THE PATTERN OF TISSUE REACTIONS TO AUTOLOGOUS AND HOMOLOGOUS MUSCULOFASCIAL TRANSPLANTS*

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

(From the Rush Departments of Pathology and Surgery, Presbyterian Hospital, in affiliation with the University of Illinois College of Medicine, Chicago)

PLATES 49 TO 53

(Received for publication, February 5, 1952)

It is generally accepted that homologous transplants of most normal tissues do not survive in rabbits when the transplants are in contact with the subcutaneous tissues of the recipient. The reason for this incompatibility has not been determined but there is evidence that mechanisms of immunity are involved and that these mechanisms are responsible for deterioration of the transplant (1). The present experiments were designed to inquire into this problem with three objectives in mind. First, we wanted to find whether it is possible by histologic methods to distinguish between autologous and homologous musculofascial transplants in rabbits. Second, we wanted to define the nature and locus of the differences, if any. Third, we wanted to determine whether successive transplants from the same donor to the same recipient would elicit systemic changes that might be reflected in modifications of a characteristic reaction to a single transplant.

Methods

Young male New Zealand white rabbits of the same strain were used. Hair was removed from the midline of the back and the skin prepared with strict aseptic technique. It was necessary to prepare three well separated operative fields in animals in which successive transplantations were done at weekly intervals. In other animals only one field was generally used. When three fields were required they were spaced at equal intervals along the midline of the back between the level of the apices of the scapulae and the crests of the ilia. Successive transplants were made in a cephalad-to-caudad direction. Single transplants were made in the middle field. In making the transplants, two animals were operated on simultaneously. Under local procaine dermal anesthesia a transverse incision was made over the vertebral column. The skin and superficial fascia were reflected to expose the erector spinae muscles. Incisions at a distance of 2 to 3 cm. to the right and left of the midline were made through the dorsal fascia and perpendicularly into the muscles. Cuboid masses of muscle with their attached overlying fascia undisturbed were then resected. These measured about 15 mm. in

* This study was supported by grants from the Otho S. A. Sprague Memorial Institute, The Surgical Section and National Heart Institute of the National Institutes of Health, and The Blind Service Association of Chicago.

length and breadth and 5 mm. in thickness. One block of muscle was transplanted to the opposite side in the site from which the second block of muscle had been removed. This was the control autologous transplant. The second piece of muscle was transplanted to the paired recipient animal. This was the homologous transplant. Transplants were sutured in place with four monofilament nylon sutures which drew the fascia of the transplant into close approximation with the muscle fascia of the recipient site. The dorsal fascia overlying the transplant and then the skin were closed with plain catgut and nylon sutures. A sterile dressing was then applied. This procedure involved, therefore, four simultaneous transplants, two being autologous and two homologous with one of each in paired animals.

This basic transplantation procedure was varied for control and experimental purposes. The animals were divided accordingly into four groups. Group I consisted of ten pairs of animals in which single autologous and crossed homologous transplants were done. These animals were sacrificed at the end of 2 weeks. Group II consisted of seven pairs of animals in which three successive autologous and crossed homologous transplants were made at weekly intervals. Of these, six pairs were sacrificed at the end of 3 weeks, so that the ages of the three paired transplants were 3, 2, and 1 week, respectively. One pair of animals was sacrificed at the end of 4 weeks so that the ages of the transplants were 4, 3, and 2 weeks, respectively. Group III consisted of three pairs of animals in which single crossed homologous transplants without simultaneous autologous transplants were done. These animals were sacrificed at the end of 2 weeks. Group IV consisted of four animals in which double autologous transplants without simultaneous homologous transplants were done. These animals were sacrificed at the end of 2 weeks.

After sacrificing each animal by injection of procaine into the cisterna magna, skin was reflected from the back and viscera examined through a ventral incision. The body was then immersed in 10 per cent formalin. After 4 days of fixation in formalin, the sites of transplantation were cut in serial thin blocks in a direction perpendicular to the muscle fascia. The serial blocks of tissue, including the residual transplant and all tissues adjacent to it, were prepared for routine microscopic study in sections stained with hematoxylin and eosin.

RESULTS

Gross examination of transplants after formalin fixation showed that they were firmly healed into the transplantation site by the end of the 1st week (Fig. 1). All space in the transplantation site was closed and there was no suppurative necrosis except in the presence of infection, which occurred at times. During the 2nd week, pallor of the muscle of the transplants increased and the total dimensions of the transplants decreased. Transplants remained fused with the regional tissues but sharp distinction between transplants and adjacent tissue was always apparent (Fig. 1). During the 3rd week, the muscle of transplants was rapidly absorbed so that at the end of this time only a small fraction of muscle persisted (Fig. 1). The disappearance of muscle was accompanied by increasing opacity of the muscle sheath, which often was slightly contracted, thickened, and wrinkled. Throughout this 3 week period of deterioration and resorption, no gross differences between homologous and autologous transplants of the same age could be detected. It was evident, however, that the optimum age at which transplants could be most suitably compared was 2 weeks. Too little of the transplant usually remained in recognizable form after the 3rd week.

Microscopic study showed that transplants were composed initially of a superficial layer of connective tissue, representing the sheath of the muscle, and a subjacent layer of voluntary muscle. The sheath of the muscle consisted of a superficial thin layer of collagen with fibrils arranged in the plane of the surface of the sheath. Beneath this, the sheath was composed of a relatively thick layer of collagen, arranged in primary and secondary bundles. The delicate fascicles of collagen around these bundles were continuous with the collagenous tissue surrounding the voluntary muscle fibers. The musculofascial junction, where muscle fibers lay adjacent to the sheath, showed no notable features. The voluntary muscle fibers at the periphery of all transplants, except in the musculofascial zone, had been transected and placed in apposition to transected recipient muscle fibers surrounding the transplantation site.

With the passage of time autologous and homologous transplants showed several similar sequences in degeneration and concomitant organization by tissue growing from the margin of the site of transplantation (Figs. 2, 3). The sheath of collagen overlying the degenerating muscle thickened as a result of retraction and swelling of collagenous fibrils. Initially, the sheath had no intrinsic vascular network but by the end of the 2nd week a rich vascular network had ordinarily developed. This network arose from the vessels in an overlying pannus of granulation tissue which grew centripetally from the margin of the transplantation site along the superficial aspect of the transplanted fascia (Figs. 2 to 4). Between this pannus and the overlying subcutaneous tissues of the host, a space resembling a bursa was formed (Fig. 3). The buds of capillaries arising from the vessels of the pannus grew perpendicularly through the muscle sheath at regular intervals along the delicate septa enclosing the primary bundles of collagen (Fig. 6). Upon reaching the musculofascial zone they branched into a capillary network which grew in volume and extent as the degenerating muscle in the musculofascial zone became replaced by immature fibroblastic tissue (Fig. 4). Muscle also underwent degeneration and resorption at the margins of the transplant. This was again a centripetal process with vascularized granulation tissue from the margins of the transplantation site occupying the zone vacated by resorption of degenerating muscle (Figs. 2, 3). Deep penetration of granulation tissue to the center of the degenerating transplant did not occur. The penetration seemed to await the resorption of degenerating muscle. This was equally true of the migration of significant numbers of inflammatory cells into the transplant (Figs. 2, 3).

These conditions of degeneration and repair created a well protected locus in which reactions between the vascularizing tissues of the host and degenerating or viable tissues of the transplant could be studied. This locus lay in the musculofascial zone, protected above by fascia and below by muscle of the transplant.

Three types of reaction in the musculofascial zone were distinguished in autologous transplants. The reaction (AI) consisted of one in which changes in the fascia were insignificant. The musculofascial zone was narrow. Early vascularization had occurred but fibroblastic proliferation and collagen deposition were minimal. In the musculofascial zone there were a few elongated multinucleated cells of immature appearance (Fig. 5). These had long intracytoplasmic fibrils but no well developed cross-striations. These cells at times seemed to extend from within the fascia into the musculofascial zone toward the margins of degenerating muscle fibers. We shall call these cells atypical myocytes without knowledge of their origin or significance. Along with these cells, a few infiltrating polymorphonuclear leukocytes were found but multinucleated giant cells of "myophagic" type, mononuclear leukocytes, and lymphocytes were absent or scarce.

The autologous type of reaction (AII) was characterized by mild penetration of the fascia by capillaries arising from the superficial pannus (Figs. 4, 6). These capillaries, following penetration of the fascia, branched and anastomosed throughout the abundant fibroblastic tissue in the musculofascial zone. Collagen was conspicuous between the fibroblasts (Figs. 4, 5). Atypical myocytes were numerous. Inflammatory cells, except for scattered eosinophils and occasional multinucleated "myophages" applied closely to degenerating muscle fibers, were inconspicuous.

The autologous type of reaction (AIII) was the most characteristic (Figs. 2, 4, 6). Here, the vascularized pannus over the fascia was well formed. From this, capillaries, and at times larger vascular channels, had grown directly through the fascia to vascularize the broad musculofascial zone (Figs. 4, 6). This zone consisted of scattered fibroblasts and many atypical myocytes imbedded in a dense collagenous matrix. A few lymphocytes and mononuclear leukocytes were usually found but signs of active inflammation were minimal.

The reactions encountered in homologous transplants were also divided into three types. The reaction (HI) was characterized by vasodilatation, edema, and polymorphonuclear cellular infiltration in the pannus overlying the fascia. The new vascular channels passing perpendicularly through the fascia into the musculofascial zone were dilated. The fascia was edematous (Fig. 7). Many fibrocytes of the fascia were degenerating. Polymorphonuclear leukocytes were numerous between the collagenous fibrils of the fascia. The musculofascial zone was narrow with a conspicuous network of dilated blood vessels (Fig. 7). Edema was marked. Fibroblasts were not actively proliferating. Collagen was difficult to distinguish clearly and polymorphonuclear leukocytes were numerous around blood vessels. Atypical myocytes, monocytes, and lymphocytes were usually absent. Multinucleated "myophages" adjacent to degenerating muscle cells were common. The most pronounced feature was the presence of end-angeitis with fibrinous thrombi in many vascular channels

throughout the pannus, the fascia, and the musculofascial zone (Fig. 8). This was associated with acute focal necrosis and hemorrhage.

The second form of reaction (HII) in homologous transplants was subacute in nature and less intense than the first type. Fewer vascular channels were thrombosed. Congestion and edema were less conspicuous. Polymorphonuclear leukocytes were few in number while eosinophils were abundant. Lymphocytes and monocytes were scattered about in moderate numbers. Fibroblasts were more numerous but there was little collagen deposition. Atypical myocytes, although occasionally found, were never prominent. There was usually good evidence of angeitis with and without intravascular thrombosis. The angeitis was characterized by swelling of endothelial cells, proliferation of endothelium, and a discrete subacute inflammatory reaction in the perivascular tissues (Fig. 8).

The type of reaction (HIII) in homologous transplants was characterized as follows. The pannus was well formed and normally vascularized (Fig. 3). The spread of the vascularizing channels through the fascia into the broad musculofascial zone was almost perfect (Fig. 9). There was thorough vascularization of the loose reticular tissue in the musculofascial zone. Occasional intravascular thrombi were found but the important vascular changes consisted of swelling and active proliferation of endothelial cells (Fig. 10). Periangeitis with accumulation of lymphocytes and monocytes was conspicuous. But the most distinctive feature of this reaction was a compact dense infiltration of the entire breadth of the musculofascial zone with inflammatory cells (Figs. 3, 9, 10). The principal cell was lymphocytic in form. Eosinophils and monocytes were less frequent. Atypical monocytes were rarely encountered. Fibroblasts and newly formed collagen were never conspicuous. The characteristic features of this reaction were not always restricted to the musculofascial zone. When the reaction was minimal, only the musculofascial zone immediately beneath the fascia was involved. When it was of greater degree, the entire musculofascial zone was affected. When it was of greatest degree the musculofascial zone, the perivascular areas in the fascia and the overlying pannus were almost equally involved (Figs. 3, 9, 10).

The preceding descriptions apply to the various types of autologous and homologous reactions. Each type was evaluated as to the degree of the reaction. Four degrees were recognized and tabulated numerically as 1, 2, 3, or 4, the degree of the reaction increasing in rough proportion to the assigned numerical value.

Other classes of reaction occurred. These were regarded as indeterminate or due to infection. Reactions were of undetermined type when the anatomic relationships between the transplant and adjacent tissues were distorted or when the reaction from place to place in the musculofascial zone was too variable to permit classifying it as either autologous or homologous in type.

Reactions due to infection of the transplant were usually recognized because of abscesses. Whenever this occurred, characteristic autologous and homologous reactions could not be distinguished even though the musculofascial zone was often well preserved. Indeed, it seemed that acute infection in one transplant exerted some influence which increased the difficulty of classifying the types of reaction in the uninfected transplants in the same animal.

There were forty single autologous and crossed homologous transplants in paired animals (Table I). Of these, thirty-five transplants were suitable for study. One of the thirty-five transplants was infected. The remainder were

TABLE I

Relations between the Actual Class of Transplants and the Classification of Transplants Determined by Microscopic Study

Transplantation operation			Classification of transplants by host reaction															
Procedure	Transplant		Autologous reaction							Homologous reaction							Undetermined reaction	Infected reaction
	Class	No.	Type A			Degree				Type H			Degree					
			I	II	III	1	2	3	4	I	II	III	1	2	3	4		
Single paired	Autologous	20	0	3	14	0	7	9	1	1	0	0	1	0	0	0	2*	0
	Homologous	20	1	1	0	0	2	0	0	1	3	10	0	3	7	4	3*	1
Multiple paired	Autologous	42	1	3	24	0	16	12	0	2	0	0	0	2	0	0	3* 2	7
	Homologous	42	1	2	0	1	2	0	0	22	3	5	10	10	4	6	3* 1	5
Single	Homologous	6	0	0	0	0	0	0	0	0	0	6	0	1	4	1	0	0
Single	Autologous	4	0	0	4	0	1	3	0	0	0	0	0	0	0	0	0	0

* Not suitable for microscopic study.

classified as showing either homologous or autologous types of reaction. Of the thirty-four transplants which were classified, three transplants were at variance with the known classification, two homologous transplants being classified as autologous and one autologous transplant as homologous. In thirty-one instances the transplants were accurately classified. Fourteen were homologous and seventeen were autologous. The types and degrees of reaction in the two groups are shown in Table I. The principal classification in each group was Type III, Degree 3.

These results are to be compared with those obtained in paired animals in which multiple autologous and crossed homologous transplants were done at weekly intervals. In this group there were eighty-four transplants. Six contained no well oriented residual muscle or fascia so that only seventy-eight

were suitable for microscopic study. Of those that were suitable, three reactions were of undetermined type and twelve transplants were infected. The remainder were classified. Five classifications were at variance with the known composition of the transplant. Three homologous transplants were classified as showing autologous types of reaction. Two autologous transplants showed homologous types of reaction. Of the fifty-eight transplants in which the classification given was in accord with the type of transplant, thirty were homologous and twenty-eight were autologous. The type and degree of homologous and autologous reactions are shown in Table I. These data show that the principal homologous reaction was Type I, Degree 1 or 2, and the principal autologous reaction was Type III, Degree 2.

A comparison of the data obtained by a study of single and multiple successive transplants led to the following conclusions:—The type and degree of reactions in autologous transplants were essentially the same in the two groups of animals and, therefore, were not modified by multiple successive transplants. The type and degree of reaction in homologous transplants were different in the two groups of animals and, therefore, were modified by multiple successive transplants. Multiple homologous transplantation resulted in accentuation of an acute angeitic thrombotic reaction especially in the new musculofascial vascular supply to the transplant (Figs. 7, 8). This reaction superseded and apparently inhibited the characteristic form of lymphocytic and angeitic reaction encountered when only single homologous transplants were made (Figs. 9, 10).

A study of single homologous transplants, 2 weeks of age, uncomplicated by the presence in the same animal of autologous transplants, led to the classification shown in Table I.

A study of double simultaneous autologous transplants, 2 weeks of age, uncomplicated by the presence in the same animal of homologous transplants, led to classifications shown in Table I.

The data in these two small groups of animals indicated that the presence of autologous transplants in the same animal had no influence upon the characteristic reaction in homologous transplants. Likewise, the presence of homologous transplants in the same animal had no significant influence upon the development of the reaction in autologous transplants.

DISCUSSION

The musculofascial type of transplant was selected for study for several reasons. First, two discrete contiguous and fairly homogeneous types of tissue were included in the transplant. One tissue was an avascular sheet of collagen with intervening fibrocytes which normally require very little blood supply. The other tissue was voluntary striated muscle composed principally of large cells. These cells normally require a rich blood supply and when deprived of it

shrink in dimensions and disappear without exciting an undue inflammatory reaction. Second, the sheet of collagen in intimate connection with the main bulk of muscle provided a landmark for distinguishing the relations between the transplant and the regional proliferating tissues of the recipient at the site of transplantation. Third, if it could be determined that distinctive reactions to transplants occurred, attempts to discover factors responsible for the reactions might readily be made later because the voluntary muscle cell can be dissolved and its components subjected to fractionation. Fourth, the use of fascia and muscle assured us of an ample supply of tissue for transplantation or eventual fractionation. Finally, infection which complicates transplantation or fractionation of tissue such as skin could largely be avoided in using muscle and fascia. It was not our intention to seek for retention of viability or function of muscle of any transplant.

The results of this study showed that autologous and homologous transplants deteriorated and resorbed at about the same rate. It would have been desirable if the rate of resorption had been somewhat less, because rapid resorption imposed limitations on microscopic observations beyond 3 weeks. Persistence of transplants for as long as 6 weeks would have been more desirable, especially in the study of changes in reactions brought about by successive homologus transplants.

The basic pattern of organization which followed deterioration and resorption of autologous and homologous transplants was essentially the same. A pannus of vascularized granulation tissue grew from the margins of the transplantation site over the surface of the fascia of the transplant. A space resembling a bursa formed between this pannus and the overlying superficial fascia of the tissues of the host. The reason for the development of this space was not clear. The fluid within the space contained little coagulable protein and few inflammatory cells, except in the presence of infection. The pannus served as the only important source for a vascular supply to the fascia and the musculofascial zone except at the periphery where the fascia of the transplant was sutured to the fascia of the host at the margin of the transplantation site. At the periphery where the muscle of the transplant was in contact with transected muscle of the host, vascularization proceeded in granulation tissue arising from transected recipient muscle in the bed of the site of transplantation.

The mode of vascularization of the fascia and the musculofascial zone was similar in both types of transplants. Buds of capillaries accompanied by delicate fibroblastic tissue grew from the vascularized pannus perpendicularly through the fascia at regularly spaced intervals. These intervals coincided with the distribution of the septa of collagen which separated the fascia into compartments containing bundles of collagen. Lateral vascularization of the fascia did not occur. The ingrowing capillaries penetrated directly to the

musculofascial zone and spread therefrom, together with the accompanying fibroblastic tissue, throughout the musculofascial zone. The participation of fibroblasts originating from the transplant in this process seemed to be minimal, especially in homografts.

The musculofascial zone was created by the formation of granulation tissue between the fascia and the degenerating muscle. Increase in breadth of the zone kept pace with the resorption of degenerating muscle fibers. This occurred at a uniform rate along the entire breadth of the musculofascial zone.

The same process of degeneration and resorption of muscle occurred at the margins of both forms of transplants. The zone of degeneration here was progressively organized by granulation tissue arising from the contiguous transected muscle of the recipient at the margins of the transplantation site. There was little evidence that fibrocytes of the transplant proliferated in this zone of repair.

It was in the framework of repair by ingrowth of recipient tissues into the musculofascial zone that distinctive reactions to transplants occurred. The course of events in autologous transplants was one of progressive unimpeded vascularization, fibroblastic proliferation, and collagen deposition in the pannus, the musculofascial zone, and peripheral zone. The fascial structure, except for the perpendicular vascularization, persisted without much change, but immature elongated multinucleated cells with long parallel intracytoplasmic fibrils regularly appeared. At times, these seemed to arise in the fascia and project perpendicularly through the musculofascial zone toward the retreating margin of degenerating muscle fibers. The type, origin, and nature of these cells is unknown to us but they were regularly found in the autologous musculofascial zone of reaction. Although other cells such as multinucleated giant cells, called myophages by some authors, and a scattering of inflammatory cells were always found in the musculofascial zone and elsewhere, nothing of significance could be attached to them.

The course of reactions in homologous transplants was different from that in autologous transplants. The basic plan of stromal organization was about the same as in autologous transplants but there were factors which interfered with the persistence and completion of the pattern of organization. This interference was exhibited by the development of two general forms of reaction. The first form was characterized by a massive accumulation of mononuclear cells, especially lymphocytes, and few eosinophils. The preponderant accumulation of these cells was in the musculofascial zone and their most intense concentration was adjacent to the fascia, though the depth of the entire musculofascial zone was usually occupied by them. In some instances, when this greater degree of reaction occurred, the cells also were numerous around vessels penetrating the fascia and those in the overlying pannus of granulation tissue. Contrary to expectation, this type of reaction was never intense or distinctive

in granulation tissue connecting degenerating muscle of the transplant with degenerating muscle of the host at the margins of the site of transplantation.

The second general form of reaction which distinguished autologous from homologous transplants was an angeitis. This varied in degree and character. At times, the principal finding was thrombosis of small vessels. At other times, endothelial swelling and proliferation dominated the picture of end-angeitis with closure of the lumen of the vessel. At still other times, the principal locus of the reaction was around capillaries and small blood vessels. These forms of angeitis of different degrees and types, had, apparently, a deleterious effect upon completion and persistence of the pattern of organization of the musculofascial zone. Indeed, even the characteristic pattern of lymphocytic reaction either did not develop or disappeared when the acute thrombotic forms of angeitis were conspicuous. The angeitis, in whatever form found, followed the same distribution as the lymphocytic reaction described above. It was conspicuous in the musculofascial zone and in more severe instances involved the capillaries penetrating the fascia and those in the pannus overlying the fascia. Under these circumstances, an intense acute inflammation with necrosis of the fascia and adjacent tissues occurred. As with the lymphocytic response, the angeitic reaction was never distinctive or conspicuous in the granulation tissue connecting degenerating muscle of the transplant to the marginal transected muscle of the host in the bed of the transplantation site.

As the result of the superposition of angeitic and lymphocytic forms of reaction in the vascularizing granulation tissue of the musculofascial zone, the completed sequences of repair encountered in autologous transplants were not realized in homologous transplants. Fibroblasts became obscured and collagen deposition was retarded. The long striated multinucleated cells were usually absent. The fascia, most resistant of all to change, lost its fibrocytes and the collagen become converted into a hyalin homogeneous mass which was absorbed rapidly when the angeitic reaction was acute and intense. Otherwise the fascia, especially in instances of mild lymphocytic reactions, seemed to persist with as much unity of form and structure as the fascia of autologous transplants.

It is to be emphasized that the lymphocytic and mild angeitic or periangeitic forms of reaction occurred in single homologous transplants with regularity. Although the intensity and degree of the reaction varied with the source of the transplant as well as with the recipient, experiments were not designed to inquire into the reasons for these differences. We believe, however, that either muscle from different sources excited reactions of different magnitudes or the reactive response varied among different recipients. This seemed to be more a variation of degree rather than type of reaction.

The outstanding difference between reactions in different homologous

transplants was encountered only when multiple successive transplants were made from the same donor to the same recipient. The customary lymphocytic and mild angeitic reaction encountered in single homologous transplants was replaced by an acute thrombotic angeitic reaction with necrosis and widespread polymorphonuclear cell infiltration. This was most pronounced in transplants, 2 weeks of age. As yet, we are not prepared to say whether the change in the character of the reaction was due to the amount of muscle transplanted, which was three times as great in these animals as in the control group, or whether repetition of transplantation of fresh homologous muscle was a factor. In this relation, Medawar in studies of homologous skin transplantation placed emphasis upon "dosage" and repetition as influencing the time of survival of transplants (2, 3). He found that the average time of survival of skin transplants was reduced by increasing the amount of skin transplanted or by successive transplants of skin from the same donor to the same recipient.

The question arises as to whether any hypothesis can be offered to explain the results of these experiments. If we assume that the reaction of the host was due to products released by disintegration of muscle cells of the transplant, we might have expected the reaction to have been generalized about the transplant rather than localized to a particular zone. However, if these products were readily diffusible, the reaction might be localized to zones where diffusion was restricted, for instance by a collagenous fascial barrier. This idea would be in accord with our findings. On the other hand, it may be assumed that the reaction of the host was due to products elaborated by living cells of the transplant. The main bulk of living cells of the transplant persisted in the fascia. If products of their activity were important the reaction should have been conspicuous in the fascia itself or at least equal in magnitude above and beneath the fascia. In all cases, the principal reaction was in the subfascial zone. Finally, the reaction may have been due to the presence of some component of normal transplanted tissue, concentrated in a particular anatomical location. In the present instance the musculofascial zone prior to transplantation was nothing more than a line of apposition of muscle and connective tissue. It would not be reasonable to regard this junction as being chemically distinct from other collagenous muscular junctions.

Not only was the localization of the reaction in homologous transplants puzzling, but also the nature of the reaction was difficult to explain. This reaction was unlike customary tissue reactions in most inflammatory diseases of the rabbit or human. There was a "granulomatous" quality to the lymphocytic form of the reaction and at times it resembled the inflammatory reaction in chronic granulation tissue of wounds that fail to heal even in the absence of significant infection. But here the similarity ceased because in such tissue, angeitis of small vessels is seldom conspicuous. Perhaps, the angeitic

reaction has more in common with natural or experimental inflammatory diseases in which arteritis of "allergic" origin is encountered.

The present findings may be compared with those of other investigators who have studied tissue reactions of the host to transplants of normal and neoplastic tissues. Much of this work has been summarized by Loeb (1).

The studies, especially by Rous, Murphy, Loeb, Greene, and Medawar, have directed attention to the probability that acquisition of immunity by the host is important in bringing about retrogression of homologous transplants. Though it is generally agreed that the immunity is acquired by stimulation of immune mechanisms of the host by some factor or factors in the transplant, opinions differ as to the means by which the specifically immune state brings about a deterioration of the transplant. Medawar believes that a local antigen-antibody reaction is the important factor which is directly responsible for retrogression of skin transplants (3). Though this is an attractive idea, the components of the reactive system have not yet been demonstrated. After a very extensive study, Murphy and his associates concluded that the general and local lymphocytic reaction was responsible for the development of immunity (4). Loeb also emphasized the importance of lymphocytic infiltration but did not believe that the development of immunity depended on the local or systemic lymphocytic reaction (1). He concluded, however, that the strength of the lymphocytic reaction could be used as a quantitative measure of the intensity of the reaction of the host against a strange individuality differential in the transplant. He was puzzled by the variable magnitudes of lymphocytic reaction in different hosts, in different tissues, and even in different parts of the same tissue. For instance, homotransplants of cartilage and anterior hypophysis attracted few lymphocytes. The corpus luteum attracted many lymphocytes while granulosa cells of developing follicles were avoided by lymphocytes, infiltrating ovarian homotransplants. Rous recognized the importance of the lymphocytic reaction but believed that the lack of a stromal reaction, manifest by a deficient ingrowth of connective tissue and capillaries into the graft, was a mechanism through which active immunity of the host became effective against homologous transplants of embryonal mouse tissue. He stated that the immunity to embryonic tissue implanted in mice manifests itself in the same way as that for implanted tumor, namely by an absence of the stroma reaction necessary to life of the engrafted tissue. (5). Later, after a study of factors involved in the resistance to transplanted chicken sarcoma, Rous and Murphy concluded that the appearance or non-appearance of the specific supporting and vascularizing reaction in the host tissues could not be looked upon as determining the fate of grafts of the chicken sarcoma. They believed that this was also true of mammalian neoplasms (6). Woglom and Greene have concluded that the nature of stromal ingrowth plays a conspicuous part in mechanisms by which immunity affects transplanted tumors (7–8). After a study of the problem of the development of immunity against transplanted tumors in rabbits Greene concluded that the ability of a tumor to grow in resistant environments is a function of its stromal requirements and that if a specialized stroma is obligatory or if the degree of resistance prohibits any stromal response, growth does not occur and the host is said to be refractory to the tumor (8). More recently, Medawar has developed a different view of the significance of

stroma reactions. This was based upon his observation that a homograft of skin in the anterior chamber of the eye of a specifically immunized rabbit was destroyed only if the graft had been vascularized by the host's tissues (9).

The present experiments have not offered a solution to these problems but they may aid in progress toward a solution in several ways. First, a reproducible aseptic technique for semiquantitative histologic bio-assay of the host's reaction to an homologous transplant has been developed in rabbits. Second, the similarities in the initial pattern of stromal reaction to autologous and homologous transplants have been defined. Third, the superposition upon the stromal reaction of a cellular infiltration and an angeitis has been interpreted as a specific form of response of the host to an homologous transplant. When single transplants were made the cellular infiltration, lymphocytic in character and restricted primarily to the musculofascial zone of the transplant, occurred in association with mild angeitis. When multiple successive transplants were made from the same donor to the same recipient, the cellular infiltration, composed principally of polymorphonuclear leukocytes and restricted primarily to the musculofascial zone of the transplant, occurred in association with an acute thrombotic angeitis. In both instances, the superimposed cellular and vascular reactions interfered with the completion of orderly stromal organization of the musculofascial zone of homologous transplants. These observations form a basis for further experiments designed to inquire into local and systemic factors responsible for the reactions.

SUMMARY

Simultaneous autologous and homologous musculofascial transplants were made in New Zealand white rabbits. The basic pattern of degeneration and granulation tissue organization of both types of transplant was essentially identical. The superposition of two reactions in the framework of organizing granulation tissue served to distinguish homologous from autologous transplants. One reaction to homologous transplants was predominantly characterized by lymphocytic infiltration and the other by angeitis. The principal locus of these reactions was in the musculofascial zone of the transplant, and from this zone the reactions spread to a variable degree through the fascia into the overlying pannus of granulation tissue. When single homologous transplants were made, the lymphocytic and mild angeitic forms of reaction predominated, becoming conspicuous at the end of 2 weeks. When multiple successive homologous transplants were made from the same donor to the same recipient, acute angeitis with thrombosis supervened and the lymphocytic reaction failed to develop or persist. Multiple successive autologous transplants, on the other hand, did not influence the type or degree of reaction to autologous transplants in the same animal. There was no evidence that autologous transplants had any influence upon the sequence of reactions to homol-

ogous transplants or that the presence of homologous transplants influenced the nature of the reaction to autologous transplants in the same animal.

Until better methods are developed, methods of bio-assay of the type described, though lacking in quantitative precision, offer the best means for further analysis of factors which govern the incompatibility of tissues of one animal for those of another animal of the same species.

BIBLIOGRAPHY

1. Loeb, L., The Biological Basis of Individuality, Springfield, Illinois, Charles C. Thomas, 1st edition, 1945.
2. Medawar, P. B., *J. Anat., Lond.*, 1944, **78,** 176.
3. Gibson, T. and Medawar, P. B., *J. Anat.*, London, 1943, **77,** 299.
4. Murphy, J. B., The Lymphocyte in Resistance to Tissue Grafting, Malignant Disease and Tuberculous Infection, Monograph of The Rockefeller Institute, for Medical Research, No. 21, New York, 1926.
5. Rous, P., *J. Exp. Med.*, 1910, **12,** 344.
6. Rous, P., and Murphy, J. B., *J. Exp. Med.*, 1912, **15,** 270.
7. Woglom, W. H., *Cancer Rev.*, 1929, **4,** 129.
8. Greene, H. S. N., *Cancer Research*, 1942, **2,** 669.
9. Medawar, P. B., *Brit. J. Exp. Path.*, 1948, **29,** 58.

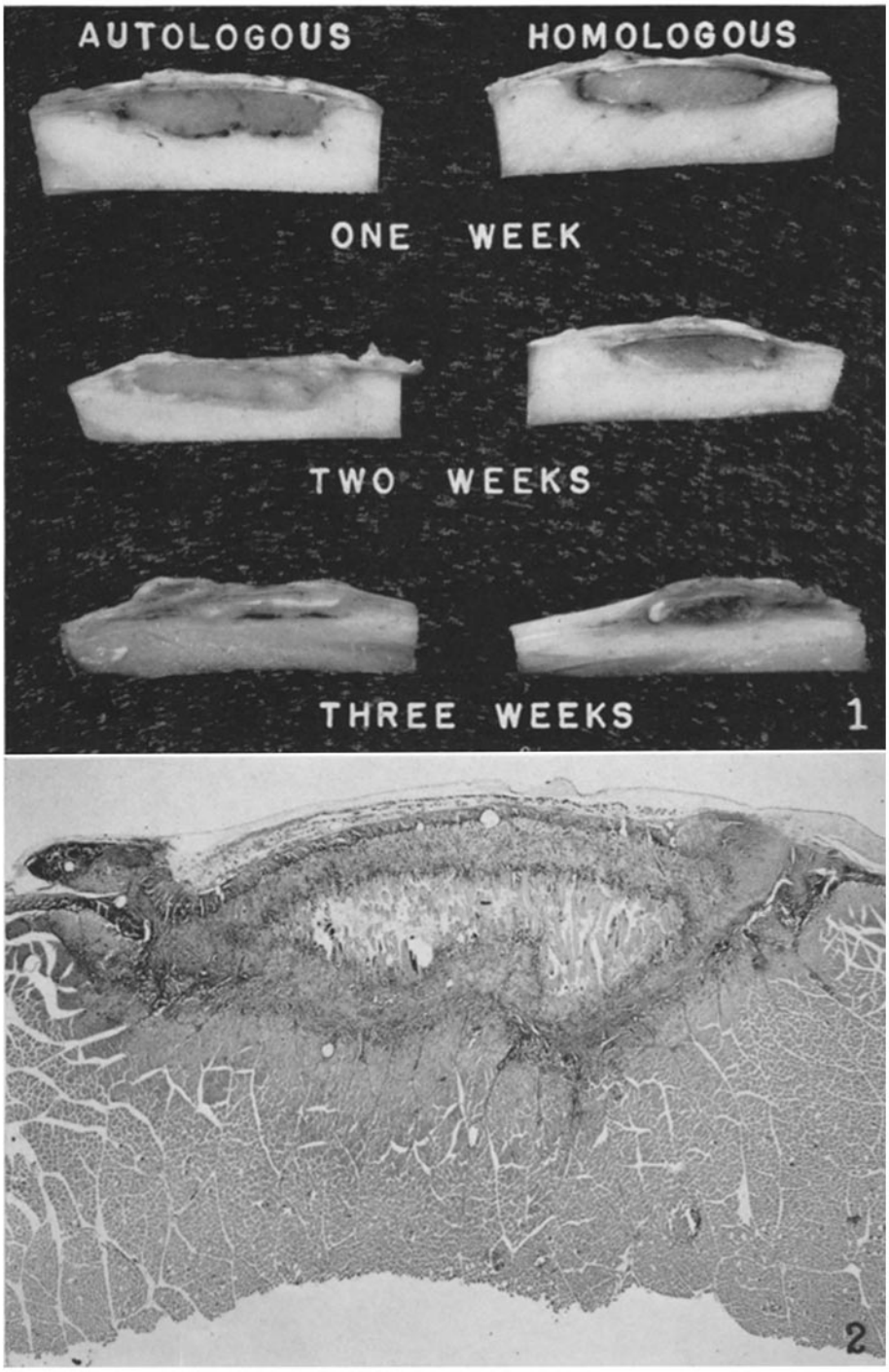
EXPLANATION OF PLATES

The microscopic sections were all stained with hemotoxylin and eosin.

PLATE 49

FIG. 1. Cross-sections of a set of autologous and homologous transplants which are 1, 2, and 3 weeks of age. The dark areas are the muscular tissues of the transplants. These are bounded above in each instance by a white band of transplanted muscle fascia and elsewhere at the periphery by the erector spinae muscle of the recipient. Note the uniform shrinkage of both types of transplants with increasing age and the fixation of the periphery of the transplants by stromal reactions of the recipient. Natural size.

FIG. 2. Low power photomicrograph of an autologous musculofascial transplant, 2 weeks of age. Note the superficial pannus of connective tissue which has grown over the surface of the fascia of the transplant. Beneath the fascia there is a broad zone of proliferating vascularized connective tissue, which has filled in the space vacated by degenerating muscle of the transplant. This is the zone of greatest interest in evaluating the forms of reaction of the recipient to the transplant. Vascularized connective tissue can also be seen, uniting the periphery of the muscle of the transplant to the muscle of the recipient in the bed of the transplantation site. × 20.

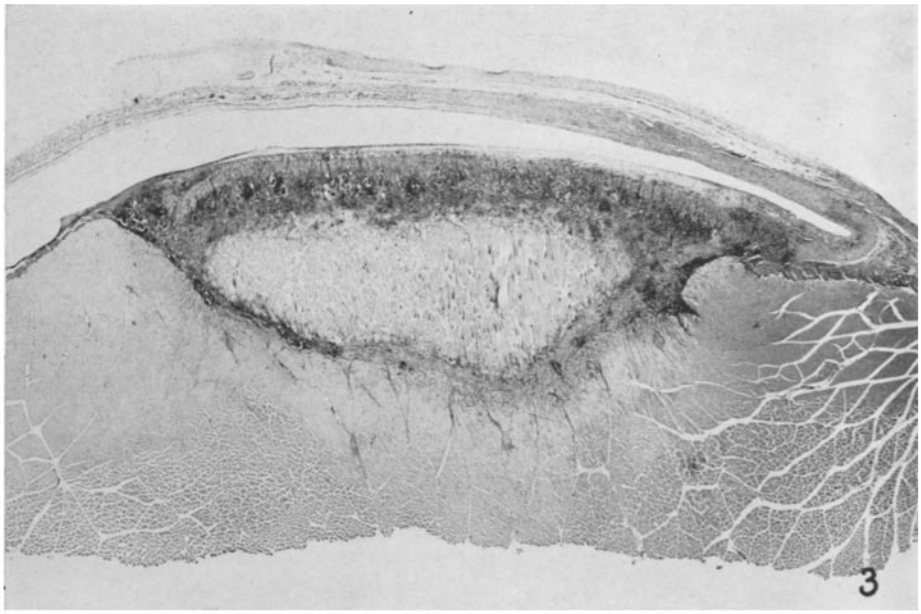


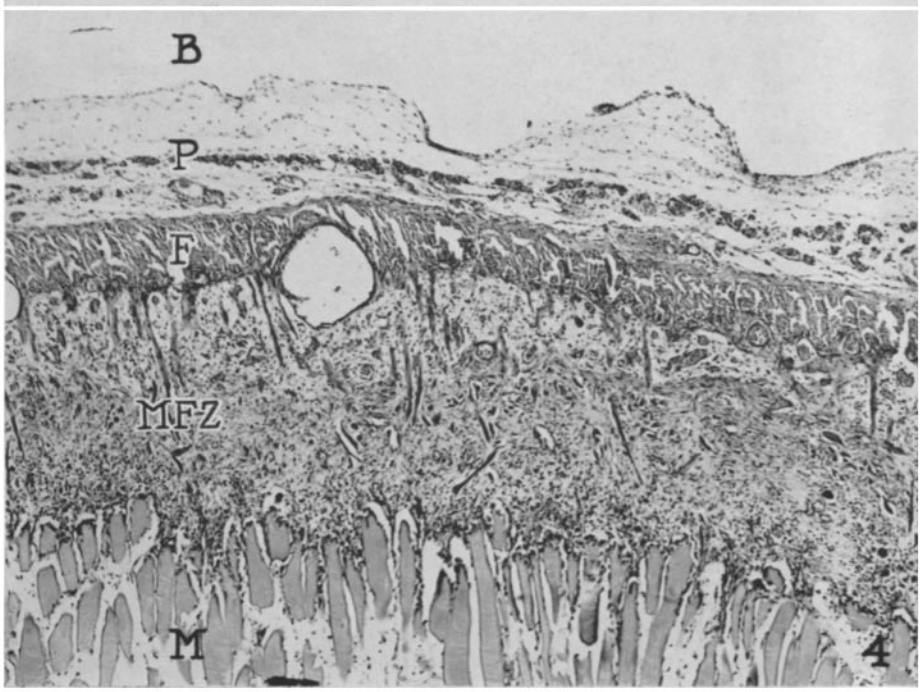
(Andresen *et al.*: Reaction to musculofascial transplants)

PLATE 50

FIG. 3. This is a low power photomicrograph of an homologous musculofascial transplant, 2 weeks of age. Note the superficial pannus of connective tissue which has grown over the surface of the fascia of the transplant and which is continuous with the wall of a bursal space which regularly formed over all types of transplants. The fascia of the transplant, just beneath the bursa and pannus, is heavily vascularized. It is less distinct than the fascia in Fig. 2, and is much darker because of an extensive infiltration with lymphocytes which also permeated the underlying broad musculofascial zone created by degenerating muscle of the transplant. The lymphocytes were characteristically concentrated in this zone and a progressive decrease in their number is indicated by the lightening grey color in the vascularized connective tissue which joins the periphery of the muscle of the transplant to the muscle of the recipient at the margin of the transplantation site. × 20.

FIG. 4. This is a medium power photomicrograph of an autologous transplant, 2 weeks of age. The empty space at the top of the photograph is part of the bursa (*B*) which formed over the transplant. The vascularized superficial pannus (*P*) of proliferated connective tissue of the recipient is shown just below the bursal space. Beneath this is the narrow collagenous band of fascia (*F*) of the transplant. Several large vascular channels arising in the pannus have penetrated the fascia and branches of these channels ramify throughout the broad musculofascial zone (*MFZ*). This zone is occupied by proliferating connective tissue and there are few inflammatory cells. Elongated strands of tissue, projecting from the fascia into the granulation tissue of the musculofascial zone are composed of immature, elongated multinucleated "myocytes." Beneath the musculofascial zone, the retreating margin of degenerating muscle (*M*) of the transplant is shown. × 100.



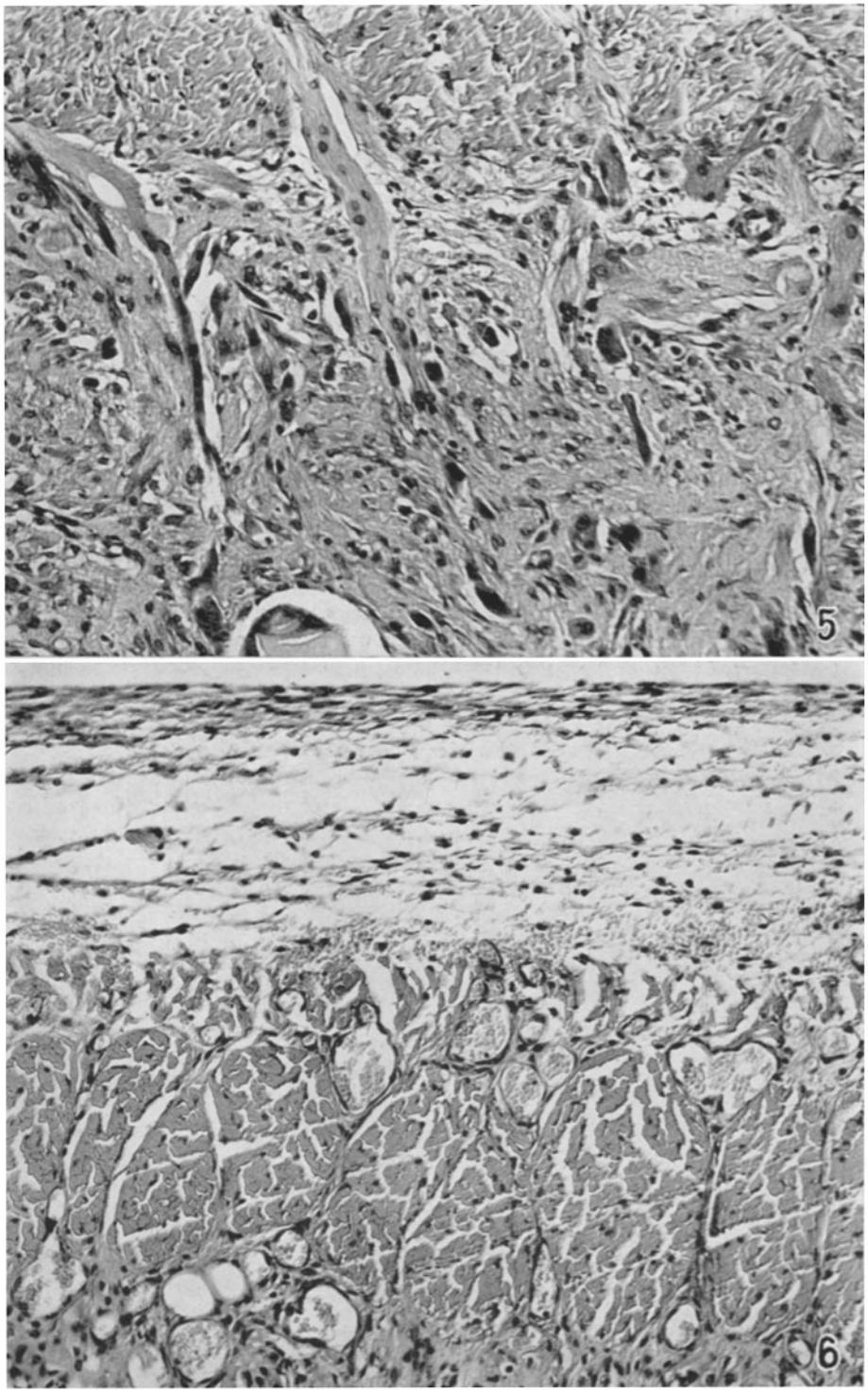


(Andresen *et al.*: Reaction to musculofascial transplants)

PLATE 51

FIG. 5. This is a high power photomicrograph of the musculofascial zone of an autologous transplant, 2 weeks of age. The upper part of the photograph shows the deep layers of the fascia at the junction with the fibroblastic tissue which has formed in the musculofascial zone. Note the scant infiltration with inflammatory cells and the multinucleated, elongated "myocytes" with a fibrillary cytoplasm. These cells were rarely found in the musculofascial zone of homologous transplants. × 450.

FIG. 6. This is a medium power photomicrograph of a part of an autologous musculofascial transplant, 2 weeks of age. The empty area above the tissue is part of the bursal space overlying the transplant. Beneath this is a pannus of granulation tissue which is fused with the surface of the transplanted fascia. Capillaries arising in this pannus project perpendicularly between the collagenous bundles of the fascia and communicate with the subfascial vascular plexus which has formed in the musculofascial zone at the lower margin of the fascia. Most vessels are empty and shown in cross-section. This represents the basic plan of stromal organization of the musculofascial zone of both types of transplants. × 450.

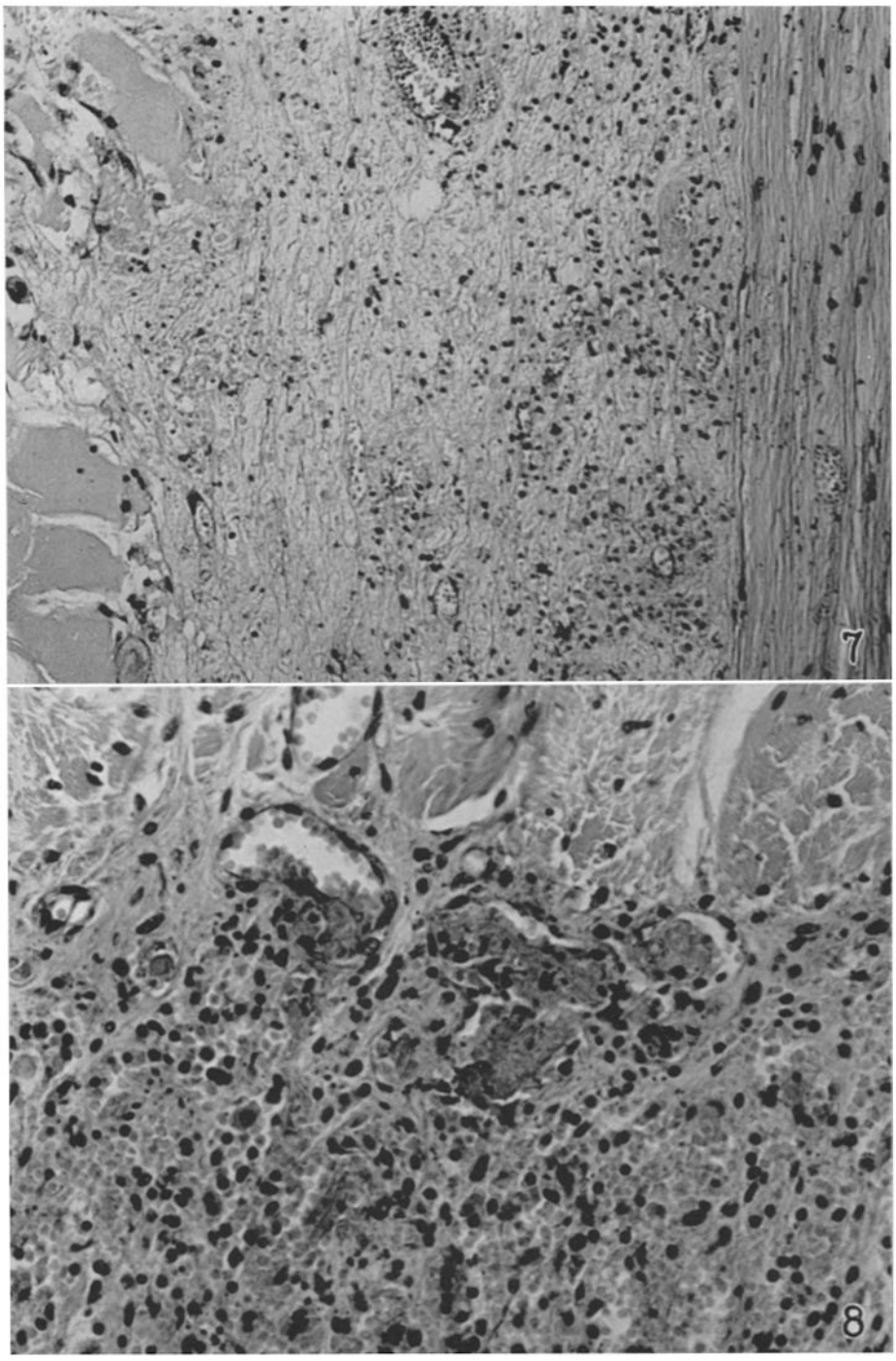


(Andresen *et al.*: Reaction to musculofascial transplants)

PLATE 52

FIG. 7. This medium power photomicrograph shows the musculofascial zone of an homologous transplant 2 weeks of age. This was the second of three successive transplants from the same donor to the same recipient. It illustrates the character of the acute necrotizing reaction which occurs in grafts under these conditions. This section is rotated through an angle of 90 degrees in relation to those of the previous figures. Note the deterioration of fibrocytes and collagen of the fascia which in this section is at the right of the photograph rather than at the top as in previous figures. The broad musculofascial zone between the fascia and the retracted margin of degenerating muscle at the left of the photograph shows vasodilatation, acute angeitic thrombosis, edema, and necrosis of the previously well formed granulation tissue. This is to be compared with Fig. 9, which illustrates the usual reaction to single homologous transplants, 2 weeks of age × 250.

FIG. 8. This high-power photomicrograph shows a part of the musculofascial zone adjacent to the fascia of an homologous transplant 2 weeks of age. This was the second of three successive transplants from the same donor to the same recipient. The lower margin of the fascia is at the top of the photograph. Just beneath this there is conspicuous acute end-angeitic thrombosis of the newly formed vascular channels in the musculofascial zone. The adjacent musculofascial zone shows hemorrhagic necrosis which often occurred in granulation tissue in this location when successive homologous transplants were made. × 450.

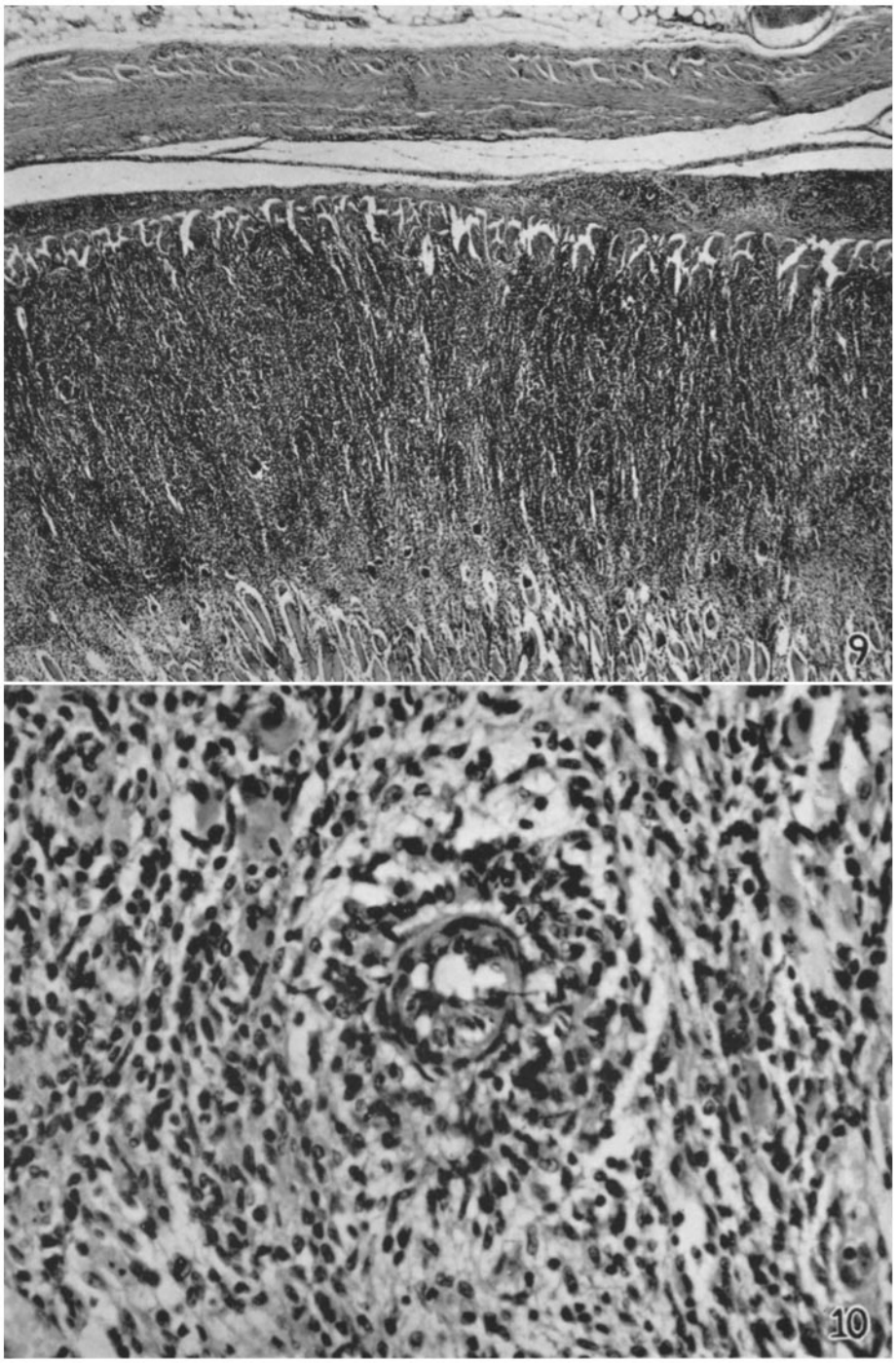


(Andresen *et al.*: Reaction to musculofascial transplants)

PLATE 53

FIG. 9. This is a medium power photomicrograph of the musculofascial zone of an homologous transplant 2 weeks of age. It illustrates the nature of the reaction when single transplants were made. Note the bursal space between the normal tissues of the recipient and the thin pannus overlying the fascia of the transplant. The musculofascial zone which lies between the fascia and the retracting margin of degenerating muscle of the transplant is dark because of a massive accumulation of lymphocytes. The infiltrating lymphocytes have also permeated the fascia along vascular channels and accumulated in large numbers in the vascularized pannus overlying the fascia. This represents the maximum intensity of lymphocytic reaction encountered in homologous transplants. × 60.

FIG. 10. This is a high power photomicrograph of the cellular reaction in the musculofascial zone of an homologous transplant 2 weeks of age. This illustrates, especially, the most characteristic form of angeitis which occurred when only one homologous transplant was made. Note the proliferation of endothelium with partial obstruction of the lumen of the vessel. In the wall and adventitia of the vessel there is a peculiar spoke-like infiltration of polymorphonuclear leukocytes and macrophages. This form of angeitis is very similar to that which has been attributed in rabbits and human beings to sensitization. × 450.



(Andresen *et al.*: Reaction to musculofascial transplants)