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PHOSPHOTUNGSTIC ACID STAIN AND EPIDERMAL CELL SURFACE

AN ELECTRON MICROSCOPIC STUDY

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In recent years the use of heavy metals such as uranyl and lead salts has been a routine procedure to introduce contrast in electron micrographs, and these electron stains have been known to serve as a general stain. On the other hand, it has been suggested by some investigators that the chemical identification of cellular components could be accomplished by the use of suitable electron stains which attach with some discrimination to the various chemical groups and lead to a local increase in electron density. The use of heavy metal salts as electron stains to identify the chemical nature of various components has recently been reviewed in detail by Zobel and Beer (1).

The authors have attempted to obtain the chemical information from the staining behavior of electron stains by applying selected heavy metals to the section on the glutaraldehyde-fixed human epidermis. An advantage of glutaraldehyde fixation in the study of electron stains is that it does not of itself introduce contrast, unlike the osmium tetroxide (OsO_4) which serves as an electron stain as well as a fixative. Furthermore, in case of OsO_4 fixation, the fixative bound to the tissue may itself react with heavy metals (2).

The staining behavior of uranyl acetate and lead hydroxide in the glutaraldehyde fixed human epidermis was described earlier (3). This paper deals with the electron micrographs of the human epidermis fixed in OsO_4 or glutaraldehyde alone and stained on the section with phosphotungstic acid (PTA).

MATERIAL AND METHODS

Normal human skin used in this study was removed from the abdomen of a 30-year-old man under general anesthesia. The tissue was cut in smaller pieces and quickly immersed in 1% osmium tetroxide buffered to pH 7.2 with phosphate-sucrose-buffer or in 5% glutaraldehyde buffered to pH 7.2 with phosphate. After fixation for two hours, the tissues were dehydrated in ethanol and embedded in Epon 812 as described by Luft (4).

Thin sections were cut with glass knives on a JUM-5 ultramicrotome and collected on 200 mesh grids coated with a carbonized collodion film. They were stained at room temperature for 60 to 90 min. by submersing totally in a solution of 10% phosphotungstic acid in 50% ethanol (pH 2.8). The pictures were taken with a HU-IIA Hitachi electron microscope.

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RESULTS

In Fig. 1, the fine structure of part of the stratum malpighii is clear. This shows that phosphotungstic acid (PTA) serves as a general stain in the osmium fixed material, although the contrast is somewhat lower than in case of uranium or lead salts.

On the other hand, an increase in density is characteristic of the material fixed in glutaraldehyde alone and stained with PTA (Figs. 2, 3 and 4). In general, cytoplasmic details are not well clarified; mitchondria, ribosomes and tonofibrils are not clearly seen. However, the nuclear structure is of somewhat higher density than mitchondria and tonofibrils. As for melanin granules, they appear to be encircled by a substance stained intensely with PTA.

The most characteristic finding in glutaraldehyde fixation and PTA staining is the presence of a substance stained heavily with PTA along the cell border (Fig. 2). As the cell membrane is not preserved in glutaraldehyde fixation and Epon embedding (5), this indicates that there is some substance which reacts with PTA on the cell surface. This intensely stained substance is also observed on the basal surface of basal cells (Fig. 3).

In the glutaraldehyde-fixed and PTA-stained section, the density of intercellular spaces is moderately high (Figs. 2 and 3). As the density of intercellular spaces is almost translucent in the uranium or lead salts-stained sections, this finding shows that the amorphous substances in the intercellular spaces have some affinity to PTA.

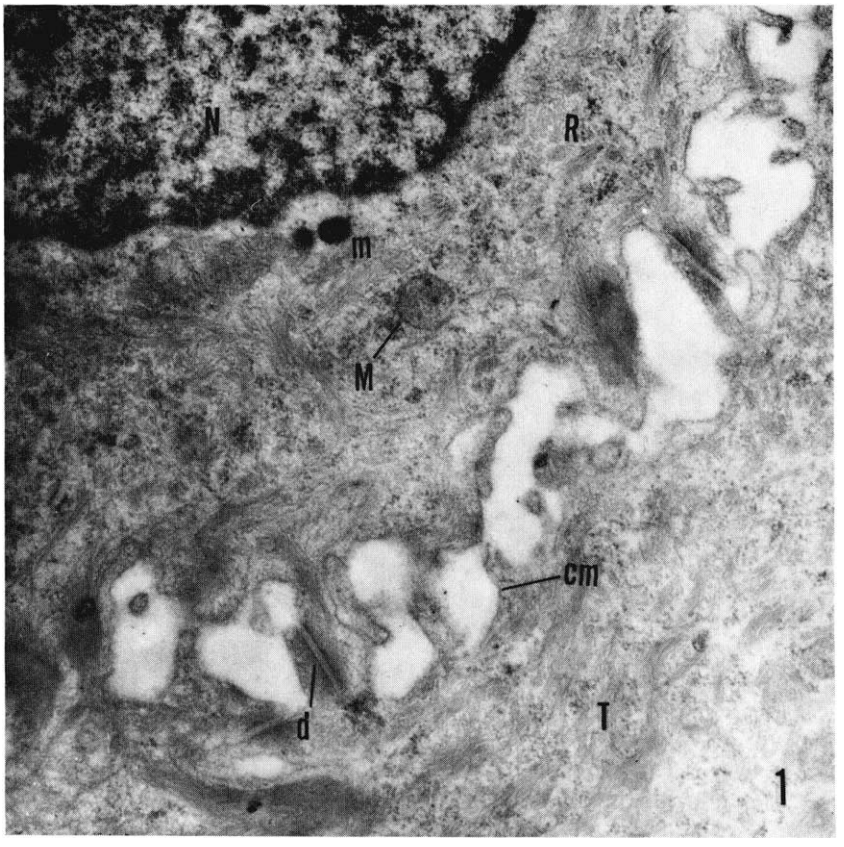


FIG. 1. Partial view of the stratum malpighii fixed in OsO_4, embedded in Epon and the section stained with phosphotungstic acid (PTA). × 25,000 PTA serves as a general stain in OsO_4 fixation, although the contrast is somewhat lower. N: nucleus, M: mitochondria, T: tonofibril, R: ribosome, m: melanin, cm: cell membrane, d: desmosome.

In desmosomes, no layer or substance is observed to stain as intensely as the substance stained with PTA along the cell border (Figs. 4 & 5). Odland's (6) attachment plaques, three thin, dense layers between the attachment plaques and the substances around the thin dense layers are mildly stained. At the dermo-epidermal junction (Fig. 3), the basement membrane is clear, but its density is not as high as that of the substance stained with PTA along the cell border. Apparently, there is no substance stained intensely with PTA to coincide with the PAS-positive basement membrane in the light microscopic observation. In the dermis, the density of collagen is rather high and its fine structure is easily seen in glutaraldehyde fixation and PTA staining (Fig. 3).

DISCUSSION

According to Pease (7), phosphotungstic acid (PTA) was originally introduced by Hall et al. in their studies of collagen, and had remained the most effective general stain until 1958 when Watson introduced lead hydroxide and uranyl acetate. At present these stains have superseded PTA, and the latter is now a somewhat selective stain.

The properties of PTA as an electron stain have been discussed only occasionally. Watson (8) observed an intense staining of the collagen in sections of osmium fixed tissues after treatment with PTA. On the other hand, Marinozzi (2) observed in mouse kidney that if the staining is preceded by oxidation of the sections with oxidants such as hydroxide or periodic acid, the collagen staining remains unaltered and only the plasma membranes among the cellular components are increased in density.

In his paper, Marinozzi cites the observations made by Steiner et al. (9) and Latta (10) that in the limiting membranes of adjacent

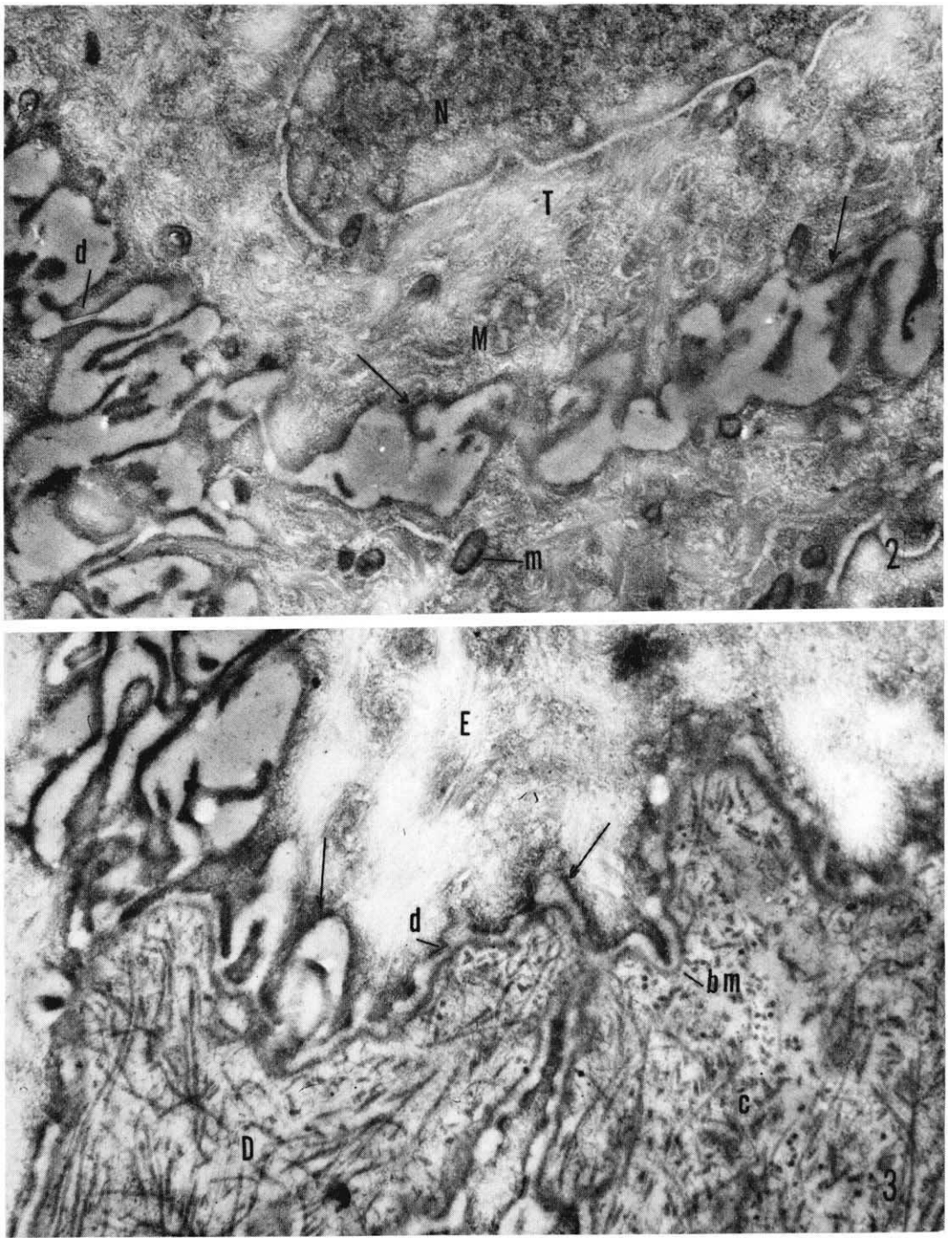
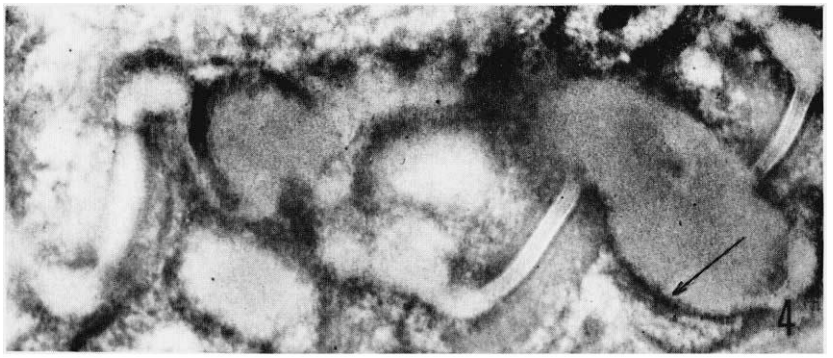
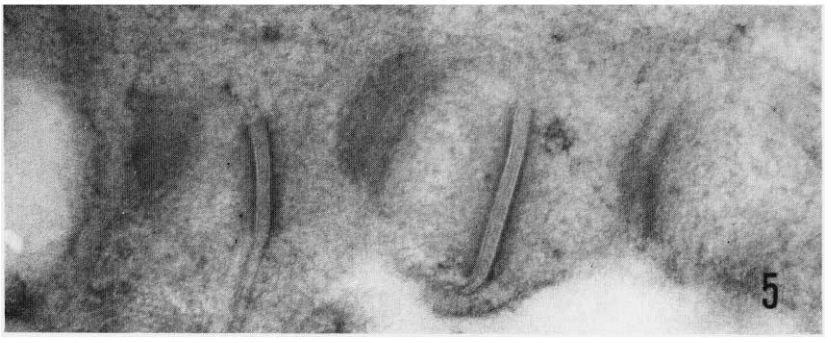


FIG. 2. Partial view of the stratum malpighii fixed in glutaraldehyde alone and stained with PTA. Note the presence of a substance stained intensely with PTA along the cell border (arrow). The density of an amorphous substance in the intercellular space is moderately high. Cytoplasmic details are not clear except for melanin granules (m). × 22,000. N: nucleus, M: mitochondria, T: tonofibril, d: desmosome.

FIG. 3. Glutaraldehyde fixation and PTA staining. × 20,000. Basement membrane (bm) and collagen (c) are well stained, but their density is not so high as that of the substance (arrow) stained with PTA along the cell border. E: epidermis, D: dermis, d: desmosome.





FIGS. 4 and 5. Higher magnification of desmosome in the malpighian layer. Fig. 4—Glutaraldehyde fixation and PTA staining. × 76,000. Note that the density of the substance between Odland's intermediate dense layers is very low, whereas the substance along the cell border is intensely stained (arrow). Fig. 5—OsO_4 fixation and PTA staining. × 64,000.

liver cells in bile canaliculi and the plasma membrane of rat glomerular epithelium respectively, there is a substance outside the membrane proper which is stained intensely with PTA. Benedetti et al. (11) also pointed out that PTA stains some selected regions of the plasma membrane after the bleaching procedure with oxidants in the osmium fixed section. These authors, however, did not mention the chemical nature of the substance stained with PTA.

Recently, Pease (12) observed that in rat kidney, duodenal mucosa and cerebral cortex prepared by his "inert dehydration" and hydroxypropyl methacrylate embedding, PAS-positive substances are vigorously stained with PTA, and states that PTA serves excellently as a seemingly specific stain for polysaccharides.

The present study shows that the section staining with PTA enhances selectively the density of some substance along the cell border of human keratinocytes fixed in glutaraldehyde alone.

When Marinozzi (2) found that the plasma membranes are increased in density after the bleaching procedure, he could not conclude whether PTA reacts with an intrinsic component of the plasma membrane or whether there is an actual difference in structure of the plasma membrane according to the cell type. The findings in this study confirm that the substance stained with PTA is not the cell membrane proper since cell membranes are not preserved in glutaraldehyde fixation and Epon embedding (5), and usually no layer or substance is stained with uranyl or lead salts along the cell border in the fixation with glutaraldehyde alone (3).

Although it is difficult to judge from the electron microscopic images of the glutaraldehyde-fixed section where the substance is located, it is more likely that the substance is located on the cell surface and not inside the cell. The reason is that the density of intercellular spaces is somewhat high after PTA staining and this seems to indicate that the substance is located on the cell surface and tends to disappear in the intercellular spaces.

In a light microscope study, Wislocki et

al. (13) described in 1951 that PAS-positive and salivary-resistant materials are present in the intercellular spaces and on the cell surfaces of skin of man and monkey. Later, Braun-Falco (14) described PAS-positive materials covering the entire surface of epidermal cells and concluded that they are neutral mucopolysaccharides. It seems possible that the substance on the cell surface of the human keratinocyte as shown by the electron microscope corresponds to this PAS-positive material.

The PAS-positive material in the intercellular spaces has been widely considered to be an adhesive cement insuring the cohesion of cells. According to Fawcett (15), this view has been altered by electron microscopic observations, and cells are now considered to be held together by desmosomes and other junctional complexes rather than by intercellular cement. He also states that the extraneous coats of cells may play a supportive or protective role by maintaining the constancy of intercellular gap and may influence the permeability of the cell surface. Pease (12), in his recent publication, states that an important significance of a polysaccharide layer at the surface of cells would be to trap and hold water.

The difference in electron density in the glutaraldehyde-fixed and PTA-stained section suggests that the so-called cementing substance at the desmosome is somewhat different from the surface substance of cells. The density of the latter is intensely increased after PTA staining, whereas the density of the substance between Odland's intermediate dense layers in desmosomes is not increased as illustrated in Fig. 4. Similarly, the difference in electron density of the basement membrane itself and of the substance between half-desmosomes and basement membrane suggests a chemical difference between these and the surface substance.

SUMMARY

Sections of normal human epidermis fixed in osmium tetroxide or in glutaraldehyde alone were stained with phosphotungstic acid (PTA) and studied with the electron microscope to evaluate the specificity of PTA as an electron stain. PTA serves as a general stain in OsO_4 fixation, but some substance on the surface of the epidermal keratinocyte is selectively stained with PTA in the section fixed in glutaraldehyde alone.

The staining behavior of PTA indicates that the substance responsible for the adhesion at desmosome and dermo-epidermal junction is chemically different from the surface substance of the cells. Also, the basement membrane is likely to be different chemically from the surface substance as its density is not markedly increased after PTA staining.

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