Studies of the Origin of the Vasculature in Free Skin Grafts

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The survival of all free tissue grafts is dependent upon the rapid re-establishment of an adequate circulation. However, despite almost a century of repeated investigations (Clemmesen, 1962; Medawar, 1944; Converse and Rapaport, 1956; Converse and Ballantyne, 1962) the origin of the blood vessels within an established skin graft remains equivocal. The question to be answered is simply: Are the graft's own vessels used for its permanent circulation, or are they of little significance, the new vascular supply originating by capillary invasion from the host bed?

Recent studies of the cheek pouch of Syrian hamsters (Billingham and Silvers, 1962) suggested that their unusual structure might facilitate the elucidation of this problem. The skin-like tissue constituting the highly vascular cheek pouch is almost transparent, being devoid of pigmentation and appendages. Thus, when transplanted to genetically compatible hosts, it offers an unique "window" through which serial observations on the developing circulation can be studied. The purpose of this paper is to report our observations on the vascularization of free grafts of cheek pouch skin and to cite evidence which suggests that the intrinsic vasculature of a free graft of normal skin is re-utilized and is mandatory for survival.

Methods and Observations

A. Preparation and Transplantation of Pouch Skin Grafts. To avoid the necessity of autotransplantation, adult hamsters of the LSH isogenic strain (Billingham et al., 1960) were used throughout. This made possible the exchange of grafts between normal animals without the intervention of complicating factors of transplantation immunity.

The entire cheek pouch (Fig. 1) was everted with forceps, carefully cleansed with 5% aqueous Dettol solution and excised. It was next drawn over the finger tip, epithelial surface innermost, and opened longitudinally (Fig. 2) avoiding the major blood vessels, producing a tongue-shaped sheet of skin approximately 5×6 cm. (Fig. 3). When transplanted to a full-thickness bed on the side of the chest of an isogenic host, pouch skin grafts healed like grafts of ordinary skin conserving their distinctive anatomical features and presenting a mitotically active, pink epithelial surface. Because of their transparency, the principal and many of the finer vessels can easily be seen.

The blood supply of the hamster cheek pouch has previously been described by us (Haller and Billingham, 1964). It consists essentially of two vessels of origin, one at the apex and the other at the mouth of the pouch. After the excised pouch has been opened longitudinally, most of the vascular continuity remains undisturbed and a distinct vascular pattern is recognizable (Fig. 3).

In a panel of 40 hamsters, pouch skin grafts were applied directly to fresh beds prepared by removing the entire thickness of skin down to the panniculus carnosus on the lateral thoracic wall. Full details of the operative technic and dressings applied have been published elsewhere (Billingham and Silvers, 1961). At the time of transplantation, the blood vessels of the pouch grafts were invisible because the blood had been extruded from them. All grafts were observed serially to record the vascular patterns as they developed. Application of a thin film of mineral oil to the graft surface augmented its transparency and facilitated observation.

Most of the original graft vessels contained blood by the second postoperative day, and all of the grafts had blood-filled vessels by the third day. No blood flow was demonstrable, however, before the fifth day. Examination of histologic sections confirmed the absence or great sparcity of red blood cells in the lumina of the vessels before the second day and confirmed their engorgement with blood from the fourth day on.

The concept that there is an initial, ambient or "Plasmatic Circulation" in a free skin graft has remained controversial since its proposal by Hübscher in 1889 (Clemmesen, 1962). Most authorities find it necessary to assume some type of interim nourishment until an adequate vascular circulation can be developed. Hynes' (1954) careful observations on skin grafts removed at short intervals after transplantation, led him to believe that the initially empty graft vessels became filled with fluid and red blood cells by capillary attraction from free fluid in the graft bed.

Our observations support the concept of an initial "Plasmatic Circulation" (Converse, 1957); but since the fluid does not clot, it is probably not true plasma. This nutritional process is mediated by passive fluid uptake and might be more accurately referred to as the "Phase of Imbibition."
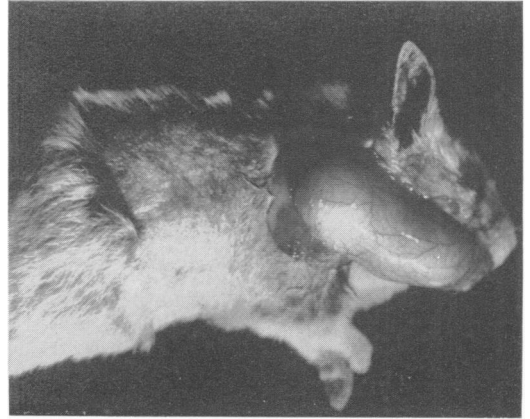

FIG. 1. Showing hamster's right cheek pouch *in situ* revealed by the removal of the overlying skin. The pouch has been packed with cotton wool to distend it. Because of the transparency of the cheek pouch even the finest vessels are clearly visible.
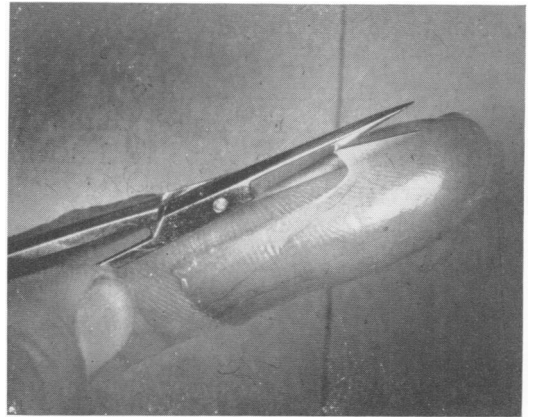

FIG. 2. Excised cheek pouch, epithelial surface innermost, drawn upon the finger to facilitate longitudinal incision.

According to an alternative theory put forward by Thiersch (Clemmesen, 1962), an early blood supply is obtained by "Inosculation." This rather naive concept of the regular occurrence of "kissing" between the severed ends of juxtaposed vessels of the graft and its bed has been difficult for some investigators to accept. To visualize the free ends of host bed vessels remaining somehow unclotted in the presence of tissue juices and lying in exact apposition to transected unclotted graft vessels defies anything short of an active imagination.
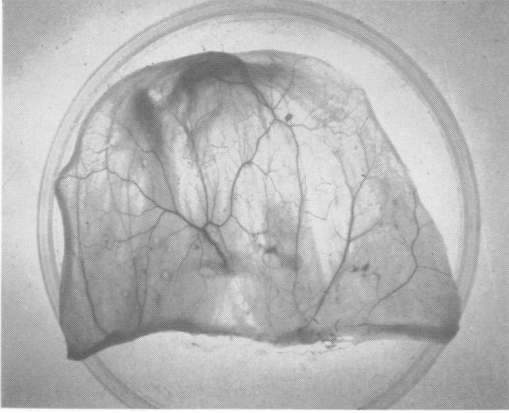
FIG. 3. Large, tongue-shaped piece of "skin" resulting from opening up an excised cheek pouch longitudinally. Note the vascular pattern.

In some of the hamsters, the blood vessel patterns of grafts were recorded by serial photography and the grafts biopsied for histologic study. In other hamsters the grafts were left undisturbed for six days after which they were serially studied. The grafts in some animals were applied with the major pouch vessels oriented at right angles to the long axis of the host vessels while in others the orientation of the graft was such that their vessels were parallel (Fig. 4, 5). In all animals the blood vessel pattern was clearly discernible from day 4 to 5 until the observations were terminated. The patterns of the functional blood vessels in established grafts were consistently identical to those of the original graft vessels. The size and maturity of these vessels was such as to exclude the possibility of an invasive *de novo* origin from the capillary bed of the host in such a short period of time—active blood flow was observed in the graft vessels by the fifth day.

B. Transplantation onto the Beds of Mature Granulation Tissue. A critical test of the Inoculation Theory would be to transplant skin to a bed which does not contain large, open vessels. Granulating wound beds which furnish only a capillary base would seem to answer this requirement.

Accordingly, isogenic pouch grafts were transplanted onto beds of mature, dense granulation tissue of six to eight days' duration in 20 hamsters. Histologic study of such beds showed a myriad of fine capillaries but no large surface vessels.

Under these circumstances the vessels of the grafts became filled with red blood cells just as early as the grafts placed upon freshly prepared beds, and the dynamic circulation began at the same time. In addition, the blood vessel patterns in these grafts were the same as those noted in grafts placed upon fresh beds. These observations do not support a theory of large vessel inosculation.

C. Transplantation of Pouch Grafts with Occluded Blood Vessels. Although the above observations confirmed the re-utilization of the grafts' own blood vessels, there remained the question of whether the survival of the graft depended upon the mandatory utilization of its intrinsic vasculature. The blood vessels of the cheek pouch were, therefore, occluded and the fate of the pouch grafts observed.
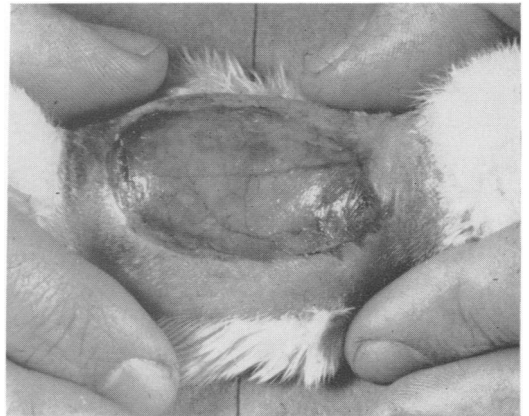

FIG. 4. Pouch skin isograft of 14 days' duration on the side of a hamster's chest. It was placed so that the orientation of its original vessels was in the long axis of the host's body. The original pattern has been faithfully conserved. A thin film of mineral oil was applied to the graft surface to facilitate visualization of the vessels.

FIG. 5. Pouch skin graft of 14 days' duration transplanted so that its principal vessels were oriented at right angles to the long axis of the host's body. Again, the vascular pattern of the graft has been maintained.

Silicone rubber * in a non-toxic solvent was injected into the carotid arteries of intact, anesthetized hamsters. With the addition of a catalyst to the solution immediately before injection, the entire blood supply of the pouch, as well as other head and neck structures, became solidly occluded by a firm cast of Silicone rubber within 15 to 20 minutes (Fig. 6). The pouches were then removed, prepared as in the other studies and transplanted.

Pouch grafts previously injected with Silicone rubber were transplanted to a panel of 20 hamsters. By the eighth day small areas of necrosis of the pouch grafts were noted in the periphery. Necrosis became uniformly confluent, with a full thickness graft loss by the eleventh day (Fig. 7). Histologic sections showed the graft vessels to be solidly plugged with Silicone rubber.

In an attempt to exclude the possibility that a diffusible toxic agent rather than vascular deprivation was responsible for the necrosis of the injected grafts, the following test was performed. Grafts of uninjected pouch skin were transplanted to ten hamsters and a wafer of filter paper,

* Silicone rubber kindly supplied by Silicone Products, Waterford, New York.

which had just been soaked in the Silicone rubber solution, was immediately placed over the center of each graft and maintained in position with the aid of dressings until primary inspection on the sixth postoperative day (Fig. 8). In no case was graft tissue damaged by the presence of the Silicone material above it. It may also be mentioned that subcutaneous injection of Silicone rubber solution caused neither inflammation nor necrosis.

Discussion

The present observations suggesting that early blood vessel filling takes place by a process of *Imbibition* rather than *Inosculation* are supported by work of Edgerton and Edgerton (1955) who studied the circulation in grafts placed in modified
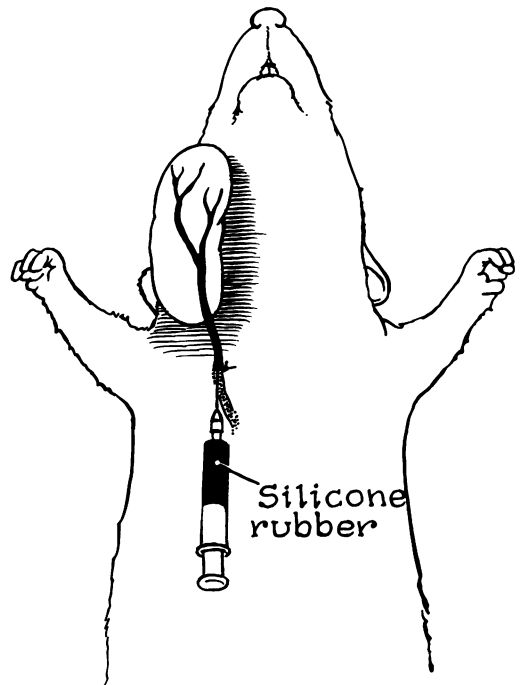
Injection of Cheek Pouch

FIG. 6. Illustrating the manner in which the principal vessels of pouch skin were occluded by injection of silicone rubber solution into the carotid artery.

Experimental Group: Silicone Injected Pouch Grafts
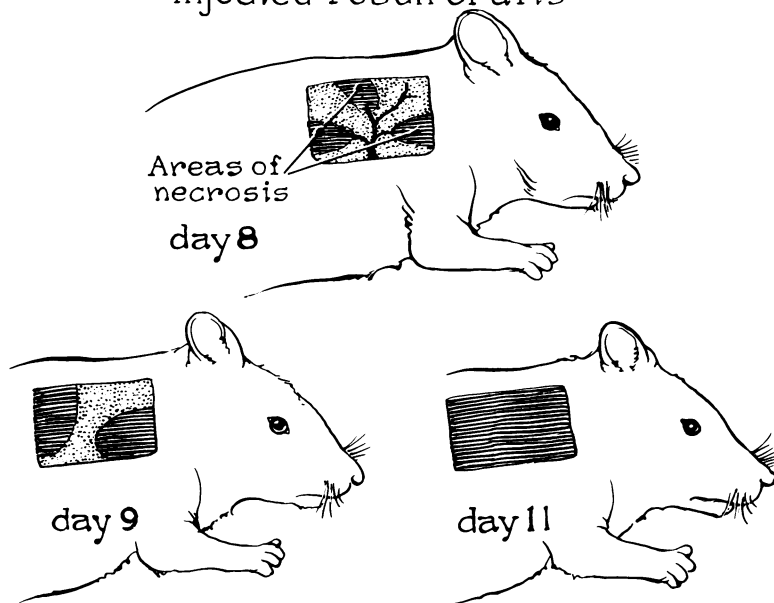
Areas of necrosis

day 8

day 9

day 11

FIG. 7. Illustrating the fate of a typical pouch skin isograft in which the principal vessels had been occluded with silicone rubber before transplantation. At 8 days there were peripheral areas of necrosis in areas of cheek pouch skin supplied by occluded branches of the artery. By the 9th day there was coalescence of areas of necrosis and by the 11th day the graft was totally necrotic.

Algire chambers. The empty graft vessels ("ghosts") first became filled with blood and finally established a sluggish circulation after the fourth day. The presence of a dynamic circulation in the hamster pouch skin grafts by the fifth day in vessels identical in size and pattern to the original graft vessels argues strongly against the possibility that these vessels arose from new capillary sprouts invading the graft from the host bed.

The strongest evidence that the original pouch vessels become the permanent vascular channels in transplanted pouch skin was the rigid conservation of an unchanged pattern of the major vessels. This was clearly visible through the transparent pouch graft. Once the graft vessels became passively filled in the Phase of Imbibition, the vessel pattern remained as the Phase of Dynamic Flow began. The pattern continued to be recognizable in grafts of 21–28 days' standing. Although these observations are based upon a peculiar type of skin, it seems reasonable to conclude that the findings have general application to free skin grafts. The failure of pouch iso-

grafts to survive when their intrinsic blood vessels were occluded with Silicone rubber indicates a mandatory utilization of the intrinsic vessels if survival is to take place. The present conclusions are entirely consistent with Rolle, Taylor and Charipper's (1959) observations on the vascular changes in skin autografts in mice. Here the pattern of blood-filled vessels was complete by 48 hours after grafting and a steady flow was generally evident throughout the graft by 72 hours. These workers concluded that not only was the time interval between grafting and the appearance of blood flow too short to allow for the formation of *new* vessels of the size observed (Hildemann and Haas, 1960); but during the initial period, there was no detectable change in the pattern of vessels from the time they were first filled with blood until restoration of circulation. Furthermore, during the initial period, before re-establishment of blood flow, there was no histological indication of degeneration of the graft vessels.

However, on the basis of a recent histochemical study of diphosphopyridine nu-

cleotide diaphorase activity in skin auto-grafts in rats, Converse and Ballantyne (1962) claim: (a) that there is collapse and loss of enzymatic activity from the native graft vasculature; and (b) that rapid and early ingrowth and development of capillaries from the host bed produces the definitive vasculature of the bed. According to them, host vessels reach the dermo-epidermal junction of full-thickness flank skin grafts within 48 hours. It may be emphasized that the present findings do not exclude the ingrowth of capillaries from the graft bed and their differentiation to form larger vessels as playing an important secondary role in vascularization of the grafts.

Numerous observers have noted the striking vascular reaction which occurs as the earliest evidence of an impending immunologic graft rejection (Taylor and Lehrfeld, 1953; Rolle, Taylor and Charipper, 1959). This reaction has been difficult to explain when it was thought that blood vessels of the growing graft were of host origin. With the recognition that the early vascular supply is intrinsic; that is, of *graft* origin, the manner in which the cellular immunity, responsible for skin homograft destruction, is put into effect become more intelligible (Waksman, 1963).

Summary

Hamster cheek pouches were removed and portions of their walls transplanted as free "skin" grafts. The intrinsic blood vessels were easily studied through this transparent skin. The blood vessels filled at once but no flow was noted before the fourth to fifth day. The blood vessel patterns in the healed-in isografts were identical to the original graft vessels. Indeed when these vessels were blocked by injections of Silicone rubber, the grafts became necrotic. Evidently the intrinsic vessels of the graft are re-utilized and are necessary for its survival.
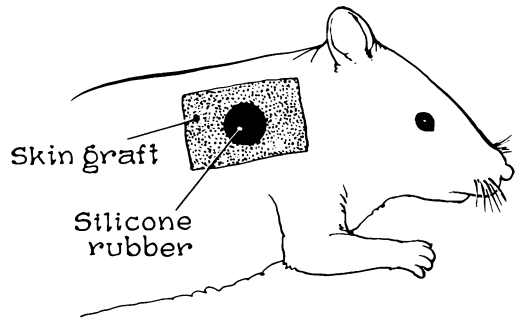

FIG. 8. Illustrating the principle of the toxicity test performed on the silicone rubber. A piece of filter paper was soaked in the silicone rubber solution and maintained in place over the central region of a freshly transplanted graft of pouch skin. The presence of this agent failed to prejudice the well-being of the subjacent graft tissue.

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