in the infant would probably be greater than 0.25 and less than 1. These relationships can then be used, within the limitations mentioned, to estimate radiostrontium levels in young infants from knowledge of their diet or of the mother's diet during breast-feeding.

Summary. 1. In 4 out of 5 normal healthy women Sr^{90}/Ca ratio in milk ranged from 0.085 to 0.13 of diet with average 0.10. These values are essentially the same as reported for lactating animals. The value for the other subject was 0.028, and it is considered most likely that this was a result of relatively low calcium intake (about 1 g/day), which probably led to marked negative calcium balance and thus invalidated the procedure. 2. If we assume relationship between Sr/Ca ratio in blood and milk is the same for the human as for cow and goat, it can be calculated that Sr/Ca ratio in these subjects would be about 0.25 that in diet consumed. This value is in agreement with other values obtained for man by methods based on other assumptions.

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Effect of Dilantin Sodium on Growth of Human Fibroblast-Like Cell Cultures.* (25778)

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Fibrous hyperplasia of the gingiva occurring as an untoward reaction following systemic administration of sodium diphenylhydantoinate (Dilantin Sodium) in treatment of epilepsy is a well recognized clinical phenomenon. Microscopic examination of this hyperplastic tissue characteristically shows increased fibroblastic activity with pronounced formation of new collagen fibers. Shapiro(1) has reported that administration of Dilantin to non-epileptic patients increased rate of healing of experimentally induced gingival wounds through increased connective tissue activity as well as accelerated epithelialization. In support of this clinical finding, Shafer *et al.*(2) noted that administration of Dilantin Sodium to rats produced a dramatic increase in tensile strength of healing skin wounds. They suggested that this was probably a function of increased collagenization, the same reaction which occurs in the gingival hyperplasia of patients receiving this drug. In an attempt to learn more of the mechanism for both the gingival hyperplasia and the apparent stimulation of wound healing, a study has been carried out testing the effect of Dilantin Sodium on rate of growth of human fibroblast-like cells in cell culture.

Materials and methods. The strain of fibroblast-like cells (designated as E.D.) used in these studies was originally isolated from adult human gingiva by Dr. Donald E. Flieder, St. Louis Univ. School of Dentistry and was courteously supplied by him. At the time of ini-

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TABLE I. Effect of Various Concentrations of Dilantin Sodium on Fibroblast-Like Cell Growth.

	Mean cell count (per ml of medium) $\times 10^5$	
	Initial	5 days
Control (with NaOH added equivalent to that in experimental tubes)	1.02	2.78
Control with NaCl (NaOH added + equivalent NaCl to supply Na^+ present from 200 μg Dilantin Sodium)	1.00	2.95
Dilantin (20 μg/ml of initial solution)	1.02	3.93
" (200 *Idem*)	1.00	5.90
" (1000 ")	1.03	All cells dead
" (10,000 ")	1.01	*Idem*

tial studies in our laboratory, this strain of cells was in its 17th transfer and was grown on a nutrient medium consisting of: 30% calf serum, 9% Earle's balanced salt solution (BSS) (10x conc.), 12% Eagle's basal medium (BM) (3) and triple distilled water with 100 units of penicillin, 200 μg of streptomycin and 50 units of Mycostatin (Squibb) per ml of medium. The resulting medium was adjusted to pH 7.0 with sodium bicarbonate. In the repetitive studies (I-VII,), (Table II) actively growing cultures of fibroblast-like cells were trypsinized (0.25% 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 divided into 2 bottles of 80 ml each. To one bottle was added 2 ml of Dilantin Sodium (200 μg per ml of solution) dissolved with sodium hydroxide (0.2 ml of a 1% solution per 100 ml) while to the other batch was added 2 ml only of sodium hydroxide of the same concentration. The suitable concentration of Dilantin Sodium was determined by a pilot study with results as shown in Table I. In all studies in Table II except I and II, the cells were exposed to the drug for 20 minutes at room temperature (22-23°C) centrifuged, washed and resuspended in fresh medium containing Dilantin Sodium before dispensing into the culture tubes. In studies I and II, this step of prior exposure to the drug was omitted. In study VII, concentration of the calf serum was lowered to 20% and Eagle's BM to 6% in an attempt to decrease growth rate of the cells and thereby permit possible magnification of the Dilantin effect. After obtaining viable cell counts of control and experimental suspensions and adjusting initial pH in both suspensions to pH 7.0, 4 ml portions of each of the control and experimental suspensions 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, cell contents of 4 tubes picked at random from each group were suspended by addition of 4 ml of 0.25% trypsin to each and viable cells counted in a hemocytometer after staining with trypan blue (0.5%). Each value expressed in Tables II and III is the average count on 4 tubes with occasional exceptions. The effect of Dilantin Sodium was also tested on the growth of HeLa cells by an identical procedure, except that the cells were grown in 63% mixture 199, 25% lactalbumin hydrolysate (5%), 10% calf serum, and 2% sodium bicarbonate (2.8% solution) plus the usual concentration of antibiotics. In addition, the effect of Dilantin Sodium was tested on growth rate of cells isolated from a fibrosarcoma induced in a rat and courteously supplied by Dr. David F. Mitchell, Indiana Univ. At time of testing with Dilantin Sodium, the tumor was in its 17th transfer. Growth medium consisted of 5% calf serum, 7.5% Eagle's basal medium (3×), 10% lactalbumin hydrolysate (5%), 5% whole egg ultrafiltrate† and antibiotics.

Results. These studies (Table II) indicate that Dilantin Sodium produces a remarkable *in vitro* stimulus to growth of the fibroblast-like cell under investigation. After a period

† Microbiological Assoc., Washington, D.C.

TABLE II. Effect of Dilantin Sodium on Growth of Human Fibroblast-Like Cells at Varying Time Intervals.

		Control		Dilantin		
Series	Day	Mean cell count (per ml of medium) $\times 10^5$	Stand. dev. $\times 10^4$	Mean cell count (per ml of medium) $\times 10^5$	Stand. dev. $\times 10^4$	p*
I	0	1.00	.71	.98	.75	
	1	1.30	.82	1.55	.55	$<.01$
	2	1.51	.85	1.67	.53	$.02 < p < .05$
	5	3.65	4.12	6.05	4.20	$<.01$
II	0	.98	.33	1.01	.53	
	1	1.23	.73	1.51	.82	$<.01$
	2	1.54	.82	1.74	.44	"
	5	3.48	4.43	6.10	1.82	"
	7	5.15	2.52	6.35	3.41	"
III	0	.88	.20	.87	.28	
	1	1.11	.82	1.36	.44	$<.01$
	2	1.40	.39	1.61	.46	"
	5	2.93	1.71	4.98	1.71	"
	7	4.80	2.00	5.55	3.12	$.02 < p < .05$
IV	0	1.00	.48	1.03	.53	
	1	1.07	.22	1.26	.71	$<.01$
	2	1.41	.35	1.58	.33	"
	5	2.98	1.71	4.97	.98	"
	7	4.94	1.37	5.83	1.71	"
V	0	1.01	.22	1.02	.33	
	1	1.13	.46	1.37	.26	$<.01$
	2	1.44	.44	1.57	.53	$.01 < p < .02$
	3	1.55	.41	1.69	.30	$<.01$
	4	1.70	.17	1.89	.22	"
VI	0	.98	.14	.98	.17	
	1	1.12	.32	1.39	.17	$<.01$
	2	1.46	.35	1.60	.26	"
	4	1.72	.30	1.92	.07	"
	7	5.96	.45	6.43	1.74	"
VII	0	.99	.53	.98	.64	
	1	1.01	.14	1.03	.22	.3
	4	1.99	.46	4.59	2.74	$<.01$
	6	3.12	.54	5.44	1.82	"
	7	4.00	.79	6.10	.82	"

* Calculated by Student's "t" test.

TABLE III. Effect of Dilantin Sodium on Growth of HeLa Cells at Varying Time Intervals.

		Control		Dilantin		
Series	Day	Mean cell count (per ml of medium) $\times 10^5$	Stand. dev. $\times 10^4$	Mean cell count (per ml of medium) $\times 10^5$	Stand. dev. $\times 10^4$	p*
I	0	1.05	1.01	1.04	.48	
	1	1.09	.26	1.18	.17	$<.01$
	2	1.46	.44	1.63	.14	"
II	0	1.10	.71	1.10	.44	
	1	1.12	.35	1.26	.45	$<.01$
	2	1.51	.32	1.66	.30	"
	3	1.65	.25	1.79	.26	"
	4	1.72	.26	1.92	.32	"
III	0	1.02	.32	1.00	.26	
	1	1.16	.37	1.26	.45	$.02 < p < .05$
	2	1.54	.36	1.68	.35	$<.01$
	4	1.78	.17	1.99	.14	"
	7	6.05	.32	6.86	.36	"

* Calculated by Student's "t" test.

of only one day, the treated cell cultures invariably showed increased growth in comparison with control cultures, as determined by cell counts. In nearly all series, the effect on growth reached its maximum after 4 or 5 days of incubation, and in most cases, interestingly, this difference was reduced by the 7th day. Some series exhibited nearly a 2-fold increase in the cell count at the 4 to 5 day incubation period. These increases are statistically significant at levels shown in the table.

Investigation of HeLa cells revealed a similar but less remarkable increase in cell population induced by Dilantin Sodium (Table III). The fibroblasts isolated from the fibrosarcoma evidenced no reaction to the Dilantin Sodium; in fact, growth was retarded for several days.

The explanation for this apparent *in vitro* stimulation of fibroblast-like cells is not known. It is apparently caused by the diphenylhydantoin structure (or some metabolic product) rather than to the additional sodium ions present because addition of gram-equivalent amounts of sodium did not significantly stimulate growth.

Summary. Addition of sodium diphenylhydantoinate (Dilantin Sodium) to tissue culture of human fibroblast-like cells resulted in stimulation of their proliferation. A similar but more limited stimulation to proliferation occurred with HeLa cells but there was no detectable effect on cells isolated from rat fibrosarcoma.

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Gas-Liquid Chromatography of Methyl Esters of Fatty Acid from Human and Chicken Brain Lipids.* (25779)

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Purification of individual lipid fractions followed by analysis of component fatty acids involves laborious procedures while constituent highly unsaturated acids may suffer oxidative degradation(1-5). It appears beneficial if fatty acid composition patterns of total brain lipids could be surveyed simply and rapidly by gas chromatography before more detailed analyses were attempted. In the present study analyses for a number of normal saturated, unsaturated and α hydroxy fatty acids from samples of human and chicken brain lipids are described.

Methods. Preparation of fatty acids from brain lipid. Samples of human cerebrum obtained on autopsy† and pooled whole chicken brains were extracted with 2:1 chloroform-methanol solution according to procedure of Folch *et al.*(6). The lipid-containing solution was concentrated by evaporation of solvent under vacuum. The concentrate was poured into 4 times its volume of ice cold acetone containing 1 ml of 5% $MgCl_2$ in 94% ethanol/100 ml of acetone(7). The precipitated lipid was then filtered off under N_2, washed several times with acetone-magnesium chloride solution, dried under vacuum, and weighed. This precipitation procedure removes acetone-soluble cholesterol which, with certain exceptions, occurs in brain as free sterol only. Lipid thus obtained had a negative reaction to Liebermann-Burchard test for β-ol sterols. Dried lipid was subjected to methanolysis by refluxing 24 hours with super-dry half-saturated methanolic HCl. Methyl esters were removed from the solution by 4 extractions with diethyl ether. The combined ether ex-

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† Male aged 65, cause of death cerebral hemorrhage. Involved area of brain was not used for analysis.