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EFFECT OF LIPID SOLVENTS ON PROTEIN, DNA, AND COLLAGEN SYNTHESIS IN HUMAN SKIN: AN ELECTRON MICROSCOPIC AUTORADIOGRAPHIC STUDY

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The effect of acetone and kerosene on the synthesis of protein, DNA, and collagen was studied by electron microscopic autoradiography using [^{3}H]leucine, [^{3}H]thymidine, and [^{3}H]proline as tracers in human skin. Quantitative analyses following concomitant administration of tritiated leucine and acetone or kerosene demonstrated, at 90 min, a marked decrease in silver grains as compared to control or nonexposed areas. Incorporation of tritiated thymidine is moderately stimulated only by acetone, whereas radioactive proline distribution is not significantly affected. Electron microscopic autoradiograms revealed that tritiated leucine is distributed over all epidermal cells, mostly in the stratum spinosum of control epidermis; a marked decrease of silver grains from [^{3}H]leucine followed both lipid solvent exposures. The autoradiographic reaction is specifically located over cytoplasmic organelles, such as polysomes, endoplasmic reticulum, and especially tonofilaments. Tritiated thymidine resulted in silver grains mostly over nuclear chromatin and these were moderately increased after acetone application, whereas the incorporation of radioactive proline in the fibroblasts and collagen fibrils were not significantly influenced. These investigations indicate a dissociated effect of lipid solvents on protein, DNA, and collagen synthesis in human skin.

In previous investigations, we found that acetone and kerosene exerted a strong effect on human epidermis [1]. The cellular damage occurred mainly in the upper layers (stratum corneum and spinosum) and consisted of large paranuclear vacuoles after acetone administration, and a marked cytolysis of epithelial cells after kerosene. In order to study the intrinsic mechanism of these injuring agents at the cellular level and because the resolution of light microscopy is not sufficient to detect the specific subcellular sites of protein, DNA, and collagen synthesis, we employed electron microscopic autoradiography. This sensitive and accurate method has been used extensively in the last decade for the study of ribonucleic acid (RNA), protein, and deoxyribonucleic acid (DNA) synthesis in different organs. Epithelial protein synthesis has been the subject of investigations in recent years in different species [2–5] including man [6], using labeled amino acids ([^{3}H]histidine, [^{3}H]cystine, [^{3}H]methionine). However, most of these studies were carried out by biochemical procedures [2,4] or light microscopic autoradiography [5–8]. A few authors [9,10] used electron microscopic autoradiography for the study of keratohyaline granule formation in newborn rat epidermis and collagen synthesis with [^{3}H]proline in experimentally wounded guinea pigs. This study deals with comparative high-resolution autoradiographic investigations regarding the effect of lipid solvents on protein, DNA, and collagen synthesis in human skin.

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MATERIALS AND METHODS

Materials

The experiments were performed after informed consent had been obtained from each of the subjects. In order to study protein synthesis, uniformly labeled [^{3}H]leucine, specific activity 30–50 Ci/mM 0.01 N HCl (New England Nuclear, NEN, Boston, Mass.) was injected intradermally (dose of 20 μCi), simultaneously with acetone or kerosene topical administration on the forearm skin of 6 healthy volunteers. For the study of DNA synthesis, [^{3}H]methylthymidine, specific activity 6.7 Ci/mM (NEN) was injected intradermally (20 μCi) concomitant with lipid solvent topical administration. For the study of collagen synthesis, [^{3}H]proline, specific activity 30 Ci/mM (NEN) (20 μCi) was injected intradermally simultaneous with acetone or kerosene topical administration.

Experiments

Lipid solvents (acetone and kerosene) were administered in the following manner. Five glass tubes (5 mm

diam) containing approximately 1 ml of acetone were topically applied on the right forearm and maintained in vertical position by adhesive tape for 90 min. Kerosene was applied in the same manner to the left forearm. The tubes were then removed. No cleansing of the test sites was done either before or after the procedure. Specimens were removed at 90 min under local anesthesia by punch biopsy. They were fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.3 and 300–400 milliosmoles) for 2 hr, postfixed in 1% phosphate-buffered osmium tetroxide (OsO_4) for another 2 hr, dehydrated in ascending series of ethanol washes, and flat embedded in a mixture of Epon and Araldite. Thick sections (1 μ) were cut using a Porter-Blum ultramicrotome, stained with 1% toluidine blue, and used in order to select a suitable area for further study. Thin sections (60–90 nm) were cut using a LKB ultrotome equipped with diamond or glass knives. The grids with sections were covered with Nuclear Emulsion-L_4 diluted with distilled water 1:1, using a wire loop procedure according to Caro and Tubergen [11]. The filmed grids were exposed for 6 to 10 weeks in refrigerated (4°C) dark boxes containing Drierite. They were developed in Microdol-X for 5 min or D_{19} for 3 min, fixed, stained with uranyl acetate and lead citrate, and examined in an Hitachi HS-8 electron microscope at 5,600–9,300×.

TABLE. *Effect of acetone and kerosene on [^3H]leucine, [^3H]thymidine, and [^3H]proline incorporation in human skin at 90 minutes*

	[^{3}H]leucine[a]	[^{3}H]thymidine[b]	[^{3}H]proline[c]
Controls	76 ± 3.8	34 ± 3.2	42 ± 5.2
Acetone	42 ± 3.6*	44 ± 3.5**	40 ± 4.8
Kerosene	24 ± 1.7*	34 ± 3.6	35 ± 5.0

[a] Expressed as a mean ± SE (standard error) of developed grains (DGR) over 100 cm² of cytoplasm.

[b] Expressed as a mean ± SE of DGR over 100 cm² of chromatin.

[c] Expressed as a mean ± SE of DGR over 100 cm² of fibroblasts and fibrils.

* Significantly ($p < 0.001$) less than controls.

** ($p < 0.05$) greater than control.

Three series of electron microscopic autoradiographs were carried out from the same material. Quantitative analyses of electron microscopic autoradiograms were performed according to the procedures of Loud, Barany, and Pack [12]. Electron micrographs were randomly taken and printed on 5 × 6 inch paper to facilitate counting. Each micrograph was counted twice. A circle was drawn around each developed grain (DGR) with a radius of approximately 50 nm, having a 95% probability of including the source of DGR [13]. The background was essentially zero. Only fields containing 8 to 14 silver grains were counted. The grids heavily and diffusely labeled were discarded.

The cells and extracellular areas were divided as

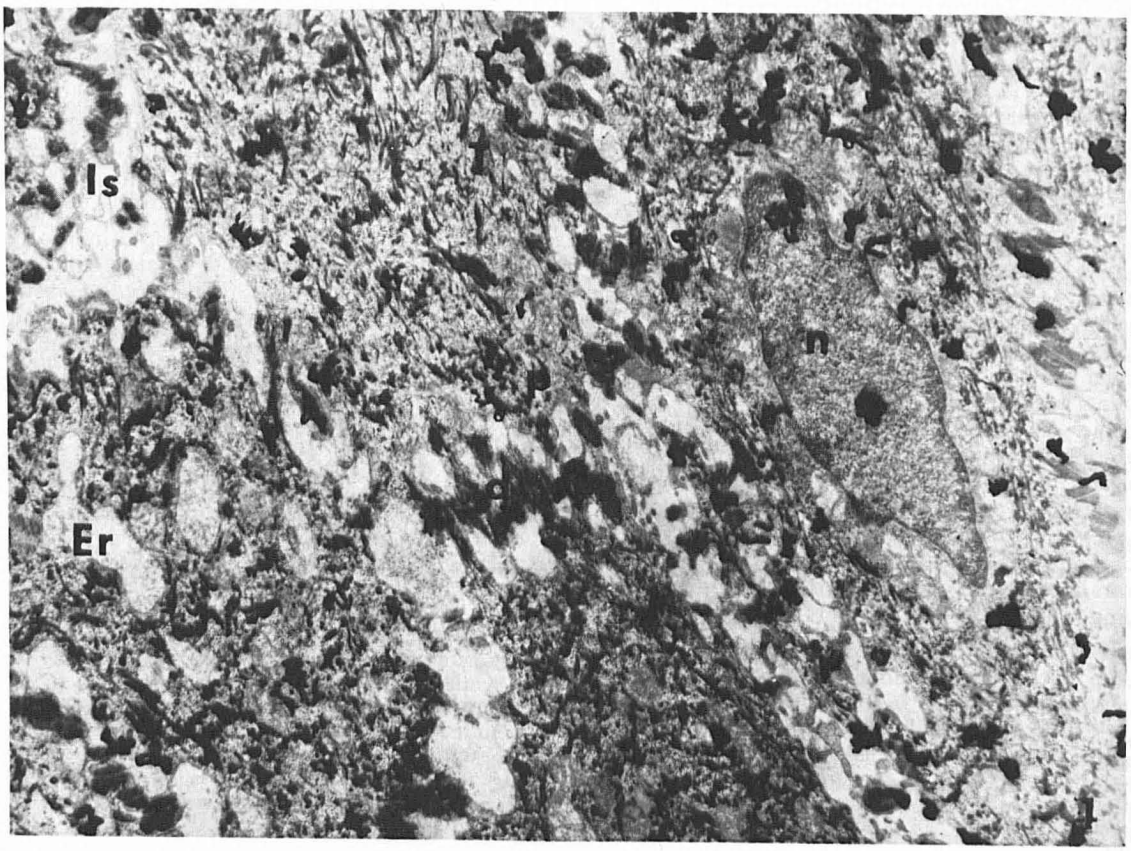

FIG. 1. Intense incorporation of [^{3}H]leucine over the cytoplasm of spinous cells in control epidermis at 90 min. Polysomes, *p;* intercellular space, *Is;* tonofilament, *T;* nucleus, *n;* desmosome, *d;* endoplasmic reticulum, *Er* (× 9,000).

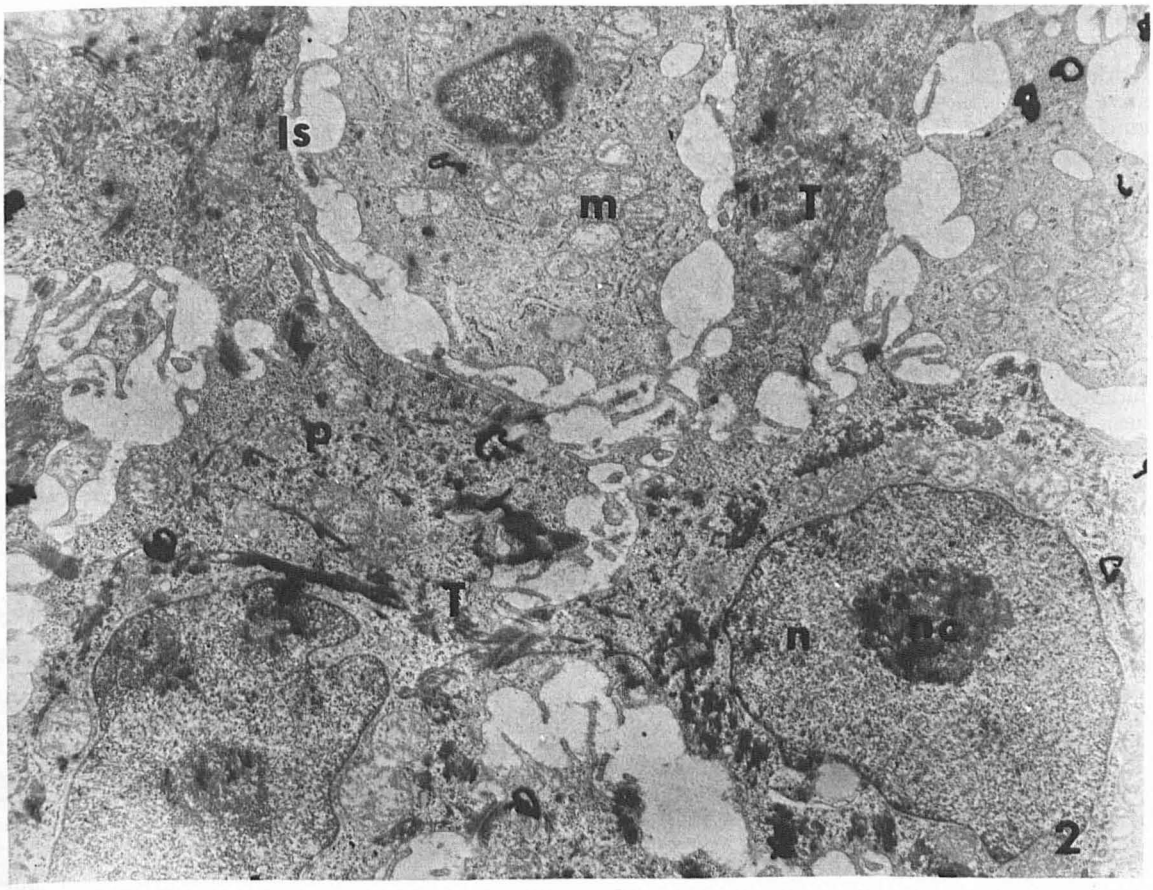

FIG. 2. Marked reduction of [^{3}H]leucine incorporation following kerosene. Few silver grains can be seen. Tonofilament, *T*; polysomes, *p*; intercellular space, *Is*; mitochondrion, *m*; nucleus, *n*; nucleolus, *nc* (× 9,000).

follows: cytoplasmic structures (polysomes, endoplasmic reticulum, and tonofibrils); nuclei (chromatin); and collagen (intracellular fibroblasts and extracellular fibrils). Thus, [^{3}H]leucine incorporation is expressed as a mean number of DGR per 100 cm^2 of cytoplasm at 90 min as compared to controls; whereas, [^{3}H]thymidine incorporation is expressed as a mean of DGR number per 100 cm^2 nuclear chromatin, and [^{3}H]proline incorporation was evaluated as a mean of DGR per 100 cm^2 of fibroblasts and collagen fibrils. DGR/100 cm^2 represents a mean of total DGR distributed over the cytoplasmic organelles or nuclei of all epidermal cells (granular, spinous, and basal) in at least 50 micrographs (average magnification 9,000×) containing a total of 400 to 1,000 grains per experimental group (mean ± SE).

RESULTS

The quantitative evaluation of [^{3}H]leucine distribution revealed that acetone and kerosene markedly decrease [^{3}H]leucine incorporation into the cytoplasm of epithelial cells at 90 min as compared to controls; however, kerosene exerts a stronger effect than acetone. [^{3}H]Thymidine incorporation is moderately increased only after acetone administration. Neither acetone nor kerosene significantly interfere with [^{3}H]proline incorporation into fibroblasts or collagen fibers (Tab.)

The autoradiograms, show incorporation of radioactive leucine as silver grains over the cytoplasmic organelles of control epidermal cells (polysomes, endoplasmic reticulum, and tonofilaments) (Fig. 1). A decrease of [^{3}H]leucine distribution occurs following acetone administration. Some silver grains are located near paranuclear vacuoles, within nuclei of spinous cells; others are distributed over endoplasmic reticulum. A marked decrease of autoradiographic reaction is observed following kerosene application with an advanced degree of cytolysis and few developed grains which are seen in the cytoplasm of spinous cells (Fig. 2).

DISCUSSION

The study of protein synthesis, DNA, and collagen synthesis in human skin is of great biologic and pathologic significance. Electron microscopic autoradiography is a valuable tool in cell biology and provides us with a better visualization of amino acid distribution in cell organelles. It has been extensively used in the last decade for the study of protein synthesis in different organs, such as exocrine pancreas, neural tissue, or thyroid [14].

The findings from the present investigations

reveal that protein synthesis is markedly impaired following acetone and kerosene administration at 90 min, the latter exerting a greater effect. Quantitative estimations and electron microscopic autoradiograms show a significant decrease in the incorporation of tritiated leucine over cytoplasmic organelles, such as polysomes and endoplasmic reticulum cisternae which are the cell machinery for protein synthesis. Meanwhile, [^{3}H]thymidine is only moderately increased after acetone application. No significant changes in tritiated proline incorporation and distribution were observed. Some authors [5–7] consider that leucine-rich proteins are synthesized in the lower layers of the epidermis, whereas histidine-rich proteins are synthesized mostly in upper layers (granular layer). Freedberg and Baden [2], based on their studies in vitro, concluded that epidermal proteins are predominately synthesized in the basal layer and that a parallel exists between protein synthesis and cell differentiation. It is of interest that both lipid solvents inhibit leucine incorporation and protein synthesis, which may explain their degenerative effects on cytoplasmic organelles. Tritiated proline was never found in our investigations to be incorporated in upper layers, but was present mostly in the lower basal layer, fibroblasts, and collagen fibrils of dermis.

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