IMMUNOMORPHOLOGIC ANALYSIS OF G_2 CHALONE LOCATION DURING HISTOGENESIS OF THE RAT EPIDERMIS

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Much factual information has now been obtained about the chalones, which are responsible for tissue-specific regulation of proliferative processes, so that a number of hypotheses have been put forward on the mechanisms of action of these biologically active substances on definitive tissues [3]. Meanwhile data on the effect of chalones on developing tissues remain scanty and highly contradictory [4] and, in particular, the times of appearance of chalones during individual development remain almost completely unstudied. Only in one recent survey [4] is mention made of the discovery of epidermal G_2 chalone in the skin of rat embryos starting from the 17th day of embryogenesis, made by Okulov by the immunodiffusion method. At the end of embryogenesis and during the first days of postnatal development considerable changes are known to take place in the epidermis in connection with the formation of functionally mature tissue. In rats this takes place toward the 8th-12th day of postnatal development [7].

The object of this investigation was to determine the exact times of appearance of G_2 chalone during histogenesis of the epidermis and also to make a more detailed study of its localization in the definitive stratified squamous epithelium by means of an immunomorphologic method.

EXPERIMENTAL METHOD

Experiments were carried out on albino rat embryos (at the 16th, 17th, 18th, 20th, and 21st days of development) and young albino rats (newborn animals and rats aged 2, 3, 5, 6, 7, 8, and 9 days) during early postnatal development. Pregnancy was dated by the usual method [1]. At each time pieces of skin were taken from the dorsal region of three animals. The material was frozen in isopentane, cooled with liquid nitrogen, after which frozen sections 6 μ thick were cut. The immunomorphologic investigation was carried out by the indirect Coons' method. Monospecific rabbit immune serum against rat epidermal G_2 chalone was prepared and the preparations treated in accordance with the scheme described previously [6].

EXPERIMENTAL RESULTS

The first weak fluorescence, characterizing the presence of G_2 chalone, appeared in the surface layers of the epidermis of the dorsal skin of 17-day rat embryos. A sharp increase in the intensity of fluorescence of the cytoplasm was observed in 18-day embryos in cells of the intermediate layer of the epidermis and, to a lesser degree, in the peridermis; cells of the basal layer in these cases were not fluorescent (Fig. 1: a-c). During the next 3 days the epithelial layer grew thicker. Under these circumstances G_2 chalone was absent as before from the cells of the basal layer but was present in all the upper layers. Its distribution remained similar in character also in the epidermis of newborn rats (Fig. 1b). At this period, however, G_2 chalone also appeared in the cytoplasm of individual cells of the basal layer. Later its content in the basal layer increased, and on the 2nd-5th days after birth the intensity of its fluorescence became equal in all layers of the epidermis. Starting with the 3rd day, besides areas of uniform distribution of G_2 chalone in the epithelium, areas of epidermis with stronger fluorescence of cells of the basal layer were observed. On the 6th-

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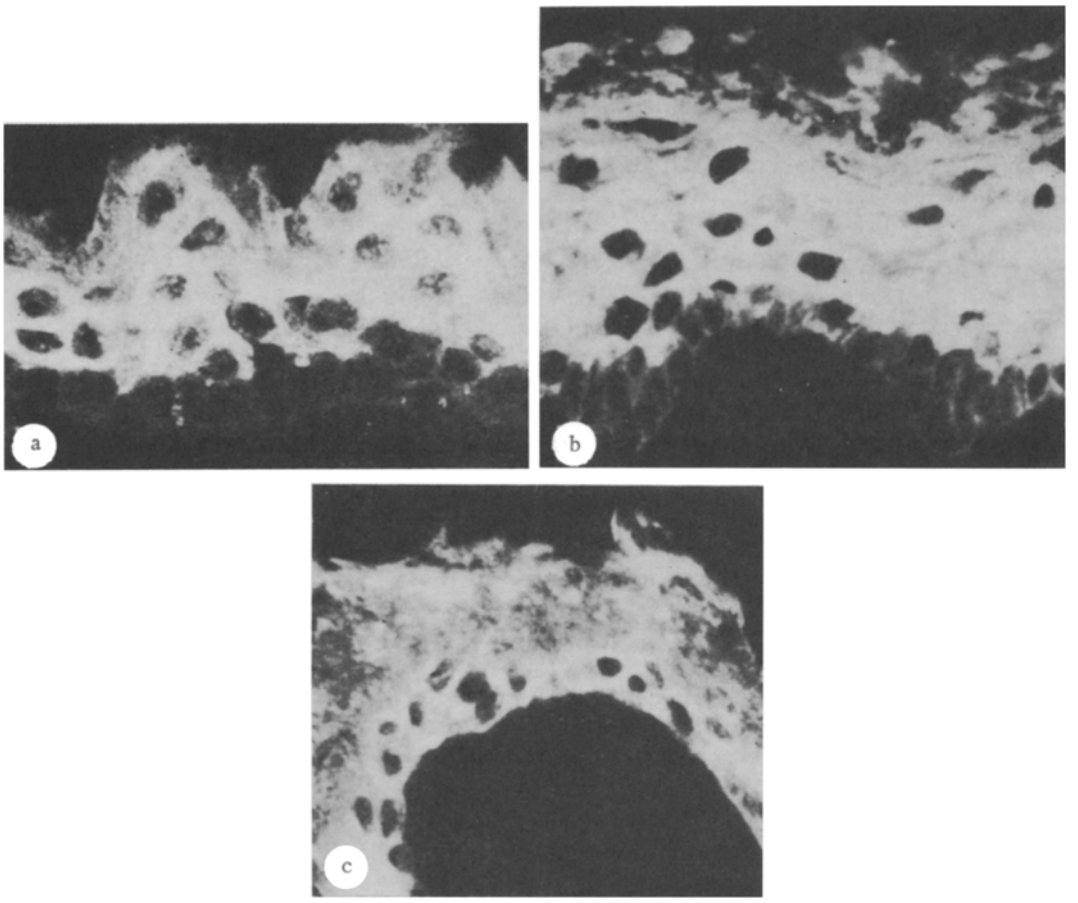

Fig. 1. Frozen sections through rat skin treated with monospecific immune serum against epidermal G_2 chalone: a) skin of 18-day embryo: reaction with cytoplasm of cells of intermediate layer of epidermis and peridermis; b) skin of newborn rat: reaction with cytoplasm of cells of upper layers of epidermis and of a few cells in basal layer; c) skin of rat at 8th day of postnatal development. Reaction mainly with cytoplasm of cells of spinous and basal layers of epidermis. Indirect Coons' method. Objective 40, water immersion, ocular 10.

9th day of postnatal development G_2 chalone now predominated in spinous and basal cells (Fig. 1c), corresponding to the distribution of epidermal G_2 chalone in the definitive tissue [6].

According to Bullough's hypothesis [9] chalones are synthesized by differentiated tissue cells but they act on proliferating cells. It has been shown that differentiated cells appear in the surface layers of the epidermis on the 17th-18th days of embryonic development [8, 10]. We found G_2 chalone in the cytoplasm of these cells at these same times. Evidently its synthesis begins with the appearance of the first differentiated cells in the epidermis. Meanwhile it is known that differentiation of cells of the basal layer intensifies in the course of histogenesis of the epidermis [2]. Corresponding to this process is a gradual appearance of G_2 chalone, which, as the functional maturity of the epidermis becomes established, begins to be formed mainly in the spinous and basal layers. Naturally the more differentiated cells are to be found in the upper layers of the mature epidermis, but synthesis of G_2 chalone in keratinizing epithelia of cutaneous type is a function of cells at a certain level of differentiation, located in the basal and spinous layers [6].

The sensitivity of epidermocytes to the action of chalone obtained from the skin of adult animals is not established until the 8th day of postnatal development [5], i.e., when histogenesis of the epidermis is complete in rats [7]. Meanwhile the appearance of G_2 chalone on the 17th day and the sharp rise in its content on the 18th day of embryonic development may probably be attributed to the sharp fall in proliferative activity observed soon after (19th day) in the epidermis [5]. The possibility of the presence of chalone (endogenous) regulation of proliferation of epidermocytes actually during embryogenesis cannot thus be ruled out.

Allowing for this hypothesis and also for data showing that chalones obtained from newborn skin may have an inhibitory effect on proliferation of the epidermis in adult animals [11], whereas chalones isolated from mature skin act only on the definitive tissue [5], it can be postulated that in the course of histogenesis of the epidermis maturation of the G_2 chalone molecule takes place. Evidence in support of this hypothesis is given by data showing that chalones isolated from the skin of newborn and adult animals differ in molecular weight and in certain properties of their action [12]. To judge from the data described above, they have common antigenic determinants, for chalone was found in the embryonic epidermis with the aid of antibodies obtained to G_2 inhibitor from adult skin.

In the process of histogenesis of the epidermis of the rat skin reversal of the localization of G_2 chalone is thus observed. In embryos aged 17-21 days and in newborn animals it is absent in the basal layer, on the 2nd-5th days of postnatal development it is found in all layers of the epidermis, but on the 6th-9th days it begins to be found chiefly in spinous and basal cells. In other words, together with the completion of histogenesis of the epidermis, the distribution of G_2 chalone characteristic of mature tissue also is established.

LITERATURE CITED

1. A. P. Dyban et al., in: Objects in Developmental Biology [in Russian], Moscow (1975), pp. 505-566.
2. A. A. Zavarzin, DNA Synthesis and the Kinetics of Cell Populations in Mammalian Ontogeny [in Russian], Leningrad (1967).
3. S. A. Ketlinskii, Arkh. Anat., No. 1, 29 (1980).
4. S. A. Ketlinskii, Arkh. Anat., No. 2, 90 (1981).
5. S. A. Ketlinskii, "Tissue-specific regulation of cell proliferation and differentiation under normal and pathological conditions," Author's Abstract of Doctoral Dissertation, Leningrad (1981).
6. S. A. Ketlinskii and A. S. Simbirtsev, Arkh. Anat., No. 6, 58 (1981).
7. F. M. Letuchaya and S. A. Ketlinskii, Tsitologiya, No. 2, 176 (1980).
8. F. W. Bauer, Dermatologica, 145, 16 (1972).
9. W. S. Bullough, Cancer Res., 25, 1683 (1965).
10. J. Hanson, J. Anat., 81, 174 (1947).
11. J. M. Marrs and J. J. Voorhees, J. Invest. Derm., 56, 353 (1971).
12. S. Rothberg and B. C. Arp, Natl. Cancer Inst. Monogr., 38, 93 (1973).

ACTION OF PERTUSSIS VACCINE ON MOUSE HEMATOPOIETIC STEM CELLS

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The immunostimulating properties of many adjuvants have now been studied in detail. Microbial endotoxins, Freund's complete adjuvant (SCA), and pertussis vaccine are well known and widely used. However, much remains unexplained in respect of the mechanisms of the stimulating action of adjuvants on the immune response. Immunogenesis and hematopoiesis are intricately interconnected. However, whereas many aspects of the effect of bacterial endotoxins and FCA hematopoiesis have now been explained [8, 12, 13], the action of pertussis vaccine on hematopoietic cells has virtually not been studied.

The object of this investigation was to study this problem, which may be important for the development of approaches to the specific regulation of immunity against both infections and tumors.

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