HISTOCHEMICAL STUDY OF THE SULFHYDRYL GROUPS IN THE CYTOPLASM OF FIBROBLASTS DURING HISTOGENESIS OF THE CONNECTIVE TISSUE OF THE SKIN

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Many histochemical investigations have recently been made of the proteins in normal skin and during its regeneration [4,8,9,12]. In these investigations, attention was concentrated on the changes in the course of the protein reactions in the fibrous structures of the skin and only a comparative study was made of the intensity of staining of the fibers and cells. In the histochemical study of the reacting groups of the protein molecules in the connective-tissue cells during development, the author showed that a gradual increase in the intensity of staining of the cytoplasm is observed in the fibroblasts. This increase in the staining properties is explained by the accumulation of a protein of collagen type in the cytoplasm of the fibroblasts and it coincides in time with the process of collagen formation [5].

The object of the present investigation was to study the dynamics of the sulfhydryl groups of the protein molecules in the cytoplasm of the fibroblasts during histogenesis of the connective tissue of the skin.

EXPERIMENTAL METHOD

The dermis from the dorsal surface of the trunk of the golden hamster was studied in embryos on the 12th-16th day of development, in newborn animals, and in animals during the postnatal period of development (from 1 to 30 days, and on the 35th, 45th, 60th, 90th and 365th days). The sulfhydryl groups were detected by a method using p-nitrobromacetophenone [11], and using monobromoacetate as a control [1]. The intensity of the reaction was assessed visually in accordance with a five-point system.

EXPERIMENTAL RESULTS

A distinctive pattern of changes in the intensity of the reaction for sulfhydryl groups in time was found in the fibroblasts of the developing connective tissue. In the embryonic period the cells of the fibroblastic series stained only weakly, (traces of a reaction), but at the end of the embryonic period of development the intensity of staining of the cytoplasm of the fibroblasts increased, and in the newborn animals it became perfectly clear. In the dermis of the 14-16-day embryos and in the animals in the first week of life, collagen fibers accumulated rapidly. In the first week of the animal's life, the intensity of staining of the cytoplasm of the fibroblasts increased (+2). This intensity of staining of the cytoplasm persisted until the 9th day of life, after which it diminished until the 13th day of the postnatal period of development, when only traces of it remained.

Starting with the 14th day of the postnatal period, the single collagen fibers of the dermis joined together to form bundles. The formation of bundles of collagen fibers took place on a large scale after the 21st day of the animal's life, and by the 28th day all the fibers of the dermis were collected into bundles. In the dermis of the 14-day-old animal, the staining of the cytoplasm of the fibroblasts again increased in intensity (+1). During the subsequent development of the dermis, the intensity of staining of the cytoplasm of the fibroblasts gradually increased and by the 28th day of life it reached its maximal degree (+3). This intensity of staining of the cytoplasm of the fibroblasts persisted until the 45th day of the postnatal period of development, after which it fell appreciably (see figure).

Hence, in the process of connective-tissue formation in the skin of the golden hamster, two periods of an increase in the intensity of staining of the cytoplasm of the fibroblasts are observed when tests are made for sulfhydryl groups. The first period, starting during embryonic development and lasting until the 13th day of the animal's postnatal development, coincides with the formation of collagen fibers in the dermis. The second period of an increase

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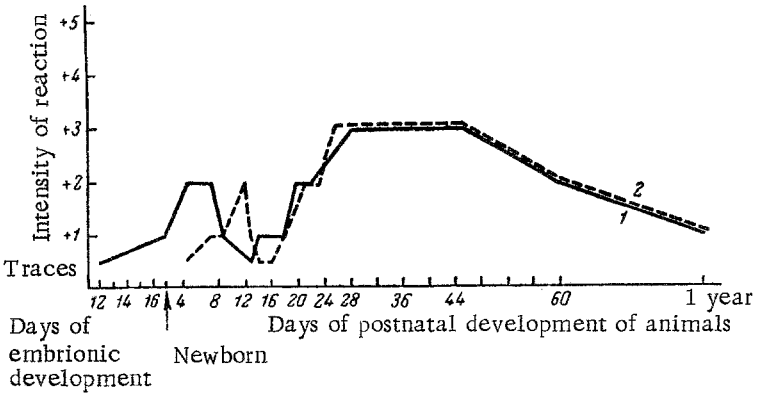

Dynamics of changes in intensity of staining of the cytoplasm of the fibroblasts during tests for sulfhydryl groups and carbonic esterases in the process of development of the connective tissue of the skin in the golden hamster: 1) sulfhydryl group; 2) nonspecific esterase.

in the intensity of the reaction for sulfhydryl groups is longer than the first, and coincides with the formation of bundles of collagen fibers in the dermis.

The sulfhydryl groups of the protein molecules are known to possess high biochemical activity and they are present in the active centers of many intracellular enzymes [3,7]. To discover the dynamics of the changes in the composition and activity of the enzymes in the process of histogenesis of the skin, a series of histochemical studies has been undertaken in recent years [13,14,15]. These have shown that the enzyme composition in the cell structures of the skin of adult animals and embryos is similar, but the activity of the enzymes so revealed was different. For instance, during determination of phosphatases the intensity of the enzyme reaction was higher in the embryonic cells, but during determination of the dehydrogenases it was higher in the skin of the adult animals, and the choline esterase activity was independent of the animal's age. Studies in the author's laboratory [6,10] have demonstrated the relationship between nonspecific esterase and the process of collagen formation both during regeneration of the connective tissue and during its normal development. It was found that the dynamics of the nonspecific esterase activity in the cytoplasm of the fibroblasts in developing connective tissue is similar to the change in the intensity of the reaction demonstrating the sulfhydryl groups of the protein molecules (see figure).

It is impossible by means of histochemical methods to determine into which protein the sulfhydryl groups demonstrated are incorporated, although there are reports in the literature that only sulfhydryl groups bound to protein can be detected by histochemical methods [2]. However, bearing in mind the similarity between the periods of activation of nonspecific esterase and of the sulfhydryl groups, and also the fact that of all the enzymes in the skin detected by histochemical methods only nonspecific esterase gives a similar periodicity of activity, it may evidently be concluded that the sulfhydryl groups discovered in the cytoplasm of the fibroblasts are associated with nonspecific esterase.

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