The Effect of of Dilantin upon Fibroblast Proliferation[1] (36277)

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In 1958, Shapiro (1) demonstrated an increased rate of gingival wound repair in nonepileptic patients who had been receiving Dilantin (5,5-diphenylhydantoinate). Shafer *et al.* (2) also demonstrated an increase in tensile strength of surgically created wounds in the rat after treatment with Dilantin. This effect could not be confirmed in the rabbit (3) despite the report that healing in the mandible of the rabbit was also accelerated by Dilantin administration (4). Other studies have indicated that the corneal wound will heal more rapidly in Dilantin-treated animals (5) and a longitudinal, double blind and cross-over study using Dilantin in patients with various types of leg ulcers demonstrated a significantly increased rate of repair with Dilantin administration (6).

Shafer (7) using a variety of presumably heteroploid cells and a gingival derived "fibroblast-like" cell showed that Dilantin administration at concentrations of 200 μg/ml would increase the rate of proliferation of these cells. Subsequently, the same author, focusing on his human "fibroblast-like" gingival derived cells, found that the optimal rate of growth of these cells in culture was at approximately 5 μg/ml of Dilantin (8).

In Shapiro's (1) original description of increased gingival wound healing with Dilantin administration, he mentioned both increased fibroplasia and collagenization as contributing markedly to this increased rate of repair. The increased collagenization was also in part implied by Shafer's demonstration of increased tensile strength of healing wounds in Dilantin-treated rats (2). We have also reported that administration of large amounts of Dilantin to rats, although they would not produce gingival hyperplasia, will increase the apparent concentration of insoluble collagen in the skin of these animals (9–11).

Therefore, it was decided to explore the effect of Dilantin administration upon the rate of collagen synthesis by diploid human fibroblasts in culture.

Materials and Methods. Cells were obtained from two normal volunteers by cutaneous biopsies and subsequent culturing of the explants. The karyology of these cells was confirmed normal and they were found to have a population doubling time of 35 $\pm$ 2 hr (12). Approximately 10^6 cells in 20 ml of Eagle's minimum essential medium were seeded into disposable plastic flasks (Falcon Plastics, Los Angeles, CA) having a gross surface of approximately 75 cm^2. The medium was changed after 48 hr and again after 5 days. For the collagen anabolism studies, on day 8 when these cells had reached confluency, Dilantin sodium (Parke, Davis and Co.) was added to the cultures at a final concentration of 10^{-4}; 10^{-5}; and 10^{-6} *M* (27; 2.7; 0.27 μg/ml). A special solvent supplied by the manufacturer to solubilize the Dilantin was also added to the control cultures. At the same time, sodium ascorbate was added to both experimental and control media to a final concentration of 50 μg/ml.

L-Proline-3,4-^{3}H (5 μC) (New England Nuclear Corp.; 5.1 μC/mmole) was added to each culture immediately following the change of medium every 3 days.

Harvesting was carried out every 3 days at the time of medium change. Two cultures were harvested for each experimental condi-

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tion. Harvesting and assay procedures for collagen hydroxyproline and total protein have been previously described (13). All assays were performed in duplicate.

Results. For the population doubling time experiments, 0, 2, 5, 10, and 20 μg of Dilantin were added/ml of medium 24 hr after seeding the culture in Leighton tubes (12). The results of the chemical determination of collagen in these confluent monolayers are shown in Table I. Statistically, all concentrations of Dilantin had no significant effect on cellular collagen synthesis compared with controls. The data in Table I compare the mean values with the range of mean values for chemical hydroxyproline and radioactive hydroxyproline from each of the three Dilantin concentrations used.

The effect of similar concentrations of Dilantin upon the rate of proliferation or population doubling time was determined in these cells in a manner previously described (12). These results are summarized in Table II, and indicate that as little as 2 μg/ml of Dilantin will significantly reduce the time required for diploid human fibroblast populations to double. It would appear that 5 μg/ml is probably the optimal amount of this particular drug in terms of decreased population doubling time. Separate experiments indicated that Dilantin had no significant effect in overcoming the "contact inhibition" of these fibroblasts when they had reached confluency. Finally, Dilantin when added to the media in the absence of serum did not stimulate the cells to divide.

TABLE I. The Effects of Dilantin (10^{-4}; 10^{-5}; 10^{-6} *M*) upon the Mean[a] Collagen Hydroxyproline Content in Diploid Human Skin Fibroblast Confluent Monolayers.

	Hydroxyproline/mg of culture protein			
	Chemical (nmole/mg)		Radioactive (dpm × 10^{-3}/mg)	
Days	Control	Dilantin[b]	Control	Dilantin[b]
0	3.6	3.6	0.5	0.5
3	16.5	13.9–19.1	7.6	6.2– 9.0
6	30.5	25.9–36.1	13.4	11.4–15.3
9	27.3	21.2–33.4	13.5	10.8–16.2

[a] Each value represents two cultures; duplicate assays on each culture.

[b] The range of means from all three Dilantin doses.

TABLE II. Effects of Dilantin upon the Population Doubling Time of Diploid Human Fibroblasts *in Vitro*.

	Doubling time (hr)
Serum alone	35.0
Serum + Dilantin (μg/ml)	
2	25.1
5	21.8
10	25.9
20	33.1

Discussion. From these data, it is clear that Dilantin will significantly increase the rate of proliferation of diploid human fibroblasts in culture while it has no effect upon the rate of collagen synthesis determined either chemically or by the isolation of radioactive hydroxyproline which has been converted from labeled proline. Thus, it appears unlikely that Dilantin has any influence upon collagenization *per se*. It is possible that if the rate of proliferation of fibroblasts could be stimulated significantly *in vivo* (the doses of Dilantin *in vitro* are less than those usually found in the circulation of patients receiving the drug in the normal course of therapy) then these cells might reach a critical concentration in a wounded area more rapidly than in patients who had not received this drug. Thus, the initial lag of wound repair (*i.e.*, proliferation of fibroblasts) might be reduced somewhat and this may express itself as an increased rate of wound repair.

Summary. Dilantin, at a concentration of 2 to 5 μg/ml, has no effect upon the rate of collagen synthesis by diploid human fibroblasts *in vivo*. Dilantin will stimulate the rate of proliferation of these cells reducing their population doubling time from 35 to 22 hr.

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