OBSERVATIONS OF NERVE TERMINALS IN HUMAN LABIAL SALIVARY GLANDS

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INTRODUCTION

The existence of a motor innervation to the secretory and myoepithelial elements of salivary glands has been supported amply by physiological and pharmacological studies extending over a century (reviewed in Babkin, 1950; Emmelin, 1967). However, the exact morphological nature of this innervation at the acinar level is less well established, and has been the subject of several conflicting reports. The earliest electron microscopic investigations of rodent salivary glands (Scott and Pease, 1959) demonstrated the presence of axons between parenchymal elements, deep to the basement membrane. These observations have been confirmed by Shackleford and Schneyer (1964) for the rodent salivary gland, by Ruskell (1968) for the monkey lacrimal gland, by Yamamoto (1966) for the guinea pig nasal gland, by Yamauchi and Burnstock (1967) for the sheep lacrimal gland, and by Watari (1968) for the exocrine pancreas of several different vertebrates. Tandler (1965) has illustrated a direct contact between an axon and a myoepithelial cell in the human submandibular gland. All of these studies have shown that a space of 200–250 A exists between the neural membrane and the effector cell membrane when they are in a "direct" contact (Grillo, 1966). On the other hand, "indirect" contacts, with a space of 0.1 μ or more between the neural element and the acinar element, together with the interposition of basement membrane, have been described in the salivary glands of dogs (Fujita et al., 1964), cats (Garrett, 1966), rats (Tamarin, 1966), and man (Garrett, 1967).

The present report describes the appearance of several intra-acinar axons and axonal varicosities in human labial salivary glands and thus supports the concept of a direct-contact innervation of the secretory and contractile elements of these glands.

MATERIALS AND METHODS

Specimens of human labial salivary glands were obtained by biopsy from 32 people. Tissues were prepared for electron microscopy by methods described previously (Tandler et al., 1969 *a*).

OBSERVATIONS

The general organization of the human labial salivary gland has been reported (Tandler et al., 1969 *a*, *b*). The periacinar connective tissue contains many small nerves. These nerves are both myelinated and unmyelinated, and the latter have the typical appearance of Schwann cell envelopment. The unmyelinated axons range from 0.4 to 1.6 μ in diameter and have not been observed to have any enlargements or varicosities.

Within the acinus, deep to the basement membrane, naked axons of smaller size range are seen. Their diameters vary between 0.3 and 0.8 μ and they exhibit enlargement along their length (Figs. 1–4). In this intra-acinar location, the Schwann cell envelopment is lost, and the axons course in intimate relation to the acinar cells, being separated from the latter by a gap of about 200 A devoid of any basement membrane material or cellular processes (Figs. 3 and 4). In some sections, the axons lie within invaginations of the secretory cell itself (Fig. 1). The axons are characterized by the presence of an electron-lucent axoplasm and numerous tubules about 200 A in diameter (Figs. 1 and 3). These axons were observed not only at the periphery of the acinus (just within the basement membranes (Fig. 4)), but they also were seen penetrating deeply between the acinar cells (Fig. 3). Where ever they are seen, the axons always lie close to a myoepithelial cell.

The enlargements or varicosities, which may be as much as 1 μ in diameter, contain many small vesicles and mitochondria. There are several such varicosities along the course of a single axon, thus resembling terminations "en passage" (Figs. 1 and 3). The vesicle population of these areas is of two types: a small agranular vesicle 350 to 450 A in diameter, and a larger granulated vesicle about 800 A in diameter (Figs. 2 and 3). Vesicles were seldom seen in the cylindrical portions of

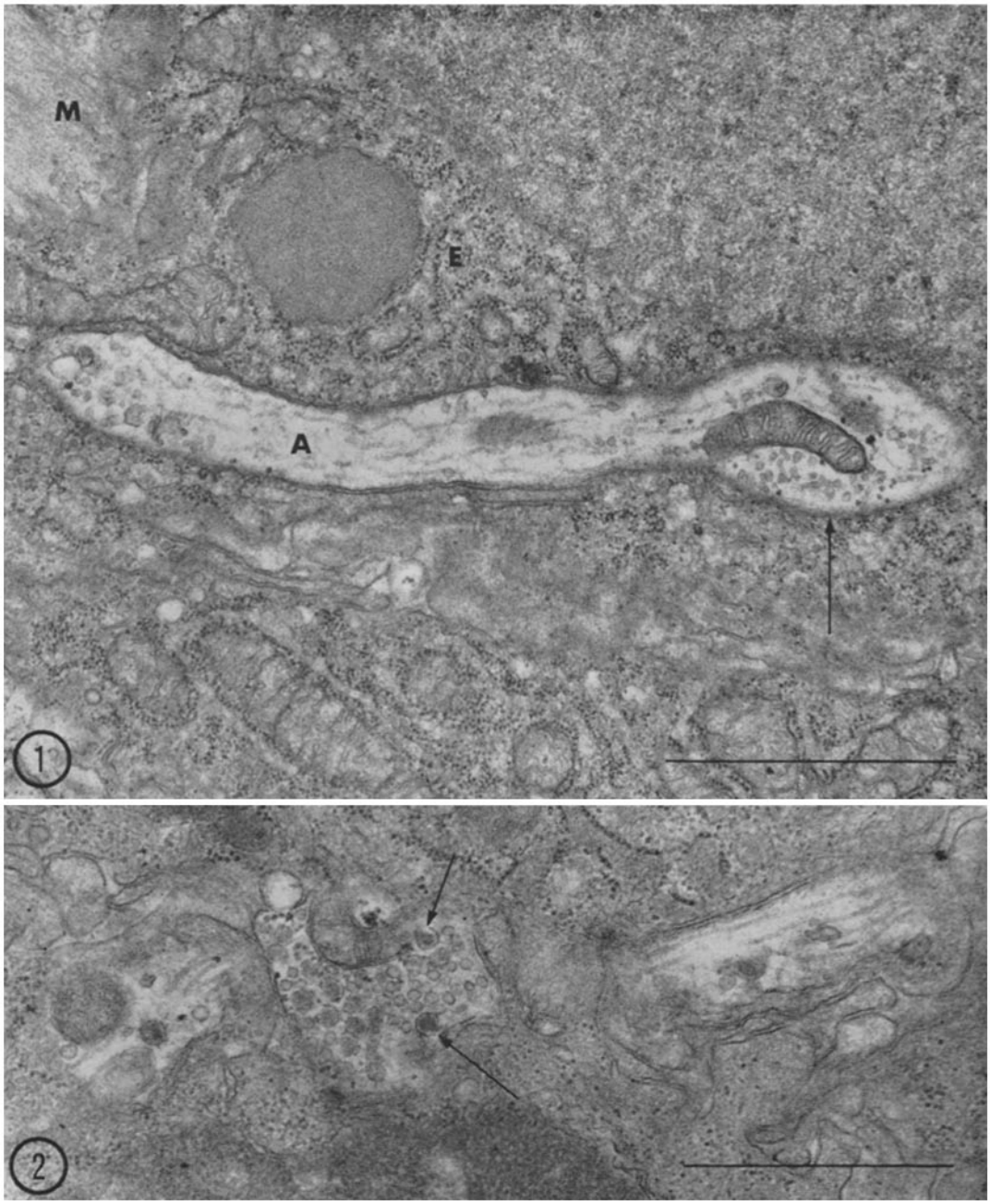

FIGURE 1 This micrograph illustrates a portion of an axon (*A*) in longitudinal section deeply penetrating a glandular epithelial cell (*E*) within the acinus. A portion of a myoepithelial cell (*M*) is shown in the upper left corner. A varicosity of the axon (arrow) contains a small mitochondrion and many small nongranular vesicles. Neurotubules extend longitudinally in the rest of the axon. × 33,600.

FIGURE 2 A nerve varicosity appears in the center of the micrograph. Some of the vesicles are of the larger, granular type (arrows), while others are of the smaller granular and nongranular variety. Other portions of axons can be seen to either side. × 37,000.

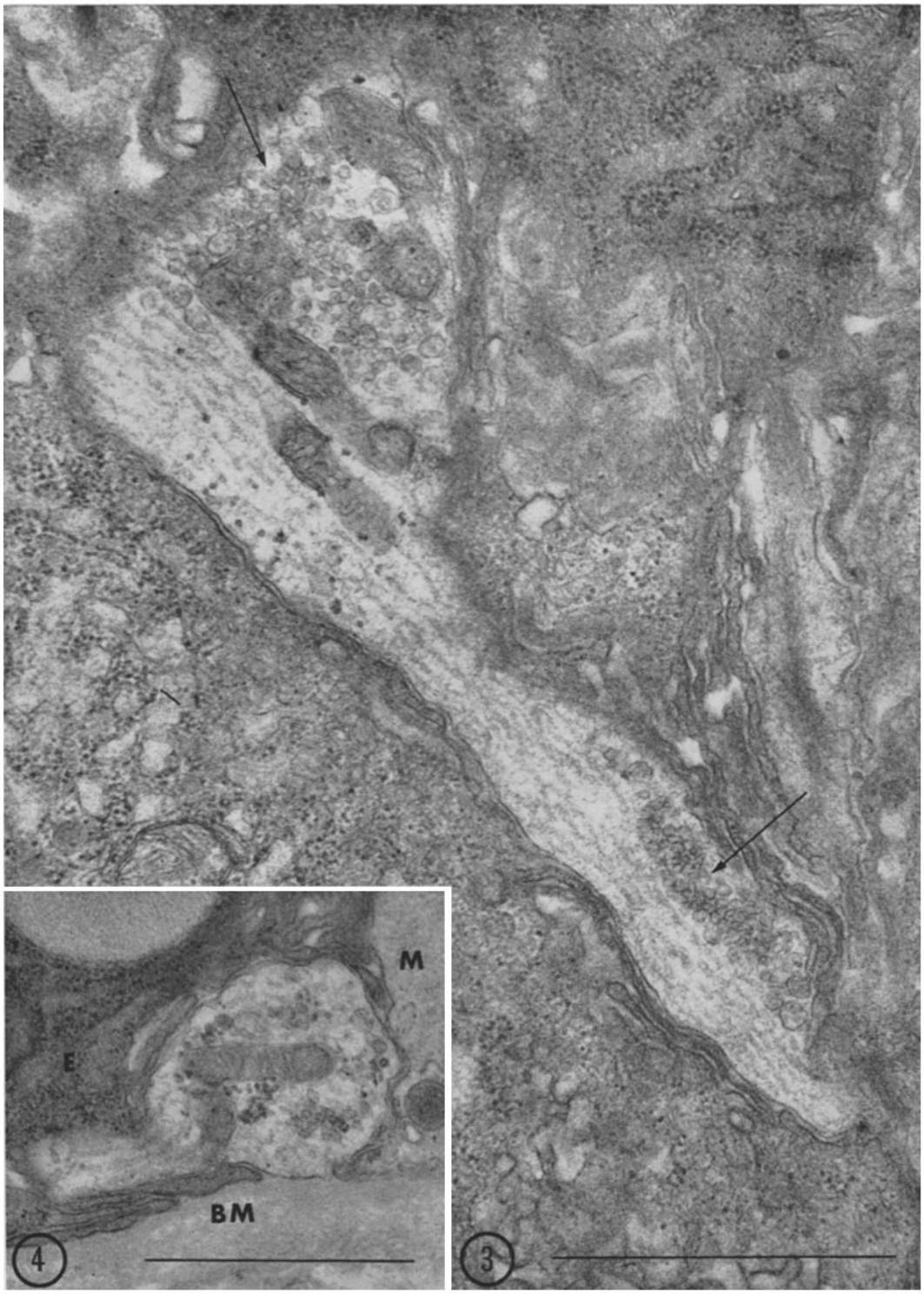

FIGURE 3 A micrograph illustrating an axon deep within the acinus, extending longitudinally across the field. In its course the axon is closely related to glandular cells, and at two sites (arrows) dilatations can be seen. The larger of the dilatations contains several small mitochondria and a cluster of small vesicles, mostly of the nongranular type. × 52,000.

FIGURE 4 A micrograph showing an axon lying within the basement membrane (*BM*) of the acinus and in immediate contact with both glandular epithelial (*E*) and myoepithelial (*M*) cells. × 40,000.

the axons, but were generally confined to the varicosities. On the other hand, the neurotubules are absent from the varicosities. In these regions, as along the rest of the axon, the neural and parenchymal elements are separated by a clear space of about 200 A. No membrane thickenings were seen, nor is there any evidence of cytoplasmic specializations.

DISCUSSION

The morphological criteria for identifying functional contacts between an autonomic axon and an effector have been detailed by Grillo (1966). In essence, these contacts are beaded enlargements or varicosities containing clusters of vesicles and mitochondria. A Schwann cell sheath is usually absent and the surface of a varicosity is always exposed to the effector cell membrane. Membrane thickenings are absent, as are pre- or postsynaptic organelles. By these standards, therefore, the structures described in this report represent terminal autonomic neuroeffectors, and may be added to the growing list of presumed functional contacts which have been described in a variety of exocrine organs.

The principal discordant note in this list is the location of the nerve terminals. Garrett (1966, 1967) and Tamarin (1966), among others, have not observed intra-acinar axons and have thus maintained that while terminals exist, they occur only in the interstitial tissue, forming the ground-plexus. This method of innervation by indirect contact implies a diffusion of any released transmitter from the axon and through the connective tissue elements (basement membrane) to the parenchymal elements. Direct contacts with axons penetrating between the acinar elements have been described in a number of exocrine glands (Ruskell, 1968; Tandler, 1965; Watari, 1968; Yamamoto, 1966). In these situations a more intimate relationship between axonal varicosity and effector cell is established and, while contact specializations do not exist, a restricted area of transmitter release is implicit in the morphologic appearance of these terminations "en passage." At the very least, the transmitter material would presumably be confined to the acinus by the basement membrane, thus making neural control more selective. The importance of this relationship for smooth muscle has been discussed by Richardson (1964). The observations of the present study support the concept of direct contact within the acinus. It may be, as Ruskell (1968) has pointed out, that two different systems are involved, an interstitial and a parenchymal autonomic plexus, each with different functions.

The vesicle population of the varicosities is heterogeneous. While the small, 450 A vesicle predominates, the method of fixation employed does not permit conclusions to be drawn about its granularity. To determine whether the terminal is adrenergic or cholinergic would require fixation with permanganate (Richardson, 1966; Hökfelt, 1967).

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