A Cytochemical Study on the Sweat Gland of the Human Axilla

(On the coupled tetrazolium reaction)

By

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Preface

There have been several reports on the distribution of proteins and amino acids in the sweat gland. Kagawa (1956) and Ihijima (1958) have made investigations on them, employing the mercuric bromphenol blue method (Magia, 1953). There have been very few investigations in which the proteins and amino acids with specific radicals in them, have been treated, except the one reported on the sulfhydryl groups or the disulfide groups by Montagna, Eisen, Rademacher and Chase (1954). In this study, the authors reported on the distribution of tyrosine, tryptophan and histidine in the sweat gland of the human axilla.

Materials and Methods

The skin of the axillary region of the patients who suffered from osmidrosis axillae were employed. The specimens taken by the surgical operation were fixed with 10% formalin and Zenker-formalin solution, embedded in paraffin and were cut in serial sections of 5μ in thickness. The frozen sections of the unfixed materials were also prepared. The coupled tetrazolium routine was employed in demonstrating the proteins with phenol radical. (Pearse's revised routine of Danielii's method, 1954). To ascertain the precise localization of histidine, tyrosine and tryptophan, the sections were stained with coupled tetrazolium routine after the preliminary treatment with 2,4-dinitrofluorobenzene for ten hours,
with benzoyl chloride for ten hours and with performic acid for ten minutes, respectively.

Observations

The positive results for the tetrazolium reaction can be recognized as brownish yellow or reddish yellow coloration.

1. Apocrine gland.

In general, the cytoplasm of the apocrine glandular cells presents feeble brownish yellow coloration. Occasional cells have no reaction. There is no difference in the stainability between the apical and basal cytoplasm. The hyaline apical border demonstrates week yellow coloration. The brush border demonstrates scarcely any reaction. The granules stained brownly are encountered in the apical cytoplasm and are probably corresponding with the so-called mature secretory granules. Sometimes, the accumulation of the small granules with feeble stainability is noticed inside of the large granules. Some granules encountered in the Golgi area and in the supra-nuclear area present a variation of the color from feeble yellow to brown. In occasional cells, negative images of the granular structures are recognizable. These granular structures are supposed to coincide with the immature secretory granules. The Golgi area remains unstained. The karyoplasm shows feeble yellow coloration, and both the nuclear membrane and nucleoli present a brownish coloration. The fine granular structures which demonstrate feeble yellow coloration are scattered in the infra-nuclear and basal cytoplasm. The cell border is not clearly demonstrated. The luminal contents are as brown as the apical cytoplasm and as the mature secretory granule. The myoepithelial cells are remarkably stained and present deep brownish coloration. The basement membrane is strongly reactive but presents a little weaker coloration than that of the myoepithelial cells. Two layers are distinguishable in the basement membrane. The outer layer gives a stronger reaction than the inner layer.

2. Ecorine gland.

The cytoplasm of the dark cells demonstrates uniformly brownish coloration. The granules occurred in the apical cytoplasm and stained markedly with PAS method and with iron-hematoxylin demonstrate dark brownish coloration. The nuclei are feebly reactive and the
nucleoli present yellow coloration. The stainability of the cytoplasm of the clear cells is weaker than that of the dark cells. Some clear cells have scarcely any coloration and others show feeble reaction only in the juxta-nuclear and basal cytoplasm. There is no positive reaction in the intercellular secretory canaliculi. The so-called ectoplasm of the clear cell is free of reaction and demonstrates a belt-shaped unstained zone. The reaction of the nuclei is barely distinguishable. The myoepithelial cells are as intensely reactive as those in the apocrine gland.

**Discussion**

The tetrazolium reaction is thought to reveal the proteins such as tyrosine, tryptophan and histidine and some substances other than protein. The mechanism of the tetrazolium reaction is due to the reason as following. The diazonium hydroxide, for example HO·N·N<sup>∞</sup>, makes some colored azo-groups in the alkaline side, after it reacts with phenol radical of tyrosine, indol radical of tryptophan and imidazol radical of histidine. This reaction is taken place when the tetrazolized benzidine combines with three amino acids mentioned above at 4°C, pH 9.0. According to Pearse (1954), the subsequent coupling of residual free diazo group could combine with phenol or with aromatic amines. This reaction is also sensible to purin- and pyrimidin-bases in the nucleic acid, muco-polysaccharides and mucoprotein.

Danielli (1947) employed the treatment of 2,4-dinitrofluorobenzene, benzoyl chloride and performic acid followed by the coupled tetrazolium reaction, to decide the precise localization. After the treatment of the periodic acid, cystine, methionine and tryptophan are oxidized. So, tyrosine and histidine remain unoxidized to be reactive to the coupled tetrazolium reaction, unlike triptophan which loses the sensibility to the reaction. The localization of tryptophan is indirectly distinguishable by the disappearance of the stainability.

The treatment with 2,4-dinitrofluorobenzene (DNFB) causes a reaction between the aromatic hydroxyl group of tyrosine and DNFB. In general, as the compound produced by the reaction between tyrosine and DNFB is colorless, the site which decreases the stainability for coupled tetrazolium reaction after the treatment with DNFB may indicates the negative image of the localization of tyrosine. But, DNFB is presumed to combine with SH groups, free α-amino
groups, imidazole groups of histidine and partly with ε-amino groups of lysin. Though the aromatic OH of tyrosine reacts more specifically with DNFB after SH and NH groups in the same tissue are blocked with naphthoquinone than without any preliminary treatment, still histidine has the possibility to react with DNFB. Accordingly, the sites where the stainability falls after the preliminary treatment with DNFB, indicates mostly the localization of tyrosine but partly that of histidine. After the sections are treated with benzoyl chloride, tyrosine, tryptophan and histidine are seemed to be unreactive to the coupled tetrazolium reaction. According to Danieelli, histidine is not always dissolved after benzoylation. He reported that the substances which resist to the benzoylation are purine- and pyrimidine-containing compounds, as chromatin, nucleoli, ribonucleoprotein, collagen and reticulum, mucopolysaccharides. Nevertheless, the mucin distributed in the brush border of the duodenum and the free mucin in the stomach are positively reactive to the PAS method even after the benzoylation, but unreactive to the coupled tetrazolium reaction after the same procedure. The results obtained after the benzoylation depend on the variety of the physical factors. Applying the above mentioned chemical reactions to both kinds of the sweat glands, following findings are obtained.

In the first place, in the apocrine sweat gland, the large granules (the so-called mature secretory granules) in the apical cytoplasm present the same intensity of the tetrazolium reaction even if it was preliminary treated with DNFB and performic acid. But after the benzoylation for ten hours, the intensity of the reaction decreased. Accordingly, these large granules are expected to contain histidine. However, such substances as purine and pyrimidine in the nucleic acid, mucoprotein and mucopolysaccharides also do not lose the sensitivity to the reaction after the benzoylation. So, these granules are abundant with RNA as reported by Ito, Tsuchiya and Ishige (1954), positive to the PAS reaction and contain mucoprotein as reported by Yasuda et al., (1960-b). Besides, histidine, though it is very little amount, is also contained in the granules.

In the second place, the middle sized and small granules which are encountered extending from the infra-nuclear cytoplasm to the basal, do not display any changes in the stainability by the treatment of DNFB, but they do slightly decrease with performic after the benzoylation treatment. From these data, the localization of
tryptophan and histidine is indicated in the granules. Nevertheless, the main element in the granules sensible to the coupled tetrazolium reaction is attributable to histidine from the arguments that the granules give the sensibility to the alkaline tetrazolium reaction (Yasuda et al., 1960–a), but tryptophan and tyrosine are unreactive to the alkaline tetrazolium reaction. Of course, the granules also contain nucleic acid and mocroprotein.

Though the cytoplasm of the glandular cell presents a reddish yellow coloration with the coupled tetrazolium reaction, the stainability is unchangeable even using the above mentioned three kinds of regents. Accordingly, it is probable that the positive result in this portion is mainly caused by the nucleic acid. The hyaline apical border is slightly stained with thionin and the PAS method, but with the coupled tetrazolium reaction, it presents about the same sensibility as the cytoplasm does. Besides this reaction is unchangeable after the treatment with three kinds of regent. Therefore it is sure that in this portion the nucleic acid substance is present but it is also presumed that the localization of histidine which was not digested by the benzoylation is recognizable in this portion.

The nucleus gives a medium or strong intensity to the coupled tetrazolium reaction, but the reaction is unchangeable with the treatment of three kinds of drugs, so that it may depend on the content of the nucleic acid. After the benzoylation some increasing image of reaction is encountered but this reason is unknown.

The myoepithelial cells are intensively sensible to the coupled tetrazolium and even they are preliminarily treated with some kinds of drug, they do not loose the sensibility to the reaction. Moreover, from the data that they display very intensive stainability with thionin staining, this reaction probably depends on the nucleic acid.

The basement membrane reacts intensively. With the PAS method, the inner layer of the membrane gives reaction but the outer layer functions weakly (Montagna, 1956). With the tetrazolium reaction, on the other hand, the outer layer presents an intensive function. Collagen and reticulum are indicated as the fibers which are positive to the coupled tetrazolium reaction but if it is collagen, the inner layer, not the outer, must present strong reaction, because the collagen fibers are positively reactive to the PAS method. Nevertheless, the reason why the outer layer gives a strong reaction, is still owing to the collagen, though it is contrary
to the PAS reaction. In the third place, in the eccrine sweat gland, the granules in the superficial cells are intensively sensible to the coupled tetrazolium reaction and also the reaction is unchangeable with the preliminary treatment using three kinds of regents. Namely it is thought, that these granules do not contain histidine but they mainly consist of mucoprotein and nucleic acid protein. From the result that the cytoplasm of the dark cells are decreased in reaction with the benzyolation, the cytoplasm may contain histidine. Moreover, the cytoplasm probably contains tryptophan, as the result of the decrease in the reaction with performic acid. However, it is much necessary to research in regard to this point.

The clear cells give a very faint reaction to the coupled tetrazolium and with the preliminary routine using three kinds of regents the reaction is unchangeable. They are intensively positive with PAS staining and do not give any coloration with thionin. In regard to this point, it may be thought that the clear cells do not contain tyrosine, tryptophan and histidine, but mostly contain polysaccharide, especially glycogen alone and partly scarce nucleic protein.

Conclusion

Protein with phenol radicals was demonstrated in both apocrine and eccrine sweat glands, applying the coupled tetrazolium reaction to the sweat glands of the human axilla.

1. Apocrine sweat gland.

The mature secretory granules contain a small quantity of histidine as phenol radicals. The immature secretory granules also contain a small quantity of histidine. Both kinds of granules do not contain tyrosine and tryptophan.

In the cytoplasm of the glandular cell, purine and pyrimidine bases in the nucleic acid are sensible to the coupled tetrazolium reaction. But, occasionally, histidine is also contained in the cytoplasm. The nucleus and nucleolus are not distributed with phenol radicals. The cytoplasm of the myoepithelium is positive to the reaction. However, this positive image is attributable to nucleic protein but not to phenol radicals.

2. Eccrine sweat gland.

The cytoplasm of the dark cells contains histidine other than nucleic acid. The localization of tryptophan is unknown. The
granules which are visible in the luminal side of the dark cells are not provided with phenol radicals and are positive to the coupled tetrazolium reaction. This positive reaction is attributable to nucleic acid and to mucoprotein. The clear cells do not contain phenol radicals but mainly glycogen.

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References


Explanation of Figures

Fig. 1. The transverse section of the secretory tubule of the apocrine gland.
Fig. 2. The same as above.
Fig. 3. The transverse section of the secretory tubule of the eccrine gland.
Fig. 4. The same as above.
Fig. 1.

Fig. 2.

Fig. 3.
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Fig. 4.