A Histochemical Study on the Skin of Human Embryo

by

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Up to the present day, a number of histochemical studies on the skin of the adult have been reported (Montagna, 1955; Montagna and Formisano, 1955; Beckett et al., 1956; Moretti and Mescon, 1956; Ellis and Montagna, 1958; Yasuda et al., 1958; Yasuda and Montagna, 1960; Montagna and Yun, 1961; and Adachi and Montagna, 1961). Histochemical studies on the sweat glands of axilla from the newborn child to the adult have been introduced by Yasui and others (1960 and 1961). Further, the eccrine glands in the palm and sole of the human embryo have been histochemically studied by Kaga et al. (1961) and Goto et al. (1961). The sweat glands in the auricle of the human embryo have been investigated by Ihijima and others (1958). However, the author cannot find out reports on the skin of the human embryo except those on the cholinesterase in the finger of the human embryo studied by Beckett et al. (1956). The author has conducted histochemical observations on the skin of the human embryo, paying special attention to the distribution of various kinds of enzymes and the change of reactions induced by these enzymes in the course of the development of the human embryo.

Materials and Methods

Specimens were removed from fresh embryos immediately after clinical abortion. Materials used were the skin of human embryo, ranging from the 4th to the 10th fetal month. The skins from the following regions were examined by histochemical procedures; the scalp, eyelid, cheek, lip, breast, back, axilla, external genitalia, perianal region, palm and sole. Paraffin sections of tissues, having
been fixed in Helly's solution, were stained with (a) H e i d e n h a i n's iron-hematoxylin, (b) Mallory's azan staining and (c) PAS staining. Cytochrome oxidase (B u r s t o n e, 1959 et 1960), succinic dehydrogenase (F a r b e r et al., 1956), monoamine oxidase (G l e n n e r et al., 1957) and phosphorylase (T a k e u c h i et al., 1955) were demonstrated in frozen sections of unfixed tissues. Other enzymes were studied in frozen sections fixed for 4 hours in 10% formalin: (1) alkaline phosphatase (G o m o r i, 1952) and acid phosphatase (B u r s t o n, 1954); (2) alpha-naphthol esterase (G o m o r i, 1952) and naphthol-AS esterase (P e a r s e, 1960); (3) aminopeptidase (B u r s t o n e et al., 1956); (4) acetylcholinesterase and butyrylcholinesterase (K o e l l e et al., 1949).

Observations

A) Succinic dehydrogenase, Cytochrome oxidase and Monoamine oxidase

(1) Epidermis (Fig. 1, 4, 11, 13, 17, 18 and 23)

The activities of the succinic dehydrogenase, the cytochrome oxidase and the monoamine oxidase in the epidermis increase in accordance with the advance of the fetal month. In the 4th fetal month, the Malpighian layer shows weak reactions of the succinic dehydrogenase, the cytochrome oxidase (Fig. 1) and the monoamine oxidase. These reactions in the palm and sole however are stronger than those in the other portions of body surface. In the 5th fetal month, activities of three kinds of enzymes were stronger in the Malpighian layer without regional differences (Fig. 11). In the 6th fetal month, the cytochrome oxidase and the succinic dehydrogenase show strong reactions in the Malpighian layer. Thereafter, activities of the succinic dehydrogenase and the oxidase increase in proportion to the advance of the fetal month. Through the whole course of development, the succinic dehydrogenase activity is stronger in the palm and sole than in the other portions of the body surface studied.

(2) Pilary System (Fig. 13 and 23)

In the 4th fetal month, weak activities of the cytochrome oxidase, the monoamine oxidase, and the succinic dehydrogenase in the hair follicle are seen. The stainabilities of the succinic dehydrogenase and the cytochrome oxidase in the 5th fetal month are more intensive than those in the 4th fetal month. And after then, the activities of both the succinic dehydrogenase and the cytochrome oxidase in the hair follicles increase in accordance with the develop-
ment of the human embryo. But the hair follicles from the 4th to the 7th fetal month show a weak reaction of monoamine oxidase. After then, the stainabilities gradually get stronger. The activities of the succinic dehydrogenase and the monoamine oxidase are stronger in the external root sheath than in the internal root sheath (Fig. 23), while those of the cytochrome oxidase are found diffusely in the whole portion of the hair follicle. The regional differences of the hair follicle are not recognized by means of both dehydrogenase and oxidase reactions.

(3) Sebaceous Gland

In the 4th fetal month, the primordium of the sebaceous gland is encountered. The primordium shows weak reactions to the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase. In the 5th fetal month, the activities of the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase are much stronger in the sebaceous glands and also in the Meibomian glands. The stainability of the secretory portion of the sebaceous gland is more intensive than that of the excretory duct. From the 6th to the 10th fetal month, the activities of both the succinic dehydrogenase and the cytochrome oxidase increase step by step in both kinds of the sebaceous and the Meibomian glands. But the curve of the monoamine oxidase activity slants down in the 6th fetal month, and after then that goes up in proportion to the advance of the fetal month. The activities of the monoamine oxidase in the sebaceous glands is stronger in the 10th fetal month than in any other fetal month. The regional differences of the body surface on the sebaceous glands are not encountered.

(4) Eccrine Gland (Fig. 1, 4, 11, 13, 17 and 18)

In the 4th fetal month, the primordia of eccrine glands are found only in the palm and sole, but not in any other portion of the body surface. The primordia of the eccrine glands, being lentiform or papillary, are weakly reactive to all reactions of the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase (Fig. 1). In the 5th fetal month, these three kinds of enzymes in the clavate-like primordia of eccrine glands are much more active than those in the 4th fetal month (Fig. 4). And they are stronger in the swollen portion of apex of the primordium than in the cord portion of the same. From the 5th to the 10th fetal month, the activities of both the succinic dehydrogenase and the cytochrome oxidase increase with the development; and especially in the 10th fetal month, the stainability of the succinic dehydro-
genase is remarkably intensive in the secretory portion of the eccrine gland. In contrast, the activity of the monoamine oxidase declines in the eccrine glands only in the 6th and the 7th fetal month. After the 8th fetal month, the reactivity to the monoamine oxidase is stronger again in the eccrine glands. In each fetal month after the luminal formation of the eccrine gland, the activities of the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase are stronger in the secretory portion than in the excretory duct. Moreover, it has been noted that the activities of these three kinds of enzymes in the eccrine glands are more intensive in the palm and sole than in any other portion of the body surface after the 5th fetal month.

(5) Apocrine Gland (Fig. 13 and 23)

The primordia of the papillary form of the apocrine glands are encountered in the axilla and the lip in the 4th fetal month. And that primordium has weak activities of the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase. The stainabilities of these kinds of enzymes are stronger in the clavate-like primordium of the apocrine gland in the 5th fetal month than in the papillary-like primordium of that in the 4th fetal month. Although the activities of both the succinic dehydrogenase and the cytochrome oxidase increase in the apocrine glands with the advance of the fetal month, these enzymatic activities are stronger in the eccrine gland than in the apocrine gland. The curve of the activity of the monoamine oxidase in the apocrine gland in the course of development is irregular in part. Namely; from the 4th to the 6th fetal month, the activity of the enzyme increases gradually, but in the 7th fetal month, the activity declines. Again in the 8th fetal month, the activity of the monoamine oxidase is stronger; and in the 10th fetal month, the enzyme shows the strongest activity in the apocrine gland. It is common that the activities of the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase are stronger in the secretory portion than in the excretory duct in each fetal month after the 6th fetal month when the lumen of the apocrine gland are formed (Fig. 13 and 23). Moreover, the activities of these three kinds of enzymes are more intensive in the eccrine gland than in the apocrine gland through all the fetal months. The regional differences of the apocrine glands are not observed by means of both the reactions of dehydrogenase and oxidase.

(6) Nerve Fiber, Blood Vessel, Adipose Tissue and Muscle

Nerve Fiber: Nerve fibers are reactive to the succinic dehy-
drogenase, the monoamic oxidase and the cytochrome oxidase. These enzymatic activities in the nerve fibers show no relation to the advance of the fetal month. After the 7th fetal month, the cytochrome oxidase is strongly reactive in the inner bulb of Vater-Pacini's corpuscle.

Blood Vessel: In all the fetal months, the endothelium of the blood vessel shows positive reactions of the succinic dehydrogenase, the monoamine oxidase, and the cytochrome oxidase.

Adipose Tissue: From the 4th to 10th fetal month, the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase are active in the immature adipose tissue of the human embryo (Fig. 13).

Muscle: Although the activities of the monoamine oxidase and the cytochrome oxidase are stationary from the 4th to the 10th fetal month, the activity of the succinic dehydrogenase increases in the muscle fibers in accordance with the development.

B) Phosphorylase

(1) Epidermis (Fig. 10)

The phosphorylase activity in the epidermis, especially in the stratum Malpighii, is very strong in both the 5th and the 6th fetal month (Fig. 10). After the 7th fetal month, that phosphorylase activity decreases gradually; and it is not observed in any other portion of the body surface except in the axilla and in the transitional portion between the mucous membrane of the eyelid and the skin of that. In the 10th fetal month, the activity of phosphorylase becomes negative in the epidermis throughout the whole body surface.

(2) Pilary System (Fig. 10 and 16)

The hair follicle of an embryo 4 months old is reactive to the phosphorylase. The hair follicles are stained more strongly by the phosphorylase staining in the 5th than in the 4th fetal month. In addition, the activity of the phosphorylase is stronger in the external than in the internal root sheath. Thereafter, the enzymatic activity becomes particularly strong in the apex of hair follicle (Fig. 10). In the 7th fetal month, the activity slopes down; but again in the 8th fetal month the hair follicles are strongly stained (Fig. 16). In the 10th fetal month, the phosphorylase activity becomes stronger in the middle and one-third portion of the hair follicle than in the apex of the hair follicle.

(3) Sebaceous Gland (Fig. 10 and 16)

In the 4th fetal month, the primordia of sebaceous glands react
strongly to the phosphorylase. When the embryos reach the age of 5th fetal month; the phosphorylases in the eyelid, the lip and the axilla are strongly reactive in the whole portion of the sebaceous gland, but those in the cheek are weakly reactive only in the peripheral portion of that gland. In the 6th fetal month, the reaction to the phosphorylase in that gland is strong only in the lip, but weak in the other portions of the body surface. From the 7th to the 10th fetal month, the reaction to that enzyme in all the portions on the body surface is completely negative in the central portion of the sebaceous gland, but is positive in the peripheral portion. The phosphorylase in the Meibomian gland of an embryo from 5 to 10 months old is not stained in the secretory portion, but is stained in the excretory duct. The activity of the phosphorylase in the sebaceous glands decreases with the advance of the fetal month.

(4) Eccrine Gland (Fig. 15)

In the 4th fetal month, the activity of the phosphorylase are strong in the papillary-like primordia of the eccrine glands on the general body surface, and are stronger in the clavate-like ones in the palm and sole than in any other portion. The reaction to the enzyme is strong in the cord portion of the clavate-like primordium, and is weak in the apical portion, whose outer-layer cells do not react to the phosphorylase. In the 5th fetal month, the activity of the phosphorylase in the eccrine glands is stronger in the cord portion of the primordium than in the swollen portion of the apex. The enzymatic activity in the cord portion is stronger in the area toward the epidermis than in the area toward the supposed secretory portion. In this developmental stage, the cells corresponding to the myoepithelial cells give a negative reaction to the phosphorylase. In the 6th fetal month, the reaction to the phosphorylase is strong in the eccrine gland without regional defferences throughout the body surface. In addition, the enzymatic activity is stronger in the excretory duct than in the secretory portion. At this stage, the secretory portions of the eccrine glands are stained strongly in the cytoplasm facing the Lumen. Both kinds of the superficial and the basal cells are not discriminated by the phosphorylase reaction in the 6th fetal month. The myoepithelial cells show the negative reaction to the phosphorylase. The enzymatic activity is strong in the excretory duct, especially in the portion facing the lumen of the duct. For the first time in the 7th fetal month, the reaction to the phosphorylase in the secretory portion of the eccrine gland
is stronger in the basal cell than in the superficial cell. In the 8th fetal month; the enzymatic activity is strong in the basal cells (Fig. 15), but are weak or negative in the superficial cells. At this stage, the excretory duct is composed of both the phosphorylase strong-positive cell and the phosphorylase weak-positive cell. In addition, the myoepithelial cells do not react to the phosphorylase even in the 8th fetal month. After the 8th fetal month, the distribution of the phosphorylase in the eccrine gland of the human embryo is similar to that in the eccrine gland of the adult. Through the entire fetal months, regional differences in the body surface on the eccrine gland are not observed.

(5) Apocrine Gland (Fig. 16)

In the 4th fetal month, the papillary-like primordia of apocrine glands in the axilla and the eyelid are reactive to the phosphorylase. In the 5th fetal month, the activity of the phosphorylase in the clavate-like primordia of the apocrine glands is stronger in the cord portion than in the swollen portion of the apex. In the 6th fetal month, the reaction to the phosphorylase in the secretory portion of the apocrine gland is positive in the glandular cells, but negative in the myoepithelial cells. The excretory ducts are more weakly reactive to the phosphorylase in the 6th than in the 5th fetal month. In the 7th fetal month, the enzymatic activity in the secretory portion of the apocrine gland is almost as strong as that in the excretory duct of that. The activity of the phosphorylase in the apocrine gland is weaker in the 8th than in the 7th fetal month. In addition, the Mo l l's glands of an embryo 8 months old are weakly reactive to the enzyme. Although the activity of the phosphorylase in the glandular cells of the apocrine glands in this stage is weak, non-typical cells that are phosphorylase strong-positive are encountered in the secretory portion of the apocrine gland in the lip. The myoepithelial cells of the apocrine glands are not reactive to the phosphorylase like those of the eccrine glands. From the 8th to the 10th fetal months, the reaction to the phosphorylase in the apocrine glands is stronger in the excretory duct than in the secretory portion. Although the secretory portion of the apocrine gland in the adult is unreactive to the phosphorylase, the glandular cell of the secretory portion is stained faintly by the phosphorylase staining. Moreover, the enzymatic activity is weak in the Mo l l's gland even in the 10th fetal month. The regional difference of the body surface on the apocrine gland is not observed by means of the phosphorylase reaction.
(6) Nerve Fiber, Blood Vessel, Adipose Tissue and Muscle

Nerve Fiber: The reaction to the phosphorylase in the nerve fibers is stationary from the 4th to the 10th fetal month (Fig. 16).

Blood Vessel: Through all the fetal months, the media of the blood vessel is strongly reactive to the phosphorylase.

Adipose Tissue: The activity of the phosphorylase decreases in the adipose tissue of the human embryo in accordance with the advance of the fetal month. In the 10th fetal month, the reaction to the phosphorylase in the adipose tissue is completely negative in all portions of the body surface.

Muscle: From the 4th to the 10th fetal month, in every portion of the body surface, the activity of the phosphorylase is very much strong in the muscular tissue (Fig. 15 and 16). Sometimes, a great number of striations that are phosphorylase strong-positive are clearly observed in the muscle fibers (Fig. 15).

C) Acetylcholinesterase

(1) Epidermis

In the 4th fetal month, the nerve fibers containing acetylcholinesterase reach just below the epidermis in the eyelid, the lip and the axilla; while the photographs, showing these nerve fibers inside the epidermis of the palm, are observed. In the 5th fetal month, the acetylcholinesterase-positive nerve fibers are also just below the epidermis in all portions of the body surface except the palm and sole. From the 6th to the 10th fetal month, the nerve fibers containing acetylcholinesterase branch just below the epidermis in every portion of the body surface, and these nerve fibers penetrating into the epidermis are not encountered even in the palm and sole. The nerve fibers containing acetylcholinesterase increase in number with the advance of the fetal month; so in the 10th fetal month, a great number of ramifications of the acetylcholinesterase-positive nerve fibers are observed in the dermal papilla.

(2) Pilary System (Fig. 8, 9, 12 and 14)

In the 4th fetal month, the nerve fibers containing acetylcholinesterase are near the apex of the primordium of the hair follicle or in contact with that. But already at this stage, the cilia in the eyelid are wrapped with the acetylcholinesterase-positive nerve fibers. In the 5th fetal month; while the hair follicles are wrapped with these nerve fibers only in the eyelid and the lip, those are not encircled in the axilla, the external genitalia and the perianal region. For the first time in the 6th fetal month, the hair follicles are wrapped with the acetylcholinesterase-positive nerve fibers in all
portions studied, such as in the eyelid, the lip, the breast, the axilla, the external genitalia and so on (Fig. 8). From the 7th to the 10th fetal month, nerve fibers containing acetylcholinesterase around the hair follicles increase in number in proportion to the development of the human embryo (Fig. 12). The nerve endings of hair follicles are positive to both the acetylcholinesterase and the butyrylcholinesterase reactions (Fig. 9, 12 and 14).

(3) Sebaceous Gland (Fig. 8 and 14)

The primordia of the sebaceous glands are not wrapped with the acetylcholinesterase-positive nerve fibers in the 4th fetal month, and only the Meibomian glands are encircled with a few nerve fibers containing acetylcholinesterase at this stage. The distribution of the acetylcholinesterase around the sebaceous gland in the 5th fetal month is similar to that in the 4th fetal month. Initially in the 6th fetal month, the sebaceous glands are wrapped with the acetylcholinesterase-positive nerve fibers in the breast, the axilla and the external genitalia (Fig. 8), but not in the lip. In the 7th fetal month, the sebaceous glands in all portions studied are encircled with the nerve fibers containing acetylcholinesterase. The number of the nerve fiber containing acetylcholinesterase increases in the sebaceous gland and the Meibomian gland with the development of the human embryo.

(4) Eccrine Gland (Fig. 5, 8 and 12)

The nerve fibers containing acetylcholinesterase reach the apex of the primordium of the eccrine gland in the palm of an embryo 4 months old. In the 5th fetal month, the eccrine glands in all portions studied are wrapped with the acetylcholinesterase-positive nerve fibers (Fig. 5). From the 6th to the 10th fetal month, the nerve fibers containing acetylcholinesterase in the eccrine glands increase in number in conjunction with the advance of the fetal month (Fig. 8 and 12). Through all the fetal months, the acetylcholinesterase-positive nerve fibers wrap the secretory portion of the eccrine gland, but not the excretory duct.

(5) Apocrine Gland (Fig. 9 and 14)

The apocrine glands are not encircled with the nerve fibers containing acetylcholinesterase from the 4th to the 7th fetal month (Fig. 9). In contrast, the secretory portions of apocrine glands in such specialized regions as the perianal region and the axilla are wrapped with the acetylcholinesterase-positive nerve fibers from the 8th to the 10th fetal month (Fig. 14).

(6) Nerve Fiber, Blood Vessel, Adipose Tissue and Muscle
Nerve Fiber: Distributions of the acetylcholinesterase in the skin of the human embryo are encountered in the large myelinated nerve trunks and in the thin non-myelinated nerve fibers (Fig. 5). The nerve fibers containing acetylcholinesterase increase in number with the development of the human embryo. For the first time in the 5th fetal month, Meissner's corpuscles that are positive both to the acetylcholinesterase and to the butyrylcholinesterase reactions are observed in the palm. Moreover, the mucocutaneous endings in the lip are encountered at this stage.

Blood Vessel: From the 4th to the 10th fetal month, the blood vessels in the skin are wrapped with the acetylcholinesterase-positive nerve fibers and these nerve fibers often penetrate into the large blood vessels.

Adipose Tissue: The adipose tissues of the human embryos themselves are unreactive to the acetylcholinesterase, but the nerve fibers containing acetylcholinesterase make a branch inside the adipose tissue.

Muscle: From the 4th to the 10th fetal month, the muscles of the human embryo are reactive to the acetylcholinesterase, and especially the motor endplates are stained strongly in a dotted line. The muscles are strongly reactive to the acetylcholinesterase, but weakly to the butyrylcholinesterase. Therefore, the muscles of the human embryo are presumed to be containing plenty of acetylcholinesterase and a small quantity of butyrylcholinesterase. Moreover, the ramification of the acetylcholinesterase-positive nerve fibers inside the muscle of the human embryo is observed.

D) Alkaline and Acid Phosphatase

Epidermis

From the 4th to the 10th fetal month, the epidermis of the human embryo is not stained by both kinds of alkaline phosphatase stainings, the azo dye method and the Ca-Co method. However, the activity of the acid phosphatase is strong in the epidermis from the 4th to the 10 fetal month. Moreover, the stainability of the acid phosphatase in the epidermis is stronger in the stratum granulosum and the upper portion of the Malpighian layer than in the lower portion of the Malpighian layer.

Pilary System (Fig. 2 and 3)

The activity of the alkaline phosphatase in the hair follicle decreases in inverse proportion to the advance of the fetal month. Namely, the stainability of the alkaline phosphatase is remarkably strong in the apex and the outermost portion of the primordium
of the hair follicle in the 4th fetal month (Fig. 2 and 3). The activity of the alkaline phosphatase in the hair follicle is stronger in the 4th than in the 5th fetal month. And in the 5th fetal month, the stainability of the alkaline phosphatase is observed only in the apex of the hair follicle in all portions of the body surface studied. In addition, the alkaline phosphatase-positive collors in the hair follicle are encountered only in the eyelid of an embryo 5 months old. From the 6th to the 10th fetal month, the activity of the alkaline phosphatase in the apex of the hair follicle declines gradually with the development. In the 10th fetal month, the apex of the hair follicle shows the weakest reaction to the alkaline phosphatase. The distribution of the alkaline phosphatase in the hair follicle shows no difference between by the azo dye method and by the Ca-Co method. In the 4th fetal month, the primordium of the hair follicle is stained weakly by the acid phosphatase staining. On the contrary, the activity of the acid phosphatase in the hair follicle from the 5th to the 10th fetal month is strong. The reaction to the acid phosphatase in the hair follicle is stronger in the internal than in the external root sheath, through all the fetal months.

(3) Sebaceous Gland

The reaction to the alkaline phosphatase in the sebaceous gland and the Meibomian gland is negative through all the fetal months. But the alkaline phosphatase-positive blood capillaries are encountered around the Meibomian gland. The curve of the activity of the acid phosphatase from the 4th to the 10th fetal month is irregular. Namely, the activity of the acid phosphatase in the sebaceous gland is weak in the 4th fetal month, is stronger from the 5th to the 7th fetal month, and again gradually weaken from the 8th to the 10th fetal month. In the Meibomian gland, the activity of the acid phosphatase is stronger in the excretory duct than in the secretory portion.

(4) Eccrine Gland (Fig. 6)

In the 4th fetal month, the activity of the alkaline phosphatase is strong along the central portion and in the apex of the primordium of the eccrine gland. In the 5th fetal month, the stainability of the alkaline phosphatase in the eccrine glands in the palm and sole is strong in the excretory duct and in the supposed cytoplasm facing the lumen of the secretory portion. In any fetal month after the 6th, in any other portion except the perianal region, the eccrine glands do not show reactions to the alkaline phosphatase. Even in the 10th fetal month, the activity of the alkaline phosphatase is
observed only in the region facing the lumen of the excretory duct of the eccrine gland in the perianal region. Through all the fetal months, the stainability of the alkaline phosphatase is not encountered in the myoepithelial cells both by the azo dye method and by the Ca-Co method. The activity of the acid phosphatase in the primordia of the eccrine glands of an embryo 4 months old is weak. From the 5th to the 7th fetal month, the stainability of the acid phosphatase declines in the secretory portion of the eccrine gland, but do not declines in the excretory duct just below the epidermis. From the 8th to the 10th fetal month, the reaction to the acid phosphatase is completely negative in the other portions except the excretory duct just below the epidermis.

(5) Apocrine Gland (Fig. 2)

The activity of the alkaline phosphatase in the apocrine glands, like that in the eccrine glands, decreases with the development of the human embryo. In the 4th fetal month, the reaction to the alkaline phosphatase is encountered along the central portion of the primordium of the apocrine gland (Fig. 2). In the 5th fetal month, the activity of the alkaline phosphatase is observed linearly along the central portion in the cord region of the clavate-like primordium of the apocrine gland. From the 6th to the 10 fetal month, the reaction to the alkaline phosphatase is completely negative both in the secretory portion and in the excretory duct. The strong activity is observed only in the regions facing the lumens of both the secretory portion and the excretory duct of the Moll’s gland of an embryo 8 months old. Through all the fetal months, the myoepithelial cells in the apocrine glands are unreactive to the alkaline phosphatase. The activity of the acid phosphatase in the apocrine glands, like that in the eccrine glands, decreases in accordance with the advance of the fetal month. In the 4th fetal month, the primordia of apocrine glands are faintly reactive to the acid phosphatase. From the 5th to the 10th fetal month, the activity of the acid phosphatase makes weaker gradually, but one part of the duct opening into the hair follicle is stained more strongly than the other parts of the duct. In the 10th fetal month, the activity of the acid phosphatase is observed only in the part of the duct connecting the hair follicle.

(6) Nerve Fiber, Blood Vessel, Adipose Tissue and Muscle

Nerve Fiber: The nerve fibers of the human embryo are reactive neither to the alkaline phosphatase nor to the acid phosphatase.

Blood Vessel: The capillaries are stained very strongly by alkaline phosphatase stainings, both by the Ca-Co method and by
the azo dye method (Fig. 2 and 3). And the blood vessels larger than the capillaries are unreactive to the alkaline phosphatase reaction of the azo dye method. On the contrary, sometimes the activity of the alkaline phosphatase are observed in the endothelial cells of large blood vessels only by means of the Ca-Co method. The reaction to the acid phosphatase is negative in the blood vessels.

Adipose Tissue and Muscle: The activities of the alkaline phosphatase and the acid phosphatase are not encountered both in the adipose tissue of the human embryo and in the muscular tissue of that.

E) Nonspecific Esterases

(1) Epidermis (Fig. 19)

In the 4th fetal month, the activities of the nonspecific esterase are demonstrated in the epidermis. In addition, these enzymatic activities are stronger in the upper part of the Malpighian layer and the stratum granulosum than in the lower part of the Malpighian layer. The stainabilities and the distributions of the nonspecific esterases in the epidermis in the 5th and the 6th fetal months are similar to those in the 4th fetal month. In the 7th fetal month, the epidermis in all portions of the body surface studied is stained weakly by the naphthol-AS esterase staining, but strongly by the alpha-naphthol esterase staining. In the 8th fetal month, the activities of both the naphthol-AS esterase and the alpha-naphthol esterase are strong in the epidermis (Fig. 19). In the 10th fetal month, the epidermis of the human embryo is stained rather more strongly by the alpha-naphthol esterase than by the naphthol-AS esterase staining. Generally, the activity of the naphthol-AS esterase in the epidermis is stationary in spite of the development of the human embryo, but the activity of the alpha-naphthol esterase in the epidermis increases with the advance of the fetal month.

(2) Pilary System

Although the activity of the naphthol-AS esterase in the hair follicle is stationary regardless of the development of the human embryo, the reaction to the alpha-naphthol esterase increases with the advance of the fetal month. Namely, the hair follicles through all the fetal months are stained to the same degree by the naphthol-AS esterase staining, but the activity of the alpha-naphthol esterase in the hair follicles is stronger in the latter half of the fetal life than in the former half. In addition, the stainabilities of both kinds of enzymes are stronger in the internal root sheath than in
the external root sheath. The time when the hair collors stained by the nonspecific esterase staining first make appearance is the 7th fetal month. In addition, these hair collors are encountered only in such portions as the axilla, the perianal region and the external genitalia.

(3) Sebaceous Gland

Although the sebaceous glands are stained strongly by the naphthol-AS esterase staining through all the fetal months, the activity of the alpha-naphthol esterase in the sebaceous glands is weak from the 4th to the 6th fetal month, and is strong from the 7th to the 10th fetal month. The reactions to the nonspecific esterases in the Meibomian glands are stronger in the secretory portions than in the excretory duct throughout the fetal months. Moreover, the stainabilities of the nonspecific esterases are stronger in the peripheral portion than in the central portion both in the sebaceous gland and in the Meibomian gland.

(4) Eccrine Gland (Fig. 19 and 22)

In the 4th fetal month, the activity of the naphthol-AS esterase is weak in the primordia of the eccrine glands in every portion of the body surface. From the 5th to the 10th fetal month, the activity of the naphthol-AS esterase becomes stronger only in the secretory portions of the eccrine glands in the palm and sole (Fig. 19 and 20), and is much stronger in the secretory portion than in the excretory duct (Fig. 19). In contrast, the stainability of the naphthol-AS esterase is constantly weak both in the secretory portion and in the excretory duct of the eccrine gland in all portions of the body surface, studied at this time, except the palm and sole through all the fetal months. The myoepithelial cells of the eccrine glands are unreactive to the naphthol-AS esterase. The activity of the alpha-naphthol esterase in the eccrine glands is encountered weakly in all portions of the body surface studied, including the palm and sole, both in the 4th and in the 5th fetal month. For the first time in the 6th fetal month, the alpha-naphthol esterase shows strong reaction only in the secretory portion of the eccrine gland in the palm and sole. And this strong enzymatic activity continues constantly in the secretory portion of the eccrine gland till the 10th fetal month. In addition, the stainability of the alpha-naphthol esterase in the eccrine gland in each portion of the body surface except the palm and sole increases gradually after the 7th fetal month. Generally, the activity of the alpha-naphthol esterase is stronger in the palm and sole than in any other portion. The
distribution of the alpha-naphthol esterase is similar to that of the naphthol-AS esterase in the eccrine glands.

(5) Apocrine Gland

The activity of the naphthol-AS esterase in the apocrine glands is weak from the 4th to the 7th fetal month. The apocrine glands from the 8th to the 10th fetal month are strongly reactive to the naphthol-AS esterase. The activity of the naphthol-AS esterase in the apocrine glands shows no difference between in the secretory portion and in the excretory portion. The reaction to the alpha-naphthol esterase in the apocrine glands is weak from the 4th to the 6th fetal month, but that increases gradually after the 7th fetal month. Alpha-naphthol esterase, like naphthol-AS esterase, shows no difference of the activity between in the secretory portion and in the excretory duct of the apocrine gland. The activities of both the naphthol-AS esterase and the alpha-naphthol esterase are comparatively strong in the secretory portion and the excretory duct of the apocrine gland. The stainabilities of both naphthol-AS esterase and alpha-naphthol esterase are more intensive in the secretory portion of the eccrine gland than in that of the apocrine gland.

(6) Nerve Fiber, Blood Vessel, Adipose Tissue and Muscle

Nerve Fiber: The nerve fibers of the human embryo are stained constantly both by the naphthol-AS esterase staining after the 8th fetal month and by the alpha-naphthol esterase staining after the 7th fetal month. Nonspecific esterases are stained in Meissner's corpuscle after the 7th fetal month and in Vater-Pacini's corpuscle after the 5th fetal month.

Blood Vessel: The activities of the nonspecific esterases are encountered in the region facing the lumen of the blood vessel. And the enzymatic activities in the blood vessels are stronger by alpha-naphthol esterase than by naphthol-AS esterase.

Adipose Tissue: The adipose tissue of the human embryo shows a weak or negative reaction to the nonspecific esterase.

Muscle: The stainabilities of the naphthol-AS esterase and the alpha-naphthol esterase are observed faintly or none at all in the muscular tissue of the human embryo. But only the M. sphincter ani in the perianal region is strongly reactive to the nonspecific esterase. Moreover, the motor endplates in the muscle show the strong activities of the nonspecific esterases throughout the fetal months.

F) Leucine Aminopeptidase
(1) Epidermis

From the 4th to the 10th fetal month, the activity of the leucine aminopeptidase is observed weakly in the Malpighian layer of the epidermis. The stainability of the leucine aminopeptidase in the epidermis shows no regional difference of the body surface.

(2) Pilary System

The activity of the leucine aminopeptidase in the hair follicles decreases with the development of the human embryo. Namely, the activity of the leucine aminopeptidase is strong in the hair follicles in all portions of body surface studied from the 4th to the 6th fetal month. In the 7th fetal month, the stainability of the leucine aminopeptidase in the hair follicle is intensive only in the lip, but that is faint in the other portions of the body surface studied. From the 8th to the 10th fetal month, the reaction to the leucine aminopeptidase declines in the hair follicles in all portions of the body surface. Generally, the apical portion of the hair follicle has the strong activity of the leucine aminopeptidase throughout the fetal months. The activity of the leucine aminopeptidase in the hair follicle is stronger in the internal than in the external root sheath.

(3) Sebaceous Gland

The activity of the leucine aminopeptidase in the sebaceous glands is weak from the 4th to the 6th fetal month. And the stainability of this enzymes is not encountered in the sebaceous glands from the 7th to the 10th fetal month. In addition, the Meibomian glands are unreactive to the leucine aminopeptidase throughout all the fetal months.

(4) Eccrine Gland

In the 4th fetal month, the activity of the leucine aminopeptidase is strong in the primordia of the eccrine glands, especially in the apex of the primordium. In the 5th fetal month, the stainability of the leucine aminopeptidase is strong in the swollen portion of the apex, but weak in the cord region of the primordium. In the 6th fetal month, the secretory portion of the eccrine gland in the palm is strongly reactive to the leucine aminopeptidase, while the secretory portions in the axilla, the lip and so on are less weakly reactive than those in the palm. Moreover, the excretory ducts of the eccrine glands are stained faintly by the leucine aminopeptidase staining at this stage. After the 7th fetal month, the activity of the leucine aminopeptidase in the secretory portion of the eccrine gland increases in accordance with the advance of the fetal month. Generally, the secretory portions of the eccrine glands are more
strongly reactive to the leucine aminopeptidase in the palm than in the other regions of the body surface studied. The excretory ducts of the eccrine glands are weakly reactive to the leucine aminopeptidase from the 4th to the 10th fetal month. The activity of the leucine aminopeptidase in the secretory portion of the eccrine gland is equally strong among the superficial cell, the basal cell and the myoepithelial cell.

(5) Apocrine Gland

The stainability of the leucine aminopeptidase in the apocrine gland is faint from the 4th to the 7th fetal month, but that in only the secretory portions of the apocrine glands increases gradually after the 8th fetal month. In addition, the Moll’s glands, like the apocrine glands, are more strongly reactive to the leucine aminopeptidase after the 8th fetal month. Generally, the activity of the leucine aminopeptidase in the apocrine glands is stronger in the secretory portion than in the excretory duct. The reaction to the leucine aminopeptidase is stronger in the secretory portion of the eccrine gland than in that of the apocrine gland.

(6) Nerve Fiber, Blood Vessel, Adipose Tissue and Muscle

Nerve Fiber: The nerve fibers are weakly reactive to the leucine aminopeptidase through all the fetal months. The activity of the leucine aminopeptidase is encountered in the inner bulb of Vater-Pacini’s corpuscle and in the peripheral portion of that.

Blood Vessel: The stainability of the leucine aminopeptidase is observed constantly in the portion facing the lumen of the blood vessel through all the fetal months.

Adipose Tissue: The immature adipose tissue of the human embryo is unreactive to the leucine aminopeptidase.

Muscle: From the 4th to the 10th fetal month, the activity of the leucine aminopeptidase is weak in the muscular tissue.

Discussion

Generally, the activities of the succinic dehydrogenase, the monoamine oxidase and the cytochrome oxidase increase in the epidermis, the hair follicle, the sebaceous gland, and both kinds of sweat glands in accordance with the advance of the fetal month. The distributions of the succinic dehydrogenase (Montagna et al., 1955), the monoamine oxidase (Yasuda et al., 1960), and the cytochrome oxidase (Montagna et al., 1961) in the skin of the adult are similar to one another, but only in the myoepithelial cells they
are not similar. The distributions of succinic dehydrogenase, mono-
amine oxidase and cytochrome oxidase in the skin of the human
embryo are similar to one another even in the myoepithelial cells.
These enzymatic activities are stronger in the skin of human
embryo than in the skin of man. The time when the distributions
of these kinds of enzymes in the epidermis and the cutaneous
appendage of the human embryo are similar to those of man is
variable in each enzyme and in each organ. Namely, that time is
the 5th or the 6th fetal month in the epidermis, and is the 6th
fetal month in the sebaceous gland by means of succinic dehy-
drogenase, monoamine oxidase and cytochrome oxidase stainings.
Moreover, that time in the hair follicle is after newborn by the cyro-
chrome oxidase staining, is the 10th fetal month by the monoamine
oxidase staining, and is the 6th fetal month by the succinic dehy-
drogenase staining. After the 8th fetal month, the secretory
portion and the excretory duct of the eccrine gland in the human
embryo show such a distribution of cytochrome oxidase as those in
the eccrine glands in man. Although only the excretory ducts of
the apocrine glands in man are negative to the cytochrome oxidase,
both the secretory portion and the excretory duct of the apocrine
glands in the human embryo are stained by the cytochrome oxidase
staining till the 10th fetal month. Therefore, it is presumed that
the excretory ducts become negative to that reaction after birth,
but not negative in fetal life. The cytochrome oxidase-positive
Vater-Pacini's corpuscles, which are observed in the palm of
man, are encountered even in the palm of an embryo 7 months old.
The distribution of the monoamine oxidase in the sweat glands of
the human embryo, being similar to that in the sweat glands of
man, is encountered in both the secretory portion and the excretory
duct of the eccrine gland in the 5th fetal month, and is observed
in both the secretory portion and the excretory duct of the apocrine
gland in the 10th fetal month. In addition, the monoamine oxidase-
positive nerve fibers are demonstrated in the skin through all the
fetal months. The distribution of the succinic dehydrogenase in the
eccrine glands after the 6th fetal month is similar to that in the
eccrine glands of the adult, with the exception of the strong en-
zymatic activity in the cytoplasm facing the lumen of the secretory
portion of the human embryo. Moreover, the distribution of the
succinic dehydrogenase in the apocrine gland after the 8th fetal
month, except the strong stainability in the area facing the lumen
of the secretory portion, is similar to that in the apocrine gland
of man. The activities of the respiratory enzymes increase in the epidermis and the cutaneous appendage with the development of the human embryo. The stainabilities of the respiratory enzymes in the skin of the human embryo are more intensive in the epidermis and the secretory portion of the eccrine gland of the palm and sole than in those of the other portions of the body surface studied.

The activity of the phosphorylase in the epidermis till the 6th fetal month is observed strongly in the Malpighian layer like that in the epidermis of man, but that enzymatic activity decreases gradually after the 7th fetal month. The distribution of the phosphorylase even in the 10th fetal month is observed in the middle and one-third portion of the hair follicle, so it is presumed that the distribution of the phosphorylase in the hair follicle after birth becomes similar to those in the adult (Ellis et Montagna, 1958). The activity of the phosphorylase in the peripheral portion of the sebaceous gland after the 8th fetal month is as strong as that in the adult. The reaction to the phosphorylase in the eccrine glands of man is positive in the basal cells, but negative in both the superficial cells and the myoepithelial cells (Yasuda et al., 1958). Although the secretory portion of the eccrine gland in the human embryo is stained diffusely in both kinds of glandular cells except the myoepithelial cells till the 6th fetal month by the phosphorylase staining, the activity of the phosphorylase in the eccrine gland in the 7th fetal month becomes stronger in the basal cells than in the superficial cells. In addition, for the first time in the 8th fetal month, the distribution of the phosphorylase in the eccrine gland of the human embryo is similar to that in the eccrine gland of man. According to Tsuchiy'a's paper (1954), both kinds of glandular cells (superficial and basal cells) of the eccrine gland in the axilla are discriminated by the glycogen staining in the 7th fetal month. The apocrine glands of the human embryo are reactive to the phosphorylase through all the fetal months. Therefore, it is presumed that the secretory portion of the apocrine gland after birth becomes negative to phosphorylase reaction like that of the apocrine gland in man. According to Yasui and others (1961), the time when the secretion granules being positive to all stainings of PAS, iron-hematoxylin, protein, Giemsa and toluidin blue make appearance is after the age of 12 years. After the 8th fetal month, the phosphorylase strong-positive cells thought as Yasuda's non-typical cells (1959) are encountered in the secretory portion of the apocrine gland of the human embryo.
Beckett et al. (1956) studied the distribution of the cholinesterase in the finger of the human embryo and man. The specimens they used are the fingers of embryos from the 4th to the 7th fetal month, while the author's specimens are the human embryos from the 4th to the 10th fetal month. The author's studied distribution of cholinesterase in the palm of the embryo is in agreement with that in the finger of embryo (Montagna, 1960) in the following points; the cholinesterase-positive myelinated nerve trunk, the specific cholinesterase-positive nerve fibers inside the epidermis of palm in the 5th fetal month, Meissner's and Vater-Pacini's corpuscles containing pseudocholinesterase, and the secretory portion of the eccrine gland wrapped with the nerve fibers containing the specific cholinesterase. The cholinesterase-positive nerve fibers penetrating into the epidermis of the human embryo are not observed even in all portions of the body surface studied except the palm in the 4th and 5th fetal month. In the embryo after the 6th fetal month, also in man, the epidermis in all portions of the body surface studied, including the palm and sole, does not have the nerve fibers containing cholinesterase. Although only the hair follicles in the perianal region and the external genitalia in man are wrapped with the nerve fibers containing cholinesterase, the cholinesterase-positive nerve fibers wrapping the hair follicles of the human embryo are observed in such portions as the eyelid, the lip, the cheek, the axilla, and the external genitalia in the 10th fetal month. It is presumed that the hair follicles wrapped with the nerve fibers containing cholinesterase are localized only in such specialized portions as the external genitalia and the perianal region after birth. According to Montagna (1960), the sebaceous glands are not encircled with the nerve fibers containing cholinesterase in the skin of man. However, the Meibomian gland and, only the sebaceous gland in the eyelid are wrapped with these nerve fibers. According to the author's study, the sebaceous glands of the human embryo after the 6th fetal month are wrapped with the nerve fibers containing cholinesterase in the eyelid, the lip, the breast, the axilla, the external genitalia and the perianal region; and the Meibomian glands after the 6th fetal month are also wrapped. The nerve fibers containing cholinesterase around the eccrine glands of the human embryo through all the fetal months encircle the secretory portions, but not the excretory ducts. According to Avvik (1955), the secretory portions of the apocrine glands are wrapped with nerve fibers containing cholinesterase; according to Hurly, Shelley
and Koolie (1953), they are not wrapped with them; and according to Montagna (1960), the nerve fibers containing cholinesterase wrap the apocrine glands in the Negro, but not in the Caucasian. The author encountered the interesting result that the apocrine glands are wrapped with nerve fibers containing cholinesterase in the human embryo. Namely, the apocrine glands in the human embryo from the 4th to the 7th fetal month are not wrapped with nerve fibers containing cholinesterase. But the secretory portions of apocrine glands of the embryo after the 8th fetal month are encircled with nerve fibers containing cholinesterase only in such specialized portions as the perianal region and the axilla. Yasuda and others (1963) observed the cholinesterase-positive nerve fibers wrapping the apocrine glands in the axilla of osmidrosis of the Japanese. Although the apocrine glands are wrapped with the cholinesterase-positive nerve fibers in the above written regions of the human embryos, it is presumed that the nerve fiber networks containing cholinesterase around the apocrine glands become coarse after birth and become invisible in adult.

Stratum granulosum of the epidermis in man is slightly reactive to the alkaline phosphatase of the Ca-Co method (Montagna, 1956), but the epidermis in the human embryo is not stained by alkaline phosphatase stainings of both the Ca-Co method and the azo-dye method. The activity of the alkaline phosphatase in man is strong in the hair papilla of the hair follicle (Fisher and Glick, 1947), and the activity in the human embryo from the 4th to the 10th fetal month is also strong in the apex of the hair follicle. Although the stainability of the alkaline phosphatase (the Ca-Co method) in the sebaceous glands of man is observed in the immature cells, the reaction to the alkaline phosphatase in the sebaceous glands of the human embryo is negative (the Ca-Co method and the azo dye method). The distributions of the alkaline phosphatase both by the Ca-Co method and by the azo dye method in the eccrine and the apocrine gland in the human embryo are completely different from those of alkaline phosphatase by the Ca-Co method in both kinds of sweat glands in man. The activities of the alkaline phosphatase (Ca-Co method and the azo dye method) in the human embryo after the 4th fetal month are remarkably strong in the blood capillaries.

Moretti and Mescon (1956) studied the distribution of the acid phosphatase in the skin of man by means of Gomori's method, Rutenberg et Seligman's method and Burton's method. The author studied the distribution of the acid phosphatase
in the skin of the embryo from the 4th to the 10th fetal month. The time, when the distribution of the acid phosphatase in the skin of the human embryo is similar to that in the skin of man, is variable in the epidermis and the cutaneous appendage of the human embryo. That time is after the 5th fetal month in the epidermis, after the 7th fetal month in the sebaceous gland, and is after the 5th fetal month in the excretory ducts of both kinds of sweat glands. But the distributions of the acid phosphatase in the secretory portions of both kinds of sweat glands and in the hair follicles of the human embryo are not similar to those of man; because the cytoplasm facing the lumens of the secretory portions of both kinds of sweat glands in embryo are not stained so strongly as those in man, and the enzymatic activity in the hair follicles in the embryo is not so strong in the internal root sheath as in the external root sheath.

The activities of the nonspecific esterases in the sebaceous gland in the human embryo after the 7th fetal month are strong in the peripheral portions of the acini and in the sebum of the excretory duct like those in man. Although the distributions of the nonspecific esterases in the secretory portions of the eccrine glands in the human embryo are similar to those in man, the excretory ducts of the eccrine glands in the human embryo are stained by the nonspecific esterase staining unlike those of man. The distributions of the nonspecific esterase in the secretory portion and the excretory duct of the apocrine gland in the human embryo are not similar to those in man. From a physiological point of view, the mechanism of sweating in the eccrine glands of the palm and sole is not different from that in the eccrine glands of other portions of body surface (Rothman, 1954; Kuno, 1956). From a histochemical standpoint, the activity of the naphthol-AS esterase in the eccrine glands of the palm and sole is much stronger than that in the eccrine glands of the other portions of the body surface studied. In addition, the activity of the alpha-naphthol esterase is stronger in the eccrine glands of the palm and sole than in those of the other portions of the body surface after the 6th fetal month. The results described above are histochemical and regional differences between the eccrine glands in the palm and sole not containing the sebaceous glands and the eccrine glands in the other portions of the body surface containing the sebaceous glands. The activities of the nonspecific esterases in the sebaceous glands of the human embryos are stronger in such portions as the
 decide the perianal region, and the external genitalia than in the
other portions of the body surface. In addition, the activities of the
nonspecific esterases in the muscle of the human embryo are more
intensive in the M. sphincter ani than in the muscle of the other
portions of the body surface.

The localization of the leucine aminopeptidase in the skin of man
was studied by A d a c h i a n d M o n t a g n a (1961). That enzymatic
activity is stronger in the epidermis of the palm than in that of the
general body surface, and is particularly strong in the stratum
guanulosum of the epidermis in all portions of the body surface.
The stainability of the leucine aminopeptidase in the human embryo
is observed in the Malpighian layer through all the fetal months,
but not in the stratum granulosum. The reaction to the leucine
aminopeptidase in the apex of the hair follicle is as strong in the
human embryo from the 4th to the 10th fetal month as in man.
The distributions of the leucine aminopeptidase in the secretory
portions and the excretory ducts of the eccrine glands in the human
embryo after the 5th fetal month are similar to those in man. In
addition, the distribution of that enzyme in the secretory portion
of the apocrine gland in the human embryo after the 8th fetal
month is similar to that in man. The excretory ducts of the
apocrine glands are reactive to the leucine aminopeptidase even in
the 10th fetal month. The activity of the leucine aminopeptidase
is stronger in the eccrine gland than in the apocrine gland in man,
and are quite same in the human embryo through all the fetal
months. The activity of the leucine aminopeptidase in the human
embryo, like that in man, is stronger in the apex of the hair
follicle and in the secretory portion of the eccrine gland than in
any other organ of skin. In addition, the stainability of the leucine
aminopeptidase in the secretory portion of the eccrine gland is
stronger in the palm and sole than in the other portions of the
body surface studied.

In brief,

(1) Generally, it is thought that the activities of such enzymes
as cytochrome oxidase, monoamine oxidase, succinic dehydrogenase,
acetylcholinesterase, and nonspecific esterase increase in the skin
with the development of the human embryo. Especially, the activity
of phosphorylase in the eccrine gland increases with the development,
but that in the apocrine gland decreases with the development.
Moreover, the activity of leucine aminopeptidase is stronger in both
kinds of sweat glands, but the stainabilities of alkaline and acid
phosphatases are weaker in both the eccrine and the apocrine glands.

(2) The activities and distributions of enzymes are different between in the human embryo and in the man. The noticeable different points of the activities and distributions of enzymes between in the human embryo and in the man are as follows:

a) The reaction to cytochrome oxidase is positive in the excretory duct of the apocrine gland in the embryo, but negative in man.

b) The activity of succinic dehydrogenase is strong in the cytoplasm facing the lumens of both kinds of sweat glands in the embryo.

c) The stainability of phosphorylase is encountered in the secretory portion of the apocrine gland in the embryo.

d) The acetylcholinesterase-positive nerve fibers wrap the hair follicle only in the perianal region and the external genitalia in man, but they do wrap hair follicles in the eyelid, the lip, the axilla, the external genitalia, the perianal region, the cheek and so on in the embryo. Only the sebaceous gland in the eyelid in man are wrapped with the acetylcholinesterase-positive nerve fibers, but the sebaceous glands in such many regions as the eyelid, the lip, the breast, the axilla, the external genitalia, and the perianal region in the embryo are encircled with the nerve fibers containing acetylcholinesterase. In addition, the secretory portions of the apocrine glands in the human embryo are wrapped with the cholinesterase-positive nerve fibers only in the perianal region and the axilla.

e) The reaction to the alkaline phosphatase is positive in the peripheral portion of the sebaceous gland in man, but completely negative in the sebaceous gland of the human embryo. In addition, the distributions of the alkaline phosphatase in both kinds of sweat glands in the human embryo are absolutely different from those in man.

f) The reaction to the acid phosphatase is positive in the cytoplasm facing the lumens of both kinds of sweat glands in man, but negative in the secretory portions of both kinds of sweat glands in the embryo.

g) The reaction to the nonspecific esterase in the excretory duct of the eccrine gland is negative in man, but positive in the embryo. In addition, the activities of the nonspecific esterases of the apocrine glands in the embryo are different from those in man.

h) The activity of the leucine aminopeptidase in the epidermis is strong in the stratum granulosum in man, and in the stratum
Malpighii in embryo.

Conclusion

(1) The activities of cytochrome oxidase, monoamine oxidase and succinic dehydrogenase tend to increase in the epidermis and the cutaneous appendage with the development of the human embryo.

(2) The number of the nerve fiber containing acetylcholinesterase increases in the hair follicle, the sebaceous gland, the eccrine gland, and the apocrine gland with the advance of the fetal month. Especially, nerve fibers containing acetylcholinesterase are observed in the eccrine glands through all the fetal months studied. Moreover, the nerve fibers containing acetylcholinesterase in the apocrine glands appear for the first time in the 8th fetal month and do not disappear till the 10th fetal month.

(3) The activities of the nonspecific esterases, naphthol-AS esterase and alpha-naphthol esterase, increase in the epidermis and the cutaneous appendage with the development of the human embryo. In addition, the activity of the naphthol-AS esterase in the palm and sole is much stronger than that in any other portion of the body surface studied.

(4) Although the activity of the phosphorylase increases in the secretory portion of the eccrine gland and in the hair follicle in course of the development of the human embryo, that decreases in the epidermis, the sebaceous gland and the secretory portion of the apocrine gland. In addition, the superficial cells and the basal cells in the secretory portion of the eccrine gland are discriminated clearly by their phosphorylase activities in the 8th fetal month. The enzymatic activity in the secretory portion of the apocrine gland is observed through all the fetal months.

(5) The activity of the alkaline phosphatase decreases in the hair follicle, the eccrine gland and the apocrine gland with the advance of the fetal month, but does not in the epidermis and the sebaceous gland.

The activity of the acid phosphatase decreases in the sebaceous gland, the eccrine gland and the apocrine gland, but does not in the epidermis and the hair follicle.

(6) The activity of the leucine aminopeptidase is stationary in the epidermis through all the fetal months studied. This activity, however, decreases in both the hair follicle and the sebaceous gland,
and increases in both the eccrine and the apocrine glands with the advance of the fetal month.

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References

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Explanation of figures

Fig. 1. Skin from the sole of an embryo 4 months old, showing strong cytochrome oxidase activity in the ridges of the epidermis. But the primordium of the eccrine glands is stained weakly. x100

Fig. 2. Alkaline phosphatase in the skin of the perianal region of an embryo 4 months old. The enzymatic activity is strong in the apocrine gland and in the blood capillary. x100

Fig. 3. Skin from the eyelid of an embryo 4 months old, showing strong alkaline phosphatase activity in the apex and the outermost portion of primordium of the hair follicle. x200

Fig. 4. Skin from the palm of an embryo 5 months old, showing strong monoamine oxidase activity, both in the ridges of the epidermis and in the supposed secretory portions of the eccrine glands. x100

Fig. 5. Specific cholinesterase in the nerve fibers of the skin of the palm in the 5th fetal month. A large nerve trunk in the middle portion and many nerve fibers around the eccrine glands in the lower portion are strongly reactive. x110

Fig. 6. Alkaline phosphatase in the eccrine gland from the axilla; the cord region of the gland shows strong and linear reaction to the enzyme in the central portion of the gland. x400

Fig. 7. PAS-positive cell of the secretory portion of the apocrine gland in the axilla of an embryo 6 months old. x200

Fig. 8. Section of the external genitalia of an embryo 6 months old, showing nerve fibers containing cholinesterase around the eccrine gland, the hair follicle and the sebaceous gland. x200

Fig. 9. Skin from the eyelid of an embryo 6 months old, showing the primordium of the apocrine gland. The apocrine gland is not wrapped with the nerve fiber containing acetylcholinesterase. x75

Fig. 10. Phosphorylase activity in the eyelid of an embryo 6 months old. x45

Fig. 11. Skin from the palm of an embryo 7 months old, showing the strong cytochrome oxidase activity both in the epidermis and in the secretory portion of the eccrine gland. x100

Fig. 12. Section of the perianal region of an embryo 7 months old, showing the nerve fibers containing specific cholinesterase around the eccrine gland in the left-lower portion and the pseudocholinesterase containing end-organs around the hair follicle in the middle portion. x200

Fig. 13. Cytochrome oxidase in the epidermis, the hair follicle, the immature adipose tissue, the eccrine and the apocrine gland of an embryo 7 months old. x45

Fig. 14. Skin from the perianal region of an embryo 8 months old, showing nerve fibers containing specific cholinesterase around the apocrine gland in the right-lower portion and pseudocholinesterase containing end-organs around the hair follicle in the middle portion. x120

Fig. 15. Phosphorylase in the lip in the 8th fetal month, showing strong activity both in the basal cells of the eccrine gland and in the myofibrils. x330

Fig. 16. Skin from the lip of an embryo 8 months old, treated with the phosphorylase technique. Enzymatic activity is observed in the hair follicle, the myofibril and the apocrine gland, while the sebaceous gland is unreactive. x100

Fig. 17. Succinic dehydrogenase activity in the palm of an embryo 8 months old. x100

Fig. 18. Skin from the palm of an embryo 8 months old, showing the strong succinic
In the skin of human embryo, succinic dehydrogenase activity is observed both in the epidermis and in the eccrine gland. ×50

Fig. 19. Naphthol-AS esterase activity in the palm of an embryo 8 months old is strong in the epidermis and the secretory portion of the eccrine gland. ×75

Fig. 20. PAS positive cells (basal cells) in the eccrine gland of an embryo 8 months old. ×200

Fig. 21. Iron-hematoxylin staining in the eccrine gland of the axilla in the 10 fetal month. ×200

Fig. 22. The eccrine gland of the palm in the 10th fetal month, treated with naphthol-AS esterase technique. Enzymatic activity is demonstrated in the gland cells except the myoepithelial cells. ×430

Fig. 23. Succinic dehydrogenase activity in the apocrine gland of the axilla of an embryo 10 months old. ×100
Plate IV

H. Machida