MEMBRANE SPECIALIZATION AT AN INSECT MYONEURAL JUNCTION

JACK ROSENBLUTH

From the Departments of Physiology and Rehabilitation Medicine, New York University School of Medicine, New York 10016

ABSTRACT

Myoneural junctions were examined in the asynchronous basalar flight muscle of the beetle *Pachnoda ephippiata*. The outer surface of the postjunctional membrane exhibits an array of prominent projections spaced at ~200 Å intervals which arise directly from the outer dense lamina of the plasma membrane and extend part way across the junctional cleft. The projections follow irregularities in the contour of the postjunctional membrane precisely and they end abruptly near the edge of the junctional region. No separation can be resolved between the projections and the underlying trilaminar plasma membrane after a variety of preparative methods, and the projections therefore appear to be a component part of the membrane. This specialization, which is distinctly different from that at desmosomes and hemidesmosomes, occurs nowhere else on the surface of the muscle and is interpreted as a mosaic of specialized membrane subunits which probably include the receptor sites for the transmitter.

INTRODUCTION

Recent studies of cholinergic myoneural junctions have demonstrated a distinctive postjunctional membrane specialization consisting of hexagonal arrays of subunits in the muscle membrane which project from its outer surface and can be demonstrated by selective staining methods (14, 15). The number of subunits per square micron in the specialized region approximates the estimated concentration of receptor sites and acetylcholinesterase sites at cholinergic junctions elsewhere, and on this basis it was suggested that the periodically disposed elements may correspond to either or both of these components (14).

This report concerns noncholinergic myoneural junctions in a beetle flight muscle (5). Although insect myoneural junctions are generally similar to cholinergic junctions in form and function, they differ in that inactivation of transmitter is thought to be accomplished through uptake by surrounding processes rather than by enzymatic breakdown (4) and therefore a high concentration of a degradative enzyme corresponding to acetylcholinesterase would not be expected at insect myoneural junctions. The beetle junctions examined here were nevertheless found to have a projection-bearing postjunctional membrane similar to that occurring at cholinergic junctions in dimensions, periodicity, and staining properties, and it is inferred, therefore, that this membrane specialization probably represents the location of receptor sites for the transmitter.

MATERIALS AND METHODS

The basalar flight muscles of the beetle *Pachnoda ephippiata* were exposed and flooded with a fixative consisting of 5% glutaraldehyde in 0.15 M cacodylate buffer (pH 7.0–7.2) with 5% sucrose added. The muscles were left *in situ* for approximately 2 h under the fixative and then were partially dissected out and soaked overnight in the same solution. They

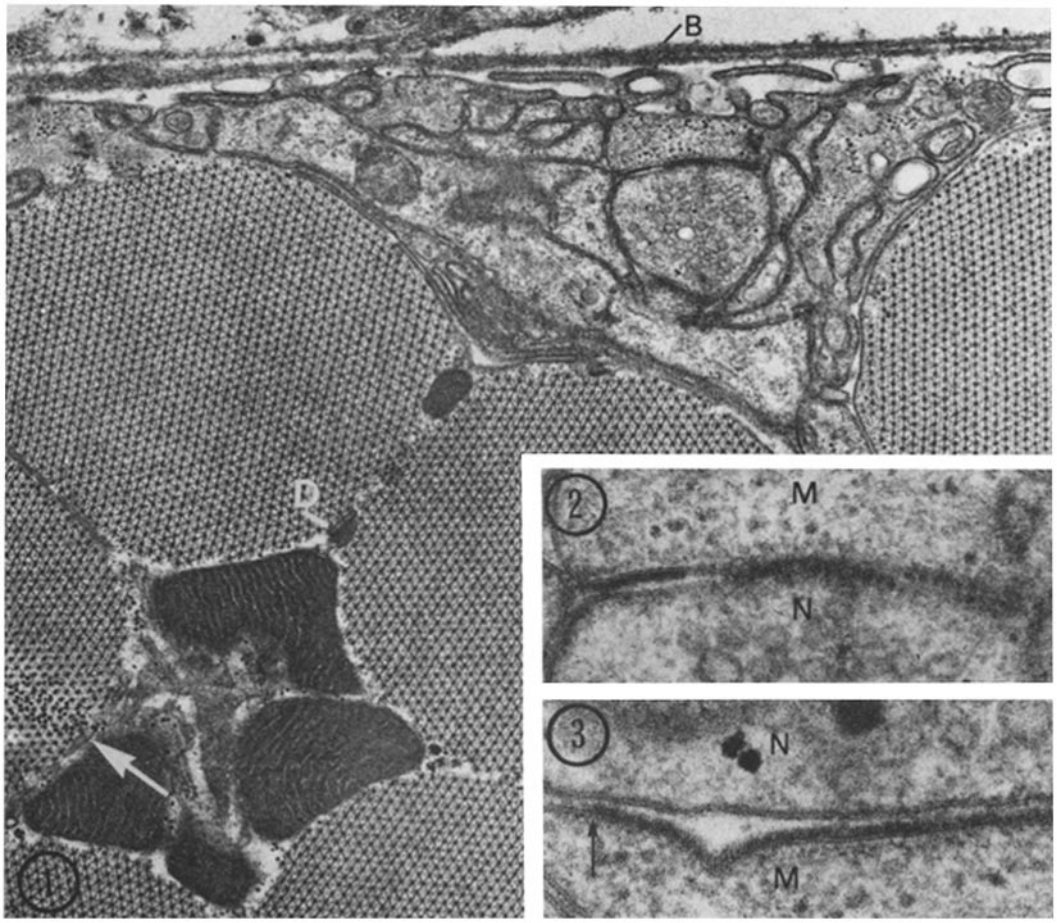


FIGURE 1 Survey picture of muscle fiber and myoneural junction. A small nerve ending containing clear vesicles and glycogen particles is embedded in the peripheral sarcoplasm. Several myofibrils are shown, one of which contains glycogen particles in the M-band region (arrow). *B*, basement membrane; *D*, dyad. × 25,000.

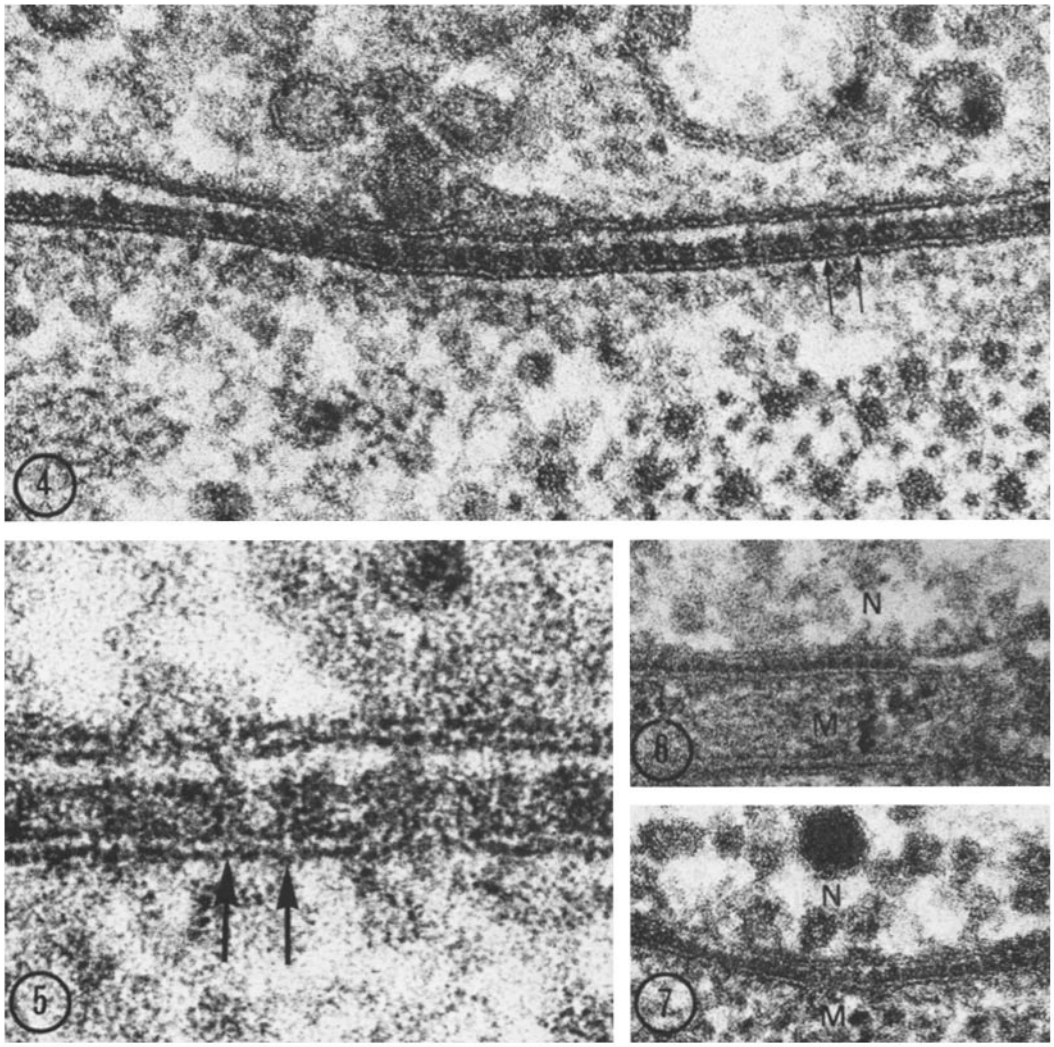
FIGURE 2 Detail of myoneural junction. The membrane of the muscle process (*M*) appears to be coated by a dense material which at the center and right is sectioned tangentially and exhibits a periodicity. *N*, nerve ending. × 80,000.

FIGURE 3 At this junction the "coating" on the postjunctional membrane follows the membrane into a V-shaped depression and ends abruptly at the arrow. *N*, nerve ending; *M*, muscle process. × 94,000.

were trimmed further the following day and then stored in this fixative. After periods varying from approximately 1–5 mo, muscles were removed from this solution and rinsed in saline and then were either postfixed in 2% osmium tetroxide in cacodylate buffer and dehydrated and embedded in Araldite, or were dehydrated and embedded directly after rinsing without postosmication. Transverse sections were cut through the entire muscle and well-fixed areas then selected for thin sectioning. Sections were stained with either uranyl acetate followed by lead hydroxide or with potassium permanganate followed by a citrate rinse and then hot alcoholic uranyl acetate (14). Electron microscopy was carried out with a Philips EM 300 instrument operating at 60 kV.

RESULTS

The basalar muscles are composed of large caliber fibers which have the structural characteristics of asynchronous flight muscles (cf. reference 11 for review). In regions where myoneural junctions occur the muscle cell membrane is thrown up into



FIGURES 4–7 Details of postjunctional membrane.

FIGURE 4 The postjunctional membrane is covered with regularly spaced projections some of which are arrow shaped (arrows). No separation can be resolved between the projectons and the outer dense lamina of the plasma membrane. At the right very fine filaments extend between the projections and the axonal membrane. Part of a myofibril is visible at the bottom right. × 200,000.

FIGURE 5 Both the prejunctional (above) and postjunctional (below) membranes are trilaminar. Arrows point to the *inner* dense leaflet of the postjunctional membrane which is continuous. Above the arrows gaps can be seen in the *outer* dense leaflet, which appears to be reflected onto projections having low density cores. × 570,000.

FIGURE 6 Unosmicated specimen stained with uranyl acetate and lead hydroxide. *N*, axon; *M*, muscle. × 100,000.

FIGURE 7 Unosmicated specimens stained with permanganate and uranyl acetate. Here as in Fig. 6, no separation can be resolved between the projections and the trilaminar muscle membrane. *N*, axon; *M*, muscle. × 130,000.

processes that surround the terminal axon closely (Fig. 1), so that the junctions do not occur at the very surface of the muscle fiber but are, rather, buried (16). Although the continuity between the muscle processes surrounding the axon terminals and the body of the muscle is difficult to follow, such connections can occasionally be traced (Fig. 8) and in some instances the axon is directly apposed to a portion of the muscle cell containing obvious myofilaments (Fig. 4). The thick basement membrane surrounding the muscle fiber does not extend into the junctional cleft (3).

The most distinctive feature of the junction is the postjunctional membrane. In appropriately oriented specimens it exhibits a clear trilaminar "unit" membrane structure (12) from which an array of projections extends ~160 Å towards the facing axolemma (Fig. 4). No separation can be detected between the projections and the outer dense lamina of the membrane even at high magnification and the projections therefore appear to be continuations of the membrane and not merely superimposed on it. Apparent interruptions in the outer dense lamina of the membrane are sometimes seen in relation to the projections (Fig. 5), but these "gaps" do not involve the inner dense lamina (Figs. 4–7). The projections are spaced at ~200 Å intervals except in the unosmicated specimens where the spacing is 10–20% less. Although the projections themselves do not reach completely across the junctional cleft, a few very thin filaments (Fig. 4) sometimes extend from their tips to the axolemma.

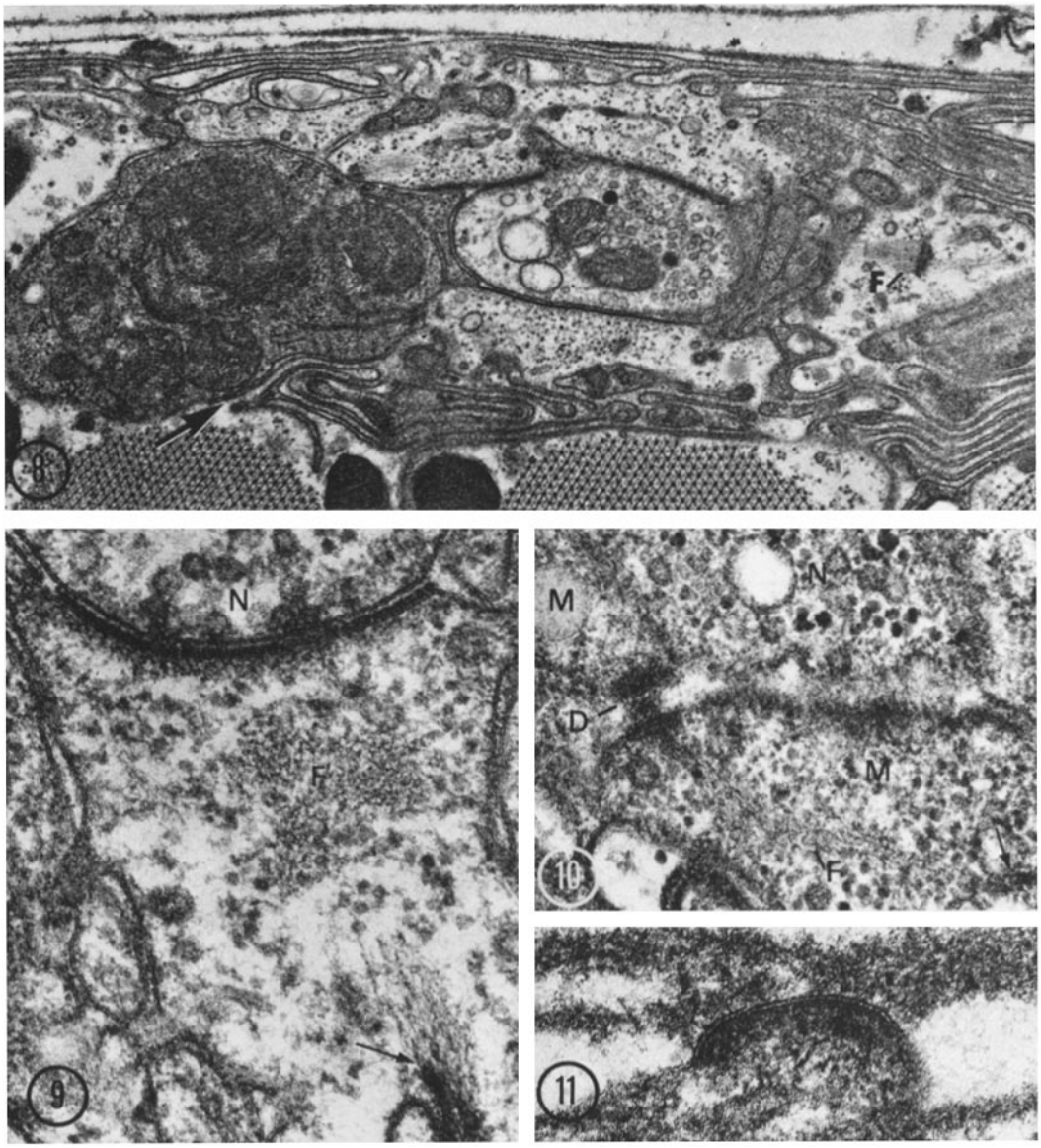
The projections are usually confined to muscle cell membrane directly apposed to the axon membrane but occasionally they may extend slightly beyond the edge of the axon. They follow irregularities in the contour of the postjunctional membrane faithfully, e.g., into V-shaped indentations (Fig. 3), and have never been seen lifted away from the underlying membrane. In contrast to basement membranes their attachment to the postjunctional membrane thus appears to be very firm. The projection-bearing muscle membrane ends abruptly near the edge of the junction (Figs. 2, 3) and has not been seen on any other portion of the muscle. As in the case of myoneural junctions in annelids (14) the projections are distinct in specimens that have not been postfixed with osmic acid but have been stained with permanganate and uranyl acetate (Fig. 7). In the beetle equivalent results were also obtained with a uranyl acetate-lead hydroxide stain on unosmicated specimens (Fig. 6). No distinction could be made between the projections and the outer dense lamina of the membrane in either case.

The cytoplasmic surface of the postjunctional muscle membrane is not coated by dense material although an ill-defined filamentous layer sometimes appears a small distance subjacent to it (Fig. 4). This membrane is thus easily distinguished from membranes specialized for adhesion at desmosomes and hemidesmosomes where a thick, dense, feltlike material is directly apposed to the cytoplasmic surface (Figs. 10, 11), but is similar to the membrane at septate junctions. Many of the postjunctional muscle processes contain glycogen particles and bundles of fine filaments which extend from desmosomes at the surface near the junction to nodal densities in the cytoplasm (Figs. 8–10). Few "aposynaptic" vesicles were seen in the postjunctional cytoplasm and it appears likely that most if not all of these structures, which were described in earlier reports, may originate from the breakdown of the paired infolded plasma membranes, which are so common in this region, under certain preparative conditions (13).

Physiologic studies indicate that the anterolateral region of the muscle is polyneuronally innervated (D. Ballantyne, personal communication), but thus far it has not been possible to subclassify the junctions found on the basis of qualitative morphological differences. Quantitatively, the nerve terminal profiles vary from ~0.5 to 2.0 μm in diameter and correspondingly in contact surface area. In addition some of the axon terminal profiles contain only clear vesicles, mitochondria, and glycogen particles while others contain a number of dense-cored vesicles and larger vacuoles as well (Figs. 1, 8). The number of junctions found was, however, insufficient for statistical analysis in order to assess whether these apparent variations in size and vesicle content reflect the existence of two or more morphologically distinguishable populations of endings, or are merely random sections though members of a single population (cf. reference 7).

DISCUSSION

This report describes an insect postjunctional membrane specialization in more detail than has been presented previously. It shows that this projection-bearing membrane is similar to that



FIGURES 8–10 Details of postjunctional cytoplasm.

FIGURE 8 The muscle process beneath the nerve fiber contains numerous glycogen particles but only one membranous vesicle. Arrow indicates connection between this process and the sarcoplasm immediately adjacent to a myofibril. Another muscle process at the right contains a bundle of fine filaments (*F*) leading to a nodal density. × 24,000.

FIGURE 9 A nodal density (arrow) appears at the bottom right in a postjunctional process. A bundle of fine filaments (*F*) is sectioned transversely above this. *N*, nerve ending. × 80,000.

FIGURE 10 Myoneural junctional cut tangentially. The nerve ending (*N*) is surrounded by muscle processes (*M*) which form a desmosome (*D*). A bundle of fine filaments (*F*) extends from this desmosome toward a nodal density at the lower right (arrow). × 72,000.

FIGURE 11 Hemidesmosome at the outer surface of a muscle fiber. The plasma membrane is convex outwards and, like the desmosomal membrane in Fig. 10, is coated on its cytoplasmic surface by a thick layer of dense material. The muscle cell basement membrane (top) is adherent to the outer surface. ×120,000.

recently described in annelids in dimensions, periodicity, and staining properties. Moreover, since neither the nerve nor muscle basement membrane enters the junctional cleft in insects it is clear in this case that the projections on the postjunctional membrane cannot represent basement membrane components. In both annelid and vertebrate somatic muscle junctions, basement membrane does extend into the cleft, and, it might be argued, could contribute to the projections. In addition, the *Pachnoda* postjunctional membrane differs from that of annelids in not having a dense coating on its cytoplasmic surface. It is therefore clearly distinguishable from the membrane at desmosomes and hemidesmosomes, which have heavy cytoplasmic coatings, and is presumably unrelated in function.

Earlier studies of insect myoneural junctions have reported a dense material within the junctional cleft in some cases related specifically to the postjunctional membrane (6, 16) and in some cases in the form of indistinct projections (10, 17). A periodically disposed coating has been found associated with the postjunctional membrane in crustaceans (1, 7, 9); however, the trilaminar structure of the membrane could not be visualized well enough in any of these to determine the precise relationship of the coating to the membrane. In annelid junctions (14, 15), hexagonally arrayed projections occur on the postjunctional membrane and on the basis of special staining methods, it is clear that the projections arise within the outer dense lamina of the membrane. The present paper offers evidence that this is true at an insect myoneural junction as well and that here too the projections probably do not merely represent a coating superimposed on the plasma membrane in the manner of a basement membrane but rather a component part of a specialized plasma membrane.

The postjunctional membrane may be viewed as a mosaic of macromolecular subunits each of which includes a projecting portion probably composed of protein or glycoprotein. The subunits of this specialized membrane presumably contain the receptor for the transmitter and perhaps also the transduction mechanism responsible for the end-plate potential, but not degradative enzymes in view of the evidence for transmitter inactivation by uptake rather than enzymtic breakdown in insects (4) as in crustaceans (8).

If the projection-bearing membrane does prove to be the location of the receptors then it would appear that the receptive surface in these animals is concentrated into "hot spots" rather than interspersed to a degree with nonspecialized membrane. A similar conclusion has also been drawn from the results of iontophoretic studies (2, 18). The mechanism by which the integrity of such sharply demarcated specialized patches is maintained in a semifluid membrane is a general problem confronting cell membrane physiologists. In this case it may be the axon terminal that maintains the localization of the specialized postjunctional membrane perhaps by way of the fine filaments that extend between the projections and the axolemma.

The extent of the projection-bearing postjunctional membrane may prove useful as a more accurate measure of the size of the receptive surface than the total nerve-muscle contact area and also as a visible gauge of changes in the receptive surface under different physiological and pathological conditions.

The specimens were obtained and fixed by the author at the Zoology Department, Oxford University. I am indebted to Professor J. W. S. Pringle for making the facilities of the department available and to Mr. David Ballantyne for discussions of his unpublished physiologic data on this muscle and for his help in obtaining the specimens.

This study was supported by grant NS 07495 from the United States Department of Health, Education, and Welfare.

Received for publication 9 April 1973, and in revised form 19 June 1973.

REFERENCES

1. ANDERSON, M. E., and D. SMITH. 1971. *Tissue Cell.* **3**:191.
2. BERANEK, R., and P. L. MILLER. 1968. *J. Exp. Biol.* **49**:83.
3. EDWARDS, G. A. 1959. *J. Biophys. Biochem. Cytol.* **5**:241.
4. FAEDER, I. R., and M. M. SALPETER. 1970. *J. Cell Biol.* **46**:300.
5. GERSCHENFELD, H. M. 1973. *Physiol. Rev.* **53**:1.
6. HAMORI, J. 1963. *Acta Biol. Acad. Sci. Hung.* **14**: 231.
7. HOYLE, G., and P. A. MCNEILL. 1968. *J. Exp. Zool.* **167**:523.
8. IVERSEN, L. L., and E. A. KRAVITZ. 1968. *J. Neurochem.* **15**: 609.
9. KOMURO, T. 1970. *Z. Zellforsch. Mikrosk. Anat.* **105**:317.

10. OSBORNE, M. P. 1967. *J. Insect Physiol.* **13**:827.
11. PRINGLE, J. W. S. 1972. *In* Structure and Function of Muscle. G. H. Bourne, editor. Academic Press, Inc., New York. 2nd edition. **1**:491.
12. ROBERTSON, J. D. 1959. *Biochem. Soc. Symp.* **16**:3.
13. ROSENBLUTH, J. 1963. *J. Cell Biol.* **16**:143.
14. ROSENBLUTH, J. 1972. *J. Cell Biol.* **54**:566.
15. ROSENBLUTH, J. 1973. *Anat. Rec.* **175**:428.
16. SMITH, D. S. 1960. *J. Biophys. Biochem. Cytol.* **8**: 447.
17. SMITH, D. S., and B. SACKTOR. 1970. *Tissue Cell.* **2**:355.
18. USHERWOOD, P. N. R., and P. MACHILI. 1968. *J. Exp. Biol.* **49**:341.