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Cellular Dynamics

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BIOLOGY is now in an exciting phase marked by the confluence of different disciplines of research in the attack on focal problems. But the enthusiasm raised by the spectacular results of combined physical and chemical approaches to biology sometimes has outraced people's ability to keep pace conceptually with the technical developments. As a result, we frequently try to fit our questions to the very limited answers which our fragmentary knowledge has been able to provide, instead of boldly facing the much broader questions posed by living systems and phrasing them in such a way that still more penetrating answers may be obtained in the future. In order to do this, one needs to focus on the real living objects, rather than on the somewhat fictitious and oversimplified models that one is prone to formulate, as intended targets for physical and chemical attack. Models are necessary, but they must bear more than a coincidental resemblance to the real object if they are to serve as meaningful aids to analysis.

As the result of this extensive use of overly simple models, notions about the cell have become at times slightly vague and unrealistic. It would be presumptuous in one single chapter to try to do more than to just give a few illustrative examples of what the real cell is like. The best that can be hoped is to show the change that has occurred in our thinking about the cell from the static to the dynamic—that is, from static organization to organized behavior. Much of the knowledge of what the cell is has come from ruling out erroneous conceptions of what it is not. Progress has come from narrowing the margin of error. By being exposed to a few examples of the living cell in action, the reader can judge for himself whether or not his mental picture of the cell corresponds to the real thing.

The first example deals with one of the most prominent characteristics of the cell—its shape. Figure 1(a) shows a textbook picture of a particular cell found in the cerebellum. One sees that the cell body has elaborate ramifications. This is the way one usually learns about a cell—through pictures in a book; and since the picture looks the same in all of the thousands of copies of a textbook of microscopic anatomy, one forms the notion that all such cells are like tin soldiers stamped out according to a standard pattern. Thus, the mental habit of cell form as something static and rigid becomes engrained. The truth is, however, that no two cells are ever strictly alike, nor is any one cell quite the same at different times of its life history. It is this history which a static textbook picture fails to reveal. To stress this fact, Fig. 1(b) shows by comparison a Chinese brush drawing of some shrub. Here common experience tells us that the bush has not been stamped out in the shape in which one finds it. It has grown into that shape from seed. So, what one sees as pattern in the shrub is merely the residual record of prior activities of that particular protoplasmic system. In other words, shape is simply an index of antecedent processes by which that shape has come about.

Something else is lacking, however, in both of the pictures besides the account of prior events. The objects are portrayed against a blank background as if they were in a vacuum. Again, in the case of the plant, one knows from daily life how vital the invisible air is for its existence as a provider of chemical necessities.

Now, in the case of the cell, the medium is involved even more intricately: it provides not only chemical components for nutriment, but also a physical framework that integrates the separate cells into a structural continuum. The existence of this continuum usually remains unrecognized because staining techniques deliberately leave the substratum out of sight. Yet, to the classical morphologist only seeing was believing and, what is worse, not seeing amounted to not believing. This attitude is undergoing radical change.

Processes as such are not visible. What is visible is a constellation of elements at different stages of the process. Visible form, a pattern at any one stage, must be viewed as the product of antecedent formative processes. The cell thus appears as a system of highly complex, but ordered, molecular populations grouped in a hierarchy of supramolecular complexes, in constant interactions among themselves and with their environment (that is, the space beyond the cell border), leading to features, some permanent, others transitory, the visible expression of which is recorded as shape. If the environmental conditions are reasonably constant for a group of cells of the same type, the behavioral history of the latter will be reasonably similar so as to end up

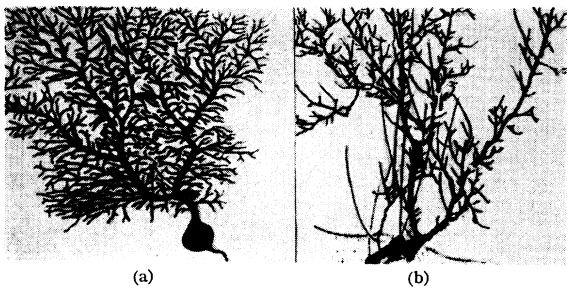

FIG. 1. Comparison between a nerve cell (a) and a plant (b).

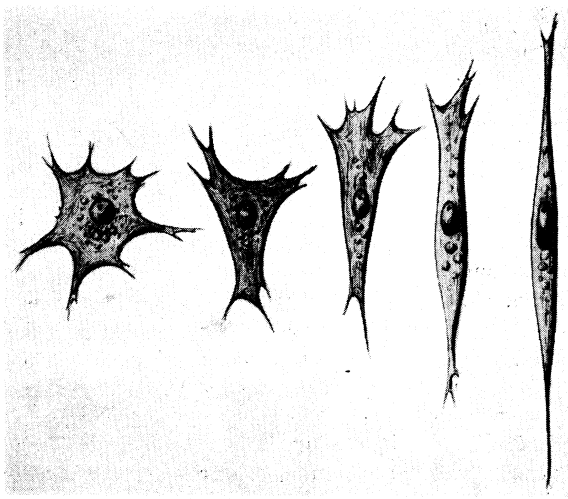

FIG. 2. Samples of shapes assumed by cells of the same connective-tissue cell strain in tissue culture.

with reasonably similar and classifiable shapes of the sort that have made it possible for sciences of microscopic anatomy and microscopic pathology to develop. As soon as there is a change in the conditions, the behavioral response of the cell likewise changes and the familiar shape derived from normal standard conditions ceases to be a diagnostic sign.

A most dramatic illustration of this situation is seen when cells are taken out of their normal site in an organism and transferred into an extraneous medium in tissue culture. As an example, a time-lapse phase-contrast microcinematograph* of human-liver cells spread on glass in horse serum (film made in my laboratory by A. Cecil Taylor and Albert Bock) shows up impressively the lack of fixity and the incessant reshuffling of cell content and contour. No static description can do justice to this vivid record of ever-changing activity. These liver cells in culture look quite different from those one would be used to seeing in stained sections through an intact liver. Except for shape, however, they still possess most of the essential properties of liver cells. In a third type of setting, for instance, in suspension, they would assume still other shapes.

Thus, one realizes that there is no way of getting a fully valid description of a cell except by studying its behavior under as wide a spectrum of conditions as is feasible. Cells of different kinds behave differently. While the transfer to tissue culture alters their morphological expressions markedly, they do retain their constitutional distinctions of behavior.

In conclusion, one is led to the thesis that cell shape is the result of a distinctive behavioral reaction of a living cell to its environment.

To make this concrete, consider a specific example. A colony of fibroblast cells cultured in dilute blood plasma yields a wide spectrum of shapes. The same cell can appear in any of the series of forms pictured in Fig. 2, ranging from the bipolar spindle at one extreme [Fig. 2(e)] to the multipolar star at the other [Fig. 2(a)]. The shape thus depends upon the number of directions in which the cell border shows radial extensions. The tips of these extensions are the active mobile organs of the cell. They push outward and thus distort the originally rounded surface of the cell. Evidently, if there are only two processes tugging in opposite directions, the cell body is drawn out between them into the shape of a spindle [Fig. 2(e)]. If there are three major protrusions, the cell assumes a tricornered shape [Fig. 2(b)], and with even more processes along its circumference it approaches more and more a star shape [Fig. 2(a)].

Must one accept this spectrum merely as a given descriptive fact, or can it be explained causally in the way physical systems are treated? The answer is that, to a certain extent, the whole series can be expressed in terms of a single function derived from a study of cellular behavior. Cells do not live in a structural vacuum as is the illusion created by standard histological preparations, which, by stressing only those features which happen to be stained, obliterate the structural continuum within which the cells reside. In tissue culture in a blood-plasma clot, for instance, this continuum is provided by a network of fibrin fibers—aggregates of molecular chains of varying diameter from submicroscopic to microscopic dimensions, the meshes of the network being filled with serum (Fig. 3). It is in this fibrous jungle that these cells live and move, applying themselves to the interfaces between the fibers and the liquid medium as to a trellis. As was mentioned before, the shape of these cells is determined by the number of protrusions from their surface. One can go one step further and prove that the number of such processes, in turn, is a function of the fibrous constitution of the medium.

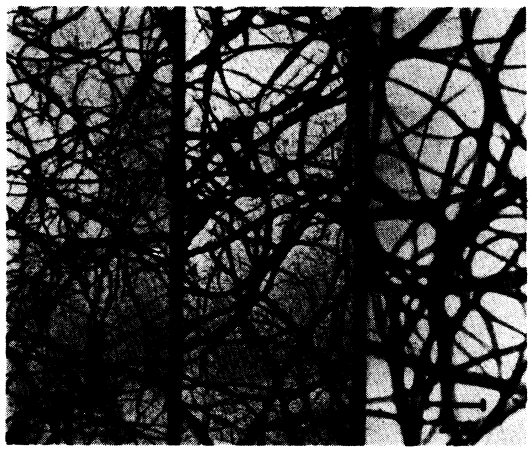

FIG. 3. Electron micrograms of plasma clots coagulated at different *p*H values (from left to right: alkaline, neutral acid).

* The motion pictures referred to in the text were shown at the Study Program in Biophysical Science in conjunction with the lecture on which this article is based.

The relevant interaction is between the cell surface and the fibers in its microenvironment. To understand such surface reactions, one must give up in the first place the outdated notion that the cell surface is a sort of static cellophane-like bag. This may be true of some specialized cell types, for instance, the cellulose membrane of a plant cell, the capsule of a bacterial cell, or the envelope of a red blood corpuscle. But in most types of cells, the surface is far from stable and is by no means of identical composition and state all over the cell. In tissue culture, this state of disequilibrium manifests itself in the continual thrusting forth and withdrawal of surface processes at the expense of cellular energy, showing great variations of the contractile force along the surface. Temporarily weaker points along the surface thus become outlets for thrusts. Such microleaks or "herniations" may occur at random or they may be determined systematically by outside factors, of which one of the most important is the encounter of a fibrous-liquid interface with the cell surface. The fiber contact, in a sense, pricks the cell surface locally. The strength of the resulting strain can be shown to vary with the size of the fibers. Hydrodynamic, viscous, and elastic competition for outflow favors fibers which have a larger diameter (Fig. 4). Consequently, the prevalence of a few major protrusions over minor ones may be expected to be the greater, the larger the average fiber size is in the medium.

These predictions have been tested (jointly with B. Garber) by culturing cells in plasma clots containing fibrin fibers of different average dimensions. The average diameter of such fibers is a function both of *p*H (Fig. 3) and of plasma concentration, larger fibers being formed at either lower *p*H or higher plasma concentrations. It actually was found, in line with expectations, that the ratio of bipolar cells (few processes) over multipolar stellate forms (many processes) increased as a steady linear function as the plasma concentration was raised

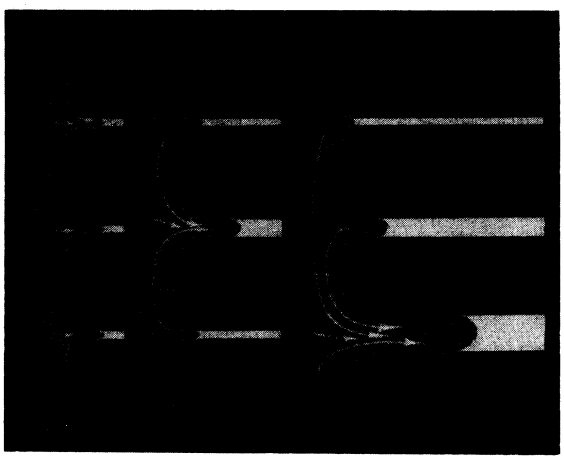

FIG. 4. Microherniations of cell content at intersections of fibrin fibers of various sizes with cell surface. Arrows indicate protoplasmic outflow.

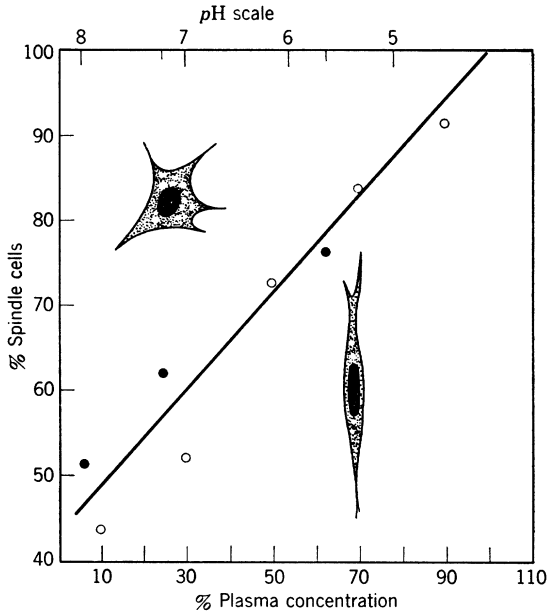

FIG. 5. Distribution of cell shapes in populations cultured in plasma clots of different average fiber sizes.

or as the *p*H during clotting was lowered (Fig. 5). Other criteria of cell shape, such as the ratios of length over width of the cell bodies and of the cell nuclei, showed correspondingly systematic changes. In other words, the whole gamut of shapes displayed by this particular cell strain could be written in a single formula derived from insight into the mechanisms by which deformations into one or another shape come about. The point to stress is that one gets further by studying realistically the formative process rather than by dwelling upon pictorial samples of forms already achieved.

At the same time, it must be stressed that the formula is a probabilistic one, for to predict just what any individual cell will look like is impossible because of the accidental nature of the details of its surroundings. The microclimate and the microenvironment of the individual cell are unique, unknown in each particular instance, and this establishes a certain degree of variance for the actual expressions within each cellular system which is built into its nature.

In a different medium, the same cell strain would give different responses. For instance, these cells, when suspended in a liquid medium without interlaced fibers, would manifest the inequalities along their surfaces by blunt herniations rather than by the pointed protrusions noted along filaments. As a result, such cells appear to be blistering and boiling along their surfaces as can be seen in cinematographs of unattached single cells, and is well known from the loose cells in the late stages of cell division. Conversely, cells of different tissue types would show morphological responses different from the strain just exemplified. The more a cell is given to producing internal structures that serve as a cytoskele-

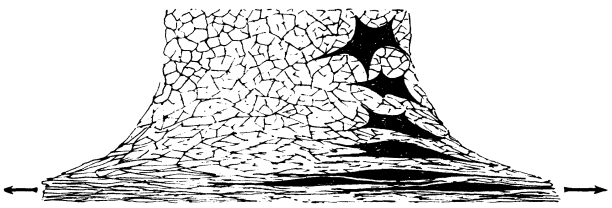

FIG. 6. Effect of regionally varying degrees of tensions on the organization of a fibrin network and, through it, on the morphology and orientation of enclosed cells.

ton—such as in sperm cells, higher forms of protozoans, or muscle fibers—the less will its shape be codetermined by physical constellations in its environment, although even in the most extreme case the polarization of the axiated system of the cell is presumably still a response to external gradients or to other inhomogeneities of the environment. There is an enormous task laid out here for future detailed investigations on the physical factors involved in cytogenesis and morphogenesis.

As a further example of the complexities involved, one can again cite experiments with connective-tissue cells in tissue culture. A long time ago, I became preoccupied with the role of external factors in guiding the oriented movements of cells. In 1927, I succeeded in orienting cells in tissue culture by applying stretch to the blood-plasma clot; the cells assumed a common orientation along the lines of tension. The analysis of this phenomenon over the years has led to the following summary conclusion: The primary effect is on the orientation of molecular chains which become aligned in the direction of the stretch. When cells are contained in such a medium, their shape—that is, behavioral deviation from a sphere—simply reflects the degree of structural organization of the underlying submicroscopic fibrous network. Where all fibers run parallel and, therefore, where one direction only is open to the cells, they naturally become bipolar, all pointing in the same direction (Fig. 6). With decreasing amounts of stretch and correspondingly less rigorous orientation of the fibrin, cell forms grade over from the strictly determined spindles at one extreme to the only probabilistically describable cells of the multipolar sort mentioned in the earlier example. In the present case,

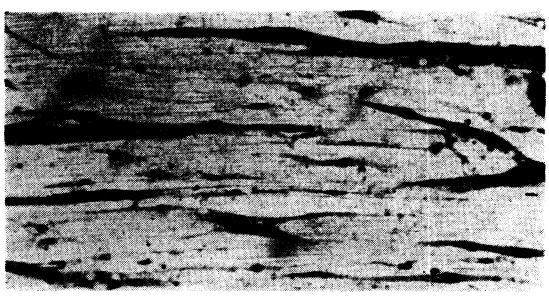

FIG. 7. Elongation of erstwhile-round connective-tissue cells placed on a sheet of parallel collagen fibers from the interior of a fish scale.

however, the average shape does not vary from clot to clot in accordance with the average constitution of the clot, but varies locally within the same clot in accordance with a systematic variation of an extrinsic factor, namely, stretch.

However, the immediately relevant thing for the orientation of the cells is the orientation of the fibrous pathways which they are bound to follow, and it is immaterial that, in those earlier experiments, the agent for producing such fibrous orientation was stretch. Even though the normal organism frequently uses stretch in that capacity, other forces also are at work to produce oriented fiber patterns, which likewise act as guides for cells. The inside of a fish scale, for instance, contains layers of collagen fibers beautifully arrayed in parallel lines, and when loose spherical cells are deposited on such a sheet they immediately assume spindle shapes, with the axes strictly aligned along the fibrous substratum (Fig. 7). Therefore, to carry the analysis

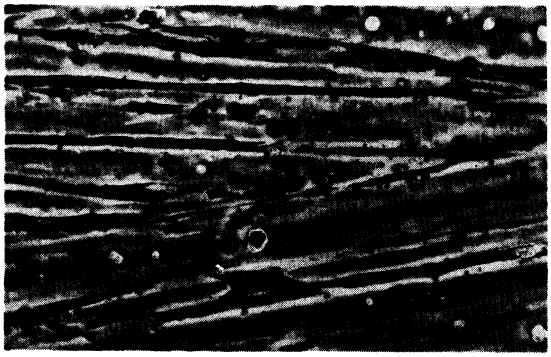

FIG. 8. Phase-contrast photomicrogram of cells which have become elongated along streaks of silicone paste on glass (round white and dark splotches are clusters of cells over nonadhesive silicone where they have been unable to attach themselves).

further, one must study why a round cell on a linear track becomes correspondingly deformed.

A clue to the mechanism operating in this case may be obtained from the following experiment. A glass slide first is streaked with silicone paste or with cholesterol. A loose cell suspension then is placed on top in an appropriate liquid medium. As the cell surface does not adhere to cholesterol but does adhere to glass, the cell becomes drawn out along the striplets of bare glass; hence, it is oriented parallel to the streaks (Fig. 8). Shape is determined here by the differential adhesion of different parts of the cell surface to the substratum. An even more impressive example of this kind is provided by cells which have been seeded out on glass scored with a microlathe (experiments performed with A. Cecil Taylor). These cells become deformed from a spherical to an elongate shape in the direction of the microgrooves (Fig. 9).

What then precisely is the mechanism of this response? To understand it, consider an older experiment in which spindle-shaped cells were made to spread out

flat when they were forced to splash against a smooth surface from above. Such a surface offers the cell innumerable directions in which to radiate at the same time. Thus, the periphery of the cell actually expands concentrically, flattening the cell into a disk (Fig. 10). This transformation is accompanied by profound changes in function—the cell becomes phagocytic—and in the distribution of intracellular materials, further emphasizing the intimate dependence of chemical activity on the physical constellation in a cell. Similarly, a freshly isolated cell set down on scored glass spreads out at first in all directions, but only those sectors of the cell border which lie in a linear direction of the substratum retain a foothold and continue to advance. The other sectors are retracted and become consolidated as cell flanks.

Each cell surface thus acquires radically different properties at the ends and along the flank. The two "engines," one at either end, remain active and by extending in opposite directions simply draw the cell out

FIG. 9. Cells which have become elongated along the microgrooves of scored glass.

into an elongate form. Such elongation again has further consequences for the cell. For instance, the mitotic spindle preparatory to cell division mostly will become aligned with the long axis of the cell so as to make elongation a major factor in the orientation of cell growth. But because of the opposing tugs, to which this elongate cell is subjected from its two active ends, there is no net forward movement. Such a cell simply shuttles back and forth about a stationary position, comparable to Brownian motion, depending upon which one of the two ends happens to have the upper hand at the moment.

Thus, although the foregoing has brought some deeper understanding of cellular orientation, it tells nothing about the mechanism of cellular locomotion, which remains one of the basic unsolved biophysical problems. Something is known about those cases in which cells have special locomotor organs, such as cilia or flagella, but when it comes to cells moving with their free, unstable surfaces devoid of structural specializations that could be related to motility, ignorance is profound. Cyclosis in plant cells, the gliding of slime molds, the

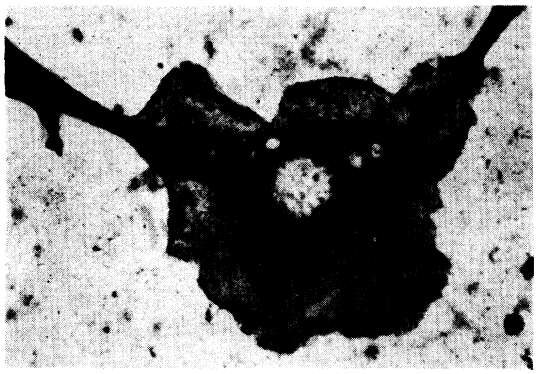

FIG. 10. Spindle cell expanding into a flat disk on impact with glass surface.

shift of a sheet of skin to cover a raw wound, the invasion of tissues by metastasizing cancer cells, or the penetration of leucocytes through capillary walls to converge on a focus of infection—in none of these cases is it known just how the cell achieves these movements, except that it is suspected that gelation-solvation cycles or contraction-relaxation alternations may somehow be involved. This is one of the most neglected areas of physical approaches to biology. Not only is the mode of locomotion shrouded in ignorance, but also there is equal uncertainty about the reasons why a free cell, which can extend in many directions, often advances steadily in one direction to the exclusion of others. As was just said, a cell left to its own devices in an isotropic environment strays at random with no net dislocation.

There have been many theories and speculations about the directive movement of free cells. Once again, one can illustrate how progress has come from eliminating among such competing concepts the ones ruled out by factual analysis. Turning again to the sample object of cells in tissue culture, contrary to the scattered population of stationary isolated cells discussed before, the cells of a solid fragment of tissue explanted into culture behave quite differently. They move in droves from the explanted piece into the empty medium, giving rise to the well-known phenomenon of "peripheral outgrowth." Why do they move centrifugally? For a long time, I had considered this question synonymous with that of cell orientation and had invoked the fibrous guide rails as explanations of "oriented movement." But, as was just explained, orientation and displacement are two different things, and for the displacement there has been no crucial explanation. "Tropisms" and gradients of various kinds have been proposed to explain the phenomenon. It has been assumed that cells respond either positively or negatively to differential concentrations of hypothetical "attractive" or "repelling" substances emanating from point sources, even though it has never been possible to demonstrate just how a cell could translate such directional cues into actual convection towards or away from the source. Recent observations on our tissue-culture

strain have, however, turned up a wholly different story, and it is this.

The only way to get locomotion of a cell with two motor engines at opposite ends is to stop one of the engines, at least for a while. Then the remaining engine can tow the cell away without opposition. This is precisely what happens in tissue cultures whenever free tips of two cells make accidental contact with each other: the colliding ends become temporarily paralyzed. A wave of retraction runs over the affected processes, which become partly detached from the substratum, and the two cells thus come under the exclusive pull from their remaining motile ends facing away from each other. Thus, they move apart in opposite directions. After some time, the paralyzed ends gradually recover their motility and again take hold on the ground, so that the cells are stalled once more, but at a greater distance from each other.

Extrapolating this process to a cell population with a gradient of densities, such as a tissue explant, it is evident that, of a pair of cells moving apart, the one shifting peripherally has a lower probability of encountering another cell than has the one which moves toward the explant. Statistically, this leads to a prevalence of outward migration even though cells are actually free to move in any direction. Eventually, a situation obtains in which the cells have become so widely spaced that random collisions are no longer likely; at this point further migration ceases. Thus, the only gradient which plays any role is a gradient of population density. The phenomenon is formally comparable to the diffusion of molecules from regions of higher to lower concentration, except that one deals with the random collisions not of molecules but of complex entities which may be treated as units for the purposes of description. Again, a close observation of the behavior of the real living object has brought answers far more concrete than what could be anticipated from such generalities as "attractive" or "repulsive" forces between cells. Parenthetically, it should be stressed that the type of contact separation between cells mentioned above is characteristic of the species of connective-tissue cells here described, as well as of several similar strains, but it does not apply to other cell strains, for instance of the epithelial variety, where reaction of two cells on contact can be just the opposite—namely, their drawing much closer together, provided they are both of the same kind. Further details on this behavior are given by Weiss (p. 449).

As still another instance of the dangers inherent in dealing with the living cell in terms of broad generalities, one may once more cite the behavior of cells of diverse shapes observed in plasma clots of different pH or concentrations. As was reported before, such cells have a variable number of processes, each of which now may be thought of as a train behind an "engine." In such cultures, it is possible to calculate the average rates of advance of the various cell types in a given direction by dividing the total distance spanned in a given period by the time elapsed. Since such cells move neither steadily nor in a straight course, however, any such average value for "rate of advance" is wholly unrepresentative of the true velocity of cell motility. As plasma concentration is increased, the cells tend to become bipolar. This means, inevitably, that the average rate of locomotion increases simply because the course is less tortuous and the cell is stalled less frequently by simultaneous divergent pulls from multiple processes to which multipolar cells are subjected. Consequently, in the lower concentration range, where most cells are multipolar, there is a progressive increase of the average rate as the number of processes declines toward the liminal value of two (Fig. 11). Once the great majority of cells have attained bipolarity, the average rate of advance remains constant, expressing more nearly the true velocity. By comparing Fig. 11 with Fig. 5, it can be seen that, at a plasma concentration above 50%, where the "rate" curve levels off, more than three-fourths of the cell population are actually bipolar. Just as one cannot tell the true speed of a railroad train if the times of departure and arrival at the terminal are the only data available and the frequency and duration of station stops on the way are not taken into account, so the mere establishment of the average rate of locomotion of a cell under various conditions has little practical meaning. Yet, the literature is full of examples in which such average rates have been used to assess the effects of a variety of agents or drugs on cells, without due attention to the effects of these agents on the medium, which may alter the whole setting in which

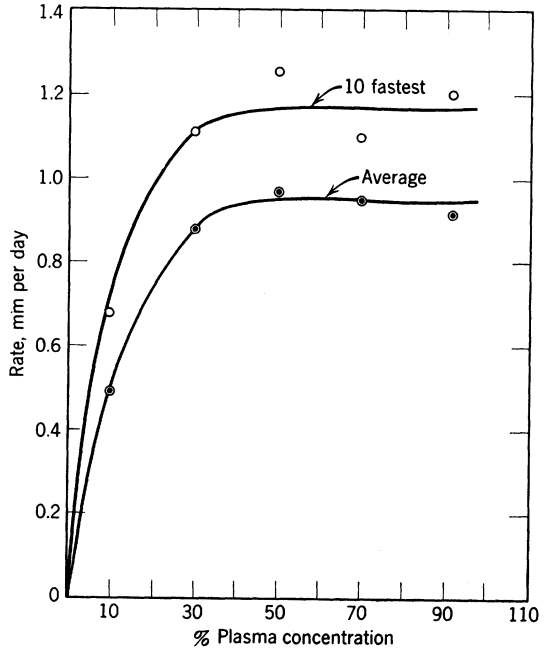

FIG. 11. Rate of progress of connective-tissue cells in plasma clots of different constitution.

the cells move. Without knowledge of these effects, no meaningful comparisons can be made. This illustrates some of the hazards of operating with *average* quantities when one deals with systems such as cells, the composition and behavior of which are inhomogeneous in space and time.

The foregoing discussion has illustrated how a controlled modification of the medium can indirectly modify cell morphology and behavior, including locomotion. It has explained the orienting effect of tensions on the fiber systems of the medium; how such structures evoke conforming organization in the cell population residing in them; and how cell-to-cell interaction and, in the last analysis, population dynamics govern cell locomotion. In this chain of events, an outside experimenter applying tensions to a culture medium appeared as the primary agent. This, of course, immediately raises the question as to what factors serve this organizing function within the living body. The sole agency of the body is its own cells and it was comforting, therefore, to find that cells, by their own activity, can create the type of orderly structural patterns in the intercellular spaces which had been imitated crudely by extraneous tensions. The cells engender in their own environment physical conditions and orderly restraints which then in turn play back on them as guides and regulators of their own behavior. Thus, a further step of complexity is added to the picture. There are innumerable examples, mostly poorly understood, all showing how, through an enormous variety of mechanisms, the same basic principle is served; which is that the cell population, through its products and interactions, sets up conditions modifying the behavior of the enclosed cells, and thus often leading to new settings and interactions which may cause further alterations of the cells, and so forth, in sequences of interactions of ever increasing complexity.

To illustrate this, consider a piece of tissue embedded in a fibrous network and let the boundary of the tissue

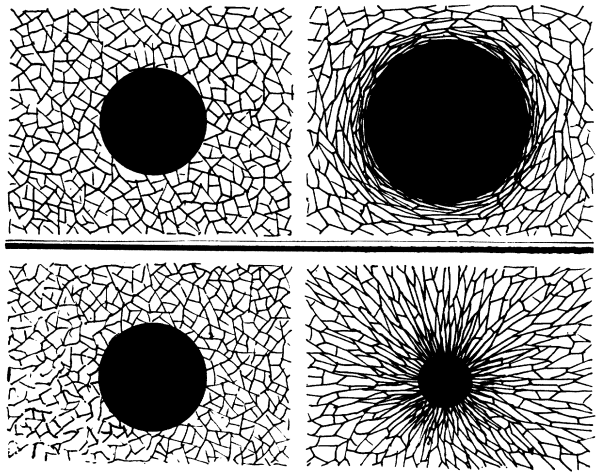

FIG. 12. Effect of an expanding (top) or contracting (bottom) center on the architecture of the surrounding meshwork.

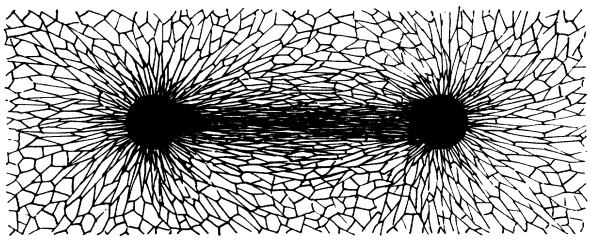

FIG. 13. Effect of two simultaneously contracting centers on a common meshwork.

expand, as happens, for instance, in a vesicle or tube or cyst swelling by the secretion of liquid into its closed lumen. Evidently, as shown in Fig. 12, top, the meshes of the fibrous medium become circumferentially compressed so as to assume a predominantly tangential orientation around the fragment. Cells happening upon such a territory would obviously be forced to circle round and round producing an envelope or tunic. Many of the connective-tissue sheaths and capsules in the meshwork of the body owe their origin to this mechanism. By contrast, if somewhere in the meshwork there arises an area which contracts, the fibrous components will be gathered toward that center in a predominantly radial orientation (Fig. 12, bottom). Cells in such zones then become likewise disposed radially. This type of effect obtains frequently in the vicinity of rapidly proliferating cell groups. It seems that chemical agents, as yet undefined, are being discharged by such cells which cause intensive synaeresis of the surrounding colloids, which means condensation of the fibrous components with loss of bound water, the resulting local shrinkage being much greater than the gain of mass by cell growth. In other words, a purely *scalar* change in a piece of tissue—increase or decrease of volume—can, through the intermediary of a fibrous continuum, translate itself into marked *vectorial* effects, establishing well-defined geometrical and structural patterns. A further degree of ordered complexity is introduced if two or more cell masses are explanted together in a common clot (Fig. 13). Through their joint constriction effects, they deflect the fibrous meshes of the intervening medium into a line connecting the active centers, and this, of course, constitutes a path for direct cell traffic between them. The cells growing out from the two centers simply follow the submicroscopic bridge which has been laid down for them automatically by the tension-engendering chemical activity of their sources (Fig. 14). Here is one primitive example of how chemical action can translate itself into physical organization.

Extrapolating briefly from these model systems of living cells, one may assume that the same sort of intimate interdependence shown here for the microscopic dimension repeats itself both in submicroscopic and in higher supracellular dimensions. One is led to the conclusion that there is a tie between physical structure and chemical activity which is indissoluble and which

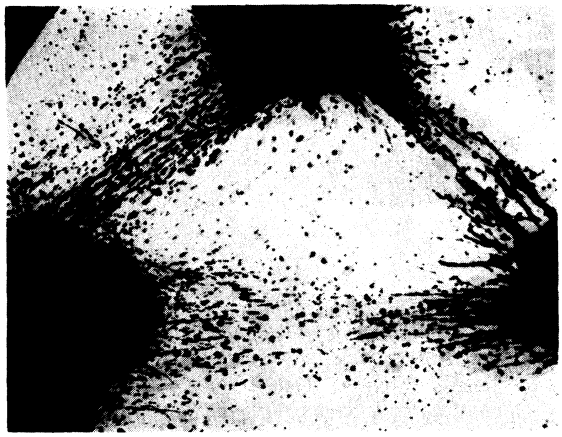

FIG. 14. Automatically established triangular cell-bridge connections between three separate tissue cultures (dark masses) in a common blood-plasma membrane.

it is not enough to assert, but which has to be explored systematically. Where, in order to study a particular metabolic reaction in isolation, the biochemist provides optimum conditions for that process, the cell produces the unique prerequisites for that same reaction only in certain strictly confined localities. It does it through the metabolic products of other chemical reactions going on in another equally confined sample of cell space. Thus, one system feeds another and, in turn, is fed by a third, in a vast system of mutually interdependent, symbiotic, and harmonized partial reactions subsidiary one to another. This interdependence, characteristic of the living state, is what I once termed "molecular ecology." As a field of investigation, it barely has reached infancy. Yet its significance is pointed up by the fact that, in the living cell, the various biochemical partial systems coexist in a common space, without preformed rigid partitions, but rather compartmentalize themselves by structural effects of their own activities of the sort exemplified in crude and elementary fashion by the case just cited. Physical structure and physicochemical conditions limit the types of enzymatic and other chemical reactions that can go on in a given spot —for instance, along interfaces of fibrillar, lamellar, or corpuscular systems—while the resulting reactions, in turn, modify the physical substratum; and by continual interplay of this sort between physical structure and chemical action, the cell system passes first through its progressive developmental transformations and then is stabilized in the steady state of maturity. To some extent, physical structure then is frozen into static arrangements of cytoskeletons, but even then physical structures still are regenerated continuously by cellular activity, leaving at least part of the cellular system in a state of incessant development and self-renewal.

A realistic concept of cell behavior also must take into account the limits set to interactions between distant parts by the formation of compartments within compartments. A diagram of the organism (Fig. 15) would represent it as a system of concentric shells, with the gene in the center enclosed in the chromosome, which is enclosed in the nucleus, which is enclosed in the cytoplasm, which forms part of a tissue, which forms part of the organism, which is surrounded by the external environment; for simplicity, cytoplasmic particles are omitted. No outer agent can influence any of the inner shells except through the mediation of the shells in between, which may or may not modify that factor during its inward passage. Conversely, products of inner systems may not reach outer shells as such, but may be significantly screened and altered in transit. The arrows in the diagram indicate the complex network of relations that one must bear in mind. Oversimplified mental pictures form when, for instance, the simple statement is heard that a gene "controls" a particular feature of the organism, without allowing that it can do so only through interactions with the outer shells, which in themselves have become progressively modified in their long developmental history by countless chains of interactions with other shells, including, of course, the innermost, the gene. Since it has been my assignment to sketch the living cell as it truly is in all its highly ordered complexity, I feel compelled to caution against the illusion that a simple statement, such as, "a gene controls a character" reflects any similar degree of simplicity in the phenomenon covered by the statement.

A final example projects the principle of interaction between physical structure and chemical activity upward into the realm of supracellular order, a field so baffling in its problems that many an investigator prefers to look the other way when he encounters them. The example is chosen from an almost diagrammatically simple object which, because of this, holds at least some promise of more penetrating analysis by the combined physical and chemical tools now at one's disposal. It refers to the origin of the internal architecture of cartilage. Cartilage is formed by groups of cells producing

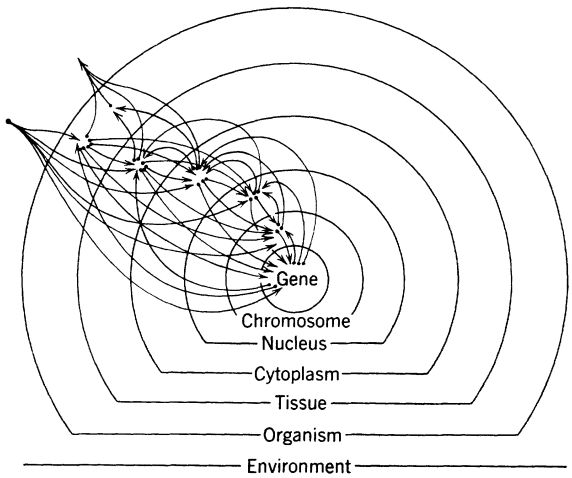

FIG. 15. Diagram of network of possible interactions in an organism.

ground substance between them. That ground substance has been identified partly as a mucopolysaccharide—chondroitin sulphuric acid—and collagen. But cartilage is not just chemical substances; it assumes characteristic shapes and configurations, depending upon its sites, owing to developmental processes under genic control. The nature of the problem becomes clear when one contemplates that the peculiar pattern of convolutions of one's earlobes, which are but covered cartilage, is an individual inherited characteristic. How do such patterns form? Cartilage of the limb is different from cartilage around the eye; the former grows in compact, whorl-shaped masses, the latter develops as a flat plate. It has long been known that, if the cell group that is destined to form limb cartilage is reared in tissue culture, it will grow according to the standard limb pattern, giving rise to recognizable skeletal elements. Similarly, the author observed years ago (jointly with R. Amprino) that the precursor cells of the scleral cartilage around the eye, after explantation as an intact group, go on to form cartilage in the shape of a plate. Evidently, in either case, the explanted tissue complex contained some physical properties that guided the cells contained in it into their respective typical arrangements and growth patterns. The crucial property differentiating between limb and eye cartilage had to be thought of as inherent in the block of tissue as a whole, the further development *in vitro* merely amplifying some distinctive architectural pattern already present in the tissue fragments at the time of their isolation from the embryo.

To test this conjecture, the author (with A. Moscona) recently resorted to a technique that permits one to disintegrate a tissue into its constituent cells and thereby to destroy any intercellular structures and supracellular arrangements that could have acted as guides for subsequent development. Cells can be separated from one another and from their matrix by trypsin, washed in a loose suspension, and then seeded out in a tissue culture where they can aggregate into random clusters. More about the manner of aggregation is reported in another article (Weiss, p. 449). When a piece of prospective limb cartilage and a piece of prospective eye cartilage were dissociated in this manner into their component cells and the cells of each type were permitted then to aggregate in tissue culture and to continue with their actual cartilage-forming activities, it turned out that the cells from the limb site produced cartilage of the typical whorl-shaped massive limb pattern (Fig. 16, top), while the cells that had originated at the eye site produced the plate-shaped laminated structure to which they would have given rise prior to their disaggregation (Fig. 16, bottom). In other words, the "blueprint" of the architecture of the group performance is ingrained in each individual cell and not just carried over into the culture by the block of tissue as a whole. Now, how to convert this figure of speech into concrete terms is problematical. But one may speculate that the architecture lies in the specific

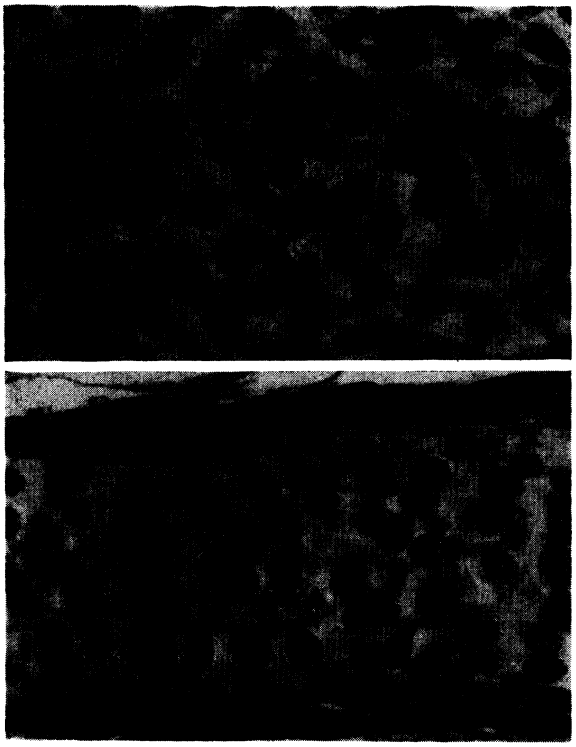

FIG. 16. Cross sections through cartilages developed in tissue culture from dispersed and reaggregated precartilage cells from a prospective limb (top) and from a prospective eye coat (bottom) of the chick embryo, forming whorl-shaped and lamellar structures, respectively.

ground substances secreted by the cells. One would have to suppose that each cell type secretes a complex ground substance of a distinctive pattern of macromolecular stacking or crystallinity of such a kind that it would predispose a planar array of the mass in the form of layers in one case, and a more massive isotropic arrangement in the form of three-dimensional whorls in the other. The cells then would dispose themselves in conformity with these structural patterns of ground substance for which they had furnished the elements in the first place.

This is sheer conjecture. No facts are known that would either support or contradict it. Yet this very uncertainty helps to point up the immensity of ignorance in matters of supracellular organization. If one is convinced that such higher-order organization is to be explained solely in terms of cellular dynamics, then one must raise one's sights to the domain where those phenomena occur, which is no longer the intracellular microcosm.

The study of tissue architecture, used here as an example, may be one of the easier inroads into the maze of perplexing problems presented by the orderliness of the complex living organism, as against the relative disorder and simplicity of its shattered fragments, on which one preferably concentrates, mostly after homogenizing them either physically or conceptually.

The lessons of this story repeat themselves as the viewpoint is shifted from the organization of the body in its structural aspects to the supracellular coordination and ordering systems of its functional activity, whether this be the homeostatic maintenance of blood composition, the integrative action of the nervous system in behavior, or the mobilization of defense and repair mechanisms in response to pathological disturbances. Of course, the operative tools in all of these performances are the individual component cells, which in turn operate through physically ordered subsystems in incessant chemical interaction. But unless one remains cognizant of the fact that the level of the organism is reached from the level of molecular biology not in one single jump over the conceptual gap customarily bridged by the word "organization," and unless one learns to think in terms of a hierarchy of ordered systems, each one with some degree of identity and stability even on supramolecular levels, one obscures rather than enucleates the problems to which thinking and research must be directed. The most impressive feature of a cell is not the constant flux, reshuffling, and variability of its population of molecules and particles (of which our cinematographs presented at the Study Program have given a clear expression), but the fact that, in spite of this ever-present change, each cell remains so remarkably invariant in its total behavior; that indeed, as an entity each behaves so much like millions of other entities equally variable in inner detail, that one comes to recognize them as essentially alike. Such relative invariance of the whole presupposes the harmonious subordination of the behavior of the parts to the conditions of the collective group. It presupposes that the free interactions among the subunits are subject to restraints, the nature and direction of which vary adaptively with the state of the system as a whole. I can think of no more propitious introduction to a biophysics program than by re-emphasizing that the restraints in question are not the sole province of chemistry, especially the stoichiometric branch, but that they include crucial restraints of *physical* nature, with nonrandom molecular arrays a most prominent and analytically most promising feature.

Awareness of the existence of these problems in no way detracts from the pragmatic value of analyzing each elementary component process in its own right and of using whatever models, however simplified, may be found to be constructive as aids to understanding. But it is precisely the phenomenal advances made in the study of the partial and isolated system, in contrast to the dearth of information on the *organized interactions of such systems in the living cell and organism*, that lead me to conclude with a plea for a more balanced effort and more extensive occupation with the latter problems than there are at present. Cellular and tissue dynamics are population dynamics of species of molecules, of cellular subunits, and of cells. Such group dynamics cannot be derived solely from looking at the members of the population in isolation, but only from a study of the ecology and technology of their behavior in the group. It is hoped that this modest effort at presenting the case of the living cell not only has given a fair introduction to the problem but also has shown that practical techniques for its eventual solution are at hand, with physical and chemical approaches indissolubly interwoven.

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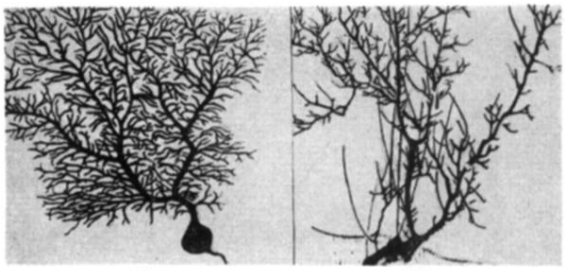

(a) (b)

FIG. 1. Comparison between a nerve cell (a) and a plant (b).

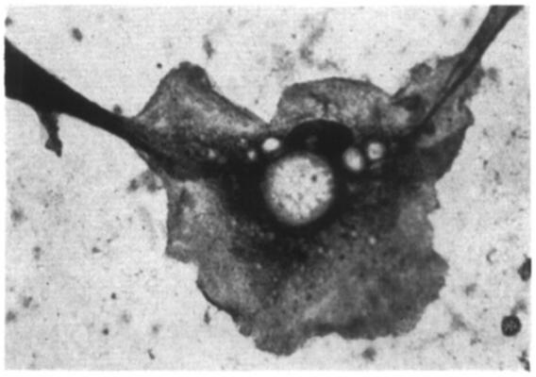

FIG. 10. Spindle cell expanding into a flat disk on impact with glass surface.

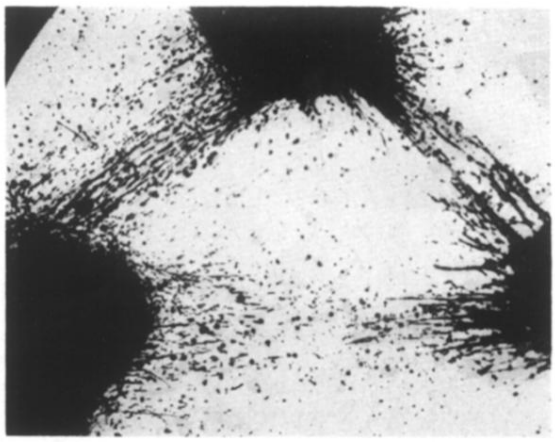

FIG. 14. Automatically established triangular cell-bridge connections between three separate tissue cultures (dark masses) in a common blood-plasma membrane.

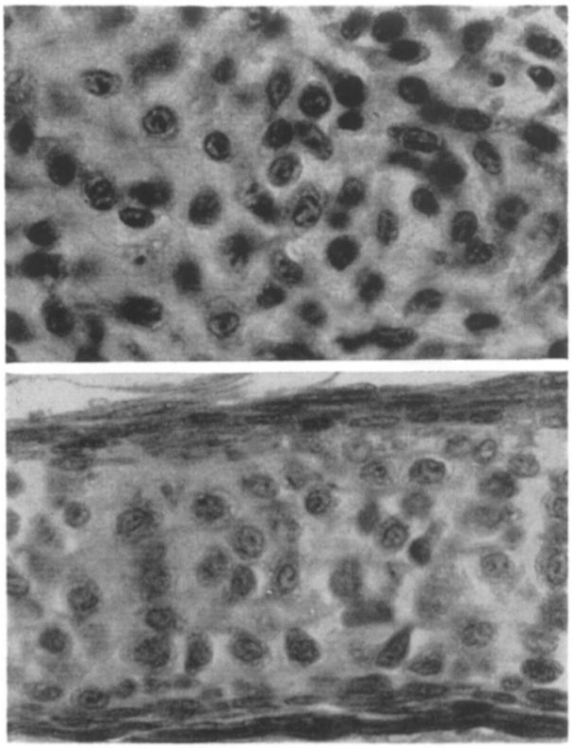

FIG. 16. Cross sections through cartilages developed in tissue culture from dispersed and reaggregated precartilage cells from a prospective limb (top) and from a prospective eye coat (bottom) of the chick embryo, forming whorl-shaped and lamellar structures, respectively.

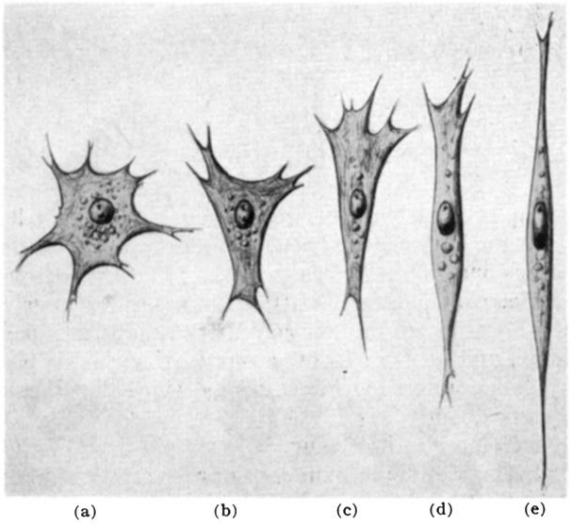

FIG. 2. Samples of shapes assumed by cells of the same connective-tissue cell strain in tissue culture.

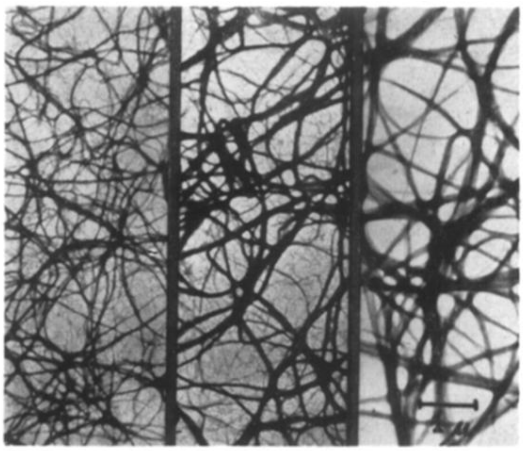

FIG. 3. Electron micrograms of plasma clots coagulated at different *p*H values (from left to right: alkaline, neutral acid).

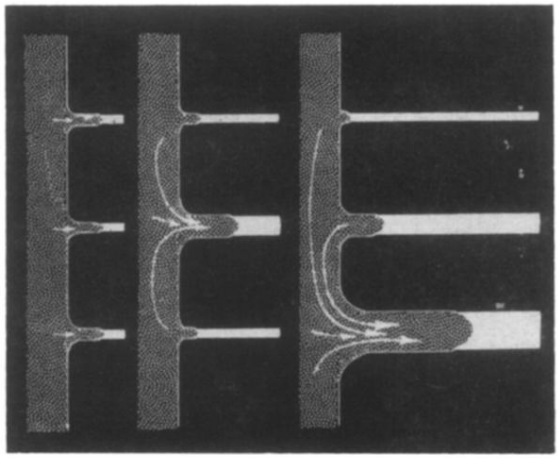

FIG. 4. Microherniations of cell content at intersections of fibrin fibers of various sizes with cell surface. Arrows indicate protoplasmic outflow.

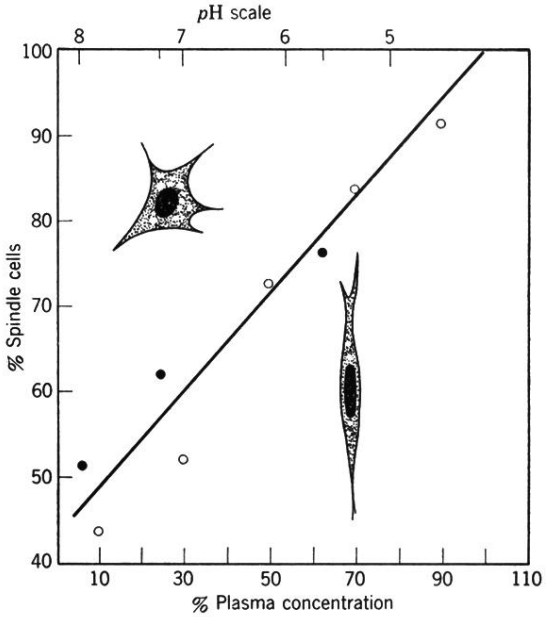

FIG. 5. Distribution of cell shapes in populations cultured in plasma clots of different average fiber sizes.

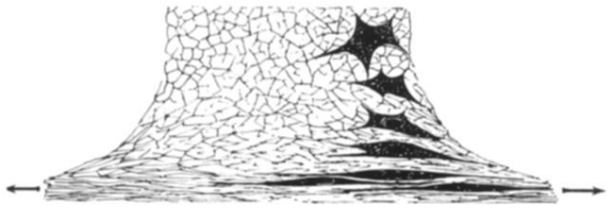

FIG. 6. Effect of regionally varying degrees of tensions on the organization of a fibrin network and, through it, on the morphology and orientation of enclosed cells.

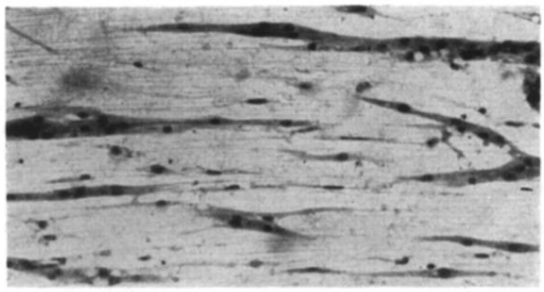

FIG. 7. Elongation of erstwhile-round connective-tissue cells placed on a sheet of parallel collagen fibers from the interior of a fish scale.

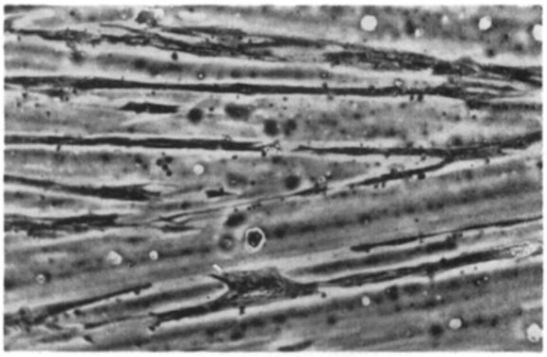

FIG. 8. Phase-contrast photomicrogram of cells which have become elongated along streaks of silicone paste on glass (round white and dark splotches are clusters of cells over nonadhesive silicone where they have been unable to attach themselves).

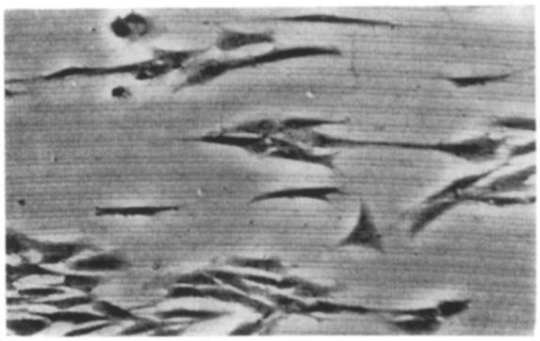

FIG. 9. Cells which have become elongated along the microgrooves of scored glass.