FURTHER COMPARATIVE STUDIES IN OTHER FISHES OF CELLS THAT ARE HOMOLOGOUS TO THE LARGE IRREGULAR GLANDULAR CELLS IN THE SPINAL CORD OF THE SKATES

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TWO PLATES (THIRTEEN FIGURES)

INTRODUCTION

It was pointed out by Dahlgren (1) that there exists in the spinal cord of the skate a series of large remarkable cells of peculiar structure. The complete morphology, location, and distribution of these cells together with some experimental results have been published by the writer in a previous paper (2). This present paper deals especially with the occurrence and appearance in other fishes of cells homologous to these cells of Dahlgren.

In the skate, *Raia*, the cells are present in the anterior horn of gray matter on each side of the central canal, their exact position, however, being rather variable. They are located in the posterior portion of the spinal cord only, being placed in *Raia ocellata* in the posterior half of the tail (i.e., extending from the level of the sixty-fourth vertebra to the tip of the tail, approximately the one hundred and twentieth vertebra).

The cells are of enormous size, a large one that was measured being about 300μ long (antero-posterior measurement), 200μ wide (medio-lateral measurement) and 176μ thick (ventro-dorsal measurement). At first sight of one it is difficult to believe that it is a single cell (fig. 9). It is irregular in outline and contains a huge nucleus of the distributed, branching type extending to all parts of the cell. In sections this often appears to be multiple in character, causing the cell to resemble a fusion of a number of

cells—a syncytium. A study of the embryonic history, however, shows clearly that these cells are not syncytia, but that each is developed from a single cell. The chromatin is scattered throughout the nucleus in the form of large granules. No definite plasmosome is visible. The cytoplasm of a resting cell is homogeneous, but in active stages small and large vacuoles appear. Secretion appears in these and finally the contents of the vacuoles are discharged in the form of granules of various sizes which remain in the tissues of the cord for some time. Often the vacuolar membrane does not break as the secretion material is discharged, but remains intact and holds the enclosed granules together for some time after they have become separated from the secreting cell. A section through the lower part of the spinal cord of the skate in the region of these active cells will be characterized by these deeply staining groups of granules which are distributed chiefly in the ventral portion of the gray matter.

This granular material is protein in character and not easily soluble. It was found that increase in volume of granular material occurred after stimulation of the spinal cord by electricity and pilocarpine, but not after the administration of atropine. The evidence, therefore, both morphological and experimental, indicated that the large cells of Dahlgren in the skate were glandular in nature. The exact function of the secretion, however, was still unknown. It was thought that an extensive comparative study of homologous cells in other fishes might reveal some other forms in which the cells were as unusual as they were in the skate, and that more light might be thrown on the exact nature and function of the cells.

MATERIAL

The fishes were obtained for the most part at Woods Hole, Massachusetts, during the summer months. Thirty genera were studied, comprising most of the common salt-water forms and a few fresh-water ones. These include representatives of the teleosts, elasmobranchs, and ganoids. Examination was also made of the ventral nerve cord of the lobster and horseshoe crab, and of the spinal cord of the lamprey, newt, and mud-puppy. A complete list of the forms examined follows:

List of fishes examined

First group

1. Alewife, *Pomolobus pseudoharengus*
2. Eel, *Anguilla chrysypa*
3. Minnow, *Fundulus heteroclitus*
4. Toadfish, *Opsanus tau*

Second group

5. Butterfish, *Rhombus triacanthus*
6. Catfish, *Amiurus nebulosus*
7. Cunner, *Tautoglabrus adspersus*
8. Dogfish, smooth, *Mustelus canis*
9. Dogfish, spiny, *Squalus acanthias*
10. Garpike, *Lepidosteus osteus*
11. Goosefish, *Lophius piscatorius*
12. Mackerel, *Scomber scombrus*
13. Menhaden, *Brevoortia tyrannus*
14. Puffer, *Sphaeroides maculatus*
15. Sand-dab, *Bothus maculatus*
16. Sandshark, *Carcharias littoralis*
17. Scad, *Decapterus* sp.
18. Sculpin, *Acanthocottus* sp.
19. Scup, *Stenotomus chrysops*
20. Sea-robin, *Prionotus carolinus*
21. Shark-sucker, *Echeneis naucrates*
22. Swordfish, *Xiphias gladius*
23. Sting-ray, *Dasyatis* sp.
24. Tomcod, *Microgadus tomcod*
25. Torpedofish, *Torpedo* sp.
26. Tautog, *Tautoga onitis*
27. Whiting, *Melucius bilinearis*
28. Winter flounder, *Pseudopleuronectes americanus*

Third group

29. Summer flounder, *Paralichthys dentatus*
30. Skate, *Raia*
 - Barndoor skate, *Raia laevis*
 - Italian skate, *Raia punctata*
 - Spiny skate, *Raia radiata*
 - Summer skate, *Raia erinacea*
 - Winter skate, *Raia ocellata*

Other forms examined

- Horseshoe crab, *Limulus polyphemus*
- Lobster, *Homarus americanus*
- Lamprey, *Petromyzon* sp.
- American newt, *Diemyctylus viridescens*
- Mud-puppy, *Necturus* sp.

OBSERVATIONS

For purposes of description the fishes may be conveniently divided into the three groups indicated in the list above. In the first group cells homologous to the Dahlgren cells are lacking. In the second group the cells are present and are of moderate size, though varying somewhat. In the third group the cells are enormous and highly modified.

In regard to the fishes of the first group, the alewife, eel, minnow, and toadfish, I know of no special reason why the cells of Dahlgren should be lacking. I have already suggested that they may be looked upon as having been derived from modified embryonic nerve tissues. A study of their development in skate embryos shows that they are derived from the same cells which give rise to nerve tissues. In these four fishes it would seem that there had never been any modification of the embryonic nerve tissues in the direction of the Dahlgren cells in this region, or so slight a modification that it is not easily noticeable.

The second group varies somewhat with reference to the Dahlgren cells, both in size and appearance. In forms like the mackerel and goosefish the cells bear some resemblance, perhaps, to nerve cells. They are larger than nerve cells, but still relatively small. One cell of about average size in the mackerel measured 60μ in length, 52μ in breadth, and 39μ in thickness. The cell processes are not numerous. The nucleus is usually only slightly lobulated, and may be almost spherical. One or more plasmosomes may be distinguished, the number often depending upon the number of lobes of the nucleus. In each of the cells of figures 1, 2, 3, and 4 taken from the mackerel one plasmosome is present (although in figure 2 it is not shown in the section represented). In the cell shown in figure 5 taken from the butterfish two plasmosomes are present. In one cell from the goosefish, the nucleus of which had three lobes, three plasmosomes were distinctly present, one in each lobe. Other forms, such as the catfish, cunner, garpike, menhaden, butterfish (fig. 5), puffer, sea-robin, scad, scup, shark-sucker, tautog (fig. 6), tomcod, and whiting, show somewhat greater lobulation of the nucleus, plasmosomes sometimes being present, sometimes not. The cell-body is also larger in these forms.

The cytoplasm of the cells in most of the forms was homogeneous. In the cell from the sandshark (fig. 7), however, there may be seen at the periphery a vacuole containing a fine granular precipitate. This is probably a secretion of the cell. Well-defined granular precipitate of secretion contained in definite vacuoles at the periphery of the cell was also seen in the shark-sucker. It has previously been pointed out that in the Dahlgren cells of the skate such vacuoles containing fine precipitate are of common occurrence. These vacuoles often are outside of the cell in the neighboring tissues of the spinal cord. Granular material associated with the cells, but outside of them, has been noticed in the dogfish, flounder, menhaden, and possibly tautog. Another interesting feature in connection with the appearance of the cytoplasm of the Dahlgren cells is that it is often pierced by one or more capillaries (especially in the case of the shark-sucker), so that in cross-section the capillary with its endothelium and enclosed blood corpuscle appears to lie within the cytoplasm of the cell.

In the case of the other fishes of the second group, the sting-ray (fig. 8), the dogfish, and torpedo-fish, the cells are larger, the volume of nuclear material relatively larger in comparison to the amount of cytoplasm and the nucleus more lobulated. The cells in these forms, indeed, almost make a series of transition stages leading up to the third group. In general, it may be said that the Dahlgren cells are larger and their nuclei more highly branched in the elasmobranchs than in the teleosts or ganoids. The one notable exception to this rule is the summer flounder. In none of the cells of the second group were Nissl bodies seen, although in the neighboring muscle motor nerve-cells they could be clearly made out.

The cells from the fishes of the second group, as arranged in figures 1 to 8, make an interesting comparison with the cells in embryo skates in various stages of development. The two series are very similar. In other words in the development of the complex type found in the adult skate simpler stages are passed through. These simpler stages may be found in the adults of the fishes which have been placed in the second group.

The third group includes only the flounder and the skate. The cells in these two forms are the most highly modified in size and differentiation yet found. The skate cells have already been described and a typical cell is shown in figure 9. This cell as seen in a cross-section of the spinal cord is elongated in two directions (ventro-medially and laterally), and at each of the ends is seen a number of granules, the secretion material of the cell. If the serial sections passing through this cell be followed, first in an anterior direction and then in a posterior direction, it will be found that the number of granules and volume of granular material both increase. The volume of granular material is greater at the anterior and posterior ends of the cell than at the middle region. The amount shown in the figure, therefore, represents only a small percentage of the total amount associated with the cell (much less than a tenth). The skate differs from all other forms that have been examined in the presence of large amounts of this granular material associated with the cells.

Besides the skate, the flounder is perhaps the most interesting form. In this animal the spinal cord terminates posteriorly in an enlargement, the terminal enlargement, which extends anteriorly for a short distance, forming a sort of cap over the dorsal part of the spinal cord. In this region, and for about an inch anteriorly, the spinal cord instead of appearing macroscopically as glistening white in color appears as a rather transparent gray. It is here that the Dahlgren cells are most numerous and of greatest size, the gray color of the spinal cord being due to their presence together with the lack of many myelinated nerve fibers.

The cells are very large, about as large as in most species of skates. In the terminal portion of the spinal cord they are present in great numbers closely grouped together so that in both longitudinal and transverse sections they easily form the most conspicuous part of the section (figs. 12 and 13). A characteristic collection of the Dahlgren cells, as seen in longitudinal section, is shown in figure 12, a low-power microphotograph. It is interesting to note that in many of the cells in this section the nuclear material is located not at the center of the cell, but at the periphery. For this reason, the cell looks somewhat like a

syncytium, bearing a superficial resemblance at least to the giant-cells which occur in certain diseases (as in miliary tuberculosis) that have been formed by a fusion of many endothelial cells. Figure 13 is a low-power microphotograph showing the general appearance of a cross-section of flounder spinal cord near the posterior end. Six or more of the large Dahlgren cells may be seen. The terminal enlargement appears here as a large cap on the dorsal side of the spinal cord proper. The central canal is located near the ventral side. The horns of gray matter are not well defined this far posterior in the spinal cord. In the terminal enlargement may also be seen two or three small Dahlgren cells, or pieces of cells. The terminal enlargement is really a continuation of the spinal cord (although in this cross-section it appears to be separated from it), but it contains practically no gray matter. These cells or pieces of cells, therefore, which are included in it seem rather out of place.

As may be seen from the figures, the cells are enormous. In actual size a fairly large flounder cell was found to measure 240μ ventro-dorsally, 160μ antero-posteriorly and 110μ medio-laterally. The average-sized cell is somewhat smaller than this. The details of structure of a typical cell may be seen in the cell shown in figure 10. The similarities in appearance between this cell of the flounder and the one of the skate are evident. The cells of the flounder, however, seldom have either vacuoles or granules associated with them, at least in appreciable amounts. In some cases I have found vacuoles near the edge of a cell containing a fine granular, or threadlike, or rod-like precipitate or secretion which apparently has been elaborated by the cell. A typical one of these is shown in figure 11. Similar thread-like or fine granular secretion in vacuoles either inside or outside the cell is also often found in the case of the skate cells. In the flounder, however, in spite of the huge size of the cells, I have never seen any of the large-sized granules similar to those which form such a characteristic part of the secretion of the cells of the skate.

The observations of the cells of the flounder, then, indicate that in spite of their enormous size they do not elaborate any appreciable amount of granular material, as is the case in the

skate. It was thought that it might be of interest in this connection to see whether or not the administration of pilocarpine or stimulation with electricity would have any effect in stimulating the cells. Accordingly, a solution of pilocarpine hydrochloride (0.1 per cent) was injected into each of four flounders by way of the peritoneal cavity and by way of the vertebral canal. This was allowed to act for from ten to fifteen minutes. Examination of these animals showed that no marked increase in granular secretion or in vacuole formation had taken place. Electrical stimulation of the spinal cord of each of four animals gave a similar result. If the Dahlgren cells of the flounder are at all glandular in nature they are certainly very sluggish, far more so than the homologous cells in the skate.

It has already been pointed out in a previous paper (2) that the conclusion that the cells of Dahlgren in the skate were of glandular nature applied only to the skate. At that time only a few kinds of fishes had been examined and the appearance of homologous cells in these forms did not warrant the conclusion that they were glandular in these other forms. It seems to me that the structure of the cells from a comparative standpoint suggests a series of transition stages from primitive nerve tissue to glandular tissue. In the skate the cells are markedly glandular. In the cells of the barndoor skate, *Raia laevis*, a very large amount of granular secretion is elaborated; in the winter skate, *Raia ocellata*, and the Italian species, *Raia punctata*, there is not quite so much produced; while in the spiny skate, *Raia radiata*, and the summer skate, *Raia erinacea*, still less secretion material is to be found. In the flounder, shark-sucker, dogfish, sandshark, and menhaden the cells seem to have only a slight glandular activity. In the other forms no secretion material at all was seen.

Examination was also made of the spinal cord of the lamprey, *Petromyzon*, the American newt, *Diemyctylus*, the mud-puppy, *Necturus*, and of the ventral nerve cord of the lobster, *Homarus*, and the horseshoe crab, *Limulus*. In none of these were cells found that were homologous to the Dahlgren cells of the fishes.

SUMMARY

1. Cells homologous to the large irregular glandular cells of Dahlgren in the skate are present in the large majority of fishes. Of thirty genera examined the Dahlgren cells were found in twenty-six. They have not been found in any other group of animals. They are always located (when present) in the posterior portion of the spinal cord.

2. Morphologically, many gradations are found. In some of the fishes the cells, though somewhat larger, still bear some resemblance to nerve cells, the nucleus being almost spherical or slightly lobulated and containing one plasmosome, as in the mackerel. In other fishes, as in the goosefish and butterfish, the nucleus is more lobulated with two or three plasmosomes often present. In others, as in tautog, sandshark, sting-ray, and dogfish, the cells are larger and the nucleus still more lobulated. In some a few vacuoles containing precipitate or secretion are sometimes found associated with the cells. The extreme size is reached in the cells of the flounder and skate. In addition to the enormous size of cell and nucleus, a large amount of granular secretion is also usually present in the skate with occasional vacuolation of the cytoplasm. In the flounder a very few vacuoles containing granular or thread-like precipitate may be found occasionally associated with the cells.

3. The cells have been found in elasmobranchs, teleosts, and ganoids. They are relatively larger in elasmobranchs than in teleosts (with the exception of the summer flounder) and ganoids.

4. Vacuoles and secretion material are not present to any great extent in any of the fishes other than the skate. Small amounts of fine granular material have been found associated with the cells of the flounder, sandshark, shark-sucker, scup, menhaden, and dogfish. Secretion granules of large size, however, are typical of the skate cells alone. In none of the other forms, therefore, is there evidence of marked glandular activity.

5. In size of cell, lobulation of nucleus and secretory activity the Dahlgren cells of the skate represent the most extreme type

found in any of the fishes. The Dahlgren cells of most of the fishes examined resemble very much the early developmental stages of the Dahlgren cells in skate embryos and young skates.

LITERATURE CITED

- 1 DAHLGREN, U. 1914 On the electric motor nerve centers in the skates (Rajidae) *Science*, vol. 40, no. 1041, pp. 862-863.
- 2 SPEIDEL, C. C. 1919 Gland-cells of internal secretion in the spinal cord of the skates. Pub. 281, Carnegie Inst. of Wash.

PLATES

ABBREVIATIONS

c.c., central canal

gr., granular secretion

nuc., nucleus

pl., plasmosome

sp. c., spinal cord proper

ter. en., terminal enlargement

vac., vacuole

D. c., Dahlgren cell

PLATE 1

EXPLANATION OF FIGURES

All of the figures of plate 1 were drawn with camera lucida to the same scale of magnification ($\times 390$).

1 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. The nucleus is almost spherical and a plasmosome is present. The diameter of this cell is about twice that of an ordinary muscle motor nerve-cell in the same animal. This cell and the cells of figures 2, 3, and 4 were all present in the same cross-section.

2 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. The nucleus is slightly elongated and lobulated. A plasmosome is present, but is not shown in the section represented.

3 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. The nucleus has three lobes.

4 Dahlgren cell from the spinal cord of the mackerel, *Scomber scombrus*. This section shows the nuclear material enclosing a central core of cytoplasm.

5 Dahlgren cell from the spinal cord of the butterflyfish, *Rhombus triacanthus*. The nucleus is more highly branched than in the mackerel, and in this section it appears to be multiple. Two plasmosomes may be seen. The size of the cell is also somewhat larger than in the mackerel.

6 Dahlgren cell from the spinal cord of the tautog, *Tautoga onitis*. Note the two greatly elongated nuclear masses. These are, however, in reality parts of a single nucleus.

7 Dahlgren cell from the spinal cord of the sandshark, *Carcharias littoralis*. Note the increase in lobulation of the nucleus. Note also the vacuole at the edge of the cell containing some fine granular material. Apparently the vacuole has just burst and discharged most of its contents. Plasmosomes are not distinguishable in this cell nor in any of the cells of the succeeding figures.

8 Dahlgren cell from the spinal cord of the sting-ray, *Dasyatis*. This shows further increase in size of the cell and size and lobulation of the nucleus.

9 Dahlgren cell from the spinal cord of the skate, *Raia ocellata*. Both the cell body and nucleus are of enormous size. Note especially the characteristic groups of granules of various sizes at the ends of the cell. Each group of granules represents the original content of a single vacuole. Only a small percentage of the granular secretion is shown in the section represented, most of it being present in the sections near the ends of the cell (anterior and posterior ends).

10 Dahlgren cell from the spinal cord of the summer flounder, *Paralichthys dentatus*. The size of the cell is about the same as that of the skate, *Raia ocellata*. No large granules, however, have ever been found associated with these cells of the flounder, such as are present in the skate.

11 Typical vacuole sometimes found associated with the cells of the summer flounder, *Paralichthys dentatus*. This vacuole contains a fine granular or thread-like precipitate which is apparently elaborated by the cell. It is similar in appearance to the vacuoles containing fine secretion material which are often found associated with the Dahlgren cells of the skate (both inside the cell and outside).

PLATE 2

EXPLANATION OF FIGURES

12 Longitudinal section of the posterior terminal portion of the spinal cord of the summer flounder, *Paralichthys dentatus*. Note the large collection and the great size of the Dahlgren cells in this region. In many of the cells the nuclear material may be seen peripherally arranged about a central core of cytoplasm. The 'terminal enlargement' of the spinal cord appears here as a cap over the dorsal side of the spinal cord proper. Low-power microphotograph. $\times 60$.

13 Cross-section of the spinal cord of the summer flounder, *Paralichthys dentatus*. The 'terminal enlargement' appears here as a cap on the dorsal side. Five or more of the large Dahlgren cells may be seen in the spinal cord proper. A couple of small Dahlgren cells or pieces of cells may be seen in the 'terminal enlargement.' The Dahlgren cells occupy about one-quarter of the cross-section area of the spinal cord. Low-power microphotograph. $\times 70$.