HOST-HOMOGRAFT TISSUE INTERACTIONS FOLLOWING EXCHANGE BLOOD TRANSFUSIONS IN RABBITS*

By RICHARD H. ANDRESEN, M.D., CLARENCE W. MONROE, M.D., GEORGE M. HASS, M.D., AND DOROTHY A. MADDEN

(From the Rush Department of Pathology and Laboratory of Surgical Research, Presbyterian-St. Luke's Hospital, Chicago)

PLATES 3 TO 7

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Previous studies of rabbits of the same species and strain united in aural parabiosis showed that the cross-circulation established at 3 to 4 days after anastomosis was progressively interfered with so that at 13 days it was no longer demonstrable (1). The loss of the cross-circulation seemed related to development of a characteristic inflammatory reaction along the line of surgical union. This reaction persisted as long as the homologous tissues remained in apposition. In spite of loss of cross-circulation and continuance of the inflammatory reaction, the united tissues of the parabionts failed to separate from one another. The persistence of the union was due to a collagenous matrix mutually deposited during the early period of healing and resistant to the destructive effects of the incompatibility reaction permeating the scar.

When rabbits were placed in parabiosis a second time by uniting the remaining ears, the cross-circulation was rarely reestablished and the ears usually separated spontaneously 6 to 7 days after anastomosis. This observation led to a consideration of the postparabiotic state by microscopic study of host-graft tissue interactions during this period. This study showed that paired cross-transplants of musculofascial tissues during the postparabiotic period elicited two unique forms of host-graft interactions which were reproducible between pairs of parabionts for as long as 90 days after parabiosis (2). The first type of postparabiotic tissue reaction consisted of a collagenous encapsulation of the homograft unaccompanied by vascular penetration by the host. Collagenous deposition and fibroblastic proliferation in the graft were impaired. The inflammatory aspects of the classical homograft reaction were absent. The second type of postparabiotic tissue reaction was similar to the first with the exception of the development of a peculiar angiomatous pattern of vascularization beneath the fascia of the homografts. In spite of

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this redundant vascularization, inflammation did not occur and there was impairment of autolytic mechanisms normally active in the organization and resorption of musculofascial grafts. These host-graft interactions were different from those observed in non-parabionts with initial cross-grafts or following repeated free grafts from the same donor.

Since the development of a cross-circulation which was a conspicuous feature of the healing between parabiotic twins led to prolonged exposure of each parabiont to one another's blood, a study of the effect of exchange transfusions of whole blood upon host-graft interactions in non-parabionts was undertaken.

Methods

Twelve pairs of male New Zealand rabbits weighing 2.5 to 3 kg. were subjected to controlled cross-circulation by one or the other of the following methods:—

Interarterial Extracorporeal Cross-Transfusion.—Under precautions for sterility and with nembutal anesthesia (1 ml. of 6 per cent solution given intravenously), a femoral artery was exposed in each of a pair of animals. Each animal then received 0.25 ml. intravenously (marginal ear vein) of heparin sodium (Organon, Inc., 1000 U.S.P. units per ml.). After occluding the exposed femoral artery of each animal with clamps adjacent to Poupart's ligament, one-half the circumference of the arterial wall was divided, a polyethylene catheter (2 mm. in diameter) was inserted into the lumen and, following release of the clamps, pushed up into the aorta. The catheters were connected to a three-way Luer-Lok stop-cock with an attached syringe of 20 ml. capacity. 20 ml. quantities were withdrawn from one animal and injected directly into the other. The catheters were flushed with 2 ml. of the solution of heparin after each withdrawal. New syringes were used for each withdrawal and transfusion. The exchange of blood which amounted to 280 and 320 ml. (140 and 160 ml. to each animal of the two pairs) required 30 minutes. Following completion of the cross-transfusion, each animal was given 0.25 ml. of a 1 per cent protamine sulfate solution intravenously. The catheters were then withdrawn and the arteries ligated. The wounds of the thighs were closed in layers.

Intravenous Extracorporeal Cross-Transfusion.—A procedure similar to the one described above was used in establishing cross-circulation between the inferior vena cavae. A 20 gauge needle with attached equipment was inserted into the inferior vena cava of each animal just inferior to the renal veins. Following completion of the cross-transfusion, amounting to 100 to 120 ml. in each animal of ten pairs, the needles were withdrawn and bleeding controlled by momentary pressure. The abdominal wounds were closed in layers.

Seven to 10 days after the exchange transfusions, the animals were cross-grafted as follows. Cuboid masses (2.5 x 1.5 x 0.5 cm.) of erector spinae muscle with attached fascia were resected from the lumbar regions and cross-transplanted. Two weeks later, the animals were sacrificed by injection of a 1 per cent procaine solution into the cisterna magna. Complete autopsies were performed. The skin was removed from the back and the tissues fixed in 10 per cent formalin solution. Following fixation, the grafts and regional tissues were cut into serial blocks for microscopic study. The sections were stained with hematoxylin and eosin.

RESULTS

The gross appearance of cross-homografts at 2 weeks of age indicated that they were well healed in place. The bursal space which customarily develops over this type of transplant formed as usual. The fascia of the transplant was

abnormally white with some reddish streaks located in the thin transparent pannus which had grown over the fascia. These grafts were similar in appearance to those noted previously in postparabiotic animals (2).

Microscopic study of each cross-homograft showed it to be well healed in place. The pannus which had grown over the fascia of the graft consisted mostly of collagen containing a few vascular channels. These vessels frequently penetrated the fascia and joined large patent vascular channels which filled in the region between the fascia and muscle of the graft. Inflammatory aspects of the classical homologous incompatibility reaction were absent. Though vascularization was rich, fibroblastic proliferation and collagen deposition in the musculofascial zone were inconspicuous (Figs. 4, 6).

A study of the other organs and tissues disclosed no significant changes.

DISCUSSION

Transplants of most adult normal tissues of mammals fail to survive when implanted into another unrelated adult host of the same strain and species. There is evidence that mechanisms of immunity are responsible for the deterioration of the transplants but the nature of the mechanisms remains obscure. Inquiry into this problem by microscopic study has led to characterization of different patterns of host-graft interactions which occur under various experimental conditions. The use of deep musculofascial transplants rather than superficial grafts has aided in standardization of this study by provision of aseptic conditions in a uniform protected environment (3). The autologous musculofascial transplant, 2 weeks after implantation, showed a consistent pattern of degeneration and organization. The transplant was well healed in place. A well developed pannus of vascularized granulation tissue arising from the host had grown over the fascia of the transplant. From this pannus numerous vascular channels penetrated the fascia to terminate in the broad musculofascial zone created by progressive absorption of the muscle of the transplant. This zone acquired a richly vascular network imbedded in a matrix of proliferating fibroblasts and newly formed collagen. Curious multinucleated striated cells indicative of proliferative tendencies by muscle of the transplant were frequently observed in this zone. These sequences of healing proceeded to completion without significant inflammation. The lateral and inferior aspects of the transplant showed similar sequences of degeneration and organization. We have assumed that this host-autograft interaction characterized by prompt non-inflammatory stromal organization and absorption within the musculofascial zone represents a state of mutual compatibility of the tissues of the host and transplant.

Homologous musculofascial transplants in animals which had not been grafted before underwent similar sequences of degeneration and organization except for superposition of inflammation, impaired fibroblastic proliferation,

and reduced collagen deposition within the musculofascial zone (Figs. 1, 2). Angiitis involving newly formed vessels arising in the host and penetrating the grafts was a conspicuous feature of this classical host-homograft reaction. Autolysis and resorption of the muscular component of homologous transplants appeared to proceed at the same rate as in comparable autologous transplants. This host-homograft interaction was defined as one showing prompt vascularized stromal organization complicated by gradual development of a characteristic type of inflammation (3).

Previous transplantation of musculofascial tissues from the same donor to the same recipient resulted in deviation from the pattern of classical host-homograft interactions (3). This consisted of an acute necrotizing reaction involving particularly the musculofascial zone of the transplant. Fibrinous thrombi in newly formed vessels and widespread hemorrhages were conspicuous. Fibroblastic proliferation and collagen deposition were more seriously impaired than in the classical host-homograft interaction due to an initial graft. Proliferation of cells of the transplant was negligible. Autolytic and resorptive mechanisms, however, were active. This altered pattern of stromal organization of homologous musculofascial transplants was interpreted as a specific type of reaction excited by repeated free grafting of the same type of tissue from the same donor to the same recipient. It was designated as an acute, sensitized, host-homograft interaction.

The sequences of healing at surgical planes of anastomosis between parabionts were comparable in many ways to those occurring in musculofascial grafts (1). Initially, healing proceeded normally between the parabiotic twins. Dermal epithelial surfaces became continuous. Capillary connections were established between the parabionts. An abundant matrix of collagen and reticulum was mutually deposited to unite the apposed homologous tissues. As this occurred, an inflammatory reaction similar to that encountered in free grafts appeared and gradually increased in intensity. This reaction appeared to interfere with the cross-circulation so that within a few days blood no longer passed between the parabionts. The dermal surfaces became discontinuous, but the homologous tissues still remained firmly united owing to persistence of the collagenous matrix formed during parabiosis. This matrix remained "immune" to the effects of the inflammatory reaction as long as the homologous tissues remained apposed which in prolonged experiments was several months.

Further studies disclosed that if parabionts were once separated and again anastomosed to one another the second anastomosis failed to heal. Seldom did these animals develop a second cross-circulation. Spontaneous separation of the anastomosis usually occurred by the 6th day. Microscopic studies of these second serial junctions were difficult to interpret because of infection accompanying poor healing and early separation. This modification of healing

between parabiotic twins at their second union seemed to correspond to the acute necrotizing "sensitized" reactions which characterized host-graft interactions in animals which had received previous transplants from the same donor.

In view of the change in the reactivity of parabionts toward one another on reanastomosis in the postparabiotic period, a possible change in their reactivity toward musculofascial cross-transplants during the postparabiotic period was postulated. Appropriate studies in this connection disclosed that two modifications of the standard host-homograft interaction occurred. The first consisted in the development of a peculiar angiomatous pattern of vascularization through and beneath the fascia of the graft (Figs. 3, 5). Inflammation was conspicuously absent. Collagen deposition and fibroblastic proliferation were negligible. Autolytic and resorptive mechanisms, normally active in the musculofascial zone of grafts, were suppressed despite the abundance of newly formed vascular channels in this region. The second modification consisted of a collagenous encapsulation of the transplant without penetration by vessels arising from the host. This host-homograft interaction resembled the one occurring in homografts treated *in vitro* to extremes of temperature and x-irradiation prior to implantation (4).

The present studies involved exchange of sufficient blood between pairs of rabbits so that about 40 per cent of each animal's final blood volume was foreign blood.[1] Subsequent cross-grafting led to a host-graft interaction identical with the first type of interaction in cross-grafts in parabiotic twins during the postparabiotic period (Figs. 4, 6). The reaction was reproducible, consistent, and presumably independent of differences in blood groups of rabbits, for animals were paired at random for cross-transfusions (5). Following exchanges of the large volumes of blood there was excessive endothelial proliferation with formation of large vascular channels in organization of the graft. There is reason to suppose that the vascularizing stimulus of the transplant to the stroma of the host may be unrelated to the proliferative activity of cells of the transplant. Furthermore, the evidence indicates that the stimulus to fibroblastic–collagenous repair may differ from the stimulus to formation of vascular channels in repair.

One of the first curious effects of cross-circulation was reported by Lillie to be due to hormonal influence between bovine twins of the opposite sex with a cross-circulation *in utero*. Under these conditions the female twin showed abnormalities in development of the reproductive tract (6). More recently Owen studied the consequences of intrauterine vascular anastomosis between bovine twins and concluded that a heifer whose blood type is the same as that of her twin brother will probably be a freemartin (7). Further work by

[1] In more recent experiments, positive results have followed the presence of 1 to 5 per cent foreign blood in each of the pair of cross-transfused animals.

Owen *et al.* has shown that intermingling of fetal circulation can cause changes in the hematopoietic tissues so that one twin will produce cells of the other twin for the rest of its life (8). The presence of two blood types, presumably due to fetal cross-circulation between twins, has since been reported in man by Baron and by Dunsford *et al.* (9, 10). Studies by Anderson *et al.* of skin grafts between hosts which have cross-circulated *in utero* showed that most dizygotic twins were tolerant of one another's skin grafts. It was suggested that this tolerance had the same origin as their conformity of blood types (11).

The effect of injecting adult blood into an adult host has been investigated in relation to grafts of tumor and normal tissues. Barrett working with mammary carcinomas in mice found that resistance to these tumors could be induced in susceptible mice by subcutaneous injections of defibrinated blood "homologous to the tumor." The degree of resistance appeared to vary with the genetic relationships of the host, the tumor, and the donor of the blood (12). On the other hand, Medawar investigating skin grafts found that the intradermal injection of homologous leukocytes conferred typical immunity toward skin subsequently grafted from the donor of the leukocytes. The immunizing effect of leukocytes was at least 18 times more effective when given intradermally rather than intravenously (13).

The relation between the data recorded in the literature and our findings is not yet clear. Suffice it to say that, if reactions involving homografts are based on current concepts of immunology, we are at a loss to explain our findings without recourse to unjustified speculation.

CONCLUSIONS

Under ordinary conditions musculofascial cross-grafts made between pairs of rabbits of the same strain and species elicited classical host-homograft tissue interactions. When the cross-grafting was done 7 to 10 days after exchange transfusions leading to introduction of about 40 per cent of foreign blood, the classical host-homograft reaction failed to develop. In its stead there was an harmonious interaction characterized by abundant vascularization of each graft, with minimal stromal replacement and without a trace of inflammation. This reaction resembled a common type previously described in cross-grafts made between postparabiotic twins but lacked some conspicuous features of the reaction of an animal to grafts of its own tissues.

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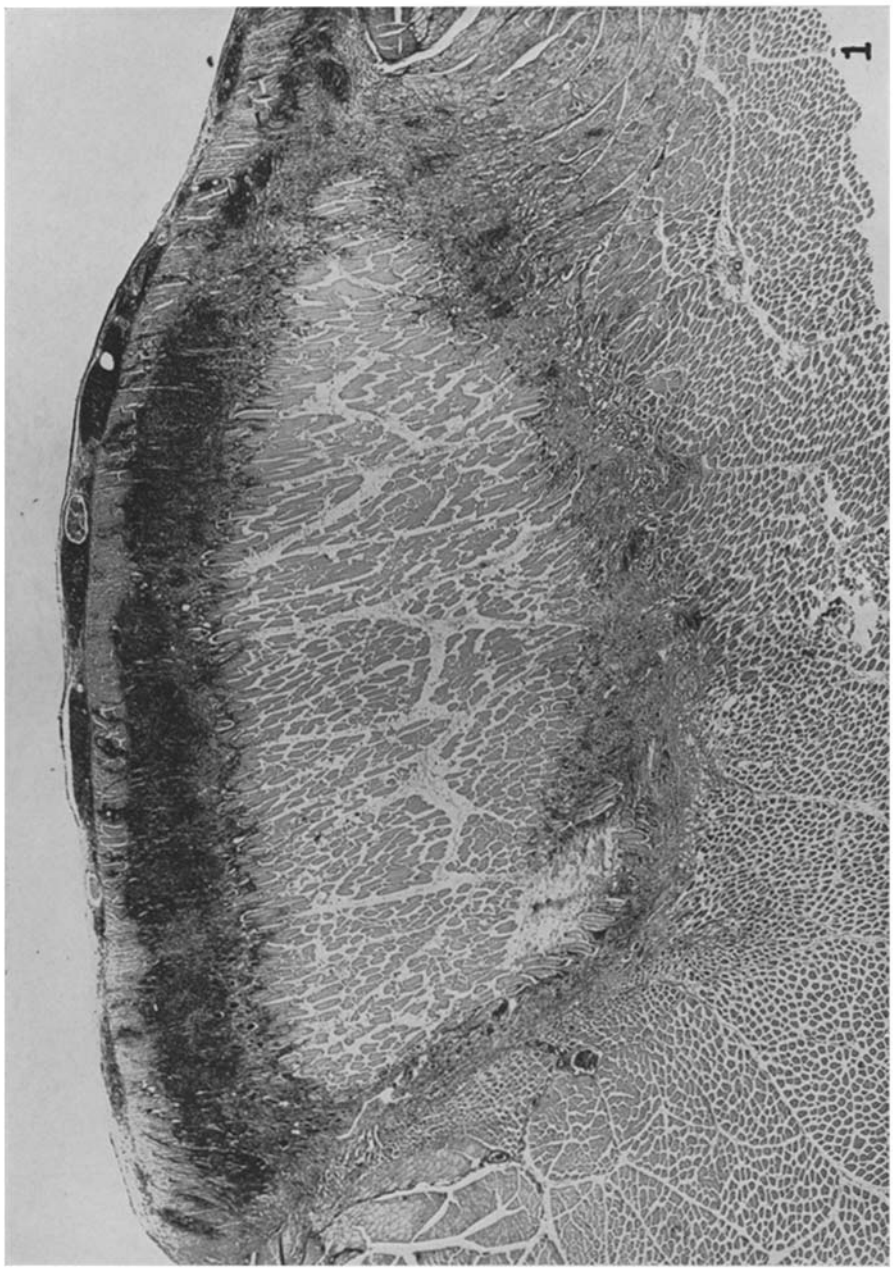
EXPLANATION OF PLATES

PLATE 3

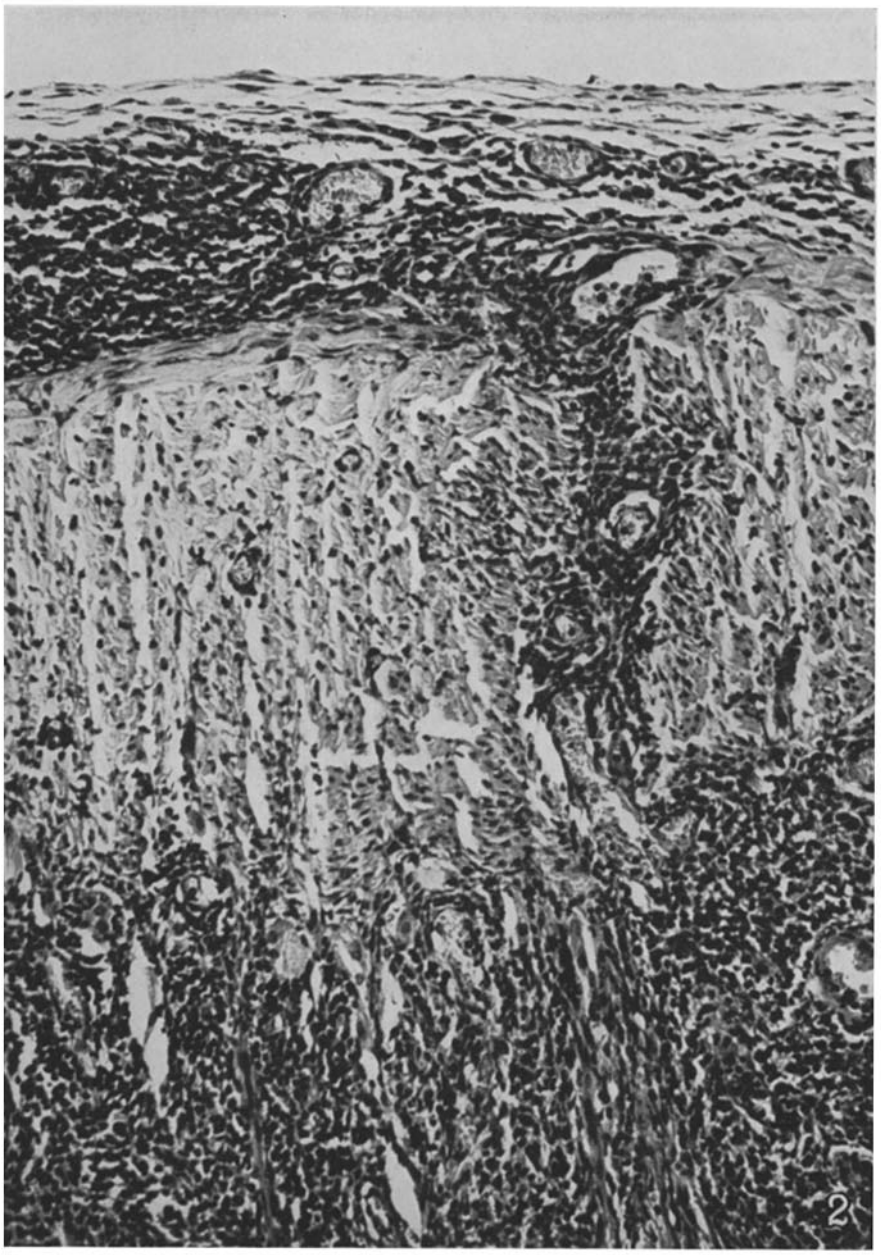
FIG. 1. This is a low power photomicrograph of an homologous transplant, 2 weeks of age. The empty space above the tissue is the bursa which forms regularly over musculofascial transplants. The pannus which forms the floor of the bursal space contains dark areas which are foci of lymphoid cells originating from the recipient. The fascia of the transplant is well supplied with vessels which have grown down from the overlying pannus of the host's granulation tissue. Beneath the fascia is the musculofascial zone, appearing darkly stained because of the massive accumulation of lymphocytes. The muscle of the transplant is undergoing degeneration and absorption at the margins in a way similar to that occurring in autologous transplants. The lateral and inferior aspect of the transplant bed contains vascularized granulation tissue arising from the host and replacing regions vacated by the absorbing muscle of the transplant. **Hematoxylin and eosin stain. × 20.**



(Andresen *et al.*: Host-homograft tissue interactions)

PLATE 4

FIG. 2. This is a medium power photomicrograph of a part of the homologous musculofascial transplant shown in Fig. 1. The space above the tissue is the bursa. The floor of the bursa is formed by host's granulation tissue which is well vascularized and infiltrated with lymphocytes. Vessels from the pannus have grown down through the fascia of the transplant to terminate in the broad musculofascial zone. This zone occupies the lower one-third of the illustration and contains degenerating and proliferating muscle cells between which are dense infiltrations of lymphoid cells and a delicate vascularized matrix of collagen. Hematoxylin and eosin stain. × 325.



(Andresen *et al.*: Host-homograft tissue interactions)

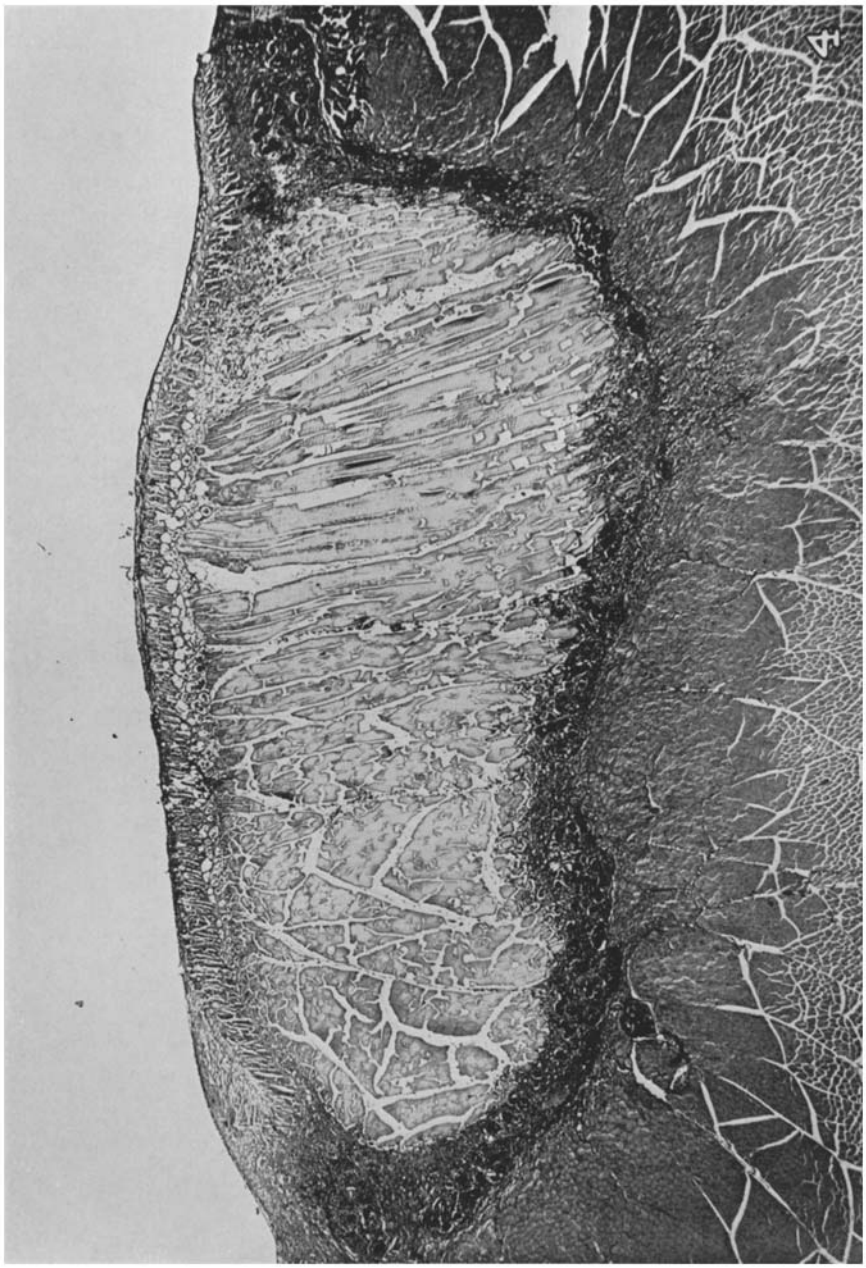
PLATE 5

FIG. 3. This is a low power photomicrograph of an homologous musculofascial graft, 2 weeks of age, transplanted from one postparabiont to its twin. The empty space above the tissue is the bursal space which has formed as usual. The floor of the bursal space is formed by granulation tissues of the host which have grown over the fascia of the transplant. The fascia contains large vascular channels which terminate just beneath the fascia of the transplant. Degeneration and resorption of muscle are retarded beneath the fascia. The lateral and inferior margins of the transplant bed contain newly formed vascularized granulation tissue arising from the recipient. There is no inflammation. Hematoxylin and eosin stain. × 20.

(Andresen *et al.*: Host-homograft tissue interactions)

PLATE 6

FIG. 4. This is a low power photomicrograph of a musculofascial cross-graft, 2 weeks of age, transplanted from one animal to its partner that has undergone an exchange transfusion. The empty space above the tissue is a bursa which is similar to those formed adjacent to all types of musculofascial transplants. The pannus arising from the host is well developed and firmly attached to the subjacent fascia of the transplant. The fascia contains large new vascular channels which terminate in an angiomatous plexus just beneath the fascia in the same pattern noted in postparabiotic homologous cross-transplants (see Fig. 3). Degeneration and absorption are delayed. The junction between the margins of the transplant and the transected recipient's tissues contains vascularized granulation tissue which shows focal dark areas due to the pathologic calcification of skeletal muscle fibers. Hematoxylin and eosin stain. × 20.

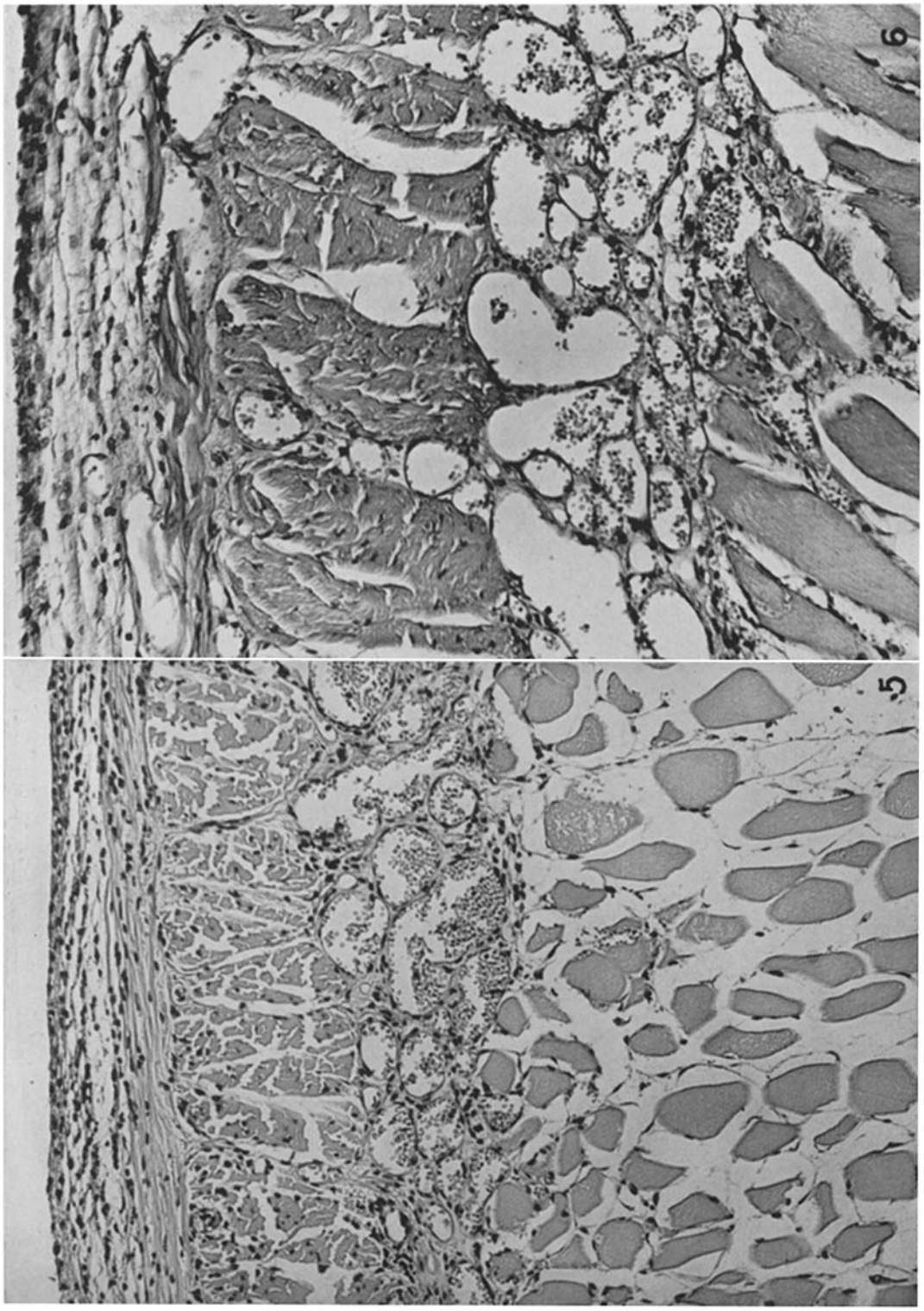


(Andresen *et al.*: Host-homograft tissue interactions)

PLATE 7

FIG. 5. This is a medium power photomicrograph of a part of the postparabiotic homologous cross-transplant shown in Fig. 3. The empty space above the tissues is the bursa. A well formed pannus of granulation tissue arising from the host is firmly adherent to the fascia of the transplant. The fascia is free from inflammation and contains new vascular channels which terminate in a peculiar new angiomatous network of vessels growing within the narrow musculofascial zone. Normal erythrocytes from the host fill these channels. Proliferative skeletal muscle is absent and degenerative changes in muscle of the transplant are negligible. The customary inflammatory and destructive effects of the classical homologous incompatibility tissue interaction illustrated in Figs. 1 and 2 are conspicuously absent. Hematoxylin and eosin stain. × 275.

FIG. 6. This is a medium power photomicrograph of a part of the posttransfusion homologous cross-transplant shown in Fig. 4. The pannus is well developed and contains new vascular chanels which penetrate the fascia in perpendicular fashion to terminate in a new angiomatous plexus beneath the fascia of the graft. The vascular channels contain blood from the host. The skeletal muscle of the graft at the lower margin of the illustration shows minimal degeneration and absorption and there is minimal fibroblastic-collagenous repair in the graft in spite of the richness of blood supply. The classical host-homograft reaction illustrated in Figs. 1 and 2 is absent. There are no apparent differences between the postparabiotic homologous cross-transplant reaction and the posttransfusion homologous cross-transplant reaction. Compare Figs. 5 and 6. Hematoxylin and eosin stain. × 275.



(Andresen *et al.*: Host-homograft tissue interactions)