AN ANALYSIS OF DESMOSOME SHAPE, SIZE, AND ORIENTATION BY THE USE OF HISTOMETRIC AND DENSITOMETRIC METHODS WITH ELECTRON MICROSCOPY

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ABSTRACT

The probable shape, size, and orientation of desmosomes of the cells comprising the secretory tubules in rat submaxillary gland was determined by statistical and algebraic methods applied to electron micrographs. It was concluded that these desmosomes are discrete ellipsoidal discs whose principal axes are in the order of 4100 and 2500 angstrom units, and that they are preferentially oriented with their long axis more or less parallel to the base-apex axis of the cell. Densitometric interpretation agrees with the statistically based reconstruction of desmosomal shape. By densitometric analysis it was also determined that the peak to peak distances between layers within these desmosomes are in essential agreement with other reported findings. The approach described may have general applications to problems in the analysis of submicroscopic morphology.

Desmosome-like structures have been described for a great variety of cells (4–8, 10–13, 15, 16, 18). Analyses of the structure of desmosomes invariably place emphasis on the cross-sectional pattern while their planar aspect receives relatively little attention. The reasons for this seem to be that these structures have low contrast in plane projection, and also that, with the exception of epidermis and oral mucosa, desmosomes account for a proportionately small volume of any given section, and are seldom, if ever, seen in an aspect that would suggest what the appearance of this other dimension may be. The planar aspect of desmosomes in human epidermal and mucosal epithelial cells reveals that these structures are "ovoid" in outline (Odland, 16; Stern, 17) and therefore it is apparently assumed that this form may be a generalized one true for these structures in other cell types as well.

Desmosomes are a prominent feature of cells forming the secretory duct portion of the rat submaxillary gland (See Figs. 1 and 2). It has been taken for granted that these desmosomes, too, are ovoid in their planar aspect. However, in sections where these tubules are cut longitudinally (Fig. 1), the desmosomes are arrayed along opposite longitudinal sides of the cells in a manner suggesting that their three dimensional form could be ribbon-like, arranged more or less perpendicular to the long axis, and surrounding the cell in a way similar to that suggested by Hama (11) for desmosomes of frog mesothelial cells. An objective verification concerning the nature of desmosomes in these tubule cells, therefore, seems desirable. The probability of getting a fortuitous planar section of a tubule cell desmosome is remote; in fact, no such figure was recognized in hundreds of electron micrographs. Because of this, the problem was pursued in other ways.

If desmosomes assume a ribbon-like form more

or less parallel to the base of the cell, then those seen in sections perpendicular to the long axis of the cell should on the average be longer than those seen in sections parallel to the long axis. On the other hand, if the desmosomes are randomly oriented, discrete ovoid plaques, one would expect no difference in their lengths whether the cell is sectioned longitudinally or transversely. A statistical comparison was therefore made between desmosome lengths in cells cut more or less in cross-section (Fig. 2) and those in cells cut more or less longitudinally (Fig. 1). Since this type of evidence is essentially circumstantial, another form of evidence which would either support or contradict the statistical findings was sought. The second approach to the interpretation of desmosome shape was based on the asymmetric nature of elliptic segments under certain conditions. The reasoning for this is developed in the Discussion. The cross-sectional morphology of tubule cell desmosomes was also analyzed densitometrically and compared with reported findings.

METHODS AND MATERIALS

Rat submaxillary gland tissue was removed under light ether anesthesia, fixed in 3 per cent osmium tetroxide, buffered to pH 7.42 with *s*-collidine at 4°C (Bennett and Luft, 2), dehydrated in graded alcohols, and embedded in Araldite (Luft, 9) which was then polymerized at 60°C for 15 hours. Sections were cut on a Porter-Blum microtome with glass knives. Section thickness, as judged by interference colors, was between 500 and 800 A. Sections were mounted on carbon-coated grids, stained with lead after the method of Millonig (14), and observed with a modified RCA-EMU-2-C.

Densitometric analyses were made with a Joyce microdensitometer on micrographs printed on Kodak fine grain positive transparency material.

Microscope magnification was calibrated with a Fullum standard diffraction grating replica (28,800 lines per inch). It could be shown that the error in linear measurement, from grid to grid, due to microscope magnification changes was kept under 10 per cent. The degree of photographic enlargement was determined by comparing point to point distances on the prints with those on the negatives, using a vernier gauge accurate to 0.05 mm.

Measurements of desmosome lengths were made only on cells cut approximately parallel or perpendicular to the longitudinal axis. The criterion for approximately parallel sections was that some of the apical and basal cell membrane must be visible in the same cell. The criteria for approximately perpendicular sections were that neither apical nor basal cell membranes be visible and that the over-all cell boundaries be relatively undistorted in any single direction. A great number of fields were examined in order to get a sampling which fulfilled the above criteria. The measurements were finally determined from a total of eleven grids representing five different blocks of tissue taken from two animals.

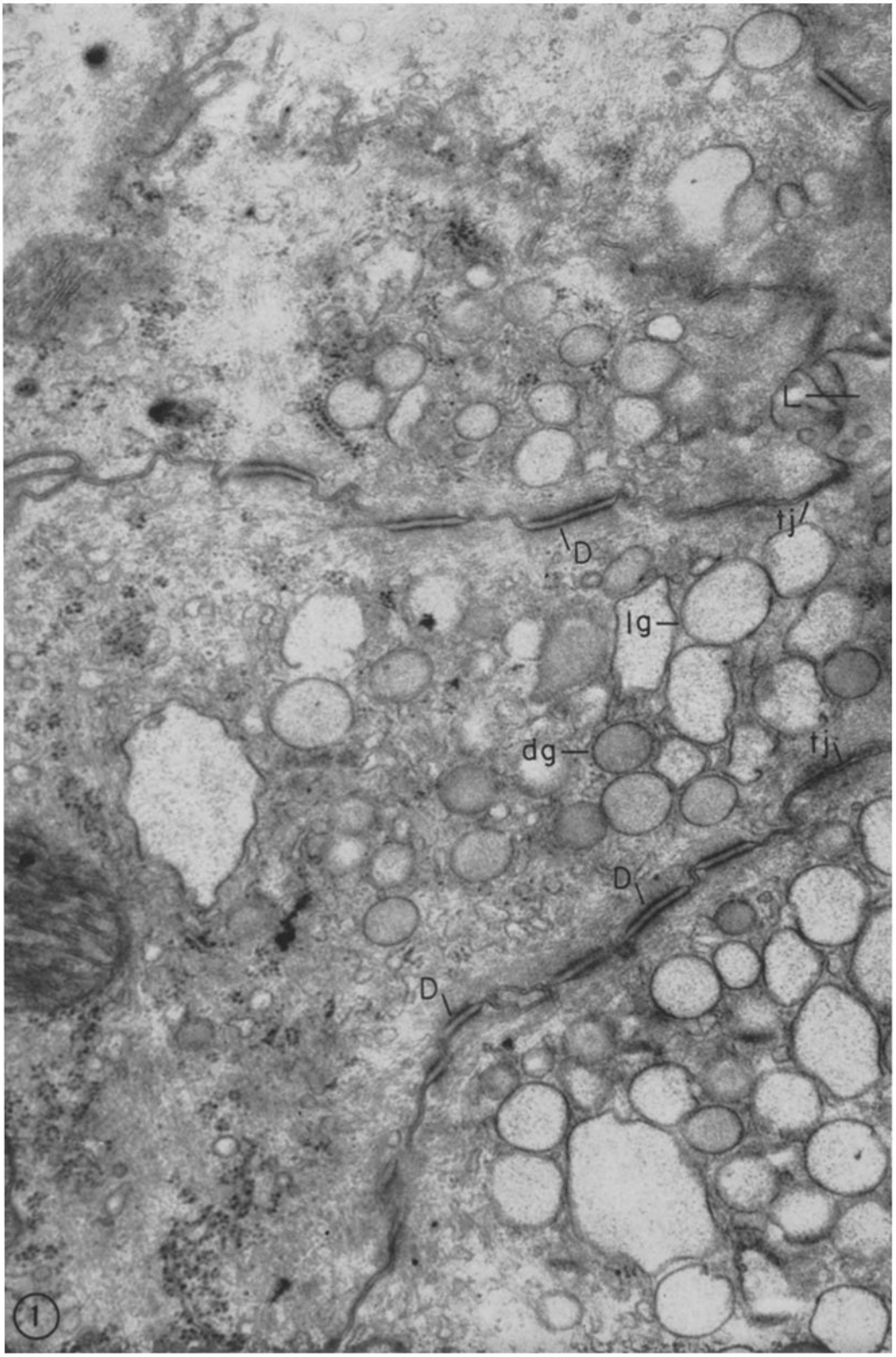
RESULTS AND DISCUSSION

A comparison of lengths of 40 desmosomes from "longitudinally" sectioned cells with 38 from "transversely" sectioned cells was made by the unpaired t test. The mean length of desmosomes from "longitudinal" sections was 3832 A (SD = $\pm$210.5) while the mean length from "transverse" sections was 2364 A (SD = $\pm$198.6). The difference between the two groups was significant ($t = 31.6$; $P < 0.001$). The F test on these data, however, was not significant ($F = 1.12$; $P > 0.10$). This statistically supports the premise that we are dealing with one population. The significance of the t test, therefore, must be due to the difference in cellular orientation of the two groups.

The above result may be interpreted in a number of ways. A mesh-like arrangement of narrow longitudinal and wider transverse "ribbons" crisscrossing the cell surface could account for the desmosome section's being wider when cells

FIGURE 1

The apical third of a rat submaxillary gland secretory tubule cell sectioned longitudinally. Desmosomes (*D*) are arrayed along opposite sides basal to "tight junction" (*tj*) attachment zones which invariably occur adjacent to the luminal (*L*) aspect of the cell. Membrane-bounded light (*lg*) and dark (*dg*) granules are characteristic of these cells. × 21,500.


L
tj
D
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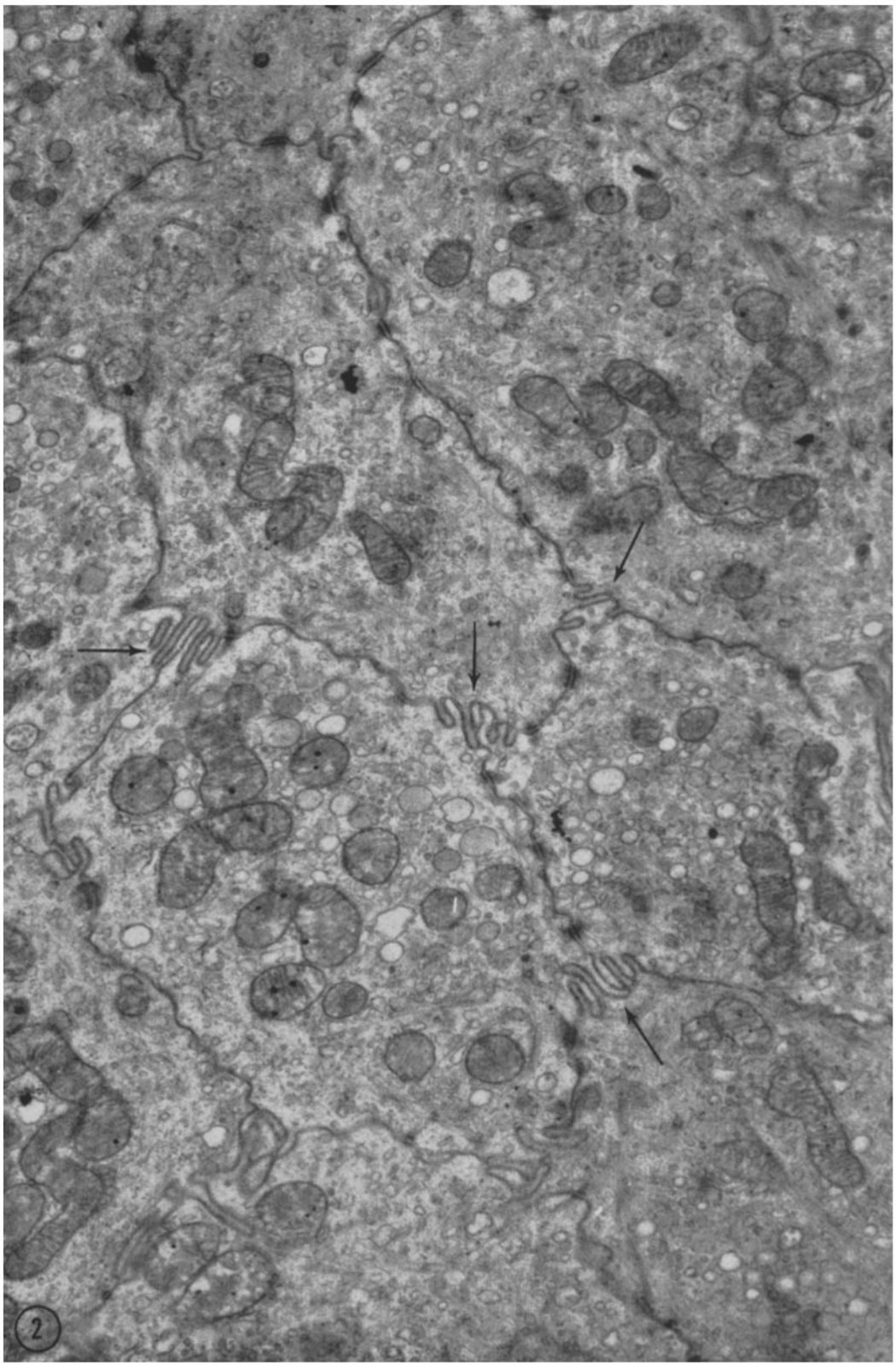
1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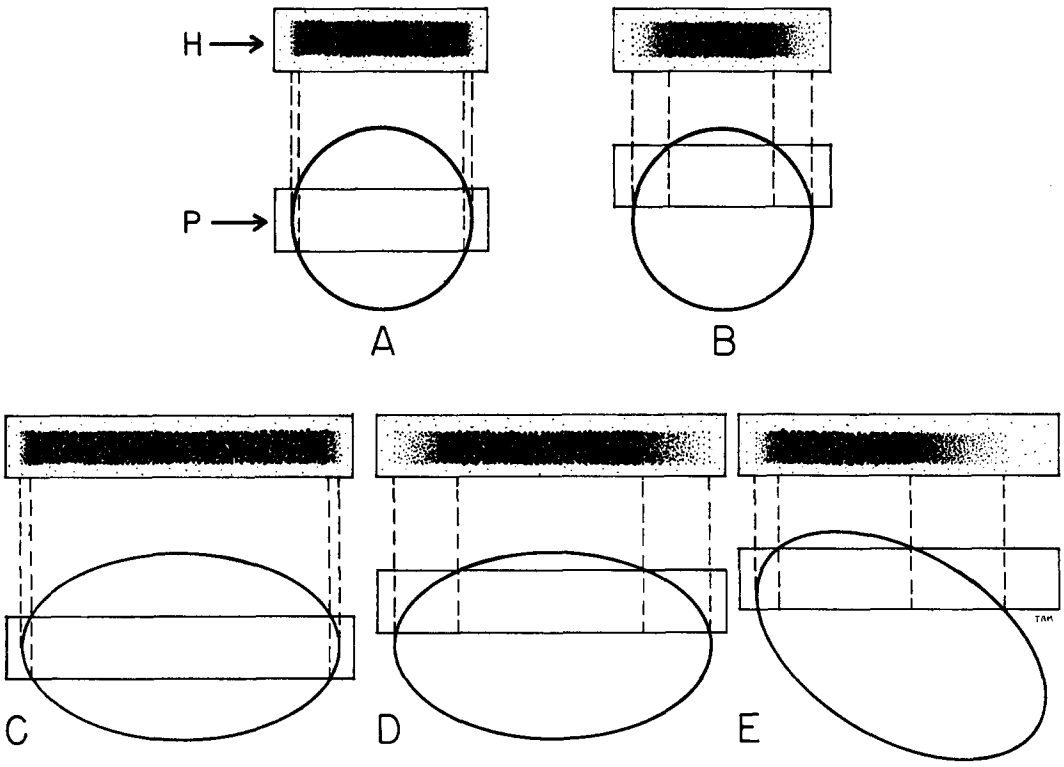

FIGURE 3

Drawing of projected photodensities of perpendicularly sectioned circular and ellipsoidal discs at different relationships to the centers.

H, projected horizontal plane view of section, showing structure and background density.

P, edge view of section perpendicular to face of disc.

Conditions *A* and *C*: maximum contrast at limits of structure.

Conditions *B* and *D*: bilaterally symmetrical fall-off of contrast.

Condition *E*: asymmetrical fall-off of contrast.

are sectioned longitudinally. Another arrangement which could account for the difference would be a series of bands or spiral-like ribbons with sinoidal undulations, the vertical phase of which is narrower than the horizontal phase. The above explanations seem untenable because one would expect to see an occasional, extremely long figure, and also, serial sections would almost invariably reveal a desmosome in a continuous predictable location. This has not been the experience.

Another interpretation seems reasonable. The desmosomes of these cells are indeed discrete ellipsoidal disc-like structures, but with their long axis oriented more or less parallel to the longitudinal axis of the cell.

Since the "ribbon-like" forms are rejected on the basis of negative evidence, it seems desirable to corroborate the "ellipsoidal form" in another way. If a perpendicular section includes the center of a circular disc or an oval disc along one

FIGURE 2

Rat submaxillary gland secretory tubule cells. The plane of section is transverse, approximately through the basal portion of the apical third of the cells. Desmosomes are dispersed along cell membranes except at the interdigitations where three cells meet (arrows). × 10,500.


I
ic
dl
gp
tf
II
III
P
IV
V

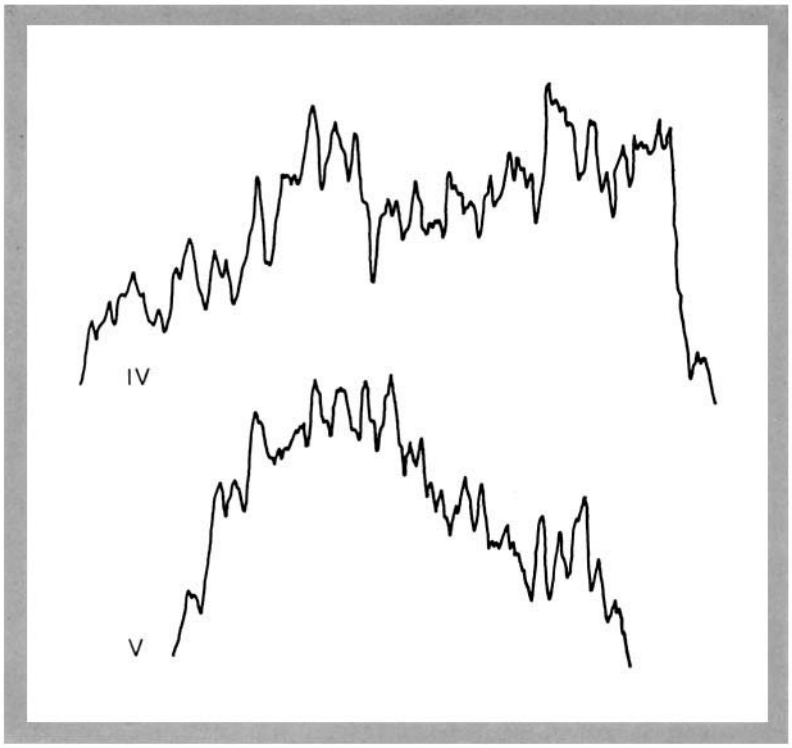

FIGURE 5

Densitometric tracings of desmosomes IV and V in Fig. 4, taken lengthwise over the attachment plaque zone, demonstrating longitudinal asymmetric photodensity comparable to condition *E* in Fig. 3.

of its principle axes, the projected image should have good definition and contrast out to the limits of the structure (Fig. 3 *A* and *C*), but if the section is much off center (still perpendicular) the density (mass) falls off towards the edge; (as the arc falls off) and the resulting projection, although well defined in the middle, will be less dense towards the ends (Fig. 3 *B* and *D*). In the case of a sectioned

FIGURE 4

Portions of two rat submaxillary gland tubule cells sectioned longitudinally. Desmosome II is sectioned close to its center and perpendicular to its surface, *i.e.* analogous to condition *C*, Fig. 3. Demosome I does not quite fulfill condition *C*, Fig. 3, in that the plane of section is not quite perpendicular to its surface nor quite through its center. Desmosomes IV and V are analogous to condition *E*, Fig. 3, although not sectioned at exact right angles to their surfaces. The densitometric tracings (Fig. 5) are of the dense zones making up the right sides of these two desmosomes. The plane surface of desmosome III is too far off from perpendicularity to allow resolution of its boundaries.

The various layers comprising desmosome "II" are clearly visible:

tf, tonofilament striation layer
ap, attachment plaque
dl, intermediate dense layer
ic, intercellular contact layer

The interspersion of a cell process from outside the plane of section is seen at *P*. × 111,000.

circle, this decreasing density and definition will always fall off symmetrically. This is also true of ellipses, providing the section is parallel to one of the principal axes, or at an angle to the axes but including the center. However, if an off-center section is not parallel to one of the principal axes of an ellipse, the definition and density will fall off asymmetrically (Fig. 3 *E*) because the elliptical arc is not bilaterally symmetrical when the transecting cord is not parallel to one of the principal axes (compare Fig. 3 *D* with 3 *E*).

The appearance of an asymmetrically dense desmosome is not infrequent. In a visual evaluation of 105 consecutive ductal desmosomes from randomly selected micrographs, 32 (30.5 per cent) appeared less dense at one end than at the other. The pattern of lengthwise densitometric measurement of desmosomes IV and V (Fig. 4) is shown in Fig. 5. The definite skewness of density is analogous to condition *E* (Fig. 3), and lends credence to an interpretation of these desmosomes as ellipsoidal discs, similar to the "oval plate" suggested by Odland (16) for the shape of epidermal attachment plaques.

The preceding evidence regarding the general morphology and orientation of these desmosomes may be misleading as to their probable size and degree of ellipticity (eccentricity ratio).

The mean cross-sectional measurement for bodies distributed in three dimensional space and sectioned randomly is not the best possible estimate of their actual mean size. In the case of spheres and oriented ellipsoids a correction factor may be calculated. (Randomly oriented ellipses would present a systematic error inherent in oblique cuts, wherein the apparent dimensional difference between major and minor axes would be reduced.) Abercrombie's (1) approach to this problem, although applicable to material sectioned for light microscopy, does not seem suited for material the section thickness of which is appreciably less than structure thickness.

To solve the problem for this material, the following approach was taken: It has already been shown that the desmosomes are probably ellipsoidal in shape and have a preferred orientation. Thus, planes (sections) cutting through these "ellipses" at random, but perpendicular to one of the axes, have as much chance to occur between the center and a point half-way to the periphery as they do to occur between the periphery and this point (see Fig. 6). Theoretically, therefore, the median (med) values for desmosomes in cells cut longitudinally and transversely should closely approximate the mean value for chords perpendicular to the minor and major axes, respectively, at points midway between the center and the circumference. By algebraic computation it is possible to reconstruct the approximate "true" size and shape of the desmosomes.

A general formula for the ellipse is $\left(\frac{x}{m}\right)^2 + \left(\frac{y}{M}\right)^2 = 1$, where m = the minor axis, and M =

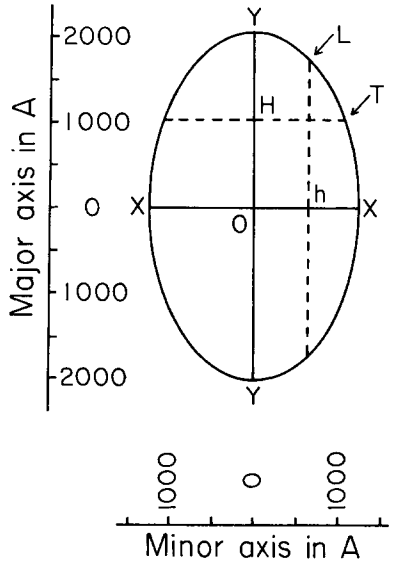

FIGURE 6

Computed mean size and shape of rat submaxillary gland secretory tubule cell desmosomes: Major axis (M) = 4106 A; Minor axis (m) = 2557 A; Eccentricity ratio =

$$\frac{\sqrt{M^2 - m^2}}{M} = 0.78.$$

YY, major axis
XX, minor axis
O, center
H, half distance from *Y* to *O*
h, half-distance from *X* to *O*
YH = *OH*: therefore the number of perpendicular cords randomly transecting *YH* should equal the number transecting *OH*. (The same holds true for cords perpendicular to the minor axis: *Xh* = *Oh*, etc.) The median values of the samples therefore correspond to the perpendicular cords intersecting *H* and *h*.
L, sample median from longitudinally sectioned cells. (3556 A)
T, sample median from transversely sectioned cells. (2215 A)

the major axis (see Fig. 6). When $y = \frac{1}{2} M$, x becomes m_{med}; and when $x = \frac{1}{2} m$, y becomes M_{med}; therefore $\left(\frac{m_{med}}{m}\right)^2 = \frac{3}{4}$, and $m = \sqrt{4/3 \cdot m_{med}^2}$. This applies to the major axis as well, *i.e.*, $M = \sqrt{4/3 \cdot M_{med}^2}$.

In non-mathematical terms, the true value of the major or minor axis is equal to the square root of $1\frac{1}{3}$ times the median value squared. The median value for the desmosomes cut longitudinally is 3446 A·(SE_{med}* = ± 41.7), while for those cut in cross-section it is 2215 A (SE_{med} = ±40.4). By the application of the above formula, the corrected values for major and minor axes are 4106 A and 2557 A, respectively, and the ellipse will have an eccentricity ratio of 0.78 (Fig. 6). The chances are 19 to 1 (95 per cent confidence limits) that the calculated mean principal axes lengths for desmosomes from other samples of like cells examined under the same conditions will fall between 4196 A and 4007 A for the major axis and between 2646 A and 2463 A for the minor axis.

An objection may be raised to the use of the sample median measurements as representing a true median. Admittedly, one cannot see (and therefore cannot measure) the end desmosome segments which fall entirely within the section and are too thin, in relation to section thickness, to be resolved. This, naturally, would bias the sample against the smallest chords and would tend to give the "median" a deceivingly greater numerical value. However, the relationship between structure thickness, section thickness, and resolution (Cosslett, 3) would tend to cause an under measurement of segments sectioned away from the center for the very same reason; *i.e.*, the ends become extremely thin in relation to section thickness and therefore are not resolved. The result is that these figures yield a deceivingly small numerical value. Since these two effects cause bias in opposite directions, it seems reasonable that even if their effects are not equal they do tend to cancel each other and thus the sample median is indeed a close approximation of the true median.

An additional observation of interest is that properly sectioned tubule cell desmosomes in good focus (*e.g.*, II in Fig. 4) are multilayered figures similar to that described by Odland (16). However, no light zone in the center of the attachment plaque layer, as Odland suggests in his halftone diagram, could be found. Measurement of peak to peak density in a number (10) of cross-section densitometer tracings yielded the following means for the spacing of the desmosome layers.

Attachment plaque to attachment plaque	332 A (SD = ±13)
Tonofilament striation layer to attachment plaque	84 A (SD = ±20)
Attachment plaque to intermediate dense layer	76 A (SD = ±10)
Intermediate dense layer to intercellular contact layer	90 A (SD = ±11)

This study could not unequivocally demonstrate an actual continuity of the attachment plaque and intermediate dense layer with the paired dense layers of contiguous plasma membrane, as shown by Karrer (6) for human cervical epithelium.

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* The standard error of the median = $\sqrt{\frac{\pi}{2N}} \times$ standard deviation of the sample.

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