1961

Paul Weiss, 1959

The Rockefeller University

THE BIOLOGICAL FOUNDATIONS OF WOUND REPAIR*†

PAUL WEISS

Member and Professor, The Rockefeller Institute, New York, New York

TO present a Harvey Lecture is not only a privilege, but a real opportunity: an invitation to take leave from the minutiae of laboratory work and to present instead, in bold perspective, a synthesis and forward projection of trends of research and thought. The boldness—or crudeness—of such a bird's-eye-view of a field of study is in direct proportion to either our power of abstraction or our blurred vision in not having perceived the details to begin with. Valid abstraction brings great intellectual and esthetic satisfaction, compared to which the mere correction of defective vision seems an uninspiring task. Nevertheless, in dealing with the living organism, the more we learn about it, the more we realize that a view from ever greater distance only too often leads to generalities, rather than useful generalizations; that there is no alternative to a realistic study of its detailed mechanisms; and therefore, that progress often will hinge on taking a closer, rather than a more distant, look. In this presentation, I mean to document this fact; foregoing thus the tempting opportunity to treat myself to an exposure of the broader and bolder generalizations in which I have at times indulged.

A living organism is an immensely complex machinery. Merely to state this is a platitude. Yet, to resolve that complexity remains the perennial challenge and task of analytical research; resolve it, not just obliterate it or gloss over it by physical or mental homo-

* Lecture delivered October 15, 1959.

† Elements of this lecture were previously presented in a Litchfield Lecture at the University of Oxford, and in lectures at Albany Medical College and at the Istituto Superiore di Sanità in Rome. Some of the experimental investigations referred to have been aided by grants from the American Cancer Society and the National Institutes of Health (United States Public Health Service).

genization. Success with disentangling the complicated machinery of the organism is rapidly advancing our knowledge of its composition, structure, and functioning. Compared with this, our insight into the mechanisms of development—the understanding of just how that complex machinery elaborates itself in orderly fashion from the infinitely simpler egg, and once matured, keeps itself continuously in renovation and repair—is still in a most fragmentary state. Perhaps there has been too much of a monopoly of attention given to the egg and early germ. Development continues throughout life, and many of its components appear in more elementary form, hence lend themselves more readily to analysis, in later stages. The repair of tissue damage by healing and regeneration is one of these.

Not only is wound healing an important source of knowledge about development, but what we learn about development, in turn, furthers the medical practices of wound repair. Which brings me to a major thesis of my talk: that in essence, biology and clinical medicine are but the two ends of a continuous spectrum, inseparably connected and interacting to mutual benefit. I shall try to illustrate this thesis by samples of experimental work from my own laboratory, some new, some old, touching on several basic problems of development. In reviewing this work, I think with deep gratitude of the integral part that my collaborators and students have played in its execution, foremost among them my long-time colleague, A. C. Taylor, with his unbounded resourcefulness. But whatever interest the factual results may command, my prime purpose in presenting them is to convey a message of overriding importance in the scene of contemporary biology and medicine, and it is this:

Let us ever be on guard lest we glibly accept a generality for knowledge, or an appealing term for insight. Developmental biology is full of general terms—"induction," "genic control," "hormonal integration," "correlative differentiation," and what not—which name the problems, but do not solve them and are no substitutes for disciplined and realistic analysis. A machine is built not by a string of words, but by the stepwise assemblage of real things. Consequently, no real understanding of the construction process can be gained except by reconstructing, step by step

and factually, the true chain of events. There is no short cut to knowledge. To come to the point, just contrast some of the labels attached to wound healing with the dearth of factual information about their content. We speak of "trauma," "wound hormones," "metabolic products," without specifying and identifying any of them. We invoke "healing tendencies" and "tropisms," unmindful of their mechanisms, and end up in doctoring a lesion with tinctures and salves empirically, without much of a notion of why and how they are supposed to work. My plea is that in the study of mechanisms—and development works through mechanisms—concreteness take precedence over abstraction.

The surgeon, like the rest of us, is faced with organs and tissues on a macroscopic scale. Skin looks not too different from leather; a tendon or a nerve looks like a string. But when a bruised or burned skin is to heal, or a cut tendon or nerve is to be mended, the relevant events take place not in the macroscopic, but in the microscopic and submicroscopic realm. The sole performers in the reparative process are the microscopic cells. For them to cross a gap of a few centimeters between the retracted ends of a cut nerve or tendon is about the same feat as is to us the crossing of a mile-wide range with rivers, lakes, and mountains. Therefore, to understand and improve wound healing, we must scale ourselves down to the dimensions of the cells and acquaint ourselves with their world in their own terms, as Gulliver had to do with the Lilliputians. This we shall now attempt.

For simplicity, we turn to an elementary kind of skin—that of larval amphibians. It consists of a smooth epithelial sheet, just two or three cells high, resting on a tough, fibrous cell-free membrane (Fig. 1). Yet even in this simple structure, repair of injury involves a combination of processes and maneuvers almost as complex as a military campaign. This is what happens after an injury (Fig. 2) (see Weiss, 1959). The wound edge retracts, and a colloidal exudate covers the lesion. Then the epidermal cells, which had been firmly attached to the basement membrane, become detached and mobilized. They then start spreading over the wound. As they advance, the next rank of cells behind them is mobilized, then the next one, and so forth, until the whole marginal area is on the march. It is this migration, rather than

growth, that establishes the primary closure of a skin wound. The converging fronts finally meet head-on and almost instantaneously stop in their tracks. Growth with mitotic cell division continues and makes up for the loss of emigrated cells. This likewise drops back to normal as tissue continuity is restored. There follows then a phase of functional adaptation, during which earlier irregularities and scars are remodeled in harmony with the mechanical and physiological conditions of the old skin. There are many more chapters to this story—e.g., the so-called wound contracture (see Grillo *et al.,* 1958) or the rebound of a lesion on the general metabolic and endocrine state of the body, which in its turn then affects the various local components of the healing process (see Moore, 1958)—but these few may suffice to indicate the composite nature of the healing process. We shall now look at each one of these separate steps somewhat more closely. Each one presents peculiar problems, defying any attempt to embrace them all in a single general formula.

First, as for the early exudate, not much is known about it. Save for a few beginnings (e.g., Friedenwald *et al.,* 1945), no one seems to have studied it effectively. Presumably it is a mixture of coagulated tissue juice, blood, cell debris, and cell discharges. The presence of blood fibrin and other fibrous units in this primary wound cover is important because, as I shall explain shortly, such fibers act as pathways for the moving cells.

The second major step is cell detachment. In stationary skin, the epidermal cells are rather firmly applied to the underlying membrane. This attachment used to be considered a simple matter

---

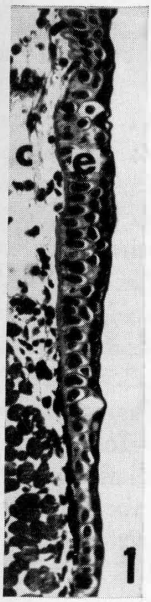
FIG. 1. Survey section through dorsal crest of amphibian larva, showing the simple skin coat (*e*) over the enclosed gelatinous connective tissue (*c*). (Original: J. Overton.)

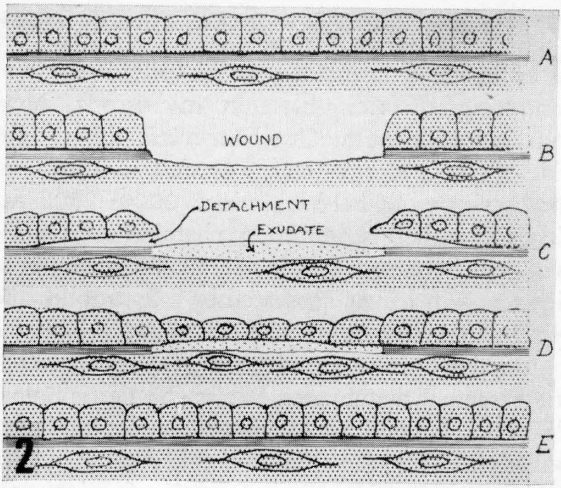
FIG. 2. Schematic representation of sequence of main steps in the healing of a skin wound in amphibian larvae.

FIG. 3. Electron micrograph of underside of epidermal cell attached to basement lamella by "bobbins." From Weiss (1958).

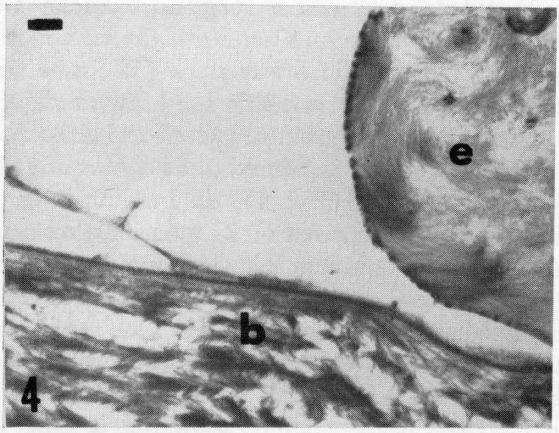
FIG. 4. Electron micrograph of epidermal cell (*e*) detached from underlying basement lamella (*b*), 1 hour after wounding of neighboring skin. Note the free "bobbins" in the detached surface. (Original: Weiss and Ferris.)

FIG. 5. Diagram of tissue culture in hanging drop of blood plasma.


c
e
1


A
WOUND
B
DETACHMENT
EXUDATE
C
D
E
2


e
3


e
b
4

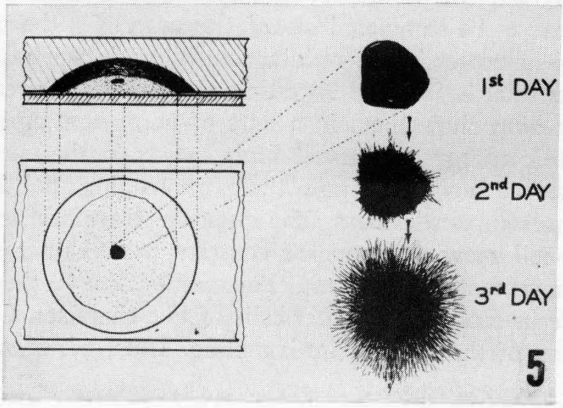

1st DAY
2nd DAY
3rd DAY
5

PLATE I

of adhesion in terms of general colloid-physical properties of the contacting surfaces. Electron microscopy, however, suggests a much more elaborate mechanism. It shows (Fig. 3) the underside of the epidermal cell to be dotted with submicroscopic bodies in the shape of "bobbins" about 2000–2500 Å high (Weiss and Ferris, 1954a). Combining chemical and enzymatic treatments with electron microscopy, we could dissect these bodies into two disks rich in lipids, separated by a hydrophilic belt, the outer disk lying in the cell surface (Weiss, 1958). This cell surface rests on a granulated layer, about 500 Å thick, which binds the cell to the basement membrane (Weiss and Ferris, 1954b). Being readily dissolved by salivary amylase, it presumably contains much carbohydrate. When an epidermis cell is made to shrink—for instance, by hypertonic solutions or formalin fixation—all its basal surface retracts from the substratum with the exception of the lipid-rich bobbins: they remain firmly stuck (Weiss, 1958). From this we judge that they are veritable hold-fast mechanisms. The devices for attachment are thus quite special and elaborate.

Now, when skin is injured, the bobbins of cells near the wound give up their hold (Fig. 4), the binding layer becomes free, and the whole cell, deprived of its moorings, rolls and glides off in a sort of ameboid motion. Its migratory phase begins.

This makes us ask at once, what actuates these cells to change from sedentary to migratory life? One used to think in terms of a positive stimulation, perhaps by wound hormones released from damaged cells. As I shall prove later on, this view is incorrect. In fact, the whole concept behind it is fallacious. Cells do not have to be stimulated in order to move. On the contrary, motility, as an expression of cellular instability, is a primary feature of any cell that is free and unrestrained. Motion pictures of isolated cells *in vitro* show them in a state of permanent agitation. The metabolic energy of the cell finds outlets in the ever fluctuating cell surface now here, now there, protruding mobile pseudopods in incessant restlessness. The question, therefore, is not what makes a cell move, but, on the contrary, what causes such autonomous movements ever to stop. For epithelial cells, the answer seems to lie in contact with other cells of like character. Only the free portion of the cell surface is mobile. Thus, when an epithelial cell is

completely girded by fellow cells, it becomes immobilized. And the cells at the edge of a wound resume motion for no reason other than that their surface has been deprived of its former contact with fellow cells (Weiss, 1950).

Now, one may ask, is this mobilized cell front at the wound border the only active motor in wound closure, dragging the cohesive epithelial sheet passively along? The answer is, no. Close observations in our laboratory (Lash, 1955, 1956) have shown that in their movement over the wound, the epidermal cells do not hang together but move individually, each of its own accord, much as a herd of animals. Then, what is it that orients their advance, so that they will not just crawl off in all possible directions, but move straight into the wound? This raises the more general problem of cell orientation, to which I have applied myself for more than thirty years, endeavoring to fill symbolic terms like "chemotaxis" and "thigmotaxis" with concrete meaning. Time will allow only a very brief summary. Most of this work was done in tissue culture (Weiss, 1929, 1933).

The now familiar method of tissue culture consists of transferring a small group of living cells into a medium containing nutrients and other life necessities in some sort of glass chamber, sealed for protection and transparent for optional microscopic inspection. A culture of the older standard type is shown in Fig. 5. The medium here consists of coagulated blood plasma. In this medium, cells from an explanted piece of embryonic tissue move out promptly, migrating, growing and dividing, surrounding the original fragment with a dense corona of emigrated cells. The margin of the explant behaves quite like the margin of any wound in the body.

Left alone, the outwandering cells move essentially at random. The locomotor apparatus of a free cell consists of blunt or pointed protrusions from its surface. These are the engines that drag the cell body around. Without directional cues, their numbers and directions are wholly a matter of chance. Figure 6 shows this lack of orientation near the margin of an ordinary tissue culture. Free cells are as erratic as Brownian motion.

However, one can readily and at will turn such randomness into rigorous orientation. One recent example is shown in Fig. 7

(Weiss, 1958). A suspension of isolated cells was deposited in liquid nutrient on a glass slide scored with microscopic grooves. The disk-shaped cells have become elongated in the direction of the grooves, the cell body being drawn out into spindle shape by its two mobile ends proceeding in opposite directions. The shape of these connective tissue cells thus results from their deformation along an axis imposed by their environment.

A study of this morphogenetic event in time-lapse films made under the phase microscope by my collaborators, Dr. Taylor and Mr. Bock, has indicated the mechanism of this elongation: it seems that the mobile cell contour clings more firmly to the rough groove than it does to the smooth surface of the glass (Fig. 8). In this manner the cells are bound to preformed tracks or pathways. Since they move blindly, guided solely by contact, I have named this principle of orientation "contact-guidance."

Of course, the grooved glass in tissue culture serves merely as a model of many similar linear track systems provided in the body by the reverse of grooves; namely, fibers. My earlier experiments in plasma cultures have made this clear. A blood plasma clot is a sponge of fibrin fibers imbibed with serum. Figure 9 is an electron micrograph of dried blood plasma (Hawn and Porter, 1947). The fine, cross-striated elementary filaments of fibrin form bundles of various sizes, but without any definite orientation. It is these solid fiber systems that serve as climbing ropes to moving cells. Evidently, having to cling to such a tangled mess of fibers, as in this picture, cells would be lost like wanderers in a dense jungle.

FIG. 6. Stroma cells near the margin of a culture, in random orientation. (Original: A. C. Taylor.)

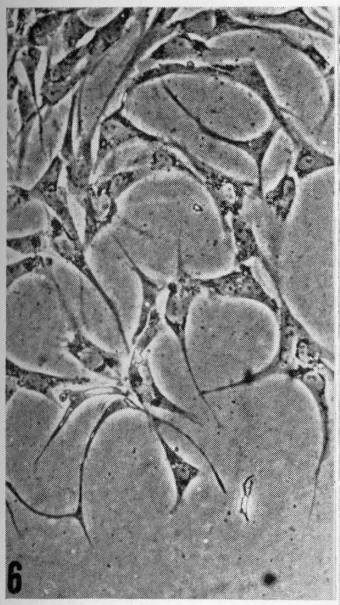
FIG. 7. Orientation of free cells in the direction of microgrooves of scored glass. From Weiss (1958).

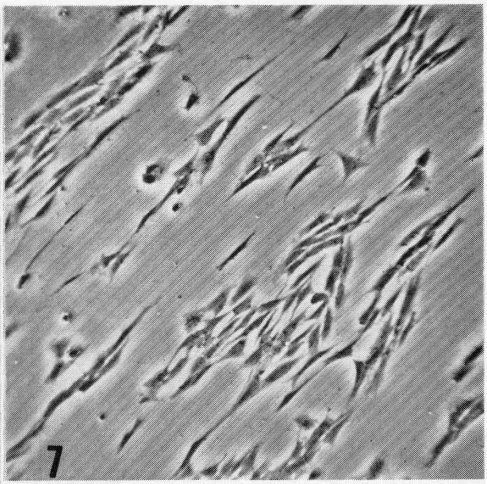
FIG. 8. Four stages from motion picture of spherical stroma cell elongating on grooved glass support. From Weiss (1958).

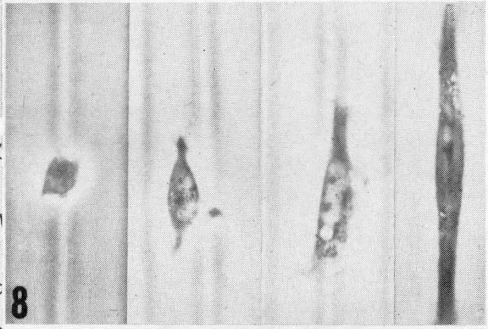
FIG. 9. Electron micrograph of dried blood plasma, showing fibrin net. From Hawn and Porter (1947).

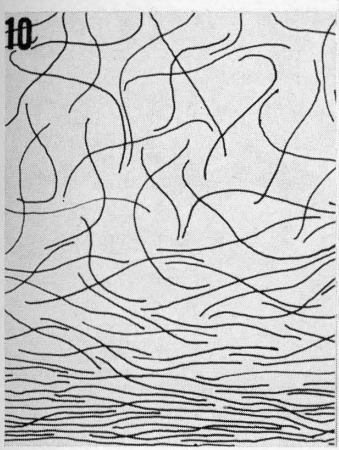
FIG. 10. Diagram of orientation imposed by tension upon a net of filamentous molecules.

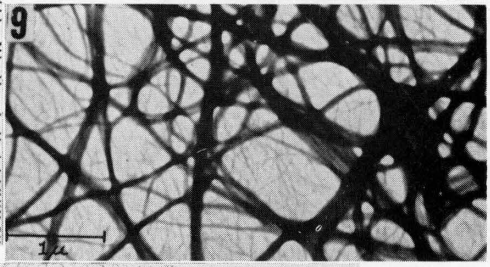
FIG. 11. Diagram of progressive orientation of a fibrous medium by stretch (in the direction of the arrows) and the conforming orientation and configuration of the cells contained therein. From Weiss (1949).











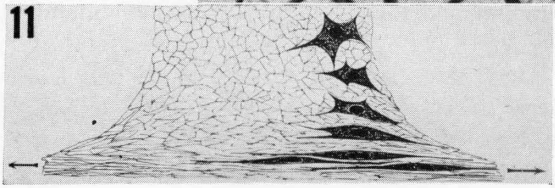
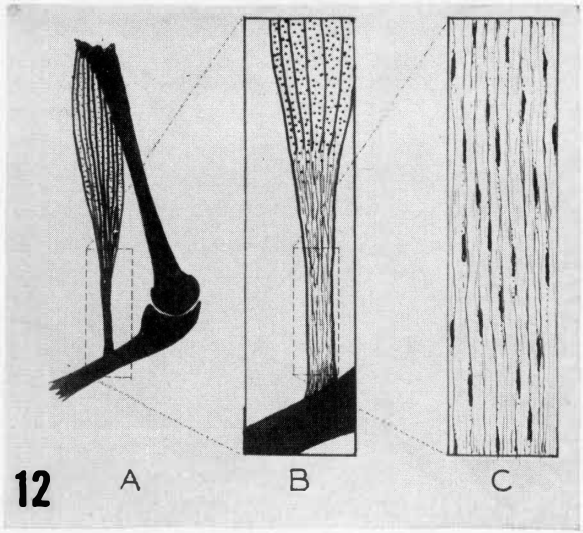


PLATE II



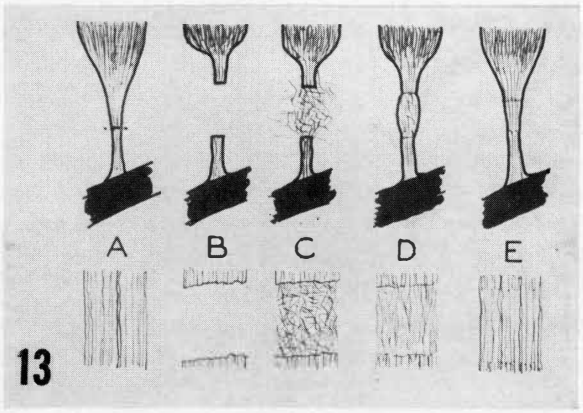


PLATE IIIA. FIGS. 12 and 13. See facing page for legends.

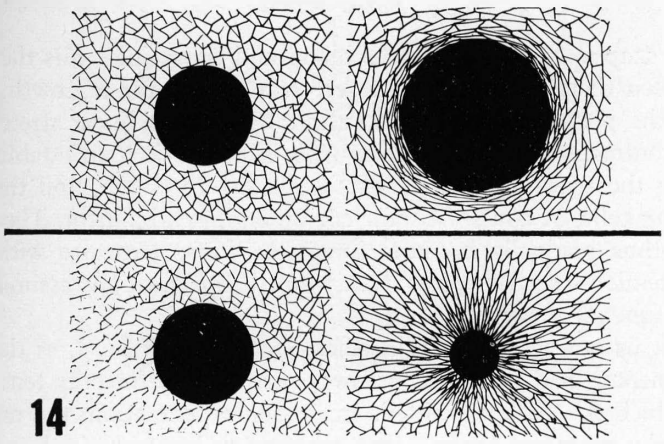


PLATE IIIB.

FIG. 12. Diagram of linear (ropelike) arrangement of tendon cells and fibers along tension lines.

FIG. 13. Diagram of tendon regeneration, indicating structural ordering of tissue linking the stumps.

FIG. 14. Patterns of tension in network developing around centers of expansion (upper row) and contraction (lower row). From Weiss (1949).

But rod-shaped macromolecules, like fibrin or collagen, can be forced into alignment by a variety of external forces. For instance, if an irregular network of filamentous molecules is stretched, as in the lower portion of Fig. 10, the molecules are straightened in the direction of the stretch. Aligned, they tend to aggregate into submicroscopic, and eventually, microscopic fibers.

Thus, tensions orient fibers, and fibers orient cells. Figure 11 shows the results. The better oriented the medium, the more rigorously are the cells aligned. Since the tips of nerve fibers likewise follow contact guidance, the direction of nerve growth can be similarly controlled (Weiss, 1934, 1945). All this has been found out by experimental work motivated by nothing but the urge to understand developmental mechanisms.

But here we also come upon a direct link between basic research and surgical practice. For these experiments are a fair, if simplified, model of the way a cut tendon is restored. A tendon (Fig. 12) is a rope of countless parallel fibers, interspersed with equally aligned slender cells, embedded in a ground substance. The ropelike structure is essential for the safe transmission of pull from muscle to skeleton. When a tendon is cut (Fig. 13), the

ends snap apart, and a clot of blood and tissue juice fills the gap between them. This clot then serves as matrix for regrowth, just like the plasma clot in tissue culture. Subject to linear stretch by the contractions of the muscle, a fibrin bridge is soon established along the tension lines, connecting stump to stump, and the invading cells, of course, assume corresponding alignment. The new link thus gradually becomes integrated in its structure with the old tendon—a messy scar transformed and fittingly assimilated into functional tissue.

Let us now go one step further: In tissue culture, I, as the experimenter, had furnished pathways or pathway-forming tensions, to which the cells submitted. In tendon regeneration, the muscle was the source of tension. But what orienting force is there in a skin wound converting the underbrush of a wild scar into converging pathways to lead cell streams toward the center? Perhaps the following considerations can guide our thinking.

The fibrous network pervading a tissue is a continuum. That is to say, tensions originating or released at any point can affect the stress pattern of the whole net. The two most elementary cases are exemplified in Fig. 14. The black disks on the left represent a piece of tissue enmeshed in fibers. When it expands, as in the upper half, it deforms the surrounding meshes circumferentially. This may explain, for instance, why tunics of connective tissue around growing or dilated ducts and organs, as well as capsules around foci of inflammation, assume concentric patterns. On the contrary, if an area shrinks, as in the lower half of the diagram, the meshes are gathered in radial directions toward the center of contraction.

Now paradoxically, such shrinkage happens in the body matrices around areas with a high rate of cell proliferation. Proliferat-

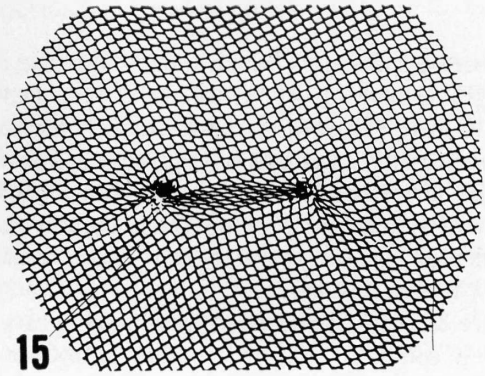
---

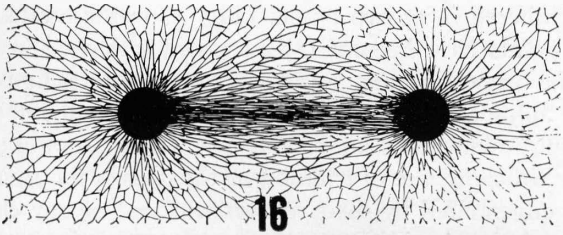
FIG. 15. Model of tensions (indicated by distortion of meshes of net) engendered by two centers of contraction.

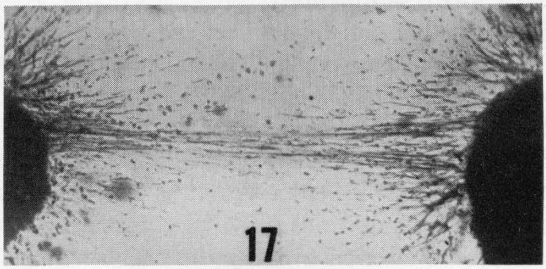
FIG. 16. Structural effect of two growing centers on fibrous matrix. From Weiss (1949).

FIG. 17. Cell and fiber bridge formed automatically between two spinal ganglia grown in thin blood plasma membrane. From Weiss (1934).

FIG. 18. Cell bridges formed automatically between three growing cultures in thin blood plasma membrane. From Weiss (1949).







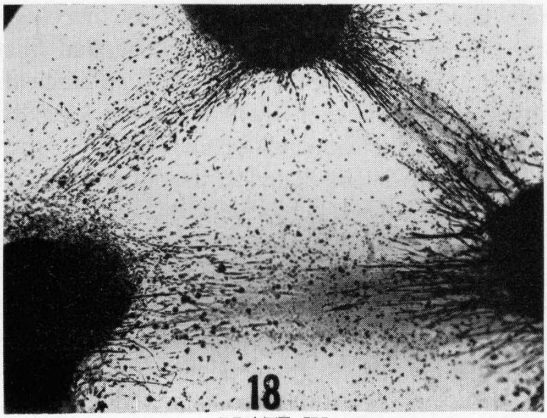


PLATE IV

ing cells liberate substances which dehydratize the colloids in the surroundings (Grossfeld, 1934; Weiss, 1929, 1934). As interstitial fluid is lost, the solid components become condensed, and since this nucleus of condensation and contraction exerts centripetal pull on the rest of the net, the meshes are gathered into a radial pathway system for moving cells. By this device, an erstwhile unoriented growth process can itself create patterns of orientation (Weiss, 1949a).

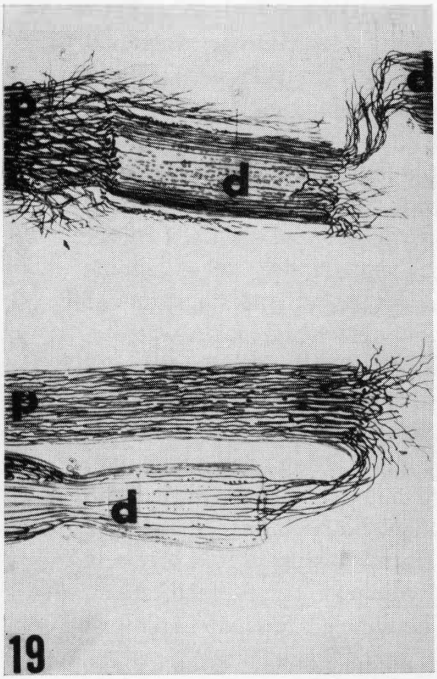
Even more striking is the effect produced by a pair of centers growing in a common clot, where they introduce two separate points of shrinkage. What to expect, is shown by a model of netting gathered in at two points (Fig. 15): the meshes between the two points have become conspicuously stretched in the direction of the connecting line. Similarly, two proliferating cell groups establish automatically a fibrous bridge between them (Fig. 16) so that outgrowing cells are conducted straight from one group to the other. In short, the orientation of the cells is the result of contractions engendered by their sources, and not of any chemotactic attractions by their destinations. Figures 17 and 18 show such cell bridges formed automatically between widely separated growing cultures without any outside direction. I shall refer to this as the "two-center effect" (Weiss, 1952).

The "two-center effect" is another instance where laboratory experience offered itself for ready translation into surgical practice—in nerve repair. When a nerve is cut, the distal stump degenerates, and reconnections between the centers and the muscles or skin can be effected only by the outgrowth of new nerve fiber sprouts from the proximal stump. Such nerve sprouts, as I said before, follow contact guidance. Degenerated fiber segments of the distal stump can serve as tracks. Yet, to reach these tracks, the growing sprouts must first traverse the trackless gap between the stumps—in cellular dimensions, an immense space in which to get lost. To minimize the distance, the surgeon sews the stumps together, achieving what macroscopically looks like neat apposition. But viewed in the dimensions of microscopic cells, the landscape there appears as a confounded wilderness: Crushed fiber ends caked with extravasated blood, create a maze of crisscrossing guide ropes—the notorious nerve scar—in which the blind new

nerve sprouts get hopelessly tangled up and trapped. A volume of new nerve sprouts that would be fully adequate for functional reconnections, if it were properly guided, thus exhausts itself in the futile meandering through this trackless jungle.

However, the lessons of tissue culture have shown us how to turn futility into success. We simply have to imitate the two-center effect by letting the two nerve stumps themselves create their own connecting bridge. Ramón y Cajal had shown (1928) in classic experiments (Fig. 19) that regenerating nerve sprouts indeed tend to converge upon a distal degenerating nerve stump as if attracted chemotactically by its orifice. His observation was correct, but the explanation was not. What actually is involved, is a two-center effect. When we explant two pieces of adult rat nerve, side by side, into a thin plasma clot, as in Fig. 20 (Weiss, 1952), Schwann cells proliferate from the open ends, and these two growing points produce again quite automatically a fibrin, and consequently, cell bridge between them. Nerve fibers, if present, would thus be guided straight from stump to stump. It is precisely this automatic self-orientation that one must try to facilitate in nerve repair, and here is how it can be done (Weiss, 1943; Weiss and Taylor, 1943; Weiss, 1944).

Instead of forcing the nerve ends together by suturing, we join them by an elastic tube of some material to which blood will not adhere (Fig. 21). In practice, we have used arterial segments, fresh or frozen, or cuffs of tantalum. The point is to let the blood clot in the gap adhere to the nerve ends, but not to the sleeve, so that tensions can act in only one direction, namely, along the line connecting the two stumps—the nerve axis. The tensions involved arise from the retraction of the stumps and the syneretic shrinkage of the clot itself. As a result, a perfectly oriented linear pathway system of parallel fibrin tracks forms in the blood bridge between the two stumps, which guides the outwandering Schwann cells and new nerve sprouts straight across the gap toward their destinations. Figure 22 illustrates the drastic contrast between an ordinary and a tubulated union in two branches of a rat nerve, 5 days after transection, the lower one left untreated, the upper one reunited by an arterial sleeve. The former shows already an irregular scar, with cells and nerve fibers crisscrossing at random,



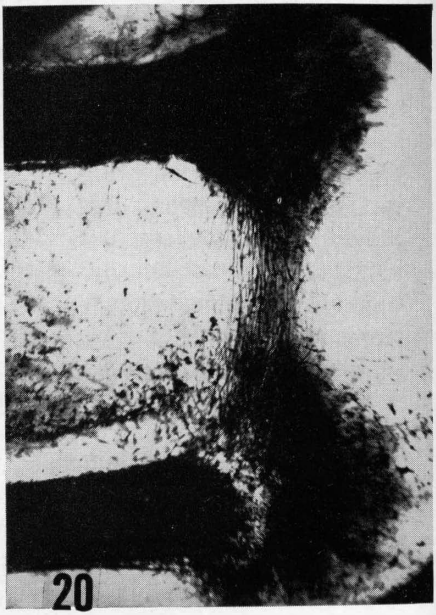
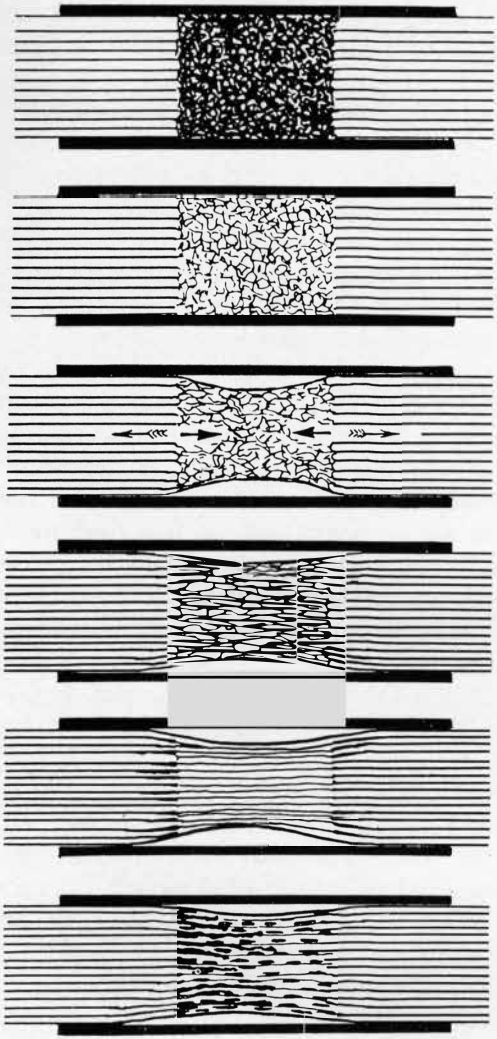


PLATE VA. FIGS. 19 and 20.

FIG. 19. Deflection of nerve fiber regeneration from proximal stumps (*p*) "toward" degenerated nerve fragments (*d*). From Ramón y Cajal (1928).

FIG. 20. Mushroomlike proliferation of Schwann cells from open ends of two rat nerve stumps in blood plasma clot, with bridge of cells connecting the two centers. From Weiss (1952).

FIG. 21. Diagram of progression of orienting effect of splicing nerve stumps by nonadhesive sleeves. From Weiss (1944).



21

PLATE Vb. FIG. 21. See facing page for legend.

whereas the sleeve has led to an orderly straight connection, with nerve fibers and cells passing directly and unimpeded from end to end.

By contrast, if the blood clot in the gap is subject to the least lengthwise compression, rather than extension, as shown in Fig. 23, which is a common occurrence in tight sutures, the fibrin net forms crosswise, constituting a most effective barrier to the transit of regenerating nerve sprouts from stump to stump. A comparison between these two cases gives an impressive demonstration of how supposedly inconsequential details of manipulation may be of the most crucial consequence for eventual success or failure.

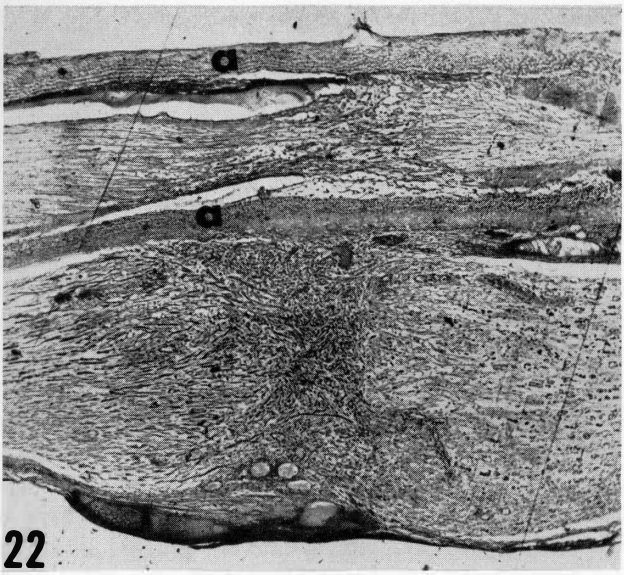
In applying these lessons now to skin wounds, one could assume that it is the gradual contracture of the primary colloidal wound exudate or granulation tissue that generates radial fiber tracks by which the cells invading from the perimeter would automatically be made to converge upon the wound center. Unfortunately, there are no actual data on this fundamental problem. If our conjecture is correct, it is evident that relatively subtle variations in the mechanical and chemical configuration can make all the difference between whether a mobilized cell mass will either radially invade a raw wound and cover it or, on the contrary, circle around it, leaving an open sore (cf. Fig. 14).

Eventually, the invading wound margins meet head on. This rather promptly makes them stop and settle down. One could conclude that as soon as equilibrium of tissue continuity is restored, migration and further growth are automatically suspended. But what counts as "tissue continuity" in complex lesions involving more than one kind of tissue? Will merger among any two tissues, even foreign ones, satisfy the provision? It has long been the experience of surgeons that there is a tendency for each tissue to heal with its own kind, avoiding nonmatching combinations. Biological experimentation has proved this discriminatory behavior to be a general property of tissues, usually referred to as

---

FIG. 22. Regeneration of two adjacent rat nerves, 5 days after transection; the upper one with ends sheathed in arterial sleeve (*a-a*), the lower one with ends simply apposed but unsheathed. From Weiss and Taylor (1943).

FIG. 23. Transverse fibrous block between two nerve ends in arterial sheath, but pressed together. From Weiss and Taylor (1943).



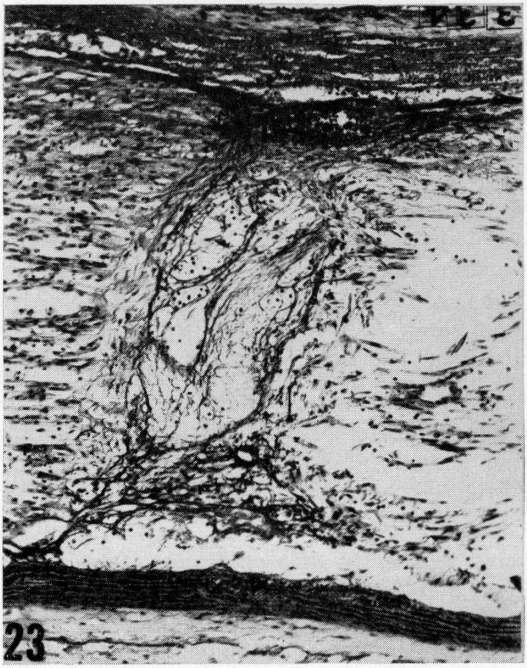


PLATE VI

tissue "affinity." Holtfreter (1939) has shown its embryonic origin, and experiments in our laboratory have tried to clarify its nature.

In one test (Weiss and Andres, 1952), embryonic precursor cells of pigment cells, injected into the blood stream of young chick embryos of an unpigmented race, cropped out as black colonies in the precise locations typical as residences for pigment cells, and nowhere else (Fig. 24). Obviously, after their random dissemination throughout the body, they had been fished out and lodged selectively at their matching sites. This broadcasting of cells through the blood stream, followed by selective lodging, has since assumed great practical promise, as it forms the basis of the reseeding of radiation-damaged bone marrow by injected healthy stem cells.

In another test, more directly bearing on wound healing (Chiakulas, 1952), various epithelia were grafted into a surface wound of skin. If the graft was likewise skin or tissue normally adjoining skin, such as cornea, the two advancing wound margins merged smoothly. However, if the graft was foreign to epidermis —for instance, esophagus or gall bladder or lung—the edges did not join, but the epidermis kept on moving either over or under the graft, not satisfied until it met another epidermal edge. Similarly, cartilages from a common source, either mesoderm or neural crest, will fuse, but cartilage from either of these sources will not join with cartilage from the other (Chiakulas, 1957).

Cells thus establish a high degree of structural order by their own discriminative associations. To study the phenomenon more closely, we have used the technique of dissociated cells in culture, designed by Moscona (Moscona and Moscona, 1952) and later elaborated by him in our laboratory. He observed that when a scrambled mixture of cells from different embryonic organs, for instance, kidney and cartilage, was explanted, the cells would sort themselves out according to kind, forming separate clusters of pure kidney and pure cartilage (Moscona, 1956). Just how they did this, was not clear. To find out, we have now taken direct cinematographic records of cell encounters *in vitro*. They show how an epithelial cell behaves when it meets another epithelial cell of either the same or a different type.

Here are the main conclusions (Weiss, 1958): After settling on the glass surface, the epithelial cells move about at random. They show no conspicuous reactions to other cells, either attractive or repulsive, so long as there is no direct contact interaction. As soon as two cells do collide perchance, they join at first. But having done so, a crucial difference appears, depending on whether they are of matching or nonmatching tissue origins. If of a matching kind, they draw together and remain associated; if of nonmatching kinds, they withdraw the apposed margins and thus become disengaged from each other. In other words, unlike cells do not shun mutual contact, but after having perceived each other's strangeness, they actively separate. The active principle is not the junction of like cells, but the disjunction of unlike ones. The nature of the discriminatory reaction is still under investigation, but the phenomenon as such is spectacularly clear.*

We evidently are faced here with a very basic biological principle, instrumental in maintaining order in the organism. It not only explains the self-ordering of complex tissues in the healing process, but also has a direct bearing on the cancer problem. For the emigration of the metastasizing malignant cell from its mother tissue and its invasion and lodging in foreign locations must surely be related to its having become so alienated that its surface no longer matches that of its sister cells (Weiss, 1949b). But to dwell on this would be going far beyond the scope of this lecture.

We should go on next to discuss the problems of compensatory cell multiplication, which returns the reduced cell population to its normal density. Basically, the problem is the same for wound healing as for growth control in general, which manifests itself, for instance, in the compensatory hypertrophy and hyperplasia of kidney, liver, and other organs after partial removal (see Weiss,

* The behavior here described has been observed in the following cell types in various combinations, freshly dissociated: embryonic chick liver, epidermis, lung, heart, kidney; embryonic mouse liver, lung; neonate rat liver. Embryonic chick and mouse livers merge, but embryonic and neonate livers do not. Cells from cultured strains of human conjunctiva, human liver, or rabbit kidney do not coalesce with any of the fresh tissues listed above, but do associate with one another. Macrophages show complementary affinity to lung epithelium. Mesenchyme cells, on the other hand, retract from each other on contact (Abercrombie and Heaysman, 1954; Weiss, 1958).

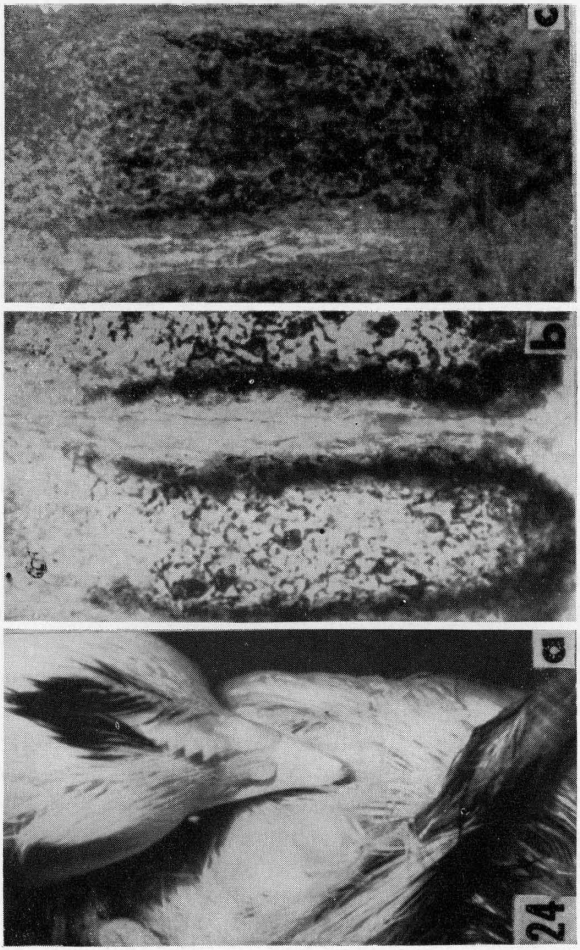


PLATE VIIA. FIG. 24. See facing page for legend.

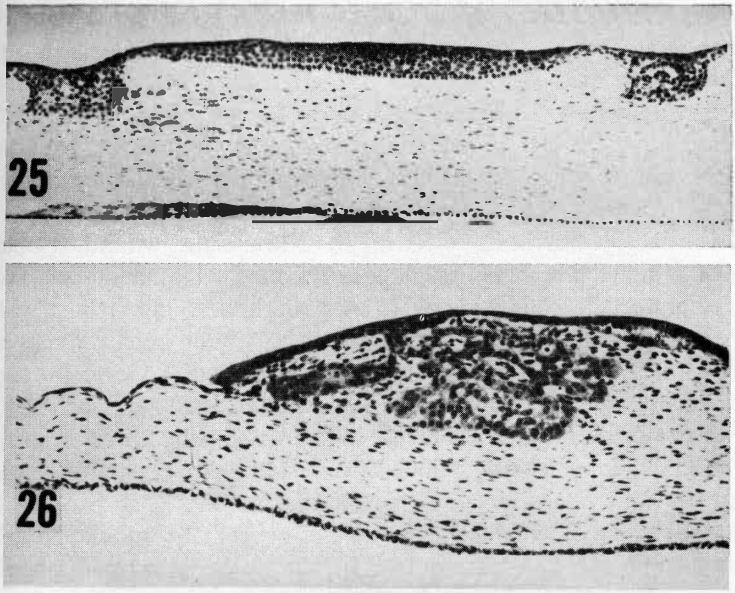


PLATE VIIB. FIGS. 25 and 26.

FIG. 24. Colonization of unpigmented chick host embryo by prospective pigment cells from colored donor disseminated after injection into the blood stream. a. Pigment islands in skin and feathers on head and leg. b. Specific location of pigment cells at base of young feathers of control donors. c. Precise localization of disseminated donor cells at feather bases of unpigmented hosts. From Weiss and Andres (1952).

FIG. 25. Embryonic chick cornea healed 4 days after wounding on sixteenth day of incubation; former wound margins marked by epidermal plugs. From Weiss and Matoltsy (1959).

FIG. 26. Margin of wound unhealed 4 days after wounding on ninth day of incubation, showing denuded stroma on the left and adenomatous penetration of proliferating epidermal border into the underlying stroma on the right. From Weiss and Matoltsy (1959).

1955). But this is such a complicated problem that it would require a separate lecture (see Weiss and Kavanau, 1957). Instead, let me just briefly report some recent results which have revealed that the activation of cell multiplication in wound healing is, in any event, not dependent upon the act of cell migration, as either can go on quite well without the other (Weiss and Matoltsy, 1959).

We found that the chick embryo in its early stages conveniently dissociates growth from migration after the wounding of skin or cornea. Any wound made at any time after the tenth day of incubation, whether in the embryo or in the hatched bird, is promptly closed by epithelial cells moving across the lesion and dividing mitotically. Figure 25 for instance, shows a corneal wound completely covered, even hyperplastically, 3 days after an injury made in the later embryonic period. Yet, if the same wound is made prior to the tenth day (Fig. 26), the wound edge grows, but fails to migrate: the cells proliferate into the underlying stroma, with cystic adenomatous extensions, while the wound area remains an open sore. Both skin and cornea behave alike. Yet, even in such stationary wounds, as soon as the injured embryo reaches the critical tenth day, migration sets in spontaneously, whereupon the wound is rapidly covered. These results prove not only that growth is independent of migration, but also that the act of wounding itself, with all its traumatic byproducts, cannot be held responsible for the initiation of the migratory process. Just why the epithelium of skin and cornea in the early embryo fails to heal, is still a mystery, for we have found that even the youngest stages can heal quite promptly when transferred from the embryo into tissue culture. We have reason to suspect that the deficiency in the early embryo is related to the absence of functioning endocrines prior to the critical age, but we have no proof. At any rate, the case surely epitomizes the composite nature of the healing process.

Let me now add to this a final complication. Not only must we learn to gauge macroscopic situations on the microscopic scale of

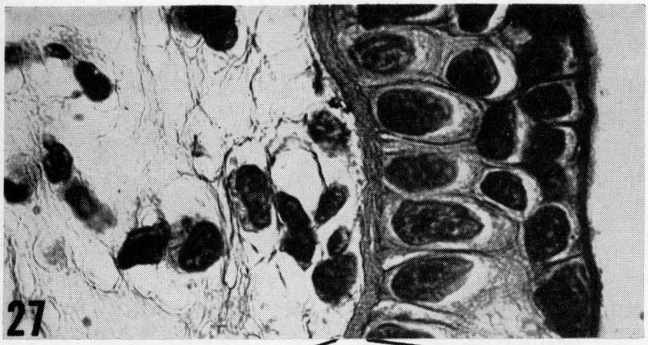
---

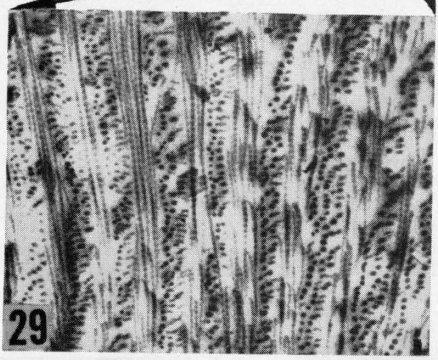
FIG. 27. Section through skin of urodele amphibian larva, showing (from right to left) epidermal cells, basement lamella, and underlying loose connective tissue.

FIG. 28. Electron microgram of stratified basement lamella under the epidermis of urodele amphibian larva.

FIG. 29. Electron microgram of section through basement lamella. From Weiss and Ferris (1956).

FIG. 30. Diagrammatic representation of two phases in the restoration of the basement lamella (*l*) after wounding (left: earlier phase; right: later phase). *e*, Epidermal cell; *f*, fibroblast.





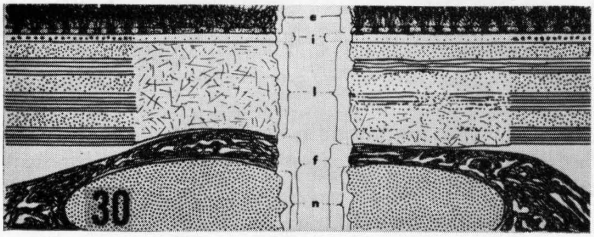


PLATE VIII

cell behavior, but cell behavior itself must be projected downward to the ultramicroscopic, macromolecular, realm. This leads me to my last example.

I mentioned in the beginning the fibrous membrane of the amphibian skin (Fig. 27). The electron microscope (Weiss and Ferris, 1954b) shows it to be laminated (Fig. 28), and higher magnification (one micron corresponds to sixteen mm.) reveals an amazing regularity (Fig. 29): There are about twenty layers, each containing a set of cylindrical collagen fibrils, 500 to 600 Å. wide, in strictly parallel array. But from each layer to the next, this orientation changes by 90 degrees.* Therefore, in Fig. 29, proceeding from layer to layer, one sees the fibers alternately in profile and in cross section.

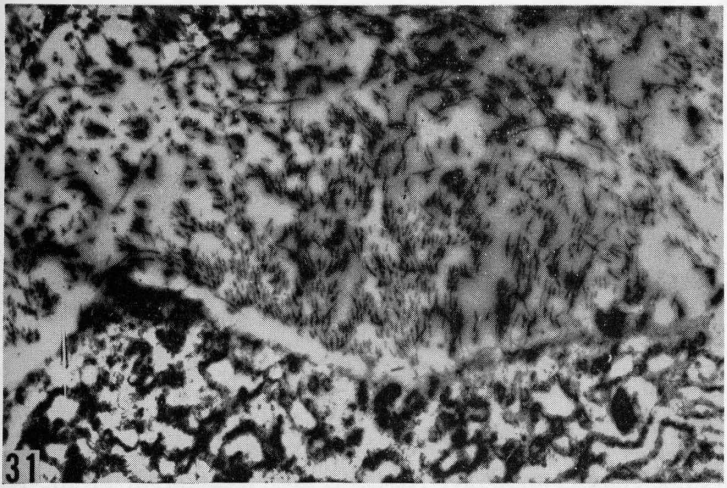
How does such a masterpiece of fine-structural architecture arise? We approached the problem by studying whether and how damage is repaired (Weiss and Ferris, 1956). Briefly, a hole made in the membrane is mended as follows (Fig. 30): After the epidermal cells have covered the wound, fibroblasts of the connective tissue underneath shed immature collagen fibrils into the gap at random, like trucks dumping building material for a house. These young fibers form a messy tangle. Within the second week, however, a new order begins to spread over this tangle, from the epithelial sheet downward, orienting the young fibers progressively into the mature layered arrangement: the erstwhile disoriented fibers are now given their definite orientations alternating between layers by right angles. Figure 31 shows the initial deposit of immature collagen units devoid of order. But just a few days later (Fig. 32), a tangential section through the skin shows the

* Clear indications of this orthogonal texture had already been observed under the light microscope by Rosin (1946) and Mizuhira (1951) in amphibians and by Fauré-Fremiet (1938) in fishes.

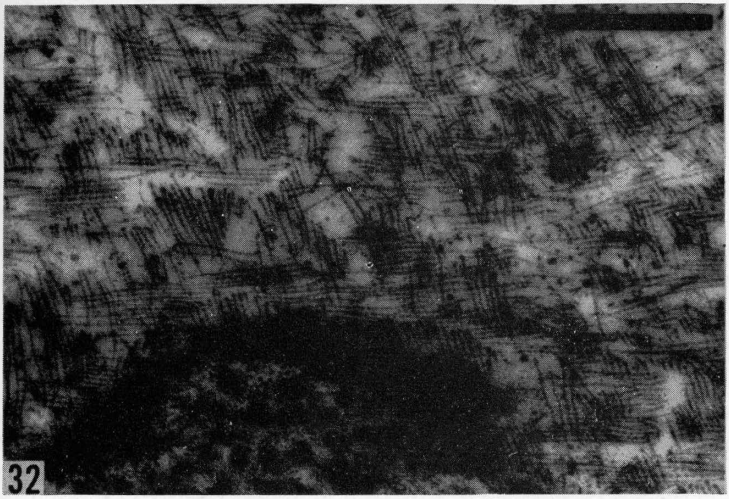
FIG. 31. Electron micrograph of unoriented collagen mat in early stage of restoration of wounded basement lamella. From Weiss (1956).

FIG. 32. Electron micrograph of beginning orthogonal ordering of collagen fabric in later stage of healing wound of basement lamella. From Weiss (1956).

FIG. 33. Hypothetical lattice of ordered alignment and stacking of collagen fabric in the restoration of the orthogonal structure of the basement lamella. From Weiss (1956).

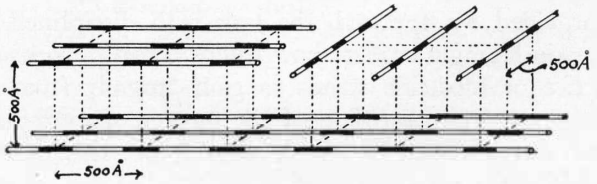


31



32

A B



33

PLATE IX

beginning of the rearrangement of the fibers in a regular checkerboard pattern, anticipating the final mature structure of the rewoven fabric. The ordering of the filamentous units seems to occur in a space lattice of cubic structure with 500- to 600-Å spacing between nodal points (Fig. 33). We witness here living organization in its workshop, but this is not the right occasion to dwell on the fascinating vistas of macrocrystallinity in biological systems which these results open and which may bring biology and modern solid state physics into close conceptual relation.

Our round trip has returned us to the ultramicroscopic realm from which we had started. Every step brought unforeseen disclosures: new facts, new concepts, modified expectations. Does this not counsel modesty, restraint, and recognition of the fragmentary state of current knowledge about life, and a more fervent determination to find out more before complacently closing our accounts? If I were to sum up the experience of my forty years of scientific work in one sentence, I would say that my ignorance has grown faster than has my knowledge. We shall never see the end; science is unending approximation.

Wound healing had seemed like a simple phenomenon, amenable to simple explanations. I have tried to show that it is neither simple nor will submit to any sweeping formula. Of course, we all appreciate broad generalizations; as I said at the outset, I have contributed my share of them, like "biological field theory" or "molecular ecology." But as thermodynamics does not tell us how to go about repairing a stalled automobile, so the great biological principles of evolution, gene action, homeostasis, and the like, don't tell us how to repair a damaged tissue. To repair an engine, we must know its mechanism, part for part. I hope to have shown that insight into the mechanisms of wound healing is within our grasp, provided we approach the task with disciplined realism, looking painstakingly hard at how things actually work; and that the practice of medicine stands to gain tangibly from such an analytic course. The profusion of the medley of examples that I presented was intended to convey some impression of the enormous multiplicity, diversity, and complexity of the processes involved even in reputedly simple vital mechanisms—as antidote to

our contemporary impatience and flair for oversimplified magic formulas and verbal trappings.

REFERENCES

Abercrombie, M., and Heaysman, J. E. M. (1954). *Exptl. Cell Research,* **6,** 293–306.

Chiakulas, J. J. (1952). *J. Exptl. Zool.* **121,** 383–417.

Chiakulas, J. J. (1957). *J. Exptl. Zool.* **136,** 287–300.

Fauré-Fremiet, E. (1938). *Arch. anat. microscop.* **34,** 219–230.

Friedenwald, J. S., Buschke, W., and Crowell, J. E. (1945). *J. Cellular Comp. Physiol.* **25,** 45–52.

Grillo, H. C., Watts, G. T., and Gross, J. (1958). *Ann. Surg.* **148,** 145–152.

Grossfeld, H. (1934). *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **131,** 324–332.

Hawn, C. van Z., and Porter, K. R. (1947). *J. Exptl. Med.* **86,** 285–292.

Holtfreter, J. (1939). *Arch. exptl. Zellforsch. Gewebezücht.* **23,** 169–209.

Lash, J. W. (1955). *J. Exptl. Zool.* **128,** 13–28.

Lash, J. W. (1956). *J. Exptl. Zool.* **131,** 239–256.

Mizuhira, V. (1951). *Arch. Histol. (Okayama)* **2,** 445–462.

Moore, F. D. (1958). *Harvey Lect. Ser.* **52,** 74–99.

Moscona, A. (1956). *Proc. Soc. Exptl. Biol. Med.* **92,** 410–416.

Moscona, A., and Moscona, H. (1952). *J. Anat.* **86,** 287–301.

Ramon y Cajal, S. (1928). "Degeneration and Regeneration of the Nervous System." Oxford Univ. Press, London and New York.

Rosin, S. (1946). *Rev. suisse zool.* **53,** 133–201.

Weiss, P. (1929). *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **116,** 438–554.

Weiss, P. (1933). *Am. Naturalist* **67,** 322–340.

Weiss, P. (1934). *J. Exptl. Zool.* **68,** 393–448.

Weiss, P. (1943). *A. M. A. Arch. Surg.* **46,** 525–547.

Weiss, P. (1944). *J. Neurosurg.* **1,** 400–450.

Weiss, P. (1945). *J. Exptl. Zool.* **100,** 353–386.

Weiss, P. (1949a). *In* "Chemistry and Physiology of Growth" (A. K. Parpart, ed.), pp. 135–186. Princeton Univ. Press, Princeton, New Jersey.

Weiss, P. (1949b). *Proc. Natl. Cancer Conf. 1st Conf. 1949,* pp. 50–60.

Weiss, P. (1950). *Quart. Rev. Biol.* **25,** 177–198.

Weiss, P. (1952). *Science* **115,** 293–295.

Weiss, P. (1955). In "Hypophyseal Growth Hormone, Nature and Actions" (R. W. Smith, O. H. Gaebler, and C. N. H. Long, eds.), pp. 3–16. McGraw-Hill, New York.

Weiss, P. (1956). *Proc. Natl. Acad. Sci. U.S.* **42,** 819–830.

Weiss, P. (1957). *J. Cellular Comp. Physiol.* **49** Suppl. **1,** 105–112.

Weiss, P. (1958). *Intern. Rev. Cytol.* **7,** 391–423.

Weiss, P. (1959). *In* "Wound Healing and Tissue Repair" (W. B. Patterson, ed.), pp. 1–9. Univ. of Chicago Press, Chicago, Illinois.

Weiss, P., and Andres, G. (1952). *J. Exptl. Zool.* **121,** 449–487.
Weiss, P., and Ferris, W. (1954a). *Exptl. Cell. Research* **6,** 546–549.
Weiss, P., and Ferris, W. (1954b). *Proc. Natl. Acad. Sci. U. S.* **40,** 528–540.
Weiss, P., and Kavanau, L. (1957). *J. Gen. Physiol.* **41,** 1–47.
Weiss, P., and Matoltsy, A. G. (1959). *Develop Biol.* **1,** 302–326.
Weiss, P., and Taylor, A. C. (1943). *A.M.A. Arch. Surg.* **47,** 419–447.