HISTORICAL PERSPECTIVE:

The Discovery, Proof and Reproof of Neurosecretion

(Speidel, 1917; Scharrer and Scharrer, 1934)

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SUMMARY: Seventeen years before the Scharrers demonstrated neurosecretory activity of neurons within the brains of vertebrates and invertebrates and convinced the scientific world of the existence of a neuroendocrine system, Carl Caskey Speidel (1917) had identified glandular neurons in the spinal cord of the skate, postulated a neurosecretory function, and performed experiments to prove his hypothesis. The correct conclusions that he formulated from morphologic observations were not believed by biologists until 'proved' by the Scharrers, who acknowledged his pioneering contributions. The Scharrers studied many species and even demonstrated neurosecretion in nemertine worms, now believed to be closely related to the ancestors of all vertebrates. Evolutionary theorists had speculated on neuroglandular function as early as 1900, and the contributions of comparative neuroanatomists to this field have resulted in a major medical advance.

RÉSUMÉ: Dix-sept ans après Speidel, les Scharrers ont démontré l'activité neurosecrétoire des neurones dans le cerveau des vertébrés et des invertébrés et convaincu le monde scientifique de l'existence du système neuro-endocrinien. En 1917 cependant Carl Caskey Speidel avait identifié des neurones glandulaires dans la moelle épinière, postulé une fonction neurosecrétoire de ces neurones, et même fait des expériences pour prouver son hypothèse. Les conclusions correctes qu'il avait formulées à partir des observations morphologiques n'ont pas été crues par les biologistes jusqu'aux travaux des Scharrers qui n'ont pas hésité à reconnaître ses contributions princeps. Les Scharrers one étudié plusieurs espèces et même démontré la neurosécrétion chez les vers que l'on croit maintenant être les ancêtres de tous les vertébrés. Les théoriciens de l'évolution avaient depuis 1900 spéculé sur la fonction neuroglandulaire et les contributions des neuroanatomistes comparatifs à ce champ des connaissances ont résulté en des progrès médicaux d'importance.

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The central nervous system was not regarded as an organ of internal secretion until the mid-1930's, when the pioneering research of Berta and Ernst Scharrer (1928, 1932, 1933, 1934a,b, 1941a,b,c,) conclusively demonstrated that a neuroendocrine function indeed existed in the brains of both vertebrates and invertebrates. The Scharrers are widely accredited with the discovery of neurosecretion. However, another comparative neuroanatomist, Carl Caskey Speidel, described neurosecretory cells and correctly interpreted their function as early as 1917. His thesis at Princeton University was entitled 'Gland-cells of internal secretion in the spinal cord of the skates' and was later published in 1919 by the Carnegie Institute, but neither these papers, nor Speidel's (1922) later work confirming and extending his observations in other species of fishes were believed by the scientific community until his findings were reproved some 17 years later by the Scharrers, and his hypothesis restated. The Scharrers expressed surprise at the incredulity, rejection, or indifference shown by other neurobiologists. In a review article in 1945, they acknowledged "Speidel was the first to formulate this concept on the basis of a large amount of material. It is difficult to understand why Speidel's work did not receive more attention".

The vacuolated secretory neurons of the caudal part of the spinal cord of the skate (Species of *Raia*) had been noted briefly in 1914 by Dahlgren. He described the inclusions as ". . . the formation of series of vacuoles which coalesced into larger vacuoles that finally condensed and precipitated their contents into a number of heavy, homogeneous granules which were discharged from the cell in a ventral direction and became distributed through and around the tissue of the gray matter . . ." Despite these observations, Dahlgren was not prepared to accept that these cells actually were glandular, and did not even indicate a conviction that they were neurons: ". . . yet their cytological character was such that it could scarcely be believed that they were nerve cells at all . . . ". Dahlgren believed that the secretory cells were related to the animal's electric organ because of their anatomic proximity, but his skepticism about their identity as nerve cells is an inconsistency in his interpretation because he knew that it already was proved that electric organs of certain fishes were innervated by spinal motor neurons.

One student of Dahlgren in 1914 was Carl Caskey Speidel. Speidel's descriptions of these same cells in 1917 were much more detailed than those of Dahlgren. He systematically surveyed many species of fishes, amphibians, and even lobsters and

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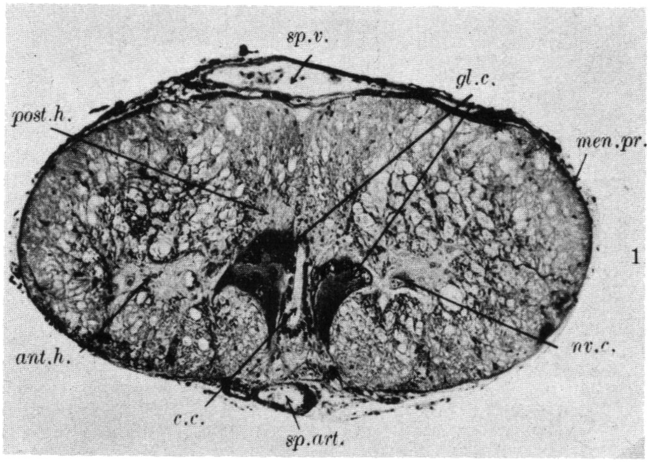

Figure 1 — "Cross-section of spinal cord of *Raia ocellata*. Two of the large gland-cells are present, one on each side of the central canal and close to it. In the anterior horn to the right is a nerve-cell, a comparison with which will bring out the large size of the gland-cells. Note the peripheral distribution of the nucleus of the cell to the right. Photograph X146." (Speidel, 1917)

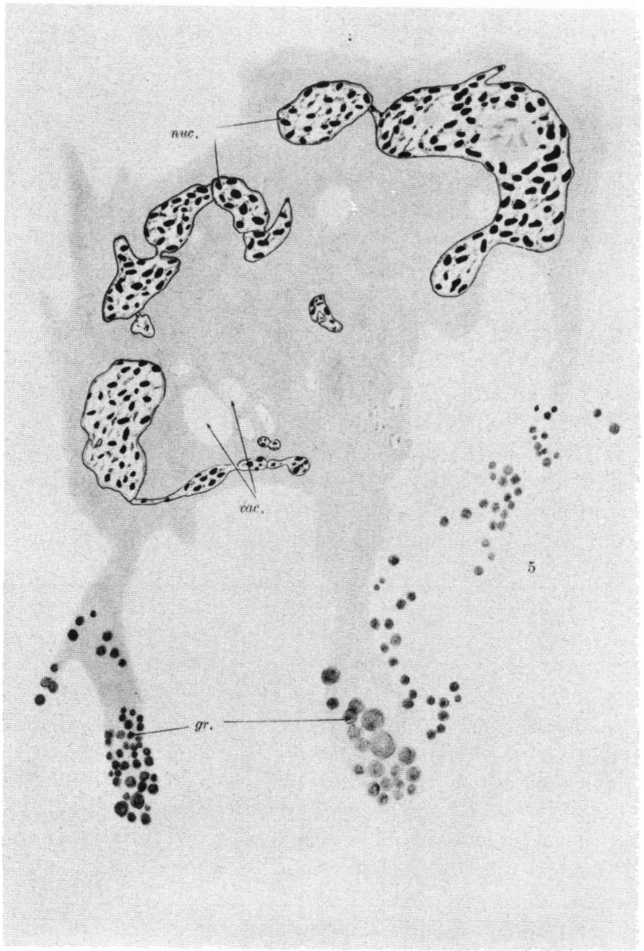

Figure 2 — "A single large cell from spinal cord of *Raia loevis*. Vacuoles and granules are present in the cytoplasm. Some of the discharged granules may be seen near the cell. The nucleus, apparently multiple, is in reality a single one. X1028." (Speidel, 1917)

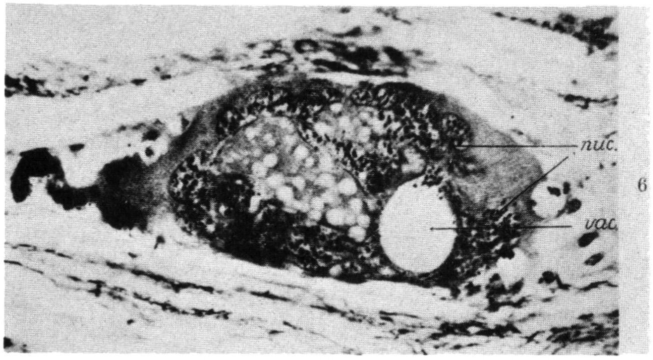

Figure 3 — "A cell from longitudinal section of spinal cord of *Raia punctata*, showing many small vacuoles in the central masses of the cytoplasm and one large one. Photograph X734." (Speidel, 1917)

crabs for comparison. He described the histologic appearance of the secretory cells using several different stains, including supravital dyes, trying to ascertain the identity of the material in vacuoles. He extended his observations to experimental conditions, noting changes in morphology and orientation of the neurosecretory cells and the fate of their cytoplasmic vacuoles and secretory granules after electrical stimulation of the exposed spinal cord in living animals and after injection of various drugs such as atropine and pilocarpine into the central spinal canal of the freshly amputated tail. These experiments may seem crude by today's standards, but were an imaginative use of the scientific method to prove an hypothesis in Speidel's time.

The following is extracted from Speidel's now classic thesis of 1917, in which he shows precocious insight into the functional significance of his morphologic observations. Figures 1-5 are reproductions of his illustrations, including the original legends. Of additional historical interest is the high quality of his photomicrographs in an age when camera lucida drawings were the accepted method of illustrating histologic findings.

> "GLAND-CELL HYPOTHESIS. Assuming, then, that these large, peculiar cells of the skate are transformed nerve-tissue, the question at once arises as to what their present function is. The cytoplasm of the cells seems to undergo vacuolation and partial liquefaction, with the production of some precipitate and granular material. This, of course, suggests glandular activity. The granules may represent some specific secretion that is being produced by the cells. The granules, after they have been manufactured and discharged, are not at once absorbed by the blood, but seem to persist in the tissues for some time. Most of them make their way down past the central canal toward the ventral side of the spinal cord. It is not known what causes most of the granules to gather in this region. Perhaps gravity pulls them in this ventral direction, or the movements of the neurolymph. Just ventral to the central canal a network of blood-vessels may often be seen. Here most of the granules are probably absorbed.
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> The whole process seems to indicate that these large cells are gland-cells of internal secretion. That such glands should be located in the anterior horn of the spinal cord seems to be remarkable; yet we know that other parts of the central nervous system have become

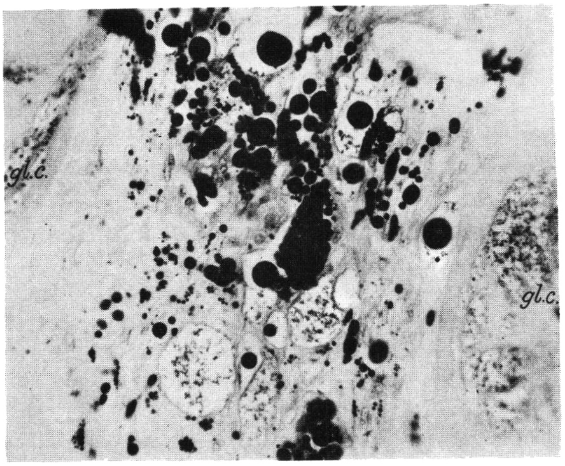

Figure 4 — "Longitudinal section of spinal cord of *Raia loevis*, showing vacuoles, precipitate, and granules outside the gland-cells in various stages of development. There are present in this section vacuoles precipitate, vacuoles with small and large granules, and granules of various sizes that have been discharged by vacuoles and are now in the tissues of the cord. The end of a large gland-cell may be seen at each side of the figure. A much larger amount of granular material is normally present in *Raia loevis* than in *Raia ocellata*. Photograph X438." (Speidel, 1917)

Figure 5 — "Cross-section of spinal cord of *Raia ocellata*, after stimulation with electricity for 2 minutes. A part of one of the large gland-cells has in some way entered the central canal and fills it up almost entirely in this section. Note also how the cell-body and even the chromatin granules of the nucleus have been drawn out ventrally, as if the cell had recently moved in this direction. Photograph X530." (Speidel, 1917)

profoundly modified. At various points in the brains of vertebrates are found places where the wall has been invaginated into long branching and anastomosing tubes of simple epithelium showing glandular activity. Blood-vessels have followed these tubes and occupy their centers so that only a single layer of epithelium separates blood and brain fluids. Through this layer of epithelium substances are probably removed from, or passed into, the brain-cavity fluids. These structures are choroid plexuses.

The pituitary body is another well-known example of the way in which nerve-tissue may become modified . . ."

Between 1928 and 1932 the Scharrers made a series of observations of nerve cells containing secretory products within the brains of fishes. Although they were not yet ready to conclude that the purpose of such neurons was primarily hormonal, they clearly were attracted to the hypothesis, as indicated by the following statement by E. Scharrer in 1932:

> "As to the role of such secretory activity of nerve cells in the brain we have no information. In former publications the question was discussed whether there might be in the diencephalon a production of hormones influencing color change in fishes. That assumption was based on the fact that blinded minnows are able to perceive light stimuli by means of the diencephalon (Scharrer, '28) . . . But as to the function of the group of gland cells in the midbrain, which have just been described, there is no observation."

In 1933 and 1934(a,b), E. Scharrer proposed that the cytoplastic granules in neurons of the paraventricular and supraoptic hypothalamic nuclei were secretory hormones transported via the axons to the pituitary. Subsequent investigations in many species of vertebrates supported these conclusions originally based wholly on the Scharrers' interpretation of microscopic morphology (reviewed by Scharrer and Scharrer, 1939, 1945).

Rejection of the concept of neurosecretory cells was expressed as late as 1940 by some respected authorities. Le Gros Clark (1939) continued to emphasize the pathologic or 'pseudo-pathologic' histologic appearance of supraoptic and para-ventricular cells because of the eccentric position of the nuclei and peripheral location of Nissl substance. The resemblance of central chromatolysis resulted from the cytoplasmic storage of secretory 'colloid' material. Finley (1939), stated: "Scharrer

SPEIDEL'S KEY TO ABBREVIATIONS IN LEGENDS OF FIGURES

ant. h.	— anterior horn of gray matter
c.c.	— central canal
gl. c.	— large gland-cell
gr.	— granules secreted by large gland-cell
men. pr.	— meninx primitiva
nuc.	— nucleus
nv. c.	— nerve-cell
post. h.	— posterior horn of gray matter
sp. art.	— ventral spinal artery
sp. v.	— dorsal spinal vein
vac.	— vacuole

believes that the nerve cells of the paraventricular and supraoptic nuclei have an endocrine as well as a neural function because of the character of the cells and their cyclic changes. But it does not seem that nerve cells whose axons terminate in an endocrine organ should themselves have an endocrine function." Other authors insisted that the microscopic appearance of secretory neurons was due to fixation artifact or postmortem change, that they really were glial cells, or that the intracellular secretory granules were a degenerative process in the cells, having no physiologic meaning (Scharrer and Scharrer, 1939). The Scharrers were still in a defensive position in convincing the scientific world of the validity of their hypothesis of neurosecretion by the paraventricular and supraoptic nuclei of the brain of man and other vertebrates.

Neurosecretion subsequently has been demonstrated to be a widespread function of neuroepithelial cells, found universally among all animals possessing a nervous system. Evidence for an endocrine capability is encountered in the simplest of multicellular invertebrates, such as the hydra (Westfall and Kinnamon, 1978), a coelenterate whose nerve celle are not yet even aggregated to form a ganglion. A neurosecretory system is well developed in advanced invertebrates, such as insects and other arthropods, but the story of its discovery was similar to that of neurosecretion in vertebrates. The demonstration by Kopeć (1922) that a hormone secreted by the brain was necessary for metamorphosis in insects was generally ignored until its

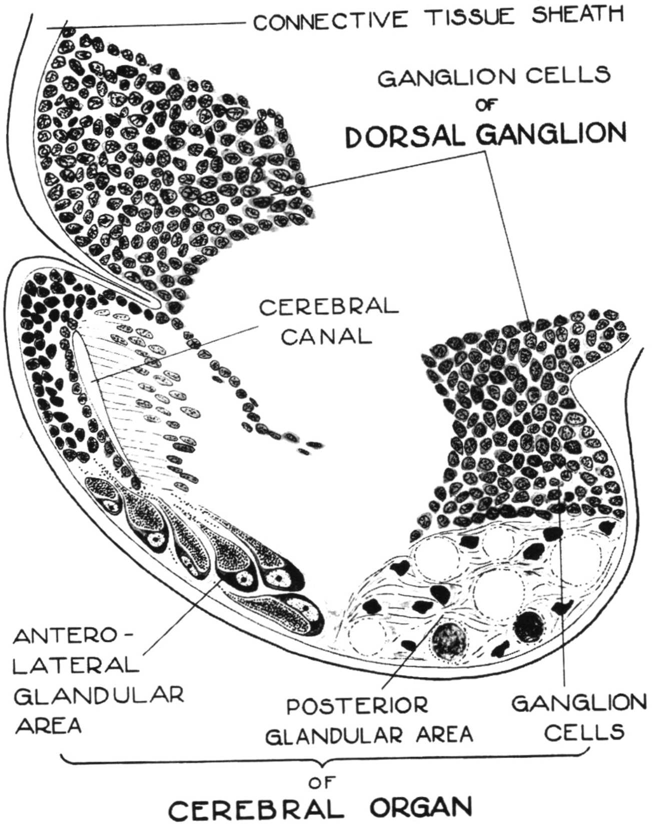

Figure 6 — This illustration of neurosecretory cells in a nemertine worm predated the development of the hypothesis that these obscure animals are little-evolved descendents of ancestral prevertebrates. "Drawing of a horizontal section through the cerebral organ and part of the dorsal ganglion of Cerebratulus lacteus. Note uninterrupted transition of nerve cells from cerebral organ into dorsal ganglion. Bouin, nitrocellulose, 20u, van Gieson X300." (Scharrer, 1941c)

rediscovery and confirmation by Wigglesworth (1940) and Scharrer (1941b). Scharrer (1941b) demonstrated neurosecretory cells in the ganglia of the cockroach and of many other species representing most invertebrate phyla (Scharrer and Scharrer, 1945).

All classes of vertebrates possess neurosecretory activity within the central nervous system, including even the jawless cyclostome fishes (Bentley and Follett, 1962; Sarnat and Netsky, 1981) and the ancient group of cartilagenous sharks and skates, as shown initially by Speidel (1917, 1919, 1922). The early ancestor of all vertebrates probably was a marine nemertine worm; surviving species of this little-evolved phylum have a 'brain' which is essentially a secretory organ (Lechenault, 1963; Willmer, 1975; Sarnat and Netsky, 1981). This discovery of neurosecretion in nemertines was made by Scharrer (1941c; Fig. 6), years before evolutionary theorists pointed out that certain unique anatomic structures of these obscure worms are potentially homologous with a dorsal notochord, a primordial thyroid, and with other vertebrate organs, and postulated an ancestral role in the evolution of vertebrates (Jensen, 1960; Willmer, 1975; Sarnat and Netsky, 1981).

The idea of neurosecrectory cells in primitive animals had occurred to biologists even before Speidel. Tunicates are protochordates, simple animals with many organs resembling those of embryonic vertebrates more than those of invertebrates. Among these is a neural gland, a primordial vertebrate neurohypophysis arising from the tunicate brain. In 1900 Metcalf commented:

> "It is remarkable to find at all, as we do in the tunicates, a gland arising by the transformation of nerve cells. It is still more remarkable to find in some species of tunicates (the Salpidae) the homologous nerve cells not giving rise to gland tissue, but remaining as part of the definitive brain . . ."

Comparative neuranatomy and evolutionary theory have made many contributions to an understanding of the human nervous system. The discovery (and rediscovery) of neurosecretion is among the most important, exemplifying the potential or imaginative interpretation of microscopic anatomy to reveal function.

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