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SURVEY FOR MITOCHONDRIAL-DESMOSOME COMPLEXES IN DIFFERENTIATING EPITHELIA*

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Summary. Mitochondria are frequently found to be closely associated with the plaques of desmosomes in a variety of columnar or cuboidal epithelia of fetal or early postnatal mammals (mouse, rat, human being). The organs in which mitochondrial-desmosome complexes were found include stomach, small intestine, pancreas, kidney, epididymis, seminal vesicle, coagulating gland, thyroid gland. The association has not been observed in simple squamous epithelium (vascular endothelium). Mitochondria lie quite close to desmosomes in the stratum spinosum of stratified squamous mucous epithelium of fetal animals and also to axo-dendritic synapses in still poorly differentiated central nervous system. Mitochondria have also been detected close to attachment sites in ectoderm of the early frog gastrulae. Here there is as yet no visible plaque material.

We suggest that the mitochondria may provide energy or some chemical for the formation of the plaque. This hypothesis does not explain why the complexes are not found in poorly differentiated epithelia from older animals.

Introduction

We first noted close association of mitochondria and desmosomes in electron micrographs of the columnar epithelium of seminal vesicles in prepuberal mice (between birth and 4 weeks of age) (DEANE and WURZELMANN, 1965a) and subsequently found them also to occur in the coagulating glands and epididymides from animals in the same age group (DEANE and WURZELMANN, 1965b). In the organs of the male reproductive tract from older animals, no such proximity was demonstrable. In mice, the epithelia lining these organs, albeit composed of columnar cells at birth, are still quite unspecialized and become well differentiated during the third and fourth postnatal weeks. Furthermore, during the first month, desmosomes appear to become progressively more common along the lateral margins of the cells, being at first essentially restricted to the apical junctional complexes, immediately below the terminal bar (FARQUHAR and PALADE, 1963).

The timing of the mitochondrial-desmosome association, coupled with the fact that electron-dense material or microtubules lie between the outer mitochondrial membrane and the thick dense plaque on the inner aspect of the plasmalemma at the desmosome, suggested that mitochondria might somehow be associated with the formation of the desmosomal structure. As an initial step in exploring this hypothesis, we therefore undertook to survey a variety of epithelia from young mammals of different ages, both pre- and postnatal, to learn whether mitochondrial-desmosome complexes occur in organs outside the male reproductive tract. We also sampled nervous tissue as an ectodermal derivative. As an example of

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early embryonic epithelium, we examined early gastrulae of the frog. The present paper summarizes the results of our survey. A brief report has been submitted elsewhere (DEANE and WURZELMANN, 1966).

Material and Methods

The initial observations were made on tissues obtained from male mice killed between the first day after birth and maturity, i.e., about 3 months of age. Developing seminal vesicle and coagulating gland, plus in many instances caput epididymidis, were obtained from over 50 young mice (DEANE and WURZELMANN, 1965a, 1965b). Samples of gastric, duodenal and colonic wall from young adult mice (DEANE, 1964) were also examined.

Subsequent observations were made on the following mammalian epithelia, which were selected quite arbitrarily: Forestomach and glandular stomach, small intestine, pancreas and epididymis of 20-day-old fetal rats; small intestine of 19-day-old fetal mice; medulla of neonatal mice; thyroid gland, forestomach, glandular stomach, and small intestine of suckling mice of about 18 days of age. In addition, Dr. JAY BERNSTEIN of the Department of Pathology, A. Einstein College of Medicine, provided us with several blocks of kidney cortex from two different human fetuses of about 16 weeks gestational age, obtained at therapeutic abortion.

We also examined a number of segments of mouse seminal vesicle that had been removed from 18-day-old animals and grown in organ culture for 3 or 6 days thereafter on a semidefined medium, with or without added testosterone propionate; these were provided by Dr. ILSE LASNITZKI of the Strangeways Research Laboratory, Cambridge, England. The culture method was the same as the one she used earlier for rat ventral prostate (LASNITZKI, 1965).

Finally, samples of animal-pole tissue from early gastrulae (Stage 12) of the frog, *Rana pipiens*, were taken from eggs from which the vitelline membrane had just previously been removed either manually, or was earlier digested away with papain. In two instances, the ectodermal cells had been dissociated with EDTA in calcium- and magnesium-free medium (MORRILL and KOSTELLOW, 1965), then allowed to reaggregate in a complete cell culture medium at 12° C for 12 to 18 hours (STEARNS and KOSTELLOW, 1958). Such reaggregates form sheets that remain intact when lifted with a spatula, suggesting that firm cellular adhesions have developed.

The samples were fixed variously in (a) 1 or 2% OsO_4, generally buffered with 0.1 M phosphate, pH 7.4 (DEANE, 1963), or (b) Dalton's chrome-osmium mixture (GLAUERT, 1965), or primarily fixed in (c) 10% buffered formalin, made freshly from paraformaldehyde as described by PEASE (1965). The aldehyde-fixed tissue was then refixed in an OsO_4 solution. The blocks were dehydrated through ethanol and propylene oxide and embedded either in LUFT's (1961) Epon resin mixture or in an Araldite mixture (GLAUERT, 1965).

Thin sections cut at approximately 75 mμ were stained successively with 0.25% uranyl acetate in 50% ethanol and the lead citrate solution devised by REYNOLDS (1963). The preparations were studied in an RCA EMU-3E or 3G.

Observations

Of the examples of incompletely differentiated mammalian organs examined, we have quite readily been able to find mitochondria associated with desmosomes in a number of simple cuboidal and columnar epithelia. (We have never seen the association in squamous epithelia, as, for example, in the endothelia of blood vessels.)

The organs in which we found mitochondrial-desmosome complexes include the epithelium of fetal rat glandular stomach (Figs. 1, 2), pancreas (Fig. 3) and epididymis (Fig. 6); fetal mouse intestine (Fig. 4); fetal human renal proximal tubules; and young postnatal mouse epididymis (Fig. 5), seminal vesicle, coagulating gland, and thyroid gland (Fig. 8). Mitochondrial-desmosome complexes also occur in early secretory seminal vesicle maintained for nearly a week in organ culture (Fig. 7).

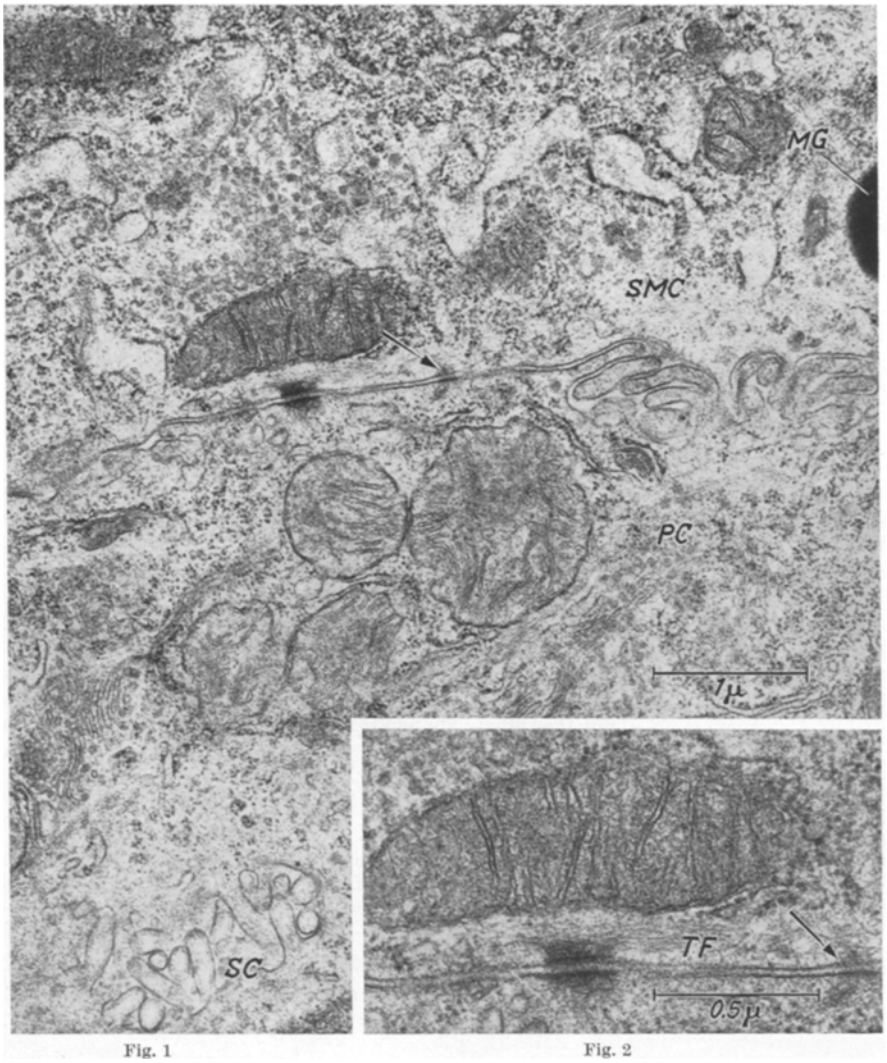

Fig. 1 Fig. 2

Fig. 1. Glandular stomach of a 20-day fetal rat, showing apposing portions of a surface mucous cell (*SMC*) and a parietal cell (*PC*); apical regions of cells to the right. *SC* secretory canaliculus of the parietal cell; *MG* mucous granule. A well developed desmosome attaching the two cells possesses one closely associated mitochondrion. Tonofilaments course into the desmosomal plaque. A second desmosome appears to be developing to the right (arrow). Fixed in Dalton's chrome-osmium mixture; Epon. × 19500

Fig. 2. Higher power micrograph of the mitochondrion, tonofilaments (*TF*) and desmosomes shown in Fig. 1. × 42000

In these examples, sometimes a single mitochondrion lies adjacent to one plaque of a desmosome (e.g., Figs. 1, 2, 4, 5, 6, 7). Just as frequently, mitochondria flank both sides (Figs. 3, 5, 8). In some instances, electron-dense material seems to extend between the outer surface of the mitochondrion and the plaque (Figs. 3, 6, 7, 8). Tonofilaments are detectable coursing through the intervening space in some specimens (Figs. 1, 2; see also DEANE and WURZELMANN, 1965a; Figs. 18, 20).

We have extensive series of developmental stages only for seminal vesicle and epididymis. In the youngest specimens from both mouse and rat, mitochondrial-

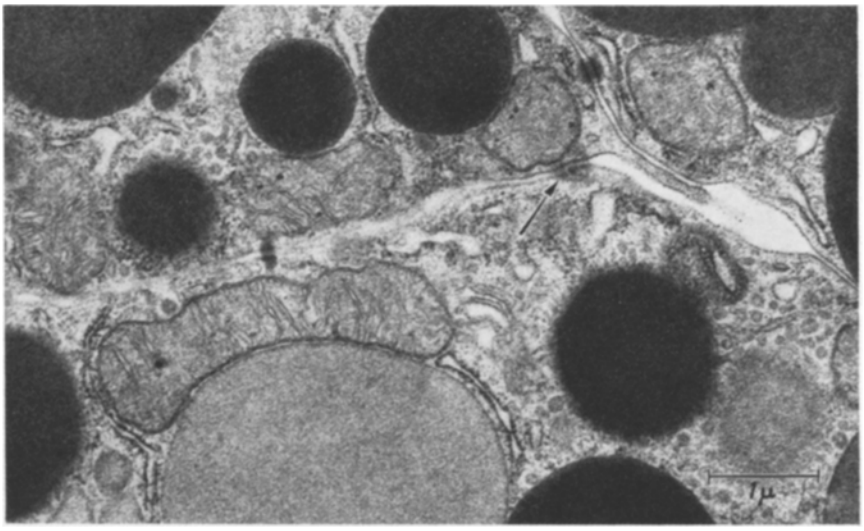

Fig. 3. Exocrine pancreas of a 20-day fetal rat, showing cross sections of the apical portions of three acinar cells filled with secretory droplets of varying density. The desmosome in the center of the field is closely flanked by two mitochondria; electron-dense material seems to extend between the mitochondrial surfaces and desmosomal plaques. A less fully differentiated desmosome to the right (arrow) has one nearby mitochondrion. Mitochondria are farther from the third desmosome close to the top of the picture. Fixed in Dalton's fluid; Epon. × 14000

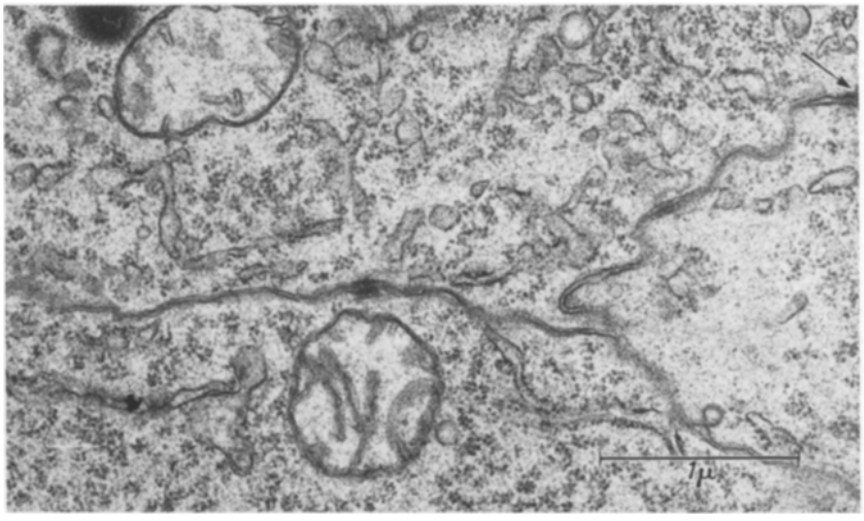

Fig. 4. Small intestine of a 19-day fetal mouse, showing segments of three absorptive cells. The desmosome in the center of the fields is flanked on one side by a mitochondrion. A newly forming desmosome (arrow) lies at the edge of the picture to the far right. Fixed in 2% OsO_4 in phosphate buffer; Epon. × 26000

desmosome complexes are limited to the apical portions of the cell — i.e., to the desmosomes comprising elements of the junctional complex as defined by FARQUHAR and PALADE (1963) (see Fig. 6; also DEANE and WURZELMANN, 1965a, Figs. 4, 7, 9). The same organs from older animals show mitochondrial-desmosome complexes also lying lower in the cells (Fig. 5; DEANE and WURZELMANN, 1965a; Figs. 15, 18, 19). In specimens of these organs and of the others cited here, of course, many

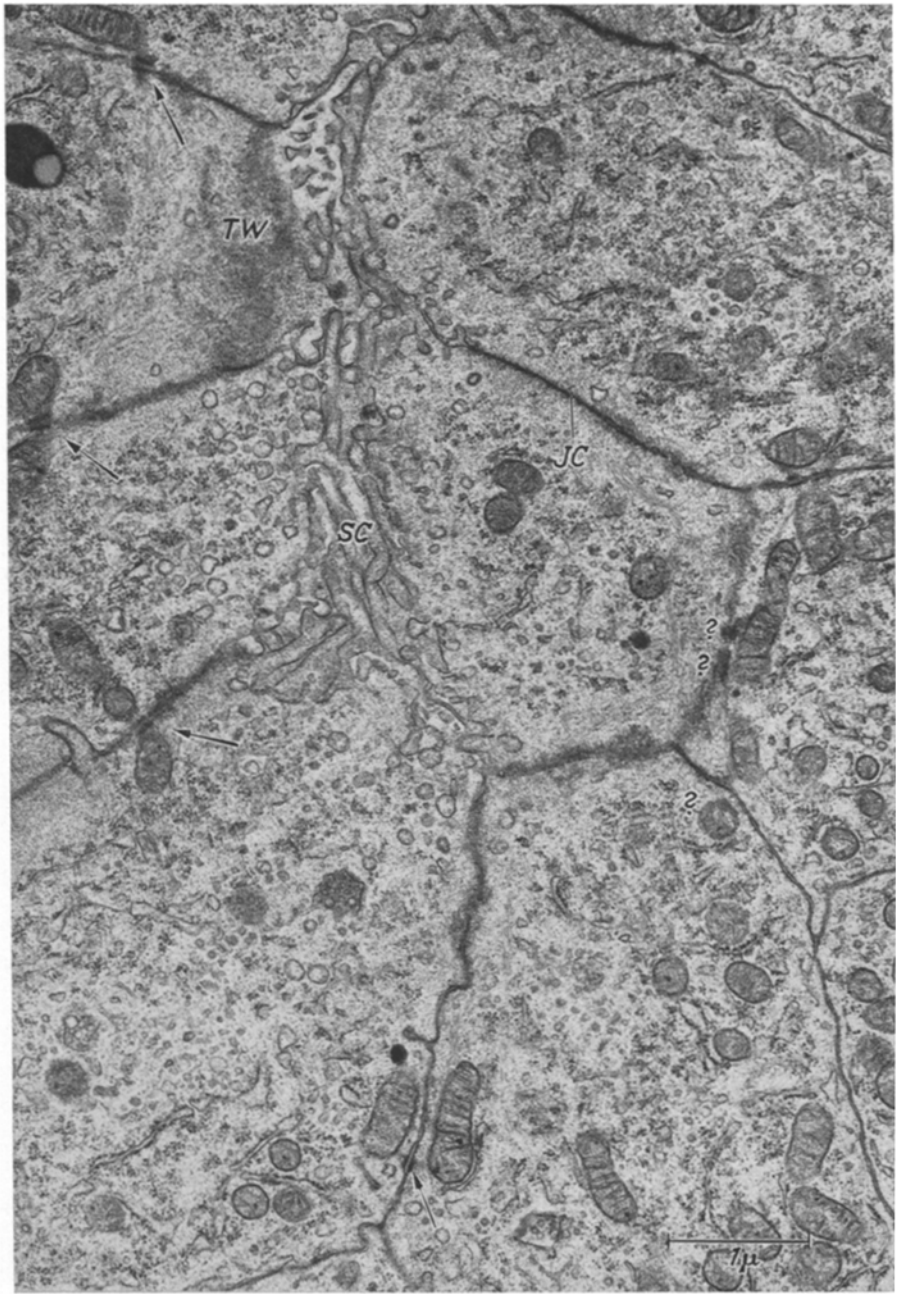

Fig. 5. Somewhat oblique section through the apices of epithelial cells of the caput epididymidis of a 22-day old weanling mouse. Five desmosomes in the field, marked with arrows, are flanked by one or two mitochondria. Others, marked with question marks, exhibit nearby mitochondria. Labels: *SC* immature stereocilia; *JC* terminal bar portion of the junctional complex; *TW* terminal web. Fixed in 2% OsO_4 in phosphate buffer; Epon. × 18000

desmosomes show no associated mitochondria. In neither reproductive organ studied extensively have the complexes been found in mice or rats exceeding a month

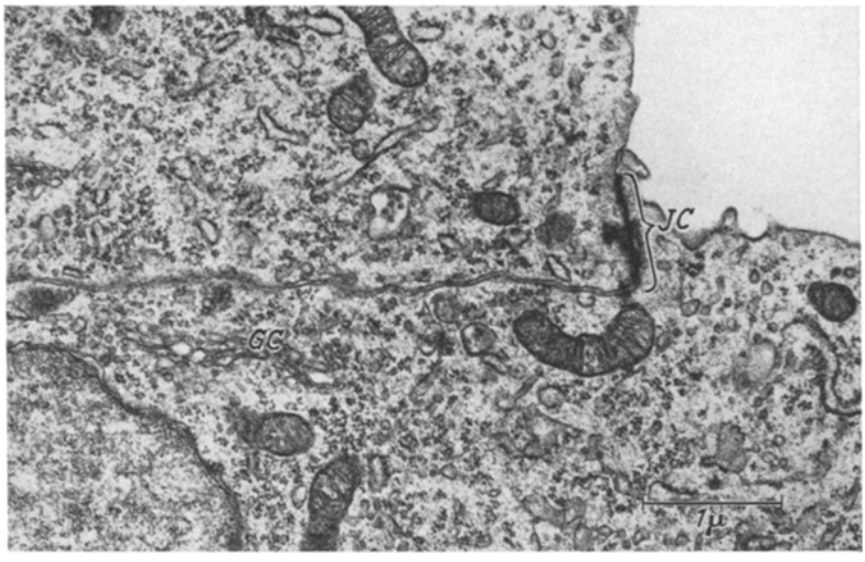

Fig. 6. Epididymal epithelial cells of a 20-day fetal rat. The desmosome of the junctional complex (*JC*) has a single associated mitochondrion. *GC* Golgi complex. Fixed in Dalton's solution; Epon. × 17600

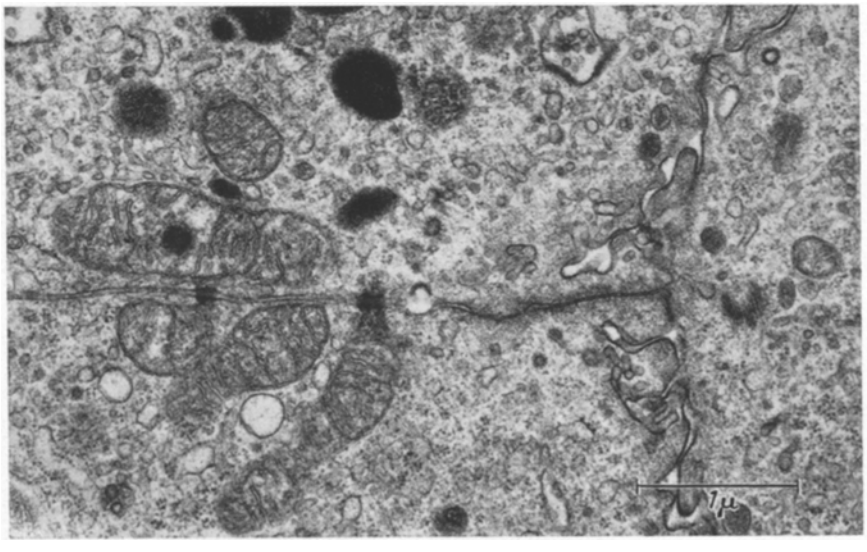

Fig. 7. Seminal vesicle cells of gland removed from an 18-day-old mouse and grown for 6 days in organ culture on semidefined medium (LASNITZKI, 1965). Two desmosomes below the junctional complex exhibit associated mitochondria. Particularly in the case of the more apical one, dense material appears to stream from the mitochondrion to the plaque. (Large internal granules characterize the mitochondria of these cultured cells.) Fixed in Dalton's solution; Araldite. × 20700

in age. Likewise, we have not detected the association in various parts of the gut of postnatal animals.

In addition to simple epithelia, we have examined two types of stratified epithelia and also nervous tissue to see whether such complexes could be found. The forestomach of fetal and suckling rats and mice served as an example of a

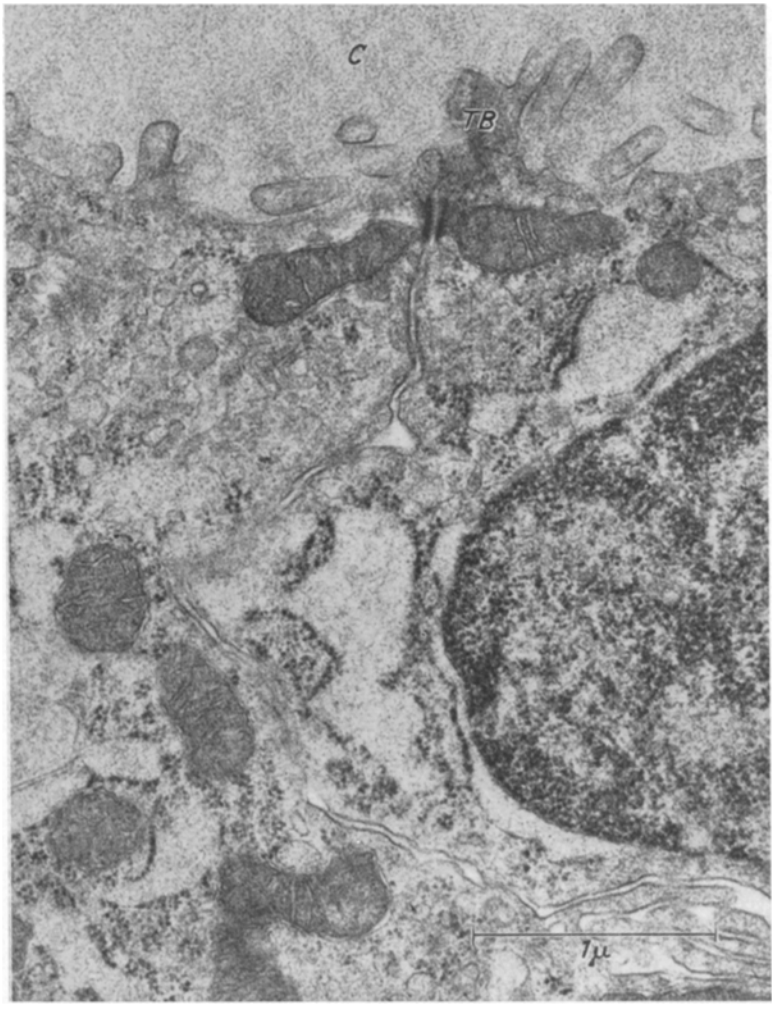

Fig. 8. Thyroid gland of an 18-day old mouse, showing portions of two follicular cells. *C* colloid. The desmosome below the terminal bar (*TB*, cut obliquely) is flanked by two mitochondria; dense material lies between the mitochondria and plaques. Fixed in Pease's formalin followed by 1% OsO_4 in phosphate buffer; Epon. × 32000

multilayered mucous epithelium. Even in the sample from a 20-day fetal rat, the epithelium is very thick, with the cells near the base showing well developed desmosomes and abundant clusters of tonofilaments (Fig. 9). In this specimen, we have detected quite a few instances in which mitochondria lie within a half-micron of a desmosome. We have failed to find cases even this closely associated in the postnatal specimens. We have never found mitochondria associated with the basal hemidesmosomes.

As an example of a far less well organized multilayered epithelium, but one in which the cells are loosely adherent to one another on all sides, we looked at the animal-pole tissue from several early gastrulae of *R. pipiens*. The cells show alternating attachment sites and gaping intercellular spaces (Fig. 10). In several cases,

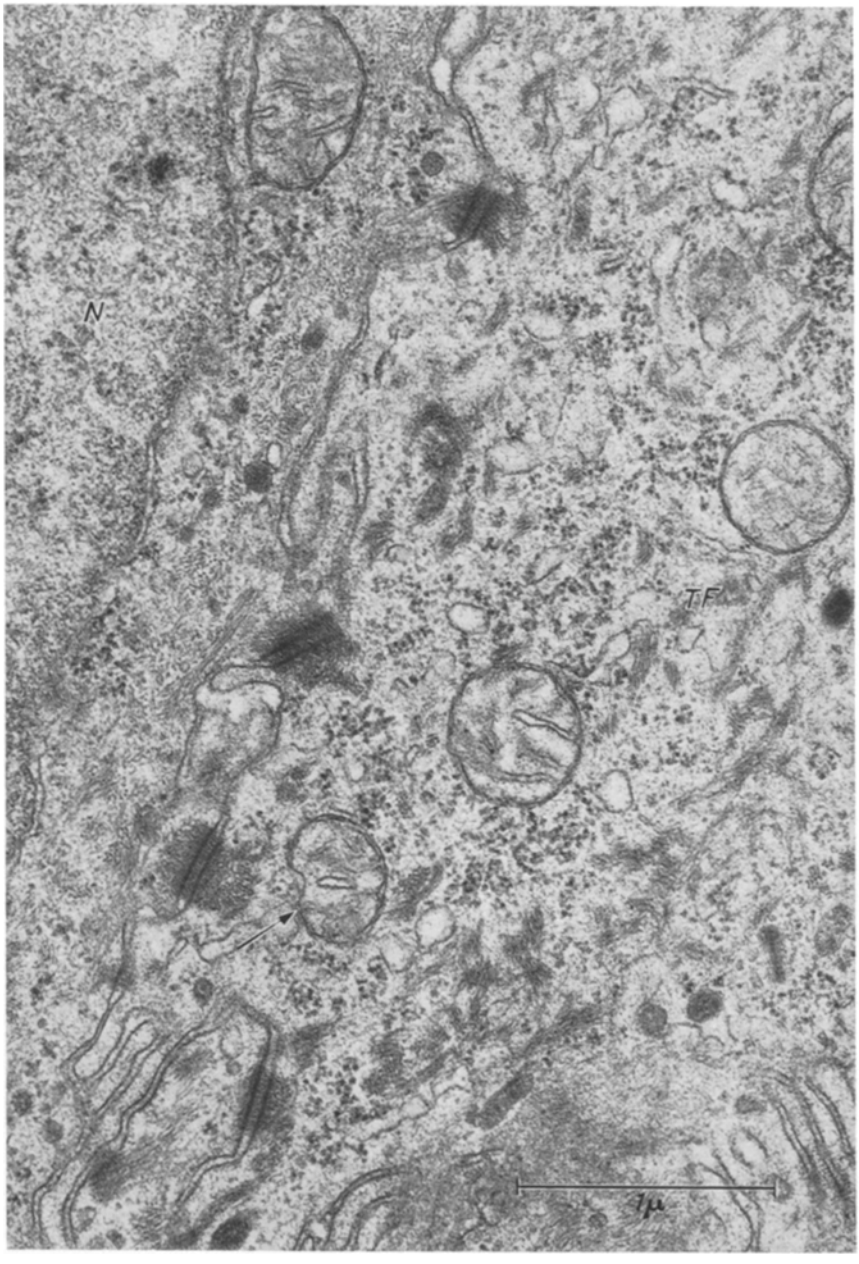

Fig. 9. Forestomach of a 20-day fetal rat, showing two adjacent "prickle" cells (stratum spinosum) attached by numerous desmosomes. The mitochondrion marked by an arrow lies close to a desmosome. *N* nucleus of an epithelial cell; *TF* tonofibril. Fixed in Dalton's fluid; Epon. × 34400

derived both from intact gastrulae and from animal-pole tissue that had been dissociated and allowed to reaggregate, examples of mitochondria may be found close to an adhesion site. It is noteworthy that in these young specimens, whether fixed first in formalin and then in osmic acid, or in osmic acid alone, there is only

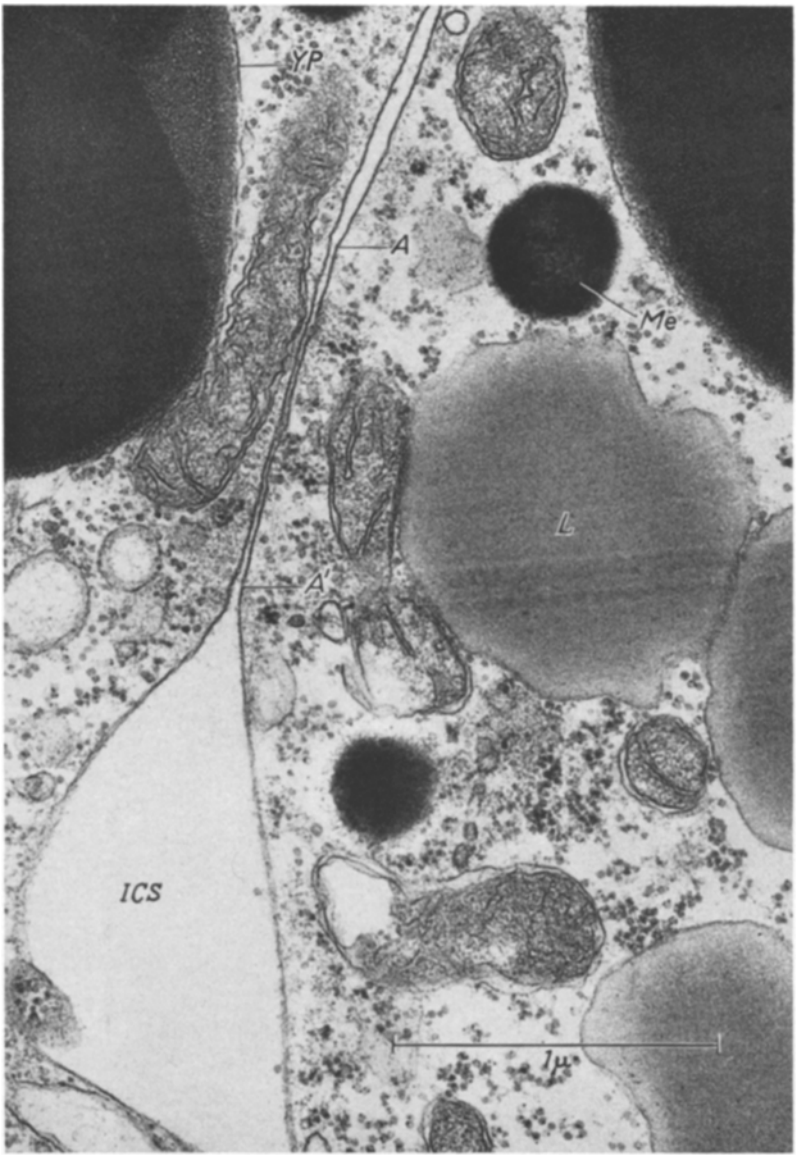

Fig. 10. Portions of two animal-pole cells from a frog gastrula, showing an attachment site (*A-A'*) flanked by two mitochondria. The cytoplasm near the attachment site is somewhat denser than elsewhere, but true plaques have not developed. *Me* melanin granule; *YP* yolk platelet; *L* lipid droplet; *ICS* intercellular space. Fixed in Pease's formalin followed by 1% OsO_4 in phosphate buffer; Epon. × 42000

the slightest hint of dense plaque material along the inner aspect of the plasmalemma at areas of attachment. Therefore, strictly speaking, these probably should not yet be considered desmosomes. There is some sign of accumulating dense substrate, but this is intermixed with ribosomes.

Finally, for the counterparts of desmosomes in neurectoderm, we examined synapses in gray matter from the medulla oblongata of the newborn mouse. In

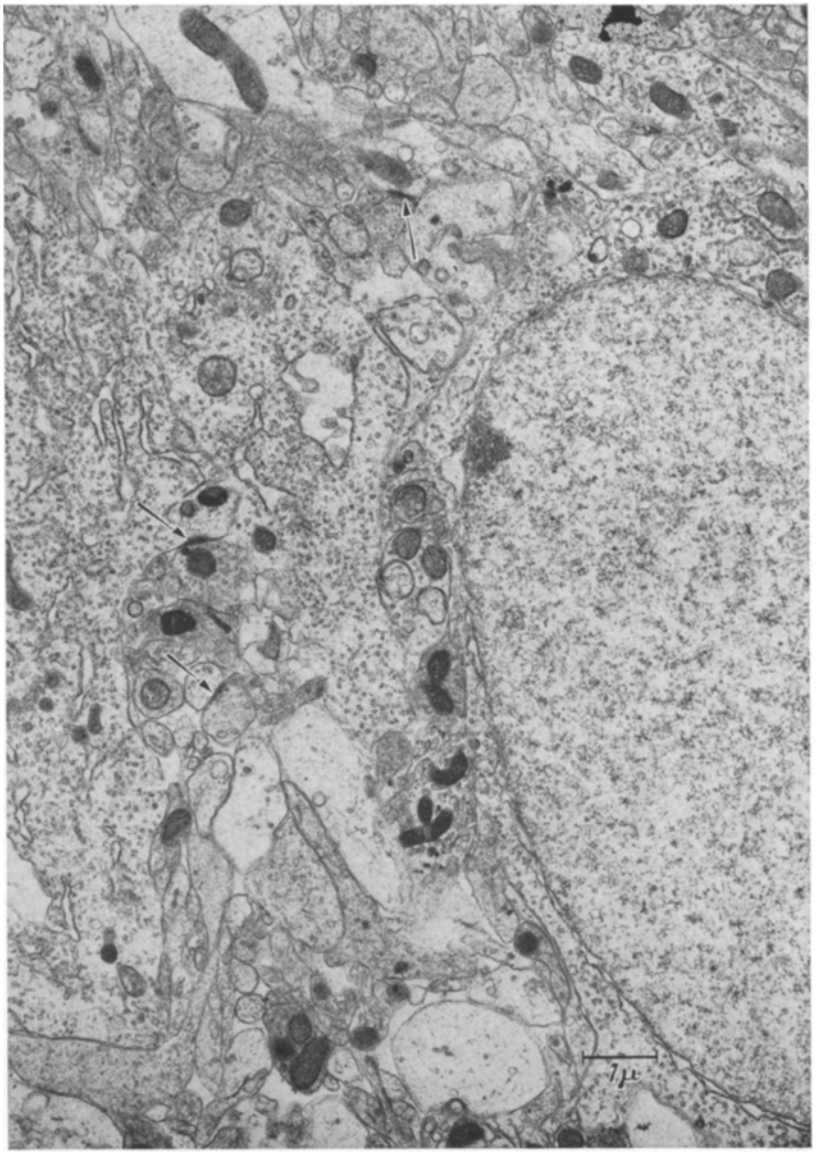

Fig. 11. Medulla oblongata of a neonatal mouse, with three axo-dendritic synapses in the field (arrows). The upper two each show a closely associated mitochondrion. Fixed in Pease's formalin followed by 1% OsO_4 in phosphate buffer; Araldite. × 9800

fact synapses still proved quite rare, but we did find a few examples and in several of these a mitochondrion may be seen associated closely with the plaque. Fig. 11 shows a low-power field containing a small cell and a surrounding cluster of nerve and glial processes; there are three clearly identifiable synapses, two having adjacent mitochondria. Of course, mitochondria are generally quite abundant near nerve terminals, but the examples here illustrated lie unusually close to the plaque.

Discussion

From the results of this survey, we would now predict that mitochondria are associated with desmosomes at some early stage in the development of most if not all columnar and cuboidal epithelia. The association may also characterize the cells in the lower layers of stratified epithelia. Among the mammals studied (mouse, rat, human being), such complexes are common in the relatively unspecialized tissues of fetal or early postnatal individuals. They have not been seen in tissues from adults, even when the tissues are relatively unspecialized, as, for example, at the bases of intestinal glands or in the basal layers of the gastric forestomach. Also, the complexes have not been detected at the joints of squamous epithelia, but these may well resemble terminal bars rather than desmosomes.

It is exciting that the association of mitochondria with a firm attachment site appears also to occur at immature nerve-to-nerve synapses, which are counterparts of desmosomes, occurring in a tissue derived from typical epithelium. It would therefore be of considerable interest to see whether they also occur at some time in the development of the nerve-to-muscle junction. A final site that might be investigated is the intercalated disk of cardiac muscle — the most conspicuous example of firm cell-to-cell attachment in a tissue that is neither epithelioid nor of epithelial origin.

The finding of these mitochondrial-desmosome complexes in a varied sampling of organs does not help us understand the basis for the association. The two principal possibilities seem to be that the mitochondria may provide energy for some synthetic process, or they may be the source of some essential chemical substance for the desmosomal structure. We know very little about the substances composing elements of the desmosome. There appears to be a concentration of mucopolysaccharide between the cells at desmosomes and similar adherent sites (references, KELLY, 1966). [For an alternative hypothesis of the nature of the intercellular material, see FARQUHAR and PALADE (1965).] In addition, as has been long known to embryologists, calcium ions are apparently involved in cell-to-cell adhesion and are necessary for reaggregation (CURTIS, 1962). Compatible with this fact is that treatment of early embryonic and some other epithelia with EDTA causes cell dissociation (e.g., SEDAR and FORTE, 1963; MORRILL and KOSTELLOW, 1965). Even less seems to be known about the nature of the electron-dense intracellular plaque apposed to the plasmalemma at the attachment site. Its electron density would be compatible with a lipoprotein nature, among other possibilities, and the fact that desmosomes stain with Sudan dyes and acid hematein serves to support this hypothesis (WISLOCKI, 1951). However, additional substances may be present, and the role of the plaque remains unclear. From KELLY's (1966) recent study of embryonic and larval amphibian epidermis, the plaque (or associated substances) seems to serve in some undefined fashion as an anchoring point for clusters of tonofilaments (tonofibrils) as they loop past.

In this connection, it may be pertinent that mitochondria appear to flank attachment sites before the plaques have developed, or at least before the plaque material can be preserved in an electron-dense state. Such pairings were seen in some seminal-vesicle cells in an earlier study (DEANE and WURZELMANN, 1965a), and were also particularly evident here in the animal-pole tissue of the frog gastrula. These associations seemed no more abundant in ectoderm that had just under-

gone reaggregation than in intact animal-pole tissue. This lack of difference suggests that the mitochondrial association may be related to plaque formation rather than to cell attachment *per se*. How to test this hypothesis remains the problem. This hypothesis also fails to explain the absence of the complexes from incompletely differentiated tissues of adults in which desmosomes and their plaques are forming.

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