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EXPERIMENTS ON CELL AND AXON ORIENTATION IN
VITRO: THE ROLE OF COLLOIDAL EXUDATES
IN TISSUE ORGANIZATION¹

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TWENTY-ONE FIGURES

INTRODUCTION

The experiments reported in this paper may be regarded as a continuation of the classical studies of Harrison ('14) on the reaction of cells to solid structures, commonly referred to as "thigmotaxis" or "stereotropism," which is one of the fundamental phenomena of morphogenesis.

For the last several years we have been engaged in extensive studies on nerve growth and cell growth in tissue culture. This program, mainly focussed on questions relating to nerve regeneration and nerve repair, has yielded much insight into the mechanisms of cell elongation, cell migration, cell orientation, cell aggregation and cell transformation. The present article presents an attempt to single out from the large stock of data accumulated in the course of this work, those bearing on the problem of cell orientation. The general observations are based on the study of nearly 5,000 tissue cultures, including 376 experiments specially designed to analyze the response of cells and axons to different substrata.

The movements of nerve fibers and spindle cells are dealt with jointly because experience has shown that they follow the same rules. In both cases the prime motive mechanism lies at the free advancing tip, the only difference being that in the case of the spindle cell, the whole cell body is dragged behind the advancing tip, while in the case of the nerve fiber, the cell body remains anchored in its original place, yielding only part of its substance to the advancing process. Past experiments have shown that both spindle cells and axons advance along oriented interfaces in the ambient medium (Weiss, '29, '34), but the

¹This work was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago. It was also aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

manner in which they do this has remained unknown. It is on this point that the new observations have a bearing. We shall advisedly use the term "advance" or "locomotion" for the so-called "outgrowth" of the cells and axons in tissue culture, because the term "growth," under which these phenomena usually go in the literature, is grossly misleading. The term "growth" should be reserved for phenomena involving an actual increase in the amount of living matter, with or without cell multiplication, as distinguished from the spreading or migration of a given mass of cells over a larger area, which is merely a matter of redistribution. The literature is full of examples of confusion due to the lumping of phenomena of migration and of actual growth. The best safeguard against such mistakes is to specify whether one deals with cell multiplication, or increase of cell size, or merely cell locomotion. The following report deals with cell locomotion, particularly the dependence of its orientation and rate upon the physical character of the medium.

The techniques employed varied so much that no over-all description is possible. Details of importance will be given in connection with the individual experiments where needed. In studying adhesiveness or other reactions of cells to solid bodies, the treatment given to the test surfaces deserves special care. The results may vary considerably depending on the cleaning process; on whether the surface is polished, dried by evaporation or wiped; whether the part is sterilized in an autoclave or dry oven; whether in pure air or air containing paraffin smoke or traces of volatile oils; and many other circumstances. Most of the experiments were done in standard cover slip cultures, but some were also made according to Maximow's technique. Non-coagulable liquid media were held confined by a circular wall of paraffin. In these cases, the tissue fragment was always placed on the dry support and the liquid medium was added a few seconds later, after the tissue had become stuck (Weiss, '44a). Liquid media consisted of either buffered Tyrode solution, or blood serum, or a mixture of both in a ratio of from 2 to 10 parts of the former to one part of serum, sometimes with an added trace of embryo extract. Coagulated media were prepared of the standard mixture of blood plasma and embryonic extract diluted with Tyrode solution.

All cultures were studied in the living or in stained preparations. The latter were total mounts treated either with silver according to Bodian, or with haematoxylin.

Sources of cells and axons were the following. Nerve fibers were obtained from spinal ganglia of chick embryos of from 8 to 17 days of

incubation, or from medulla oblongata of 8-day embryos. Schwann cells were obtained in most cases from fragments of adult predegenerated rat nerves. For this purpose, sciatic nerves of rats were transected high in the thigh, and the distal stumps were then left in place for periods of from a few days to several weeks. During this period, Wallerian degeneration occurred and the nerve fibers transformed into solid cords of Schwann cells. Fragments of these nerves of about 1 mm. in length, when explanted, yield large numbers of migrating sheath cells from both cut ends (Ingebrigtsen, '16; Murray and Stout, '40, '42; Abercrombie and Johnson, '42). Their transformation into macrophages has been described on a previous occasion (Weiss and Wang, '45).

Many phases of this work were carried out with the competent assistance of Drs. Agnes S. Burt, Hsi Wang and A. Cecil Taylor, and their cooperation is gratefully acknowledged.

EXUDATES IN LIQUID MEDIA

Most everyone familiar with tissue culture techniques recognizes that "culture in liquid medium" is a misnomer as far as animal tissues are concerned. No spindle cell or nerve fiber will ever actively emigrate from a tissue fragment that is completely enveloped by fluid. Cell migration occurs only if the culture is in contact with a surface. Since so-called surfaces are always boundaries between two media, it seems more appropriate to refer to them as "interfaces." The interface may be the boundary between a solid and a liquid, or between two immiscible liquids, or between a solid and a gas, or between a liquid and a gas. Nerve fibers and spindle cells can extend only along such interfaces.

It has been commonly held that the tissue in liquid cultures spreads along the interface between the supporting cover glass and the liquid medium, and occasionally, the interface between the liquid and the surrounding air. Grossly speaking, this is correct. However, as will be shown presently, the actual contact between the cells and the medium on either side is effected through a delicate intermediary film of organic substance which exudes from the explanted tissue fragments and settles along the macroscopic interfaces ahead of the migrating cells. With the discovery of this film, the main missing link in the explanation of cell behavior in "liquid" cultures seems to have come to light.

The presence of this exudate was first noticed in silver-impregnated cultures of embryonic spinal ganglia in liquid blood serum, diluted with Tyrode's solution (1:3 to 1:5) and a trace of embryo extract, on glass,

mica or celloidin. Fixed after 3 or more days, these cultures showed the central explant surrounded by a distinct network of very fine fibrils, grading from cca. 0.0005 mm. down to the limit of the resolving power of the microscope. This silver-stained reticulum, which will henceforth be referred to as "ground mat," forms a coherent circular zone around the explant and is set off sharply against the outlying parts of the



Fig. 1 Axons and cells on ground mat. Explant: spinal ganglion of 11-day chick embryo. Substratum: mica. Medium (liquid): serum diluted with Tyrode's 1:5 with trace of embryo extract. Period of cultivation: 3 days. Silver impregnation. $\times 660$. Some axons are indicated by arrows. Note wider meshes (some indicated by asterisks) due to proteolytic detachment of ground mat from sides of cells.

medium, in which silver precipitate has been deposited in the usual granular form. Figure 1 shows a sector of this ground mat at the surface of the cover slip, with cells and nerve processes.

Though undoubtedly modified by the histological treatment, the reality of the ground mat as such is safely established, first, by its location — it is strictly confined to within a certain radius from the explanted piece; second, by its reticulated appearance, which is markedly dif-

ferent from the ordinary granular silver "artifacts"; third, by additional supporting evidence presented below; and fourth, by the definite relations it bears to the cells in its area. These relations are the following:

1. The terminal cell filopodia, which are the actively moving parts of spindle cells and nerve fibers, always coincide with fibrils of the ground mat. Protoplasmic processes evidently have extended only along these fibrils and have avoided the exposed glass or mica surface in the meshes between the fibrils.

2. In no case have spindle cells or axon tips advanced beyond the limits of the ground mat. Those that reach the margin are deflected into a tangential course. The ground mat thus keeps the moving units within its bounds.

3. Proteolysis along the surfaces of certain cells often dissolves the adherent fibrils and thus frees the cells partially from their entrapment. Such liquefaction occurs along the smooth, sagging sides of fibroblasts and Schwann cells, and most notably, at the circumference of macrophages. As a result, large holes appear in the elastic ground mat at the site of those cells. Figure 1 shows some examples marked by asterisks.

The appearance of the ground mat suggests that its elements are filiform protein molecules which have aggregated into fibrillar chains. A final diagnosis must await further analysis. The origin of the substance forming the ground mat is likewise unknown. As these cultures were made in diluted blood serum, one might think of fibrin, formed from residual traces of fibrinogen under the influence of thrombic enzymes exuding from the tissue fragments. But since the original medium contained a potent thrombic factor in the admixture of embryo extract, this suggestion can be discounted. All we can say about the substance is that after diffusing from the explant, it becomes adsorbed to the glass-liquid and liquid-air interfaces.

The ground mat joins the living units enmeshed in it into a common fabric and also binds them to the substratum. It thus confers upon what otherwise would be isolated units, the character of a coherent tissue. Its fibrils serve as pathways for the advancing cell processes. It is at the tips of their processes that spindle cells and ground mat adhere most intimately, while the middle portions of the cells may become detached and lose contact with the substratum. Axons likewise cling to the ground mat with their tips, but tend to become detached along much of the rest of their length, with elastic tensions straightening the free portions between points of adhesion. This explains the familiar

polygonal pattern of nerve cultures in "liquid" media (Lewis and Lewis, '12; Levi, '34; Weiss, '34, '41). Only macrophages of the round-cell form (G_1 or G_2 of Weiss and Wang, '45) liquefy the ground mat all around and thus escape from the meshes. They adhere to the substratum directly or float in the liquid above.

To sum up, colloidal substances exuding from explants become adsorbed to the existing interfaces and there form a fibrous mat over which spindle cells and axons extend.

EXUDATES IN CLOTTED MEDIA

Even more conspicuous exudates were observed in cultures of fragments of adult nerve. Most of these cultures were made in plasma clots, and these form the basis for the following description. Figure 2 shows the end of such a nerve fragment after cultivation in a blood plasma clot, the margin of which is far beyond the limits of the picture. Strands of Schwann cells have migrated from the nerve into the medium. Surrounding the nerve end, one sees a more darkly stained area of granulated appearance, ending with a sharp border. This dark area is exudate which has seeped from the end of the nerve into the capillary space between the plasma clot and the mica cover slip. This nerve exudate has been briefly mentioned on previous occasions (Weiss, '43a, b). It is presumably a mixture of endoneurial fluid and degradation products of nerve fibers which have undergone Wallerian degeneration. Similar exudates have recently been reported by Friedenwald, Buschke and Crowell ('45) in wounds of the cornea, and have been shown to contain phospholipids. In view of the presence of disintegrated myelin in nerve fragments, it is not unlikely that the nerve exudate likewise contains phospholipids in addition to the fibrous compounds referred to below. Blood constituents can be discounted as essential parts of the exudate, because washing all blood out from the nerves by perfusion with Ringer's solution prior to explantation does not alter the character of the exudate. The culture in figure 2 had been treated in this manner.

The exudate begins to spread within the first day of cultivation and its margin extends slowly into the periphery. It has a head start over the migrating cells, which follow after an initial lag. After a few days, the exudate stagnates. Cell migration occurs throughout the thickness of the plasma clot. However, while those cells that lie in the interior of the clot follow the fibrin fibers in the usual manner, the ones that are in contact with the glass-plasma or plasma-air interfaces show again a marked dependence upon the exudate which has seeped out along those

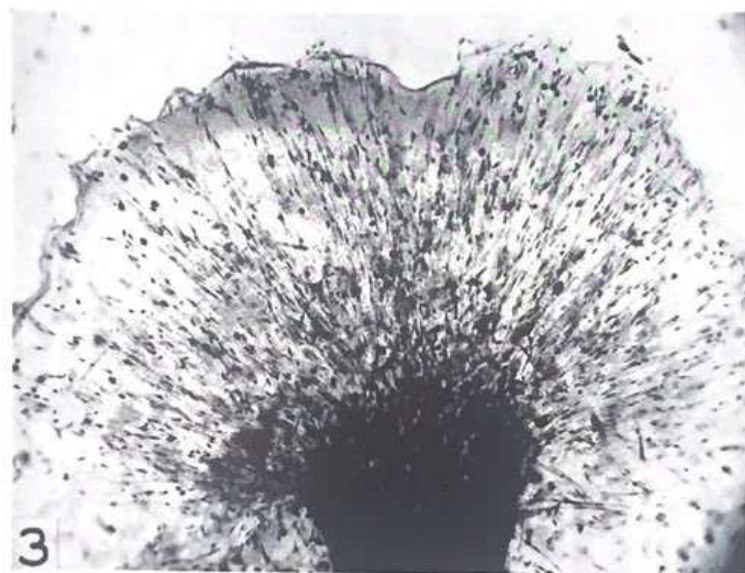
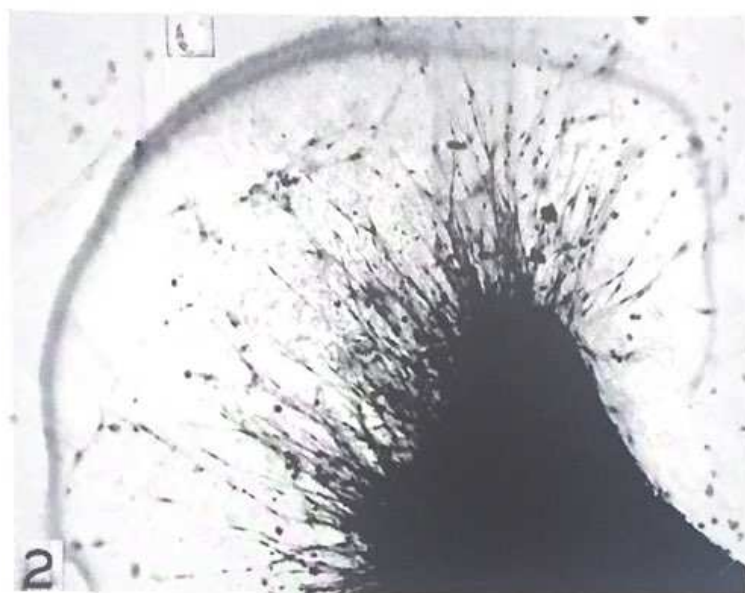


Fig. 2 Exudate from explanted peripheral nerve. Explant: rat nerve after 14 days of predegeneration; blood removed prior to explantation by perfusion with Ringer's solution. Medium (clot): chicken plasma with embryo extract. Period of cultivation: 7 days. Harris hematoxylin. $\times 72$. The exudate forms the sharply outlined, more darkly stained semicircle around the open end of the nerve; it has spread around the corners, but there is no exudation from the sides of the nerve.

Fig. 3 Coextensiveness of cell population and exudate. Explant: rat nerve, 18 days predegenerated. Medium (clot): chicken plasma and embryo extract. Period of cultivation: 5 days. Hematoxylin. $\times 61$. Note confinement of cells to the stained exudate zone, sharply outlined against the surrounding unstained plasma zone.

interfaces. As in the case of liquid cultures, all spindle cells remain strictly confined within the area of the exudate. This confinement, illustrated in figure 3, becomes evident as soon as the cells reach the margin of the exudate, that is, the line in which the three phases, substratum, plasma and exudate, meet. Upon arriving at this border, the cells are deflected from their radial into a tangential course (fig. 4), a configuration comparable to that arising at the border between two plasma clots of different densities (Weiss, '34). All observations point

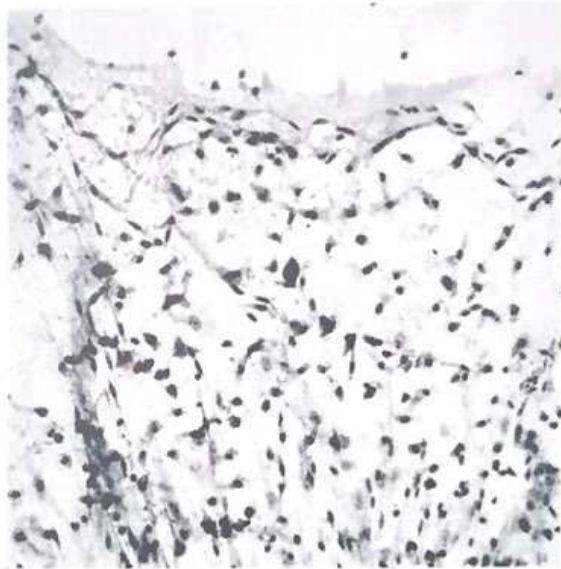


Fig. 4 Deflection of cells at border of exudate. Explant: rat nerve, 26 days predegenerated. Medium (clot): chicken plasma. Period of cultivation: 4 days. Hematoxylin. $\times 116$. Note the straight radial course of the Schwann cells in lower part of picture; their deflection into a tangential, arcading, pattern near the rim; and the advance beyond the exudate border of individual cells, accompanied by protuberances of exudate.

to the fact that the plasma surrounding the exudate becomes gradually condensed so that it finally arrests the spread of the exudate, and that the marginal region of the exudate assumes a circular ultrastructure, which stops further radial migration of the cells by forcing them into a tangential course.

Occasionally, an individual cell appears to have advanced beyond the exudate into the plasma zone. Closer inspection has revealed that this occurs whenever cell processes breach the retaining wall of the plasma border, allowing a tongue of exudate material to flow through the gap, with the cell body following. A few examples can be seen in figure 4.

The histological appearance of the exudate is again that of a reticulum of fibrillar compounds. The radial course of the cells in the inner zone (figs. 2, 3, 4) can be accounted for by the radial expansion of the exudate, which forces the fibrous elements into a prevailing radial orientation. The cells then simply follow the radiating fibrils of the ground mat.

This group of experiments thus supplements the preceding series in showing (a) the presence of a ground mat formed by substances exuding from the tissue and spreading along the existing interfaces, and (b) the formative function this matting exerts by guiding and confining the cells adhering to it. Along the macroscopic interfaces between glass and plasma, and plasma and air, sufficient amounts of this material have become adsorbed to be demonstrable by ordinary histological stains. It would seem plausible to assume that similar adsorption of exudate occurs on the microscopic and submicroscopic interfaces inside the plasma clot in amounts not detectable under the microscope. This would mean that the fibrin framework of the plasma clot would likewise become coated with substances emanating from the cells. Generally speaking, it would seem indicated from now on to examine each case of cell adhesion from the standpoint of whether the cells adhere to their substratum directly or through intermediary substances. Such substances may be discharges from living cells or products of cell disintegration.

TISSUE ORIENTATION THROUGH DEFORMATION OF EXUDATES BY CAPILLARY FORCES

If cells and nerve fibers follow the configuration of the ground mat, it should be possible to guide them into different patterns by forcing the ground mat to assume the appropriate guiding patterns. In the preceding series, the spreading of the exudate was left to chance and, consequently, it occurred rather evenly, concentrically from the openings of the nerve. The following experiments deal with differential spread brought about by forcing the exudate to advance faster in some directions than in others. Capillary action proved to be a very effective tool for this purpose.

One method consisted of placing the explant on a freshly made scratch in the surface of a mica lamella, and covering with liquid medium. Figure 5 shows the patterns of scratches used in these experiments. Cultures were placed at the intersections of cross scratches, and in some cases additional explants were placed in unmarred areas, as indicated

in figures 5D, F, H. Embryonic spinal ganglia were used as explants, and serum diluted with Tyrode solution as medium.

The results were uniformly the same. A typical example is illustrated in figure 6, showing four spinal ganglia, placed at the crossing points of two pairs of intersecting lines and cultured for 4 days. As one can readily see, new tissue has spread from the explanted pieces (dark) in form of tongues extending along the scratches. Each tongue tapers

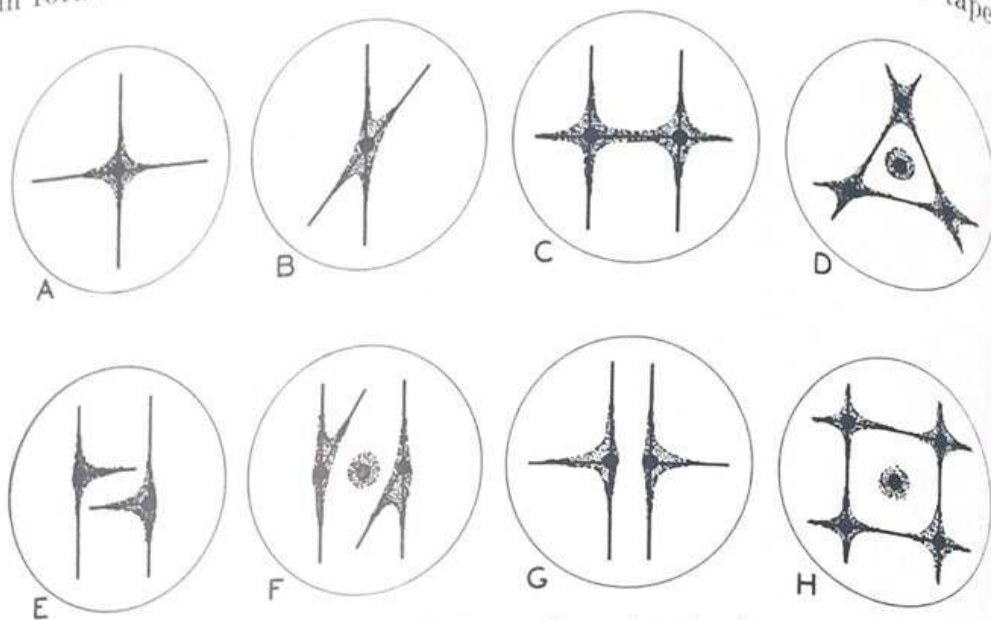


Fig. 5 Diagrams, illustrating the main types of experiments with grooved mica. Circles indicate area of liquid culture medium; heavy lines, scratched grooves; dark areas, explanted ganglia; stippled areas, new tissue after several days of cultivation.

toward an apex which lies directly on a scratch. Some tongues extending from neighboring centers have merged at their tips. As the apex marks the point of farthest advance, the tissue has obviously progressed fastest along the scratches. The depicted configuration is precisely that of the living culture and by no means a result of retraction due to the fixation process. Closer inspection of these cultures (fig. 7) reveals the newly formed sectors to be composed of a fine network of innumerable nerve processes and some sheath cells, most of the latter converted to macrophages. The edge of each sector is sharply outlined, and no nerve fibers penetrate beyond it. The over-all direction of the nerve fibers is from base to apex, giving the sectors the appearance of tents, with the scratches as poles. In favorable preparations, a fine silver-stained mat co-extensive with the sectors of tissue can again be recognized.

The immediate impression from watching the development of these cultures is that some strong pull sucks the tissue out in the direction of the scratches. The intensity of these tensions may be judged from the fact that even the compact original pieces become distorted in the direction of the scratches, as can be seen in figures 6 and 7, and more strikingly, in figure 8. Some cases were observed in which this tug from opposite directions actually tore the explanted ganglion apart in the middle.

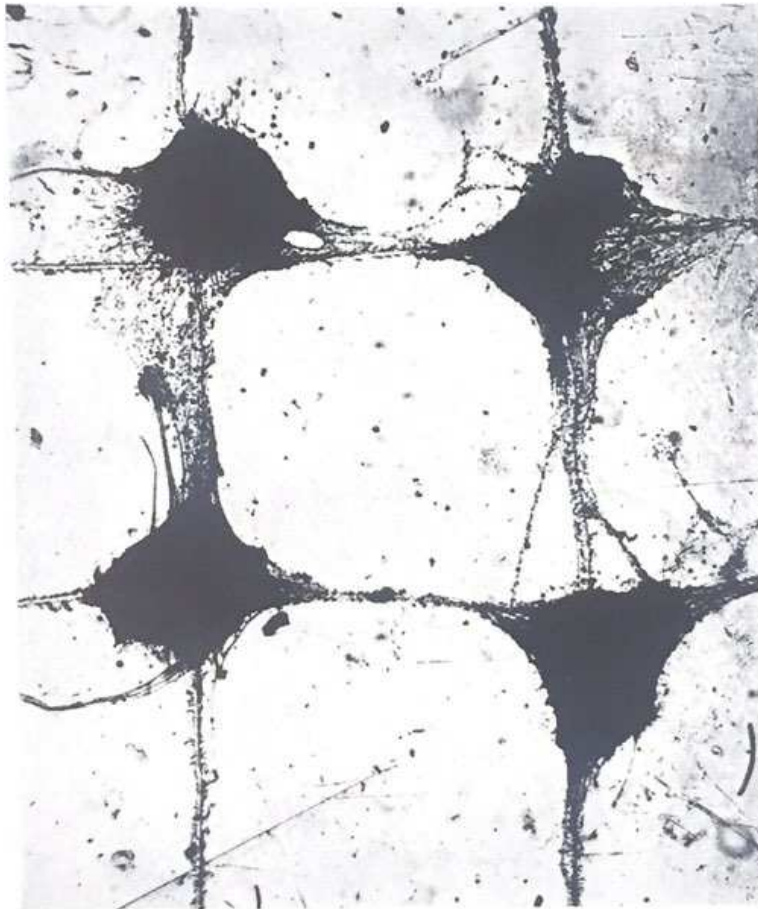


Fig. 6 Oriented nerve and cell extension along grooves of mica substratum in liquid medium. Explants: 4 spinal ganglia of 14-day chick embryos. Substratum: mica plate with two pairs of scratches intersecting at right angles (fig. 5H, without center piece). Medium (liquid): serum and Tyrode's 1:4 with trace of embryo extract. Period of cultivation: 4 days. Silver impregnation. $\times 30$.

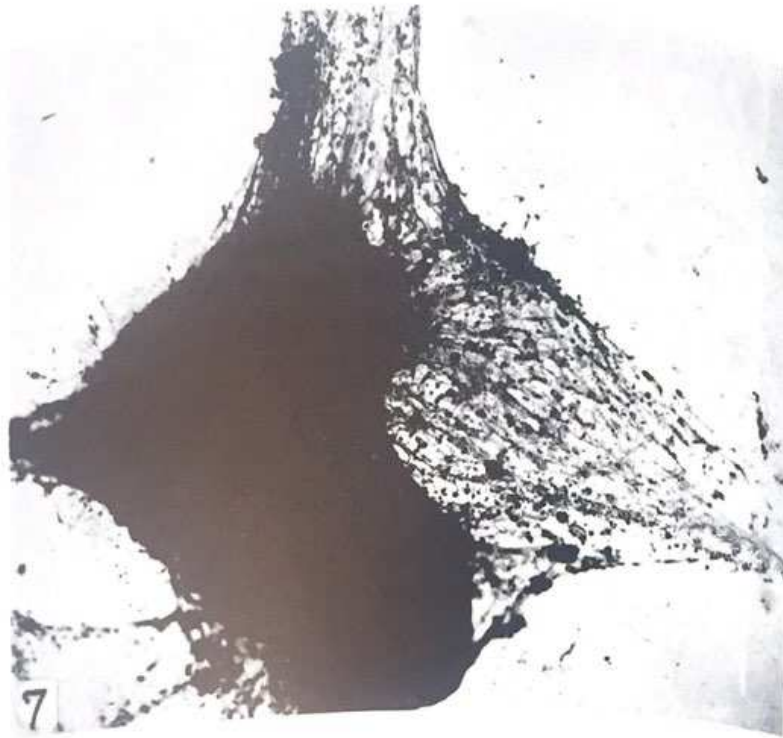


Fig. 7 Detail from figure 6 (right upper corner) at higher magnification ($\times 70$) to show texture of the new web of axons, cells and ground mat along grooves.

Fig. 8 Distortion of explants by tensions from tissue advancing along grooves in opposite directions. Explant: spinal ganglion, 11-day chick embryo. Substratum: mica plate with groove pattern, figure 5D (only one apex of triangle is shown here). Medium (liquid): serum and Tyrode's 1: 5, with trace of embryo extract. Period of cultivation: 3 days, Silver impregnation. $\times 77$. Note extension of new tissue along the four grooved channels accompanied by some "suction" of the old explant itself into those directions. The separate strand of axons lying between the two main branches in the left half of the picture is formed by fibers whose tips had moved out along the left lower groove but which have become detached from the substratum except at tips and bases.

The agent producing both the distortion of the original piece and the oriented advance of the new sectors is, in last analysis, capillary force, operating as follows.

In scratching a mica plate, one cuts across many of its constituent lamellae. They cleave slightly and thus open up countless microscopic fissures, which then constitute a capillary space of very large total surface extending sideways from the scratches. When a droplet of liquid is placed over such a scratch on a dry mica plate, its rapid seepage along the groove can be directly followed. This same phenomenon repeats itself in the actual experiments. In these, the amount of liquid medium used was large enough from the beginning to cover and wet the whole surface evenly (cf. fig. 5). Thus, instead of a mica-air interface, we are now dealing with a mica-liquid interface. But otherwise the conditions are identical, with the part of the wetting drop simply being taken over by exudate becoming adsorbed. Consequently, just as a drop of liquid was sucked into the fissures of the dry mica plate, so the exudate is now sucked into the large adsorptive mica-serum interface along the scratches. Due to this drainage into the preformed grooves, the exudate spreads much faster there than it does in the intervening quadrants, with the dual result that its contour becomes pointed and that its fibrils will be drawn out and oriented toward the corners as the points of fastest advance. Then, when the nerve fibers and cells move out, they merely trace this ground mat. The nerve fiber pattern shown in figures 6 and 7 is, therefore, essentially a replica of the features of the underlying ground mat. There are other adjuvant factors, such as the tendency of advancing macrophages to become trapped along the jagged edges of the scratches; since axons are frequently attached to, and taken in tow by, macrophages, this phenomenon favors the tent-like convergence of axons upon scratches. But the capillary adsorption phenomenon is evidently the main factor.

This concept is borne out by further experimental evidence. First, the deeper the scratches, the more marked is the orientation effect. This is directly attributable to the fact that the number of fissures, hence, the total adsorptive surface, increases with the depth of the groove. Second, only fresh scratches give satisfactory results. Plates with old scratches, which have been washed, cleaned and sterilized repeatedly, are much less effective, presumably because traces of paraffin smoke have penetrated into the cracks and reduced the adsorptive faculty of their surface. Third, scratches in the surface of non-flaking ma-

terials (glass, stainless steel, Vitallium, columbium, tantalum)² do not affect the shape of tissue cultivated on those substrata in the least. Nerve fibers and cells of such cultures behave as if they were on plain surfaces. Grooves as such, therefore, are of no consequence, unless they exercise capillary action.

In conclusion, the shape of tissue moving out from explants in liquid media can be controllably influenced by the presence in the substratum of channels with strong capillary suction. The primary effect is on the exudate film, which forms a mat for the subsequent movement of cells and nerve fibers. Bound to this matting, the individual migratory cells are by no means as isolated and physically independent as is commonly surmised.

This series of experiments has also provided an additional test of the absence of any "chemotactic" effect in cell and nerve fiber orientation. Though the past evidence on this point has been fully conclusive (Weiss and Taylor, '44), the possibility of placing contact guidance in direct competition with hypothetical chemotropic attractions gives the present experiments a key role in the argument. Figures 5D, F and H explain the experimental set-up, in which explants were placed at intersection points of scratches and, in addition, were confronted with a central explant not connected with the others by capillary channels. From the outlines of the resulting tissue migrations, indicated in the figures by stippling, it can readily be seen that the central explants have had absolutely no effect. There has been no mutual attraction between central and peripheral cultures even though they lay within close range. That the formation of tissue bridges between cultures connected by grooves in figures 5C, D and H (see also fig. 6) is a purely mechanical effect, and not the expression of any mutual attraction, is evidenced by the fact that the distances between any two cultures at the corners of the triangle (fig. 5D) or the square (fig. 5H) are greater than the distance of any of them from the center explant, towards which they have sent no processes. Moreover, new tissue may extend just as far, or even farther, in the direction away from the nearest culture as towards it (note examples in fig. 6). It should be emphasized, however, that the observation that no "bridge" forms between two neighboring cultures unless they are connected by a capillary groove, applies only to liquid cultures and not to plasma clot cultures, in which, as previous experiments have shown (Weiss, '29, '34), contractile forces attending growth produce a structural connecting path. Even then,

² The cooperation of Dr. I. R. McCall of the Tantalum Defense Corporation in providing samples of these metals is gratefully acknowledged.

however, the resulting cell bridge is the product of a guiding structure, and not of chemotactic attraction.

CELL ORIENTATION ALONG FIBERS

In 237 cultures, fibers of various descriptions were placed in contact with the explants. Culture media were either liquid or clotted. We shall consider the latter first because of their more crucial character. Of the many fiber types used, we shall mainly discuss glass wool, which was used in two sizes, 8 micra and 26 micra thick.

Figure 9 shows a typical case after 4 days of cultivation. The explant was a nerve fragment, the medium a blood plasma clot. The vast majority of the emigrated Schwann cells can be seen lined up along the glass fibers. Figure 10 shows the sharp contrast between the dense settlement of cells along the glass fibers and their sparseness in the plasma clot proper. Figure 11 illustrates the deflecting effect of a glass fiber laid crosswise over outleading rails.

The general results, of which these illustrations furnish examples, were the following. (1) Many more cells move out along the glass threads than move into the surrounding plasma clot. (2) Cells along glass fibers are oriented with their long axes parallel to the glass fiber axis. (3) Cells move more rapidly along the glass fibers than along the fibrin fibers of the clot (see below). (4) Cells extend farther along the glass threads than they do in other parts of the clot. (5) Cells making contact with a glass thread through one of their processes are readily sucked on to the glass, whereas the reverse movement, from glass into plasma, meets with considerable resistance.

Two facts in these experiments are especially noteworthy, as they both prove the inadequacy of the conventional concept of "thigmotaxis," implying merely a tendency of a cell to apply itself to a solid surface.

The first fact is that the glass fibers are not surrounded by liquid—in which case they would be the sole structures offering passage to the cells—but are firmly embedded in a plasma clot, offering alternative routes of advance. This is no longer merely a matter of cells clinging to the only interfaces available, but of their extension along one particular type of interface in preference to another.

The second fact of significance is that all cells proceed in the direction of the longitudinal axis of the threads—a fact clearly reflected in the strict longitudinal orientation of all nuclei. The cells are equally straight and well oriented whether they are single or connected in chains. These relations are illustrated in figure 12, which shows three cells on a glass fiber in profile. The cell bodies are in contact with the

glass and their axes are strictly parallel to the axis of the glass fiber. Where there are no cells, the plasma clot adheres firmly to the glass. Where there are cells, a narrow space appears between glass and plasma, due to the detachment of the plasma from the glass in the wake of the cells. The cells have wedged in between glass and plasma.

The conditions in these experiments differ significantly from those of the earlier experiments of Harrison, and from related observations reported since, in which cells were found to follow fibers suspended in a liquid medium. Since neither spindle cells nor nerve fibers can extend into a liquid medium, the solid fibers offered them the only available hold. In the present experiments, however, the plasma clot is well able by itself to support cell migration; if, nevertheless there has been such a marked difference between the reactions of the cells to fibrin fibers and to glass fibers, we must conclude that instead of plain adhesiveness, differential adhesivities are involved which make protoplasm adhere to one kind of interface more firmly than to another kind.

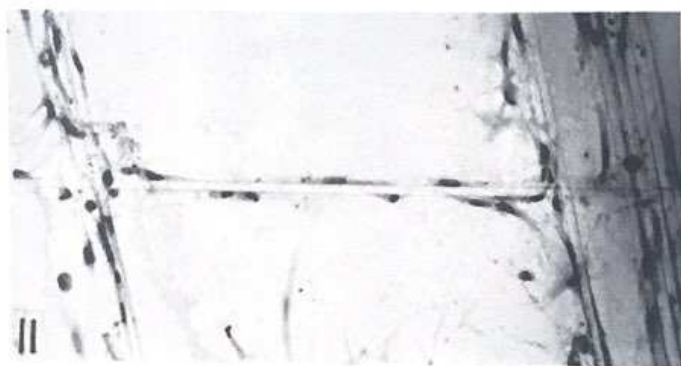
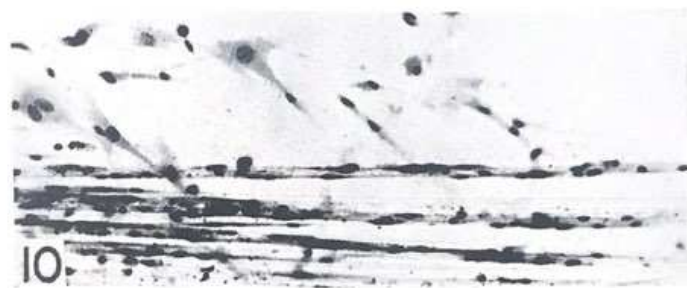
Schwann cells are bound to the glass-serum interface much more firmly than they are to the fibrin-serum interfaces inside the clot. This explains why glass fibers act as traps. The clearest demonstration of this fact is seen when the cells reach the end of a fiber (figs. 13 and 14). In figure 13, fifteen cells can be seen crowded around such an end. Richly arborized pseudopodia have extended from the terminal cells into the plasma clot proper. Yet, their pull in most cases is not strong enough to drag the main cell body off the glass. One of the rarer cases in which they did succeed, is shown in figure 14, where in a number of cells, the mass of the cytoplasm including the nucleus has given up its hold on the glass and followed the filopodia into the plasma clot. However, in this particular case, the cells are so densely crowded at the tip

Fig. 9 Migration of Schwann cells along glass fibers embedded in a plasma clot. Explant: rat nerve, 16 days predegenerated. Medium (clot): chicken plasma, containing glass fibers of 8μ diameter. Period of cultivation: 4 days. Hematoxylin. $\times 91$. Note dense settlement of cells along interfaces between glass fibers and plasma clot.

Fig. 10 Orientation of cells along glass fibers in plasma clot. Explant and medium (clot): as in figure 9. Period of cultivation: 3 days. $\times 130$. Cells with small dark nuclei are Schwann cells, those with large light nuclei are fibroblasts.

Fig. 11 Deflection of cells by cross bars. Explant: rat nerve, 41 days predegenerated. Medium (clot): as in figure 9. Period of cultivation: 5 days. $\times 163$. Note deflection of cells from their original course (from bottom to top along glass fibers near margin of picture) into transverse course (along the glass fiber in center).

Fig. 12 Schwann cells extending along interface between glass fiber and plasma clot, in profile. Explant: rat nerve, 12 days predegenerated. Medium (clot): as in figure 9. Period of cultivation: 2 days: Harris hematoxylin. $\times 360$. These three cells are part of a long tandem chain of similar cells moving from left to right.



that this fact in itself has conceivably helped to force some of them off the glass.

In conclusion, if we ascribe the adhesion between cell processes and interfaces to a force designated non-committally as "interfacial tension," the experiments prove that the interfacial tension for protoplasm along a fibrin-serum interface is much weaker than along a glass-serum interface. In speaking of a glass-serum interface, we must bear in mind that the actual contact between the cells and the glass is presumably again effected through an intermediary ground mat (see below).

Even selective adhesivity, however, is insufficient to explain the observed phenomena fully. It accounts for the fact that cells stay on the glass, instead of moving off into the plasma clot, but leaves unexplained why the cells move with a definite orientation. If merely surface attachment were involved, the cells could be expected to follow any random course in that surface, meandering or winding themselves around the cylindrical glass fibers. Instead, they take a nearly straight course down the length of the fibers (compare figs. 10, 11 and 12, and those cells in figs. 13 and 14 which have not been dislocated by crowding). If the glass fibers were of dimensions commensurable to the cell processes, so that a fiber would be nearly enveloped by the cell, orientation would present no problem. However, in the present experiments, the diameter of the glass fiber (8-26 μ) is up to 100 times that of the advancing cell filopodia (cca. 0.2 μ according to Weiss and Wang, '45). Since the filopodia are the decisive factors in determining cell orientation, the critical contact area between protoplasm and glass is thus narrower than one hundredth of the total available circumference. How then do these filopodia manage to keep on a straight course parallel to the axis of the fiber?

Several explanations might be suggested: (1) The glass surface might have a definite molecular orientation in the longitudinal direction which somehow could serve as directional clue to the protoplasmic processes. (2) The fibrin in the blood plasma next to the surface of the glass might assume a longitudinal orientation, and this fibrin orientation might be the guiding factor. (3) The advancing filopodia might be sensitive to the curvature of the cylindrical surface and move along the line of least curvature. (4) A ground mat, forming from an exudate film, might assume a longitudinal structure, which would serve as pathway.

All but the last of the mentioned possibilities can apparently be discounted, for the following reasons:

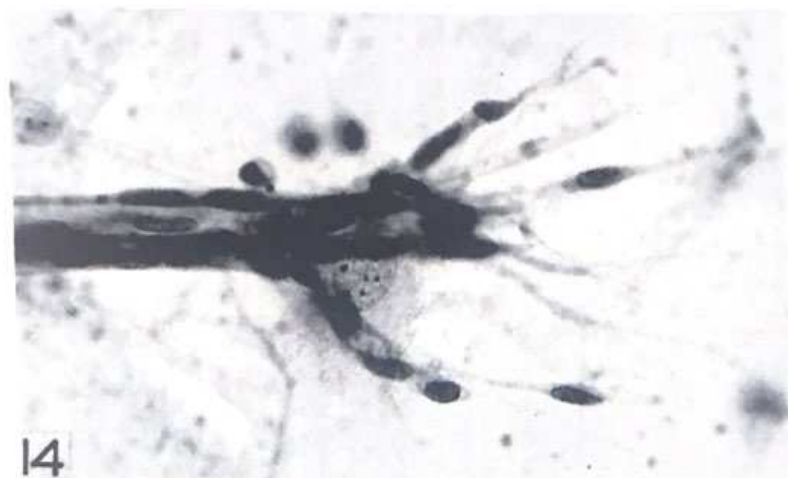


Fig. 13 Crowding of cells at end of glass fiber in plasma clot. Explant: rat nerve, 16 days predegenerated. Medium (clot): as in figure 9. Period of cultivation: 2 days. $\times 530$. Note arborizing processes of terminal cells extending into clot without locomotor success.

Fig. 14 Passage of cells from glass fiber to plasma clot. Explant: rat nerve, 14 days predegenerated. Medium (clot): as in figure 9. Period of cultivation: 3 days. $\times 530$. Note numerous filopodial extensions of terminal cells into plasma clot; in a few instances, the main nucleated parts of the cell bodies have followed.

1. In order to test possible influences of the molecular surface organization, a variety of fibers other than glass were tested. These included textile rayon, Cordura, copper ammonium rayon, cellulose acetate, nylon, aralac, ramie, methyl methacrylate, vinylite, and several others.³ Some of these fibers could be obtained both in the stretched and unstretched state, that is, with more regular or less regular molecular orientation. Cultures were confronted with these fibers both in liquid media and in clots. Summing up the results, there was no significant difference either in the orientation or density or rate of advance of

³ For the supply of samples of these materials, the author is indebted to the Experimental Station of E. I. DuPont de Nemours & Co., and particularly to Dr. M. M. Brubaker.

spindle cells and axons depending on the type of fiber used. Particularly, cell behavior on oriented and unoriented surfaces was the same within the range of variability. These results discourage the assumption that molecular orientation in the fiber surface is instrumental in guiding the cell processes.

2. Orientation of the fibrin surrounding the glass can be discounted as the guiding factor because cells are equally well oriented when moving over fibers in a liquid medium (see below).

3. The criterion of curvature, which could be pertinent in the case of fibers of small dimensions, loses its validity in view of the fact, reported farther below, that cells and axons still take a longitudinal course even when the rods along which they move are so large as to make curvature negligible in comparison to the dimensions of the cell. Moreover, some of the fibers mentioned under point (2), had geometrically irregular surfaces, with curvatures varying greatly from point to point.

4. The concept of an oriented ground mat acting as guide to the migrating cells seems at present the only interpretation consistent with the facts. It is supported by the following considerations.

Let us first call attention to the capillary effect of a glass fiber on exudate spreading along the mica-plasma interface. Figure 15 shows a nerve culture in a plasma clot with four glass fibers inserted. Of these, the two outer ones were in contact with the cover slip, while the two inner ones slanted slightly upwards into the plasma clot. It can be seen that the exudate seeping out in the substratum-plasma interface has been drawn out much farther along the glass fibers than in other directions. This is a capillary effect. To imitate it, one has simply to place a glass rod over a drop of water on an otherwise dry plate, and watch the water run out along the rod. The water corresponds to the exudate, and the plate-air interface to the substratum-plasma interface in our experiment; just as the water tends to wet the glass rod, so the exudate tends to coat the glass fibers and thereby is sucked distally. The glass fibers thus have much the same effect as did the grooves scratched in mica in the liquid medium cultures described above.

This oriented flow can be assumed to have forced the molecular chains of the colloidal exudate into a corresponding orientation, that is, parallel to the axis of the glass fiber. Such orientation would result from the shearing stresses between the substratum and the adhesive mass moving over it; and the longitudinal distortion of the coagulated fibrils of the ground mat undergoing progressive elongation. Evidence of an elongate pattern of the meshes of the ground mat has been seen, but is difficult to reproduce photographically.

The formation of an exudate meniscus between glass fiber and cover slip, as in figure 15, permits visible amounts of exudate to accumulate. Going one step further, we may assume that exudate tends to coat all glass fibers, including the ones fully surrounded by plasma, but in amounts not easily discernible under the microscope. Actually, in favorable cases, a ground mat has been clearly seen on such free glass fibers. The situation is then this: Exudate spreads from the culture distally, extending mainly along the glass fibers. The strains of elongation in the direction of flow force the meshes of the forming ground mat into a lengthwise orientation. Cell and axon processes then simply trace the longitudinal fibrils of the ground mat, and thus give the impression that they had followed the orientation of the glass fibers directly.

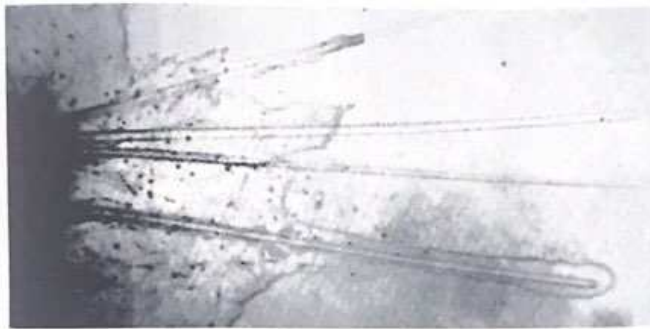


Fig. 15. Capillary extension of exudate along lines of contact between glass fibers and cover slip in plasma clot. Explant: rat nerve, 3 days predegenerated. Medium (clot): as in figure 9. Period of cultivation: 7 days. $\times 34$.

In conclusion, it would seem that the longitudinal orientation of cells in cylindrical surfaces can be explained on the basis of a coat of fibrillar material spreading ahead of the cells and assuming an over-all orientation in the direction of its movement.

If this interpretation is correct, the experiments with different natural and artificial fiber types mentioned above lack conclusiveness, because we then have tested not the reaction of the cells to the surface condition of the various fibers, but rather the spreading of exudate along the fibers. Since this spreading is influenced as much by curvature, polish, surface treatment, acquired electric charges, etc., as by the intrinsic properties of the fiber, and since the two sets of factors cannot be varied independently for experimental purposes, it is not surprising that a better correlation between the nature of the fibers used and the cell behavior along their surfaces has not been observed.

CULTURES ON FIBERS OF LARGE DIAMETER

The final test of the hypothesis of a capillary origin of tissue orientation along a cylindrical interface has come from cultures in contact with relatively large fibers in liquid medium. Fibers of about 0.5 mm. in diameter were drawn from glass or from methyl methacrylate or polystyrene. Two or three of these threads were fastened on a mica cover slip, a fraction of a millimeter apart from each other. Embryonic spinal ganglia were then placed in straddling position over the threads, and a drop of liquid medium was put over the whole system. The difference from the experiments described in the first part of this article is that the migrating cells are faced with a cylindrical instead of with a planar contact surface.

In all cases, the advancing Schwann cells and nerve fibers took a longitudinal course. They formed a coherent tissue, which enveloped the glass fibers more or less completely. However, though oriented lengthwise, this tissue was not straight, but slightly twisted. Figure 16 gives two views of such a new tissue sheath around a glass rod, view A focusing on the lower, view B on the upper side of the same portion of the glass rod. The tissue, consisting of a dense layer of cells with interspersed axons, can be seen to have described a right hand spiral. Figure 17 shows another culture composed mainly of nerve fibers and some macrophages, also forming a right hand spiral.

The origin of the spiralling remains obscure. It is not due to mechanical accidents, such as slide-slipping of the explant or rotation of the rod, for the sense of the spiralling was the same in both directions from the explant; if the explant had been dislocated, the spirals would obviously have been of opposite sense. Out of thirty cases, twenty-seven showed right hand spirals both ways, and only one showed a change of direction at the explant; in two remaining cases, the twist was not marked. The twisting does not seem to be caused by the glass surface, for special care was taken to avoid any torsion in making the glass fibers, and furthermore, glass rods deliberately twisted in a left hand spiral did not alter the direction of the twist of the tissue. One might suspect some asymmetry in the elementary fibrils of the exudate, but it would be idle to speculate about the matter pending further experimentation.

Except for the deviation due to spiralling, the tissue is oriented longitudinally. Thigmotaxis, that is, the mere tendency to adhere to the surface of the glass, would have left these cells and nerve fibers still free to take random courses on the surface. To explain their common orientation, we must again turn to one of the four alternatives

suggested in the previous section. The assumption of a guiding molecular orientation of the glass surface is contradicted by the experiments just described in which the creation of asymmetrical strains in the glass by torsion during cooling had no effect on the orientation of the tissue. Fibrin orientation can be excluded since these cultures were made with liquid medium. The argument of curvature is likewise untenable. The circumference of the glass rod measures nearly 2 mm.,



Figure 16

Fig. 16 Envelopment of thick glass fibers in liquid media by tissue coat with spiral organization. Explant: spinal ganglion, 14-day chick embryo. Substratum: glass fiber 0.15 mm. wide. Medium (liquid): serum and Tyrode's 1:5, with trace of embryo extract. Period of cultivation: 3 days. Silver impregnation. $\times 213$. The same segment of the glass cylinder was photographed both in high focus (B), showing the upper side, and in low focus (A), showing the underside. Note right-handed twist.



Figure 17

Fig. 17 Spiraling of axons on surface of glass fiber. Conditions as in figure 16. $\times 163$.

the diameter of the advancing cell tip about 0.2μ , i.e., only about one ten-thousandth of the circumference. A tip turning off from its straight course at right angles and moving in the direction of maximum curvature for a distance ten times its diameter would describe an arc of only about one-third of one degree, which is so nearly a straight line that the protoplasmic tips could not conceivably discern the difference.

An oriented ground mat formed by exudate seeping from the ex-plants again furnishes the most plausible explanation. The presence of this ground mat can be demonstrated after silver treatment. Figure 18 presents an example of the silver stained reticulum between nerve fibers (same culture as shown in fig. 17). The exudate spreads distally over the glass as a cylindrical film, its fibrils become oriented, and the tips of cells and nerve fibers follow the fibrils. As soon as the new tissue elements have merged into a continuous sheath, the free advancing margin of the latter exerts further pull upon the more proximal parts, and this leads to a further straightening of the cells and nuclei in the direction of the prevailing movement.



Fig. 18 Ground mat with axon filaments on surface of glass fiber. Detail from culture shown in figure 17. $\times 750$.

RATE OF CELL ADVANCE AS A FUNCTION OF THE ORIENTATION OF THE MEDIUM

In the first description of the orienting effect of the medium on cell growth (Weiss, '29), it was reported that cells advance faster and in larger numbers in regions in which the clot is oriented, than in less well organized regions. The difference was ascribed to the differences in the ease of liquid transfer, and hence, supply of nutrients. This explanation might hold for rates of multiplication, but not of sheer migration.

In the present series of experiments, a direct quantitative comparison was made between the rate and extent of oriented cell migration along glass fibers and the rate and extent of the more irregular migration inside the plasma clot under otherwise identical conditions. Nerve fragments were embedded in plasma clots as usual, and glass fibers were placed in contact with one end. Figure 19 shows the result after 2 days. It can readily be seen that the cells have spread much farther along the glass fibers than they have along the fibrin framework of the clot. In fifty-four cultures of this type, fixed on the second, third, fourth and fifth days, the maximum distances covered by cells along glass and in the plain plasma clot were measured. The values obtained

for each day were averaged and are given in the graph, figure 20. The dotted line indicates the means of the maximum distances covered in plain plasma, while the solid line gives the means of the maximum advances along the glass. Distances are given in micrometer units, one unit corresponding to 172 micra. The curves show that the cells have moved more than twice as fast and far along the glass than they have inside the clot. The difference is particularly marked during the first

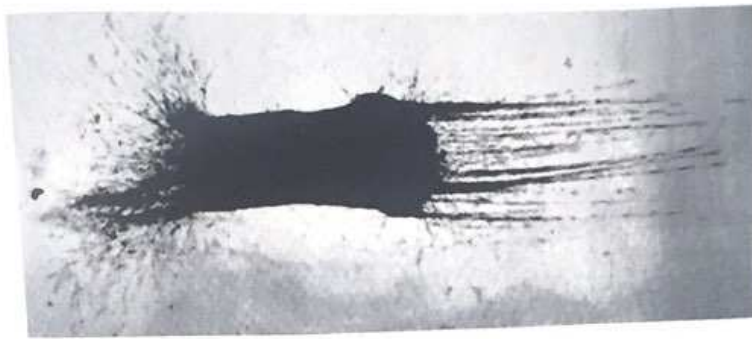


Fig. 19 Comparison between extent of cell advance inside of plasma clot (left) and along glass fibers (right). Explant: rat nerve, 12 days predegenerated. Medium (clot): chicken plasma and embryo extract. Period of cultivation: 2 days. Hematoxylin. $\times 30$.

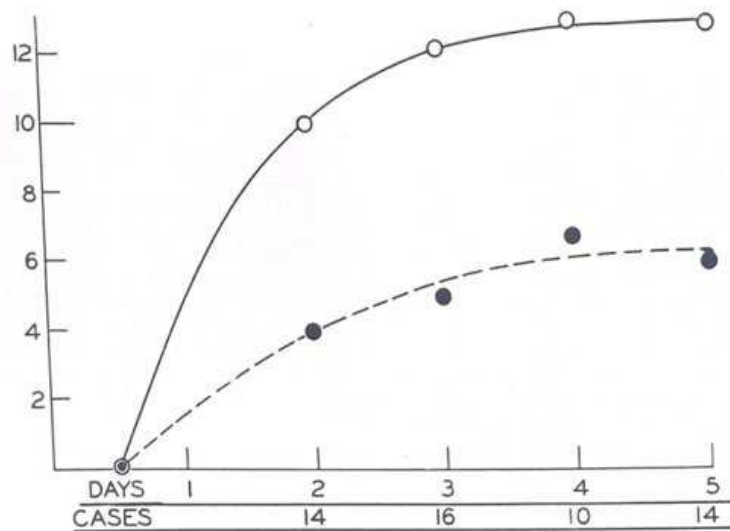


Fig. 20 Graph, giving the maximum distances reached by cells of the same cultures migrating in plasma clots and along glass fibers (compare figure 19). Abscissa: days after explantation (with number of averaged cases). Ordinate: maximum migration in micrometer units (1 unit = 172 micra) —o—o— Cells on glass; ---•---•--- cells inside plasma.

2 days, when migration is more active. Towards the fourth day, the advance slows down considerably in both sets of conditions. An explanation of the facilitation of cell movement along the glass-plasma interface will be presented in the discussion.

LATERAL CELL AGGREGATION

It has long been noted that nerve fibers advancing in plasma clots tend to remain single, whereas those along the interface between a solid and a liquid medium tend to become assembled into bundles ("fasciculation"; Weiss, '41). Evidently, nerve fibers are apt to stick together once they happen to have come in contact. But while the probability of their making chance contact is extremely low inside



Fig. 21 Lateral aggregation of cell cords along glass fiber. Explant: rat nerve, 12 days predegenerated. Medium (clot): chicken plasma, containing glass fibers. 26 μ thick. Period of cultivation: 5 days. Hematoxylin. $\times 175$.

the plasma clot, where they move in all three dimensions of space, the incidence of encounters will be high, where all fibers move in a single common plane. A continuous solid-liquid interface, therefore, is conducive to fasciculation simply because it increases the chances of the fibers to meet. The same conditions that favor fasciculation of nerve fibers favor lateral aggregation of spindle cells. Liquid spaces in the medium are, therefore, preferred sites of fasciculation as well as of cell aggregation.

New illustrations of this principle were observed in the present series of experiments. Often in dense cultures, the file of cells on glass fibers had not remained single, but additional layers had become joined to them laterally. Figure 21 gives an example. The method by which such solid cell cones develop around glass cores is apparently the following. When the first cell layer wedges in between the glass rod and the plasma, it leaves a narrow liquid space in its wake (fig. 12).

According to what was said before, this liquid space gives later cells an opportunity to glide out in direct contact with the surface of the earlier ones. This is another example of the far reaching morphogenetic effects of liquefaction in a colloidal matrix.

DISCUSSION

The facts reported above have confirmed the guiding action of oriented interfaces in the locomotion of cells and nerve fibers (principle of "contact guidance"; Weiss '41). They have, in addition, furnished some data regarding the mechanism through which this orientation is effected. Earlier experiments (Harrison '14; Weiss, '29, '34) had demonstrated the fact that spindle cells and axon tips follow microscopic and even submicroscopic fibrils in their orientation. In those cases the guiding fibrils could be assumed to be of the same order of magnitude as the advancing cell processes themselves; provided thus with straight guide fibers as climbing ropes, the cells could take no other but a straight course. The present experiments, however, show that the direction of cell movement still remains oriented in the direction of the axis of a cylindrical structure, even when the diameter of the latter is several thousand times that of the cell processes. This obviously could be no longer a matter of direct guidance of the cell by the supporting fiber as a whole.

The observations reported in this paper pointed to a solution of the problem, based on the realization that cells are not actually guided by the gross structures to which they appear to be applied, but by a fibrous exudate coating those structures and containing fibrils of dimensions very close to those of the cell processes. The orientation and arrangement of spindle cells and axons is then, in the main, determined by the features which this fibrous mat has assumed during its formation, when it was subjected to the shearing and elastic forces created by its spreading.

Thus, if the exudate spreads uniformly from a center with a concentrically advancing margin, the prevailing orientation will be radial. This explains why centrifugal cell movement predominates even in liquid cultures. Both in liquid and in plasma clot cultures, the prevailing radial cell orientation is a result of tensions. In clots, these tensions arise from contraction within a stationary margin; in liquid cultures, from the expansion of the exudate from a stationary center.

If the exudate film spreads predominantly in a single direction, the forming fibrils will become oriented in the same direction, and so will the cells and nerve fibers following them. The result is essentially the

same whether the linear extension of the exudate is caused by the capillarity of a scratch in a mica plate or of a glass fiber.

The exudate of peripheral nerve cultures can be seen to emanate from the cut ends of the nerve fragments. To judge from its staining reaction, it contains fibrous units, presumably proteins, and perhaps also phospholipids, analogous to those identified in corneal wounds by Friedenwald, Buschke and Crowell ('45). It may contain debris of disintegrated cells as well as discharges from living cells and lysed intercellular material, and its composition probably differs in different organs.

The action of the exudate in our experiments is perhaps merely a special manifestation of a much broader class of morphogenetic mechanisms, namely, colloidal surface coatings in general. Wherever in the body a new interface develops, it will present conditions favoring the adsorption and retention of certain species of molecules in preference to others, and from the mixed molecular population of the ambient fluids those preferred species will emerge and be concentrated along the interface as a film or coat. They, in turn, may serve as foundation for the stacking up of further layers of the same or related kinds, and the character of the coat may thus change with time. Under these circumstances, it is doubtful whether any surface, natural or artificial, organic or inorganic, can for any length of time stay uncoated when exposed to body fluids. After that, the reactions of cells to that surface will no longer be determined by the nature of the surface, but by the nature of its coat. The formative role of surface coats in development has recently been emphasized by Holtfreter ('43) and Lewis ('44). Schmitt ('41) views intercellular cement as mechanism of adhesion in epithelia (see also Chambers, '40). The similarity to the ground mats regulating the movements of mesenchyme cells and nerve fibers in our experiments is noteworthy. Huzella ('29) seems to have recognized these facts rather clearly, when he claimed an "organizing" action for the argyrophile "gitterfasern" of his tissue cultures. However, the rather sketchy account he gives of his experiments leaves the relation of this system to the ground mats of our experiments in doubt.

In all these instances, cells formerly thought to behave as individuals guided by "tropisms," have on closer inspection proved to be linked by a common material matrix determining the texture, orientation, as well as the behavior of the tissue as a unit. It would be interesting to examine to what extent the mass movement of slime moulds (e.g., Raper, '41) follows a similar mechanism.

The problem of direct adhesion between naked parts of cytoplasm and solid surfaces of various descriptions has received some attention

(Pfeiffer, '34). Cells with proteolytic activity deserve special consideration in this connection. Since they are apt to dissolve the protein coats in their vicinity, they would automatically detach themselves from their substrata. Our present, as well as past (Weiss, '44a) experiments, contain ample demonstrations of this fact. Those portions of the cell margin which, to judge from the holes they make in the ground mat (fig. 1), are proteolytically active, can actually be seen to retract from the substratum. Only their non-proteolyzing processes anchor these cells to the ground mat. Completely detached cells round up (types G_1 and G_2 of Weiss and Wang, '45), but may take hold again upon renewed contact with the substratum. If we ascribe to different cell types different lytic discharges (specific proteolytic, lipolytic, etc.), and assume that the various coats adsorbed to different surfaces differ in their composition, a possible mechanism of selective cell adhesion could be construed.

The studies of Holtfreter ('43, '44) on the behavior of embryonic cells on different substrata will have to be reviewed in this light. Similarly, the observations of Twitty ('44) on the migration of pigment cells under different conditions in vitro will have to be viewed with the possibility in mind that in these instances, too, there is a common ground mat of exudate along which the cells spread so that much of the behavior ascribed to the cells directly would have to be reinterpreted as due to effects on the formation and shape of this ground mat.

The observations of Friedenwald, Buschke and Crowell ('45) suggest strongly a mechanical role of the ground mat in wound healing. The authors observed that after a lesion to the corneal epithelium, there is first a spreading of exudate over the denuded area, followed only later by a radial invasion of the wound by the cells of the margin. If the exudate over the wound could be proven to contain fibrous units undergoing synergetic shrinkage, this would explain the cell movements in the healing of the wound. For the contraction of the exudate would produce radial tensions, resulting in a predominantly radial orientation of the ground mat, and this, in turn, would orient and draw the processes of the epithelial cells surrounding the lesion into converging radial courses. This is merely one example of the explanatory value of micromechanical concepts as applied to problems of tissue formation and regeneration. A systematic exploitation of this field is still a matter of the future. It would seem that many morphogenetic processes formerly ascribed to various ill-defined cell "tropisms" may find a realistic explanation along similar lines.

In our present view, locomotion of spindle cells and elongation of nerve fibers still appear as the results of interfacial tensions acting upon the mobile tips. But the interfaces in question are not those between the gross substratum and the medium, but those between the fibrils of the ground mat and the medium. The rest of the cytoplasm simply trails the advancing tip, perhaps aided by contractility of the surface coat, as suggested by Lewis ('42). There remains, however, the question as to why the cells and axons move so much faster and farther along glass fibers in a plasma clot than inside the clot itself, as shown in figure 20.

Closer inspection of the mode of cell locomotion provides the answer. Let us reiterate that it is quite misleading, though not uncommon, to speak in this connection of rates of "growth." It is cell movements we are dealing with; more precisely, the advance of cytoplasmic processes. The rate of this advance is determined by the interfacial pull exerted upon the tips and by the degree in which the cytoplasm yields to, or resists, elongation. The interfacial pull is determined by the inequality $(\overline{CS} + \overline{CL}) > \overline{SL}$, where \overline{CS} , \overline{CL} and \overline{SL} represent the interfacial tensions between cytoplasm and substratum, cytoplasm and liquid phase, and substratum and liquid phase, respectively. This formulation is non-committal regarding the origin of the interfacial tensions. The only variable in the experiments is the value of S , which refers to the ground mat in one case, and to the fibrin net of the plasma clot in the other. It is doubtful whether these values can differ enough to account for differences of migration rate of the observed magnitude.

The following explanation seems much more likely. The rates of advance of the individual filopodia need not differ markedly. However, in a medium with a less regular fibrous skeleton, the much higher incidence of intersections along the course of a cell would entail much more frequent delays, and consequently, much less rapid over-all progress, than would happen in a medium of more orderly orientation. If terminal cell filopodia extend simultaneously in several directions, there always results a tug-of-war among them, and the rest of the cell body, resisting being split, cannot proceed until one of them has won out by drawing more cytoplasm in its direction and thus gradually draining its competitors. The advance of a cell in an ordinary plasma clot is interrupted by countless such delays due to competition among multiple tips. The ground mat on the glass thread, on the other hand, being predominantly oriented in a common direction, presents far fewer intersections, hence, the occurrence of divergent terminal filopodia is much rarer and there are fewer delays. Rate of cell advance is thus merely

an expression of the degree of orientation (inversely related to the intersectedness) of the guiding interfaces.

This simple concept explains the observed results quite adequately without recourse to hypothetical stimulating and inhibitory factors. In fact, one wonders how many of the "growth promoting" or "growth depressing" effects of drugs or physical factors described in the literature on tissue cultures might simply have been effects on the structure of the medium indirectly facilitating or retarding the locomotion of the cells.

The advance of nerve fiber tips follows the same scheme, with the only difference that occasionally, instead of a single filopodium, two may come out successfully from the competition, both of which may then proceed and divide the trailing cytoplasm between them; the result is a branched axon (Speidel, '35; Weiss, '41). Accordingly, as was explained in an earlier paper (Weiss, '44b), the rate of "nerve regeneration," referring to the advance of the tips of the regenerating fibers, is not to be treated as "growth rate," but belongs in the same category as the rate of cell locomotion discussed above. The rate of nerve fiber progress in scar or otherwise unoriented tissue is much slower than it is in oriented tissue simply because of more frequent delays at the incomparably more numerous intersections in the former. The excellent nerve regeneration obtained after the "sleeve splicing" of severed nerve ends (Weiss, '44b) is largely attributable to the fact that the outgrowing Schwann cells and axons are provided with straight unbranched pathways for the transition from the proximal to the distal stump. The fact that Schwann cells and axons can be oriented into straight parallel courses by strands of parallel fibers of glass or other materials, even if the latter are embedded in a blood plasma clot, raises hopes that it might become possible to develop artificial bridges for the spanning of nerve gaps by embedding suitable fibers, properly spaced, in a cylindrical clot, sheathed by a suitable membrane.

SUMMARY

Observations on cca. 5000 tissue cultures of Schwann cells and nerve fibers in liquid or clotted media, with or without the inclusion of fibers of various kinds and dimensions (glass; textiles; synthetic resins; 8-500 μ) have led to the following conclusions concerning the mechanisms of adhesion, orientation, locomotion and association of spindle cells and axons in vitro.

1. Explanted tissue fragments give off a colloidal exudate, which slowly spreads centrifugally over all exposed surfaces and coats them

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1. Explanted tissue fragments give off a colloidal exudate, which slowly spreads centrifugally over all exposed surfaces and coats them

with a fibrous mat ("ground mat"), regardless of whether the medium is liquid or clotted.

2. The terminal processes of spindle cells and axons adhere to and follow the fibrils of the ground mat. Even in liquid media, cell migration remains confined within the limits of the ground mat. The ground mat thus binds the cells to the substratum and orients their course.

3. The orientation of the fibrils of the ground mat, in turn, is determined by the direction in which the exudate spreads. If the exudate is drawn into a linear course, the subsequent movement of the tissue will be correspondingly oriented. This effect has been produced in both liquid and clotted media by placing the explant against a groove exerting strong capillary action or in contact with glass or textile fibers.

4. Since exudate moving down the length of a cylinder from one end produces a longitudinal arrangement of the fibrils of the forming ground mat, cells and axons migrating along such a cylindrical surface assume predominantly longitudinal orientation, even if the circumference of the cylinder measures several thousand times the width of the cell processes.

5. Fibers of glass and some synthetic resins embedded in a plasma clot act as traps for cells that happen to make contact with them. Only exceptionally do cells relinquish the fiber surface and move back into the clot.

6. Cells advance more than twice as fast and as far along the interface between a glass fiber and the surrounding plasma clot as they do inside the plasma clot. This difference is attributable to the more numerous delays at the more frequent intersections in the fibrin framework of the clot. The differences of "regeneration rates" of nerve fibers according to the degree of organization of their surroundings (scar tissue vs. oriented tissue) can be explained on the same basis, without implying any differences in the rates of the intrinsic activities of movement and growth in the neurons concerned.

7. Liquid spaces in the medium, whether primary or produced by proteolysis, create favorable physical conditions for the aggregation of cells, as well as for the fasciculation of nerve fibers.

8. On the whole, the experiments have reconfirmed the principle of "contact guidance" of spindle cells and nerve fibers; have shed some light on the mechanisms of "thigmotaxis" or "stereotropism" in tissue formation; and have intimated a wider morphogenetic significance of fibrous exudates in development and wound healing.

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