AN ELECTRON MICROSCOPIC CHARACTERIZATION OF CLASSES OF SYNAPTIC VESICLES BY MEANS OF CONTROLLED ALDEHYDE FIXATION

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ABSTRACT

Examination of variables of aldehyde fixation that may affect the shape of agranular synaptic vesicles has revealed that even brief storage of aldehyde-perfused nervous tissue pieces in cacodylate buffer, prior to hardening in osmium tetroxide, has an unusually severe flattening effect on agranular vesicles of a particular type. These are the vesicles of peripheral cholinergic axon endings, and of certain central synaptic bulbs. Types of synaptic bulbs can now be further defined on the basis of shape of agranular synaptic vesicles under controlled conditions of aldehyde fixation. Previously described "S" bulbs in the spinal cord contain uniformly spheroid vesicles, which are wholly resistant to flattening. Previously described "F" bulbs contain somewhat smaller agranular vesicles that are flattened after aldehyde fixation, even when this is followed by prompt hardening in osmium tetroxide solution. A third type, previously characterized as having irregularly round agranular vesicles after the above treatment, contains only severely flattened vesicles when the osmium tetroxide hardening is preceded by even a brief wash with sodium cacodylate buffer containing sucrose. Moreover, the "third" type is characteristic of all cholinergic peripheral axon endings examined, as well as the large axosomatic ("L") synaptic bulbs of the spinal cord.

INTRODUCTION

Two new major focal points of interest were created by the electron microscope in relation to the quest for understanding of synaptic function in the nervous system. The first is the structure of the synaptic interface and its role in chemical and electrotonic transmission. The second is the nature of synaptic vesicles, those organelles unknown before electron microscopy, but now clearly one of the most characteristic, if not *the* most characteristic neuronal structure. The synaptic microvesicles, which are mostly about 300–500 A in size, and which seem to be universally present in synaptic bulbs, are moreover variable in size, in shape, and in electron opacity of contents. Correlation of these variables with specific functional characteristics of synaptic bulbs, or with different functional states, has become a major challenge of neurohistology.

Since the discovery of synaptic vesicles, the possibility that they might be associated with synaptic transmitters has been foremost. This concept has been supported by histochemical evidence for localization of norepinephrine in axon endings containing small granular vesicles (500 A), in postganglionic sympathetic nerves (1). Less direct evidence has been adduced for a similar storage role for agranular vesicles. Nevertheless, the plausibility of a role of agranular vesicles in

synaptic transmission is high, because of their universal occurrence at axon terminals, their origin at the time of embryologic onset of function (2), and their association with synaptic transmitters such as acetylcholine and noradrenalin in relatively pure synaptosome fractions of central nervous tissue (3). It must be emphasized that agranular microvesicles are prevalent not only in synaptic telodendria, but also in neuromuscular and neuroglandular telodendria, as well as in neurohypophysial axon terminals.

Since synaptic microvesicles are variable in size, shape, and electron opacity of contents, one must of course ask whether agranular vesicles of different synaptic types are regularly associated with transmitters of different types. The variation in synaptic types in the central nervous system, however, is matched by a paucity of data concerning localization of specific transmitters. In the periphery, however, a large number of cholinergic synapses are known, as well as neuromuscular and neuroglandular endings of cholinergic nature. These contain synaptic vesicles of predominantly agranular character.

The first evidence of differentiation of agranular synaptic vesicles came from observations of flattened vesicles by several workers employing formaldehyde fixation (4–8). The significance of this phenomenon was unsuspected, but Lund and Westrum, and Walberg soon reported that flattening of agranular synaptic vesicles was a result of fixation with formaldehyde or glutaraldehyde (9, 10). Synaptic bulbs containing predominantly spheroid or predominantly flattened vesicles were then shown to be regularly distributed in such a manner as to suggest that flattened vesicles might be characteristic of at least some synaptic bulbs of inhibitory function (2, 11, 12). In the next few years numerous reports described the occurrence of specific synaptic bulbs characterized by synaptic vesicles either predominantly spheroid or predominantly flattened (13–18). Average size differences of vesicles in specific synaptic bulbs were also reported (14, 15, 19).

Of additional interest is the fact that in a single functional area, the motoneuron neuropil of the monkey, a third population of agranular vesicles (L bulbs) was observed, characterized by irregularly spheroid or heterogeneous vesicles, as well as by distinctive morphological features of the synaptic bulb itself (19). The use of vesicle shape as a criterion of function of course must be applied with caution, unless separate supporting evidence is also available. This report is intended to offer data that help to characterize the "third type" of agranular vesicle population and that point to an association of this type with cholinergic telodendria.

Concurrent with numerous reports describing "spheroid" and "flattened" synaptic vesicle populations in many vertebrate nerve centers, as well as in invertebrate systems (20, 21), some uncertainty was created by reports of variation of proportion of synaptic bulbs with flattened vesicles, due to differences of fixation (9, 22). In our own attempts to improve the general quality of fixation with aldehyde perfusion of monkeys, we too became aware of variables in aldehyde fixation that

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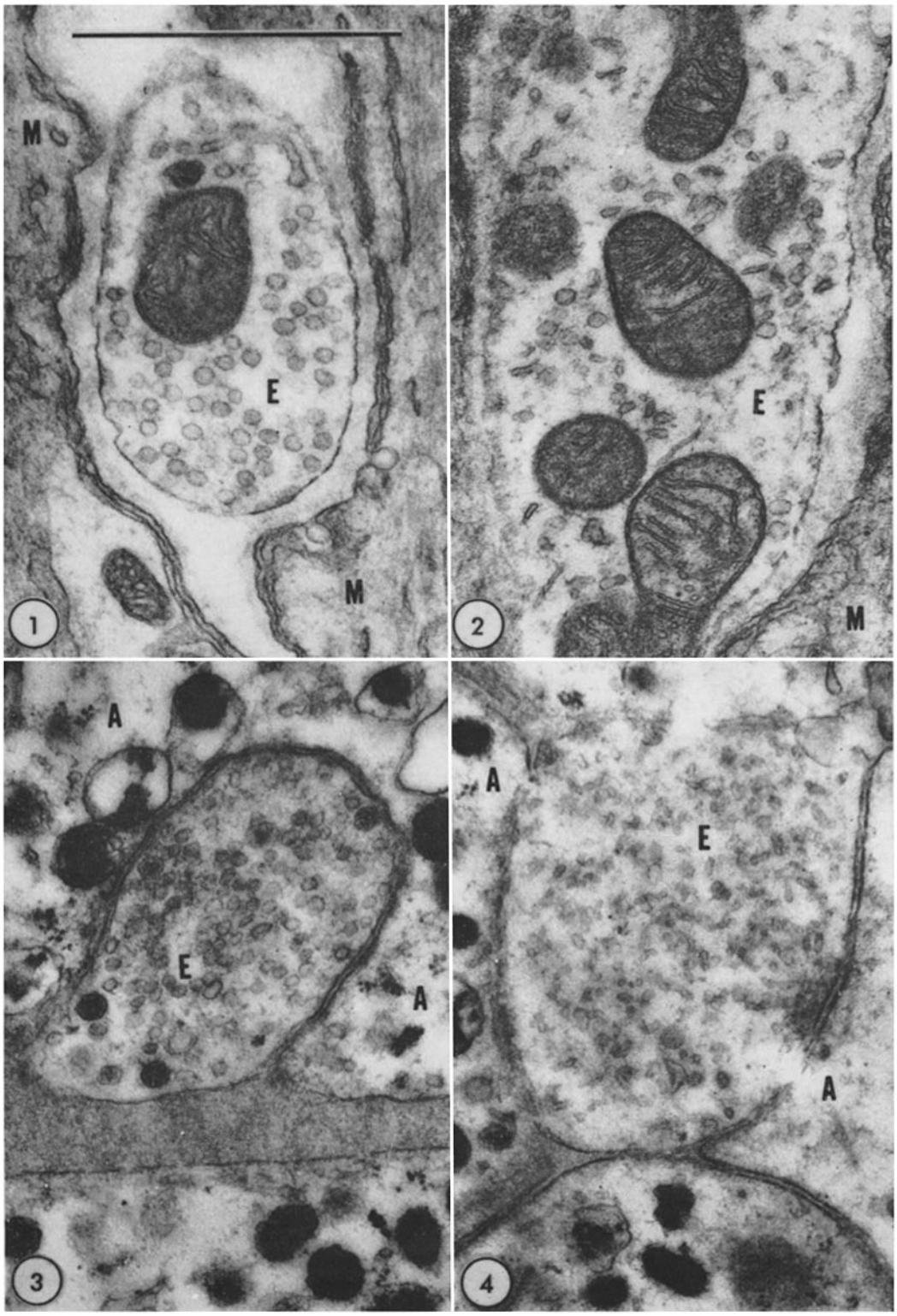
All figures at same magnification and from same animal (I 68), fixed by perfusion with 2% glutaraldehyde and 0.5% paraformaldehyde in sodium cacodylate buffer. Magnification bar indicates 1 μ.

FIGURE 1 Axon ending (*E*) in ciliary muscle of the monkey (*M*). Aldehyde perfusion, followed by osmium hardening without prior wash in buffer. × 51,000.

FIGURE 2 Axon ending (*E*), from same ciliary muscle as in Fig. 1, but tissue washed for 1 hr in buffer prior to osmium hardening. *M*, ciliary muscle. Note the flattening of agranular vesicles.

FIGURE 3 Axon ending (*E*) in adrenal medulla of monkey I 68. Tissue not washed before osmium hardening. *A*, adrenal medullary cells.

FIGURE 4 Axon ending (*E*) from same adrenal medulla as in Fig. 3, but tissue washed 1 hr in buffer prior to osmium hardening. *A*, adrenal medullary cells. Note the flattening of vesicles.


M
E
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could have a significant flattening effect on agranular synaptic vesicles. These variables affected the third type for the most part, and not the major "S" and "F" types of synaptic bulbs in the motoneuron neuropil. Cholinergic peripheral endings are also highly susceptible. Flattening of the third type is especially accentuated by even brief storage in cacodylate buffer after aldehyde fixation, prior to hardening in osmium tetroxide. Because of the indications of selective sensitivity of different populations of synaptic vesicles to flattening, an attempt was made to exploit these differences in order to better define the vesicle types.

MATERIALS AND METHODS

In an effort to gain insight into the possible sources of the morphological variation of central synaptic vesicles, we have examined a large number of synaptic loci in the central and peripheral nervous system, including sites where the cholinergic nature of the synapse has been established. Since our original work on central synaptic bulbs was done in the monkey, the same species was used in all cases, and uniform primary fixation was performed by means of aortic perfusion of aldehyde solutions at room temperature via the left ventricle (6), followed by hardening of dissected fragments in 2% phosphate-buffered osmium tetroxide for 1 hr. Various aldehyde mixtures in phosphate or cacodylate buffer, and at pH 7.0–7.2, were employed. Solutions were always hypertonic (440–500 milliosmols). Material from 20 monkeys contributed to this study. Five were considered as "standard," and these were perfused with 2½% glutaraldehyde in 0.067 M sodium cacodylate buffer plus 50 mg of $CaCl_2$/100 ml. Superior results were later achieved by using 2% glutaraldehyde and 0.5% paraformaldehyde, instead of 2½% glutaraldehyde, in the manner of Karnovsky (23). Over a period of 2 yr, in the course of other studies, other variations of procedure were also employed in attempts to improve results with aldehyde fixation, and in an effort to establish the degree of reliability of the structural marker characteristic of agranular synaptic vesicles (spheroid or flat shape).

Observations of Araldite-embedded, lead-stained sections in the RCA-3F electron microscope were made of the following tissues, in most animals: cervical motoneuron neuropil, nucleus gracilis, precentral cortex, ciliary ganglion, coeliac ganglion, last abdominal ganglion, adrenal medulla, ciliary muscle, iris constrictor, lateral rectus muscle, intercostal motor end-plates. Peripheral nerve endings in the last eight structures are of known cholinergic character.

RESULTS

Findings in this study relate to the following three essential points: (*a*) the effects of fixation on the shape of the vesicles of the major synaptic bulb types in the spinal motoneuron neuropil (S and F types); (*b*) the effects on agranular vesicles in L bulbs of motoneurons, where our initial study indicated a population of agranular vesicles of heterogeneous shape; and finally, (*c*) the effects on known cholinergic endings. With variation of aldehydes (4% formaldehyde, 2½°% glutaraldehyde, and a mixture of 2% glutaraldehyde and 0.5% paraformaldehyde), of buffer, or of osmolarity, only minor fluctuations from the appearance after "standard" fixation was noted. This was true, however, only if perfused tissue was fragmented and hardened in osmium tetroxide without prior washing. Storage for several hours in glutaraldehyde or formaldehyde fixative after removal from the perfused animal, and subsequent osmium tetroxide hardening, had little effect on the shape of vesicles as compared with the standard. However, storage for as little as ½ hr in sucrose-containing cacodylate buffer, prior to osmium hardening, as is commonly practiced (24), was found to have a profound flattening effect on agranular synaptic vesicles of all cholinergic nerve endings studied in peripheral tissues (ciliary muscle, ciliary ganglion, iris constrictor, adrenal medulla, coeliac ganglion, last abdominal ganglion, lateral rectus muscle, intercostal muscle). The effect was confirmed by comparing paired samples of tissues in four animals. All procedures were identical except that one set of fragments from each tissue was placed directly into osmium after removal from the animal, whereas a paired set was first stored in cacodylate buffer for periods of a ½ hr to overnight. Figs. 1–4 illustrate two such comparisons, in axon terminals of ciliary muscle and of adrenal medulla. Specimens shown in Figs. 1 and 3 were not washed prior to hardening, and those shown in Figs. 2 and 4 were washed in buffer for 1 hr before hardening in osmium.

The sensitivity of agranular synaptic vesicles to washing in buffer after aldehyde perfusion, resulting in severe flattening, would be of little interest were it not for the fact that such sensitivity was *not* found in the spheroid (S) agranular vesicles of certain spinal synaptic bulbs. In Figs. 5 and 6, for example, from the same animal shown in Figs. 1–4, the S bulbs contain mostly spheroid vesicles, relatively unaffected by the washing,

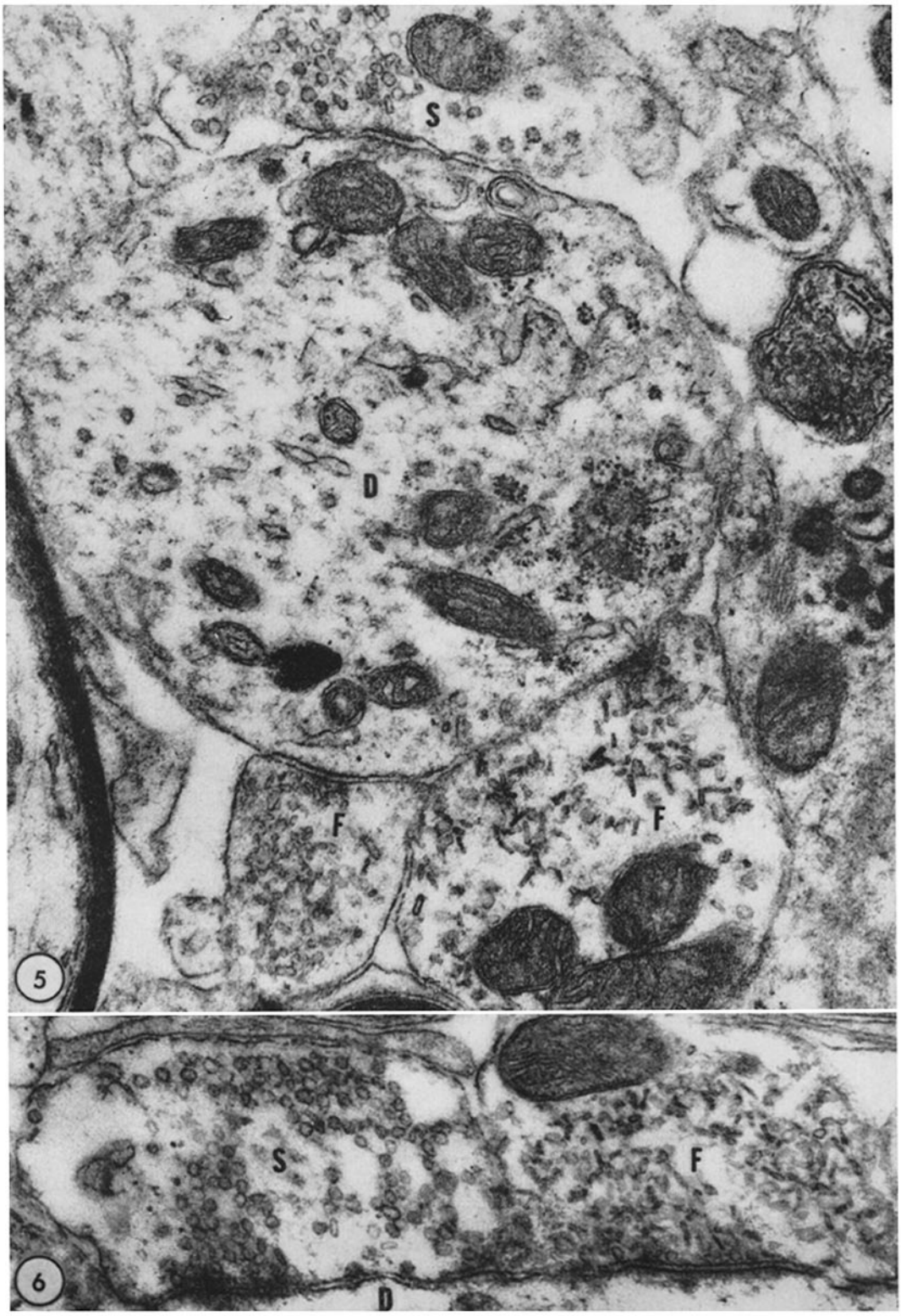


FIGURE 5 Dendrite (*D*) from cervical motoneuron neuropil of monkey I 68, bearing three synaptic bulb profiles with three distinctive agranular vesicle populations, *S*, *F* (left), *F* (right). Tissue was washed in buffer prior to osmium hardening. S vesicles mostly unaffected; *F* vesicles (left) flattened by aldehyde fixation, but similar in washed and unwashed specimens; *F* vesicles (right) elongated and apparently severely flattened by washing of tissue.

FIGURE 6 Dendrite (*D*) in cervical motoneuron neuropil of monkey I 68. Tissue washed in buffer before osmium hardening. Vesicles in *S* bulb relatively little affected. Vesicles in *F* bulb severely flattened.

whereas F bulbs show severe flattening of agranular vesicles. A certain proportion of small synaptic bulbs contain populations of agranular vesicles that are relatively flattened after standard aldehyde fixation, and that are only moderately subject to further flattening after buffer washing. These vesicles tend to be smaller than those of other F vesicle populations and may represent a distinct functional species. They are especially characteristic of the axo-axonic synaptic bulbs which are presynaptic to group A1 afferent root fiber endings (19). In cats, Khattab has reported that the axo-axonic synaptic bulbs in the spinal cord contain spherical vesicles (25). His illustration, however, indicates to us that the vesicle population of these bulbs exhibits vesicles of distinctly smaller size than typical S vesicles, and relatively flattened as well. The contrast is more marked in the monkey (Fig. 7). In Fig. 7, moreover, the F bulb, which is presynaptic to the group A1 afferent root fiber ending labeled *R*, shows additional flattening of vesicles in a washed specimen, whereas in the R bulb one can observe only a slight degree of flattening due to the washing procedure.

In contrast with the vesicle populations of the large axodendritic R bulbs, which show only moderate flattening of some vesicles after washing, and are therefore intermediate in sensitivity to washing, as compared with S or F vesicle populations, the large axo-somatic L bulbs on motoneurons are highly sensitive. After standard aldehyde fixation, these unusual synaptic bulbs contain populations of irregularly spheroid agranular vesicles, as do the R bulbs. When the sucrose-buffer wash is employed, however, the vesicles are severely flattened. Figs. 8 and 9, for example, are from paired samples of tissue, of which one (shown in Fig. 8) has not been washed prior to osmium hardening, and the other (shown in Fig. 9) has been washed for 1 hr. In each case the synaptic bulb is of the L type, also characterized by its relation to an underlying subsynaptic cistern and Nissl body in a primary motoneuron dendrite (*D*). It is therefore possible to categorize certain synaptic bulbs of the motoneuron neuropil on the basis of shape of populations of agranular vesicles under defined preparative conditions. These classes moreover may be further identified by other specific morphological and topographic characteristics.

Class 1

S-type synaptic bulbs of motoneuron neuropil and of motor cortex are in this category. Almost all agranular vesicles are relatively uniform in size, and resistant to flattening, irrespective of variations in fixing procedures, including a buffer wash prior to osmium hardening. Variations in aldehyde concentration, osmolarity, and type of buffer used in aldehyde solution also have little effect. In the motoneuron neuropil, evidence suggests that these synaptic bulbs appear first in development and are excitatory (2).

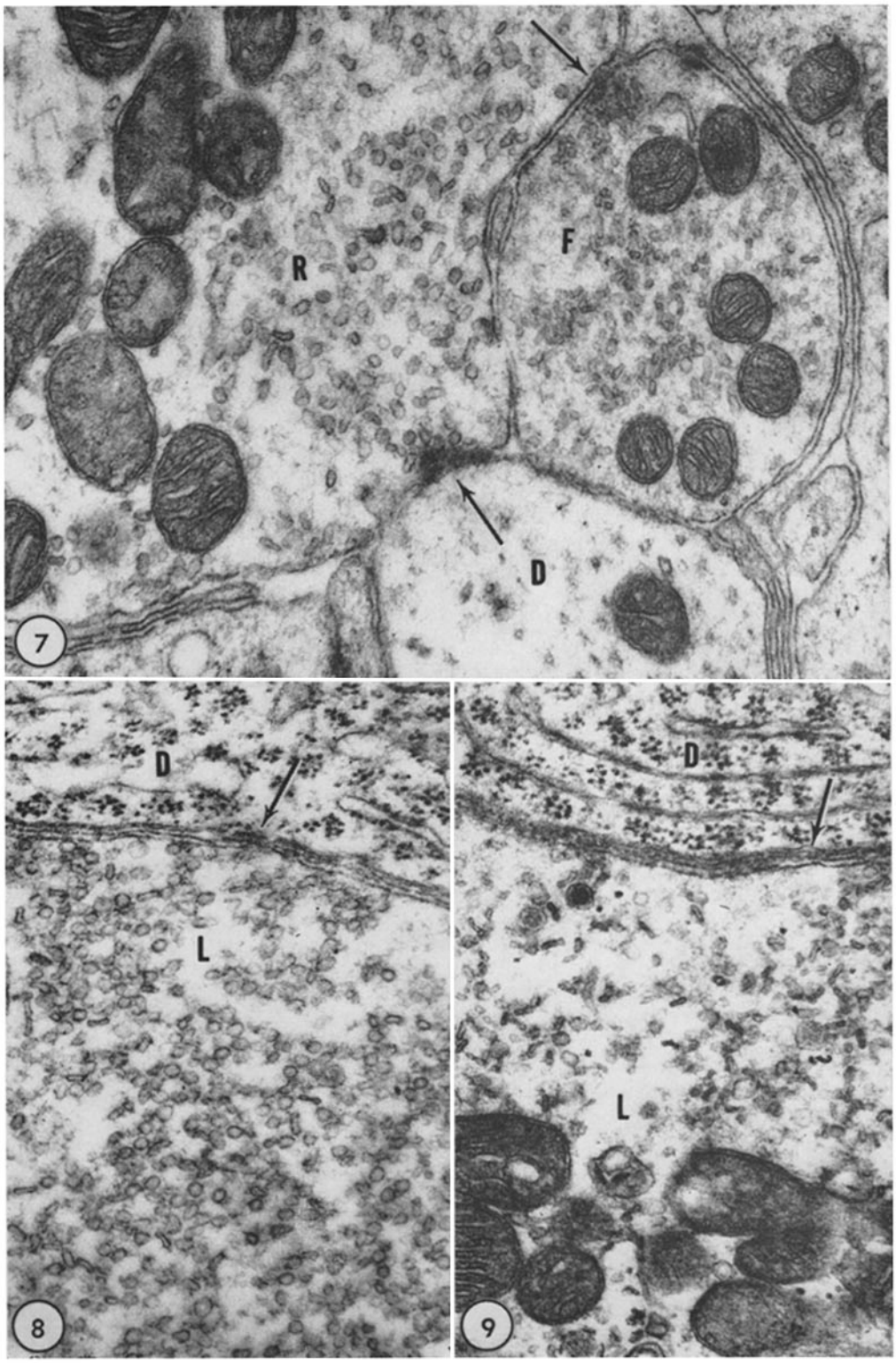
Class 2

F-type synaptic bulbs of motoneuron neuropil are in this category. Agranular vesicles may be somewhat smaller than those of the S-type and are always flattened after aldehyde fixation in mammals. Accentuation of flattening usually follows a postfixation wash in buffer. This is the second most common type found in the motoneuron neuropil (19). Similar bulbs are found in many

FIGURE 7 Group Al afferent root fiber ending (*R*) on dendrite (*D*) in cervical motoneuron neuropil. Tissue washed in buffer prior to osmium hardening. Vesicles in R bulb are partly flattened as compared with unwashed specimens. Axo-axonic bulb has vesicles of the F type. Arrows indicate serial synaptic junctional sites.

FIGURE 8 Portion of large (6 μ) synaptic bulb (*L*) in contact with primary dendrite of motoneuron (*D*). The subsynaptic cistern (arrow) and Nissl body are characteristic. Tissue unwashed prior to osmium hardening. Vesicles irregularly round, with some flattened profiles.

FIGURE 9 Portion of large (6 μ) synaptic bulb (*L*) in contact with primary dendrite of motoneuron (*D*). Same region as shown in Fig. 8, but tissue was washed in buffer for 1 hr prior to osmium hardening. Note severe flattening of most synaptic vesicles, as compared with relative resistance to flattening of R bulb in Fig. 7. Arrow indicates postsynaptic cistern.


R
F
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9

centers in the vertebrate central nervous system, and are thought to be of inhibitory character (20, 21). In snake and frog spinal cord, the flattening of vesicles of the F-type bulbs has been observed after immersion fixation with osmium (26). It is apparent therefore that gradations in sensitivity to flattening of agranular vesicles exist on the basis of animal types, as well as on the basis of fixation procedure. In the instance of F-type synaptic bulbs of the monkey spinal cord (19), it is likely that more than one functional category will be identified ultimately. The axo-axonic bulbs presynaptic to R bulbs are, for example, not so severely flattened by buffer washing as are other bulbs of class 2. The contrast is also suggested in the two synaptic bulbs shown in Fig. 5.

Class 3

Cholinergic peripheral endings and spinal cord L bulbs are in this category. Agranular synaptic vesicles are not smoothly spheroid after "standard" glutaraldehyde fixation but are irregularly round, with a scatter of flattened vesicles. Most of the vesicles in the synaptic bulb approximate the size of S vesicles with, however, greater size variation. Crystalline-like arrays of packed vesicles, such as appear with S vesicles, do not seem to occur. With the use of a wash in sucrose-buffer, even as short as ½ hr, the class 3 agranular vesicles are largely flattened. All synaptic bulbs of peripheral autonomic ganglia, of motor end plates, and of adrenal medulla appear to be of this type. In addition, the irregularly round vesicles of the large L bulbs of the motoneuron neuropil respond in similar fashion to the sucrose-buffer wash.

Class 4

R-type synaptic bulbs of the spinal cord, of group A1 afferent root fibers, are of this category (19). After standard aldehyde fixation, the agranular vesicles tend to be larger and more irregular in shape than S vesicles. The agranular vesicles are intermediate in sensitivity to flattening, as compared with other classes.

Class 5

G-type of synaptic bulbs of the spinal cord are of this category (19). The agranular vesicle population is associated with an unusually high proportion of granular vesicles of about 600–800 A. The agranular vesicles themselves are not clearly differentiatable at the present time.

DISCUSSION

It has been known for many years that formaldehyde fixation is a complicated process, in which the assets of the reagent, as a cytoplasmic fixative second only to osmium tetroxide (27), are balanced by its inability "to protect the cell from changes caused by subsequent histological treatments" (28). The latter deficiency, as is well known, may be remedied by postfixation, or hardening, in osmium tetroxide. Prior to hardening, living cells have been observed to undergo alternative shrinkage and swelling, in 5% formaldehyde, for example, indicating that simple osmosis could not be the sole factor in water movement across "fixed" cell membranes (28).

The introduction of glutaraldehyde, as a fixative with improved qualities of preservation of cellular structure and enzymatic activity (24), has not eliminated the problem of instability of aldehyde-fixed tissues prior to hardening (29). Nevertheless, fixation comparable to that obtainable with primary osmium fixation may be obtained with regularity if a standardized procedure of glutaraldehyde perfusion is followed. Moreover, such fixation, under appropriate conditions, may be used to define classes of agranular synaptic vesicles, which are quite obscure in primary osmium-fixed mammalian tissues, because of variations in membrane stability of the vesicles in aldehyde solutions.

The observation of flattened agranular synaptic vesicles in a variety of centers in the nervous system was first associated with the use of aldehyde fixatives under conditions that did not permit assessment of the significance of this findings. Subsequent work, by demonstrating the consistent distribution or morphology of synaptic bulbs containing either predominantly flattened or spheroid agranular vesicles, left no doubt that the aldehyde sensitivity of agranular vesicles was a selective and useful identifying feature of one type of synaptic ending. Our own unpublished studies indicate a moderately good correlation of synaptic bulbs containing spheroid agranular vesicles with bulbs of Gray's Type 1 (30) in both motor cortex and spinal motoneuron neuropil of the monkey, and of flattened vesicles with bulbs of Gray's Type 2.

The reports of these two types of agranular vesicle populations in synaptic bulbs in many other centers in constant proportions, by a number of investigators, has contrasted with doubts expressed concerning the reliability of the criterion of vesicle

shape in the identification of specific types of synaptic bulbs (22). The Norwegian workers correctly emphasized the need for careful definition of the character of the aldehyde used for fixation. However, our characterization of a type of synaptic bulb, with agranular vesicles of exceptional sensitivity to flattening, under appropriate conditions, indicates another possible reason for difficulty in characterizing synaptic vesicles by means of shape.

Of greater significance, however, is the finding that this type of synaptic bulb is characteristic of all of a variety of peripheral cholinergic endings subjected to test, as well as certain central synaptic bulbs with vesicles that we have previously referred to as "irregularly spheroid". An instance of unusual interest is the L bulb of the monkey spinal cord, which, usually in adjacent pairs, form a characteristically large (5–7 μ) synaptic complex overlaying subsynaptic Nissl bodies of the soma or proximal dendrites of motoneurons (19). Erulkar et al. have offered evidence that the cholinergic endings of recurrent collaterals of motoneurons may be of the size of the L bulb complex, and may represent the functional unit previously considered to be the "Renshaw cell" (31). This point is, however, at variance with the generally accepted view. It may be merely coincidental that our evidence is consistent with the concept of Erulkar et al., in the sense that agranular vesicles of L synaptic bulbs respond to buffer-induced flattening in the same way as peripheral cholinergic axon endings.

Another finding that acquires added interest as a result of our work is that of McDonald and Rasmussen (32) who report that the two types of synaptic bulb in the ventral cochlear nucleus defined by Lenn and Reese (14), can be divided into three classes on the basis of agranular synaptic vesicles. The endings with larger, spheroid vesicles are noncholinergic, and unaffected by aldehyde fixation. Synaptic bulbs with smaller, flattened vesicles in aldehyde-fixed material include some that give an acetylcholinesterase reaction and some that do not. The correspondence between these results and our own are striking, in the sense that two types of synaptic bulbs with flattened vesicles are defined. In addition to the flattening tendency of vesicles in L bulbs, there are suggestions in our material that two types of small F bulbs may be present in the spinal cord, with one type exhibiting smaller flattened vesicles than the other.

As a result of these considerations, we feel that our classification of five types of synaptic bulbs in the spinal motor neuropil, based on synaptic vesicle characteristics as well as topographic features, is strengthened. Under appropriate conditions, the five major classes can be reasonably well defined on the basis of synaptic vesicles alone. The presumption at the present time is that synaptic vesicles are somehow associated with transmitter substances. The physiological data of Graham et al. (33) offer evidence for the possible transmitter role of five substances other than acetylcholine and the catecholamines. Attempted further correlation of morphological characteristics of synaptic vesicles with specific transmitters is therefore a logical goal of further investigation.

This study was partially supported by the United States Public Health Service Grant NB07935-01, and by the National Foundation.

Thanks are due to Naomi Taylor and William D. McClaskey for their technical assistance.

Received for publication 19 June 1969, and in revised form 28 August 1969.

REFERENCES

1. Budd, G. C., and M. M. Salpeter. 1969. The distribution of labeled norepinephrine within sympathetic nerve terminals studied with electron microscope radioautography. *J. Cell Biol.* **41**:21.
2. Bodian, D. 1966. Development of fine structure of spinal cord in monkey fetuses. 1. The motoneuron neuropil at the time of onset of reflex activity. *Johns Hopkins Med. J.* **119**:129.
3. Whittaker, V. P. 1965. The application of subcellular fractionation techniques to the study of brain function. *Prog. Biophys. Mol. Biol.* **15**:39.
4. DeIraldi, A. P., H. F. Duggan, and E. De-Robertis. 1963. Adrenergic synaptic vesicles in the anterior hypothalamus of the rat. *Anat. Rec.* **145**:521.
5. Robertson, J. D., T. S. Bodenheimer, and D. E. Stage. 1963. The ultrastructure of Mauthner cell synapses and nodes in goldfish brains. *J. Cell Biol.* **19**:159.
6. Bodian, D., and N. Taylor. 1963. Synapse arising at central node of Ranvier, and note on fixation of the central nervous system. *Science (Washington).* **139**:330.
7. Bodian, D. 1964. An electron microscopic study of the monkey spinal cord. *Johns Hopkins Med. J.* **114**:13.

8. WALBERG, F. 1965. A special type of synaptic vesicles in boutons in the inferior olive. *J. Ultrastruct. Res.* **12**:237.
9. LUND, R. D., and L. E. WESTRUM. 1966. Synaptic vesicle differences after primary formalin fixation. *J. Physiol. (London).* **185**:1.
10. WALBERG, F. 1966. Elongated vesicles in terminal boutons of the central nervous system, a result of aldehyde fixation. *Acta Anat.* **65**:224.
11. UCHIZONO, K. 1965. Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. *Nature (London).* **207**:642.
12. BODIAN, D. 1966. Electron Microscopy: Two major synaptic types on spinal motoneurons. *Science (Washington).* **151**:1093.
13. HIRATA, Y. 1966. Occurrence of cylindrical synaptic vesicles in the central nervous system perfused with buffered formalin solution prior to OsO_4-fixation. *Arch. Histol. Jap.* **26**:269.
14. LENN, N. J., and T. S. REESE. 1966. The fine structure of nerve endings in the nucleus of the trapezoid body and the ventral cochlear nucleus. *Amer. J. Anat.* **118**:375.
15. LARRAMENDI, L. M. H., L. FICKENSCHER, and N. LEMKEY-JOHNSTON. 1967. Synaptic vesicles of inhibitory and excitatory terminals in the cerebellum. *Science (Washington).* **156**:967.
16. MUGNAINI, E., F. WALBERG, and E. HAUGLIE-HANSSEN. 1967. Observations on the fine structure of the lateral vestibular nucleus (Deiters' nucleus) in the cat. *Exp. Brain Res.* **4**:146.
17. COLONNIER, M. 1968. Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. *Brain Res.* **9**:268.
18. PRICE, J. L. 1968. The synaptic vesicles of the reciprocal synapse of the olfactory bulb. *Brain Res.* **11**:697.
19. BODIAN, D. 1966. Synaptic types on spinal motoneurons: an electron microscopic study. *Johns Hopkins Med. J.* **119**:16.
20. UCHIZONO, K. 1967. Inhibitory synapses on the stretch receptor neurone of the crayfish. *Nature (London).* **214**:833.
21. NADOL, J. B., JR., and A. J. D. DE LORENZO. 1968. Observations on the abdominal stretch receptor and the fine structure of associated axo-dendritic synapses and neuromuscular junctions in *Homarus*. *J. Comp. Neurol.* **132**:419.
22. HOLLÄNDER, H., P. BRODAL, and F. WALBERG. 1969. Electronmicroscopic observations on the structure of the pontine nuclei and the mode of termination of the corticopontine fibres. An experimental study in the cat. *Exp. Brain Res.* **7**:95.
23. KARNOVSKY, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. *J. Cell Biol.* **35**:213.
24. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**:19.
25. KHATTAB, F. 1968. A complex synaptic apparatus in spinal cord of cats. *Experientia (Basel).* **24**:690.
26. FUKAMI, Y. 1969. Two types of synaptic bulb in snake and frog spinal cord. *Brain Res.* **14**:137.
27. MANN, G. 1902. Physiological Histology. Clarendon Press, Clarendon, Texas.
28. CRAWFORD, G. N. C., and R. BARER. 1951. The action of formaldehyde on living cells as studied by phase-contrast microscopy. *J. Cell Sci.* **92**:403.
29. SCHULTZ, R. L., and U. KARLSSON. 1965. Fixation of the central nervous system for electron microscopy by aldehyde perfusion. II. Effect of osmolarity, pH of perfusate, and fixative concentration. *J. Ultrastruct. Res.* **12**:187.
30. GRAY, E. G. 1959. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J. Anat.* **93**:420.
31. ERULKAR, S. D., C. W. NICHOLS, M. B. POPP: and G. B. KOELLE. 1968. Renshaw elements, localization and acetylcholinesterase content. *J. Histochem. Cytochem.* **16**:128.
32. MCDONALD, D. M., and G. L. RASMUSSEN. 1969. Association of acetylcholinesterase with one type of synaptic ending in the cochlear nucleus: an electron microscopic study. *Anat. Rec.* **163**:228 (Abstr.)
33. GRAHAM, L. T., JR., R. P. SHANK, R. WERMAN, and M. H. APRISON. 1967. Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, γ-aminobutyric acid, glycine, and glutamine. *J. Neurochem.* **14**:465.