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Growth Control of Nerve Cells by a Protein Factor and Its Antiserum

Discovery of this factor may provide new leads to understanding of some neurogenetic processes.

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Long before the possibility of experimentally modifying the developing nervous system was conceived, it was realized that end organs play an all-important role on the associated nerve structures. For it is the very nature of nerve cells to establish contact with other cells such as muscle fibers, glandular tissue or other nerve cells. Such close morphological and functional connections could hardly be conceived without a bondage which would interlock the life of the two partners: both would be severely affected if such links were broken. While the mutual dependency of nerve cells and associated structures was clearly documented in the adult organism, it remained for the study of the embryo to bring into focus, in a deceptively clear and persuasive way, the role of such end organs in the growth, differentiation, and maintenance of nerve cells.

The great experimental embryologist, R. G. Harrison, first realized the advantages offered by the embryo as the object of study of the differentiating nerve centers, and it was the nervous system of the amphibian larva which was first challenged to solve problems it had never faced, such as providing for the innervation of organs and limb rudiments borrowed from larvae belonging to species of different size. In thrusting his glass needle into the soft body of the larva and in performing heteroplastic transplantations, the embryologist wanted to test the capacity of the developing nervous system to establish functional connections with foreign tissues. Even more, he wanted to see how the nerve centers and the growing nerve fibers would adjust to the different geometrical dimensions and structural configuration of the foreign organs. The results indicated a remarkable flexibility on the part of the developing nervous system which readily adapts to a new situation even to the point of accelerating the growth rate of its nerve fibers to keep pace with the fast-growing limb rudiments of a smaller species (*1*).

These results also proved that the embryonic nervous system is highly receptive to influences exerted by tissues which supply the peripheral field to the outgrowing nerve fibers. That this influence is not species-specific is documented by the growth response called forth by organs or limb rudiments of a different species. Such experiments and others dealing with heterotopic transplantations proved that peripheral fields are interchangeable, provided that some of the basic rules are respected: motor and sensory nerve fibers have their own fields of innervation; deprivation of one given sector of its motor or sensory nerve contingent does not favor invasion by the other. The same holds true with respect to somato-motor and somato-visceral fibers. The somato-motor fibers end in the voluntary striated muscles while the somato-visceral make synaptic contact with nonstriated muscles, heart, and glandular tissues. Hence embryonic nerve fibers, as well as mature nerve fibers, have no access to peripheral end organs which belong to a category different from their own. In one instance only were embryonic nerve fibers of a given type seen to make functional connections with tissues of an entirely different type from that with which they normally connect. This was the case when intrinsic nerve fibers from the exposed stump of a sectioned neural tube wandered into the surrounding tissues and established functional connections with muscles (*2*).

When Harrison presented in the Harvey lecture the results of the experimental analysis of the developing nervous system in the amphibian larvae, the report, although impressive for the ingenuity of the experiments and the talent displayed in the analysis of the response of nerve centers to all possible changes inflicted to their peripheral fields of innervation, may have raised some doubt as to the information one could gain in this way on the factors which control growth and differentiation in the nervous system. The concluding sentence in the lecture pointed to a sideline of these investigations which seemed to Harrison even more promising than the main object of the research. "By ablation and transplantation of parts of the embryo it is possible to fashion almost any kind of nervous system desired and subsequently to study its function without the disturbing effects of trauma. This is one of the most promising lines of investigation leading off from the field covered in the present lecture" (*1*).

Almost 30 years later, one must admit that these predictions did not materialize, while instead some valuable information was gained by pursuing the main line of research opened by Harrison and his students, and by carrying further the inquiry into the control mechanisms of the developing nervous system.

Considerable progress was achieved by replacing the amphibian larva with the chick embryo. It was V. Hamburger who conceived and contributed most of the work on the effects of the

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peripheral field of innervation on sensory and motor spinal systems in the chick embryo (*3–5*). Even though these same nerve structures were also the object of experimental analysis in the amphibian larvae, a considerable amount of work had centered on the causal analysis of brain structures and associated sense organs; for the vertebrate brain, with all its structural and functional complexity, holds such a fascination for man that it is difficult for him to resist the temptation of exploring it in the hope of learning more about his own brain.

Compared to the brain, the spinal cord appears to be of almost diagrammatic simplicity. How deceptive in fact this is, was to be learned through long and laborious attempts to explore the mechanisms which control the growth and differentiation of the spinal cord and of the sensory ganglia. The chick embryo proved to be particularly suited for this analysis. Its nervous system is more complex, but it lends itself better than the amphibian nervous system to the analysis, for its nerve centers are more clearly segregated and defined and their strong affinity for silver permits a visualization of the nerve structures far superior to that in amphibians. But it was a new approach to the problem which brought a significant contribution to our admittedly still very rudimentary knowledge of neurogenesis. Most of the previous work had focused on the study of the end effects of early surgical interventions on the developing nervous system. The results of heteroplastic and heterotopic organ transplantations were in fact explored in the fully developed larvae, long after the problem of adjusting to the new situation had been solved by the nerve centers and contact had been firmly established between the growing nerve fibers and the new periphery. It now became clear that this static approach would leave unanswered the main question: how does the nervous system solve the problem with which it is confronted? It is, in fact, the mechanics of developmental processes rather than their final completion that is of primary interest to the student of neurogenesis. This realization prompted a detailed analysis of the nervous system of the chick embryo from the stage of 38 hours of incubation, when the first wave of differentiation starts in the rostral part of the neural tube, to the time of hatching. The study of normal embryos was rewarding. It made clear the composite nature of apparently simple neurogenetic processes and, in so doing, brought into focus some basic problems which lent themselves to the experimental approach.

In this article I will briefly outline some of the developmental neurogenetic processes which were the objects of investigation in recent years.

At the time of its early formation, that is during the first week of incubation, the nervous system of the chick embryo (and the same is true for all vertebrates) is the stage for massive cell movements, segregation of cell groups, and degeneration of other cell populations. Migration of neuroblasts, that is, nerve cells at an early stage of their differentiation, occurs throughout the entire length of the neural tube from its apical to its caudal end. Migrating nerve cells move in the same orderly fashion as ant or termite armies and, like them, they keep in close reciprocal contact during the long journey which takes place in the dense matrix of the brain vesicles and in the spinal cord. Closely timed inspection of the developing nervous system, hour after hour and day after day, revealed the complexity and extension of these active cell displacements which may take several days to be completed (*6*, *7*). At the same time as these migratory movements occur, other nerve cell populations undergo disintegration and death and are wiped out, in a matter of a few hours, by intervening macrophages. Finally other populations form and achieve their differentiation without undergoing either degenerative or migratory processes. Thus the final product, the differentiated nervous system with its motor, sensory, and associative centers well segregated from each other and interconnected through hundreds of fiber tracts, is greatly different from the embryonic nervous system. The developmental history of each nerve center should therefore be traced back to its early inception in order to know how such a center attains its final size and position. Differentiation itself, in the central nervous system, does not seem to obey the rules which operate in other systems. Some nerve cells show all marks of morphological and functional differentiation at an early developmental stage, while others retain the characteristics of immature cells till the end of the incubation period and then suddenly transform into fully differentiated neurons. Differences in apparently homogeneous cell populations become manifest as differentiation progresses and hitherto similar cells diverge from each other in their growth rate, structure, and end connections. These differences become even more apparent under experimental conditions. Of the experimental work performed on the developing nervous system of the chick embryo, I will briefly mention here only some of the results of experiments aimed at the analysis of spinal ganglia confronted with a larger peripheral field or, conversely, deprived of their field of innervation. The main contribution of these experiments was not so much to stress the all-important role of the periphery on associated sensory centers, as to reveal the extent of these effects on the different developmental aspects which we are used to refer to with the rather general and vague term of "differentiation." The closely timed study of the response of these ganglia to the extirpation of the limb bud in two-day chick embryos, gave evidence of a sharp decrease in the mitotic activity of ganglia destined to innervate the amputated limb rudiment. Since dividing cells lack any differentiative mark and have no fiber connection with the periphery, these effects were designated with the noncommittal term of "field effects" or remote rather than direct effects (*5*). If the adverse effects on the mitotic activity already reduce the size of the sensory cell population, the same populations are even more severely affected by a destructive process which attacks cells in early differentiative stages in the same ganglia. About half of these cells undergo sudden death between the fourth and the fifth day of incubation. Dead cells are removed in a few hours by intervening macrophages, while the surviving cells undergo slow regressive changes but survive till the end of the incubation period. The striking similarity between this massive cell disintegration and the physiological disintegrative processes already mentioned, raises the question of whether in both instances, death might not follow the depletion of some agent essential to growth and differentiation of these nerve cells. Other instances of massive cell degeneration will be considered subsequently.

Thus the severe atrophy of ganglia deprived of their peripheral field of innervation is the end result of a number of detrimental effects. Why some cells should undergo sudden death while others, also deprived of their end organs, should suffer only regressive

changes compatible with life is one of the many questions still unanswered (*5*). Equally extensive and complex is the growth response of the same ganglia confronted with the task of providing the innervation of an additional limb bud. The mitotic activity sharply increases in these ganglia, and all subsequent developmental steps from early to advanced differentiative processes are likewise enhanced. While suggesting that the periphery releases some agents which control the mitotic activity as well as differentiation in the sensory nerve cells associated with these end organs, the results gave no clue concerning the nature of these agents and we would today have progressed no further than stating the problem, were it not for a fortuitous discovery which led to the identification and isolation of one of such factors.

Growth Response of Nerve Cells to a Tumor Factor

When in 1947 E. Bueker implanted a fragment of mouse sarcoma 180 into the body wall of a 2-day chick embryo, he had in mind to test the capacity of sensory and motor nerve fibers of the host to innervate a fast-growing tissue. Since the tumor, at variance with a limb bud or other embryonic organs, is homogeneous in structure, the experiment was also expected to answer the question of whether the tumor would admit all or only a given type of nerve fibers. In his 1948 paper (*8*) Bueker reported that nerve fibers from the adjacent sensory ganglia had gained access to the tumor and that these ganglia underwent what seemed to be a moderate increase in size of the same order of magnitude as that resulting from the implantation of an additional limb bud. The author saw in these results a confirmation of the hypothesis that the final size attained by the sensory ganglia is dependent upon the extension of their peripheral field of innervation. The fact that sensory but not motor fibers branched into the tumor confirmed the hypothesis of a selective affinity of nerve fibers for peripheral end organs. Finally, the observation that only sensory ganglia connected through nerve fibers with the tumor increased in size seemed to emphasize the similarity between these effects and the effects elicited by an additional limb rudiment and to give support to the hypothesis that nerve fibers are instrumental in channeling the effects of the periphery on the associated nerve centers. Viewed in this light, the results of the tumor implantation conformed to the results of limb or organ transplantation. Fifteen years had to elapse before the impact of this ingenious experiment on the classic and somewhat ill-defined concepts of "peripheral field effects" was fully realized.

Of the extensive work performed during the last twelve years, I shall consider here only that not fitting into previous schemes, thus paving the way to new experiments and to the revision of old concepts. A reinvestigation of the tumor effects (*9*) led to the discovery of new facets of the phenomenon. It was found that the growth response of the sensory ganglia contributing fibers to the tumor far exceeds the response called forth by an additional limb bud implanted in the embryo at the same developmental stage, that is, at 3 days of incubation. Whereas sensory ganglia supplying nerve fibers to an additional limb undergo a size increase ranging between 20 and 40 percent in excess of the controls, the tumor evokes an increase in volume of the same ganglia two to three times their normal size. Nerve fibers branching into the tumor make no attempt to establish synaptic contact with the neoplastic cells, but wander around in tortuous circuits, and build a dense fibrillar net around and between the cells. If the size increase of the sensory ganglia and the peripheral branching of their fibers departed already to a considerable extent from the effects evoked by an additional limb, the growth response of the sympathetic ganglia adjacent to the tumor had no precedent in any previously observed effects. These ganglia appeared in fact to be five to six times larger than controls. In addition, atypical accessory ganglionic agglomerates were partly imbedded in the tumor and partly irregularly scattered on the fringe of the neoplastic mass. Normal and atypical sympathetic ganglia sent large fiber bundles into the tumor where they intermingled with the sensory fibers and, together with them, took possession of that bizarre "peripheral field" which mimicked like a caricature the effects of normal peripheral end organs on associated nerve centers. The viscera of the embryo, which normally lack a sympathetic innervation up to the end of the third week of incubation, were now flooded with sympathetic nerve fibers from the end of the first week of incubation.

In some organs like the mesonephros, which during its short life cycle is deprived of sympathetic innervation, the density of nerve fibers was such as to force the tubules apart. All the available space in this and other organs like the ovary, the spleen, and the thyroid was filled with large nerve bundles of such size and density that the characteristic structures of the invaded organs were overshadowed.

These most unusual findings led me to propose a different interpretation of this extraordinary growth response. The observed effects could in fact be better explained by assuming that the tumor harbors a growth factor which selectively enhances the nerve fiber outgrowth from the sensory and sympathetic ganglia of the host (*10*). This hypothesis received full confirmation from experiments of extra-embryonic tumor transplantation. Fragments of sarcoma 180 or sarcoma 37 (both elicit the same effects) were grafted onto the chorioallantoic membrane of 4- to 6-day embryos. In such a position the tumors and the embryos share the circulation, but no direct contact is established between the embryonic and the neoplastic tissues. The effects compared in all respects with the effects of intra-embryonic tumor transplants, thus giving decisive evidence for the release into the blood stream of a "nerve growth factor" (NGF) produced by the neoplastic cells. In intra- as well as in extra-embryonic tumor transplantations, thick nerve bundles were also found in the lumen of large and small veins. Some vessels were in fact filled with dense nerve agglomerates to the point that the blood circulation was greatly obstructed (*10, 11*).

These discoveries, while giving new support to the hypothesis that the tumor harbors a nerve growth factor, also suggested a different experimental approach to the problem. In order to isolate and identify the active agent, a less complex system than the developing embryo was needed. The tissue-culture technique seemed to offer considerable advantages over the embryo by making it possible to test the effects of the tumor directly on isolated sensory and sympathetic ganglia. These experiments were first performed at the Biophysics Institute of Rio de Janeiro in association with H. Meyer. Sensory and sympathetic ganglia of 7- to 9-day chick embryos were confronted in vitro with fragments of sarcoma 180 or sarcoma 37.

In control cultures the same ganglia

were combined with tissue fragments explanted from chick or mouse embryos. The culture medium consisted of a drop of chicken plasma and a drop of embryonic extract, later replaced with a similar amount of amino acid solution. The cultures were incubated at 37°C and examined at 6-hour intervals. The results confirmed the expectations. As early as 5 hours after the beginning of incubation, ganglia combined with a fragment of sarcoma 180 or sarcoma 37 showed striking differences from controls. While these controls produced only few and sparse nerve fibers, ganglia adjacent to a tumor fragment were surrounded by a dense halo of nerve filters which increased in density and length during the following 24 hours (*12*). Once decisive evidence was obtained that this in vitro effect was evoked by the same neoplastic factor which enhances sensory and sympathetic nerve fiber outgrowth in vivo, the tissue-culture method replaced almost entirely the laborious and time-consuming technique of implanting the tumor in the chick embryo and analyzing the effects 2 to 3 weeks later. These results marked the turning point of the investigation. As a result, some of the picturesque and esthetically attractive features of the embryological research were lost, but the investigation itself gained in precision and depth, since for the first time the chemical approach to the problem was made possible.

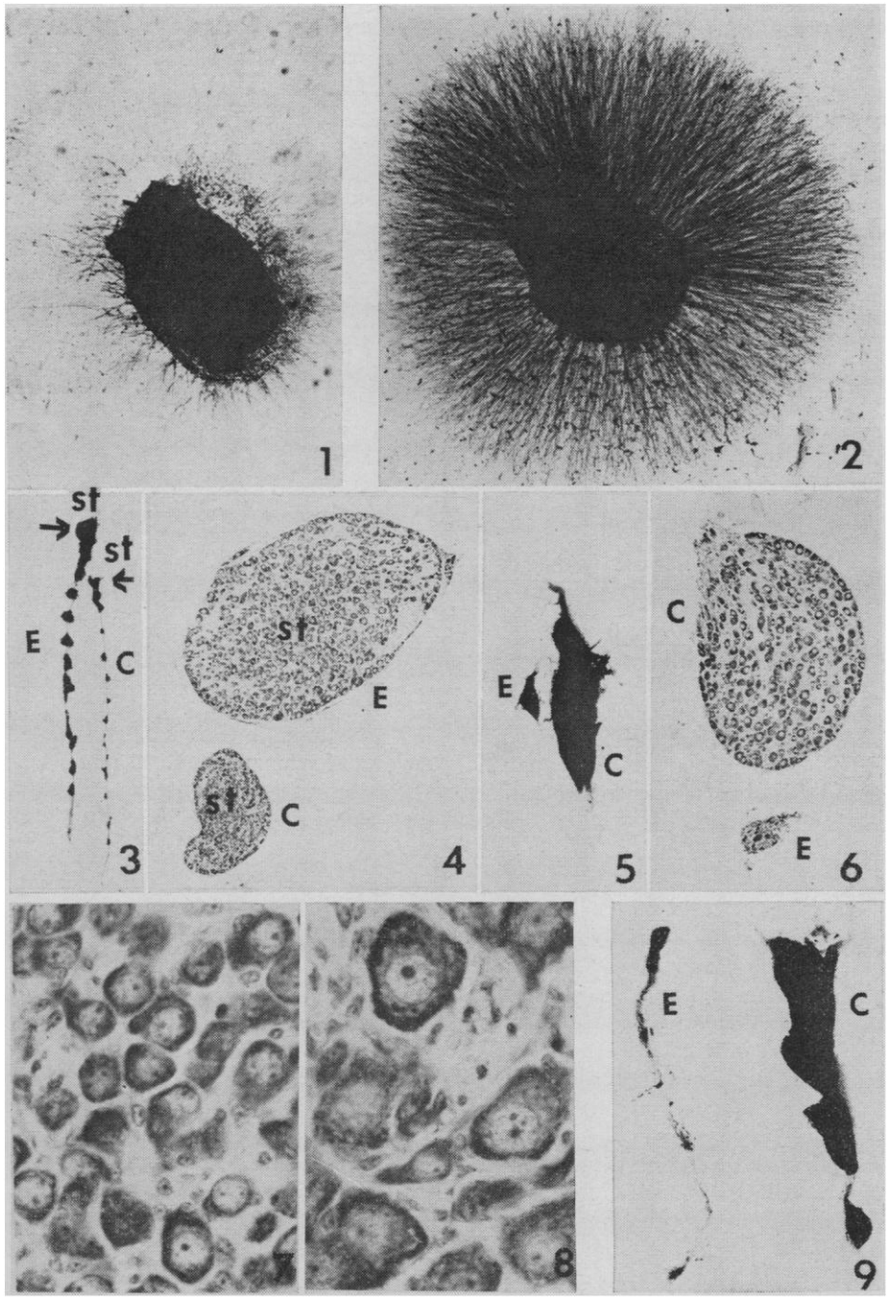
While biochemists consulted earlier had refrained from the too difficult task of identifying the tumor factor which evokes the growth effects in the embryo, the same problem presented in the simplified version of identifying the factor which promotes the nerve fiber outgrowth in vitro now seemed accessible to analysis. The biochemist, S. Cohen, agreed to join our group and undertake the task of identifying the agent. Ever since this in vitro technique was first devised in 1953, it has become the method of choice for the test of nerve growth factors in our laboratory as well as in other laboratories which are also engaged in the study of the same problem (*13*). It was only after the nerve growth factor became available in a purified form and in large quantities, that it was possible to assay its effects in the living organism. The embryos and the newborn animals then resumed their roles as the major objects of this investigation.

The Nerve Growth Factor: Its Nature, Biological Sources, and Effects

It is the rule rather than the exception in research that new leads should come from accidental findings and that these leads, when followed, would channel the main investigation into a new direction. Of all sciences, biological sciences still remain the most dependent upon such fortuitous leads to unravel some of the intricate mechanisms of life. The discovery of a potent nerve growth factor in snake venom and in the mouse salivary glands which occurred shortly after the discovery of the tumor nerve growth factor, and the recognition that both agents have many properties in common with the tumor factor, can well be listed among such fortuitous events. In 1954, Cohen had succeeded in isolating a nucleoprotein from sarcomas 180 and 37, endowed with the growth-promoting properties of the tumors and of their extracts (*14*). It was now of interest to establish whether the activity resided in the intact nucleoprotein fraction or if the nucleic acids or the proteinic component of such fraction elicited the growth effects. In this connection, snake venom was used by Cohen because of its phosphodiesterase content. Its addition to the culture medium in minute amounts was aimed at the degradation of the nucleic acid component of the active fraction. The unexpected and startling outcome of these experiments was the finding that the snake venom itself harbors a nerve growth factor. In fact ganglia cultured in a medium containing the purified venom at a concentration of 0.05 μg per ml developed an exceedingly dense halo of nerve fibers. This observation prompted a search for the venom effects in the living embryo. The results gave additional evidence for the striking similarity between the tumor and the venom effects. Daily injections of 0.5 μg of the purified venom into the yolk of embryos between the 6th and the 9th day of incubation, evoked the same nerve growth effects as intra-embryonic or extra-embryonic tumor transplantation (*15*). It now became clear why the injection of the tumor extract had failed to produce any growth effect. The specific activity of the venom is in fact about 1000 times higher than the same activity in the tumor homogenate. The intact tumor evokes the growth effects, in spite of its relatively low content of the NGF, through continuous release of this factor by living and actively dividing cells. Both the tumor and the snake venom factors are heat labile, nondialyzable, destroyed by acid (0.1*N* HCl), stable to alkali (0.1*N* NaOH), and to 6*N* urea. The chemical analysis of the venom factor was pursued farther than had been possible with the tumor factor. Upon acid hydrolysis and two-dimensional paper chromatography, the amino acid pattern was qualitatively identical to the chromatogram of crystalline bovine albumin. The activity is destroyed upon incubation with proteolytic enzymes and upon incubation with antiserum to the snake venom. The estimated molecular weight as determined with the Spinco analytical ultracentrifuge is of the order of 20,000 (*16*).

While the discovery of the growth-promoting factor in snake venom could not possibly have been anticipated, the finding of a similar factor in the submaxillary salivary glands of the mouse was the result of a planned search. The occurrence of two agents with nearly identical biological properties in mouse tumors and in the snake venom suggested the existence of other possible sources of this agent. The mouse salivary glands, being in many respects homologous to the snake venom glands, were thought of by Cohen as another possible source of a NGF factor. Experiments in vitro fully confirmed the guess (*17*). The submaxillary salivary glands of the adult male mouse harbor a factor endowed with the same nerve growth promoting activity as the sarcomas and the snake venom (Figs. 1 and 2). Its specific activity is of the order of 6000 to 10,000 times higher than the specific activity of tumors and about ten times higher than that of the snake venom. Its effects in vitro and in vivo on sensory and sympathetic ganglia of the chick embryo are qualitatively identical with the effects of the two other factors. The active agent was identified by Cohen in a protein particle that is heat labile, destroyed by acid, resistant to alkali, and nondialyzable. Like the NGF isolated from the snake venom, it is destroyed by proteolytic enzymes. Its molecular weight is of the order of 44,000 (*18*).

The high specific activity of the salivary NGF which in vitro evokes the halo effect at a molar concentration of 1×10^{-9}, suggested a test of its effects in mammals. Daily injections of the NGF into newborn mice in the amount



Figs. 1 and 2. Photomicrographs of 7-day sensory ganglia of chick embryo after 24 hours of culture in vitro. Fig. 1. Ganglion in a control medium. Fig. 2. Ganglion in a medium supplemented with the salivary NGF at a concentration of 0.01 micrograms per ml. Silver impregnation. Fig. 3. Whole mounts of the sympathetic thoracic chain ganglia of experimental (*E*) and control (*C*) mice 19 days old. Experimental mouse injected daily with salivary NGF from birth. *ST*, stellate ganglia. Fig. 4. Transverse sections of stellate ganglia (*ST*) in experimental (*E*) and control (*C*) ganglia of Fig. 3. Sections through levels indicated by arrows in both chains in Fig. 3. Fig. 5. Whole mounts of control (*C*) and experimental (*E*) superior cervical ganglia of 3-month-old mice injected for a week after birth with the antiserum to the salivary NGF. Fig. 6. Transverse sections through the superior cervical ganglia of control (*C*) and experimental (*E*) 9-month-old mice. Experimental mouse injected for three consecutive days after birth with the antiserum to the salivary NGF. Figs. 7 and 8. Comparison of cell size in control (Fig. 7) and experimental (Fig. 8) stellate ganglia represented in Figs. 3 and 4. Fig. 9. Whole mounts of stellate ganglia of control (*C*) and experimental (*E*) 9-month-old mice. Experimental mouse injected for three consecutive days with the antiserum to the salivary NGF.

of 0.5 μg per gram of body weight, evoked a striking increase of the sympathetic ganglia, already well apparent 48 hours after the first two injections. In 2- to 3-week old mice injected daily, beginning at birth, the same ganglia are four to six times larger than controls, while no changes are apparent in the parasympathetic and sensory ganglia nor in any other nerve cell population (Figs. 3 and 4). Cell measurements and cell counts show that the NGF calls forth an increase in cell number as well as increase in size of individual nerve cells (Figs. 7 and 8). When the mitotic activity comes to an end at 9 days after birth, the NGF evokes cell hypertrophy but no increase in cell number. In adult mice, sympathetic neurons attain a size two times larger than controls (*17*). The universality of the NGF effects was verified by in vitro experiments on ganglia of several species, man included. Ganglia explanted from human fetuses which became available from surgical abortions, show the same growth response to the NGF as ganglia of birds and rodents. The essential role of this protein in the life of the target nerve cells is further indicated by in vitro experiments on sensory and sympathetic nerve cells dissociated through trypsinization and explanted in liquid media. Nearly all of these cells disintegrate in the first 24 hours in a medium consisting of the Eagle solution alone or supplemented with 10 percent horse serum. The same nerve cells survive up to the third week in this medium when the NGF is added at a concentration of 0.05 μg per milliliter of solution (*19*).

Antiserum to the Nerve Growth Factor

An antiserum to the NGF was prepared by S. Cohen by injecting the purified NGF protein into the pads of rabbits together with Freund adjuvant. The antibody titer was first assayed in vitro. When it was found that its addition to the NGF solution at progressively higher dilutions inactivated the growth effects of the NGF in vitro, the antiserum was injected into newborn mice and the injection was repeated daily for the first week after birth. Three weeks later, treated and control mice of the same litter were killed. On macroscopic inspection, the sympathetic chain ganglia of the injected mice were virtually absent. Upon microscopic anal-

ysis, the diminutive ganglia consisted of satellites and a few neurons amounting to 3 to 5 percent of the normal cell population (Figs. 5, 6, and 9). Similar results were obtained with newborn rats, rabbits, and kittens. The injection of the antiserum in adult animals produces less drastic but still severe detrimental effects, while no adverse effects are apparent in other nerve cell populations or in other cell types (*20*).

Ever since these results were first reported in 1960, hundreds of mice and rats were injected with the antiserum in this and other laboratories. The nearly total atrophy of the sympathetic chain ganglia was in all instances ascertained by microscopic inspection up to two years after the treatment. Of interest is the observation that the lack of any sympathetic control is compatible with a normal life, at least under the sheltered conditions of the laboratory. The treated animals are comparable to controls in growth and in other respects. They are now the object of extensive physiological, pharmacological, and behavioral investigations.

Reevaluation of Control Mechanisms of Nerve Cells by Extrinsic Agents

One may ask whether the identification and isolation of specific nerve growth factors in some mouse sarcomas, in the snake venom, and in the mouse salivary glands, has any bearing upon the problem which promoted this search, namely the control mechanism of the end organs on the associated nerve structures. At the time of the discovery of the growth effects elicited by chorioallantoic tumor transplantations on the sympathetic system of the embryo, this response appeared quite different from the growth response evoked by additional limb buds or sense organs on the nervous system of the host. It differed from it not only in the magnitude of the effects but also in many other respects. The massive invasion of the embryonic viscera by large sympathetic nerve bundles and the penetration of these nerve fibers into the lumen of blood vessels indicated such a profound deviation from normality as to raise the question of whether the sympathetic nerve cells had not undergone a radical transformation into malignant cells. Such a hypothesis was readily dismissed but still we were not prepared to see any similarity between the effects elicited by the tumor and the effects evoked by normal embryonic tissues. The discovery of the nerve growth factor in the snake venom and in the mouse salivary glands proved that the production of the NGF is not the prerogative of neoplastic cells. A number of experiments devised and performed together with P. U. Angeletti proved that granuloma tissue, experimentally produced in a variety of mammals, releases the NGF (*21*). The same factor was detected in the sympathetic ganglia and in the serum of mammals, man included (*22*). Its presence was likewise ascertained in embryonic tissues (*13*). Hence we came to the conclusion that the nerve growth promoting protein is a normal constituent of the sympathetic cells and is normally present in the blood and body fluids of birds and mammals. Leaving open the long debated and still unanswered question of its main source of production in the organism, all evidence seems to us to favor the hypothesis that this protein which we designated as the "nerve growth factor" plays a most important role in the life cycle of the sympathetic nerve cells. As for the sensory cells, we have evidence that the same protein is required during their early developmental stages. Why the mature sensory neurons should become refractory to the NGF is one of the many unsolved aspects of this problem.

The identification of the NGF in a protein which is normally present in the developing and in the mature organism, raises the question of whether the end organs might not affect the growth and differentiative processes of associated nerve centers through the release of specific growth factors. In this way one could explain the mitotic effects and the excessive branching and altered peripheral distribution of nerve fibers subsequent to the implantation of additional organs or limb rudiments, which could not be otherwise explained. In contrast to the tumor and the purified nerve growth factors, end organs have a very restricted field of action. End organs affect only the growth of nerve centers which provide their innervation. Such differences could be of a quantitative rather than a qualitative order and could be correlated with differences in the production and release of growth factors in the two sets of experiments. Production and discharge may in fact be very limited in cases of implantation of limbs or additional organs.

In suggesting that peripheral structures and the NGF might in the final analysis operate in a similar way, we do not imply that the released growth factors should be the same. On the contrary, there is reason to believe that each nerve cell type might be receptive to only one specific factor.

Summary

Evidence is presented that sympathetic nerve cells of birds and mammals are receptive to the growth promoting effects of a protein (NGF) which was isolated from some mouse sarcomas, snake venom and mouse salivary glands. This same protein is a normal constituent of the sympathetic cells and is present in the blood and body fluids of a variety of vertebrates, man included. An antiserum to the NGF selectively destroys the sympathetic nerve cells of newborn animals without affecting other nerve cells or organs. The "immunosympathectomized" animals are comparable to controls in growth and viability.

These results give evidence for the essential role of this particular protein in the growth, differentiation, and maintenance of sympathetic nerve cells. They also suggest that other nerve cells might also depend upon specific factors for their differentiation and growth. These results are discussed in the general framework of neurogenetic problems.

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