POSTPARABIOTIC TISSUE REACTIONS OF RABBITS TO MUSCULOFASCIAL CROSS-TRANSPLANTS*

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PLATES 9 TO 14

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Previous studies of rabbits of the same species and strain united in parabiosis by the ears revealed an initial mutual participation by the parabionts in a normal process of healing by which their tissues were united along the plane of surgical anastomosis. In 3 to 4 days following anastomosis, there was anatomical and physiological proof of cross-circulation. The epithelial surfaces of the parabionts established continuity and collagenous fibrils were deposited in such a way as to hold the homologous tissues together. In 7 to 10 days following anastomosis a characteristic inflammatory reaction which appeared to interfere with the cross-circulation and continuity of the dermal epithelium developed along the line of healing. Despite this interference the ears remained healed together throughout the experimental period which at times was as long as 5 months. Apparently, the mutually deposited collagenous matrix was resistant to the ischemia, inflammation, and other features of the incompatibility reaction which persisted as long as the ears remained in apposition (1).

Further studies disclosed that if parabionts were separated following a period of parabiosis and then surgically united a second time, cross-circulation was rarely established and the ears usually spontaneously separated along the plane of the second anastomosis. This observation raised a question as to the nature of the change in the parabionts which was manifest by a difference in the tissue reactions at the site of the first and second serial parabiotic anastomoses.

It seemed reasonable to assume that the nature of this change might be further analyzed by a study of the reaction of post-parabiotic twins to cross-transplants in an aseptic intramuscular environment where the various tissue reactions might be interpreted more accurately. With this in mind, the following experiment was carried out.

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Methods

Twenty-five pairs of adult male New Zealand white rabbits of the same strain were joined in parabiosis by uniting their ears (2). The period of parabiosis was 13 to 15 days. Circulatory studies were done on the 4th day by injecting phenolsulfonphthalein or Evans blue dye as previously described. 10 to 26 days following amputation of the ears which had been healed together, thirteen of the twenty-five pairs were subjected to a second period of parabiosis by surgical anastomosis of the remaining ears of the respective partners. Circulatory studies were repeated 4 days after the second anastomosis. The second period of parabiosis varied from 5 to 15 days in length with an average of about 12 days, though most anastomoses began to separate spontaneously in 5 to 7 days. The second aural junctions were prepared for microscopic study after amputation of the ears at the bases and fixing them in a 10 per cent aqueous solution of 40 per cent aqueous formaldehyde (U.S.P.).

At postparabiotic intervals varying from 6 to 90 days, cuboid masses (2 x 1.5 x 0.5 cm.) of erector spinae muscle with attached overlying fascia were resected and transplanted between separated parabionts in the places from which tissues for grafting were removed (3). 2 weeks following transplantation the animals were sacrificed by intracisternal injection of 1 per cent procaine solution and complete autopsies performed. Following fixation of tissues appropriate sections of the transplants and organs were prepared for microscopic study.

RESULTS

Previous studies of autologous and homologous musculofascial transplants in rabbits, not subjected to parabiosis, disclosed a characteristic pattern of degeneration and organization for each type of transplant (3).

Gross observation at 2 weeks of age showed that autologous and homologous musculofascial transplants were both well healed in place. Bursal spaces were formed over the transplants and a vascularized pannus of the recipient's connective tissue grew over the fascia of each transplant.

Microscopic examination of autologous musculofascial transplants at 2 weeks of age revealed a consistent pattern or organization (Figs. 1, 3). A pannus, rich in vascular channels derived from the host, grew over and became fused with the fascia of the transplant. Vessels arising in the pannus penetrated the fascia of the graft to end in the broad subfascial zone created by progressive absorption of skeletal muscle of the transplant in this area. This zone thus soon acquired a plexus of small vascular channels supported by proliferating fibroblasts and a newly formed rich collagenous matrix. Inflammatory cells were sparse but curious multinucleated immature striated cells appeared in large numbers within the subfascial zone and thereafter gradually disappeared.

Microscopic examination of homologous musculofascial transplants, 2 weeks of age, in rabbits not subjected to parabiosis showed sequences of healing similar to those encountered in autografts with the exception of the superposition of lymphocytic infiltration and a peculiar angiitis (Figs. 2, 4). The latter involved vessels in the subfascial zone as well as those in the fascia, pannus, and adjacent bursal wall. The subfascial zone showed impaired collagen deposition. Multinucleated striated immature cells were absent or inconspicuous. This interference with the normal healing process by homologous host-graft interactions was regularly sufficient to permit us to distinguish between autologous and homologous musculofascial transplants in rabbits not subjected to parabiosis.

In the present study, gross examination of postparabiotic homologous musculofascial transplants, 2 weeks of age, revealed that they were well healed in place. The bursal space was formed as usual. However, the transplanted fascia varied in color from a pearly white to a streaked pinkish red indicating the gradations from an avascular to a richly vascularized pannus of host's tissues overlying the fascia.

Microscopic examination of parabiotic homologous musculofascial cross-transplants, 2 weeks of age, in separated parabionts showed two types of tissue reaction. The first type of reaction in separated parabionts was characterized by an avascular organization. (Fig. 6). The transplant was well healed in place with evidence of delayed absorption. The fascia, though normally cellular, was avascular and covered by a pannus of mesenchyme derived from the host but

TABLE I

Circulation Studies on the 4th Day of Parabiosis

This shows the effect of a second parabiotic union between respective partners upon the cross-circulation. The failure to establish a cross-circulation following the second parabiotic union is a characteristic feature of apposed homologous aural tissues of rabbits.

Parabiosis	No. of pairs	Method for determining cross-circulation			
		IV		IV	
		PSP	Result	Evans blue	Result
First period (14 days)	11	6 2	Pos. Neg.	3	Pos.
Second period (5–12 days later)	12	1	Pos.	11	Neg.

devoid of vascular channels. (Fig. 8). The skeletal muscle of the graft remained in close approximation with the fascia of the transplant so that absorption of the muscle occurred principally at the lateral and inferior aspects of the transplant. There was no significant inflammatory cell infiltration though small foci of plasma cells were frequently situated adjacent to the graft within the intact skeletal muscle of the host.

The second type of reaction of separated parabiotic twins to parabiotic homologous musculofascial cross-transplants consisted of a pattern of reaction which resembled the first type except for the development of an angiomatous network of dilated thin walled vessels situated between the fascia and the attached skeletal muscle of the transplant (Figs. 5, 7). Collagen formation, fibroblastic proliferation, and inflammatory cell infiltration were inconspicuous or absent in spite of the frequent richness of vascularization.

Both types of postparabiotic host-graft interactions were about equally distributed between the group of parabionts with a single period of parabiosis and the group subjected to a second period of parabiosis. Both types of reaction

were found in parabionts which had been separated for as long as 90 days prior to cross-transplantation of the musculofascial grafts.

Circulatory studies revealed failure of establishment of continuity of circulation between parabionts during the second period of parabiosis (Table I).

Complete autopsy studies of animals exposed for the second time to parabiosis failed to reveal gross or microscopic systemic changes attributable to parabiosis. Microscopic examination of the surgical junctions made to insure the second period of parabiosis showed imperfect healing along the plane of aural anastomosis. Interpretation of microscopic changes at the junctions was difficult because of the high incidence of sepsis due to spontaneous separation of the planes of surgical anastomosis. Vascular disease was not conspicuous. Temporary collagenous union occurred and tended to persist longest at points where there was minimal vascularity of the apposed tissues of the two animals.

DISCUSSION

It has long been suspected on account of the curious manifestations of "parabiotic intoxication" that surgical union of tissues of two animals may produce serious obscure systemic effects. Attempts have been made to associate these effects with deleterious immunizing reactions which have served as useful sources for ideas intended to explain various features of homologous host-graft interactions (5). However, it is generally conceded that if mechanisms of immunity are implicated in these reactions, neither the nature of the mechanisms nor the composition of the theoretical antigens and antibodies have been disclosed.

In a succession of experiments intended to explore this subject at the level of microscopic characterization of host-graft interactions, the following facts became apparent. There is a characteristic form of organization and resolution of autologous grafts of at least one standard type in a standard location. This same pattern of organization and resolution of autografts also forms the basis for host-graft interactions when homografts of the same standard type are implanted in the same standard location. In dealing with musculofascial grafts, the host-homograft interaction is complicated by the superposition of certain characteristic inflammatory changes which may be defined microscopically as distinctive features of the basic homologous incompatibility reaction. This reaction is subject to modification in several ways. It can be converted from an indolent, semigranulomatous type of inflammatory process to a severe acute thromboangiitic reaction by successive transplants of the same type of tissue from the same donor to the same recipient. This has been designated as an acute sensitized homologous incompatibility reaction. Certain corollaries exist between the basic and acute sensitized host-homograft interactions of free grafting procedures and the interactions which follow the union of genetically unrelated tissues which have individual blood supplies. This is the situation when tissues

of whole animals of a genetically impure strain are grafted to one another. The basic host-graft interaction at the junction of the parabionts is much the same as in the free grafting procedures, except that a considerable amount of tissue, largely collagenous in character, is produced locally at the common junction. This newly formed tissue is somewhat unique because it permanently resists the local incompatibility reaction which successfully destroys all other tissues of the parabionts at the anastomotic junction. We regard this resistant tissue as a common product of the parabionts and though this product is not formed at a second plane of union between two parabiotic twins, it may be formed at a second plane of union between parabionts which have not been previously united to one another.

These observations, therefore, indicated that a period of parabiosis might be used as a preliminary procedure for the further investigation of host-graft interactions with due consideration for the interesting reports of others in dealing with renal homografts (6, 7). Kamrin and Kamrin showed that parts of kidneys exchanged between inbred rats while the animals were united in successful parabiosis appeared to become functionally integral parts of the recipient while comparable grafts between rats in unsuccessful parabiosis did not unite (6). Egdahl and Hume found that cross-circulation in dogs resulted in an immune state which led to an undesirable sensitized reaction against future kidney transplants. In one pair of dogs, however, there was some evidence that the renal homotransplant reaction was slightly delayed or inhibited after periodic cross-circulation of the animals for a total of 68 hours (7).

The host-graft interactions in our experiments were consistently modified in the postparabiotic period for as long as 90 days in all eight instances in which animals were studied at this prolonged interval. This modification was the same whether the animals had been subjected to either one period or two successive periods of parabiosis. The modification was regularly specific in relation to host-graft interactions involving cross-transplants between parabionts which had been united to one another. This modification was in the direction of a "null" reaction which had none of the characteristics of autologous, basic homologous, or sensitized homologous host-graft interactions. On the contrary, the host and the homograft seemed remarkably tolerant or perhaps indifferent to one another. This was displayed by the indolent mesenchymal encapsulation and the lack of vigorous vascularizing mesenchymal penetration and organization of the graft by the host's tissues. Vascularization which did occur was of a curious hemangiectatic plexiform type which differed greatly from the usual forms of vascularization of the grafts. (Compare Figs. 3, 4, 7.) The cells of the graft were even less active in a proliferative sense than those of the host, and autolytic mechanisms leading to resorption of muscle cells were greatly retarded. In some respects, the postparabiotic homograft reactions resembled those encountered in our current study of homografts in which the viability of cells

was reduced by treatment *in vitro* with x-irradiation or undue extremes of temperature prior to implantation. Thus, the attitude may be taken that the postparabiotic host-graft relation may be one of excessive intolerance leading to reduced viability of the graft long before the sequences of stromal organization of the graft by cells of the host are initiated by events occurring within the graft. However, the absence of inflammation and the frequent occurrence of vascularization of the graft without excitation of thromboangiitic reactions in the graft are strong arguments against this idea. Likewise, it is difficult to understand why the development of a state of excessive intolerance of the host should last as long as 90 days when the "sensitized" state to successive homografts of skin disappears within 60 days (4). For the time being, therefore, we have no explanation for the observations, but recognize their utility in a further analysis of mechanisms involved in host-homograft interactions which must be controlled if homografting is to become more practical in man.

SUMMARY

Twenty-five pairs of male rabbits of the same variety and strain were joined together in aural parabiosis for 13 to 15 days and then separated from one another. Thirteen pairs of the separated parabiotic twins were surgically united for a second period of parabiosis, less successful than the first due to deficient healing and spontaneous separation of tissue in 5 to 15 days at the site of anastomosis. Within 6 to 90 days after spontaneous or surgical separation of all parabionts, the postparabiotic twins which had been united to one another were cross-grafted with musculofascial transplants. 2 weeks later, the animals were sacrificed and microscopic studies made of the organs and sites of transplantation. These studies disclosed that either one or two periods of parabiosis resulted in the same persistent modification of classical host-homograft interactions. This modification was characterized by the replacement of the customary basic or sensitized homologous incompatibility reactions by a reaction more easily interpreted as one of host-graft tolerance or indifference. The intense inflammatory aspects of the usual host-homograft interactions were absent. Penetration of the musculofascial graft by vascularized mesenchyme derived from the host was retarded. When it occurred, the vascularizing reaction was abnormal and unaccompanied by the customary proliferative, resorptive, and repairative activities of admixed cells of the host and the graft.

These observations provide another means which may be useful in seeking conditions for increasing the probability of successful homografting between members of a genetically impure species.

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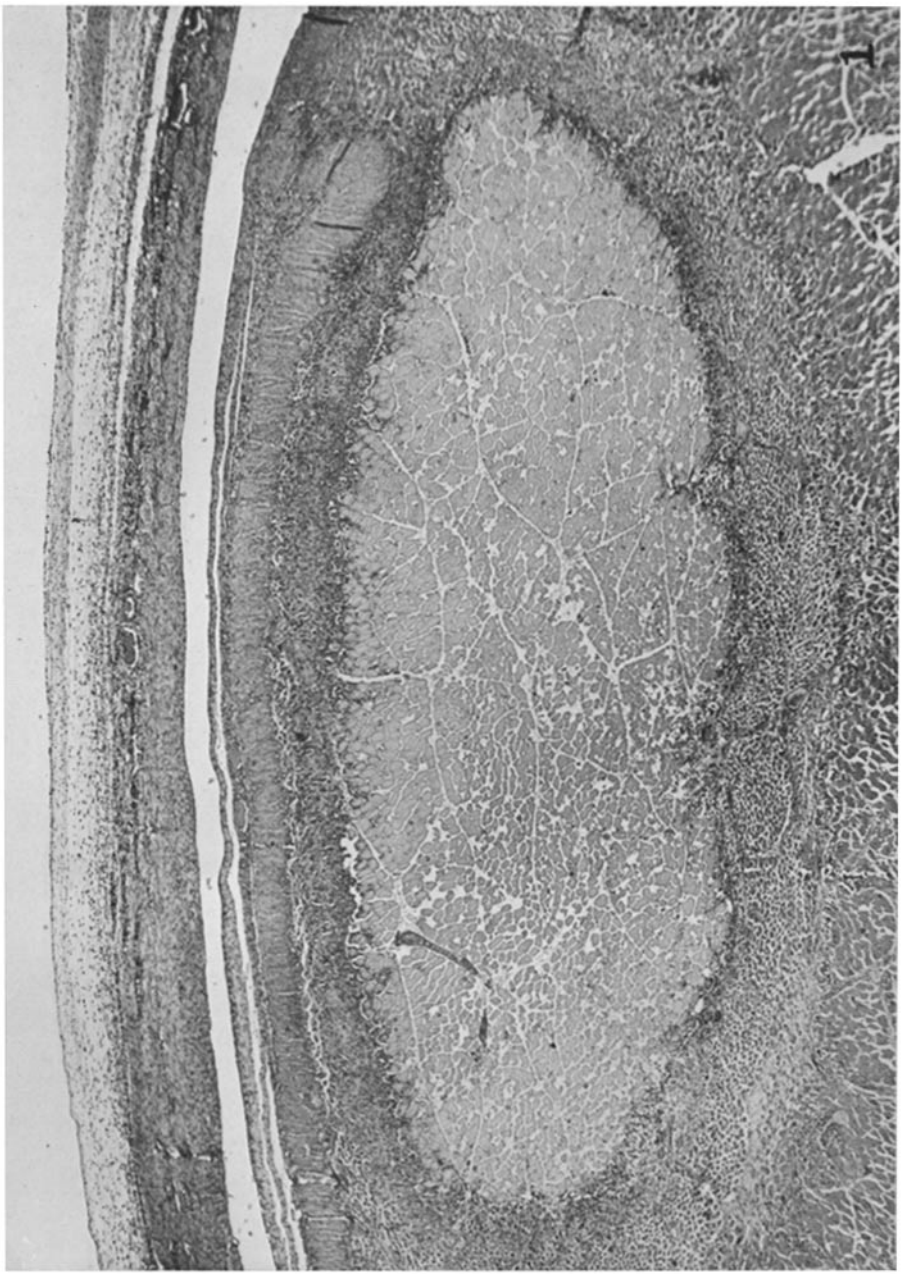
EXPLANATION OF PLATES

The microscopic sections were all stained with hematoxylin and eosin.

PLATE 9

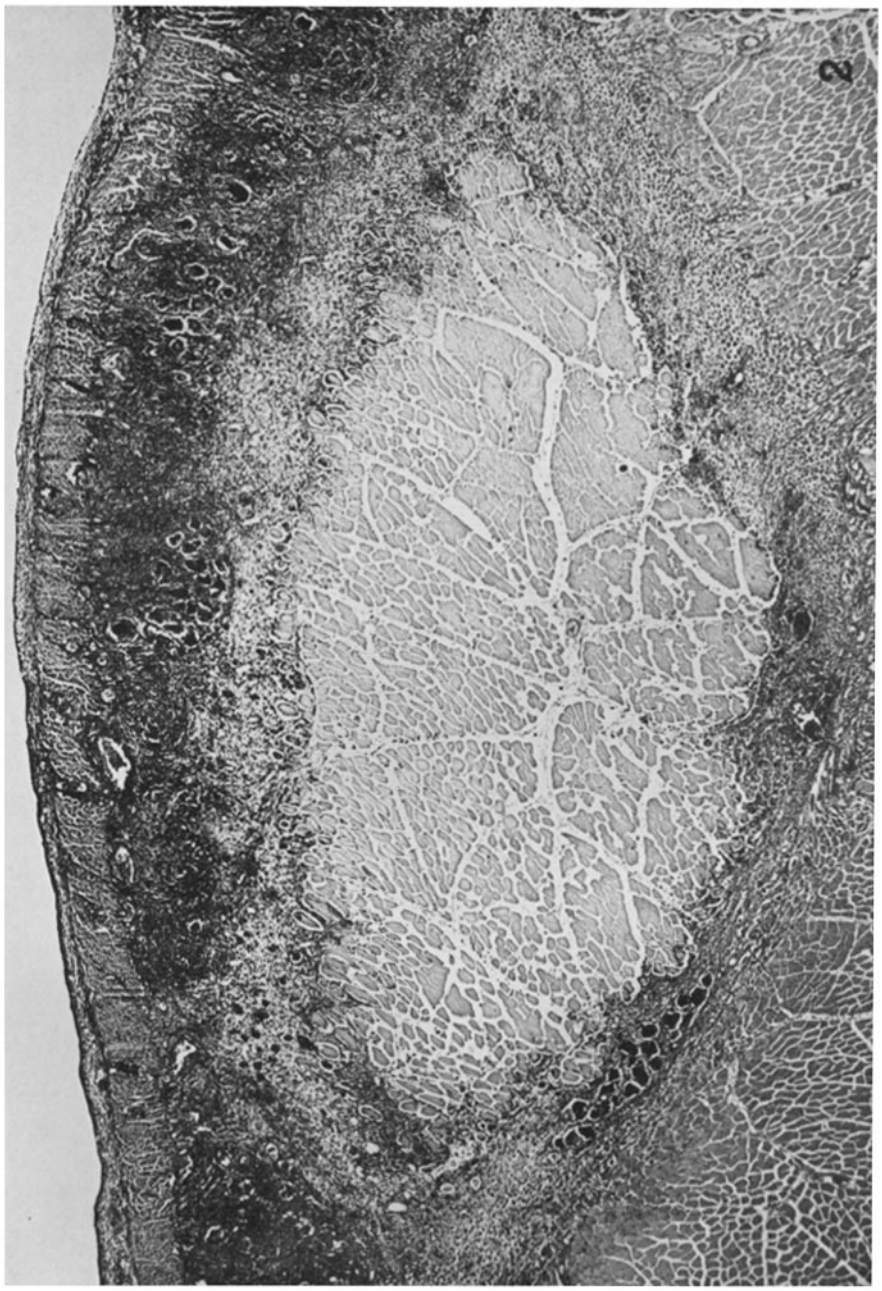
FIG. 1. This is a low power photomicrograph of an autologous musculofascial transplant, 2 weeks of age. The bursal space in the upper one-fourth of the illustration is located between the superficial fascia and the pannus. This pannus consists of a richly vascularized mesenchymal tissue which has grown over the fascia of the transplant. Beneath the fascia there is a broad zone consisting of proliferating connective tissues which have replaced the degenerating skeletal muscle bundles of the transplant. A similar pattern of vascularized connective tissue is replacing the zone vacated by the degenerating muscle of the lateral and inferior margins of the transplant. × 20



(Andresen *et al.*: Postparabiotic tissue reactions)

PLATE 10

FIG. 2. This is a low-power photomicrograph of an homologous musculofascial transplant, 2 weeks of age. The space above the tissue is the bursa which regularly formed over both types of transplants. The pannus is compact and is closely adhered to the fascia of the transplant. The musculofascial zone is now broad and is dark, owing mostly to the intense infiltration of lymphoid cells from the host. Note also areas of pathologic calcification of scattered skeletal muscle fibers of the transplant in the reaction zone. An identical process of degeneration and resorption of muscle is occurring at the margins of the transplant as is noted in Fig. 1, with the exception of varying amounts of lymphocytic infiltration. The angiitic reaction, however, was never as conspicuous or distinctive in the zones of repair about the periphery of the transplant as was encountered consistently within the musculofascial zone. × 20.

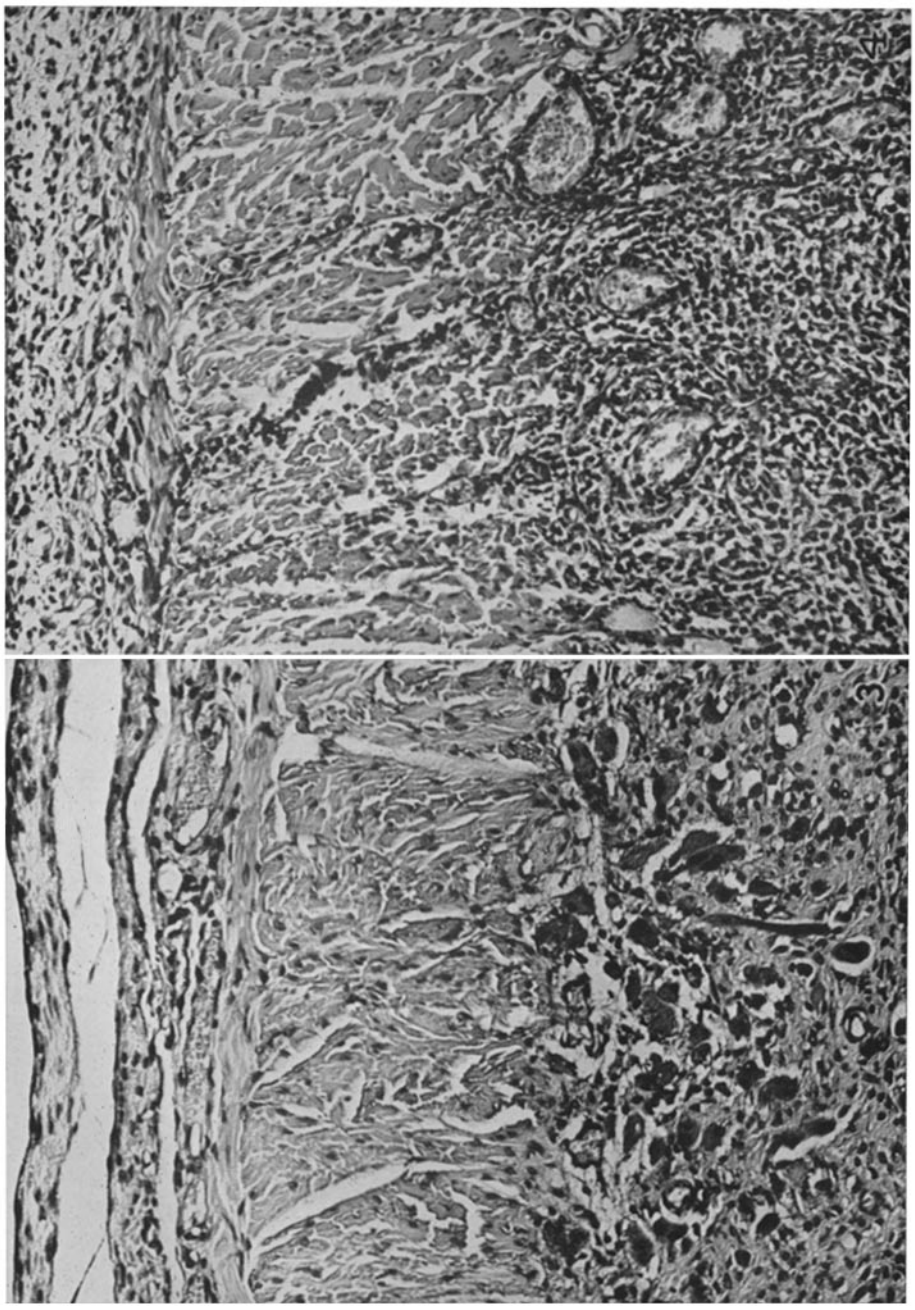


(Andresen *et al.*: Postparabiotic tissue reactions)

PLATE 11

FIG. 3. This is a medium power photomicrograph of a part of the autologous musculofascial transplant shown in Fig. 1. The empty space above the tissue is a portion of the bursal space overlying the transplant. The pannus consists of a delicate mesenchymal matrix which is penetrated by numerous newly formed vascular channels. These vessels have also permeated the fascia of the transplant to terminate in the musculofascial zone. This zone consists of absorbing and regenerating muscle of the transplant between which a delicate collagenous matrix is being deposited. Signs of inflammation are minimal. × 325.

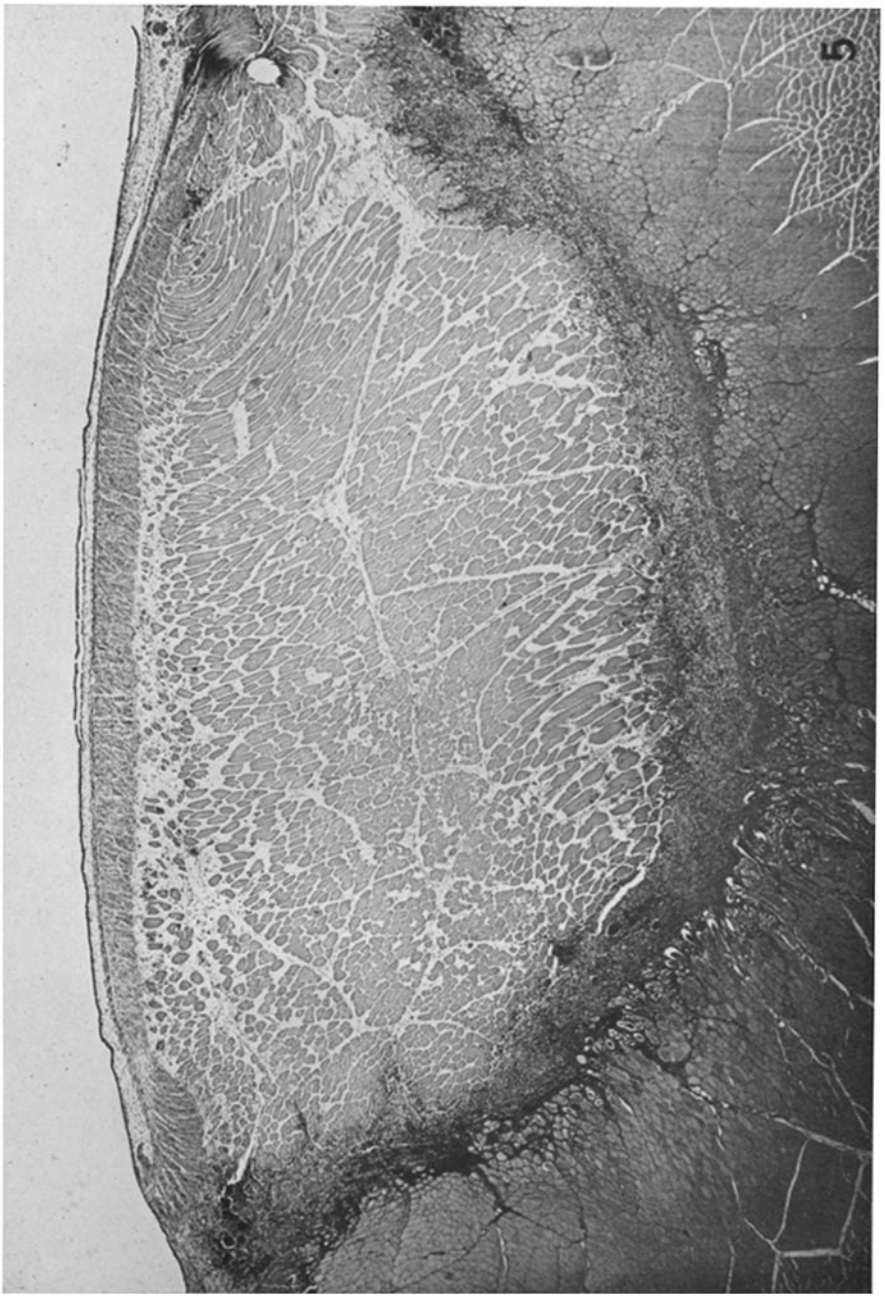
FIG. 4. This is a medium power photomicrograph of a part of the homologous musculofascial transplant shown in Fig. 2. Note the well developed pannus which is closely adherent to the fascia of the transplant. The fascia is penetrated by vascular channels which originate in the overlying pannus and terminate in the musculofascial zone below. This well developed zone shows minimal fibroblastic activity with impaired collagen deposition but an extensive lymphoid cell infiltration. Interference with the completion of the pattern of organization encountered in autografts (see Fig. 3) was characteristic of homologous host-graft interaction among the non-parabionts. × 325.



(Andresen *et al.*: Postparabiotic tissue reactions)

PLATE 12

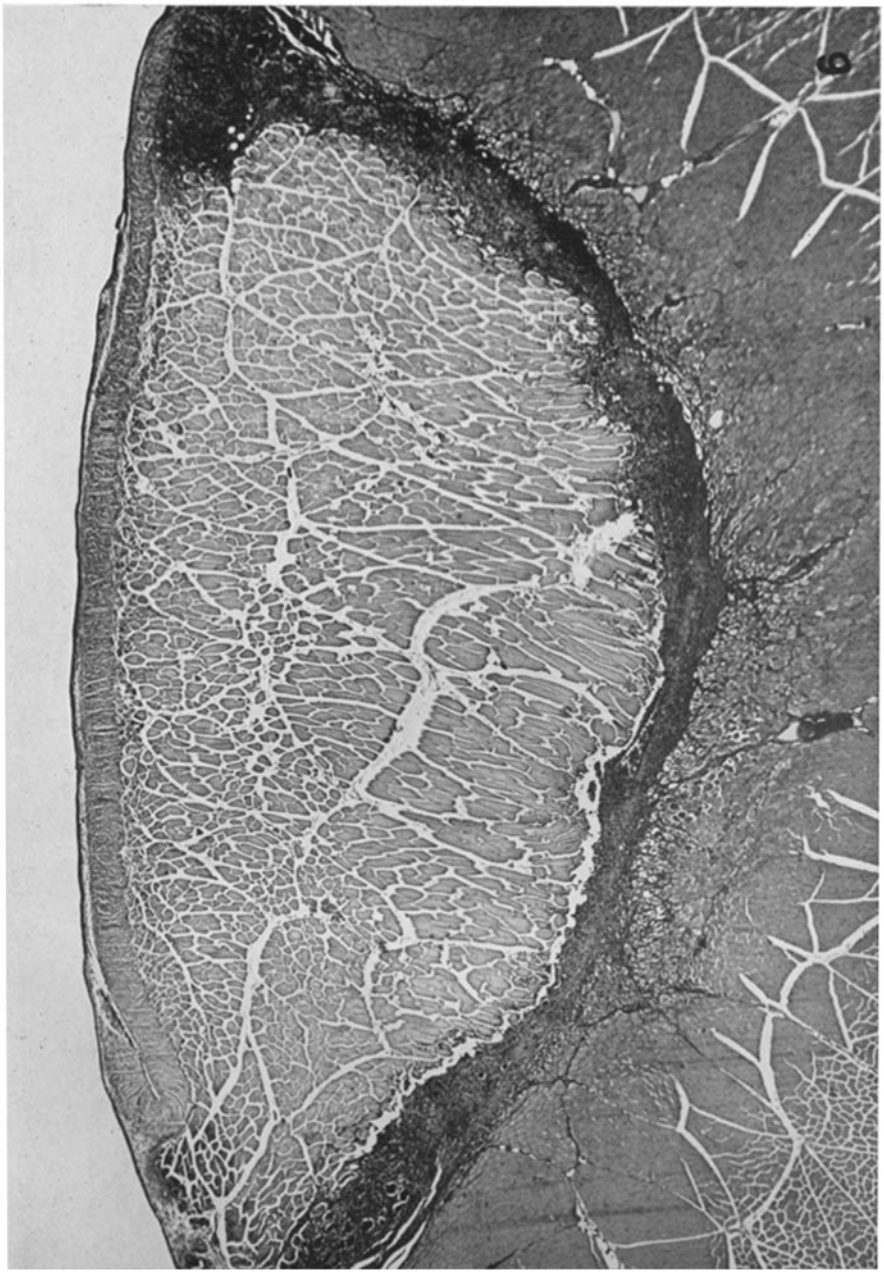
FIG. 5. This is a low power photomicrograph of a postparabiotic homologous cross-transplant, 2 weeks of age. The empty space above the transplant is the bursal sac. The floor of the bursal space consists of vascularized granulation tissue which remains closely adhered to the fascia of the transplant. The fascia is thickened and contains vascular channels which have originated from within the pannus. Beneath the fascia, there is a narrow lightly stained region which contains large vascular channels. The architecture of the skeletal muscle component is unaltered. At the periphery where the muscle of the transplant is in contact with the transected muscle of the host, vascularization has proceeded in granulation tissue arising from the transected recipient's tissue. This zone of repair contains slowly absorbing muscle bundles, patent vascular channels and scattered foci of plasma cells embedded in a collagenous matrix. Note the absence of the usual inflammatory and destructive homograft reactions. × 20.



(Andresen *et al.*: Postparabiotic tissue reactions)

PLATE 13

FIG. 6. This is a low power photomicrograph of a postparabiotic cross-transplant, 2 weeks of age. The empty space above the transplant is the bursal space which is usually encountered above all musculofascial transplants. The floor of this space consists of a thin delicate pannus of non-vascularized connective tissues derived from the host. The pannus is adhered to the fascia which is thickened and is also devoid of vascular channels. The muscle bundles remain closely approximated to the fascia. There is similar healing between tissues of the host and transplant in the bed of the transplant, as shown in Fig. 5. There is no trace of the customary homologous incompatibility reactions. × 20.

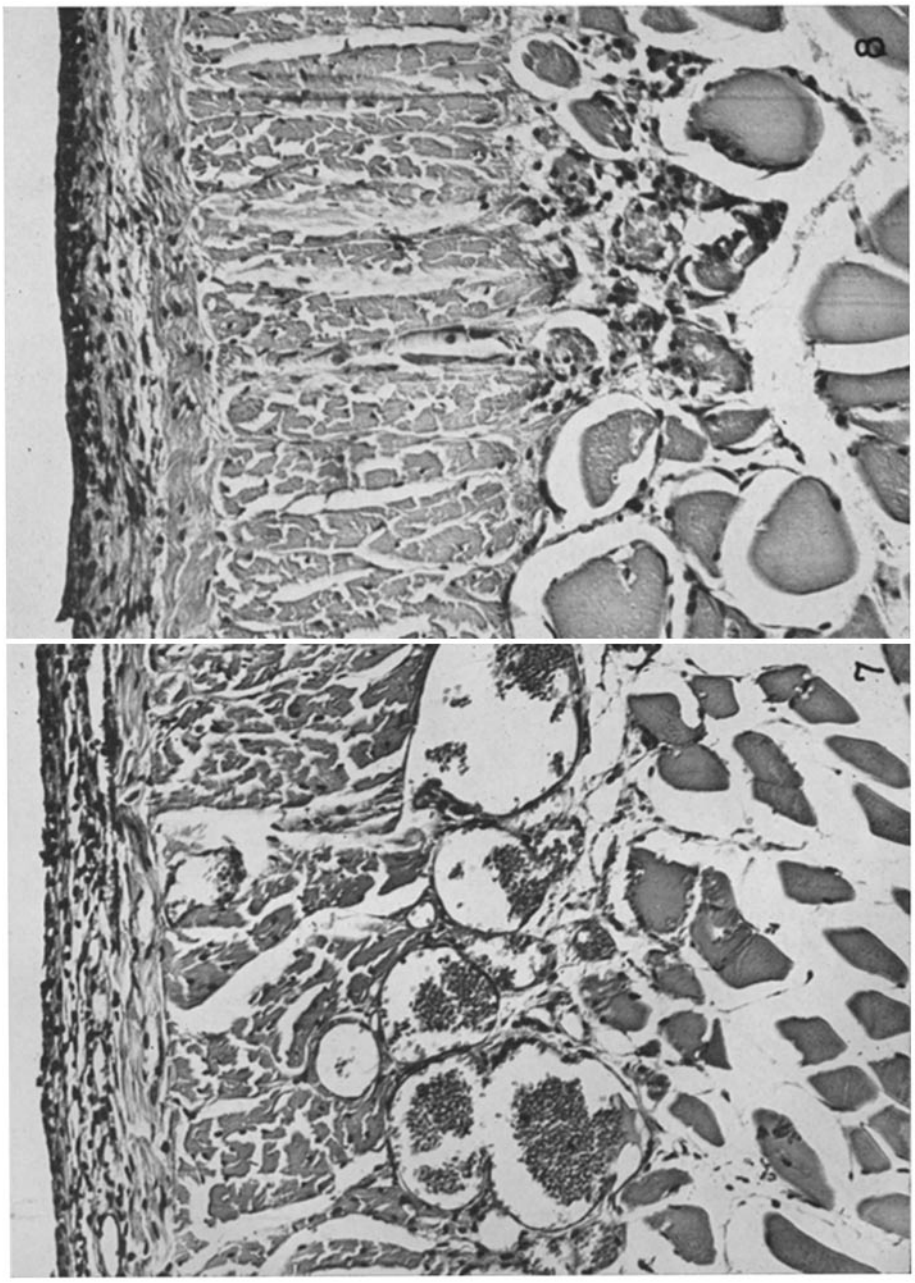


(Andresen *et al.*: Postparabiotic tissue reactions)

PLATE 14

FIG. 7. This is a medium power photomicrograph of a part of the postparabiotic homologous cross-transplant shown in Fig. 5. The empty space above the tissue is the bursal space. A well developed vascularized pannus containing rare plasma cells remains in good approximation to the fascia of the transplant. The fascia contains patent vascular channels which terminate in a peculiar angiomatous pattern within the musculofascial zone. In spite of this intense vascularization, the usual features of the homograft reaction have not developed. Also, degeneration and absorption of the skeletal muscle of the transplant are strikingly delayed. × 325.

FIG. 8. This is a medium power photomicrograph of part of the postparabiotic cross-transplant shown in Fig. 6. The transplant is covered by an avascular connective tissue pannus which has originated from the host. The pannus is fused with the fascia of the transplant. The fascia, though cellular, remains free of vascular channels. The musculofascial zone has failed to broaden and skeletal muscle bundles remain in almost their original relationship to the fascia. This represents the least active of the observed microscopic host-homograft interactions among the cross-transplants made between separated parabiotic twins. The period of parabiosis has eliminated the classical interactions. × 325.



(Andresen *et al.*: Postparabiotic tissue reactions)