ELECTRON-MICROSCOPIC CHARACTERISTICS OF THE DYNAMICS OF THYMIDINE-H^3 AND URIDINE-H^3 IN THE MOUSE EPIDERMIS

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The dynamics of distribution of uridine-H^3 and thymidine-H^3 in the epidermis of C57BL mice was studied electron-microscopically between 2 and 24 h after their separate intraperitoneal injection. A high rate of incorporation of both labeled nucleosides was discovered and their localization was found to depend on the time of injection. Depending on the character of their distribution and the rate of incorporation into the epidermis three zones of active protein synthesis can be distinguished: the boundary zone between the epidermis and dermis, the layer of basal cells, and the granular layer. The character of distribution of the label in the late period after injection confirms electron-microscopic data on the active role of the nucleus and the cytoplasmic ribosomes in fibril synthesis in the basal and prickle cells and in keratohyalin formation in the cells of the granular layer. By determining the hourly changes in the distribution of the labels the stages of transport of the injected nucleosides or their metabolites could be followed from the subepidermal region into the cells of the epidermis.

KEY WORDS: thymidine-H^3, uridine-H^3; protein synthesis; epidermis.

Although the micro- and ultrastructure of the epidermis has been studied relatively well, extremely little is known about the biochemical processes taking place in its cells. This is chiefly because of the absence of methods for isolating the epidermis without damaging its structure and for separating it into individual layers, cells, and intracellular organoids in quantities sufficient for investigation. Light-microscopic autoradiography has provided more accurate information on the rate of cell division in the epidermis and has demonstrated the zones of active protein synthesis in the basal cells and in the stratum granulosum [5, 7-9, 11, 16]. The resolving power of the light microscope is too low to enable the injected labeled precursors to be correlated sufficiently precisely with the individual cells and their organoids.

The object of this investigation was to make an electron-microscopic study of the dynamics of incorporation of thymidine-H^3 and uridine-H^3 into cells of the epidermis during the period from 2 to 24 h after injection.

EXPERIMENTAL METHOD

C57BL mice were used. An intraperitoneal injection of 200 μCi thymidine-H^3 was given to the mice of one group and of 100 μC uridine-H^3 to the animals of the other group. The animals were decapitated 2, 6, 12, and 24 h later and pieces of the plantar and abdominal skin were fixed in 3% glutaraldehyde, dehydrated, and embedded in Araldite. Ultrathin sections were coated with Gevaert 3.07 NUC emulsion by the method of Caro and van Tubergen [4] and stained with uranyl acetate and lead citrate. The sections coated with emulsion were examined in the IEM-7A (Japan) electron microscope.

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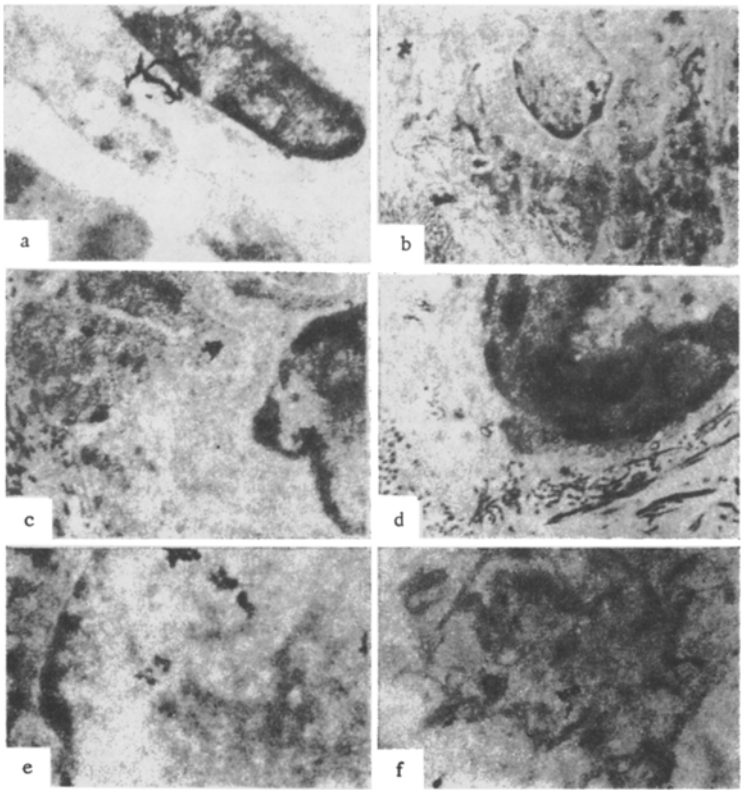

Fig. 1. Distribution of uridine-H^3 in the skin of mice at various times after injection: a) fragment of capillary wall near basement membrane: 2 h after injection uridine-H^3 migrates from the capillary lumen into the nucleus of an endothelial cell (15,000 ×); b) uridine-H^3 2 h later in the region of the subepidermal plexus, on the basement membrane, and in the zone of nuclear heterochromatin of a basal cell of the epidermis (10,000 ×); c) uridine-H^3 2 h later in the cytoplasm of a basal cell above a cluster of ribosomes in an area of newly formed primary filaments (15,000 ×); d) uridine-H^3 24 h after injection in the nucleus and cytoplasm of a fibroblast, possibly connected with synthesis of the collagen fibrils of the dermis (11,500 ×); e) fragment of nucleus of a prickle cell of the epidermis with uridine-H^3 above the nucleolus 2 h after injection (22,500 ×); f) uridine-H^3 24 h after injection above ribosomes in the cytoplasm of a prickle cell in an area of newly formed fibrils (arrow) (15,000 ×).

EXPERIMENTAL RESULTS AND DISCUSSION

The ultrastructure of all components of the epidermal cells was well preserved in the material examined.

Uridine-H^3 was detected 2 h after intraperitoneal injection in the cells of the epidermis, on the basement membrane, on the collagen fibrils close to it, and also in the region of nuclei of the capillary endothelium beneath the epidermis (Fig. 1a, b).

Uridine-H^3 was found in the basal cells of the epidermis in the nucleus beneath the nuclear membrane in the zone of heterochromatin, above the nucleolus, and in the cytoplasm above a cluster of ribosomes (Fig. 1c). In the cells of the stratum spinosum it was found in the region of the nucleolus (Fig. 1d), and in the granular cells it was seen above the keratohyalin granules and close to a cluster of granules of nucleoprotein type (Fig. 2a, I and II).

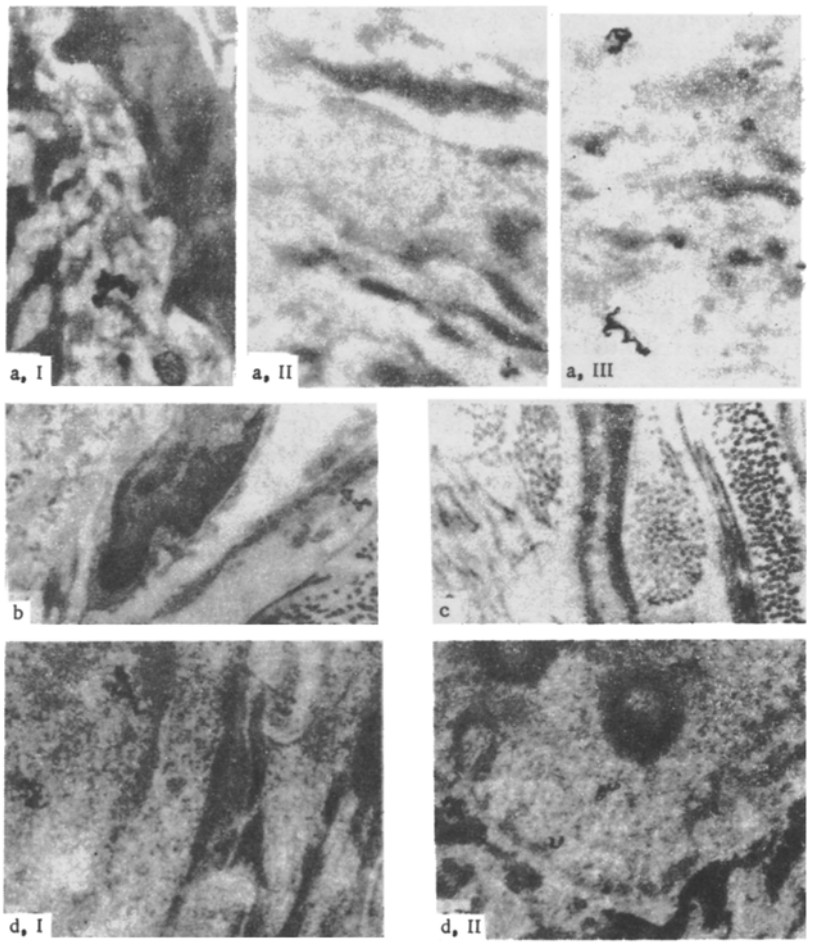

Fig. 2. Dynamics of distribution of uridine-H^3 and thymidine-H^3 in the skin of mice at various times after injection: a) uridine-H^3 2 h (I, II) and 24 h (III) after injection above a cluster of ribosomes and nucleoprotein granules close to newly formed tonofibrillary keratohyalin complexes. Magnifications: I) 11,000 ×, II) 15,000 ×, III) 12,600 ×; b) fragment of capillary wall: thymidine-H^3 2 H after injection migrates from the capillary lumen (15,000 ×); c) thymidine-H^3 in the region of the nucleus of a fibroblast in the dermis close to the basement membrane 2 h after injection (12,100 ×); d) thymidine-H^3 in the nuclei of a basal (I) and prickle (II) cell of the epidermis 24 h after injection. Magnifications: I) 31,500 ×, II) 150,000 ×.

Later the localization of the uridine-H^3 remained unchanged. After 24 h it appeared above the ribosomes close to the nucleus of the prickle cells (Fig. 1f) but it was present in an increased amount in the region of the keratohyalin granules (Fig. 2a, III), of individual connective-tissue cells, and close to the basement membrane. It could be seen both in the nucleolus of the cells and above the cytoplasmic ribosomes, the endoplasmic reticulum, and the very thin intra- and extracellular filaments (Fig. 1d).

Thymidine-H^3 was found in the plantar and abdominal skin 2 h after injection in the boundary zone between the epidermis and dermis, in the cell nuclei of the dermis and the endothelium of the capillary walls (Fig. 2b, c). Sometimes it was also found above the nuclei of the basal cells, and later of the prickle cells, where it remained until 24 h (Fig. 2d; I and II). Sometimes the thymidine-H^3 appeared to be bound with the basement membrane, giving the impression that it was passing through from the dermis into the cytoplasm of a basal cell of the epidermis.

This investigation showed that the method of autoradiography at the electron-microscopic level, as used in this case, does not give rise to any appreciable distortion or damage of the ultrastructure of the epidermal cells. It is a reasonably accurate method of determining the localization of uridine-H^3 and

thymidine-H^3 in the various cell organelles and of studying the dynamics of their incorporation and migration in the course of time after their injection.

The relatively high rate of incorporation of thymidine-H^3 and uridine-H^3 into the cells of the epidermis is in agreement with the results of other investigations [5, 11, 13, 14]. The labels of the injected nucleosides, chiefly uridine-H^3, were incorporated after 2 h not only into the nuclear and ribosomal structures of the connective-tissue cells, the capillary endothelium, and the basal cells of the epidermis, but they were also visible in the stratum granulosum. In the character of distribution and rate of incorporation of the radioactive precursors 3 zones of active protein synthesis can thus be distinguished in the epidermis: zone of the basement membrane, the layer of the basal cells, and the layer of the granular cells. The presence of active zones of protein synthesis in the basal and granular cells has also been demonstrated by other workers using tritiated amino acids [6, 7, 9, 10].

These observations showed that primary filaments 30-50 Å in diameter are synthesized in the cytoplasm of the basal cells with the active participation of the cytoplasmic ribosomes and the mitochondria, whereas collagen fibrils are formed and mature in the dermis close to the basement membrane. In the granular cells tonofibrillary keratohyalin complexes, the immediate morphological precursors of the keratin fibrils, are formed in the granular cells with the participation of nuclear nucleoproteins, released in large masses into the cytoplasm of the cells and grouped around these complexes. The localization of uridine-H^3 above the keratohyalin granules during the first few hours after injection confirms that they contain RNA [3, 12, 15]. The presence of the label in the late period after injection and also its localization in the region of concentration of nucleoprotein masses discharged from the nucleus and surrounding the tonofibrillary keratohyalin complexes, on the other hand, could be secondary in character; i.e., after primary incorporation into the nucleus the label could be discharged together with the contents of the nucleus into the cytoplasm.

Active liberation of the contents of the nucleus and nucleolus into the cytoplasm [1] was observed also in the other layers of the epidermis, including the stratum basale. This was also confirmed by the appearance of uridine-H^3 2 h after injection in the nucleolus, and 24 h after injection above the ribosomes of the cytoplasm. The appearance of cytoplasmic labels in the late period after injection of uridine-H^3 and thymidine-H^3 could also be secondary in character.

Thymidine-H^3 was found 2 h after injection in the nucleoplasm of the connective-tissue and some basal cells of the epidermis. It began to be clearly detectable in the nuclei of the basal and prickle cells only after 24 h. Having been incorporated into the interphase nucleus it could evidently be detected in the cell during division and migrated along with the cell into the stratum spinosum lying above. This is in agreement with optical microscopic observations of the incorporation of thymidine-H^3 into interphase nuclei of the basal cells of the epidermis from 15 min to 1 h after injection and the migration of individual labeled cells after division into the stratum spinosum 12 h after injection. As the results described above show, uridine-H^3 is also sufficiently specific, but it is a more promising substance with which to study metabolism and synthesis in the epidermis than thymidine-H^3. It is incorporated more rapidly into the nucleolar and ribosomal structures of the basal, prickle, and granular cells of the epidermis and the character of its distribution 2 h after injection is practically the same as that 24 h after injection, except for the liberation into the cytoplasm along with the contents of the nucleus referred to above.

Characteristically uridine-H^3 is located in individual nuclei of the epidermis not only in the region of the nucleolus, but also in what is called the Davis layer – beneath the nuclear membrane, where the ribonucleoproteins are located.

In conclusion it must be emphasized that the labels of the injected nucleosides in the first few hours were often discovered on structures with which they could not have been chemically bound (the capillary walls, the basement membrane, collagen fibrils). These findings presumably reflect stages in the transport of these substances or their metabolites into the epidermis. After intraperitoneal injection they were carried by the blood stream into the terminal capillaries of the subepidermal region, after which they migrated by transfusion through the capillary wall into the zone of the epidermal basement membrane.

Another factor, possibly with decisive importance in the interpretation of these results is the possibility of rapid breakdown of the injected labeled nucleosides [2].

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