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Developmental Patterns of Acid Hydrolases during Differentiation of Fetal Mouse Skin (39453)

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Acid phosphatase and β-glucuronidase are considered as a group of lysosomal enzymes in other tissues (1, 2). However, these enzymes demonstrate opposite distributions in the vertical strata of the epidermis. Acid phosphatase activity is greater in the upper layers of the epidermis (3, 4), while β-glucuronidase is concentrated in the lower layers (5). The differential distribution of acid phosphatase and β-glucuronidase in the epidermal strate may be correlated with progressive differentiation of keratinocytes. The inverse relationship in the development of these two hydrolytic enzymes is reflected in an increasing ratio of acid phosphatase to β-glucuronidase activity during epidermal regeneration and maturation during wound healing. The migrating epithelial tip observed during wound healing contains a relative paucity of acid phosphatase (4) but an abundance of β-glucuronidase (5). The ratio of acid phosphatase to β-glucuronidase activity appears to represent an indicator for the degree of epidermal differentiation.

The present studies were undertaken to determine whether progressive differentiation of fetal epidermis was accompanied by changing patterns of acid phosphatase and β-glucuronidase activity.

Materials and Methods. Inbred strain A/Jax mice (Jackson Laboratory, Bar Harbor, Maine) were bred, and the presence of the vaginal copulation plug was taken as Day 0 of pregnancy. Pregnant mice were sacrificed at Days 14, 15, 17, and 19 of gestation, and the fetuses were removed quickly. The fetuses and newborn mice (0–14 days) were decapitated, and the heads were frozen in liquid nitrogen. The heads of the fetuses and neonate mice were sectioned in a coronal plane at 24-μm thickness and lyophilized at −20° in a cryostat. The freeze-dried tissues were stored in a vacuum tube at −20° until the enzymes were assayed. Epidermal segments from the scalp skin were obtained from the freeze-dried sections by microdissection, weighed (0.3–0.5 μg) on a quartz fiber microbalance, and transferred into microtest tubes (2.5 × 25 mm) for subsequent enzyme analyses. Stratum cornium was excluded from the epidermal samples.

Acid phosphatase activity was assayed by the fluorometric method with α-naphthyl phosphate as the substrate (6). Optimal assay conditions for acid phosphatase activity were previously determined for the skin (3). The reaction mixture consisted of 20 m*M* α-naphthyl phosphate, 0.05 *M* acetate buffer (pH 5.3), 2 m*M* $MgCl_2$, and 0.02% bovine serum albumin. Seven microliters of reagent mixture was added to 0.3–0.5 μg of fetal or neonatal epidermis in microtest tubes and incubated for 60 min at 37°. The standard tubes contained 1–2 nmoles of α-naphthol per tube, and the blank tubes contained reagent mixture only. After incubation, a 5-μl aliquot was diluted to 1 ml with 0.5 *N* NaOH, and the fluorescence of the α-naphthol formed by the enzyme reaction was measured in a fluorometer equipped with the glass filter system. The primary filter was Corning Glass No. 5860 and the secondary filter consisted of No. 3387 and 4308.

β-Glucuronidase activity was measured by the fluorometric method with 4-methylumbelliferone β-D-glucuronide as substrate (7). Optimal assay conditions were determined previously with epidermal tissue (5) and found to be: 0.1 *M* acetate buffer (pH 3.5), 1.5 m*M* 4-methylumbelliferone β-D-glucuronide, and 0.02% bovine serum albumin. Approximately 0.5 μg of microdissected epidermal segments were incubated in 7 μl of reagent mixture for 60 min at 37°.

TABLE I. HYDROLYTIC ENZYME ACTIVITIES IN DEVELOPING MOUSE EPIDERMIS.

Age	Acid phosphatase[a] Mean ± S.D.	Range		β-Glucuronidase[b] Mean ± S.D.	Range		Activity ratio[c]
Fetal age							
Day 14				15.0		(1)	
15	0.15 ± 0.05	0.08–0.26	(8)[d]	13.0 ± 1.4	10.5–15.3	(6)	12
17	0.37 ± 0.12	0.22–0.57	(7)	6.5 ± 1.8	4.6–9.5	(6)	57
19	0.87 ± 0.08	0.75–0.96	(6)	6.5 ± 0.9	5.1–7.2	(6)	133
Postnatal age							
Day 0	1.28 ± 0.24	0.80–1.22	(14)	8.4 ± 2.6	5.0–13.6	(9)	152
2	1.48 ± 0.33	1.13–1.89	(4)	7.6 ± 0.9	6.4–8.7	(4)	194
4	0.81 ± 0.13	0.76–0.97	(4)	8.1 ± 1.6	6.2–10.5	(4)	100
7	0.77 ± 0.14	0.63–1.01	(8)	8.1 ± 2.4	4.3–12.2	(8)	95
14	0.50 ± 0.07	0.46–0.62	(4)	7.2 ± 0.5	6.4–7.6	(4)	69

[a] Activity is expressed as moles per hour per kilogram dry weight.
[b] Activity is expressed as millimoles per hour per kilogram dry weight.
[c] Denotes the ratio of acid phosphatase activity to β-glucuronidase activity.
[d] Number of animals used (each assayed in quintuplicate); number of litters used were two to nine each.

The standard tubes containing 5–20 pmoles of 4-methylumbelliferone and the blank tubes containing reagent mixture only were carried through the procedure. The fluorescence of the product of enzyme reaction was measured in a fluorometer equipped with Corning Glass No. 5860 as the primary filter and No. 3387 and 5543 as the secondary filter.

Results. Acid phosphatase and β-glucuronidase activities, as a function of developmental age, in the epidermis are summarized in Table I. The level of acid phosphatase activity was low in the epidermis on Day 15 of gestation, the activity level being only 10% of the maximum activity found in the early neonates. On the other hand, β-glucuronidase exhibited its highest activity on Days 14 and 15 of gestation. Acid phosphatase activity increased gradually from intrauterine Day 15 into neonatalhood. β-Glucuronidase activity fell to half of the Day-14 level by Day 17 of gestation and maintained the low level during the closing days of fetal development and the neonatal period. The results demonstrated an opposite developmental pattern of the two hydrolases during epidermal development.

The ratio of acid phosphatase to β-glucuronidase activity (A/G ratio) was low on Day 15 of gestation and increased markedly thereafter, achieving a maximum on Day 2 postpartum (Table I). The A/G ratio was 12 on Day 15 of gestation and approximately 200 on Day 2 postpartum. The A/G ratios decreased during the second week after birth.

TABLE II. HYDROLYTIC ENZYME ACTIVITIES IN VERTICAL STRATA OF NORMAL ADULT GUINEA PIG EPIDERMIS[a].

Layer	Acid phosphatase[b]	β-Glucuronidase[c]	Activity ratio[d]
Keratin	0.76	12.1	63
Upper half	1.35	13.5	100
Lower half	0.43	36.1	12

[a] From Ref. (4) and (5).
[b] Activity is expressed as moles per hour per kilogram dry weight.
[c] Activity is expressed as millimoles per hour per kilogram dry weight.
[d] Denotes the ratio of acid phosphatase activity to β-glucuronidase activity.

Discussion. Microanalytical studies have revealed preferential localization of acid phosphatase in the upper layers of the epidermis (4) and β-glucuronidase in the lower layers (5) (Table II). The unique pattern of acid phosphatase concentration in the granular layers of epidermis indicates indirectly that it is involved in the process of keratinization and cellular autolysis (8). The high level of β-glucuronidase activity in the lower layer suggests that this enzyme may be related to cellular proliferation and that the basal cells may be sites for more active turnover of glycosaminoglycan. Therefore, changes in the relative ratio of acid phosphatase to β-glucuronidase activity can be

correlated with progressive differentiation in whole epidermis.

Acid phosphatase activity on Day 15 of gestation is only 10% of that on Day 2 postpartum. β-Glucuronidase exhibits a high activity level on Days 14 and 15 of gestation. The activity ratio of acid phosphatase to β-glucuronidase (A/G ratio) increased 11 times from Day 15 to Day 19 of gestation, and a high A/G ratio persisted between intrauterine Day 19 and neonatal Day 2. These data may reflect a rapid expansion of epidermal differentiation during the late days of fetal development and early neonatal life.

Summary. The activities of acid phosphatase and β-glucuronidase were assayed in fetal and neonatal mouse epidermis by microanalytical methods. The level of acid phosphatase activity was low in the epidermis on Day 15 of gestation. Acid phosphatase activity increased 10-fold between Day 15 of gestation and neonatalhood. On the other hand, β-glucuronidase activity was high on Days 14 and 15 of gestation and low after Day 17 of gestation. The relative ratio of acid phosphatase to β-glucuronidase activity appeared to represent a marker for the degree of differentiation in whole epidermis.

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