THE ACTIVATION OF SKIN GRAFTS

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Most healthy tissues, when wounded, start from scratch in the task of repair: no previous happening has got them ready for the emergency. Even the epidermis, which renews itself continually under ordinary circumstances, requires several days to get proliferation well under way, and the condition of the connective tissue at many situations,—fibrous, sparsely cellular, with almost no mitoses,—is highly unfavorable to the swift knitting of the wound. It is no disparagement but a statement of fact to say that when the surgeon takes up his knife to cut into normal tissues everything has been prepared for operation except the structures immediately concerned.

There have been sound reasons for this state of affairs in the past. Tampering with tissues can only too easily bring on bacterial infection. Yet the increasing prevention and control of this latter by means of antimicrobial agents leads one to ask whether the preparation of cells to respond to a foreseen need for proliferation may not come to be a rewarding procedure.

The following observations bear on this possibility.

In experiments (1) on a tumor problem, sterile pieces of glass rod were placed day after day deep in the pectoral muscle of adult chickens, by means of a trocar, and 24 hours after the last piece had been inserted the fowls were killed and cultures were made in vitro of fragments of the tissue lying next each rod. No growth took place from those procured from about the rods inserted on the preceding day, and only large rounded cells, wandering cells attracted to the foreign body,—with a few fibroblasts in some cases,—emerged from the tissue next rods in place 2 and 3 days. But there was an immediate, profuse fibroblastic outgrowth from the tissue next the 4, 6, and 8 day rods, and so abundant was it as to be comparable with that from bits of embryo or of a spindle-cell sarcoma. The cells of the connective tissue had multiplied in response to the presence of the foreign body, and they were in such an excited state as to proliferate forthwith after transfer to the culture medium.

In a study of the cutaneous conditions favoring infection with Shope's rabbit papilloma virus, Friedewald (2) found that skin sandpapered until bare of all epidermis became covered with it again by extension of the epithelial cells from the hair follicles, and that coverage was completed several days sooner when the skin had been rendered hyperplastic by previous applications of inflammatory agents (turpentine, methylcholanthrene). Indeed the epithelium had thus been made so completely ready for the occasion that repair was far advanced after only 18 hours.

The experiments now to be reported were done to learn whether grafts of hyperplastic skin would do better than those of normal skin after transfer.
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Materials and Method

Rabbits were used as lending themselves better to standardization of the graft technique than the other creatures available. Sturdy adults of agouti strain, weighing 2.5 to 4.0 kilos, were chosen. Trials showed that skin could be most easily sliced off from the sides, in the region between thorax and haunch. Accordingly one side of each animal was swabbed in this region with an agent inducing hyperplasia, and some days later, when it had developed, pieces of the altered skin were procured, as also pieces from the corresponding area on the normal side. Each material was placed on several beds of raw corium lying near the backbone, and bandaging under pressure was done for several days. The rabbit was then anesthetized, the dressings removed, and India ink injected into the circulation to learn whether the grafts had become vascularized. Blocks were taken for later sectioning.

The technique as finally worked out was the following:

Some days before the grafting one side of the rabbit, from spine to belly, was clipped bare with an electric clipper, and the lower two-thirds of the exposed area was swabbed with a mixture of turpentine and acetone in equal parts, or with chloroform, or it was covered with a compress wet with broth. The swabbings were repeated in most instances at intervals of a few days. When the skin seemed ready for grafting the fur was clipped away from all around the trunk, the mid-dorsal line was marked with a skin pencil while the rabbit was in the normal crouching posture, and after it had been etherized the location of the pencilled line was permanently marked by stippling India ink into the corium at intervals, by means of a needle thrust through a drop. Then the skin from back to belly was gently scrubbed with tincture of green soap in warm water, dried with cloths, swabbed with 60 per cent alcohol followed by ether, and the animal was stretched on its normal side upon sterile towels over a warm electric pad.

A safety razor blade 5.5 cm. long (Durham duplex) on a metal handle with flattened cleats fitting into the slots of the blade, was used to obtain the grafts. An assistant thrust his hands under the animal's middle beneath the towels, raised the body as far as ties on the legs would permit, thus stretching the skin toward head and tail, and meanwhile grasped the trunk with his fingers and contracted his grip with result that a lateral stretching was also effected over tense underlying structures. Another assistant wet the skin with Locke's solution, levelled it and stretched it still more in the long axis of the body by pressing small boards against it, and the operator sliced off the grafts with a sawing motion of the knife. They were folded as taken, with the raw surface inwards, and placed in a Petri dish next a sponge soaked in Locke's solution, after representative portions of them had been set aside for section. The outline of the denuded area was now traced with a wax pencil on a stiff sheet of cellophane held over but not touching it, and it was dressed with a single layer of fine-meshed gauze which had been impregnated with paraffin (melting point 54°). Big rectangles of the gauze had been autoclaved in the paraffin, and while this was still hot each was gripped with forceps at two adjacent corners, held vertical for a moment to drain, and lowered to float on a dish full of sterile water which had been chilled to hasten solidification. Ordinarily the rectangles were prepared beforehand, stored in the cold, and cut to size when needed. The surface which had been next the water was smooth, and it was applied to the denuded area, and the edges of the rectangle were everywhere bound down to the neighboring skin with adhesive plaster.

Grafts of normal skin were obtained from the other side of the rabbit in the same way, and, after a tracing of the donor area had been made and paraffined gauze put on, the animal was placed on its belly with legs extended symmetrically and the stripping of the beds for the grafts was begun. They were 2.0 by 2.0 cm. square, overlying the lumbar muscles, three on each
side of the line of the backbone, as indicated by the stippled ink, and equidistant from it. To ensure that they were of identical size and that those on the right and left were at precisely corresponding situations, the corners of all were marked on the skin with dye before any incision was made. A wire instrument was used for this purpose, shaped like a trident except that it had four prongs with blunt tips so spread as to represent the corners of the square. To mark it the tips of the prongs were pressed into a gauze layer saturated with a 1 per cent alcoholic solution of brilliant green, and lightly touched to the skin. The upper sides of the squares thus dotted in were about 1.5 cm. distant from the mid-dorsal line, and each square was 1.0 to 1.5 cm. away from the next. They were stripped one after another.

The structure of rabbit skin (Fig. 1) has been well described by Medawar (3). It differs much from human skin. The epidermis is only 1 to 2 cells thick (Fig. 4), and is directly underlain by a tough and dense, exceedingly thick layer of corium. At some of the donor sites it was more than 2 mm. thick,—and 3 mm. where the graft beds were made. The hair follicles penetrate about a third of the way through it, not almost or quite to the subcutaneous tissue as in man. There are no sweat glands but tiny sebaceous ones open into the necks of the thickly scattered follicles. Firmly joined to the corium and forming the deepest part of the hide is the panniculus carnosus, a sheet of muscle with a well defined aponeurosis on its under surface but only rudiments on its upper,—to which the corium is directly attached. The nerves and many vessels course where the two join.

The hide is loosely moored to the body wall, and when it is cut through completely and a piece excised one finds merely a thin web of connective tissue overlying the muscles of the body wall proper,—a surface little suited to comparative observations on grafts because the hide shifts with each movement of the animal. Standard graft beds can be obtained however by utilization of the deep corium. Just above the panniculus its collagenous bundles are looser and smaller than they are further toward the surface (Fig. 1), and stripping at this level, when carefully done, results in an even bed.

The first step in preparing the bed was to cut the sides of the square, the incisions reaching almost to the panniculus, and then the skin was seized at the upper edge with tenaculum forceps and stripped away toward the belly,—in the direction, that is to say, which entailed least risk that the panniculus would be nicked and persistent bleeding started. When stripping was properly carried out there were no "bleeders," and the graft bed consisted of a level layer of collagenous corium which, itself nearly avascular, had many vessels ramifying not far beneath it next the panniculus carnosus. In some animals the corium was so thick that the exposed surface lay 2 mm. or more below the surrounding skin.

As soon as each bed had been got ready it was covered with a somewhat larger square of paraffined gauze, to prevent evaporation while the next was worked on, and when all six had been prepared the hyperplastic skin was put on three of them. The corresponding beds were covered later with normal skin. The placing of the two tissues was alternated from right to left of the spine, and they were handled with different instruments. When a single slice sufficed for a square, small V-shaped openings were clipped in it for drainage. Frequently one or more of the beds were covered with a mosaic of normal and hyperplastic skin, broad strips of the same size when possible, each with its long diameter transverse to the body axis. Usually

1 Medawar (3) has utilized such beds of corium in his studies of the fate of autologous and homologous pinch grafts of rabbit skin. He stripped large areas over the thorax of the animals and scattered a number of grafts on the raw surface, reporting that the thorax splinted these in an advantageous way. My experience has been that Thiersch grafts, and thick split grafts which are large enough to extend across both ribs and interspaces, fare very differently over them. Hence recourse was had to the lumbar region where an even pressure can be brought to bear against the mass of muscle.
the grafts overlapped the margin of the beds. Before they were placed the raw surface was allowed to dry for a minute or more, because otherwise they adhered poorly, and as soon as each bed had been covered a square of paraffined gauze extending beyond its edges was superimposed and sealed all around with adhesive plaster. Care had to be taken to make sure that there were no holes in the paraffin through which exudate might seep and stick to the outer dressings, for when this happened the graft was sometimes torn away from its base when they were removed. The strips of intact skin between the grafted squares provided just enough space for each to be separately dressed. A piece of gauze some layers thick, cut so that it would fit snugly into the slight depression of the grafted area, was fixed over the paraffined layer by means of a broad band of adhesive, and, when all had thus been attended to, a long rectangle of thick gauze was superimposed on the three squares on the same side of the spine, and the two rectangles were held in place with strips of adhesive running over the backbone and down to the edge of the paraffined dressings on the donor areas.

Rectangles of rubber sponge about 2 cm. thick were used to produce pressure. A long one extending across the spine was moored with adhesive plaster over the posterior squares, and a shorter one like a saddle pad was fixed on top of the two squares further forward on each side. Beveling the edges of the rubber made possible extension of the spine. A folded towel was now wrapped entirely around the body and a many-tailed binder put on which had holes for the forelegs. The tails of the binder were not tied but were cut to overlap slightly, and those opposite each other were bound together by means of broad bands of adhesive tape, after they had been drawn tight enough to produce the tension judged necessary to success of the grafts. In this way an even pressure was obtained such as knotting did not yield.

The grafts were not looked at until 2 to 6 days had passed. Then the animal was etherized again, placed on its belly with legs extended as before, the outer dressings were cut away, the adhesive strips around the paraffined squares were loosened with ether, and when all the grafted expanses had been bared, water-soluble India ink (Higgins), diluted with an equal quantity of Locke's solution and warmed, was rapidly injected into an ear vein until the animal died,—after the entrance of 25 to 50 cc. usually. Notes were made during the injection on whether and where the grafts showed ink.

Nothing further was done for some minutes to allow clotting of the blood, and then the three grafted areas on the same side of the backbone were excised with the skin about them, in a single piece, and this was stretched on a sheet of porous cardboard with care to avoid distortion. Direct tracings were made on cellophane of the dimensions of the squares, with notes on the situation and state of the individual grafts, after which the specimens were placed in a dish of acid Zenker solution. When fixation was complete several slices were taken through each square and the adjacent skin, in the direction of the long axis of the body, and the situation of these slices was marked on the tracing. Sections from the blocks thus obtained were stained with eosin and methylene blue, and in some cases every other section was colored with eosin only, to bring out more clearly where ink was present. Sometimes both sides of a block were cut.

The denuded donor areas were large, because sometimes a considerable part of the skin taken from them had to be discarded as unsuited to comparative tests; frequently they measured 10 by 5 cm. or more. At autopsy a representative slice was taken through the middle of each area, from front to rear, including the adjacent skin, to find out how far healing had progressed, and the situation of the slice was marked on the tracing made when the grafts were first procured. After fixation it was cut into blocks and sectioned.

The Fate of Grafts Rendered Hyperplastic by Turpentine

The method just described was worked out gradually, with a mixture of turpentine and acetone in equal parts to produce hyperplasia. The experiments
done by the way need not be described individually, but some of the facts they contributed deserve record.

Within 2 or 3 days after a swabbing with turpentine-acetone the rabbit epidermis builds up a layer of 5 to 10 superimposed living cells with numerous mitoses and more or less overlying keratin. The change from the normal state of affairs is so sharp that even the slightest hyperplasia can be readily perceived histologically. The corium immediately underlying the epidermis becomes turgid with fluid and somewhat more cellular, and the layer as a whole may thicken greatly if the turpentining is repeated, owing to swelling of the collagen bundles (which become more eosinophilic) and fluid accumulation between them. Yet it remains sparsely cellular in its depth unless the treatments are pushed so far as to injure its superficial portion seriously.

The hair follicles of the rabbit respond to inflammatory agents as those of man do not. They are soon much thickened by epithelial proliferation, and as this continues become widened at the mouth by accumulated keratin. They rapidly penetrate deeper in the corium, often more than twice as far down, though still not to the base of the layer. Nothing of this sort happens in human skin, which has follicles extending completely through the corium under normal circumstances. The changes are amply illustrated in Friedewald's paper (2). Because of them thick split grafts of hyperplastic rabbit skin often show on their under side a multitude of follicles cut across, whereas equally thick grafts of normal skin contain the follicles in their entirety and hence have an unbroken expanse of corium on the under surface. It follows that in comparing split grafts of hyperplastic tissue with the normal one must utilize slices of similar composition, not of similar thickness, or else induce a hyperplasia so superficial and mild that there will be no extension downwards of the follicles, or induce it just prior to the grafting, leaving no time that is to say for such extension to take place. All three methods have been employed.

Adult rabbits were found to differ markedly in their response to turpentine, the skin of some individuals becoming acutely inflamed, rugose, and several times as thick as normal after treatments which rendered that of others only mildly hyperemic, dubiously thickened, and slightly hyperkeratotic at most. These latter animals were in general the younger.

Most of the corium of normal human skin dies within a few days after grafting, as is well known. This happens even when union has been prompt with a very vascular bed, and obviously it occurs because the metabolic needs of the tissue have not been sufficiently met after transfer. The beds of rabbit corium utilized in the present work were not only poorly supplied with vessels but slow to respond with new ones and with proliferating fibroblasts. When they were stripped and not grafted, but packed with wet gauze to stimulate granulation, they showed on section almost no increase in cells after two days and very little after three. This being so, one might have predicted that grafts of normal
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rabbit skin would have a hard time when placed on such beds, and hyperplastic grafts a harder because of their increased demands. Actually the grafts of normal tissue, even very thick ones, fared well. They sat out the time, as one might say, until they were vascularized, showing no perceptible changes in the interval. Equally thick grafts of hyperplastic skin, on the other hand, often died almost in toto within 48 hours, only the epithelium in the lower part of the follicles surviving. The preliminary stimulation had not reached to the connective tissue cells along their under surface, which might have hastened a "take," but in stepping up the metabolic activities of the more superficial tissue had rendered it less capable of survival. Because of this finding most of the comparative tests had to be made with Ollier-Thiersch grafts and rather thin split grafts in which the follicles had been cut across. Occasional observations showed, however, that hyperplastic grafts thick enough to contain the follicles in their entirety would unite swiftly with the bed and succeed well if closely apposed thereto (Figs. 12 and 13).

The corium of rabbit skin is much more resistant to the knife than that of man, and the grafts of normal tissue were very difficult to obtain in some instances. Hyperplastic donor areas, on the other hand, yielded grafts readily; large sheets could be procured from animals from which only irregular small slices of normal skin could be got. The superficial turgor incidental to the hyperplasia made it possible to cut thin grafts easily, indeed just beneath the surface epithelium. Not only this, but the slices underwent relatively little contraction before they were placed on the graft bed, its amount varying inversely with the degree of hyperplasia present. As happens in man, Thiersch grafts and thin split grafts of normal skin contracted so much after they had been cut that they had to be considerably larger than the areas to be covered, and later shrinkage sometimes bared part of the beds on which they had been put. Also the thin sheets of normal tissue curled inwards at the edges. With the hyperplastic tissue, these difficulties were much less considerable. In two experiments amongst those now to be described, in which thin split grafts of turpentine, moderately hyperplastic tissue succeeded better than normal grafts of similar constitution, pantograph measurements showed that the amounts of skin of the two sorts which had to be procured to cover the standard beds were as 5 to 8. But the less the hyperplasia, the less the difference, though curling inwards of the grafts did not take place even when it was least. Very hyperplastic grafts proved friable and did not shrink at all.

After the standard grafting technique had been worked out, six experiments were done to determine the usefulness of skin prepared with turpentine. Sections of it showed in every instance outspoken hyperplasia.

Experiment 1.—Rabbit 22-15 was swabbed on one side with turpentine-acetone 10 days and 8 days before the grafts were procured. The local inflammation was subsiding when they were taken, yet the slightly scurfy skin was half as thick again as the normal on palpation.
The rabbit was killed 3 days after the grafts had been put on. Those of normal skin merely lay apposed to the beds, whereas the hyperplastic had united with them in most places and was vascularized, as ink injection proved. There was superficial purulence at some spots on the latter though.

Experiment 2.—Rabbit 22-16 was swabbed with the turpentine-acetone mixture 7 days and 5 days prior to operation. When this was done inflammation of the treated skin was on the wane, it was definitely though not markedly thickened, and showed a fine, branny desquamation. Six days later the animal was killed. The graft beds had been uneven, here deep, there shallow, and consequently apposition was poor at many places and no informative comparison of the grafts was obtained. But a singular phenomenon, never encountered with grafts of normal skin, testified to the capabilities of the hyperplastic epithelium. Where a slice of turpentine tissue with severed hair follicles along its under side had been placed over a nick in the panniculus carnosus, the epithelial cells had grown down from the follicles amidst the fibers of voluntary muscle and replaced these individually, as the cells of highly malignant carcinomas sometimes do (Figs. 3 and 9).

Experiment 3.—The skin of rabbit 22-17 was over-prepared; it had been painted with turpentine-acetone 6 days, 3 days, and 2 days prior to transfer, and had become ruddy pink and redundant. The epidermis in representative sections of the graft material was much thickened at some spots but at others was either badly damaged or actually necrotic, and there was pronounced edema of the superficial corium with increased cellularity. Grafts of the tissue thus changed were found to have died almost in toto after 2 days, and the beds on which they lay were very moist and somewhat inflamed. The grafts of normal skin on the other hand appeared unchanged morphologically, but they had not united with the underlying tissue.

Experiment 4.—Rabbit 22-18 was a well grown young animal with relatively thin skin. It was turpentined 4 days and 1 day prior to operation, and when this was done the hyperplastic donor area was bright pink and slightly thickened. The grafts provided by this latter had become attached to the beds and well vascularized 3 days later, whereas those of the normal skin were still merely apposed.

Experiment 5.—Rabbit 22-22 died of an undetermined cause 48 hours after the grafting. Its skin had been turpentined only once, 3 days previously, and was slightly thickened and fading pink. The grafts from the prepared donor area were well apposed and appeared to be alive, but none had "taken" nor had those of the normal skin.

Experiment 6.—Rabbit 22-21 was turpentined only once, yet the skin, when sliced off 5 days later, was still considerably swollen, brightly hyperemic, with a light, flaking scurf. It was procured with unusual ease in big pieces and so too with the normal skin. Some of the slices of both sorts were so thick as to contain the hair follicles in their entirety. Thick or thin, the grafts of hyperplastic tissue did far better than the similarly constituted controls. When the animal was killed after 4 days they had healed in place and were well vascularized (Fig. 12), whereas the slices of normal tissue had begun to unite with the base at a few spots only.

The animals of these experiments were killed not long after the grafting, because it seemed likely that any advantage due to hyperplasia of the cutaneous tissue would be most clearly apparent then. There were other reasons too, namely the hazards incidental to removing and replacing the dressings repeatedly, and the likelihood that any bacterial infection appearing at one spot in a graft would spread to the rest of it as time went on, if the original dressing were left undisturbed. Small foci of infection were not infrequently present after 3 or 4 days.

When the transferred tissue was kept well apposed, the beds covered with it
retained their initial size. On examination after 3 or 4 days the grafts of normal skin were found free from exudate, smooth, and more or less translucent, depending on their thickness, but were still ununited with the bed in most places. They not infrequently came away when the dressings were removed, or when blocks were cut through them and the bed after fixation, but sometimes they had “taken” at a few spots, as ink in the vessels showed. Even where they overlay the surrounding skin, or when they had been put on upside down, as occasionally happened when small pieces were fitted into a mosaic, they appeared unchanged microscopically save for a slight swelling of the epidermal cells. Occasionally a thin, migratory extension had taken place, of flat epithelial elements from the edge of the graft to an adjacent surface, like that when a cutaneous wound begins to heal (Fig. 2), or from the severed lower ends of the follicles along a cleft beneath the graft and its bed; but otherwise no epidermal activity could be perceived. Nor had any considerable extension inwards taken place of the epidermis from the side of the beds to cover raw areas from which the grafts had contracted away, although a sharp knife instead of scissors had been used to outline the squares in order to minimize the epithelial damage. The unfavorable character of the bed of corium must have been responsible for the long delay in “taking,” for, at spots where the panniculus had accidentally been cut into and the graft laid directly upon it, union had taken place within 3 days and vascularization was well under way as proven by ink-filled vessels.

Very different were the findings with the grafts of hyperplastic tissue. After 3 days they appeared thickened, semi-opaque, sometimes furry with heaped up keratin, and often moist. On ink injection they turned gray, and microscopically they proved to be well united wherever apposition had been good. The semi-opacity noted in the gross was due in part to greater initial thickness of the slices,—on comparison with those of normal skin containing the same components,—but mostly to proliferation of the epidermis during the period before the circulation was reestablished, an activity the more remarkable because the patches of skin left behind on the hyperplastic donor area underwent concurrently a considerable reversion toward the normal. Where holes had been cut in large hyperplastic grafts, as vents for possible exudates, or where the beds had been incompletely tessellated with small pieces, the epithelium had extended across bare spots in a thick layer containing many mitoses (Fig. 2). Most of the beds appeared slightly more cellular and vascular than those on which normal skin had been put, and occasionally some vessels were distended with clot.

The superiority of the hyperplastic grafts was most convincingly plain where they had been interspersed with pieces of normal skin, or where, owing to irregularities in the preparatory treatment, the same graft contained normal as

\[^{2}\] A pronounced growth of hair had occurred on the hyperplastic grafts of rabbit 22-21 (Experiment 6) killed after 4 days, and some hair also, but much less, had appeared on the grafts of normal skin.
well as hyperplastic areas. The differences are not pictured because they find better exemplification in the case of grafts rendered hyperplastic by means of broth compresses (Figs. 6 and 8). In one of the preliminary tests with turpentine a rabbit carrying a thick graft of hyperplastic tissue was allowed to live for 16 days after operation. The graft,—which contained entire hair follicles,—proved to be in excellent state then (Fig. 13). Its epithelium was still somewhat hyperkeratotic.

These were the findings where perfect apposition had been obtained. But when a bit of hyperplastic tissue had been put on upside down in making a mosaic, or where a graft of such tissue extended onto the surrounding skin, it was wholly dead after 3 days, a period well tolerated by slices of normal skin, as already remarked. Necrosis was less complete when the graft was separated from its base by fibrin or blood clot. Sometimes then the more superficial portion of the hyperplastic epidermis had died and separated, a thin, living skim of basal epithelium remaining; but in other instances only the epithelium of the deeper portion of the hair follicles survived. Follicle cells are amongst the most resistant elements in human grafts and those of the dog (4).

The dead tissue provided a nidus for infection, and this frequently took place. As may be recalled, only soap, alcohol, and ether were used to cleanse the skin, which was handled gently. Scattered purulent spots were not infrequent after 3 days on the surface of grafts of normal tissue, but they were more frequent and larger on those that were hyperplastic. The infection had usually begun at the mouths of hair follicles, which frequently were distended with keratin when the hyperplastic skin was transferred, and occasionally showed epithelial karyorrhexis and small collections of pus cells. Now and then after the grafting infection extended down into the corium although a "take" had occurred. In some cases the epidermal cells of hyperplastic grafts proliferated so rapidly beneath a layer of superficial purulence as to separate it from the graft proper, infection being outgrown in the literal sense of the word. A mass process of this sort was observed on a hyperplastic donor area in one of the experiments contributory to method. The raw surface had quickly become purulent, but soon the epithelium of the hair follicles extended so profusely to cover it that the layer of pus was cast off.

Whenever clefts existed between a hyperplastic graft and its bed, and severed follicles were present on its under side, the epithelium from these latter proliferated rapidly to fill the gaps (Figs. 2, 10, and 11). Often in such cases a thick layer of cells formed beneath transferred tissue which had "taken" well. Sometimes the follicular epithelium of thick hyperplastic grafts which had for the rest undergone massive necrosis covered the entire surface of the bed (Fig. 10).

**Chloroform as a Preparatory Agent**

Rabbit skin swabbed with chloroform becomes bright pink and slightly swollen within a few hours, and sections procured 2 or 3 days later show that
hyperplasia has taken place. Three experiments were done to learn whether
skin thus treated might not be in a better state for grafting than that prepared
with turpentine. Incidentally an effort was made to stimulate the beds of
corium by packing them with wet gauze for 1 or 2 days before the transfer to
them of normal tissue.

Experiment 7.—The injurious effect of chloroform was underestimated in this test. It was
swabbed on the side of rabbit 22-11 on the 8th, 7th, 6th, and 5th days prior to the grafting, and
the skin was twice as thick as normal and still pink, with a desquamating light scurf at time of
operation. Two days beforehand the usual six beds were stripped, with the rabbit etherized
(as in the experiments which follow), and two of the squares on each side of the spine were
packed with gauze wet with saline and sealed in with paraffined gauze. The third was merely
covered with this latter. When the grafting was done mosaics of interspersed normal and
hyperplastic tissue were put on some of the beds, whereas tissue of one sort only was placed on
others. The animal was killed 4 days later. The normal grafts were in good condition and
well apposed but had not united with the beds. The hyperplastic grafts were in contrast very
moist, purulent in spots, and the thickest ones had died in toto. But those which were thin
had “taken,” as sections disclosed, and where there were gaps between them or holes had been
purposely cut in them, the bare surface was covered with new epidermis. At some purulent
spots the epithelial cells were proliferating actively amidst the pus.

No definite increase in the cellularity of the graft beds had resulted from the preliminary
stripping, and those which had been packed with gauze did not differ histologically from those
covered merely with a paraffined sheet.

Experiment 8.—The effect of a single treatment with chloroform was tested next. Rabbit
22-19 was swabbed 4 days prior to the grafting, and the beds were bared 1 day beforehand.
Four of them were covered with paraffined gauze, the other two packed with gauze wet with
saline. When the grafting was done the erythema of the treated skin had almost faded away
but it was still somewhat swollen, and sections showed a moderate hyperplasia. Coverage was
accomplished as in Experiment 7, and 2 days later the rabbit was killed. Already the normal
skin had begun to “take” at a few points, though ink did not get into it, whereas all of the
hyperplastic grafts, most of them thin, were dead, and in the gross looked as if cooked. The
preliminary stripping and packing of the graft beds had not rendered these definitely more
cellular.

Experiment 9.—The skin of rabbit 22-20 was utilized after the acute inflammation due to
several applications of chloroform seemed to have almost entirely worn off. It had been ap-
plied on the 14th, 13th, 12th, and 11th days prior to the grafting, and 2 days beforehand the
beds were stripped, and one on each side was packed with wet gauze and the other two covered
with paraffin squares. At time of transfer the treated skin was slightly pinker than that round
about, appearing normal otherwise; yet sections showed the epidermis to be lacking at a few
spots while at others there were minute necroses, with clustered polymorphonuclear cells. In
general though the layer was hyperplastic. Mosaics were made of the normal and treated skin,
and 2 days later the animal was killed. None of the ink injected at this time got into any of
the grafts. Those of normal skin were, as usual, translucent and free from exudate, and they
may have begun to “take,” whereas the hyperplastic grafts were opaque, swollen, and wet,
purulent here and there, and the thicker ones were obviously necrotic. The thin ones, how-
ever, were uniting with the beds, the epidermal cells proliferating actively even where there
was pus. The preliminary stripping and packing had been followed by as little cellular change
as in the previous instances.

In these experiments the preparation of the skin with chloroform had disas-
trous results. Not only did thick grafts of the hyperplastic tissue die _in toto_ but purulence was frequent, even in the case of those that were thinnest. The hyperplastic epithelium showed a noteworthy ability to multiply amidst the pus.

The skin which had been left undisturbed on the donor areas prepared with chloroform appeared practically normal in the gross when the animals were killed, and where it had been sliced off repair was far along in two of the three instances. In the third the raw surface was purulent, as was also that from which normal skin had been taken.

_Preparation of the Skin with Broth Compresses_

Several investigators (5) have reported that the application of broth compresses for a few days to the skin of rabbits or guinea pigs results in a heightened local resistance to bacterial infection. Freedlander and Toomey (5) found that when compresses had been applied to the skin of guinea pigs for 48 hours the epidermis was 4 to 8 cells thick, instead of the normal 2 to 4 cells, and there was edema of both corium and subcutis, with some increase in the cellularity of these tissues and vascular dilatation. The injection of _Staphylococcus aureus_ into skin thus changed resulted in well circumscribed abscesses, whereas generalized infection and death occurred in the majority of the controls and extensive, destructive inflammation in the others. Because of these findings an experiment was done to learn the effects of grafting rabbit skin to which compresses had been applied.

**Experiment 10.—**In a preliminary test for the production of hyperplasia, a compress measuring 7.5 by 10 cm., consisting of many layers of gauze wet with broth, was placed on one side of a rabbit and covered with a rectangle of rubber sheeting bound down around the edges with adhesive plaster. A similar, larger sheet was superimposed and fixed in the same way, and a many-tailed binder was put over all. The broth, made up according to Mallory and Marble’s formula (6), contained Liebig’s beef extract and had been sterilized in the autoclave. Some leakage of it occurred along the lower edge of the dressings, and, to make up for this, 15 to 20 cc. more was run into the gauze through a rubber tube after 24 and 48 hours.

The compress was removed after 3 days. The skin under it appeared paler and felt somewhat thicker than that elsewhere. Sections of it showed a moderately hyperplastic epidermis with numerous mitoses and a hyperplastic broadening of the upper part of the hair follicles. The corium was thicker than normal, its collagen bundles were slightly swollen, and they stained deeper with eosin.

With this evidence that mild hyperplasia could be readily induced, the experiment proper was undertaken. A compress measuring 9 by 14 cm., wet with broth, was placed on the side of rabbit 22-23, covered with rubber sheets and a bandage, as in the previous test, and kept moist by running 25 cc. of broth into its upper edge each morning, and 50 cc. each late afternoon, with syringe and trochar.

When examined after 68 hours the skin was pinker than elsewhere, slightly redundant, and dubiously thicker. It was left uncovered and 24 hours later grafting was done. The pink hue of the treated area was fading by then, but the tissue sliced away seemed slightly edematous, and contracted much less after removal than did corresponding slices of the normal skin.
Large sheets were readily procured from both donor sites. Strips of both materials were placed side by side on two of the beds, whereas the others were covered with one of them only.

The rabbit was killed by ink injection after a further 3 days. A little of the ink entered the grafts of normal tissue here and there, but the microscope showed that as yet they had united with the bed at very few spots (Figs. 4-6). The treated grafts (Fig. 8) appeared semi-opaque and furry, not translucent and smooth like the transferred normal tissue, and they became gray with ink. Nearly everywhere they had joined the beds, as the microscope disclosed, and the ink was contained in numerous, large, thin-walled vessels (Fig. 8). The sections illustrating the contrast came from grafts lying side by side on the same bed. On those of both sorts there were areas of superficial purulence and these were more numerous and larger on the hyperplastic tissue. Where the latter overlay the skin surrounding the beds it had died and was purulent, whereas the normal tissue at similar situations appeared unchanged. Cultures from some of the purulent spots yielded white or lemon-colored staphylococci.

Sections showed the treated epidermis to have been moderately hyperplastic when grafted, as was also the epithelium of the hair follicles (Fig. 7). These had not as yet penetrated deeper in the corium,—which showed only a dubious increase in cellularity. The slices of normal and treated skin proved well suited for comparative tests; the hair follicles in both had been cut through near the middle at time of transfer (Figs. 4 and 7).

The epidermis of the normal skin thickened in the days after it was transferred and its cells became somewhat more numerous (Figs. 4-6), but the contrast with the treated grafts remained great. The epidermis of these latter built up still further in the days before the animal was killed (Fig. 8), whereas it reverted so rapidly toward the normal on the part of the donor area from which no slices had been taken that only a thin overlying layer of keratin attested to its previous activity.

This experiment demonstrated that mild measures would suffice to activate the skin for graft purposes. The slices of prepared tissue "took" sooner than did those of normal skin and received a notably abundant blood supply; but as against these advantages they became infected oftener.

**Healing of the Donor Areas**

Friedewald (2) has pictured the reparative changes taking place after the epidermis has been sandpapered away from rabbit skin. As already stated the raw surface becomes covered by lateral extension of the epithelium from the hair follicles. After the removal of grafts repair follows the same course, its rate depending on how many follicles have been left behind. The coverage of donor areas in human beings takes place in a similar way (7), but in its case sweat glands participate in the process.

The donor areas rendered hyperplastic in the course of the present work healed so rapidly when the skin had been sliced off through the hair follicles that they appeared dull and grayish after 3 or 4 days, because already covered with a layer of new epidermis several cells thick. They seldom became infected. The areas which had supplied normal skin on the other hand, though denuded to only the same extent, were still raw, glistening, and moist after this time, and the microscope showed that merely a thin skim of epithelium, migratory in character, was extending from the follicles. Not infrequently the raw
surface had become purulent everywhere. These differences held, irrespective of how the hyperplasia had been induced; they were pronounced in the animal prepared with a broth compress.

The follicles penetrate such a little way into the corium of normal rabbit skin that sometimes all were sliced off inadvertently here and there in taking thin grafts, and where this had happened the only source of new epithelium was the follicles left in the surrounding region where the cut had not gone so deep. Healing was exceedingly slow at such places in consequence, as under the same conditions in man (7), and local purulence sometimes developed, though more often it affected the whole raw surface, as just stated. It occurred also, though less frequently, where the knife had gone deep into hyperplastic donor areas, but ordinarily these showed no sign of infection anywhere else. Cultures of the pus yielded lemon-colored or white staphylococci.

A little more oozing took place from the hyperplastic areas after grafts were sliced away than from the almost bloodless surfaces which had been exposed on donor areas of normal skin, but pressure promptly stopped it.

The corium of the hyperplastic donor areas did not become perceptibly more cellular during the brief interval before it was covered again with epidermis.

DISCUSSION

More than three-quarters of a century ago Thiersch asked (8) whether better success might not be had in the grafting of skin if the cells of the apposed surfaces were already in an excited state. He recalled in this relation that the guild who had carried through remarkable plastic operations in India during previous centuries always pounded the skin with wooden slippers until it was considerably swollen before they utilized it. But then it was placed on a newly stripped, unprepared bed. Thiersch was convinced that grafts of normal skin did best on a bed of inflamed tissue, and he advised that the bed be treated beforehand in a way to induce inflammation whenever this had not already set in. Only experiment, so he said, could disclose what would happen if the cells of both graft and bed had been stimulated. Following up his thought, he snipped away fragments of regenerating epidermis from the edge of healing wounds and scattered them on the granulating surface with good results (9),—a procedure to which others have now and again resorted.

In this day the fact is recognized that transferred skin “takes” best, other things being equal, on a bed of vascular, responsive tissue. But no test seems to have been made of what will happen if the cellular components of the graft itself are stimulated prior to transfer. The present work makes clear that slices of rabbit skin, rendered hyperplastic by irritants, unite with the underlying tissue sooner than normal skin of the same individual and obtain a blood supply more promptly and abundantly. The activated epithelial cells proliferate practically at once to cover adjacent raw surfaces with a thick layer:
there is no lag of several days as ordinarily. Given the chance, they may invade voluntary muscle and replace its fibers individually, like carcinomatous elements (Figs. 3 and 9). No study has been undertaken of what happens to the corium of the hyperplastic graft. It has sufficed to find that union with the graft bed takes place with unusual rapidity (Figs. 5, 6, and 8).

Other advantages of hyperplastic grafts were noted. They were readily sliced off, as is not true of grafts of normal rabbit skin, and they could be cut exceedingly thin, so that they consisted almost wholly of surface epithelium,—approximating the ideal of Ollier-Thiersch grafts. Also they underwent considerably less contraction than similar slices of normal skin, and in consequence less tissue was needed to effect coverage and the grafts remained better apposed. The hyperplastic donor areas healed with extraordinary swiftness after the grafts were taken, and in so doing reduced the opportunity for bacterial infection,—which occurred with relative infrequency.1 A second crop of grafts could have been procured from the healed areas much sooner than from those which had previously been normal.

As against these advantages of hyperplastic tissue, some grave hazards and limitations have declared themselves. They will be recapitulated because they have broad implications:—

The metabolic needs of the thick hyperplastic grafts were so imperative that they often died in toto within a period readily tolerated by equally thick slices of normal skin. The latter were in a better state to “stick it out” on the bed of dense, almost avascular corium. Yet certain instances clearly showed that death of the thick hyperplastic grafts was avoidable, being due to the unresponsive character of the bed on which they had been placed. In these instances the corium had been stripped away almost to the blood vessels overlying the panniculus carnosus, and on the base thus prepared activated thick grafts did excellently (Fig. 12).

So slow was the response of the ordinary graft beds of dense corium that packing with gauze could not be kept up long enough to render them more cellular, bacterial infection supervening. For this reason the experiments have failed to meet Thiersch’s requirement, that both wound surfaces involved in the grafting be in a stimulated state. The great thickness of the corium limited the favorable effects of activation to Thiersch grafts and split grafts cut no deeper than the lower ends of the follicles,—which, though burrowing down when hyperplastic, extended little further at most than half way through the corium. In some preliminary tests for the standardization of method rabbit skin was turpentinized repeatedly until very hyperplastic, with the aim of stimulating even its deepest regions, and then was grafted in its whole thickness. Calamity followed; practically all of the transferred tissue quickly died. The influence of

1 There may have been another reason for this infrequency,—the enhanced local resistance of the cutaneous tissue, which follows upon the application of mild irritants,—including broth compresses (5).
the turpentine had not reached through the corium, and consequently no activation had been induced of the tissue on which union with the base depended. The excitation of the superficial cells of the graft had but increased their needs, rendering them more liable to die when deprived of a blood supply.

The responsive state of the hyperplastic epithelium sometimes provided a singular complication, deep lateral extension of a thick layer of cells from the hair follicles along spaces existing between the graft and its bed (Fig. 11). Spread of this sort was frequently encountered in the experiments done before a technique had been worked out to keep the grafts closely apposed, and more or less sequestration of them would inevitably have resulted had the animals been kept alive. The opportunities for deep lateral spread of the epithelium were notably great when hyperplasia had existed for some time before the grafting was done; for then the hair follicles were not only much thickened by cellular proliferation but, having extended deep, were cut across in taking even fairly thick grafts. Yet whenever good apposition was maintained no lateral growth took place from them. In some cases, of ill-placed thick grafts dying early save for the lower part of the follicles, the spread of cells from them led to such thick coverage of the underlying bed that the purpose of the graft would not have been wholly lost when it came away (Fig. 10).

It had seemed possible, when the experiments were undertaken, that hyperplastic grafts would become infected less often after transfer than those of normal tissue, because of the heightening of local resistance which results from treatment with mild inflammatory agents (5). On the contrary they became infected more often. One reason for this was the necrosis occurring whenever they were not promptly vascularized, and another was the protection afforded bacteria by surface scurf and keratin in the widened mouths of the hair follicles. The hyperplastic epithelium showed a remarkable ability to proliferate amidst pus, but whether because it had become more resistant after injury, as is the case with the regenerating epithelium of renal tubules (12), or merely because the preliminary stimulation impelled it to multiply, has not been determined.

No attempt was made to employ antimicrobial agents in connection with the grafting because of the complications they would have introduced, but instead reliance was placed on the natural resistance of the skin,—which in rabbits is considerable,—and only cursory attempts were made to sterilize it. When inflamed it could not be briskly scrubbed without injury.

Medawar (3) has reported that the revascularization of pinch grafts of rabbit skin scattered on a bed of corium is accompanied by mild inflammation, and that

4 Enderlen and Marchand (10) have reported the occurrence of deep lateral extensions under Thiersch grafts of human skin which had been separated from the beds by exudates, and according to Enderlen (11) they were derived in part from glandular structures which had been cut across. Brown and McDowell (7) have described sebaceous collections as developing under thick split grafts especially, and believe them due to accumulated secretion from the cut ends of glands, or to an inadvertent covering over of scar epithelium or of deep glandular epithelium already in situ when the grafts were placed.
this causes some of the new capillaries to become distended with stagnant blood, loss
of them following within a few days. This might very well have been the fate of
some of the many wide, thin-walled vessels to be seen in grafts of hyperplastic skin
which had taken well (Fig. 8). Medawar’s grafts succeeded, and so too did the only
hyperplastic graft of the present work which was left in situ for a considerable time.
After 16 days this graft was still hyperplastic and some of the hair follicles contained
much keratin (Fig. 13). Grafts of normal human skin become hyperkeratotic during
the first weeks after a “take,” as is well known.

The means employed to induce hyperplasia were drastic, save in the case of
the broth compresses, and they did unnecessary damage. The early death of
the grafts treated with chloroform, or too often turpentined, discloses how
largely blood supply determines the outcome when skin is exposed to injurious
chemical agents. An exposure which induces only moderate inflammation and
hyperplasia, from which recovery will be rapid under ordinary circumstances,
may be followed by necrosis if the circulation is shut off, as during the first days
after grafting. The observation is not without a bearing on the treatment of
cutaneous diseases.

From the observations as a whole, it is plain that any attempt to utilize
stimulated human skin for grafting purposes would require the utmost care to
avoid excessive stimulation or other injury to the tissue, and to prevent bacterial
infection. It may well be that activation will remain a principle, not be-
come a practicality.

SUMMARY

Rabbit skin rendered hyperplastic by various agents showed less tendency
than normal skin to contract when sliced off, and when used for grafts it united
with the bed more rapidly and was vascularized sooner. The stimulated epi-
dermis proliferated practically at once, and abundantly, to cover adjacent raw
surfaces. Also the donor area healed much more swiftly than usual and be-
came infected less often.

Certain grave limitations and hazards encountered during the experiments
with hyperplastic grafts are considered.

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EXPLANATION OF PLATES

All of the sections were stained with eosin and methylene blue. The photographs were made by Mr. Joseph B. Haulenbeek.

PLATE 5

FIG. 1. Section through the hide of a rabbit at the location of the graft beds. It consists of an exceedingly thick skin overlying and directly attached to a shallow layer of voluntary muscle (panniculus adiposus). The panniculus has a well defined aponeurosis on its lower side. The vessels have been injected with India ink.

The skin consists almost entirely of dense, collagenous, nearly avascular corium. It is very thick (almost 2.5 mm. in the present instance). Its epidermis is shallow, consisting of a layer of 1 to 2 cells, as shown by high magnification (Fig. 4), and the hair follicles extend only about a third of the way into it. Nerves and large vessels ramify in the plane where corium and panniculus meet, and just above them toward the skin surface the collagen is looser than elsewhere. The graft beds were made here. \( \times 32 \).

FIG. 2. Epidermal* extension from adjacent grafts of normal and hyperplastic skin. They had been sliced so thin as to be fenestrated, as the picture shows. The hyperplasia had been induced with turpentine (Experiment 6); specimens procured 4 days after grafting.

The epithelium covering the transferred normal skin on the left of the picture has not thickened but has extended to the under surface of the graft as a shallow layer of flattened cells which stain poorly with eosin. Its state contrasts sharply with that of the epidermis of the hyperplastic skin on the right. This has formed a thick, dark-staining layer under the graft, and has extended laterally to cover the exposed surface toward the left as far as the arrow A. In so doing it has met and checked the downward extension of normal epidermis to be seen at the center of the picture (arrow B). The difference in the staining capacity of the two is very evident where they have joined. \( \times 69 \).

FIG. 3. Replacement of voluntary muscle fibers by the epithelium of grafted skin previously rendered hyperplastic by swabbing with turpentine (Experiment 2); specimen procured 3 days after the operation. In the region shown the graft has everywhere united with its bed, and on the right of the picture it lies directly on the panniculus carnosus. Here the cells of the lower ends of the hair follicles have proliferated downwards, invading and replacing the muscle fibers individually. These latter have been cut across. An earlier stage in such replacement is shown in Fig. 9. There are several distended vessels in the panniculus, which contain ink, mostly along its lower border. \( \times 55 \).
Rous: Activation of skin grafts
FIGS. 4 to 6. To show the condition of grafts of normal skin 3 days after transfer (Experiment 10). × 93.

Fig. 4. Section of the graft material as procured. The hair follicles, which lay at a slant with the corium (Fig. 1), have been cut across this slant about midway down. The epidermis consists of a single layer of cells with a little overlying keratin.

Figs. 5 and 6. Two regions in a graft which had been in place 3 days. Arrows mark the surface of the bed. The epidermal layer appears swollen and its nuclei are closer together than in Fig. 3. In Fig. 4 the graft is somewhat thicker than the specimen of Fig. 3, whereas in Fig. 5 it is thinner. Nowhere has it acquired a blood supply, as proven by the absence of India ink from the vessels, yet it appears to be alive; it lies well apposed to the underlying corium, yet has scarcely begun to unite with this. Here and there in the bed large, thin-walled vessels can be seen containing corpuscles and ink intermixed, but its vascularization is in general scant.

To be compared with Fig. 8, of a hyperplastic graft lying next on the same bed.
PLATE 7

Figs. 7 and 8. The fate of hyperplastic skin; from the animal providing Figs. 4 to 6. The magnification is the same, × 93. The hyperplasia had been produced with broth compresses (Experiment 10).

Fig. 7 shows a slice of the graft material. The hair follicles have been cut through at approximately the same level as in the control grafts (Figs. 4 to 6), yet because of swelling incident to the hyperplasia the slice has nearly twice the thickness of the controls (Figs. 4 to 6). The epidermis is 4 or 5 cells thick, and the follicles are much broadened by epithelial proliferation. The corium is little if any more cellular than that of the normal skin.

Fig. 8. Part of a graft of the hyperplastic tissue 3 days after transfer to a bed covered for the rest with a graft of normal skin (Figs. 5 and 6). Arrows indicate the surface of the bed. There are some crevices between it and the graft yet this has “taken” well and contains numerous distended vessels with ink in them. The epidermal hyperplasia is more marked than when the graft was procured (Fig. 7), but fewer cells are visible in the corium,—in which respect the findings resemble those with the grafted normal skin.

Fig. 9. Replacement of voluntary muscle by the epithelial cells of a hyperplastic graft; an earlier stage of the process exemplified in Fig. 3, and from the same animal. The skin surface lies vertically along the left side of the picture.

The epithelial cells of the cut lower ends of the hair follicles have proliferated into an irregular mass from which they extend in tongues (as serial sections show), and are replacing the fibers of the panniculus. This had been irregularly hacked into, as indicated by the arrows which mark the surface on which the graft was put. One of the muscle fibers is surrounded and partially replaced by epithelial cells, and another deeper down, which had dwindled in size, has been wholly replaced by them. × 122.
PLATE 8

Fig. 10. Epithelial proliferation from the cut lower ends of the follicles of a thick hyperplastic graft which failed to take; specimen procured after 6 days (Experiment 2). The graft had been placed on a bed of thick corium and was poorly apposed. It died in toto except for the follicular cells. These provided a thick covering for the underlying bed by deep lateral proliferation. × 30.

Fig. 11. Section through another hyperplastic graft from the same animal. It has “taken” well on a bed deeper than that shown in Fig. 10, and its thick, living epidermis is overlain by much keratin. A profuse lateral proliferation from the cut lower ends of the hair follicles where the graft was not closely apposed now separates the latter from its base at many places. × 30.

Fig. 12. A thick split graft which has “taken” well; it contains the hair follicles in their entirety. The specimen was procured 4 days after transfer (Experiment 6). It showed numerous vessels containing ink. × 55.

Fig. 13. A thick, split graft of hyperplastic skin 16 days after transfer; from one of the preliminary experiments.

The skin had been turpentined 5 days and 3 days before it was sliced off, and the graft here shown contained the hair follicles in their entirety. It was placed on an uneven base of corium. Epidermal hyperplasia is still present. The gaps near the muscle are artefacts. × 15.