

ACID PROTEASE ACTIVITY IN HUMAN FETAL SKIN

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Abstract. We have determined the acid protease activity in crude skin homogenates of human embryos, 1 to 5 months old. The enzyme assay was performed according to Anson and hemoglobin denatured by urea was used as a substrate. During fetal development there is a permanent decrease of acid protease activity from the first to the fifth fetal month. A total of 53 determinations were made, 7 of them before 7.5 weeks, 32 from 7.5 to 11.5 weeks, 9 from 11.5 to 15.5 weeks and 5 from 15.5 to 19.5 weeks, with the average value of 261, 195, 168, and 143 micrograms, respectively, of liberated tyrosine/gww/1 min.

Acid protease activity has been studied by Wells & Babcock (7). Stütgen et al. (6) published a study dealing with the cathepsin activity of the skin at acid pH. They found acid endopeptidase of cathepsin type with higher activity than the skin dermoprotease. Klashka (3) studied the autolytic and heterolytic activity of skin proteases in normal and pathological epidermis. In the extracts of epidermis and dermis he determined cathepsin at pH 3.0-4.5. Also Krs & Lichá (4) found acid proteases in the skin with the peak of activity at pH 3.9, 4.5 and 5.5. Záruba et al. (8) published the determination of Cathepsin B in the human fetal skin.

In this paper we have studied the relationship between the development of human fetal skin and acid protease activity.

MATERIALS AND METHODS

The enzyme assay was performed according to Anson (1, 2). Hemoglobin denatured by urea served as a substrate for the acid protease activity determination. The determination of activity was made in crude homogenates of the skin. The skin was obtained from healthy fetuses from healthy women after legal abortion for social and

medical reasons. The age of the fetus was determined from data obtained from the mother (last menses, date of conception) and from inspecting the fetus using criteria of Potter (5). Most fetuses were obtained by the vaginal route. Four or six minutes elapsed between the start of anesthesia and the isolation of the skin, which was immediately placed in an ice-cold dish. The skin of the back (regio interscapularis) was carefully prepared and weighed on a torsion balance to ± 2 mg. A part of the skin was used for the histological examination and immediately put into 10% Baker solution.

The skin was homogenized for 30 sec in ice-cold bi-distilled water with a Potter-Elvehjem homogenizer having a teflon pestle. According to the expected activity, homogenates were diluted to give a linear course of the reaction and a linear relationship between the rate of substrate breakdown and enzyme concentration. The enzyme assay was performed according to Anson at pH 3.9. Activity is expressed in micrograms of substrate released during 1 min/gww at 37°C. The duration of incubation was 90 min. In the evaluation of results, the *t*-test was used.

RESULTS

The results are shown in Fig. 1. The acid protease activity decreases during the period studied. The histological examination confirmed the skin samples.

DISCUSSION

For many reasons it is difficult to explain the decrease of acid protease activity. We suppose that acid proteases play an important role in proteosynthesis but their importance decreases during the fetal development. For this reason it is possible to say that maximum of proteosynthesis and maximum of quantitative changes in the cell structures of the skin takes place during early fetal development, whereas later only qualitative

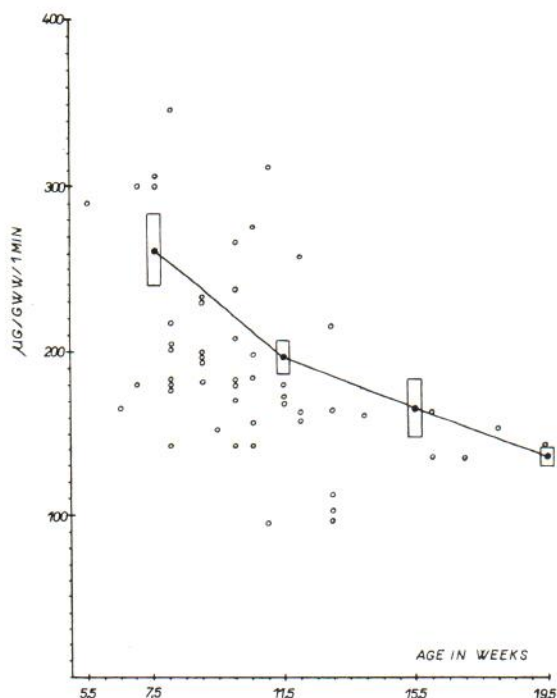


Fig. 1. Acid protease activity in crude skin homogenates. \circ , individual values. For statistical evaluation, fetuses were grouped according to age. In the first group are fetuses until 7.5 weeks (A); in the second group from 7.5 to 11.5 weeks (B); in the third group from 11.5 to 15.5 weeks (C), and in the fourth group from 15.5 to 19.5 weeks (D) of postconceptional age. \bullet , Mean values of the age groups. Small columns denote 2 s.e. Statistical evaluation: group A is significant against B, C, D for p 0.01. Group B against D for p 0.01.

changes in the sense of differentiation of the epidermis are going on. From the above it is possible to conclude that within the period of differentiation the acid proteases lose their functional importance and this is the reason for the decrease of enzyme activity.

REFERENCES

1. Anson, M.: J Gen Physiol 22: 79, 1938.
2. — Gen Physiol, 23: 695, 1940.
3. Klashka, F.: Untersuchungen zur autolytischen und heterolytischen Aktivität der Proteasen normaler und pathologisch veränderter menschlicher Epidermis und Cutis. I. Normale Haut. Arch Klin Exp Derm 215: 137, 1962.
4. Krs, V. & Lichá, K.: Acid Skin Proteases. Sborník 5. biochem sjezdu, Brno, 1968.
5. Potter, E. L.: Pathology of the Fetus and Newborn. Year Book Publishers Inc., Chicago, 1953.

6. Stütgen, G. M., Simmich & Hofman, N.: Die Proteolyse normaler und pathologisch veränderter Haut durch Endopeptidasen. Arch Klin Exp Derm 205: 381, 1957.
7. Wells, G. C. & Babcock, C.: Epidermal proteins. J Invest Derm 21: 459, 1953.
8. Záruba, F., Krs, V. & Uher, J.: The activity of Cathepsin B in human fetal skin. Acta Dermatovener (Stockholm) 48: 257, 1968.

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