaved, and cleaned with 70% alcohol. A 3 cm long incision was made through the skin and each side was retracted to expose the underlying muscle. Slightly posterior to the tip of the last rib, a 1 cm incision was made through the muscle layers, exposing the fatty tissue around the kidney. Small retractors were used to displace the fat and gently lift the kidney toward the surface. The delicate kidney capsule was lifted with a fine forceps and incised with the point of a scalpel. The implant material was then forced between the capsule and the renal cortex, being held in place by the tension of the capsule. The

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*Hyland Division of Travenol Laboratories, Inc., Los Angeles, California.

kidney was then returned to its original position, the muscle sutured, and the skin closed with wound clips. Animals were sacrificed at seven day intervals according to the schedule in Table 2. Kidneys which had received implants were removed by reopening the original incisions, lifting the kidney by the vessels of the hilus, and severing all attachments with a small scissors.

Table 2. Number of animals sacrificed on seven day intervals after implantations.

Type of implanted material	Days after implantation						
	0	7	14	21	28	35	42
Allogeneic skin graft bed	1	2	2	2	2	2	2
Allogeneic sensitized spleen		1		1			
Radioactive allogeneic skin graft bed		1	1	1		1	1
Radioactive allogeneic spleen		1		1			1
Isogeneic skin graft bed		1	1	1	1	1	1
Gelfoam		1	1	1	1		

IV. Histologic techniques

All kidneys containing implants, additional portions of graft beds and spleens used for implants, and a normal F_1 kidney were fixed in 10% buffered formalin for 24 hours, embedded in paraffin, and sectioned at 7 μ. All sections were mounted with albumin on cleaned slides, dried thoroughly, then deparaffinized with xylene and alcohol. Non-radioactive sections were stained with either 1% toluidine

blue in 70% alcohol for three minutes or with hematoxylin and eosin. Cover slips were applied with Histoclad.*

V. Radioautography

Two A/J females, to be used as hosts for skin allografts, were injected subcutaneously with tritiated thymidine before grafting. Three separate doses of one hundred μc were given at four hour intervals to each mouse. The skin grafts were made 24 hours after the final injection so that the hemopoietic tissues would be labeled and no unbound label would remain in the animal (Walker and Goldman, 1963). The skin grafting, implantation, fixation, embedding, sectioning, mounting, and deparaffinization were carried out as outlined above. Slides were then dried the second time and coated in a darkroom with Kodak type NTB_2 nuclear track emulsion**. The procedure for coating, drying, storage, and developing of slides was that detailed by Walker (1959) except that the celloidin coating was omitted. All slides were exposed for 50 days. After developing, slides were stained with 1% toluidine blue in 70% alcohol for five minutes or with hematoxylin and eosin, and cover slipped with Permount***.

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*Clay-Adams Inc., New York.

**Eastman Kodak Company, Rochester, New York.

***Fisher Scientific Company, Fair Lawn, New Jersey.

RESULTS

When dressings were removed from the skin grafts seven days after the grafting operations, all isografts and allografts had "taken" except the two allografts given to radioactively labeled animals. This was presumably due to faulty technique in grafting. The isografts appeared quite healthy and had partial regrowth of donor hair. The three allografts on non-radioactive animals had no hair regrowth, but except for this and slight flaking of the epidermis, appeared similar to isografts when observed grossly. The two allografts on radioactively labeled hosts were dried, scablike masses.

Microscopic examination of a control section of isograft revealed a thickened epidermis with 3-4 layers of healthy-looking cells. Between the epidermis of the graft and the panniculus carnosus of the host were several layers of tissue. Immediately beneath the epidermis, the bases of hair follicles were interspersed with collagenous connective tissue fibers of the dermis. Some of the fat cells of the grafted panniculus carnosus were distinguishable, but there was no distinct fatty layer as in normal skin. The layer of junction between donor and host tissue was filled with several types of cells. Since the relative numbers of cells of each type were more important in this study than the

absolute numbers, cell counts were recorded as number of cells per unit area (2500 μ^2 area of a 7 μ section) whenever possible. Absolute numbers were also recorded when they were pertinent to the results. Table 3 contains the comprehensive data of all items seen in graft beds, some of which are described below. About 60% of the cells in the dense cellular layer of the isograft bed were large cells with oval or elongated nuclei and varying amounts of basophilic cytoplasm. The rest of the cells in this area were endothelial cells, fat cells, or unidentified cells. About 60% of the muscle fibers of the panniculus carnosus beneath the graft were stained deeply with toluidine blue and had centrally placed nuclei. The diameter of these specific fibers was 5-17 μ as compared with 15-30 μ for fibers with eccentric nuclei and for those beneath uninjured skin. Beneath this muscle layer was a typical fascial layer composed of widely spaced cells, 50% of which had large, elongated nuclei and toluidine blue positive cytoplasm. The entire graft and graft bed were well vascularized, averaging 1.0 vessels per unit area. Of the vessels observed, 75% had red blood cells present, while 10% contained only white blood cells, and 15% were empty. Mast cells, totaling 20-28 cells per mm^2 area of a 7 μ section, appeared in the panniculus adiposus of normal skin at the borders of the graft as well as in the fascial layer beneath the panniculus carnosus.

A typical allogeneic skin graft, placed seven days previously, had a thin epidermis and most of the epithelial

Table 3. Comprehensive data for control sections of graft beds.

Description of items seen	Number of unit areas*	Number of items	Number of items per unit area	
			Range	Average
Isograft bed				
Basophilic cells with elongated nuclei	20	99	3-6	5.0
Neutrophils	20	0	0-0	0.0
Macrophages	20	3	0-1	0.2
Mast cells	20	6	0-2	0.3
Panniculus carnosus fibers (5-17 μ dia. and central nuclei)	60	72	0-4	1.2
Fibers with 15-30 μ dia. (eccentric nuc.)	60	24	0-3	0.4
Vessels with red blood cells	100	75	0-2	0.75
Vessels with leukocytes only	100	10	0-1	0.10
Empty vessels	100	15	0-1	0.15
Allograft bed				
Basophilic cells with elongated nuclei	20	78	3-5	3.9
Neutrophils	20	49	0-4	2.5
Macrophages	20	23	0-2	1.2
Mast cells	20	5	0-1	0.3
Panniculus carnosus fibers (5-17 μ dia. and central nuclei)	60	18	0-1	0.3
Fibers with 15-30 μ dia. (eccentric nuc.)	60	42	0-2	0.7
Vessels with red blood cells	100	27	0-2	0.27
Vessels with leukocytes only	100	14	0-1	0.14
Empty vessels	100	28	0-2	0.28
Radioactive allograft bed				
Basophilic cells with elongated nuclei				
Labeled	20	46		2.3
Unlabeled	20	16		0.8
Total	20	62	0-4	3.1
Neutrophils	20	68	2-5	3.4
Macrophages	20	10	0-1	0.5

*Selected at random from the graft bed.

cells were pyknotic and fragmented. As noted from Table 3, neutrophils were numerous in the entire graft, but especially within and just beneath the epithelium. The layer between the graft panniculus adiposus and host panniculus carnosus was filled with cells, about 40% of which had toluidine blue positive cytoplasm and large oval nuclei with from 1-4 prominent nucleoli. Of these cells, 3.9 were present per unit area in this area of the graft. About 25% of the cells in this same area were neutrophils. The remaining 15% were macrophages, endothelial cells, or cells with unidentified smaller nuclei. About 30% of the muscle fibers of the panniculus carnosus stained deeply with toluidine blue, had centrally placed nuclei, and measured 5-17 μ in diameter. The other fibers had eccentric nuclei, measured 15-30 μ in diameter, and were only lightly stained. This particular allograft contained 0.7 vessels per unit area, being not as well vascularized as the isograft described above. Of the vessels observed, 39% contained red blood cells, 20% contained only leukocytes, and 41% were empty. Mast cells were distributed throughout the graft and graft bed, numbering 20-30 cells per mm^2 area of 7 μ section. No mature plasma cells were noted in either the allograft or isograft beds seven days after grafting.

The control sections of radioactively labeled skin allografts differed from the allografts described above because of the failure of the grafts to become vascularized. The grafted tissue was entirely necrotic and was filled with

neutrophils and macrophages (see Table 3). Only a few of the neutrophils, but many of the macrophages, were radioactively labeled. The graft bed cells appeared similar to the allograft bed cells described above except that the prominent layer of cells with large, elongated nuclei and basophilic cytoplasm was located beneath the panniculus carnosus instead of superficial to it. The background fog count over the tissue was 16 grains per 400 μ^2 area. The oval or elongated nuclei measured from 17-40 μ^2 and were considered radioactive only if they were overlaid by at least five silver grains. Approximately 70% of the large cells with basophilic cytoplasm and elongated nuclei were observed to be labeled. A few other cells, 1.0 cells per unit area, with slightly smaller nuclei and no detectable basophilic cytoplasm were also labeled. The degree of labeling ranged from five to nine grains per kidney-shaped nucleus of macrophage, and from five to 12 grains per elongated nucleus of lymphocyte. Only a very few of the fibrocytes were labeled, numbering about 1% of all labeled cells. These fibrocytes were distinguished from the lymphocytes because the former had 18 to 24 silver grains per nucleus. One labeled cell per mm^2 area (about 0.1% of labeled cells) was observed to be in the process of mitosis.

Labeling on control sections of radioactive spleen was relatively light with about 4% of white pulp cells labeled, or 1.1 cells per unit area, and 10% of red pulp cells labeled, or 2.1 labeled cells per unit area.

According to the above calculations, splenic implants measuring approximately 1 mm^3 contained about 1.2×10^6 cells, of which 8.5×10^4 cells were labeled. Radioactive allograft bed implants measuring 1 mm^3 were calculated to contain about 1.1×10^5 labeled nuclei.

The implantations beneath the kidney capsule were generally quite successful. All animals appeared normal at the time of sacrifice. No local or systemic infections were encountered when the penicillin and streptomycin combination was used in the diluent. Of 33 implants, 27 could be seen grossly on the kidney at the time of sacrifice, usually appearing as a small whitish area beneath the capsule. Remnants of three additional implants were found microscopically, and three implants were not found. At the time of sectioning, it was noted that some of the kidneys had not been completely infiltrated with paraffin. Consequently, only a few good sections were obtained from some of the implanted tissues. The situation was subsequently corrected by removing part of the fibrous capsule from the kidney in an area away from the implant area before embedding.

Histological appearance of representative sections of each type of implant at each time interval are described in chronological order. The measurements of each implant are listed in Table 4. Summaries of the relative numbers of cell types seen in allograft and isograft bed implants are presented in Table 5 and Table 6, respectively, as well as relative vascularity of each implant.

Table 4. Measurement of implants in sections with maximum width of implant. Width was measured parallel and depth perpendicular to capsule.

Type of implant	Width in mm	Depth in mm
0 day allograft bed	1.3	0.5
7 day allograft bed	1.5	0.2
7 day allograft bed	1.7	0.15
7 day radioactive allograft bed	0.5	0.01
14 day allograft bed	1.1	0.1
14 day allograft bed	0.7	0.2
14 day radioactive allograft bed	0.6	0.5*
21 day allograft bed	1.0	0.2
21 day radioactive allograft bed	0.6	0.1
28 day allograft bed	0.9	0.2
28 day allograft bed	1.0	0.2
35 day allograft bed	1.0	0.2
35 day allograft bed	0.5	0.3
35 day radioactive allograft bed	0.8	0.1
42 day allograft bed	1.0	0.1
42 day allograft bed	0.9	0.2
42 day radioactive allograft bed	0.7	0.1
7 day isograft bed	1.2	0.4
14 day isograft bed	1.3	0.2
21 day isograft bed	0.5	0.05
35 day isograft bed	0.5	0.05
42 day isograft bed	0.7	0.2
7 day Gelfoam	1.5	0.5
14 day Gelfoam	0.7	0.1
21 day Gelfoam	0.9	0.1
28 day Gelfoam	0.5	0.1
7 day sensitized spleen	0.1	0.2**
7 day radioactive spleen	0.9	0.3
21 day radioactive spleen	1.0	0.5
42 day radioactive spleen	0.5	0.4

*Triangular, 0.6 mm on a side.
**Within kidney cortex.

Table 5. Number of cells and vessels present in allograft bed implants. The first column beneath each time interval contains the total number of items; the second column contains the average number of items per unit area*.

Description of item	Days after implantation												
	0		7		7**		14		14**		21		21**	
Basophilic cells with elongated nuclei													
Labeled					5	2.5			1	0.1			0	0.0
Non-labeled					3	1.5			15	1.5			10	1.7
Total	26	3.7	43	3.6	8	4.0	38	3.8	16	1.6	18	2.3	10	1.7
Neutrophils	6	0.9	12	0.9	0	0.0	3	0.3	4	0.4	1	0.1	3	0.5
Macrophages														
Labeled					0	0.0			4	0.4			2	0.3
Non-labeled					1	0.5			7	0.7			2	0.3
Total	5	0.7	13	1.1	1	0.5	11	1.1	11	1.1	18	2.3	4	0.6
Mast cells	2	0.3	5	0.4	0	0.0	4	0.4	4	0.4	4	0.5	2	0.3
Plasma cells	0	0.0	7	0.6	0	0.0	7	0.7	14	1.4	8	1.0	2	0.3
Small lymphocytes	4	0.6	11	0.9	3	1.5	9	0.9	14	1.4	15	1.9	10	1.7
Fat cells	3	0.4	5	0.4	0	0.0	3	0.3	2	0.2	2	0.3	0	0.0
Unidentified cells	29	4.1	46	3.8	4	2.0	32	3.2	26	2.6	23	2.9	14	2.3
Blood vessels with erythrocytes	0	0.0	10	0.8	0	0.0	10	1.0	6	0.6	6	0.7	5	0.8
Number of unit areas analyzed	7		12		2		10		10		8		6	

*2500 μ^2 area of 7 μ section (unit areas selected at random).
**Radioactive implant.

Table 5. Continued.

Description of item	Days after implantation											
	28		35		35*		35**		42		42*	
Basophilic cells with elongated nuclei												
Labeled					0	0.0					0	0.0
Non-labeled					15	2.1					8	1.6
Total	25	2.5	27	4.5	15	2.1	12	2.0	12	1.2	8	1.6
Neutrophils	4	0.4	3	0.5	2	0.3	2	0.3	2	0.2	0	0.0
Macrophages												
Labeled					0	0.0					0	0.0
Non-labeled					4	0.6					2	0.4
Total	8	0.8	8	1.3	4	0.6	10	1.7	13	1.3	2	0.4
Mast cells	5	0.5	1	0.2	1	0.1	2	0.3	2	0.2	1	0.2
Plasma cells	8	0.8	2	0.3	2	0.3	3	0.5	3	0.3	0	0.0
Small lymphocytes	7	0.7	14	2.3	11	1.5	12	2.0	7	0.7	6	1.2
Fat cells	3	0.3	0	0.0	10	1.4	6	1.0	15	1.5	6	1.2
Unidentified cells	24	2.4	18	3.0	12	1.6	7	1.2	28	2.8	7	1.4
Blood vessels with erythrocytes	7	0.7	3	0.5	4	0.6	4	0.7	10	1.0	3	0.6
Number of unit areas analyzed	10		6		7		6		10		5	

*Radioactive implants.
**This implant was fatty, the first 35 day non-radioactive implant was fibrous.

Table 6. Numbers of cells and vessels present in isograft bed implants at each time interval. The first column beneath each time interval contains the total numbers of items; the second contains the numbers of items per average unit area of implant.

Description of item	Days after implantation									
	7		14		21		35		42	
Basophilic cells with elongated nuclei	21	3.0	23	2.9	13	3.3	13	2.6	12	1.5
Neutrophils	1	0.1	2	0.3	2	0.5	1	0.2	1	0.1
Macrophages	5	0.7	8	1.0	3	0.8	3	0.6	8	1.0
Mast cells	5	0.7	4	0.5	1	0.3	2	0.4	4	0.5
Plasma cells	3	0.4	3	0.4	0	0.0	2	0.4	1	0.1
Small lymphocytes	12	1.7	9	1.1	6	1.5	6	1.2	10	1.2
Fat cells	3	0.4	3	0.4	1	0.3	1	0.2	8	1.0
Unidentified cells	27	3.9	32	4.0	16	4.0	16	3.2	13	1.6
Blood vessels with erythrocytes	7	1.0	8	1.0	3	0.8	3	0.6	5	0.6
Number of unit areas analyzed	7		8		4		5		8	

The normal kidney was surrounded by a capsule measuring 7-10 μ in thickness and composed of connective tissue fibers and a few widely spaced cells. Other aspects of the normal kidney are noted in comparison with kidneys which had received implants.

The allograft implant, taken for observation immediately after the implantation operation, was a folded piece of allograft bed lying beneath the capsule, directly upon the outer rim of cortical tubules (Figure 1). The largest section of the implant included the layer of densely packed cells, the panniculus carnosus, and the fascial layer of the allograft bed. This section was typical of the entire implant and a total of 3.9×10^4 cells with elongated nuclei and toluidine blue positive cytoplasm were calculated to be present in the implant. In this particular implant, no injury to the kidney tubules was evident, but this was not necessarily representative for all implants. Numbers of cells of representative types are listed in Table 5.

Both seven day allograft bed implants were somewhat flattened and appeared to have spread out slightly between the capsule and the kidney cortex. The capsule itself was thickened to 15-20 μ and contained several large cells. The average unit area of both implants contained several types of cells which are listed in Table 5. Both implants contained several capillaries and larger vessels filled with red blood cells. A .15 mm by .15 mm area of kidney cortex beneath part of each implant was filled with lymphocytes

FIGURE 1

Allogeneic skin graft bed immediately after implantation. A portion of the delicate kidney capsule is seen covering the implant and at the right and left of the picture. About 58x.

FIGURE 2

A portion of the kidney cortex beneath a seven day allograft bed implant. A portion of the implant material is present at the top of the photograph. Note the cellular infiltration, in the left center of the cortical area. Tubules near the lower and right hand borders appear normal. About 200 x.

FIGURE 1

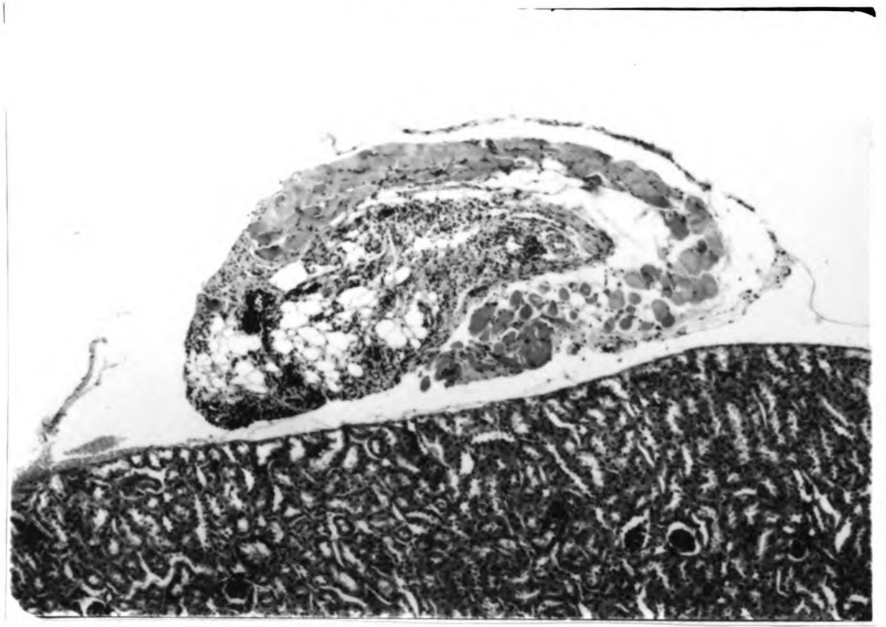
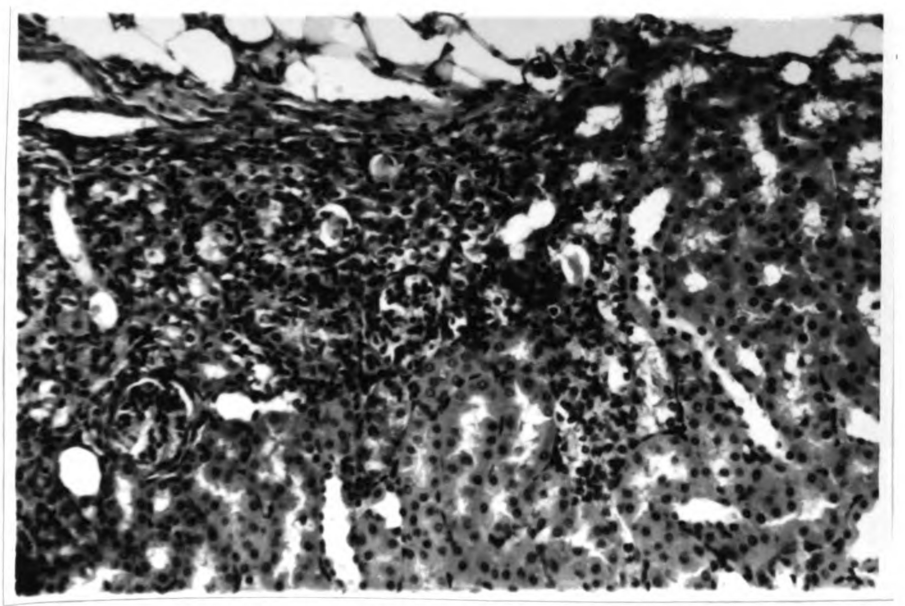


FIGURE 2



which had surrounded some of the tubules of the cortex (Figure 2). The cells of the tubules which were surrounded had vacuolated cytoplasm and light staining nuclei, and many of these tubules appeared to have debris clogging the lumen. Various sections of tubules in a direct line toward the medulla also displayed cells with vacuolated cytoplasm and light staining nuclei. Three of the six glomeruli in the cortical area immediately beneath the implant were somewhat ischemic with only a few red blood cells appearing in the collapsed capillary tufts. The other glomeruli appeared similar to those seen in the normal kidney, with capillary tufts filled with red blood cells.

The seven day allograft bed implant from the radioactively labeled graft bed donor contained only a few cells in the largest section, and the results cannot be considered representative. Nevertheless, in the implanted tissue, five out of eight large cells with toluidine blue positive cytoplasm and elongated nuclei were labeled. Three of these cells had 17-21 silver grains per nucleus, and the rest had 7-9 grains per nucleus. Other cell types noted are listed in Table 3. No invasion of kidney cortex was seen, and glomeruli and tubules beneath the implant area appeared normal.

The isograft bed which had been implanted seven days previously, had spread out only slightly between the capsule and the kidney cortex (Table 4). Table 6 lists the numbers of certain types of cells seen in the implant. Mast cells were especially prevalent in this implant (Figure 3), numbering

0.8 cells per average unit area, or approximately 7 x 10^3 cells in the entire implant. This implant had become quite well vascularized, as had the allograft bed implants, averaging 1.0 vessels with red blood cells present per unit area of implant. A small area of kidney cortical tubules, measuring .04 mm^2 at the largest area, was infiltrated with lymphocytes between the tubules. The surrounded tubules had cells with vacuolated cytoplasm and lightly stained nuclei. Only glomeruli in the area of lymphocyte infiltration appeared to lack red blood cells in the capillary tufts.

The sensitized spleen implant, observed after seven days, appeared to have been buried in the cortical tissue during implantation. A single lymphatic nodule was present, measuring 0.1 mm in diameter at the largest section, along with several megakaryocytes and a few smooth muscle trabeculae. Kidney tubules in immediate contact with the implant and tubules in a direct path toward the medulla were composed of cells with vacuolated cytoplasm and lightly stained nuclei. Only a few lymphocytes appeared between kidney tubules near the implant. Glomeruli within 0.1 mm of the implanted tissue were hypercellular and ischemic. Glomeruli at a greater distance had red blood cells in the capillary tufts. The kidney capsule was thickened to 50 μ immediately over the implant area.

The seven day radioactive spleen implant measured 0.9 mm by 0.3 mm at the largest section, and contained a lymphatic nodule and a portion of red pulp. Approximately 2% of all

FIGURE 3

Isograft bed implant after seven days. The large, darkly stained cells seen in the implant material, are mast cells, which stain heavily with toluidine blue. About 100x.

FIGURE 4

Seven day Gelfoam implant showing cellular infiltration. Both the implant (above) and the cortex (center) have been infiltrated by host cells. About 100x.

FIGURE 3

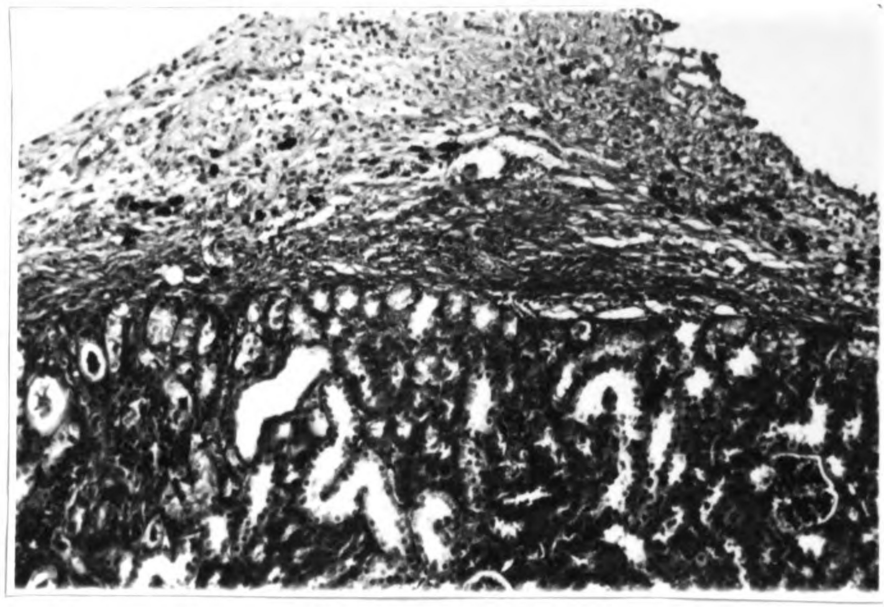
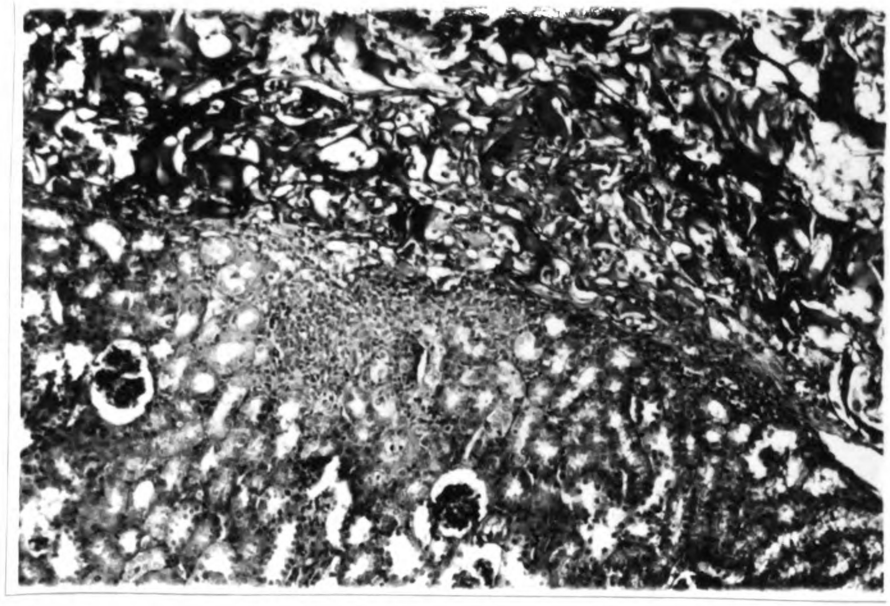


FIGURE 4



cells in the implant area were radioactively labeled. The red pulp area contained many vessels filled with red blood cells. The kidney capsule around the implant measured 20 μ in thickness, and contained several small lymphocytes and a few plasma cells, none of which were labeled. Only three or four of the outermost cortical tubules in each 7 μ section were surrounded by lymphocytes and contained cells with vacuolated cytoplasm and lightly stained nuclei. In the cortex near the implant, a vein measuring 50 μ in diameter was also surrounded by a small mass of lymphocytes. Kidney glomeruli appeared normal, having red blood cells in most of the capillary tufts.

After seven days, a Gelfoam implant was noteworthy in that it contained several types of cells (Figure 4). The average unit area contained cells of several types and vessels as listed in Table 7. No plasma cells were seen in the implant or surrounding area. Eighteen mast cells were seen in a 1.5 mm length of kidney capsule above the largest section of Gelfoam implant. The rest of the capsule, similar to the capsule of the normal kidney, contained only an occasional mast cell. A few mast cells were also scattered within the implant material. An area of kidney cortex just beneath the implant, measuring 0.1 mm square, was infiltrated with lymphocytes and the tubules which were surrounded had amorphous material filling the lumen, and the tubule cells had vacuolated cytoplasm and lightly stained nuclei.

Table 7. Numbers of cells and vessels present in Gelfoam implants. The first column beneath each time interval contains the total numbers of items; the second contains the numbers of items per average unit area of implant.

Description of item	Number of items per unit area for each time interval							
	7		14		21		28	
Large cells with basophilic cytoplasm	18	1.8	27	3.4	19	2.4	18	2.6
Neutrophils	7	0.7	9	1.1	0	0.0	0	0.0
Macrophages	8	0.8	9	1.1	15	1.9	24	3.4
Mast cells	2	0.2	2	0.3	3	0.4	0	0.0
Plasma cells	0	0.0	4	0.5	1	0.1	2	0.3
Small lymphocytes	17	1.7	20	2.5	15	1.9	12	1.7
Fat cells	0	0.0	1	0.1	3	0.4	3	0.4
Unidentified cells	20	2.0	10	1.2	11	1.4	9	1.3
Blood vessels with erythrocytes present	11	1.1	8	1.0	4	0.5	0	0.0
Number of unit areas analyzed per implant	10		8		8		7	

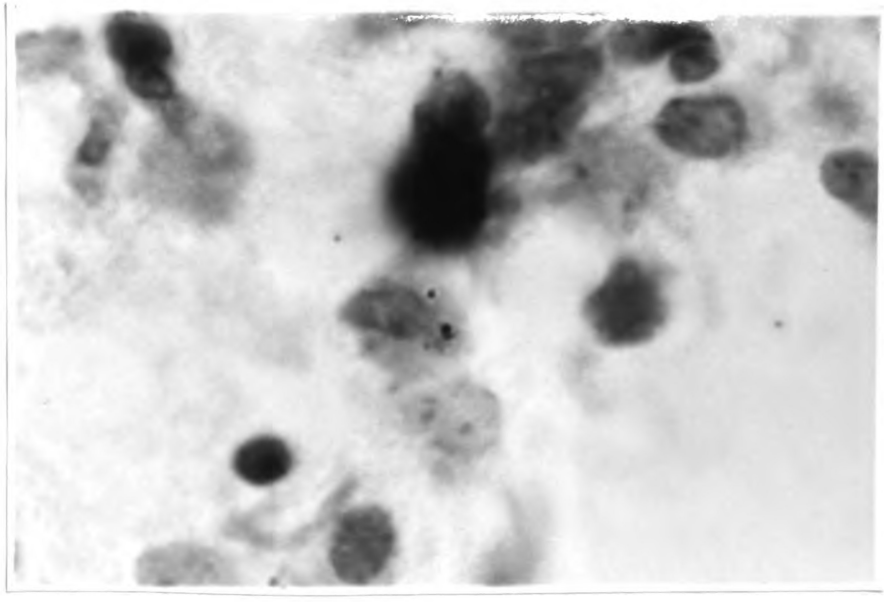
The two allograft beds implanted 14 days previously were slightly reduced in size (Table 4). The average cell counts of both implants together are listed in Table 5. Both implants had several vessels containing red blood cells. Beneath one of the implants, a small mass of lymphocytes, measuring 0.1 mm in diameter, appeared in the cortex, and the tubular cells in contact with this mass had lightly stained nuclei and vacuolated cytoplasm. Three glomeruli near the lymphocyte mass were hypercellular and had no red blood cells in the capillary tufts. Glomeruli farther outside the infiltration area contained normal amounts of erythrocytes.

The 14 day allograft bed implant from a radioactive donor appeared as a triangular mass beneath the capsule, measuring 0.6 mm on a side. Cell counts are listed in Table 5. There was a drastic reduction in the number of labeled cells in the implant area so that only a few remained. Several macrophages (0.4 cells per unit area, or about 480 cells in the entire implant) were noted to be labeled, and the radioactive material appeared to be concentrated in the cytoplasm (Figure 5). Plasma cells were present in moderate numbers, but all were clearly free of radioactive labeling. A relatively large area of kidney cortex (0.5 mm square) beneath the implant area was infiltrated with lymphocytes, none of which were radioactive. Glomeruli in this area were hypercellular and ischemic, and the remnants of cortical tubules were made up of cells with

FIGURE 5

Oil immersion of a 14 day implant of radioactive allograft bed. The cell in the center of the field is a macrophage with at least three silver grains over the cytoplasm. About 1200x.

FIGURE 5



vacuolated cytoplasm and light staining or fragmented nuclei. Outside the infiltration area, tubules and glomeruli appeared normal.

The isograft bed implanted for 14 days appeared to have spread out slightly beneath the capsule. Cell contents are summarized in Table 6, and no striking difference was noted between the cells present in this implant and those seen in the seven day isograft bed implant. Also similar was a .04 mm^2 area of kidney cortex which was infiltrated with lymphocytes, and which had tubules with vacuolated, lightly stained cells.

The most striking feature of the 14 day Gelfoam implant was a 0.5 mm square area which was infiltrated with mononuclear cells in the cortex beneath the implant. This was the largest area of infiltration seen beneath any of the implants, and the structures of some cortical tubules were completely obscured. Glomeruli within this area were hypercellular and lacked red blood cells. The implant itself was filled with cells as listed in Table 7.

One of the 21 day allograft bed implants appeared only slightly reduced in size (Table 4), and the other implant was not found. The implant that was found contained the types of cells listed in Table 5. Several vessels with red blood cells were present in the implanted tissue. A small wedge of what appeared to be implanted tissue protruded from the implant into the kidney cortex, and some of the tubules adjacent to this wedge had cells with vacuolated

cytoplasm. No invasion of lymphocytes was noted and all glomeruli beneath the implant appeared normal.

The 21 day radioactive allograft bed implant was relatively small (Table 4), and contained cells as listed in Table 5. Radioactive labeling appeared to follow the pattern of that seen for the 14 day implant of the same type. No cells could be found with labeling similar to the original implant, and several macrophages were seen with what appeared to be labeled cytoplasm. About 50% of the macrophages observed had at least two silver grains located over the vacuoles of the cytoplasm. Plasma cells and cells with elongated nuclei and basophilic cytoplasm were distinctly non-radioactive. No invasion of kidney cortex was seen, and all tubules and glomeruli appeared normal.

The 21 day isograft bed implant was seen only microscopically, the largest section measuring 0.5 mm by .05 mm. Relative numbers of cell types present are listed in Table 6. On this implant, it was not possible to distinguish the kidney capsular cells from the underlying outermost layer of implant cells. No invasion or destruction of kidney cortex was seen beneath the implant area.

The kidney containing the 21 day spleen implant was not properly embedded in paraffin, and the sections obtained were uneven. It was not possible to observe anything except that the implanted material was present beneath the capsule. No cell counts were made.

The 21 day implant of radioactive spleen was relatively large measuring about half of the esimated original implant size at the largest section (Table 4). One small nodule of densely packed cells, resembling white pulp, was present, and the rest appeared similar to red pulp. Several macrophages were present in the implant area, averaging 1.4 cells per unit area. Radioactive labeling was relatively light. Only a few scattered cells were covered by more than three silver grains. A few of the macrophages appeared to have silver grains over the cytoplasm, but the density of the vacuoles prevented accurate counting. A 1.0 mm length of capsule over the implant contained 22 mast cells, whereas the rest of the entire capsule, measuring 13 mm, contained four mast cells. Only a few lymphocytes were located beneath the implant. Kidney tubules and glomeruli appeared normal.

The 21 day Gelfoam implant was reduced in size and only a small portion of the gelatin network remained (Table 4). However, the implant did contain many cells, and was well vascularized (Table 7). Only a small area of kidney cortex was infiltrated with mononuclear cells, and only a few cells of the nearby tubules were vacuolated. Glomeruli appeared to have the normal amount of capillary tufts filled with red blood cells.

The 28 day allograft bed implants were similar in size to each other (Table 4). One implant appeared to be mainly a mass of relatively dense fibrous material. The other

was seen to contain four hair follicles as well as several fat cells. Cell counts were made and appear in Table 5. The cells of the hair follicles in the one implant appeared healthy, but there was only one layer of cells present around each portion of hair. No infiltration of kidney cortex was seen in either implant, and all tubules and glomeruli appeared normal.

No sections of 28 day isograft bed implant were found, and all sections of the kidney and capsule appeared normal.

The 28 day Gelfoam implant was infiltrated by several cell types, the greatest number of which were macrophages (Table 7). The implanted tissue itself had no blood vessels present. Alteration of kidney cortex in this case was minimal, with only a few tubules being surrounded by lymphocytes and containing vacuolated cells.

One of the 35 day allograft bed implants was heavily infiltrated with lymphocytes (Table 5), and a small lymphatic nodule was present near the implant area, but outside of the kidney capsule. The other implant of the same type could be seen only microscopically (Table 4), and consisted mainly of loosely arranged cells with several fat cells present (see Table 5). In the kidney with the infiltrated implant, a few of the outermost cortical tubules directly beneath the implant were surrounded with lymphocytes and contained vacuolated cells. Glomeruli in the cortex of both implants appeared normal.

The 35 day implant of radioactive allograft bed was reduced in size and contained only a few cells (Table 4 and Table 5). No radioactive cells were present in any area of the section. No infiltration was present in the cortex and all glomeruli and tubules appeared normal.

The largest section of isograft bed implanted for 35 days appeared to be only a thickened portion of kidney capsule (see Table 4 and Table 6). No alterations of kidney cortex were seen.

All four 42 day implants that were found were somewhat reduced in size and consisted mainly of a fat cell matrix interspersed with a few other cell types (Table 4, Table 5, and Table 6). The implant of radioactive spleen contained no trace of splenic tissue and no labeled cells. Similarly, no cells of the radioactive allograft bed were labeled. Two of the kidneys, the one with the isograft bed implant and the other which contained one of the allograft bed implants, had a small area of cellular infiltration. Beneath the allograft bed, a 0.1 mm square area contained several lymphocytes, but almost an equal number of larger cells was also present, as well as fibrous tissue. No tubular structures were discernable in the infiltration area, but all other areas of the cortex contained normal tubules and glomeruli. The infiltration beneath the isograft bed implant differed from that beneath the allograft bed, only in that it was more extensive, measuring 0.3 mm square.

DISCUSSION AND CONCLUSIONS

I. Histology of the graft beds

The results of the skin grafting and the appearance of the graft beds histologically were consistent with previous descriptions (Waksman, 1960, and Walker and Goldman, 1963). The appearance of large cells with toluidine blue positive cytoplasm can be attributed, in the case of isografts, to the proliferation of fibrocytes in response to the skin injury. In allografts, these cells represent the combination of proliferating fibrocytes and lymphocytes, the latter having infiltrated from the blood and hypertrophied beneath the graft, presumably in response to the antigenic stimulus of the allograft tissue present above it. According to the findings of Walker and Goldman (1963), these two cell types can be separated satisfactorily by radioactive labeling. They noted that only a small number of fibrocytes were labeled when the isotope was given 24 hours prior to grafting, and these had a considerably greater number of silver grains present per nucleus than the labeled lymphocytes. The results of the present experiment are in agreement with this finding. Therefore only the cells with elongated nuclei and with 5-12 grains per nucleus were considered to be of lymphocyte origin. The few cells that were seen with 18-25 grains were considered to be fibrocytes. Consequently, when labeling is done in this

way, almost all of the labeled cells were those that had infiltrated from the blood.

The observations on the vascularity of the isografts and allografts cannot be regarded as typical or representative because of the size of the sample. However, the difference in the number of vessels containing red blood cells, supports the hypothesis that both isografts and allografts become vascularized, but that the allograft blood supply is reduced during the process of rejection, by rupture of some vessels and filling of others with packed red blood cells (Taylor and Lehrfeld, 1953).

The muscle fibers of the panniculus carnosus beneath both allografts and isografts contained a number of fibers with smaller diameters and more centrally placed nuclei than normal fibers. The combination of these criteria suggested that these fibers were regenerating (Walker, 1962), probably in response to the injury received at the time of grafting. The fact that more regeneration was evident in isograft beds than in the allograft beds possibly reflected the relative vascularity, but might also have been due to a difference in degree of injury, or some other cause. The lack of plasma cells beneath the graft after seven days was in agreement with the findings of other investigators. Russell and Monaco (1965) mentioned the presence of plasma cells only in the latter stages of rejection.

II. The fate of transplanted tissue

The results of the kidney capsule implantations, described above, give rise to several interesting hypotheses. The fate of the implanted cells is difficult to determine from the relative numbers of cells present at the various time intervals. The seven day Gelfoam implant contained a slightly smaller total number of cells than was present in graft bed implants at the same interval. This fact suggested that there was a small number of graft bed donor cells still surviving at this early time interval. However, if the tissue were degenerating at this time, there probably would be a chemotactic response which would easily account for the greater number of cells infiltrating the tissue implants. The results of the seven day radioautograph of allograft bed, although indicating the presence of only a small total number of cells, did suggest the viability of at least some of the labeled cells at this time. It was not possible, from the data received, to prove that the hypertrophied lymphocytes were still present, because, even if cells were present with the expected range of labeling, these may have resulted from divisions of the few fibrocytes which were originally more heavily labeled. The data from the control sections of radioactive allograft beds compared with the radioautographs of implants prove that the radioactive cells do not remain in the implant longer than about 14 days. About 1×10^5 labeled cells were implanted, but only one labeled large cell was noted at 14 days, and none at 21, 35, or 42 days. The

macrophages of the original implant had labeled nuclei, and macrophages at 14 days and 21 days after implantation were labeled. However, the radioactive material in the cells of these latter implants appeared to be in the cytoplasm. It is concluded therefore, that the macrophages had phagocytized the remnants of at least some of the labeled cells. Another possibility exists for the fate of the labeled cells. They might have migrated to other parts of the body. Najarian and Feldman (1962b) injected (intravenously) thymidine H^3 labeled lymphatic cells, which had been sensitized to allografts, into isogeneic animals which were hosts for skin allografts. These cells were found only rarely in the graft beds and quite often in the lymphatic organs. These cells might have more of an affinity for the lymphatic organs than for the antigenic material, however it seems unlikely, in the present experiment, that the hypertrophied lymphocytes which had become established in the allograft bed would mobilize after the tissue was implanted. The possibility that the label was diluted out by repeated mitoses is unlikely, because the size of the implants as well as the cell counts decrease with time. One reason for the lack of cell survival may be that an insufficient number of cells were transplanted in order to overcome the initial shock of the operation. The present study involved 1×10^5 reactive cells, whereas other investigators (e.g. Elkins, 1964) used up to 50×10^6 cells.

III. Infiltration of the kidney cortex

Considering the difficulty of manipulating a 7-10 μ capsule without injury to the underlying tissue, it was anticipated that the majority of kidneys might sustain cortical damage by mechanical means. The manifestation of this injury was in the form of lymphocyte infiltration and tubular cell destruction, since these phenomenon occurred with some of the implants of each type of material, including Gelfoam. The fact that lymphocytes infiltrated beneath the Gelfoam indicates that the cells which infiltrated were in this case wholly of host origin, rather than having proliferated from donor cells, and since no infiltration area was greater than that beneath the Gelfoam implant at 14 days, it was concluded that the infiltration beneath tissue implants was also of host origin. The radioactive implants also support a theory of host origin for the infiltrating cells, since none of the cells in the infiltration area were overlaid by silver grains in excess of the background fog. An alternative explanation for this lack of labeling might be that the label was diluted out by repeated mitoses. But this seems unlikely, since no mitotic figures were seen in the infiltration area. Furthermore, at least four consecutive divisions would be required by each labeled cell to completely dilute the label, and this would result in at least 1.7×10^6 infiltrating cells from the original 1.1×10^5 labeled cells. No infiltration area beneath implanted tissue was seen which contained more than 5.1×10^5 cells.

Possible causes of the cellular infiltration, other than response to damage, are antigenicity of the implant or local bacterial infection. The former is unlikely because Gelfoam, advertised as non-antigenic, elicited the largest infiltration response. Furthermore, at least ten of the implants had no notable infiltration of the cortex. The possibility of a local infection is unlikely because, while practicing the implantation technique, infections were sometimes introduced but spread throughout the entire kidney by seven days, and the cellular response consisted mainly of neutrophil infiltration. Infiltrations noted in the present study were exclusively of mononucleated cells and remained localized. Therefore, it was concluded that mononuclear infiltration was the response of the host to cortical tissue damage. At 42 days after implantation, infiltration areas seemed to have a reduced number of lymphocytes and an increase of fibrous tissue and fibrocytes. This was in agreement with the findings of Elkins (1964), and probably represents the formation of scar tissue in the wounded area.

Another interesting cellular phenomenon was the large numbers of mast cells present in or near many of the implants. Possible explanations include proliferation and migration of existing mast cells, or transformation of other cell types into mast cells. By using a combination of implants and radioactive labeling of hosts as well as implant donors, this question could be resolved, as well as possibly shedding light on the function of these cells relative to such implants.

IV. Application of findings

The short survival time for transplanted cells of skin allograft beds suggests that these cells are extremely sensitive. Walker (1969) indicated that the same cells also failed to survive when transplanted within millipore chambers. However, while monitoring intact radioactively labeled allograft beds, he noted a stability of the hypertrophied lymphocytes over a long period of time. This problem deserves further investigation.

A common occurrence in renal homotransplantation is the infiltration of mononuclear cells (Kountz, Williams, Williams, Kapros, and Dempster, 1963). Many of these are undoubtedly responding to the antigenicity of the foreign tissue, but at least some of the response may be due to mechanical injury during the surgery.

The merit of this study lies chiefly in the fact that it was a preliminary experiment. It provides several interesting leads which will influence the course of further experimentation in this area. Investigations of allograft bed cell properties by transplantation in greater numbers, or by tissue culture; studies of the kidney's response to mechanical injury; and studies of specific cells (e.g. mast cells) are suggested by this investigation, and would help answer some of the questions raised.

SUMMARY

In an attempt to study properties of mouse skin allograft bed cells, minced portions of allograft beds were transplanted under the kidney capsules of F_1 hosts. Comparisons were made with control implants of isograft beds, spleen, Gelfoam, and radioactively labeled allograft beds. A total of 33 implantations were studied at various time intervals.

It was demonstrated that the implanted cells did not survive the new environment longer than about 14 days. Infiltration of mononuclear cells among cortical tubules beneath some of the implants was shown to be the host response to the mechanical damage received during implantation, rather than an antigenic reaction.

Implications of these findings relative to renal homotransplantation were suggested, as were areas for further investigation.

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LITERATURE CITED

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