A Cytochemical Study on Protein and Nucleic Acids of the Eccrine Sweat Gland in the Palm and the Sole of Human Embryos

By

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Preface

Since the classical work of Kölliker, (1889), a large number of embryological studies on the skin and the sweat gland of the human embryos have been reported by many investigators, including some Japanese such as Nagai (1936), Akagi (1939) and Ueda (1939).

Histochemical studies on the skin and the sweat gland have been carried out by Takagi (1941), Ito (1949, 1951) and Montagna (1951). Recently histochemical studies on their genesis have been done by many investigators. In Japan, Tsuchiya (1954) reported in a study on the eccrine sweat gland. In the present study, the authors examined cytochemically the protein and nucleic acids in the developing eccrine sweat gland of the palm and the sole of human embryos.

Materials and Methods

The materials for the observation were taken from the fresh skin of the palm and the sole of human embryos of the fifth to ninth foetal month, immediately after death. These materials were fixed with 80% alcohol or 10% formalin solution, embedded in paraffin, and made into sections of 5 μ in thickness. The mercuric chloride bromphenolblue method (abbreviated as Hg-BPB method) was used for the detection of protein. Nucleic acids were demonstrated by the thionin staining method (abbreviated as T-stain).
Feulgen reaction (abbreviated as F-reaction), pyronin-methyl green stain (abbreviated as PMG-stain) and the Backer and Alexander's method (1952)* (Abbreviated as B-A-method).

**Findings**

Although it has been reported by some investigators, that the development of the eccrine sweat gland begins in the fourth or the fifth foetal month, the time differs with individuals and anatomical locations. For example, the eccrine sweat glands in the palm and the sole are said to appear earliest. As our materials were all taken from embryos older than five months, the sweat glands of these localities always are at same stage of development. Therefore the authors are not going to discuss the initial generation of their rudiments. As described by previous authors, in our material of the fifth month, the rudiments of the sweat glands of both the palm and sole infiltrate deeply into corium, forming clavate cell cords with swollen ends. The swollen region later becomes the secretory region, i.e. the glandular canal. In the sixth foetal month there appears the winding of the cord region, forming an intercellular space. In the palm, the cord region distends itself gradually forming a glandular cavity. In the sole, some of the rudiments show retarded development, but their shape is nearly the same as in the palm. In the seventh foetal month, both in the palm and the sole, the glandular cavity becomes still larger. The glands of the palm become nearly fully developed in the eighth or ninth foetal month. The same thing is true about the glands of the sole. But here they still lack the complicated structure seen in the adult.

**A. Protein**

Protein was identified by the Hg-BPB method as described above. In the fifth month, the sweat gland rudiment is localized in the deeper layer of the corium both in the palm and the sole, and the cytoplasm is deeply stained. The nuclei in the cord region and in the swollen end region are stained still more deeply, and the whole rudiment of the sweat gland is coloured deeply (Fig. 1).

* The 3, 9-methyl 2, 3, 7-trihydroxy-6-fluorone, which was employed in our experiment, was given by Dr. T. Ohse, the director of the Institute of Public Health of Fukui Prefecture. This reagent was synthesized by Mr. K. Tamamura, one of his staff. The authors express their thanks for their generosity.
In the sixth month embryo, the lumen has been formed in the palm, and the secreting portion of the cord region and the swollen end also have distinct lumen. The central part of the cord region looks somewhat lightly stained because of the hollow, and the internal layer is more intensely stained than the external layer. The karyoplasm stains intensely, and the nuclei is coloured especially deep. The cell boundary zone gives a particularly intense blue coloration (Fig. 2). In the seventh month embryo, both in the palm and the sole the cord region thickens a little, and the part facing the lumen shows an intense coloration. The cytoplasm is stained fairly intensely. The cell boundary zone shows considerably intense coloration in some part. The karyoplasm is stained more deeply than any other parts of the cell. Some of the nucleoli are stained indistinctly. The cytoplasm, the karyoplasm and the nucleoli of the basal cell are generally somewhat weakly stained. In the eighth and ninth embryos, although the cord region and the secreting region in the palm and the sole are almost completely developed, yet the stainability is still the same as in the seventh month embryo, i.e. the cells of the internal layer of the cord region and the cells of the superficial layer of the secreting region both give an intense stainability (Fig. 3). The karyoplasm and the nucleoli are stained particularly deeply.

B. Nucleic acids

Nucleic acids were demonstrated by the four methods mentioned above.

In the T-stain, the sections were treated in 0.01% thionin solution for four hours at 37°C. Ribonucleic acid (abbreviated as RNA) is stained purple and desoxyribonucleic acid (abbreviated as DNA) is stained blue.

In the PMG-stain, the sections were treated with a mixture of pyronin and methyl green, which had been purified with chloroform. RNA is stained red by pyronin and DNA is stained green or purple by methyl green.

In the B-A-stain, the sections were stained with a solution of 9-methyl-2, 3, 7-trihydroxy-6-fluorone, to which alcohol and concentrated sulfuric acid had been added. RNA is stained yellow or red, and DNA is stained purple or blue black.

In the F-reaction, the sections were treated with IN solution of hydrochloric acid, stained with the Schiff’s reagent and counter-
stained with Fast green solution. DNA is stained red.

The fifth month embryo.

The cytoplasm of the cord region of the sweat gland rudiment both in the palm and the sole is coloured deep purple with the T-stain, red with the PMG-stain, and yellowish brown with the B-A-method both considerably deeply, showing the presence of RNA in considerable quantities. It is not stained by the F-reaction. On the other hand, the karyoplasm is deeply staining with any of the four staining methods, showing the presence of DNA in considerable amounts. The nucleoli, one to two in number, are fairly intensely stained with the T-stain and with the B-A-stain (Fig. 4), indicating the presence of RNA. Other staining methods could hardly make the nucleoli visible. The cytoplasm in the swollen end shows a stainability of nearly the same intensity as that of the cytoplasm in the cord region. This indicates the presence of a considerable amount of RNA. Because of the dense distribution of nuclei in this region, the F-reaction gives a general red stain of remarkable intensity. Each karyoplasm, however, indicates the presence of a medium amount of DNA.

In the sixth month embryo.

The formation of the lumen is observed in the sweat gland rudiment of the palm. In the internal cells of the cord region RNA is demonstrated in the part of their cytoplasm facing the lumen by the purple coloration with the T-stain, by the red coloration with the PMG-stain, and by the somewhat intense yellowish brown coloration with the B-A-stain. The nuclei give a metachromasia with the T-stain in places, but the distinct positive reaction to the PMG-stain, the B-A-stain and the F-reaction show the presence of DNA. In the sole, the stainability of the rudiment which forms a lumen is the same as in the palm. In the secreting portion of the palm the part of the superficial cells, facing the lumen, shows intense coloration with the T-stain and the B-A-method, indicating the presence of RNA just as in the cord region. The cytoplasm of the superficial cells shows more intense coloration with the T-stain, the PMG-stain or the B-A-method, than the cytoplasm of the basal cells. Intense coloration is seen in the nuclei with the four staining methods showing the presence of DNA, and the most intense coloration is observed in the nuclear membrane (Fig. 6). The nucleoli, one or two in number, indicate the presence of a considera-
ble amount of RNA by the distinct coloration with the T-stain. In the sole although the most of the rudiments have formed the lumina, retardation of the development is seen in some rudiments, just as in the fifth month embryo.

The seventh month embryo.

The rudiment in the sole has been enlarged almost similarly as in the palm, the cytoplasm of the internal cells in the cord region shows a purple coloration with the T-stain (Fig. 7), a red coloration with the PMG-stain, and an intense yellowish brown coloration with the B–A–stain, in the part facing the lumen. The cytoplasm of the internal cells gives more intense coloration that of the external cells. This indicates the presence of a large amount of RNA. The nucleus gives positive reactions of DNA of medium intensity to all of the staining methods. The nuclear membrane gives the most intense DNA reactions. The picture of the secreting region is also the same both in the palm and the sole. The cytoplasm of the superficial cell gives intense coloration with the T-stain, the PMG-stain and the BEA-stain in the part facing the glandular lumen. All the other parts of the cytoplasm show stainabilities of medium intensity with these methods, showing that RNA is particularly abundant in the part neighbouring the lumen. The basal cells give weaker colorations with the T-stain, the PMG-stain and the B–A–stain than the superficial cells. The karyoplasm shows coloration of medium intensity with these four methods. The nuclear membrane shows the most intense coloration. These stainabilities indicate the presence of DNA.

The eighth and ninth month embryos.

The cells have increased in size. Those in the secreting region, however are not so complex as in the adult. The superficial cells give intense coloration with the PMG-stain in the part facing the lumen, indicating the presence of RNA (Fig. 9). The cytoplasm shows somewhat indistinct granular coloration with the BEA-method (Fig. 8). There are in places unstained vacuolar areas in the upper part of the cytoplasm of the superficial cells when stained with the PMG-stain. The karyoplasm gives coloration of medium intensity with all of the staining methods indicating the presence of DNA. The F-reaction gives the most intense coloration in the nuclear membrane (Fig. 10). In the nucleoli, RNA is readily demonstrated with the T-stain, but the presence of DNA is not clearly shown.
by the PMG-stain or the B-A-method.

Discussion

As cited before, there have been reported a large number of embryological studies on sweat glands. Most of these studies, however, concern the morphological development or the time of appearance of the rudiment, or the time or mechanism of the formation of lumen. Among these reports there are the studies on the sweat glands in the various portions of the upper extremities (Nagaï, 1936), the sweat gland of trunk skin (Horikoshi, 1939), and the axillary sweat gland (Morioka, 1943). Of the sweat gland of the palm and the sole, an embryological investigation was published by Ueda (1939) in which the difference of the palm and the sole as to the nature of their glands was not discussed. Recently, however, Tsuchiya (1954) reported the development of the eccrine sweat glands in the palm, sole and axilla of 6-10 month old embryos, as well as the detection of glycogen in the glands. Tsuchiya states that the swollen part of the rudiment becomes elongated, winds and forms a fine glandular lumen in the sixth foetal month in the palm and in the seventh month in the sole, pointing out the difference between the two parts of the body. But the fact that the eccrine gland begins its development earlier in the palm and the sole than in other parts of the body, has been generally acknowledged by many investigators. Itô (1943) states that the differentiation of two types of cells, i.e. the superficial cells (darkly coloured) and the basal cells (lightly coloured), begins in the stage of the glandular lumen formation, and that these two types can be easily distinguished from each other. The authors often find the formation of the lumen already complete in the palm while some clavate, undeveloped rudiments are still remaining in the sole. Therefore the authors do not consider, that any generation can be made about the time of the lumen formation in the palm and the sole. The histological and the cytological pictures, however, are as described above.

Studies on protein are scarce. Montagna (1951) succeeded to detect protein in the gl. cerminosa of the cat with the xanthoproteic test. In our study, the Hg-BPB method was applied to this examination. The result has been described above.

In the fifth month embryo, protein is conspicuous in the cytoplasm of the rudiment, in the palm as well as in the sole, and es-
especially abundant in the karyoplasm. Both in the palm and sole of the sixth month embryo, in the rudiment, which has completed its lumen formation, protein is especially abundant in the internal cells of the cord region and in the superficial cells of the secretory portion. This picture may have some connection with Ito's opinion (1951) that the secretory granules in the superficial cells of the adult glands are probably composed mainly of protein. The picture of protein in the rudiments in the sole, whose lumen are not yet fully developed, is similar to that seen in the rodlike rudiments in the fifth month embryo. When the stage proceeds to the seventh or eighth foetal month, the protein reaction is generally intensified as the foetal age advances. In all cases, protein is more abundant in the karyoplasm.

For the detection of nucleic acids, the T-stain has been generally employed. Although it is said that the basic dyes demonstrate RNA in the cytoplasm and DNA in the nucleus, it is difficult to distinguish DNA and RNA from each other. It has been stated that the inhibition of the coloration of DNA is apt to occur easily particularly with the T-stain (Shibata, 1951). For this reason we used the F-stain, (the PMG-stain and the B-A-method as well. On the detection of RNA in the adult sweat gland cells Ito (1951) reported the results obtained by the application of the T-stain. According to Ito, RNA of the eccrine sweat gland is concentrated in the superficial cells to a considerable degree, and is hardly detectable in the basal cells. The excretory duct contains a small quantity of RNA, and the cells of the inner layer contain it abundantly. It is noted that glycogen and RNA are never found together in the same cell, therefore the external cells of the excretory duct look as unstained vacuoles. Tsuchiy a (1954) reported, in her study on glycogen in the eccrine sweat gland of the human embryo, that glycogen does not yet exist in the sweat gland in the stage of its rudiment formation, a small quantity of glycogen appears first in the sixth foetal month in the external cells of the excretory duct, and it increases gradually with the passage of time after the seventh foetal month. The basal cells of the secretory region usually look lightly coloured and contain glycogen in a diffuse manner. She also stated, that in the stage of the lumen formation, glycogen is demonstrated as scattered granules or vacuolar drops. From these findings of Tsuchiy a, and the results of Ito's investigation, it must be concluded that RNA is not present in the lightly coloured basal
cells (the secretory region) or in the external cells of the cord region. As described above, the authors have obtained in the present study the same results with the each of the four staining procedures employed. Although Tsuchiya (1954) reported that the lumen in the sole is formed one foetal month later than that in the palm, no marked difference was observed in our study. The localization of RNA, demonstrated in our study of the embryos, is the same as that observed by Ito (1951) in his study on the basophilic substance in the adult human eccrine sweat gland cells. With the passage of foetal months, the intensity of the reaction showing its presence gradually increases, but their activity is still weaker than that seen in the adult. According to Tsuchiya (1954) the glycogen content increases with the passage of the foetal months. Our results show that RNA and DNA both increase gradually when the foetal month proceeds.

Conclusion

The authors have obtained the following results from the cytochemical investigation of the genesis of skin sweat glands in the palm and the sole of human embryos, of five to nine foetal months old:

1) Protein. In the fifth foetal month, protein is demonstrated somewhat intensely in the cytoplasm of the cord region and the secretory region of the sweat gland rudiment, and markedly intensely in the karyoplasm and the nuclear membrane. In the sixth foetal month, though there are some rudiments, which show a slight retardation of development, in the sole, all rudiments in the palm and most of them in the sole have completed the formation of the lumen, and protein is demonstrated intensely in the internal cells of the cord region and in the superficial cells of the secretory region. The reaction of protein is particularly intense in the karyoplasm and in the boundaries between cells. In the seventh foetal month, protein shows a general strong positivity, which is more intensified in the eighth to ninth foetal month.

2) Nucleic acids. In the fifth foetal month, the RNA-reaction is weakly positive in the whole cytoplasm of the palm and the sole, and somewhat more intense in the nucleoli. DNA is strongly positive in the karyoplasm. In the sixth foetal month, when the internal cells of the cord region and the superficial cells of the secretory
region show strong RNA-reaction in their nucleoli and the part of their cytoplasm facing the lumen. In the karyoplasm a marked presence of DNA is demonstrated. Among the rudiments in the sole a few are found in which the lumen has not been formed, and which exhibit the same reaction as the clavate rudiments of the fifth month embryo. In the seventh foetal month, the presence of RNA and DNA is demonstrated more intensely than in the sixth foetal month. In the eighth or ninth foetal months, the whole cell bodies have grown larger and the presence of RNA in the cytoplasm and in the nucleoli and of DNA in the karyoplasm is recognized more distinctly.

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Explanation of Microphotographs

Fig. 1. Hg-BPB-stain: Embryo of the fifth month. Eccrine sweat gland of the sole. Cytoplasm is stained deep blue both in the cord region and the secretory region. Karyoplasm is especially intensely stained, looking dark as a whole. (×600)

Fig. 2. Hg-BPB-stain: Embryo of the sixth month. Eccrine sweat gland of the palm. The superficial cells of the secretory region are somewhat deeply stained. The superficial cells of the secretory region are somewhat deeply. (×1350)

Fig. 3. Hg-BPB-stain: Embryo of the ninth month. Eccrine sweat glands of the palm. The superficial cells show an especially intense coloration in the part facing the lumen. The karyoplasm shows the most intense stainability. (×1350)

Fig. 4. B-A-stain: Embryo of the fifth month. Eccrine sweat gland of the sole. The cytoplasm of the cord region universally stains brown in medium intensity. The nuclei are coloured deeply purple. The swollen and region shows the same picture. (×600)

Fig. 5. PMG-stain: Embryo of the sixth month. Eccrine sweat gland of the palm. In the secretory region the part facing the lumen stains intensely red. The nuclei stain blue. (×1350)

Fig. 6. F-reaction: Embryo of the sixth month. Eccrine sweat glands of the sole. The karyoplasm of the superficial cells and the basal cells is coloured red. Connective tissue is stained light green. (×1350)

Fig. 7. T-stain: Embryo of the seventh month. Eccrine sweat gland of the palm. The part of the superficial cell cytoplasm, which faces the lumen, stains somewhat intensely. Karyoplasm stains more intensely blue. (×1350)

Fig. 8. B-A-method: Embryo of the eighth month. Eccrine sweat gland of the palm. A weak staining of granular shape is seen in the cytoplasm of the superficial cells. Nuclei are stained deep purple. (×600)

Fig. 9. PMG-stain: Embryo of the ninth month. Eccrine sweat glands of the palm. The part of the superficial cells, which face the lumen, the cytoplasm contains similar red stained. Nuclei stain blue. (×1350)

Fig. 10. F-reaction: Embryo of the ninth month. Eccrine sweat gland of the palm. Karyoplasm alone shows intense red coloration. (×600)
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