Effect of Dilantin Sodium Analogues on Cell Proliferation in Tissue Culture.* (26287)

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Previous investigations in this laboratory have shown clearly the stimulatory effect of sodium diphenylhydantoinate (Dilantin Sodium)† on proliferation of human gingival fibroblast-like cells in tissue culture(1). It has been suggested that this stimulation of cell proliferation is related to the well-recognized phenomenon of gingival hyperplasia occurring in patients receiving Dilantin Sodium for treatment of epilepsy(1), to the increased rate of healing of gingival wounds in non-epileptic human patients receiving Dilantin(2), and to the increased tensile strength of healing skin wounds in rats receiving Dilantin(3). This finding of accelerated wound healing as judged by increased tensile strength in Dilantin-treated animals as well as animals given 3 ethyl, 5 phenylhydantoin has been confirmed by Kelln and Gorlin(4). Fibroblastic proliferation with increased collagenization would explain these *in vivo* phenomena and could be accounted for by the *in vitro* findings.

In seeking an explanation for the basic mechanism underlying this unusual cell response, a series of chemical analogues of Dilantin Sodium as well as a group of structurally related compounds have been tested for a possible similar stimulatory ability.

Materials and methods. The line of human gingival fibroblasts (E.D.) used in previously reported studies(1) was lost shortly after completion of those studies both in our laboratory and the laboratory from which it was originally obtained (Dr. Donald E. Flieder, St. Louis Univ. School of Dentistry). For this reason, another cell line maintained in our laboratory was tested in these studies with various compounds for effects on cell proliferation. This particular cell line was a human gingival fibroblast-like cell isolated and cultured from gingival tissue removed by surgical excision from a 12-year-old female patient with the condition known as fibromatosis gingivae.‡ This cell line was in its 21st transfer at time of initiation of these studies, having been maintained continuously for approximately 4 months.

The medium used for growth of this fibroblast-like cell consisted of: 30 ml calf serum, 4.5 ml Earle's basal salt solution (20× conc.), 3 ml vitamin mixture (100× conc.)§, 3 ml amino acid mixture (100× conc.)§, 3 ml glutamine solution (200 mM, 100× conc.)§, and 50 ml triple distilled water with 100 units penicillin, 200 μg streptomycin and 50 units Mycostatin (Squibb) per ml of medium. This medium was adjusted to pH 7.0 with sodium bicarbonate.

In testing the various compounds to be reported here, actively growing cultures of fibroblast-like cells were trypsinized (0.25% solution of trypsin) for 15 minutes at 37°C, centrifuged at low speed for 20 minutes, washed in single strength Earle's BSS and centrifuged once more. The cells were then resuspended in the nutrient medium and to each bottle containing 80 ml of medium was added 2 ml of an aqueous solution of Dilantin Sodium, the various analogues or structurally related compounds each prepared at a concentration of 200 μg per ml of water.

After obtaining viable cell counts of control and experimental suspensions and adjusting initial suspensions to pH 7.0, 4 ml portions of each of the control and experimental suspensions of cells were dispensed into a series of tissue culture tubes and the tubes incubated at 37°C in a stationary position. At the various periods indicated in the tables, cells of 3 tubes picked at random from each

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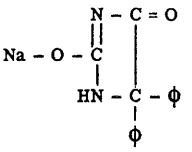
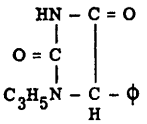
† Parke, Davis & Co., Detroit, Mich.

‡ Courtesy of Dr. Henry M. Swenson.

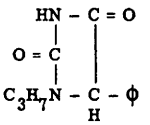
§ Microbiological Associates, Bethesda, Md.



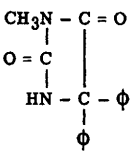
Sodium, 5, 5 diphenyl hydantointate (Dilantin)



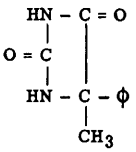
1 allyl, 5 phenyl hydantoinate



1 propyl, 5 phenyl hydantoinate



3 methyl, 5,5 diphenyl hydantoinate



5 methyl, 5 phenyl hydantoinate

FIG. 1.

group were suspended by addition of 4 ml of 0.25% trypsin solution to each, and viable cells counted in a hemocytometer after staining with trypan blue (0.5% solution).

The chemical analogues of Dilantin Sodium tested in this study were: 1) 1 allyl, 5 phenyl hydantoinate; 2) 1 propyl, 5 phenyl hydantoinate; 3) 5 methyl, 5 phenyl hydantoinate and 4) 3 methyl, 5, 5 diphenyl hydantoinate,‖ while the structurally related compounds were: 1) barbital sodium, 2) urea, 3) uric acid, 4) hydantoin, 5) allantoin and 6) alloxan (Fig. 1 and 2).

Results. The data shown in both Tables I and II clearly confirm the remarkable stimulatory effects of Dilantin Sodium on proliferation of human gingival fibroblast-like cells in tissue culture. In most cases, the counts of cells exposed to Dilantin Sodium were approximately double those of control tubes by the 4th or 5th days of incubation. Each value expressed in Tables I and II represents the average count on 3 tubes with occasional exceptions. Two of the Dilantin analogues (1 allyl, 5 phenyl hydantoinate and 5 methyl, 5 phenyl hydantoinate) stimulated cell proliferation to the same significant degree as Dilantin Sodium (Table I).

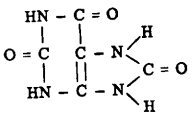
‖ The 4 chemical analogues were courteously supplied by Parke, Davis & Co., Detroit, Mich.

TABLE I. Effect of Dilantin Analogues on Proliferation of Human Fibroblast-Like Cells in Tissue Culture.

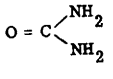
	Mean cell count (per ml of medium) × 10^5					
	Day					p (2-7 day intervals)
	0	1	2	4	7	
Control	1.01	1.18	1.65	2.23	4.00	—
Dilantin	1.01	1.20	3.03	4.26	6.04	<.01
Analogue A*	1.01	1.18	3.10	4.22	6.02	<.01
" B*	1.00	1.17	1.66	2.36	4.06	N.S.
	Day					p (2-5 day intervals)
	0	1	2	5		
Control	1.03	1.28	1.51	3.00		—
Dilantin	1.07	1.26	2.95	4.58		<.01
Analogue C*	1.04	1.27	3.00	4.82		<.01
" D*	1.05	1.26	1.50	2.90		N.S.

* Analogue A, 1 allyl, 5 phenyl hydantoinate; Analogue B, 1 propyl, 5 phenyl hydantoinate; Analogue C, 5 methyl, 5 phenyl hydantoinate; Analogue D, 3 methyl, 5, 5 diphenyl hydantoinate.

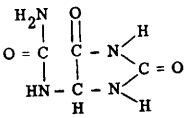
The other two very closely related compounds (1 propyl, 5 phenyl hydantoinate and 3 methyl, 5,5 diphenyl hydantoinate) were completely ineffective in producing a stimulatory response, at the periods shown, similar to



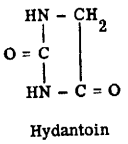
Uric acid



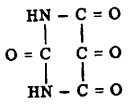
Urea



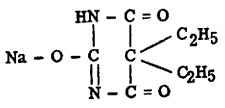
Allantoin



Hydantoin



Alloxan



Barbital sodium

FIG. 2.

TABLE II. Effect of Compounds Structurally Related to Dilantin on Proliferation of Human Fibroblast-Like Cells in Tissue Culture.

	Mean cell count (per ml of medium) $\times 10^5$		
	0	4	p (4 day interval)
Control	.96	2.02	—
Dilantin Na (.0007M)	.97	4.19	<.01
Barbital Na (.0010M)	.98	2.03	N.S.
Hydantoin (.0019M)	.98	2.02	"
Allantoin (.0013M)	.98	2.08	"
Uric acid (.0012M)	.98	2.02	"
Urea (.0033M)	.99	2.03	"
Alloxan (.0014M)	.98	2.02	"

Dilantin Sodium or the other 2 chemical analogues.

None of the structurally related compounds (barbital sodium, urea, uric acid, alloxan, allantoin or hydantoin) produced a stimulatory effect similar to that of Dilantin Sodium (Table II). At the 4-day interval, found adequate through numerous studies in this laboratory for evaluation of cell-stimulation effects, cell counts in all tubes except Dilantin Sodium did not differ significantly from those of control tubes.

Discussion. The studies here obviously confirm the cell stimulatory effect of Dilantin Sodium. However, they fail to clarify that particular portion of the hydantoin molecule or side chain or grouping of side chains responsible for this phenomenon. The phenomenon is apparently unrelated to the basic hydantoin structure, presence of phenyl groups, or presence or absence of various groups at the 1 or 3 position. It is interesting to note that, while analogues B and D and all the structurally related compounds were ineffective in stimulating cell growth, there was no evidence of growth inhibition or cytotoxicity.

Summary. Several chemical analogues and chemically related compounds of Dilantin Sodium have been used to study and clarify the cell stimulatory effects of this compound. Two analogues (1 allyl, 5 phenyl hydantoinate and 5 methyl, 5 phenyl hydantoinate) induce stimulation of fibroblast-like cell proliferation while 2 other analogues (1 propyl, 5 phenyl hydantoinate and 3 methyl, 5,5 diphenyl hydantoinate) were completely ineffective. None of the other structurally related compounds tested (barbital sodium, urea, uric acid, alloxan, allantoin or hydantoin) produced any stimulation of cell proliferation.

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A Study of Bentonite Flocculation Test for Measurement of Type-Specific Streptococcal Antibodies.* (26288)

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A reliable and sensitive technic for determining type-specific anti-streptococcal antibodies in the blood of patients recovering from Group A streptococcal infections would help greatly in investigating epidemiological and clinical problems associated with such infections and their sequelae. The mouse-protection test(1), which is generally accepted as the ultimate basis for type classification of Group A hemolytic streptococci and for detection and measurement of type-specific protective antibody, has the disadvantage that it requires strains of high mouse virulence (which are infrequent among streptococci freshly isolated from human sources),

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