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Effect of Calcitonin on DNA Synthesis in Experimental Wounds (39110)

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Recent studies using biochemical and autoradiographic procedures revealed that sex steroid hormones (estradiol, progesterone, testosterone) and some corticosteroids (cortisol) strongly interfere with deoxyribonucleic acid (DNA) synthesis (1, 2). Thus the addition of ^{3}H-cortisol to isolated rat liver nuclei induced a rapid uptake of cortisol, a phenomenon which is temperature-dependent (3). Also, cortisol and epinephrine stimulated the ^{3}H-thymidine incorporation into the nuclei of squamous epithelial cells on the mouse's forestomach. This effect was inhibited by actinomycin D. (4). At present we are not aware of similar investigations regarding the effect of calcitonin on DNA synthesis. In previous investigations, we found that salmon calcitonin exerted a marked stimulatory effect on fibroblasts proliferation and induced a hypertrophy of nuclei (5). Because the electron microscopic autoradiography is a sensitive and accurate method for the study of DNA or RNA synthesis in different cells such as pancreatic, neuron, or thyroid cells (6), in this report we investigate the effect of synthetic salmon calcitonin (SCT) on ^{3}H-thymidine uptake by the skin DNA and its intranuclear distribution in the cutaneous wounds experimentally inflicted in rabbits.

Materials and methods. Adult rabbits, weighing 4.0 kg, were used in these experiments. Four linear incisions approximately 2 cm long were made on the dorsal skin after removal of the hair and then closed with Michel clamps for 2–14 days. At 2, 5, and 14 days, biopsy specimens were removed under sodium pentobarbital (Nembutal) anesthesia. During this time, 18 rabbits were injected im with 64 MRCU (Medical Research Council Units) of SCT every other day. We selected this dose because we found in previous experiments with light and electron microscopes that SCT exerts its maximum effect on the cell structure. Another 18 rabbits, approximately the same weight, were only wounded and served as controls. Each experimental group was divided into three subgroups: (A) six rabbits wounded only 2 days, (B) six rabbits wounded 5 days, and (C) another six rabbits wounded for 14 days. Both calcitonin-treated and wounded rabbits received intradermally 100 μCi of ^{3}H-methyl thymidine (New England Nuclear, Boston) (sp act of 6.7 Ci/mM), in four wounded areas at 1, 2.5, 5, and 24 hr before they were sacrificed for the study of DNA synthesis. Two specimens were removed from each wound at 1, 2.5, 5, and 24 hr following the administration of ^{3}H-thymidine and calcitonin to all experimental groups.

Half of the specimens were transferred to a liquid scintillation cocktail and counted in a Nuclear Chicago Liquid Scintillation System (No. 724–725), efficiency 25%, and using ^{3}H as internal standard. Each sample was trimmed to a size of 1 cm^2 and 0.45 cm thick and was counted at least 10 min. The results were expressed as cpm/g of wet tissue (mean ± SEM). The remaining specimens were fixed in 3% cacodylate-buffered glutaraldehyde (GTA) for 2 hr, then postfixed in 1% phosphate-buffered osmium tetroxide

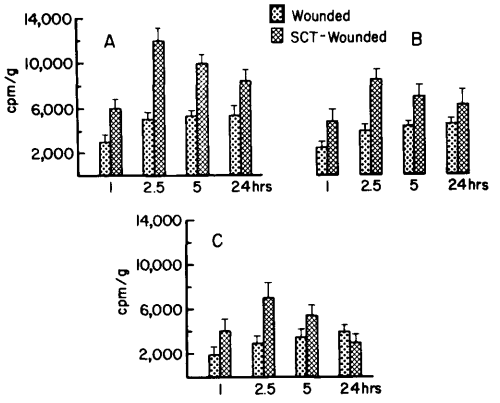
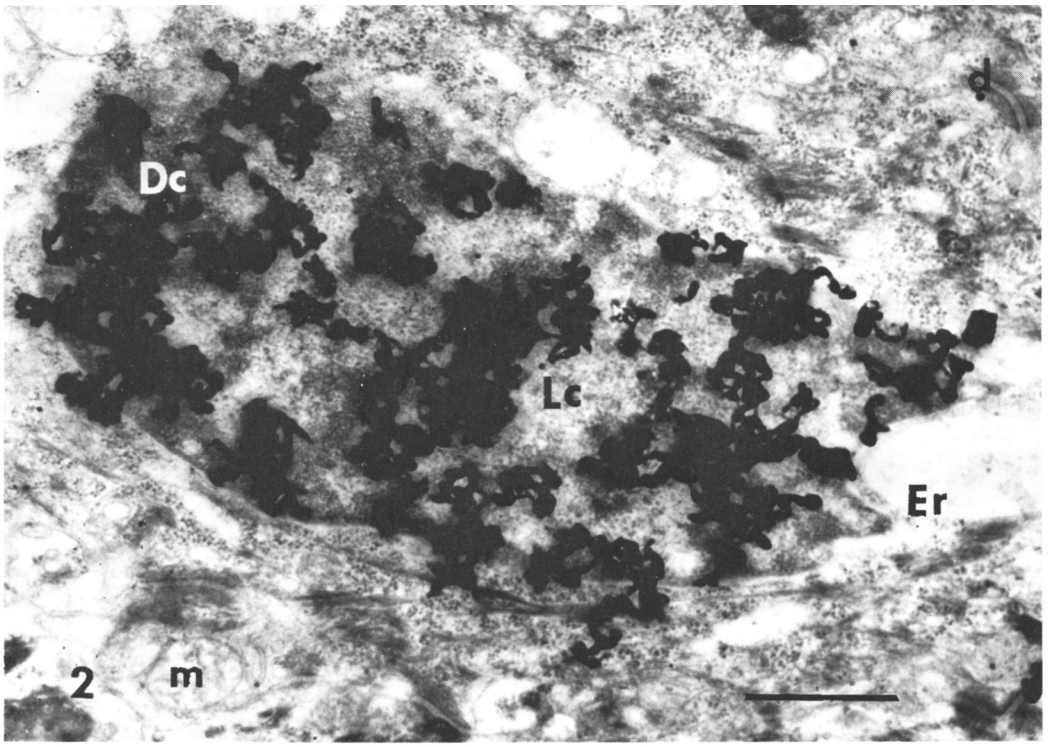


FIG. 1. ^{3}H-thymidine incorporation in the skin of wounded and calcitonin-treated rabbits (2 days, Lot A; 5 days, Lot B; and 14 days, Lot C). Each column represents the mean ± SEM from 10 specimens.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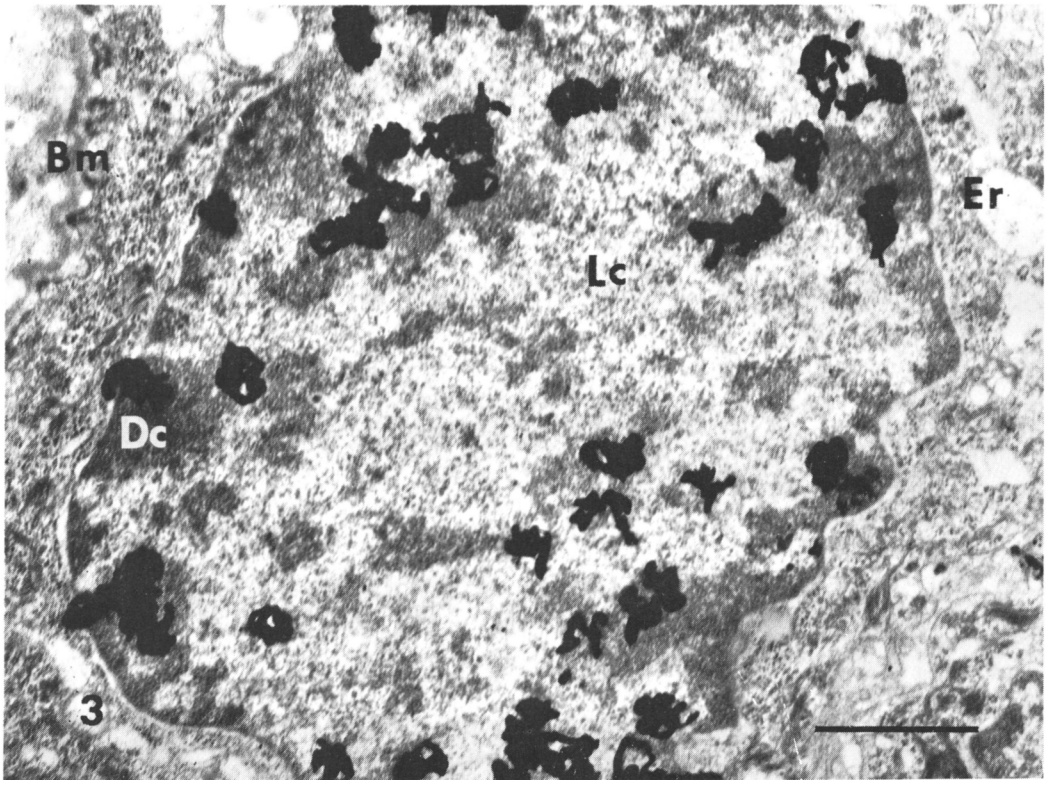
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(OsO_4) for another 2 hr, dehydrated in an ascending series of ethanol, and embedded in a mixture of Epon-Araldite. Electron microscopic autoradiography was performed on thin sections (600–900 Å) covered with Nuclear Ilford L_4 emulsion diluted 1:2 using a wire loop procedure (7). The filmed sections were exposed for 8–10 weeks at 4°C; then they were developed in Microdol-X for 5 min, fixed, washed, and stained with uranyl acetate and lead citrate. They were examined under an Hitachi HS-8 electron microscope.

For quantitative estimation of developed grains (DGR), a modified procedure was used (8). Thus, electron micrographs were randomly taken at x5,600–11,000; each micrograph was counted twice, and a circle was drawn around each DGR with a radius of approximately 2.500 Å, which provided a 90% probability of including the source of DGR. The results were expressed as DGR/100 cm^2 chromatin (mean ± SEM).

Results. Radioactivity measurements of skin specimens at different time intervals shows a marked increase of ^{3}H-thymidine uptake following long-term administration of SCT in wounded rabbits, especially in 2-day-old wounds (Group A) at 2.5 hr following isotope and SCT administration (Fig. 1). ^{3}H-thymidine incorporation moderately decreases in 5-day (Group B) and 14-day calcitonin-treated rabbits (Group C); however, the uptake still remains higher than in the wounded-only rabbits. Electron microscopic autoradiograms reveal a specific and marked incorporation of ^{3}H-thymidine over nuclear dense chromatin (heterochromatin). Thus, a heavier autoradiographic reaction (several developed grains over dense chromatin of epidermal cells) is observed at 2.5 hr in 2-day calcitonin-treated rabbits (Fig. 2) when compared to their controls (Fig. 3). No developed grains can be seen over loose chromatin (euchromatin), mitochondria, or endoplasmic reticulum profiles.

Quantitative estimations of developed grains (DGR) distribution over nuclear chromatin of epidermal cells and fibroblasts also reveal a significant increase following calcitonin administration, but it is more intense in the 2-day wounded group (A) and at 2.5 hr ($P < 0.001$) than in the control group. The incorporation decreases moderately in 5- (Group B) and in 14-day (Group C) ($P < 0.05$) (Table I).

Discussion. The present data indicate that large doses of SCT markedly enhance the ^{3}H-thymidine incorporation and distribution in the nuclei of epithelial cells and fibroblasts of scar tissue in the wounded rabbit, and, therefore, it stimulates DNA synthesis (S-phase) in these cells. The most important effect occurs at 2.5 hr following the hormone administration in recently wounded rabbits, and then it moderately decreases. Previous radiochemical studies using ^{3}H-proline demonstrate that acute as well as chronic administration of synthetic porcine calcitonin increases proline uptake in young rats (9).

It is also known from other investigations that some hormones (ecdysone, thyroxine, growth hormone, corticosteroid, and sex steroid hormones) significantly interfere with RNA and DNA synthesis under different conditions, and thus, can increase the cytoplasmic polysome population.

The steroid hormone initially exerts a regulatory influence on the transcription level and only indirectly regulates the translation of mRNA. These investigations reveal that calcitonin also can interact with DNA synthesis in the epidermal cells and fibroblasts, and thus may play an important role in cell cycle (increasing S-phase) and cell proliferation.

Summary. ^{3}H-thymidine incorporation and intracellular distribution in epidermal cells and fibroblasts of scar tissue from wounded rabbits were studied following synthetic salmon calcitonin administration. Both scintillation counting and electron microcscopic

FIG. 2. EM autoradiograph showing a specific and intense reaction of ^{3}H-thymidine over dense chromatin (Dc) at 2.5 hr in calcitonin-treated and wounded rabbits (Group A); no silver grains are visible over loose chromatin (Lc); d-desmosomes; m-mitochondrion; Er-endoplasmic reticulum. x21,000.

FIG. 3. EM autoradiograph of a control and wounded rabbit showing a specific but a weaker reaction of ^{3}H-thymidine over dense chromatin (Dc) at 2.5 hr (Group A); loose chromatin (Lc); basement membrane (Bm); Er-endoplasmic reticulum. x21,000.

TABLE I. EFFECT OF SYNTHETIC SALMON CALCITONIN (SCT) ON ^{3}H-METHYL THYMIDINE INCORPORATION IN WOUNDED RABBITS

Experimental groups	Days	Hours			
		1	2.5	5	24
SCT-treated and wounded (18)	2	30±3[a]	80±4[x]	58±3	50±3
	5	24±3	50±4[xx]	51±3	44±3
	14	18±2	30±3[xx]	27±2	22±2
Controls and wounded (18)	2	21±2	35±4	38±3	42±4
	5	17±2	30±3	33±3	38±4
	14	11±1	20±2	23±2	25±2

[a] Data are presented as developed grains (DGR)/100 cm^{2}/chromatin. Mean ± SEM. Each value is an average of at least 10 specimens from one experimental group. Number of animals per group is in parenthesis. [x]Significant ($P < 0.001$), as compared to controls. [xx]Significant ($P < 0.05$), as compared to controls.

autoradiography revealed a marked increase in ^{3}H-thymidine incorporation within nuclear dense chromatin of epidermal and fibroblasts cells, mainly in 2-day wounded rabbits and at 2.5 hr. Then, ^{3}H-thymidine uptake decreased moderately. These findings demonstrate an important role of calcitonin on DNA synthesis.

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