NERVE PATTERNS: THE MECHANICS OF NERVE GROWTH

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NERVE PATTERNS: THE MECHANICS OF NERVE GROWTH[1]

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Compared with the steady flow of comparative-neurological research, analytical studies into the "how" and "why" of nerve pattern formation have remained a mere trickle. Whenever pressure for dynamic understanding of the development of the nervous system became irresistible, the comparative neurologist turned to speculation rather than to direct experimental attack. Kappers' "neurobiotaxis," Bok's stimulogenous fibrillation, even Child's gradient theory of nerve development, are noteworthy examples. There were sporadic attempts at analytical experimentation, but a deliberate drive did not really get under way until Harrison stepped in and gave the field an aim and a method; an aim, in that he broke down untractable generalities into tangible problems, and a method, in that he developed new techniques of transplantation and tissue culture for the solution of these problems.[2] His work, aptly extended by his students, notably Detwiler ('36), has been the greatest single step towards an analytical understanding of nerve development. Much of what I am to present today has had its roots in his work.

Harrison's experiments have settled a number of controversial issues concerning the elements of the nervous system, which we may list here as points of departure: (*a*) The neurone doctrine of nerve development was confirmed (Harrison '10): the nervous system arises from the differentiation of discrete cells. (*b*) The filamentous nerve processes, axons and dendrites, arise as extensions of the protoplasm of the nerve cell. (*c*) The cell strands enveloping the peripheral nerve fibers, or sheath cells, are later additions of central origin (Harrison '24). They play an accessory but not a formative rôle.

Nerve patterns, accordingly, do not emerge from a protoplasmic continuum, as was at times suggested (Hensen, Held), but are gradually built up in a true synthesis by the activities of individual neurones in constant interplay among each other and with their non-

[1]Experimental investigations reported in this paper have been aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2]For the most recent review of Harrison's work, consult his *Croonian Lecture* (1935).

nervous environment. More specifically, the neurones multiply, differentiate, and aggregate in definite distributions, grow in definite directions, branch in a definite order, assemble into definite bundles, connect with definite organs, assume definite size relationships, and are enveloped by definite types of other, non-nervous, cells. Occurring according to a definite order in space and time, these processes produce the final nerve pattern of the adult, which is highly intricate. But the task of resolving it into simple developmental components is not as arduous as it may seem, for the following reasons: Firstly, we must keep in mind that the final pattern is of composite origin. That is to say, the pattern-determining factors, while varying from stage to stage, may at any one stage be relatively simple in themselves, the final complexity resulting from the superposition of the successive imprints left behind by each pattern in the form of definite cell groupings and fiber connections. A developed nervous system compares, in a sense, with a photographic plate upon which innumerable pictures have been exposed in succession. The second fact to be kept in mind is that the manifest features of nerve patterns are only in part active achievements of the nervous elements; partly they are the result of purely passive dislocations. Neuroblasts may actively aggregate by migration or be passively crowded together by the expansion of neighboring areas. The contorted course of a nerve fiber may indicate that the fiber took a crooked path in growing, but it may also be due to later distortions of an originally straight fiber.

Thus, resolved into its component steps, nerve development is really not so complicated that it would discourage analytical approach. With this in mind, let us now examine how far this approach has taken us.

The differentiation of a neurone proceeds roughly as follows: The neuroblast, after ceasing to divide, grows considerably in size. Next, a localized protrusion of protoplasm appears, destined to become the nerve sprout. Later similar sprouts appear on the opposite part of the surface. In the organism the first sprout usually develops into the axon, the other sprouts under considerable arborization into the dendrites. In nerve cells cultivated in vitro the distinction between axons and dendrites is frequently quite arbitrary. A nerve process elongates by amoeboid activity of its tip (Figure 3). This free end is in a constant state of unrest, sending out pseudopodia in various directions, of which one usually establishes itself, while the others are hauled in.[3] Then from the end of this pseudopodium new competing

[3]Instructive illustrations of the amoeboid growth of nerve fibers can be found in Speidel's work (1933).

feelers are stretched out into the surroundings, one of which again takes hold, and so the fiber extends farther and farther, until it is eventually arrested by connecting with other cells, by unsurmountable mechanical obstructions, or by nutritive exhaustion. In advancing, the amoeboid tip spins out the nerve fiber from the body of the nerve cell, which stays behind, anchored in the ganglionic tissue.

All nerve fibers arise in this way.[4] The first fibers to sprout out are fittingly called "pioneering" fibers. When they develop, the body is still of very small dimensions, and all tissues lie fairly close together. The greatest distances to be spanned by the pioneering fibers until they reach their destinations are at best of the order of the millimeter. Once connected with a peripheral tissue, epidermis cells or muscle buds, the roaming life of the pioneering fiber is over. Attached now at both ends, its further course is no longer one of its own choosing: the terminal tissues take it in tow and drag it along during their own extensive shifts.

The number of nerve fibers maturing early enough to act as pioneers is relatively small. Nerves at that stage consist only of a few fibers. New fibers are added as additional neuroblasts differentiate. Thus the fiber complement of a nerve is gradually built up. The road problem facing these later fiber generations is, however, quite different from that of the pioneering phase. Pioneering fibers must find their way in strange country and get orienting cues from a non-nervous environment. Subsequent fibers, on the other hand, need simply cling to the older ones to reach the same destinations. *Free* outgrowth has given way to growth by *application*.

Thus the establishment of a peripheral nerve involves three overlapping phases (Figure 1): First, the *free* outgrowth of a group of pioneering fibers through non-nervous surroundings. Second, the *bound* outgrowth of subsequent fiber generations along the line laid down by the pioneering fibers. And third, the *towing* process in which the nerve is drawn out by the growth and dislocations of its terminal tissues.

This developmental history of the neurone presents a number of clear-cut problems. Do the pioneering fibers move at random or do

[4]The idea of a peripheral rather than a central origin of nerve fibers has longest survived in the field of nerve regeneration. The autogenous regeneration of peripheral fiber fragments, advocated mainly by Apáthy and Bethe, has found adherents as recently as 1920 (Spielmeyer). However, all existing evidence is definitetly against any such view (Boeke, '35). It is true that abortive attempts at regeneration have been observed in distal nerve fragments in vitro (Levi, '34), but they are short-lived and of no practical significance.

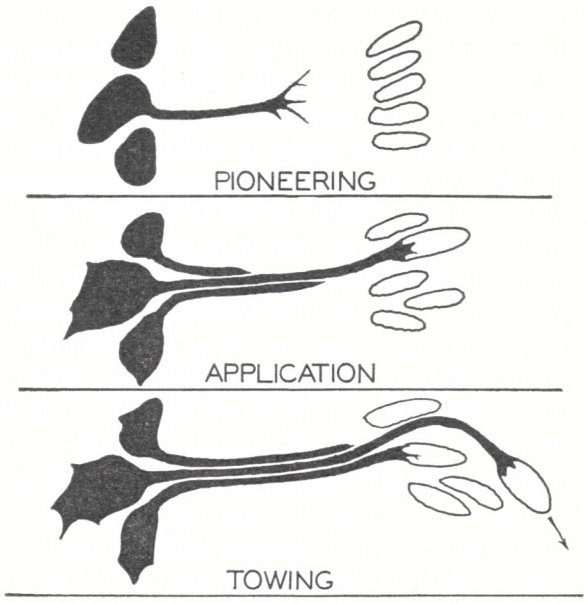


FIGURE 1
GROUP OF NEUROBLASTS IN THREE SUCCESSIVE STAGES

they follow definite courses? If directed in their outgrowth, what makes them find their way? Are they aimlessly following casual routes for better or worse, or are they aiming towards definite destinations? Do they connect with any terminal tissue, right or wrong, or does selectivity prevail in the process? Why do post-pioneering fibers follow the older fibers? And which ones do they follow?—And many more such questions.

Although the answers to some of them are beginning to emerge in their outlines, our analytical information is on the whole still very inadequate. Therefore, if I am to present to you a coherent story rather than loose scraps, I shall have to call rather liberally on hypotheses to do the cementing. Some gaps can be filled by borrowing analogies from related fields, others by thoughtful evaluation of purely descriptive data. But even so the picture will remain sketchy.

We exclude from consideration the factors that create the initial pattern of neuroblasts, as we find it in the early nervous system. The question of differentiation, i.e., of what makes embryonic cells different among one another, is one that cannot be solved for the nervous system separately. It is one of the most fundamental and

most obscure problems of all biology. We find neuroblasts of different potencies in different typical locations. These then transform into the different species of nerve cells. We do not even know whether they are already ear-marked for certain specializations when they are proliferated from the germinative (ependymal) layers, and then migrate each according to its type into a specified location, or whether presumptive nerve cells are still equivalent as they migrate into the various central districts, and then differentiate under the influence of local factors to which they become subjected in their final positions. We do not know, furthermore, what causes the proliferation of nerve cells to be confined to definite loci which shift from stage to stage and have been said to move in waves along the axis of the spinal cord (Coghill, '26, '36).

We, therefore, exclude from our discussions the problems presented by the differentiation of the early nervous system and begin at that later phase in which neuroblasts are already segregated, but nerve fibers have not yet appeared.

The first question of interest is, how does the neuroblast get polarized and oriented? It had been suggested by Child ('21) and Kappers ('17) that neuroblasts are polarized by the potential gradient of a surrounding electric field. Kappers viewed the case as one of direct galvanotropism, while Child suggested differential physiological response of the neuroblast at the anodal and cathodal poles. According to Kappers, the anodal outgrowth would become the axon, and the cathodal outgrowth the dendrite. All this is pure speculation. There simply are not sufficient data on hand to reach any decision. Péterfi and Williams ('33), who attempted to test the theory directly by placing neuroblasts into an electric field in vitro, failed to produce convincing evidence either way. One obvious difficulty of the whole theory lies in the frequent occurrence of tripolar and multipolar neuroblasts; it is hard to visualize how electric polarization could ever produce better than bipolar forms. The points at which the nerve processes emerge from the neuroblast are probably determined by some local changes in the cell surface. However, these changes need not be of electric origin. In fact, they need not even be of a single kind. In this connection Whitaker's ('40) splendid analysis of the polarization of the Fucus egg, reported at last year's symposium, deserves full attention as a possibly pertinent analogy. Similar experiments on neuroblasts are needed. Pending the outcome, we must suspend judgment.

This is not serious for our present purpose, because the further course of the nerve fiber does not directly depend upon the polarity and orientation of the nerve cell. Once the amoeboid tip of the fiber has left the cell body, it follows a course of its own. Our interest, thus, turns to the factors determining that course.

Several theories have been developed to account for the oriented outgrowth of nerve fibers. Strasser (1892) suggested the electric field as the orienting factor, and Child ('21) and Kappers ('17) considered the same potential gradients which had supposedly polarized the neuroblast as responsible for the further direction of the axonic outgrowth. However, aside from one positive claim by Ingvar ('20), which can be accounted for by deceptive experimental technique (P. Weiss, '34, p. 426), repeated attempts to demonstrate any orienting effect of the electric field on outgrowing nerve fibers, either in the embryo or in tissue culture, have consistently failed (P. Weiss '34; Karssen and Sager '34; Levi '34, p. 611; Williams '36; Gray '39). This negative evidence is strengthened by the fact that one frequently sees two nerve fibers grow out along a common path, but in opposite directions (Speidel '33). Such reciprocal fiber growth has recently been reproduced on a mass scale in experiments which will be reported below. Other experiments, also to be detailed later, have demonstrated that nerve fibers from the same source grow with equal ease in headward and tailward direction. Thus, whatever axial electrical polarity there may be in the body, has obviously no decisive effect on the direction of fiber growth. Consequently, the theory of a direct orientation of nerve fiber growth by potential gradients of an electric field finds no support in the facts.

Turning to the second group of theories, the chemical theories, or, more specifically, the concept of Cajal ('08), Forssman (1898, 1900), Tello ('23), that nerve orientation is produced by some sort of chemotropism, the existing evidence is overwhelmingly against them, too. Chemotropism implies a directive movement of an organism or part of an organism toward a distant source of chemical emanation. In order to operate, such a mechanism requires: (*a*) a constant source of chemical diffusion with a steady concentration gradient; (*b*) selective sensitivity of the growing part for that particular chemical; (*c*) an ability of the growing part to orient itself along the lines of the concentration gradient; and (*d*) a stagnant and homogeneous medium in which the concentration gradient can remain stationary during the whole process of oriented growth. These conditions are not realized

in the growing organism. There are, of course, numerous centers of chemical diffusion in the growing organism. However, nerve fibers do not follow the direction of concentration gradients, and even if they did, the body would not provide them with gradients of sufficient steadiness to serve as guides, as the agitation of the interstitial fluid between the tissues would blurr and distort the original concentration gradients beyond recognition. Furthermore, the fact of reciprocal fiber growth between two points is as fatal to the theory of chemotropism as it is to that of electrotropism, because it would mean that while one fiber runs up the concentration slope, the other one runs down. Also, in spite of several direct experimental attempts it has never been possible to demonstrate a direct attraction of nerve fibers towards a source of chemical emanation in vitro (P. Weiss, '34), not even towards degenerated nerve tissue, according to Cajal the most potent source of such action.[5]

Both the galvanotropical and chemotropical theories proceed from the assumption that the growing tips are oriented by factors acting from a distance. Nerve fibers are seen to *travel towards* certain localized regions and, hence, are assumed to have been *attracted by* those regions. For brevity, let us refer to this kind of orientation as a "distance effect." Now, before looking further into the problem, let us first check the solidity of its premises. In other words, are nerve fibers really moving towards definite destinations, or are they merely running along some preformed traffic routes?

The growth of the segmental nerves along the boundaries of the myotomes, the growth of the lateral line nerve down a longitudinal groove along the trunk musculature, the coursing of the limb nerves along the main blood vessels, and similar facts, would seem to make nerve orientation a matter of the road rather than of the destination. His (1887) has stressed the predilection of nerves for mechanical roadbeds, and Vanlair (1885) has gone so far as to claim that nerves simply take the way of least resistance. Exceptions, in which nerves seem to have gone out of their way, so to speak, in order to reach distant organs, such as the long backward loop of the vagus from the

[5]Centanni ('14) once reported "nerve growth" in vitro as oriented towards explanted tissue fragments of supposed chemotactic activity. His statement, however, contains a gross misrepresentation, inasmuch as his "nerve growth" came from isolated fragments of peripheral nerves, in which, as we know, all nerve fibers degenerate rather than regenerate. The so-called "nerve growth," therefore, can have reference only to emigration of spindle cells from the fragments, which has no bearing on the problem of nerve growth at all.

head to heart and intestine, or the long forward swing of the lingual nerves into the tongue are deceptive, in that these courses reflect extensive shifts of the terminal musculature of these nerves in the towing phase, rather than directive free outgrowth of the nerves. So, one cannot dismiss the possibility that nerves connect with their proper peripheral areas simply because these areas happen to lie at the end of those lines which the nerves find open when they start to grow out. And the problem of whether nerves are destination-bound at all, is a real one.

Experimental evidence on the point is meager but suggestive. Cajal ('08) and Forssman (1898, 1900) have tried to prove under a wide variety of conditions that nerve fibers in the process of regeneration grow actually toward a goal rather than merely along a path. Let me cite one of their classical experiments, exemplary for many others. When a nerve is cut, the distal fragment transforms into a non-nervous protoplasmic cord which persists as such, while the proximal fragment gives rise to abundant outgrowth of new fibers (cf. Boeke, '35). Whether the two stumps are directly apposed or whether they are dislocated, in either case a large number of the new sprouts is finally found trapped inside the old degenerated tubes (Figure 2). The orientation of the regenerating fibers is by no means strict. Numerous

A B

FIGURE 2

NERVE REGENERATION INTO DISLOCATED PERIPHERAL STUMPS (AFTER CAJAL)

A, Nerve fibers from a severed nerve (*A*) have partly found their way into a degenerated nerve fragment lying some distance behind the cut surface.

B, Nerve fibers from the proximal nerve stump, *A*, after pervading the scar, *B*, have partly entered, partly by-passed, the degenerated nerve segment, *C*; part of the fibers emerging from *C* have traversed the gap to the dislocated fragment *D* and grown into it.

fibers can be seen to have strayed about at random. Nevertheless, a large proportion has reached the peripheral nerve stump. In order to do this, they had to take a somewhat contorted course. Doubtless, the degenerated peripheral stump has exerted some potent influence on the pattern of regeneration.

However, in specifying this influence, we must be aware of two possibilities: One is, that the sprouting fibers were attracted by some factor emanating directly from the open end of the distal fragment. This is the interpretation given to the results by both Cajal and Forssman. In view of experiments which we shall take up in a moment, however, a second possibility seems more likely.

Suppose, the fibers stray out at random. Some strike the peripheral stump by accident. This puts an end to their period of vagrancy. Then something happens to them: As a result of their having become settled, their condition changes; changes in a fashion that will render them sticky or otherwise attractive for other nerve fibers growing out later. In this way, more and more fibers will get trapped along this preferential course. Thus, what originally was accidental encounter, becomes gradually a systematic pursuit. The successful bridging of the gap would be a matter not so much of directive fiber outgrowth, as of the fact that an accidentally successful nerve sprout becomes the center for the building up of a nerve cable. We will call this process "*selective fasciculation*" and return to it in greater detail presently. For the moment, we merely mention it to show that Cajal's experiments do not necessarily prove the case of destination-bound nerve orientation.

Experiments on the embryo, reported by Detwiler (reviewed '36), seem to be more conclusive. The nerves of a vertebrate limb are of plurisegmental origin and converge upon the limb base, where they intertwine in a plexus. When a limb bud is transplanted to a more anterior or posterior position, the innervating centers shift likewise, but less, and there is a certain tendency of nerves to slant more anteriorly in the former, and more posteriorly in the latter case. In spite of great individual variations, limb nerves, as a rule, show a more headward slant if the limb bud was transplanted more anteriorly, and a more tailward slant in the case of a posterior shift. Since the limb plexus converges likewise upon a transplanted nasal organ (Detwiler '28) a tail, or an eye (Detwiler and Van Dyke '34), the attraction, if such it is, is certainly not very specific. Nevertheless, a

general diversion of nerves toward growing organs seems to be indicated.[6]

Unfortunately, only the end stages of this process are known, and since secondary events, such as extensive shifts of musculature, plexus formation and, possibly, selective fasciculation, have distorted the original situation as it existed at the moment of the outgrowth of the nerves, it remains unknown just how potent the supposed attraction of embryonic nerves by growing organs is. However, since Burr ('32) has shown that nerves growing out from transplanted nasal organs are likewise showing definite preferences as to the point of ingrowth into the embryonic brain, and since, furthermore, Coghill ('29) has come to the conclusion that in the development of the brain nerve fiber tracts tend to grow towards centers of simultaneous differentiation, we may take it for granted, pending proof to the contrary, that growing regions of the body exert a general influence, which in purely descriptive language may be termed "directional attraction."

The acceptance of this fact puts us into a real dilemma. We admit that nerves can be attracted towards distant organs, and at the same time deny the validity of the best known mechanisms of distance action—galvanotropism and chemotropism. The alternative to distance action is what, with a general term, we may call *"contact action."* All present evidence points to the fact that nerve fibers are conducted on their way solely by "contact action," and in order to extricate ourselves from our dilemma, we would have to prove that distance effects can actually be resolved into contact actions.

This we can prove. But before doing so, we must give some closer attention to the mode of progression of the growing nerve fiber. This takes us into a much wider biological field—the problem of amoeboid movement. Amoeboid movement is produced by the protrusion of lobular or filamentous processes from the protoplasmic surface, so-called pseudopodia, which take hold on the substratum and cause the cell content to stream after. Some cells can project pseudopodia into a homogeneous liquid. Nerve fibers cannot. The nerve protoplasm needs a surface or interface along which to extend. Perhaps this need is imposed by the great length of the fibers. Since the tremen-

[6]Cases of extensive nerve detours, as if aiming to reach nerve-free limbs, described by Hamburger ('29), owe much of their impressiveness to the secondary distortion which the nerve courses have suffered during the towing phase. In view of the close apposition of the young limb buds and the closeness of their respective nerve fiber pools, contralateral innervation occurs under infinitely simpler conditions than the final developed product would make one suspect.

dous expansion of relative surface in elongation requires a large output of energy, it is possible that the task would go beyond the capacity of metabolic energy production by the cell, so that external physical structures would have to be called on for support.

However, whatever the reasons, nerve fibers positively do not grow when suspended in a homogeneous liquid medium. When cultured in liquid media in vitro, they cling to the surface of the coverslip or to the surface of the drop (Lewis '12, Levi '34). The occasional observation of nerve fibers inside the spinal canal, which is filled with cerebro-spinal fluid, is no exception, as such nerve fibers have most likely grown along the inner wall of the tube and merely become swept into the liquid in the act of fixation. Let us repeat, therefore, the pseudopodia of the growing tip of a nerve fiber extend along phase boundaries, and only along such.

These phase boundaries need not be of the gross macroscopic kind, such as coverslip–liquid, or liquid–air. In fact, they rarely are. Much more commonly they are of microscopic or submicroscopic order. In the embryo the growth of a nerve fiber along minute microscopic threads has been repeatedly described (cf. Held '09). But since, what in a microscopic preparation appears as a fibril, is often an artifact, resulting from the clotting together of identically oriented ultramicroscopic structures, it is most likely that even in these cases nerve growth in the living occurred along some ultramicroscopic rather than microscopic phase boundaries. The same is true of tissue culture in a blood plasma clot. The plasma coagulum is essentially a spongy gel consisting of a more solid skeleton of fibrin micellae in various degrees of aggregation, imbibed with a continuous phase of blood serum. Interfaces between fibrin aggregates and serum thus are present everywhere in the clot, and they furnish the necessary substratum on which the pseudopodia of growing nerves may take hold.

The question has been raised of how a microscopic body, such as a nerve fiber, can follow an ultramicroscopic interface, which is of lower order of magnitude. The answer is simply that it is not the microscopically visible nerve fiber, which plays the active part in the creeping process, but its delicate terminal pseudopodia, which may very well taper to submicroscopic dimensions.[7] We know that what

[7] Grossfeld ('34) has overlooked this fact when taking issue with the explanation of oriented fibrocyte growth in terms of the ultrastructure of the medium. Obviously, what we have stated here about nerve fibers, applies equally well to fibrocytes, which likewise advance by means of fine filamentous pseudopodia.

under the microscope appears as the tip of the pseudopodium of a nerve fiber, is not its real end; Giuseppe Levi was able to trace the filaments far beyond that point in dark-field illumination, and we have, of course, no assurance that they may not even extend beyond the limit of dark-field visibility. This makes the pseudopodial tips and the interfaces to which they apply themselves appear as of commensurable dimensions. Once established, the pseudopodium is soon enlarged to microscopic dimensions by the inflow of protoplasm, while new pseudopodia issue from its extreme tip and penetrate into the medium (Figure 3).

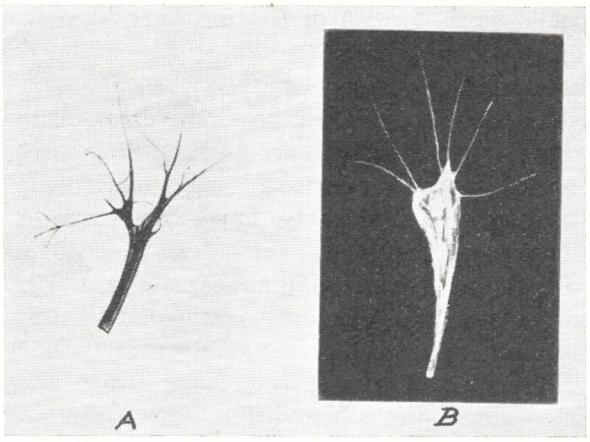


FIGURE 3

PSEUDOPODIAL RAMIFICATION OF THE GROWING TIP OF A NERVE FIBER CULTIVATED IN VITRO (AFTER LEVI)

A, Fixed and stained preparation.—X 950. *B*, Dark-field view of living specimen.

The tendency of organisms to cling, as they grow, to solid structures, is commonly referred to as *stereotropism*. This is a naturalistic term. It classifies the phenomenon, but reveals nothing about its nature. The movement of a nerve fiber falls into this class. Our analytical insight into the phenomenon does not seem to have advanced far beyond where it stood when Quincke, in 1888, explained protoplasmic movement as the work of surface forces, comparing it with the spreading of oil on a water-air interface. It is to be hoped that such studies of the mechanism of amoeboid motion as have been carried out by Mast ('31) on amoebae, by Lewis ('39) on macrophages, and by Fauré-Frémiet on amoebocytes, may lead to informa-

tion which can be applied to the nerve fiber. For the time being, however, our ideas about the process are still highly speculative.

We are probably safe in saying that interfacial tensions between the nerve fiber protoplasm and the micellar aggregates along which it extends are at work, but just how they operate is still obscure. Of the whole protoplasmic system only that fraction counts which takes part in the surface and is in actual contact with the medium. Reversible gelation of the cortical protoplasm endowing the surface film with adhesiveness, as suggested by Lewis, may be an essential element of the mechanism. But this "adhesiveness" itself can bear further analysis. Are we simply dealing with a purely colloid-mechanical phenomenon, or might we not have to postulate molecular affinities of greater specificity? I have observed some nerve fibers in vitro following a pathway which was wholly ignored by others. This and many similar experiences suggest selective "adhesivity" rather than a common kind of stickiness.

In an unoriented gel of isotropic constitution phase boundaries extend from any one point in several directions. This favors the simultaneous protrusion of several pseudopodia along different lines. (Figure 3). Which one of them is to endure, depends probably largely on local accidents. But it is reasonable to assume that the inertia of the centrifugal protoplasmic flow in the growing sprout will generally enhance the repletion of those pseudopodia that lie more or less in direct line of the axis of the sprout. We have little accurate data about protoplasmic movement inside the fiber, but Levi ('34) has observed some agitation, and Speidel's films show some kind of peristaltic motion. Once a pseudopodium has established itself and caused the mass of protoplasm to flow into it, the drain thereby exerted on the other pseudopodia produces their automatic withdrawal. Only if two pseudopodia happen to be of approximately equal strength, they may divide the protoplasmic influx between each other and thus initiate a terminal branching of the fiber (Figure 11). We shall return to this point. In a medium lacking definite orientation, the nerve processes will thus follow random courses. This is actually realized in ordinary tissue cultures as well as in many parts of the central neuropil.

However, from what we have said, it can be anticipated that if nerve fibers are confronted with a medium in which the interfaces follow a common instead of random direction, their growth, bound to retrace those interfaces, would become likewise oriented, just like

vines, growing along parallel stakes. This expectation has been borne out by tissue culture experiments. Since these have been reported at length (P. Weiss '34), we need not go into details. When nerve fibers are made to grow out in a blood plasma clot which had been stretched or otherwise put under tension, the course of the fibers follows the pattern of tension. The tension has served to turn the polarized fibrin micellae into a nearly parallel orientation, and consequently all pseudopodia were likewise drawn into a nearly parallel course.[8] To obtain the effect, the tension need not be exerted from without. Living

FIGURE 4

ORIENTED NERVE GROWTH CONNECTING TWO SPINAL GANGLIA CULTIVATED IN A THIN MEMBRANE OF BLOOD PLASMA IN VITRO (x 48, P. WEISS, '34).

[8]For illustrations of the effect, see Figures 1-4 in P. Weiss, '34.

proliferating tissue by itself has a contracting effect on the surrounding medium and thereby produces tension. Two such centers of contraction in a common medium automatically produce a pathway of tensionally oriented micellae, which, if nerve cells are present in the centers, becomes soon populated by parallel nerve fibers growing out from both ends in opposite directions (Figure 4). Incidentally, this is another example of reciprocal fiber growth, defying galvanotropic and chemotropic interpretations.

The fact that in these experiments the agent to produce micellar orientation happened to be tension, is irrelevant. For any ultrastructural orientation of the medium, no matter how attained, may have the same effect on the nerve fibers. Convection of liquids through the medium can produce such orientation, and vectorial aggregation of micellae may even occur spontaneously. On the whole, it is possible to explain the oriented outgrowth of individual nerve fibers by taking into account the colloid-physical structure of the medium, and the tendency of the nerve protoplasm to comply with it. The stereotropism of nerve fibers with regard to solid microscopic structures, as assumed by His, and emphasized by Harrison ('14), seems to be only a special case of the more general principle of contact guidance of the nerve tip by the ultra-structure of the medium. To call this contact principle "mechanical," misses the point, the more so as we do not yet know of how much selectivity the adhesiveness to the contact structure admits. It is quite conceivable that differential chemical impregnation endows certain ultra-structures with greater affinity to nerve pseudopodia than others (see below).

Nerve fiber orientation was thus explained, at least in principle. The attraction of nerves towards growing organs, nerve branching, the formation of nerve plexus, and the reciprocal outgrowth of nerves between simultaneously differentiating centers, could be reproduced in vitro. More than that, the experiments have definitely proven that nerve fibers are actually guided by contact and not attracted by agents operating from a distance. So, when nerve fibers are seen to move towards a remote destination, one may take it for granted that that goal was instrumental in laying down an oriented pathway in the medium, rather than acting on the fibers directly. Established in tissue culture, all this certainly holds true for nerves in vitro. But does it likewise apply to the living organism? And if so, does it help to explain the nerve patterns as they actually arise in the course of development?

As I have pointed out on earlier occasions ('33, '34), the conditions between tissue culture and organism are fundamentally comparable in many respects. However, the actual testing ground is the organism, and this test has thus far remained wanting. Moreover, there are certain features of nerve growth in the organism which cannot be repeated in tissue culture. There was urgent need to clarify the situation in the organism directly. During the last few years I have tried to approach this task by devising methods specially adapted to the problem. The problem was to let nerve growth occur under conditions infinitely simpler and more transparent than those under which it normally occurs and whose complexity defies direct analysis, but still in the organism.

Two different techniques were found to be successful. In both amphibian larvae are used. The first consists of producing an extensive bed of simple granulation tissue and allowing nerves to regenerate into it under various controllable conditions. Locations chosen for the purpose were the *skull cavity,* from which the brain down to the anterior end of the medulla oblongata had been removed, and the *orbit of the eye* after enucleation of the eye ball (see Figure 9, *A, C*).

Even large tadpoles survive the removal of brain of the indicated extent for six weeks or more. The skull cavity fills with a soft, loose connective tissue, which provides our testing ground for nerve growth. This nerve growth comes from four sources, the two stumps of the olfactory nerves and the two optic nerve stumps, all four belonging to nerves whose cells lie in the periphery and grow centripetally (Figure 9, *C*). Similarly, after enucleating the eye under certain precautions, the orbital space becomes filled with a simple vascularized connective tissue, and a source for nerve regeneration is made available by cutting the trigeminal nerve, which runs along the inner wall of the orbit (Figure 9, *A*). These experiments have furnished instructive information concerning the relation between nerve growth and connective tissue orientation, relations between nerves and capillaries, the non-existence of any orientation of nerve fiber growth with regard to the general body axis, as well as the non-selectivity of nerve associations (see below), but lack of space prevents us from discussing these points in greater detail at this time.

The second method may be called *deplantation.* It takes advantage of the fact that the amphibian larva contains one extensive and readily accessible tissue which in itself is so elementary that it com-

passes in simplicity almost with a tissue culture, namely, the gelatinous connective tissue of the fin. It consists (Studnicka, '38) of a gelatinous keel-shaped mass extending over most of the dorsal midline, sparsely settled with cells, and pervaded by a moderate amount of capillaries and sensory nerve fibers. Into this tissue we transplant large fragments of developed spinal cord or brain from another larva (P. Weiss, '40, '41, '41*a*). These fragments are to furnish outgrowing nerves. The transplanted mass undergoes partial resorption, but what is left is sufficient to serve as a potent nerve source. This method has the advantage that other organs can be transplanted into the vicinity of the deplanted center so that the effects of different organs on nerve growth can be analyzed, and, in fact, directly observed, because the fin tissue is sufficiently transparent to permit direct observation of the gross events.

Let us see what happens when a brain fragment and a limb are incorporated in the fin at some distance from each other. (Figure 5).

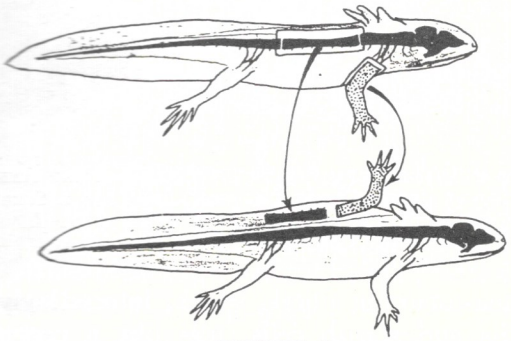


FIGURE 5

DIAGRAM OF DEPLANTATION EXPERIMENT

A portion of the spinal cord and a fore limb are excised from the donor animal (top) and implanted at some distance from each other in the dorsal fin of the host (bottom).

The result is quite impressive: Within two to four weeks after the operation a strong nerve cable forms between brain graft and limb (Figure 6). Inside the limbs these nerves follow the regular nerve channels and make functional connections with the musculature. Innervation completed, the whole grafted complex begins to exhibit spontaneous functional activity which is highly interesting from a physiological standpoint, but which to discuss is beyond the scope of this paper.[9]

Now, in these experiments we have witnessed nerve formation in

[9]The functional results have been summarized in P. Weiss, '41.

an almost diagrammatically simple form. Given: an isolated nerve center and an isolated limb in a common gelatinous matrix. Result: a direct nerve connection. That the limb is the actual objective of the outgrowth, is proved by various facts. If no limb is transplanted, only stray fibers leave the brain fragment, but no nerve cables form. Depending on where the limb was inserted, whether behind or in front or on top of the grafted nerve center, the nerve cable grows posteriorly or anteriorly or dorsad, which again proves that the polarity of the body is immaterial in nerve orientation. If two limbs are transplanted, one anteriorly and the other posteriorly, nerve connections form in both directions. Histologically, these nerve cables

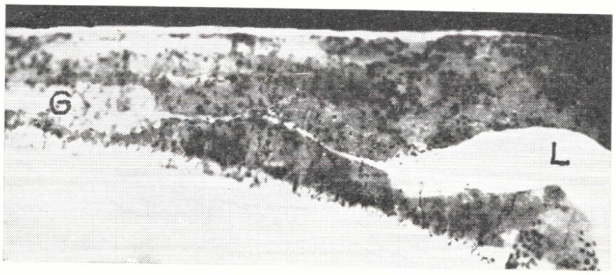


FIGURE 6

LATERAL VIEW OF THE TRANSPARENT FIN OF AN EXPERIMENTAL ANIMAL CONTAINING DEPLANT OF SPINAL CORD (*G*) AND GRAFTED LIMB (*L*): A STRONG NERVE CAN BE SEEN TO HAVE GROWN FROM *G* TO *L*

(13 weeks p. op.—cca 6 x.)

consist of non-anastomosing fibers, which, interestingly enough, are devoid of sheaths and sheath cells, just like a central fiber tract (Figure 7). It is noteworthy that they are fully functional, proving the physiological adequacy of naked peripheral nerves as conductors.

As for the analysis of the phenomenon, one technical detail must be taken into consideration. In order to introduce the deplants, a longitudinal channel was made in the fin gel; the brain fragment was deposited in the distal part of this pocket, and the limb was usually inserted into its orifice. Residues of this channel would present a road of least resistance between the deplant and the limb graft and favor nerve growth between the two structures. As you will see, this explains part but not all of the phenomenon. For, even if the limb and the brain grafts are introduced into separate pockets, without open communication, nerve connections between the two are still established. These differ, however, from the channel connections in one notable respect. Instead of a single straight nerve

cable, there are usually several nerves, and instead of being straight, their courses are somewhat crooked.

Nerve cables, though of smaller size, may form also between the deplant and underlying trunk musculature. Here, too, the presence of a connecting channel facilitates the connection, but is by no means essential.

If we focus, for the moment, on the cases without common channel, we realize from the crooked course of the nerve connections that they have not come about by directive outgrowth, but rather by differential fasciculation. More specifically, this is the sequence of events. Nerve fibers, singly or in small groups, radiate from the deplant in all directions and stray through the surrounding fin tissue. Some

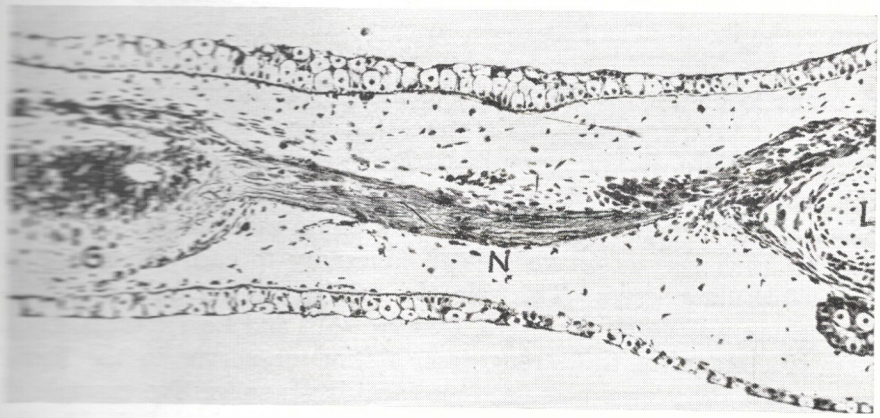


FIGURE 7

Frontal Section Through Fin of Experimental Animal Containing Deplant of Spinal Cord (*G*), Transplanted Limb (*L*), and Connecting Nerve Cable (*N*) (18 days p. op.—x 72.) Impregnated according to *Bodian*.

have reached the grafted limb, others have not. Only those nerves, however, which have entered the limb, have subsequently become filled up into a sizable bundle. This leaves only one interpretation. Obviously, those pioneering fibers which had accidently struck the limb, had thereby acquired some contact property which made their surface sticky, or otherwise a pathway of preferential application, for other fibers growing out subsequently. This is precisely what I had suggested before as an alternative to Cajal's chemotropism in the regeneration of nerves; only here we have more direct evidence. Just how the fasciculation of "successful" fiber courses comes about, has not yet been decided. Nor do we know whether the successful fiber

owes its rallying power merely to the fact that it has come to a halt as a result of terminal connection, or rather to some specific chemical impregnation which it may have received from its new end organ.

The straightness and singleness of the nerve cable in the channel experiments is easily accounted for as a special case of selective fasciculation. For the channel scar establishes a straight connective tissue bridge between the limb and the deplant, along which pioneering fibers are actually guided toward the limb, as in our tissue culture experiments. For the rest, the gradual building up of the cable occurs according to the same rule as in the other series, in which the encounter with the limb is purely a matter of chance.

A deeper analysis of the change, presumably in the surface, which distinguishes successful fibers from unsuccessful ones, is definitely within the limits of our methods. But nothing has as yet been done in that direction. One point, however, seems to emerge. That is, that in order to obtain fasciculation, the immediate vicinity of the nerve fiber must be in a fairly liquid state. This can be observed directly in tissue culture. While nerve fibers inside the solid plasma clot grow out individually, they anastomose and associate easily in liquefied parts of the medium. Nerve fibers in a liquid medium stick together because their surfaces have greater adhesivity to each other than to the surrounding serum. This rôle of liquidity for fasciculation in tissue culture was noted by myself ('34, p. 441) and Levi ('41, p. 177).

In the organism the strong fasciculation of nerves in the liquid spaces along the large blood vessels furnishes a suggestive example of the same situation. We can almost generalize and say: Inside the solid tissues—epithelium, muscles, central gray, and so forth—nerve processes tend to take individual courses. In between these tissues, however, where the fibers pass through liquid-filled spaces, they are grouped into nerves. Now, if liquidity of the medium is a prerequisite for fasciculation, part of the task of a successful pioneering fiber may consist of liquefying its immediate surroundings by proteolysis.

Indications of facultative proteolysis by nerve fibers have been observed in tissue culture.[10] Nerve fibers in old cultures, that is those

[10]This remark does not refer to the proteolysis of the plasma clot commonly observed in brain cultures (cf. Olivo '28, Levi '34, P. Weiss '34), which has been shown (P. Weiss, '34*a*) to be due to the secretory action of ependymal *cells* (and which, incidentally, has been wrongly interpreted by Levi, '41, as plasma syneresis). We are referring here to proteolytic effects of the *nerve processes*. A few examples were observed during earlier work (P. Weiss, '34, p. 434). More extensive evidence has been obtained since, but has not yet been published.

in which growth has essentially come to a standstill, frequently begin to dilute the surrounding plasma medium and, at the same time, combine into bundles (Figure 8). In favorable cases, the formation

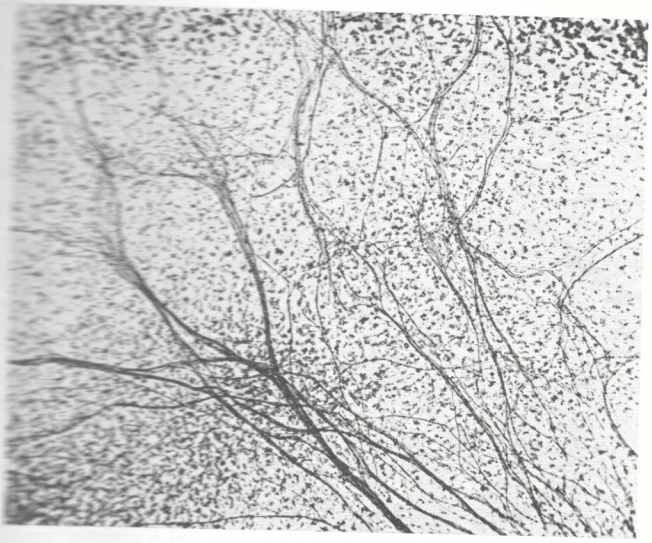


FIGURE 8

Peripheral Growth Zone of Tissue Culture of Spinal Ganglion (Chick Embryo) after Seven Days' Cultivation in Plasma Clot

Extensive bundling of nerve fibers. Impregnated according to *Bodian's* method. x 56.

of blisters in the vicinity of a nerve can be immediately observed. However, whether these cases have a real bearing on the mechanism of fasciculation in the body, remains to be seen.

But whatever the details may be, nerve fibers which have "arrived" somehow bear the stamp of success on their exterior, which induces other nerve fibers to follow them, while unsuccessful ones, lacking similar appeal, usually remain unattended. In the light of these results, the significance of primarily oriented fiber outgrowth for the development of nerve patterns seems to be greatly reduced. The problem obviously exists only during the pioneering phase of early fiber growth and regeneration; during that phase it is apparently well covered by the principle of *ultrastructural orientation*. But from there on all further development of peripheral nerves is a matter of *selective fasciculation*.

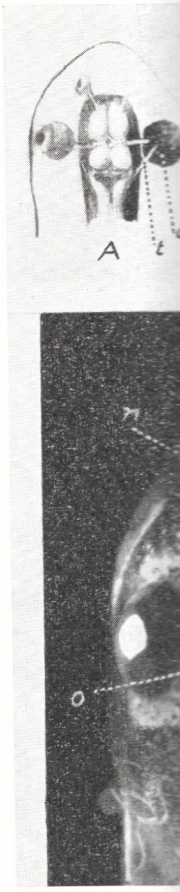
This immediately raises two questions: Firstly, the question of what degree of selectivity there is in the application of one nerve fiber to

another. And second, the question of what kind of numerical control limits the size of a nerve cable to its normal proportions.

Let us consider the problem of selectivity first. Selectivity may assume two forms: selectivity of pathway and selectivity of termination. Therefore: can nerve fibers select their courses, and can they choose the tissues with which to connect? To take up the latter point first, the notion that nerve fibers from a given source would possess a prerogative on a corresponding pre-assigned peripheral organ, is wholly untenable. Any number of cross connections between nerves and terminal areas strange to them have been successfully effected.

A brief list may indicate the range of non-selectivity. First, with regard to regeneration: Motor fibers can connect with sensory organs, and sensory fibers can form terminations in muscles (Boeke, '17). It has been shown that such connections may even be physiologically adequate: a dorsal spinal root (P. Weiss, '35) or a sensory nerve (P. Weiss '34b) forced upon a muscle can mediate its contraction. Any motor nerve will connect with any muscle. It even connects with spinal cord when inserted into it (P. Weiss '32). In the deplantation experiments, just discussed, innervation of limb and trunk muscles was obtained from all spinal cord, medulla oblongata, midbrain, and forebrain, all of which had reached functional differentiation at the time of deplantation (P. Weiss, '41a). Turning to the embryonic phase, cranial nerves were shown to penetrate limbs (Harrison, '07; Braus, '05, Detwiler, '30; Nicholas, '33) or trunk muscles (Hoadley, '25), grafted within their domain; and in fact, central fiber tracts can do the same (Nicholas '29, '30).

I can supplement this array further with an example from our recent experiments on nerve growth in the cleared orbital or cranial cavities, which I mentioned before. In the empty skull cavity the regenerating fibers of the optic and olfactory nerves pervade the granulation tissue in all directions, forming an unorganized neuroma. But in most cases there were also strong fascicular connections between at least some of the nerve stumps. Quite aside from their bearing on the problem of fasciculation, they interest us here for the weird combinations they have produced. For instance, the optic and the olfactory nerves had met head-on, merged into a common cord, whereupon the optic fibers have travelled on towards the nose, and the olfactory fibers reciprocally towards the eye (Figure 9, *D*). Their ultimate fate has not yet been studied, but the gross fact is clear. Similarly, in the enucleated orbit regenerating fibers from the severed



Method of Studying Nerve G
A, Orbital bed (*or*), cleared
fiber source.
B, View of normal brain of fro
C, Cranial bed (*c*), cleared by
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D, Experimental animal, operat
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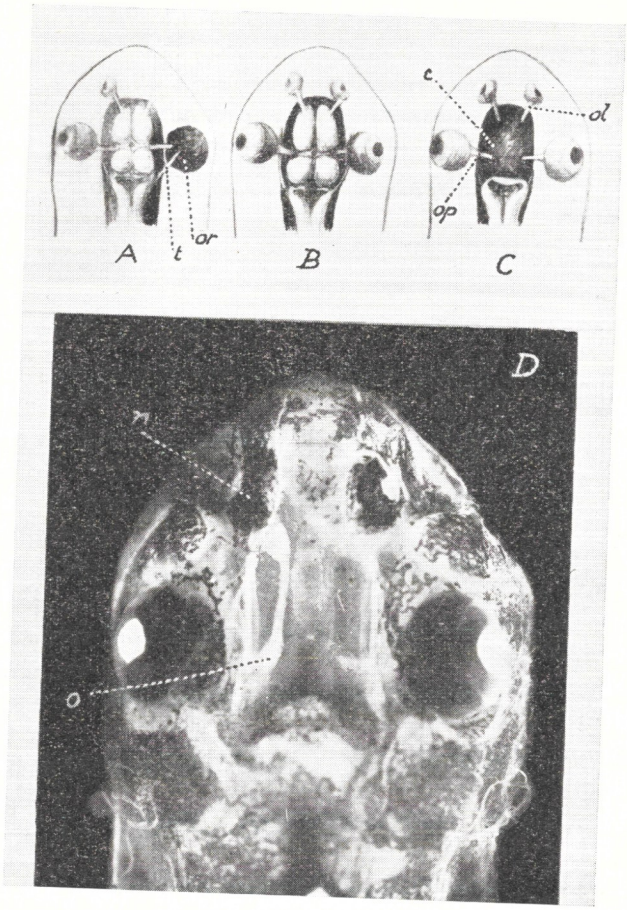


FIGURE 9

METHOD OF STUDYING NERVE GROWTH IN EXPERIMENTALLY PREPARED CAVITIES AS BEDS

A, Orbital bed (*or*), cleared by enucleation of bulb, with trigeminal nerve (*t*) as fiber source.

B, View of normal brain of frog tadpole after dorsal opening of skull.

C, Cranial bed (*c*), cleared by removal of all brain parts anterior to cerebellar lamina, with olfactory (*ol*) and optic (*op*) nerves as fiber sources.

D, Experimental animal, operated according to *C*, showing fusion of left optic (*o*) and olfactory nerve (coming from nose, *n*) into common cable.

trigeminal nerve have penetrated into the central stump of the deserted optic nerve and grown back into the brain.

In spite of this demonstrated non-selectivity of terminal connections, the fact remains that in normal development highly specific relations are somehow achieved. The simplest expression of these is

the fact that ventral root fibers connect with muscles, while fibers growing out from the spinal ganglia connect with sensory end organs, mostly in the skin. Since both travel for some distance along a common road before segregating into muscular and cutaneous branches, they present us with a really puzzling problem. This problem has not yet been brought anywhere near its solution. One might submit that there might first be indiscriminate outgrowth, followed later by the reduction of functionally inadequate connections. This contention, however, does not find support in the facts, as the relations are established very early, long before there can be any question of functional effects.

Mr. Taylor, of our laboratory, has made a thorough investigation of the innervation of hind limbs of frog larvae, which had developed in the absence of either their sensory or their motor nerve sources. Purely motor innervation was obtained by the removal of the sensory ganglia prior to the outgrowth of the limbs, and purely sensory innervation by the early extirpation of the whole spinal cord of the limb area, leaving the ganglia undisturbed. Even the very early limb bud, long before differentiating muscle and skeleton, is already pervaded by sprouts from the nerves which had been waiting at the limb base in large numbers. Later, as the limb elongates, the various nerves can be identified. In animals with purely motor centers most fibers are then found in muscular nerves, while purely sensory sources have sent most of their fibers along cutaneous pathways. The separation is not strict. Motor fibers get into the skin and cutaneous fibers into muscles, but a statistical predilection of each kind is nevertheless indicated. This bears out an earlier observation of Hamburger ('29). The fact seems clear, its explanation less so. We are still collecting evidence, and I do not want to be too positive. But one thing seems to emerge: If there is selectivity in these early stages, it seems to refer to the pathway rather than to the terminal areas. Sensory pioneering fibers would show affinity to a cutaneous nerve path rather than to skin; motor fibers to muscular nerve paths rather than to muscle. These paths might be viewed, in line with our earlier discussion as ultrastructures with differential chemical impregnation. Sensory and motor nerve fibers would have to react differentially to the two types of structure. In assuming this, we are on firmer ground. We know from anatomical and physiological experiences that motor and sensory fibers are constitutionally different. This differential might very well predispose them for different contact affinities. Of course, once the

pioneering fibers have been sorted into their proper channels, the rest may be merely a matter of selective fasciculation: Guided by surface affinities, sensory and motor fibers would apply themselves each to the corresponding type.

The fact of surface affinities among identical kinds of nerve fibers seems to be well supported. For what else can it mean when the neurologist describes the fibers which mediate special sensations, such as pain, proprioception, cutaneous sensation, and so forth, as running in their intraspinal course as separate fiber bundles? Or the fact, that even in a peripheral nerve the sensory and the motor fibers hold pretty much to themselves, although imbedded in a common nerve tract? Each bundle contains fibers of a wide range of ages, which have developed in succession; if they lie together, this must mean that the later ones have grown out along the path of the earlier ones. Oppenheimer ('41) has recently described interesting observations on the course of supernumerary Mauthner's fibers in the fish brain. The extra fibers, easily recognized by their size, have shown a marked tendency to follow the course of the original Mauthner's fiber. This is strong evidence of selective surface application. Thus, it may become necessary to concede to nerve fibers the attribute not only of being just adhesive, but of *selective adhesiveness*. This affinity would presumably be obliterated by the appearance of a myelin sheath.

The problem of selective fasciculation brings up a question of much wider biological significance, namely, that of tissue affinities in general. The intimate fusion or adhesion between tissues of different origin presupposes a compatibility between the contact surfaces which thus far has not found sufficient elucidation. Whether two protoplasmic systems can adhere to each other, depends primarily on some contact affinity which must hold at least until mechanical ties, such as basement membranes, or other fiber structures, have cemented the surfaces. Holtfreter ('39) has reported some highly instructive observations on how this contact affinity among different tissues changes in the course of ontogeny; how tissues which at one time have adhered, later separate off, as their biochemical divergence becomes greater.

I am inclined to regard this merely as one special case of a very general biophysical problem, which finds its expression in such diverse phenomena as the selective fusion of protozoan pseudopodia, depending on whether they belong to the same animal or to different animals; the fusion of individualized cells with a common syncytium and later

re-formation of individual cells within the syncytium, depending probably on variations of biochemical constitution of the respective nuclear territories (see P. Weiss, '40*a*); the different rate and ease with which grafts can be incorporated depending on their character and orientation; the differential agglutination of sperm in the presence of different types of eggs; and many other similar phenomena. We may view all these phenomena in a common light, as follows (Figure 10).

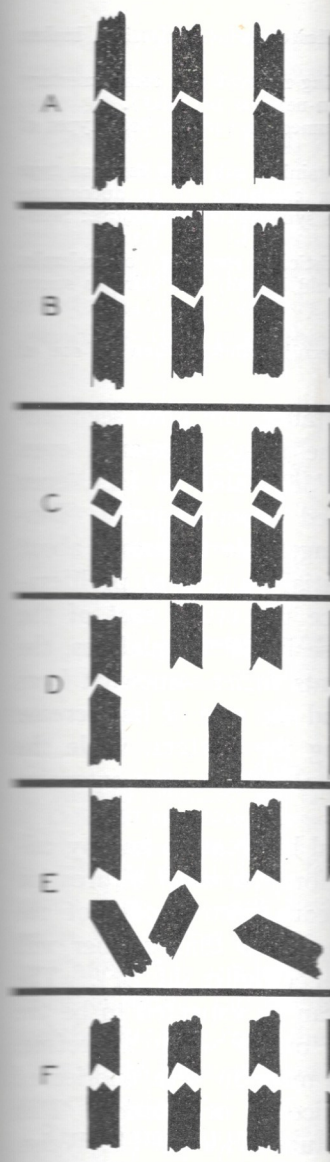
Tissue affinity may be based on the presence in the contact surface of identical (*B, C*) or complementary (*A*) types of proteins, plus the fact that these will be oriented by surface forces in such regular manner that they can interlock. Molecular attraction forces among the two films would seal the surfaces to each other.[11] Furthermore, different degrees of adhesion might be explained by differences in the spacing of the molecules on either side of the phase boundary (*D*). Any change resulting in divergence of the biochemical characteristics or the physico-chemical parameters of the two systems in contact might upset either the orientation (*E*) or the compatibility (*F*) of these films and produce a release of one system from the other. This may likewise be attained by direct proteolytic action of enzymes present at the surface.

The association by surface contact between two nerve fibers, or even between a nerve fiber and a specifically impregnated non-nervous pathway, could then be viewed in the same light. The fact that we are seemingly dealing with a problem of very general biological applicability, may give renewed impetus to its analysis on the molecular level. So much about orientation and fasciculation of nerves.

The second problem to arise in this connection, as we have said, is one of numerical control. For the size of the fiber complement of a given nerve follows a definite norm, which raises the specific question of what determines the final size of a nerve? Four factors enter into consideration: Size of the nerve source; peripheral amplification by fiber branching; peripheral reduction by anastomosing; and the size of the periphery to be innervated. Each one of these factors contributes to the number of nerve fibers ultimately found in operation.

The size of a nerve source anticipates grossly the size relations of the future nerves; that is to say, the amount of neuroblasts disposed to send out peripheral nerve fibers varies in different parts of the central nervous system, in a certain forward reference to the later

[11]Compare the paper on *Protein Patterns in Cells* by F. O. Schmitt presented at this symposium.



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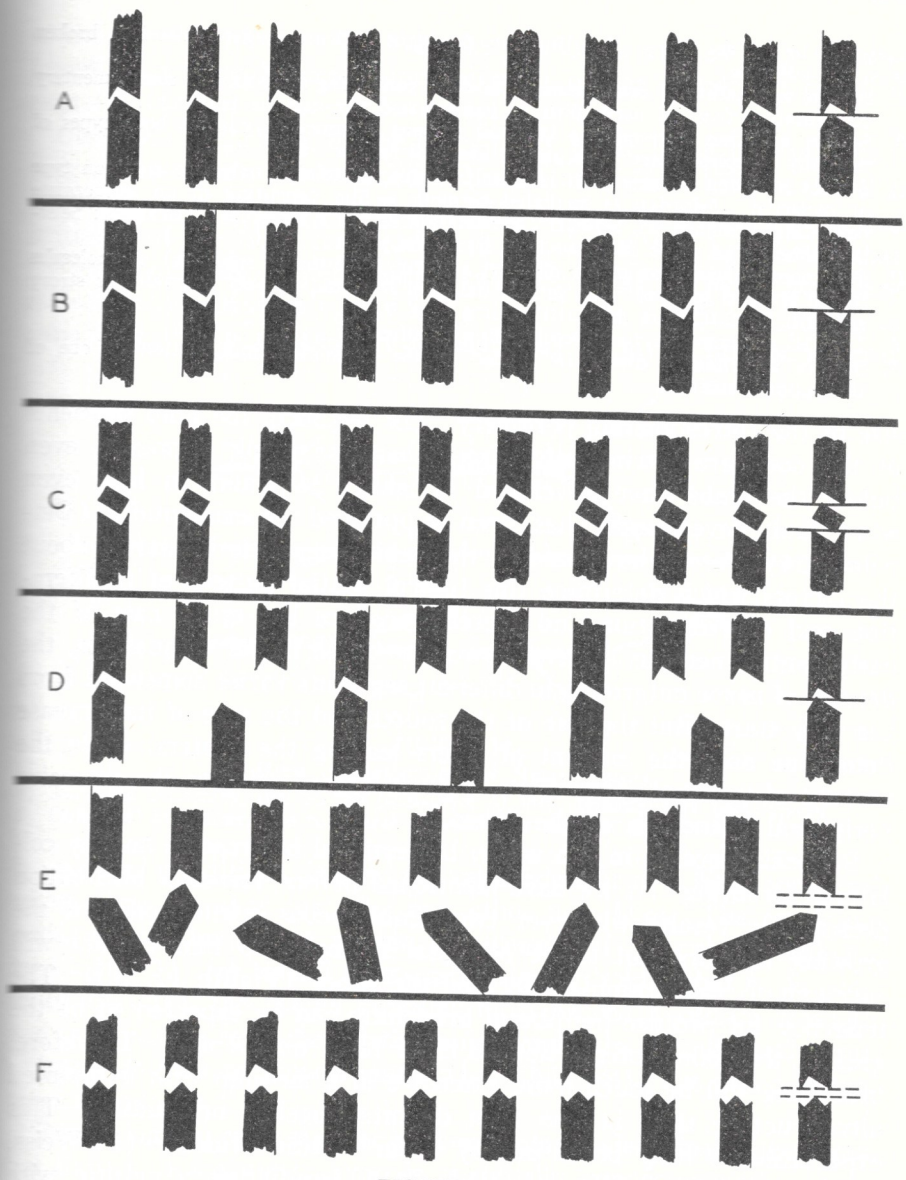

FIGURE 10

SCHEMATIC REPRESENTATION OF SELECTIVE ADHESION BETWEEN TWO ORGANIC SYSTEMS ON THE ASSUMPTION OF SPECIFIC PROTEIN CONFIGURATION

Each rod represents the polar end of a molecule. The notches and correspondingly shaped triangular protrusions symbolize complementary steric configurations in the molecules resulting in selective interlocking, according to the hypothetical analogy of antigen-antibody union. Vertical position of rods indicates parallel orientation of

molecules in surface films. Full lines on the right indicate surface adhesion, broken lines non-adhesion.

A, Surface application of one system to another containing molecules of complementary configuration (e.g., of nerve fiber to impregnated non-nervous pathway).

B, Surface adhesion between two identical systems, assumed to contain both types of mutually complementary molecules in identical ratios.

C, Surface adhesion between two identical systems, containing only one common type of molecule. Adhesion is mediated through a film consisting of an appropriate cementing substance (cf. Schmitt's presentation at this symposium). (Example: Selective adhesion of nerve fibers to their own kind.)

D, Weakened adhesion owing to different spacing (statistically speaking) of molecules in the apposed surfaces. The surface union in *D* would be only one third as strong as in *A*.

E, Lack of adhesion due to molecular disorientation.

F, Surface detachment, owing to change in the molecular configuration in one of the apposed surfaces.

size of the corresponding periphery. For instance, the limb segments of the cord are intrinsically larger than the trunk segments, even before the limbs have developed (Coghill, '36), and, in fact, even when limb development has been suppressed experimentally (Detwiler, '24). As we have said in the beginning, little is known about the factors which determine this typical spatial pattern of proliferation and differentiation and thereby produce the crude cast of the early central nervous system. Owing to these initial inequalities, the density of nerve outgrowth in different segments varies somewhat from the very start. But the size of the source and the rate of its increase determine only the amount of fibers leaving the centers, while the amount of terminal connections is much larger, owing to the extensive peripheral branching of the fibers.

Branching occurs in two ways: by terminal bifurcation and by collateral sprouting. As we have mentioned before, *terminal* bifurcation presumably results whenever two simultaneous pseudopodia of the growing tip turn out to be of equal strength, so that neither will succumb to the draining effect of the other (Figure 11, *A*). The tendency for profuse peripheral branching can be expected to be the greater the more intersections a fiber finds on its way. Therefore, branching will be much more extensive in a medium with unoriented ultrastructure than in one with definite micellar orientation. This expectation is fully borne out by tissue culture evidence (P. Weiss, '34, p. 440). According to Levi ('41), irradiating a culture with radium may greatly increase the incidence of branching, but the manner in which this occurs has not yet been analyzed. The rôle of the medium is likewise illustrated by the extensive branching which occurs in the unorganized scar around the cut end of a regenerating nerve. Perhaps the different density of the neropil in various parts

of the brain (Herrick, '34) will one day be correlated with the different density and micellar organization of the respective colloidal matrices.

Collateral branching, on the other hand, is the result of a new budding process from the consolidated stem of a fiber (Figure 11, *B*). Experiments of Peterfi and Kapel ('28) suggest that local mechanical irritation of the fiber can produce it. Speidel ('33) has shown that the agitation caused by a dividing cell on or near the nerve fiber may act similarly. Obviously, any irritant, which is sufficiently strong to remobilize the local fiber protoplasm may, if given further support

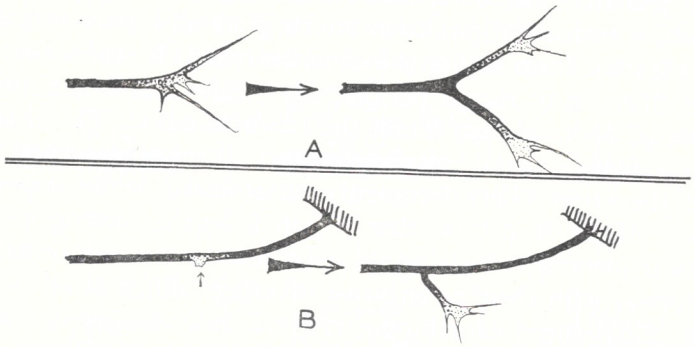

FIGURE 11

MODE OF BRANCHING IN NERVE FIBERS

A, Terminal branching, due to the persistence of two contemporary pseudopodia.
B, Collateral branching, due to lateral activation of stem of fiber.

by external conditions, lead to the establishment of a local outgrowth which then proceeds just like any other new fiber. The conditions under which this occurs remind us strongly of the rules established by Child ('27) for lateral regeneration in Hydroids; that is, in order to be effective, the wound stimulus must exceed a certain strength, and the distance from the existing apical end must be sufficiently great to insure to the new growth center what Child ('41) has called "physiological isolation."

Branching of peripheral nerve fibers occurs chiefly inside the terminal tissues. Each motor axon is eventually connected with a great number of muscle fibers through corresponding peripheral arborizations. There is evidence that muscle fibers are innervated long before they ever become muscle fibers, that is, in the state of myoblasts. This being the case, it is probable that whenever a myoblast or a young muscle fiber divides, this stimulus by itself provokes the formation of

a collateral branch from the nerve fiber, so that each new muscle fiber, as it emerges, takes its share of the neurone along. The fact that the average number of muscle fibers supplied by a single nerve fiber varies systematically for different muscles, would thus be an expression of the different rates of multiplication of the muscle fibers in different muscles.

The question of peripheral *anastomosing* of fibers by protoplasmic fusion is less clear. Boeke ('33) has emphasized the syncytial nature of the peripheral sympathetic plexus, and Stöhr ('35) has taken an even more extreme stand. Boeke ('30, '38) has also stressed the syncytial anastomosing of somatic nerves in the early phases of regeneration, a transitory condition which gradually gives way to fiber individualization from within the common mass. It seems that simple and thin fibers may merge peripherally, while the larger and more differentiated fibers always retain their individuality. This distinction is substantiated by nerve cultures in vitro. Levi ('41, p. 193) points out that the finest fibers frequently anastomose, while larger fibers never do. Might it not be that fusion is again contingent on full biochemical identity, which would exist among the primordial fibers, but disappear with their progressive divergent differentiation as they mature? This would explain the gradual individualization of fibers in regenerated nerves. However, these processes have not yet received enough attention to permit us to evaluate their significance for the final configuration of the peripheral nerve pattern.

Besides affecting nerve orientation and branching, the periphery regulates the volume of its innervation in two other ways. One of these has become familiar from the comprehensive studies of Detwiler and his co-workers (reviewed in Detwiler '36). Their experiments have shown that in urodele amphibians the size of the spinal ganglia is adjustable within limits to the actual extent of the periphery which they supply. An experimental increase or decrease of the peripheral tissue produces an augmentation or reduction in the number of spinal ganglion cells in the corresponding segments. May ('33) in the frog, and Hamburger ('34, '39) in the chick have demonstrated a similar relation between size of periphery and size of the motor nuclei of the cord. While the manner in which the expanse of the periphery reflects upon the volume of the centers is still wholly obscure, it is certain that the periphery exerts some influence on the number of fibers it is to receive right at their source.

Recently, however, we have come to learn about a second regulative

influence by the periphery which does not affect the production, but rather the admission of fibers, in that it determines what proportion of the total fiber production is actually admitted into final functional connections. It was noted that in the case of transplanted full-sized limbs innervated from a reduced nerve source (P. Weiss, '37), as well as in the case of undersized regenerating limbs innervated from a superabundant nerve source (Weiss and Walker, '34), the total amount of peripheral nerve branches bore a definite relation to the size of the limbs rather than to the size of the nerve sources. These experiments, amplified later by Litwiller ('38, '38*a*) have shown beyond doubt that the tissues of the limb exert a controlling influence on the admission of nerve fibers into the peripheral field. The experiments can be best summarized in a diagram (Figure 12). Faced with

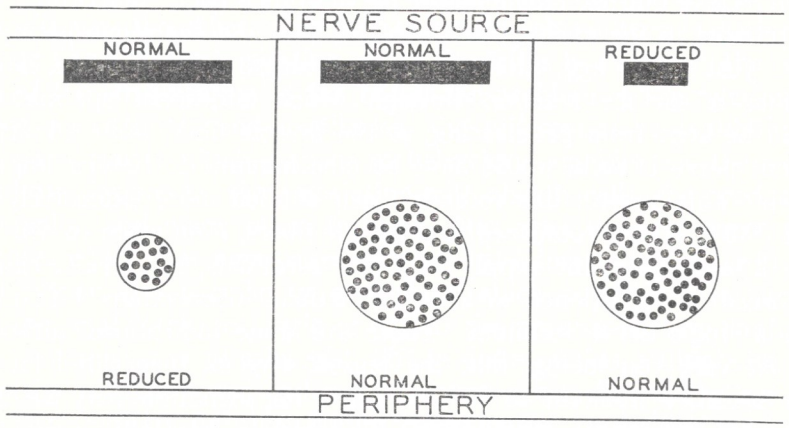

FIGURE 12

DIAGRAM, INDICATING LACK OF DEPENDENCE OF PERIPHERAL NERVE FILLING UPON SIZE OF THE NERVE SOURCE

an oversized nerve supply, the tissue can somehow prevent its becoming oversaturated with nerve fibers. Faced with an undersized nerve source, it can yet draw its full quota of peripheral branches. How it does this, is still unknown. A direct experimental demonstration of the effect has thus far been furnished only for regenerated nerves. However, the remark by Harrison ('35, p. 148), that a giant limb grown on a small body may contain a motor nerve complement proportional to its size without corresponding increase of the innervating centers, indicates that a similar mechanism of control operates in the embryo.

From these studies it would seem, that an innervated area spreads some influence which prevents the penetration of further innervation into its domain. It stakes off a territory. This may account for a well known observation. When a skin nerve is cut, the denervated area is slowly invaded by collateral nerve fibers from surrounding intact areas: the denervation has apparently removed some restraining influence previously exerted by the former occupants of the territory.

Thus receptor and effector tissues regulate the density of their innervation. The mechanism need not be the same for all tissues, and each one will have to be accorded a separate investigation. In only one case do we have more concrete information, that of the muscle. It is a well established anatomical fact that the large majority of muscle fibers receive but one single motor nerve branch each. Harrison ('10) has pointed to the analogy between this fact and the monospermy of eggs. Just as an egg, after receiving a single spermatozoan, would produce a surface reaction through which additional spermatozoa would be kept from penetrating, so the muscle fiber, after admitting its first nerve branch, would have become immune to further impregnation. This subject has recently been studied more extensively by Fort ('40). It was confirmed that muscle fibers, even when confronted with a superabundant supply of nerve terminals, as a rule, cannot be forced or conditioned to accept functional connections with more than a single nerve terminal. There are some indications, although not yet very convincing, that the reason why an innervated muscle fiber becomes resistant to further nerve impregnation may lie in a change of its surface constitution. The muscle, however, is the only case in which at least a beginning has been made to analyze the nature of what we may call the "saturation factor" of peripheral innervation.

Lack of space as well as of reliable information prevents us from going into the subject of nerve fiber *size,* the diameter of the individual fiber. One observes fibers in all gradations, from down at the limits of microscopic visibility up to those visible to the naked eye. They can be grouped into different size classes with different physiological properties (Erlanger and Gasser, '37), and different nerves contain fibers of the different classes in different proportions. These proportions seem to be essentially re-established in regeneration (P. Weiss, '37, p. 517), and the question of what determines the diameter of a nerve fiber seems to offer considerable interest. However, I know of

no single analytical examination devoted to this problem. Correlations have been established from observational data between the length of fibers and their size, between innervated area and size, and the like, but with the possible exception of observations by Detwiler and Lewis ('25), indicating that motor neurones may show size reduction in the case of an experimental reduction of their periphery, no analytical experiments are available to decide in which way intrinsic and environmental factors contribute to the determination of the final dimensions of a neurone. We may mention that in tissue culture nerve cells and fibers occur in all sizes, but their variation is too unsystematic to furnish any clues.

Let me stop here with the analytical part of the discussion. The picture I have given you has covered only the most prominent features of nerve patterns. If I were to be exhaustive, I would have to go on and discuss the finer differentiation and physiological distinction among fibers, the arborization of the dendrites in the gray, the problem of the stratification of the brain, the problem of the relation between nerve fibers and capillaries, the differential susceptibility to hormonal agents which may be of behavioral significance, the causes of plexus formation, the association with sheath cells, the production of myelin, and many other features. But even the very incomplete picture, which you have received, will have impressed you with the multiplicity and complexity of factors which participate in turning out the final intricate fabric as which the nervous system confronts the anatomist and physiologist. If, in spite of this multiplicity and diversity of agents, the end product turns out to be, on the average, a nervous system fully capable of coördinating, controlling, and integrating the functions of the body, the credit for this achievement must be ascribed to three main factors.

Firstly, to the existence in the embryo of a definite spatial and chronological organization, according to which the individual events are seriated. Secondly, to the fact that the initial organization provides only for the gross outlines of the future development, leaving sufficient latitude for direct adjustive interactions to allow for a certain amount of variation among individuals. And thirdly, to the fact that the operation of the nervous system does not require absolute structural stereotypism, so that only statistically speaking must there be something like constancy and repetition in the final developmental product.

Just a few words in amplification of these three principles. The

standardized dosing and timing of the morphogenetic processes is something the nervous system has in common with other parts of the body, and need not be discussed here (cf. P. Weiss, '39, pp. 104, 319, 486, 558).

The second point, however, deserves illustration because it gives a tangible conception of what we usually refer to as "regulation." Let us take a specific example. Remember that the formation of a straight nerve connection between a center and a peripheral organ is due to a dual process: Oriented outgrowth of the pathfinders, and the subsequent filling up of the cable by selective fasciculation. Suppose now, that owing to a genetic mutation or to developmental accidents, the pioneering fibers grew out precociously, before their supposed leading structure is ready: they will stray about and take devious routes, but some of them have still a good chance to reach the periphery. After that, selective fasciculation will do its part and produce a fair-sized nerve connection after all. The initial size deficit of the nerve may be partly corrected by the stimulative influence of the periphery which will mobilize additional neurones. But even with a constantly undersized nerve source, the peripheral field can still obtain its full quota of innervation, owing to the control it exerts over the amount of peripheral branching. The fact that each neurone will now have to carry a heavier load, is immaterial, because the size of the innervating centers is no measure of functional perfection, as is illustrated by the perfect functioning of transplanted supernumerary limbs innervated from only a fraction of the normal number of motor neurones (P. Weiss, '36). We realize, thus, that quite a few steps in the complex process of nerve formation may deviate from the norm without endangering the essential adequacy of the final product.

This situation again is characteristic not only of the development of the nervous system, but of development in general. One must remember that no developmental process is a unitary event. Innumerable independent and interdependent partial events share in the formation of an embryonic part. So, if one among the many fails to coöperate properly, the result may be only slightly off normal. This is merely translating the conclusions at which the geneticist has arrived in his multiple factor analysis from the symbolic language of genetics into the concrete terms of embryodynamics (cf. P. Weiss, '39, p. 479-486).

The latitude left to developmental processes leads us to the third point. No two nervous systems are identical or even nearly identical,

if we concentrate on details. Yet, many of the most popular schemes to interpret the physiological action of the nervous system in terms of neurone relations, are based on the assumption that every detail of design and measurement in each individual neurone is significant as such. The number of collaterals, the length of collaterals, the points where they branch off, the number of end feet, their density on the cell body, the spacing between individual endings—all such details are treated as if there were agents in the organism which could wisely see to it that each individual neurone would really be as precisely constructed as physiological theory demands.

Now, while we are still far from complete insight into the factors which operate in establishing the finer structural details of the neurone architecture—and there is no saying what surprises the future may hold—we do know certain things; and one of them is that those factors which we have discussed in this report and which certainly do operate, are not of the kind that could produce the envisaged precision machine. The finished machine can be of no greater precision than the agents by which it has been constructed. We do not mean to deny here the possible intimate correlation between structural aspects and physiological performance altogether, but we must point out that, if the neurophysiologist wants to take into account the lessons of embryology, he will have to substitute *statistical* considerations for his overemphasis on *systematic* traits.

For instance, the *average* density of dendrites or collaterals in a certain neurone group may be functionally relevant, even though the number of dendrites or collaterals on each element may fluctuate considerably about that average number. As we have said before, the statistical amount of branching is a function of the colloidal consistency of the medium, and could, therefore, be very well understood on embryological principles. On the other hand, the assumption that each and every nerve cell of the group might possess a fixed number of dendrites or collaterals is positively discouraged by what we know about developmental mechanisms.

Here is another example of how statistical properties can play as good an explanatory rôle as is usually ascribed to structural details. The phenomenon of central irradiation, first described by Sherrington, implies that, as the excitatory influx into a center increases, more and more cells become engaged, leading to a proportional increase of the response. This is usually explained on the assumption that the central cells are connected in definite chain arrangements. In

spreading from one cell to the next, synaptic resistance must be overcome. Thus, the stronger the initial charge of the first excited cell, the more hurdles it will be able to take, and the more cells of the chain will be set off. But since each cell is viewed as a link in several intersecting chains, the structural provisions prerequisite for an orderly gradation of the response would have to be infinitely complicated. In contrast to this view, I have recently obtained evidence of graded responses, increasing with the strength of the stimulus, in deplanted centers (see p. 179), in which every trace of systematic organization had vanished. In these deranged neurone pools, cells are still interconnected, but at random. If graded responses can still be obtained, there seems to be only one explanation: That is, that central cells develop as a population in which the *thresholds* of excitability vary *at random* (P. Weiss, '41). In such a population the number of elements of a given threshold class, plotted against the thresholds, would follow a near-normal distribution curve (Figure 13, *A*). A stimulus of given

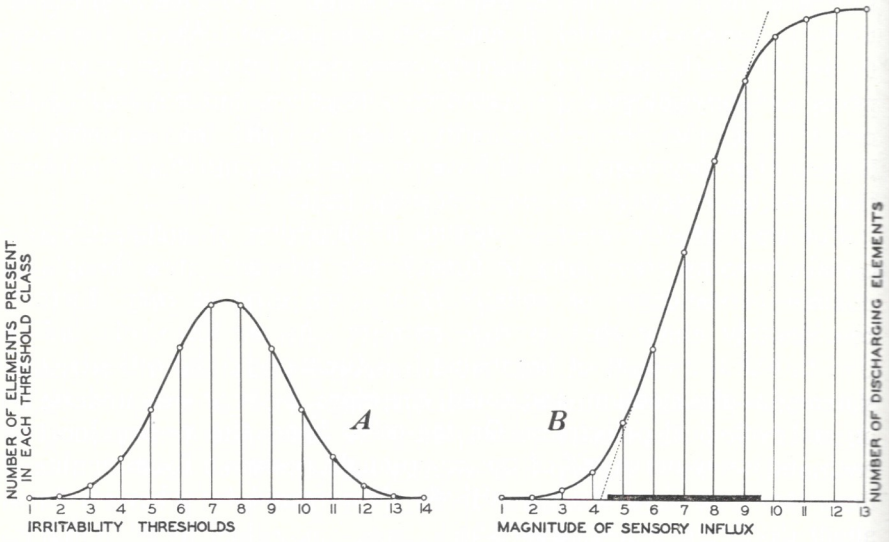

FIGURE 13

HYPOTHETICAL DISTRIBUTION CURVE OF THRESHOLDS OF EXCITATION IN A POPULATION OF NERVE CELLS

(Explanation in text.)

strength would set into action all cells of the corresponding and lower thresholds. The number of cells activated by stimuli of varying strength would be expressed by the integral of the normal distribution

curve, and this, a sigmoid curve, very nearly approaches the course of a straight line for about 80 per cent of its range (Figure 13, *B*). Thus, within that range, any increase of the stimulus would bring in a proportionally larger number of cells, without the need for any special structural provisions. It is interesting to note, that v. Brücke, Early, and Forbes have actually demonstrated that the distribution of thresholds among the α fibers in a peripheral nerve follows a normal distribution curve, in other words, expresses random fluctuation within a population of living elements, rather than any systematic provisions specially devised in development. I think Hecht ('26), in his treatment of the visual apparatus, was the first to suggest such statistical considerations.

It has been my intention in this review not only to point out the multiplicity and complexity of factors that enter into the making of a nervous system—and of any organic system for that matter—but also to indicate the analytical insight that may be gained by resolving the complex situations into their simpler components and treating these one by one. In doing this, we have seen that the resolution can sometimes even be carried to the point where the biophysicist proper may take over. Once a vital process has been reduced to terms of molecular events, nothing remains to be done by the biologist in that particular direction. Thus, after having deposited the problems of adhesivity, selectivity, ultrastructure, etc., at the doorstep of the biophysicist, we may withdraw expectantly to see what further elucidation he may be able to provide from his own province. Or better still, we may combine forces with the biophysicists to create teamwork as proficient as that of that well-known symbiosis of the fable, the blind and the lame.

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