A histochemical investigation on the sweat gland of human axilla, with special reference to the substances which increase basophilia after oxidation with periodic acid

By

Kenjiro Yasuda, Hiroshi Kagemoto and Kazuyoshi Kobayashi

Department of Anatomy, School of Medicine, Keio University, Shinjuku, Tokyo, Japan
(Director: Prof. Dr. T. Taniguchi)

Dempsey, Singer and Wislocki (1950) have reported the presence of the sulfide and sulfhydryl groups in the basophilic substances in some tissues, besides nucleic acid and mucopolysaccharide. As widely known, there are many basophilic substances in the cytoplasm of both eccrine and apocrine glandular cells. In this study, the authors aimed to pursue the existence of the sulfur containing substance in both sweat glands and to compare the constituents of the apocrine granules with those of the eccrine granules.

Materials and Methods

The materials used in this study were the skin from the axillary region of the osmidrotic patients. The small pieces of the skin taken from the patients by the surgical operation were fixed with 10% formalin, Zenker-formalin and Champy's solution, embedded in paraffin and cut in sections of 5μ in thickness. The sections were treated with 1% periodic acid, followed by the staining with toluidine blue, as described by Dempsey, Singer and Wislocki (1950).

Both thionine staining and PAS routine were also employed to ascertain the precise localization of nucleic acid and mucopolysaccharide.
Observations

1. Apocrine sweat gland (Figs. 1, 2 and 3).

The ground substances in the apocrine cells are partly metachromatically stained, but almost uniformly unstained. The basophilia occurred in the juxta-nuclear region and in the basal cytoplasm may correspond to the distribution of the mitochondria. This basophilia becomes visible only when the sections are stained after the preliminary treatment of 1% periodic acid.

The so-called mature secretion granules demonstrate deep blue coloration. The minute structure inside of these granules is not clearly distinguishable, but sometimes map-like meshwork is encountered. When the sections are stained with the same procedure mentioned above after the acid hydrolysis (IN HCL for 3 hours at 37°C), the unstained fine structure in the interspace of the meshwork is clearly demonstrated. These spaces may correspond to the sites of the nucleic acid in the secretion granules, as the coloration disappear by the acid hydrolysis. The so-called immature granules reveal themselves as less stained and smaller granular structure than the mature ones. Occasional immature granules present metachromatically violet blue coloration. The immature granules are encountered at the surroundings of both nuclei and Golgi apparati, and are often found just beneath the hyaline apical border together with the mature ones. In some cuboidal cells, the mature granules appear in the surroundings of the nuclei or in the infra-nuclear region. After the acid hydrolysis, both granules lose their affinity to the basic dye. But when the sections are hydrolyzed with 1% hydrochloric acid and stained by basic dye after treated with periodic acid, neither of the granules show metachromasia. This fact indicates that both granules contain nucleic acid, mucopolysaccharide and sulphur containing substances in themselves. The fine granules distributed around the Golgi area and in the basal cytoplasm demonstrate dark blue coloration and probably coincide with the distribution of the mitochondria. They are not stained with basic dye after acid hydrolysis, even if they were treated with periodic acid. They presumably contain no sulfide and sulfhydryl groups.

Occasionally, the hyaline apical border and the apical cytoplasm present metachromatic violet coloration. Sometimes the hyaline apical border swells and contains greater accumulation of the basophilia. With the exception of the cases described above, the
hyaline apical border is usually unstained with basic dye and only the cuticular border is metachromatically stained. As the swollen apical border and the cuticular border are also positively reactive to the PAS method and resist to the diastase digestion, they may contain mucopolysaccharide.

The Golgi area is uniformly and weakly stained. The "Basalfilament" on which Brinkmann (1912) reported, is never encountered in the basal cytoplasm.

The nuclei demonstrate deeply blue coloration. The karyoplasm has weak violet coloration, the chromatin presents dark blue coloration. The nuclei are unstained with toluidine blue after the preliminary treatment with hydrochloric acid but gain the stainability again with the oxidation by 1% periodic acid to demonstrate euchromatic blue color.

In regard to the myoepithelial cells, they present occasional metachromasia, sometimes no coloration and often they have so narrow cytoplasm that it is very difficult to distinguish the cytoplasmic stainability.

Double membranous structure can be recognized in the basement membrane. The outer layer gives metachromatically violet coloration, while the inner layer presents euchromatically dark blue coloration.

The luminal contents are partly stained, uniformly blue but partly violet.

2. Eccrine sweat gland (Figs. 4 and 5).

The cytoplasm of the dark cells, especially the small granules arranged themselves in the apical cytoplasm, exhibit metachromatic purple coloration, in the control section which is not previously treated with periodic acid. The metachromasia increases after the treatment with periodic acid, specifically in the granules in the apical cytoplasm. This increased metachromasia is never encountered in the apocrine cytoplasm and in the secretion granules. The granules in the superficial cells are more strongly metachromatic than the cytoplasm, when the sections are stained with basic dye after oxidation with 1% periodic acid. The Golgi area of the dark cells is somewhat weaker in the stainability than the surrounding cytoplasm and is recognized as a negative image. In occasional dark cells, only the granules are intensely stained metachromatically, while the cytoplasm demonstrates feeble basophilia.

The luminal surface of the dark cells shows, in general, some-
what coarse structure and often those which are similar to the cuticular border in the apocrine glandular cells. The free border of the dark cells stains metachromatically to the toluidine blue. This is attributable to the existence of the mucopolysaccharide.

The cell border between two superficial cells reveals itself as feeble linear structure with weak stainability.

The clear cells are, generally, hardly stained with basic dye. But, sometimes, there are some amorphous substances which are stained metachromatically. As these amorphous substances are unstained with toluidine blue after acid hydrolysis followed by oxidation with 1% periodic acid and they are also not stained by thionine but reactive to PAS method, these substances may be mucopolysaccharide, especially glycogen.

There is no difference in the stainability between ectoplasm and endoplasm of the basal cells. The inter- or intra-cellular secretory canaliculi have feeble stainability. The linear structures which are visible between two basal cells and have weak stainability may consist partly of cell border or intercellular canaliculi but sometimes of the cytoplasmic stalks protruded from the dark cells to reach the basement membrane.

The myoepithelial cells are sometimes unstained but occasionally stained metachromatically. Some vacuolated structures are encountered in the cytoplasm which demonstrates strong metachromasia. The coarse fibrous structure is metachromatically stained in the unstained cytoplasm of occasional myoepithelial cells.

The stainability and the structure of the basement membrane is similar to those in the apocrine gland.

Discussion

Dempsey, Singer and Wislocki (1950) stated that the periodic acid not only promotes the reaction of Schiff's reagent to polysaccharide but also increases the affinity of pure proteins and of tissue components for basic dyes, and strongly brings about the formation of acid, basophilic groups.

These acid groups may be formed in regions of high sulfur content. Moreover, they said that the increased metachromasia may be attributable to the sulfonic acid yield after the oxidation of the sulfide and sulfhydryl groups. This result resembles the Suter's report (1944) in which he insisted the formation of the sulfonic
acid after the oxidation of sulfide and SH groups.

The structures which demonstrate basophilia after the oxidation by 1% periodic acid are as follows:

Apocrine gland:

1) Euchromatically stained structure; mature secretory granules, nuclear membrane, karyoplasm, nucleolus, mitochondria, inner layer of basement membrane.

2) Metachromatically stained structures; immature secretory granules, occasional karyoplasm, hyaline apical border, cytoplasm of myoepithelial cell, some parts in the cytoplasm, outer layer of basement membrane.

Eccrine gland:

1) Euchromatically stained structures; nucleus, clear cells.

2) Metachromatically stained structures; dark cell, secretory granules in the apical cytoplasm of the dark cells.

After the acid hydrolysis with IN hydrochloric acid, whole the structures mentioned above loose their affinity to the basic dye, except the basement membrane and the cytoplasm of the myoepithelial cells. Sometimes, feeble stainability appears in the cytoplasm of the apocrine cells. This is considered to be immature secretory granules. When the sections are stained by toluidine blue solution at pH5.7 after the preliminary treatment with IN hydrochloric acid followed by the oxidation with 1% periodic acid, almost all the structure in the apocrine cells are stained euchromatically, with the exception of the granules in the dark cells of the eccrine gland, the cytoplasm of the clear cells of the eccrine gland and the basement membrane. The karyoplasm is free from the stainability, except the nucleolus and the chromatin net. Dempsey et al (1950) asserted that the hydrolysis with hydrochloric acid would move out the nucleic acid (both DNA and RNA) completely and also mucopolysaccharide incompletely. So the substances which are stained with toluidine blue after the preliminary treatment with IN hydrochloric acid and 1% periodic acid are thought to be the residuary mucopolysaccharide, sulfide and sulfhydryl groups and some other unknown substances.

Within the substances described above, the residuary mucopolysaccharide is rich in hyaline apical border and the cuticular border of the apocrine cells and in the secretory granules of the dark cells of the eccrine cells, and poorly encountered in the cytoplasm
of the eccrine clear cells. The mature granules of the apocrine gland and the nucleoli of both glandular cells contain greater amount of ribonucleic acid. As the stainability to toluidine blue, after the previous treatment with IN hydrochloric acid followed by the oxidation with 1% periodic acid, is markedly in the former, while never encountered in the latter, it is presumed that the former contains both ribonucleic acid and sulfide components but the latter does nothing but the ribonucleic acid.

Ito et al (1951) reported that the immature apocrine granules do not contain ribonucleic acid and are not stained with basic dyes. In this study, they reveal themselves only when the sections are stained with toluidine blue after the treatment in the periodic acid, and present weak metachromasia. After stained with the same procedure followed by the hydrolysis with hydrochloric acid, they demonstrate euchromatic blue. So the immature granules are considered to have same kind of proteins which contain sulfide and sulfhydryl groups. In proportion as the immature granules give rise to the mature ones, the ribonucleic acid and PAS-positive substances are presumed to predominate over the sulfide and sulfhydryl groups. The mitochondria-like structures occurred in the infra-nuclear region are never stained with basic dye after the hydrolysis with hydrochloric acid, even though previously treated with 1% periodic acid. As Ito et al (1951) state, the capsule consisted of ribonucleic acid is solved out by the acid hydrolysis to keep the mitochondria-like structure unstained.

The granules in the dark cells of the eccrine gland are strongly reactive to PAS routine, never influenced by the acid hydrolysis and stained deeply with thionine. They may contain greater amount of mucopolysaccharide, less amount of nucleic acid and no sulfide groups.

As the basophilia of the cytoplasm of the superficial cells in the eccrine gland is never recovered in the sections which are oxidized by periodic acid followed by toluidine blue staining, after the treatment with hydrochloric acid, it may be attributable to the nucleo-protein only.

The clear cells of the eccrine gland present weak metachromasia, when they are stained with toluidine blue after preliminary treatment with hydrochloric acid followed by the oxidation with periodic acid. These cells are unstained with thionine and strongly reactive to PAS routine, so they may contain larger accumulation of
A histochemical investigation on the sweat gland of human axilla

glycogen and less amount of mucopolysaccharides.

Regarding the stainability of the myoepithelial cells, it may be due to the existence of the ribonucleic acid or to the reaction of the myofibrils, on which Dempsey et al (1950) reported that the Z-discs and anisotropic segments of the muscle fibers could be selectively stained with their method no matter where they were located in the section.

The basophilia of the basement membrane is not clearly understood, but it may be attributable to the existence of the collagenous fibers, because Dempsey et al (1950) stated that the collagenous fibers increased their dye-binding capacities after the oxidation by pericodic acid.

We should like to record our thank to Prof. Dr. T. Taniuchi for his helpful guidance and criticism.

Conclusion

The authors observed the distribution of the substances which increase basophilia after oxidation with periodic acid, in both apocrine and eccrine sweat glands of the human axilla. At the same time, they compared the nature of the granules in both glands.

1) The mature granules in the apocrine glandular cell contain greater amount of ribonucleic acid, appreciable amount of mucopolysaccharide and less amount of sulfide and sulfhydryl groups.

2) The immature granules in the apocrine cells contain no ribonucleic acid and mucopolysaccharide but sulfide and sulfhydryl groups and some other substances.

3) The granules in the dark cells contain much mucopolysaccharide and moderate amount of ribonucleic acid. They are free from the sulfide and sulfhydryl groups.

4) The clear cells of the eccrine gland have a little amount of polysaccharide, not to mention glycogen.

5) The myofibrils in the myoepithelial cells have sulfide and sulfhydryl groups.
Kenjiro Yasuda, Hiroshi Kagemoto and Kazuyoshi Kobayashi

References

I to, T., K. T a u c h i y a and K. I w a s h i g e: 1951. Studien über die basophile Substanz (Ribonukleinsäure) in den Zellen der menschlichen Schweissdrüsen. Arch. hist. jap., vol.2 n. 3. s. 279.

Explanation of figures

Plate I
Fig. 1 The epithelial cells of the apocrine gland.
Fig. 2 The same as above.
Fig. 3 The same as above.

Plate II
Fig. 1 The epithelial cells of the eccrine gland.
Fig. 2 The same as above.
Fig. 3 The excretory duct of the eccrine gland.