A MORPHOLOGICAL AND FUNCTIONAL STUDY ON A CLUSTER OF IDENTIFIABLE NEUROSECRETORY CELLS IN THE ABDOMINAL GANGLION OF *APLYSIA CALIFORNICA*

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Because of the large size of its cell bodies, the abdominal (visceral) ganglion of the sea hare, *Aplysia*, has proven a useful preparation for biophysical and pharmacological studies of central neurons (1, 2, 7, 18). However, the systematic study of the organizational properties of this ganglion has been seriously limited by an inability to recognize more than a very few specific cells from one preparation to another (3, 9, 17, 19). In order to overcome this difficulty we have identified twenty-nine of the largest cells in the ganglion. The criteria for identification include: location of the cell in the ganglion, peripheral distribution of its efferent axon, its synaptic input, spontaneous activity, synaptic connections with other identifiable cells, and its appearance under the light and electron microscopes. The usual position of each of the 29 identified cells is indicated in Fig. 1, which is an idealized drawing of the dorsal and ventral surfaces of the ganglion. The availability of these identifiable cells, which can now be studied with both physiological and morphological methods, makes it possible to correlate cellular structure and function in this ganglion. The present report describes a combined electrophysiological and morphological study on a cluster of distinctive identified cells. A more detailed report on all the identifiable cells is in progress (8).

Under the dissecting microscope all neurons on the left side of the ganglion, identified as well as unidentified, appear translucent and orange-colored. On the right side, in addition to the colored neurons, there are a number of cells which are opaque and white. The white cells are stippled in Fig. 1 and are designated R3 to R15. Eleven of these cells, R3 to R13, form a cluster in the rostral part of the ganglion. The number of clearly visible white cells in this cluster may vary, in different ganglia, from six to eleven. Two solitary white cells, R14 and R15, are found in the caudal part of the ganglia. Because they differ in certain respects from the white cells in the rostral cluster, the two caudal white cells will be described in more detail in the later paper (8).

In *Aplysia californica*, the white cells contain droplets that are stained faintly by chromalum hematoxylin or paraldehyde fuchsin, two basic stains for the demonstration of neurosecretory material in the light microscope. In 1935 and 1937, Berta Scharrer reported that some cells in the abdominal ganglion of either *Aplysia limacina* or *Aplysia depilans* contained many droplets that were presumed to indicate neurosecretion (13, 14). It is likely that the white cells in *A. californica* are homologous to the neurosecretory cells described by Scharrer in the European species of *Aplysia*.

In osmium tetroxide-fixed and plastic embedded material, the white cells can be located by their large size and distinctive position. It is possible to further characterize them by the presence of cytoplasmic granules. The granules are round, vary in diameter from 1000 to 4000 A, and possess large osmiophilic cores (Fig. 2). Thus, they resemble the "elementary neurosecretory granules" seen in unidentified cells in this ganglion by Rosenbluth (12) and Simpson et al. (16), or in the cells of established neuroendocrine systems such as the supraoptico-hypophysial system in vertebrates (11). It is presumed that these granules, either

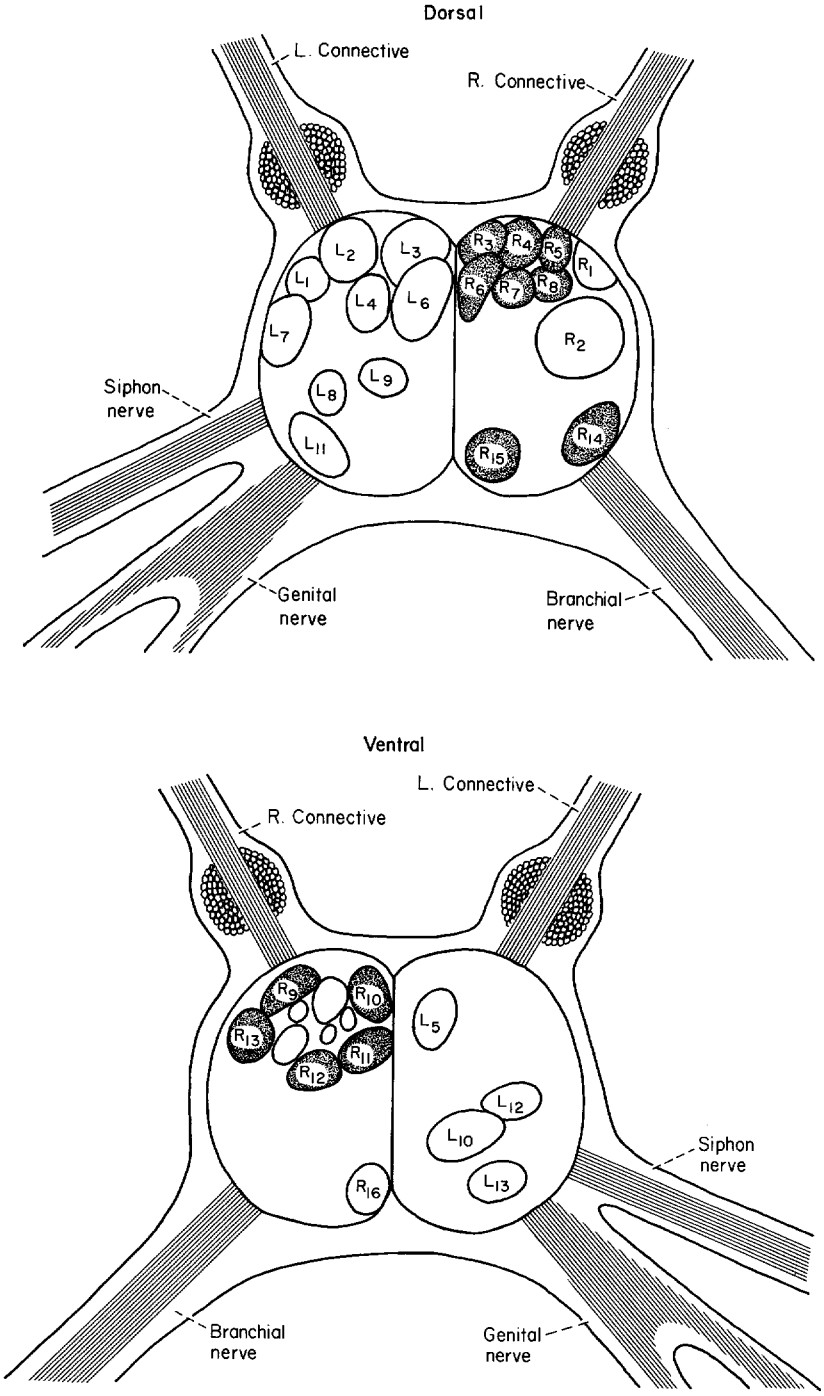

FIGURE 1 Idealized drawings of the dorsal and ventral surfaces of the isolated abdominal ganglion of *Aplysia californica* indicating position of the identified cells. The white cells are stippled. Cells R3 to R13 form the rostral white cell cluster; R14 and R15 are the two caudal white cells.

singly or in clumps, stain and are then identified as "neurosecretory" droplets under the light microscope.

In order to provide additional evidence for the neurosecretory nature of these cells, the destination of their processes was followed. Each rostral white cell was found to send an axon into the branchial nerve, and many fingerlike processes into the sheath which surrounds the ganglion (Fig. 2, inset). Both the processes and the axons are filled with the same type of granules which characterize the cell body. Serial sections for the light microscope revealed that the processes end within the sheath and do not travel to the periphery or reenter the nervous system. Thin sections taken at points just beyond the termination of these processes, as demonstrated by light microscopy, show that the granule-filled processes end in the sheath and do not continue as axons too small to be seen with the light microscope. Extensive search by both light and electron microscopy did not reveal any contacts between the granule-filled processes and muscle or other effector cells.

It was further possible to demonstrate that the sheath and the cell processes which terminate within it have the morphological characteristics of a neurohaemal organ. The outer surface of the sheath lacks a continuous cell layer (6), and only the extracellular components of the sheath separate the granule-filled processes of the white cells from the blood within the haemocele in which the abdominal ganglion lies. Furthermore, it was possible to demonstrate, by serial sections of ganglia after injection of opaque materials into the aorta, that arterial blood is emptied directly into the interstices of the sheath, and that there it comes into contact with the granule-filled processes (6). The presence of granule-filled neuronal processes that end without establishing contact with other axons or with effector cells in a vascularized sheath establishes this as a "neurohaemal organ" (4). These findings all support the notion that the rostral white cells are neurosecretory.

The availability of a clearly identifiable cluster of large cells which are neurosecretory by several morphological criteria made it possible to investigate their neurophysiological properties using intracellular recordings. This is of especial interest, in view of the paucity of detailed data on the electrophysiological properties of neurosecretory cells in other invertebrates. Frequently, two white cells or one white cell and a neighboring nonwhite cell were impaled simultaneously in order to obtain comparative data on neurosecretory and nonsecretory cells.

The electrophysiological properties of the white cells in the rostral cluster were quite uniform. The cells tended to fire spontaneously at a slow and highly regular rate of about 0.5 to 1 impulses per second. The regularity in rate was due to an absence of spontaneous synaptic potentials which modulate the discharge pattern of most actively firing cells in the ganglion. The action potential had a duration of about 20 to 25 msec, which was twice that of most neighboring cells. The spike was usually followed by a relatively small, hyperpolarizing afterpotential which differed from the larger after-hyperpolarization typical of neighboring nonneurosecretory cells and of most spontaneously firing, identifiable neurons in the ganglion. All of these white cells, without exception, could be activated antidromically by stimulating the branchial nerve, confirming the morphological evidence that a neurosecretory tract emerges in that nerve (Fig. 3).

In addition to lacking spontaneous synaptic input from interneurons, the white cells also gave relatively weak synaptic responses to stimulation of the major axon pathways into the ganglion. Single shocks delivered to the two connectives or to the genital or siphon nerves usually produced insignificant or slight effects on the spontaneous firing rate. Single stimuli to the branchial nerve did produce slowing, but this was probably attributable to the hyperpolarizing afterpotential following the antidromic spike. This slowing could be simulated by interjecting a directly initiated spike. Trains of stimuli (6 to 10/sec, for 1 sec) did produce changes in firing rate of most white cells, consisting of a slight increment followed by slowing (Fig. 3). But even the responses to stimulus trains were minor when contrasted with those which occurred in all identified pigmented cells and in the many unidentified cells, including those which form the outer margin of the white cell cluster (Fig. 3 and reference 8).

A weak response to nerve stimulation was also seen in some small cells which lie with the white cell cluster on the ventral side (unlabeled cells in Fig. 1). It is possible that these are nonneurosecretory cells that have some of the same physiological properties as the white cells, but it is also possible that the cells in question are neurosecretory, by the morphological criteria outlined above, and do

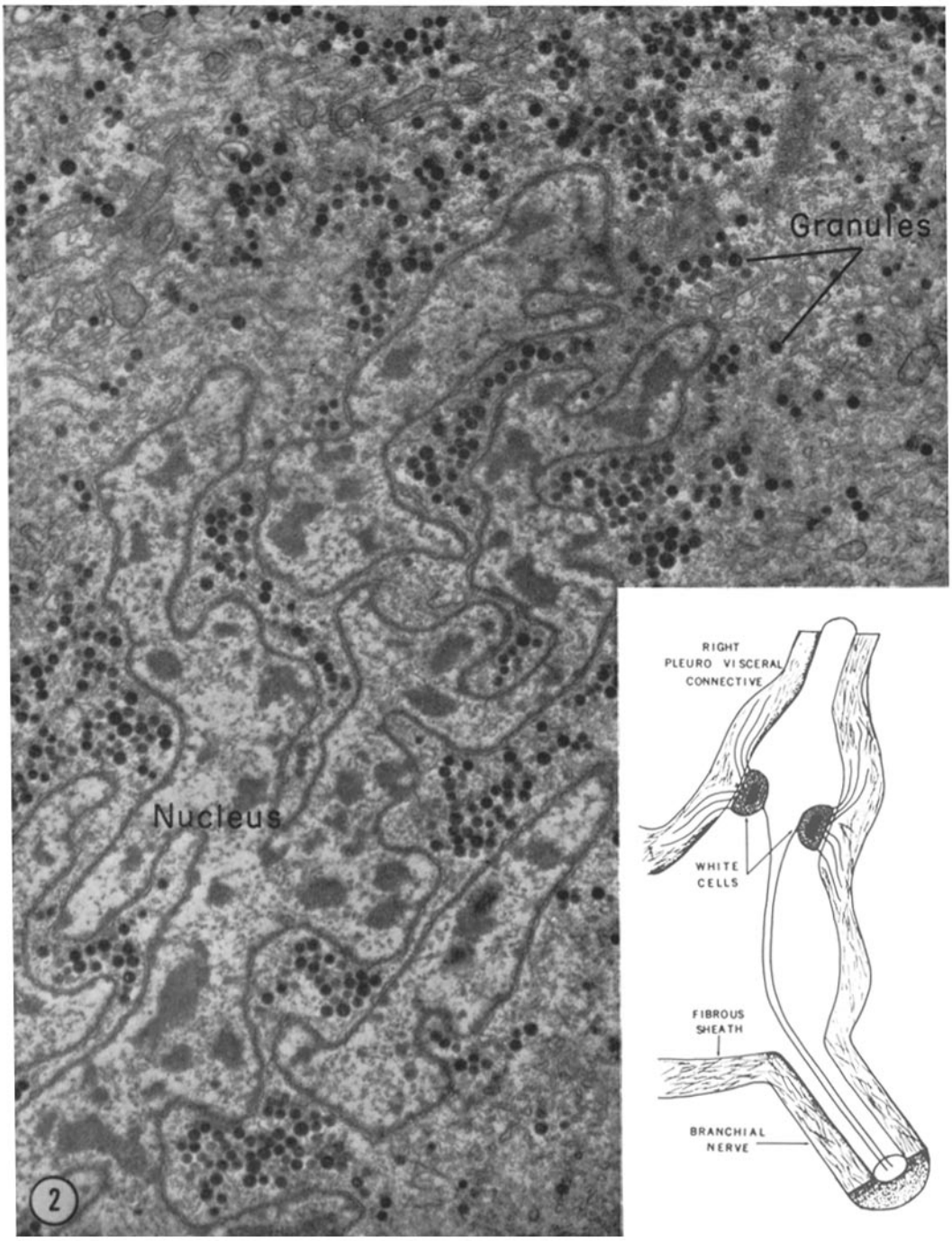

FIGURE 2 An electron micrograph of the nucleus and perinuclear cytoplasm of a white cell. Note the electron-opaque neurosecretory granules. The insert is a drawing of two typical white cells, showing the multiple processes ending within the sheath, and the single axon that enters the branchial nerve. The fine processes within the sheath, and the axon in the branchial nerve, are also filled with the electron-opaque granules.

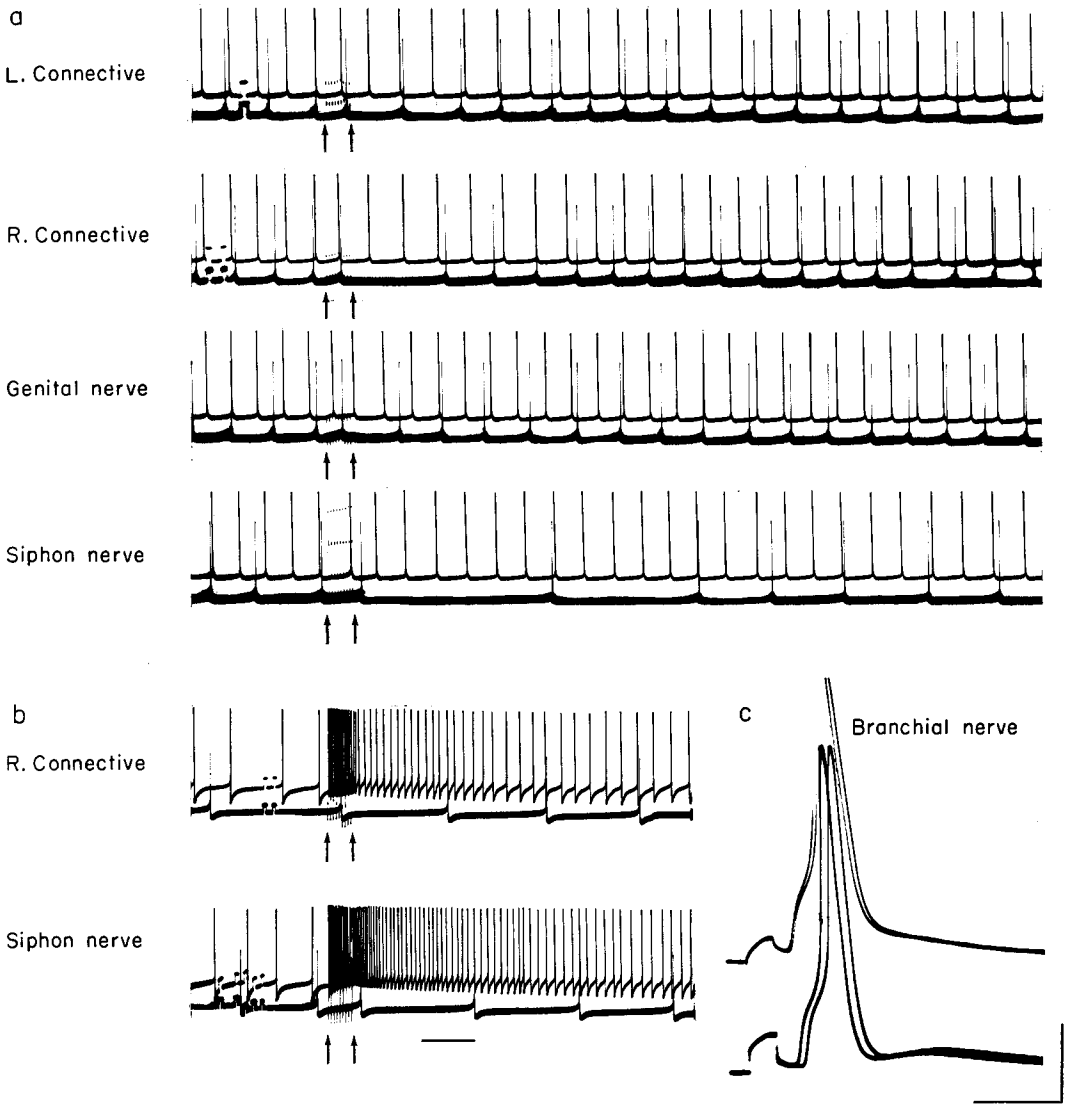

FIGURE 3 Responses of white and pigmented cells to stimulus trains (8/sec for 1 sec) applied to afferent nerves and connectives. The duration of the train is indicated by the arrows. Fig. 3 *a* is a simultaneous recording from two white cells in the rostral cluster. fig. 3 *b* is a simultaneous record from a white cell in the rostral cluster on the dorsal surface (lower trace) and from a neighboring pigmented cell (upper trace). Note that the stimulus trains produce only weak responses in the white cells (Fig. 3 *a* and lower trace of Fig. 3 *b*) and brisk responses in the pigmented cell (upper trace in 3 *a*). Responses of the white cell ranged from practically nothing (upper trace in *a* and lower trace in *b*) to a slight or moderate slowing (lower trace in *a*). Fig. 3 *c* is a simultaneous recording from two white cells showing the antidromic action potentials which follow stimulation of the branchial nerve. The voltage calibration at the beginning of the sweep in *a* and *b* is 10 mv. The time calibration in *b* applies to both *a* and *b* and is 2.5 sec. The calibrations for *c* are 20 mv and 40 msec.

not appear white because they are small and/or deep to other cells and/or do not contain as high a proportion of elementary neurosecretory granules. To resolve this question, it will be necessary to mark these cells with recording microelectrodes and then examine them in the electron microscope.

One of the most interesting features of this cell cluster is that the cells are an opaque white, in contrast to the vast majority of neurons which are transparent and pigmented. The white appearance is particularly evident when the ganglion is illuminated by reflected light. A white or bluish white appearance of neurosecretory cells and neurosecretory organs is well known in invertebrates (4, 5, 15, 20). Since the perikarya of the rostral white cells in *Aplysia* differ from the perikarya of neighboring cells mainly by their content of innumerable elementary neurosecretory granules, it is probable that it is the granules which cause the cells to appear opaque and white. A similar conclusion was previously reached by Maynard (10) in a study of neurosecretory cells in crustacea. Maynard (10) proposed that the distinctive appearance of the neurosecretory cells was due to the presence of small particles, presumably the elementary neurosecretory granules, which refracted light. It would still be desirable, however, to corroborate this conclusion by showing that the white cells become transparent when their content of granules is depleted.

In summary, a group of identifiable neurons in the abdominal ganglion of *Aplysia californica* is grossly recognizable by the white color of its cells. These cells are neurosecretory by several morphological criteria. They differ in their electrophysiological properties from the surrounding pigmented cells. Of particular interest is the fact that white cells have a relatively weak response to neural input, which suggests that their activity may be regulated by changes in the ionic or humoral environment of the ganglion. The large size and easy recognition of these cells makes this cluster advantageous for further experiments designed to reveal the function of these cells.

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REFERENCES

1. ARVANITAKI, A., and CARDOT, H., *Bull. Histol. Appl. Physiol. et Pathol. et Tech. microscop.*, 1941, **18**, 133.
2. ARVANITAKI, A., and CHALAZONITIS, A., *J. Physiol.*, 1958, **50**, 122.
3. ARVANITAKI, A., and TCHOU-SI-HO, *Bull. Histol. Tech. micr.*, 1942, **19**, 244.
4. BULLOCK, T., and HORRIDGE, G. A. (editors), Structure and Function in the Nervous System of Invertebrates, San Francisco, W. H. Freeman & Co., Publishers, 1965. (See especially Chapter 6, Neurosecretion, by H. A. Bern and I. R. Hagadorn.)
5. CARLISLE, D. B., and KNOWLES, S. F., Endocrine Control in Crustaceans, Cambridge, Cambridge University Press, 1959.
6. COGGESHALL, R. E., in preparation.
7. FRANK, K., and TAUC, L., *in* Cellular Functions of Membrane Transport, (J. F. Hoffman, editor), New Jersey, Prentice-Hall, Inc., 1964, 113–135.
8. FRAZIER, W. T., KANDEL, E. R., KUPFERMANN, I., WAZIRI, R., and COGGESHALL, R., in preparation.
9. KANDEL, E. R., and TAUC, L., *J. Physiol.*, 1965, **181**, 1.
10. MAYNARD, D. M., *Gen. and Comp. Endocrinol.*, 1961, **1**, 237.
11. PALAY, S. L., *in* Ultrastructure and Cellular Chemistry of Neural Tissue, (H. Waelsch, editor), New York, Hoeber-Harper, 1957, 31–44.
12. ROSENBLUTH, J., *Z. Zellforsch. u. Mikr. Anat.*, 1963, **60**, 213.
13. SCHARRER, B., *Pubbl. Staz. Zool. Napoli*, 1935, **15**, 132.
14. SCHARRER, B., *Naturwissenschaften*, 1937, **25**, 131.
15. SCHARRER, E., and SCHARRER, B., *in* Recent Progress in Hormone Research, (G. Pincus, editor), New York, Academic Press Inc., 1964, **10**, 183.
16. SIMPSON, L., BERN, H. A., and NISHIOKA, R. S., *J. Comp. Neurol.*, 1963, **121**, 237.
17. STRUMWASSER, F., *in* Circadian Clocks, (J. Aschoff, editor), Amsterdam, North Holland Publishing Co., 1965, 442–462.
18. TAUC, L. J., *Gen. Physiol.*, 1962, **45**, 107.
19. TAUC, L., and GERSCHENFELD, A. M., *Nature*, 1961, **192**, 366.
20. WILLIAMS, G. M., *Harvey Lectures*, 1962, **74**, 126.