sein has antigoitrogenic properties(8). Precisely the reverse may be seen for the data from rats fed diet YST. Their mean thyroid weight was significantly greater than those of rats fed diet REM ($P = .05$). Furthermore, their thyroid iodine content was only half as great as those of animals fed diet REM. Thus the low iodine 40% yeast diet, which had supported normal growth, did not exert an antigoitrogenic effect as do low iodine casein diets such as diet CAS. Indeed in this experiment diet YST had a significantly positive goitrogenic effect, over and above that to be expected from its low iodine content.

Discussion. Dry yeast has been used frequently in experimental animal diets, but rarely if ever at levels as high as the 40% employed in this study. This high level of yeast, together with supplementation with methionine, enabled diet YST in the present study to support a rate of gain in body weight similar to that supported by a standard laboratory rat chow or a semipurified diet based on casein. As the dry yeast had a low iodine content (0.06 μg/g), it was possible to use it in a low iodine diet. Furthermore, as seen in this study, the yeast did not have an "antigoitrogenic" effect aside from its iodine content, such as has been attributed to animal proteins like casein.

Hence diet YST, or similar ones, may prove useful in experimental goiter studies with the rat. The literature on dietary goitrogenesis does not clarify the apparent positive goitrogenic effect, over and above that attributable to low iodine content, seen with diet YST in this study.

Summary. By using 40% of brewers type dry yeast as the principal protein source, a low iodine diet has been designed that supports a good rate of body weight gain and also produces large goiters in rats. This diet appears to have a positive goitrogenic effect, over and above that attributable to its low iodine content.

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Effect of Dilantin Sodium on Various Cell Lines in Tissue Culture.* (27038)

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Previous studies from this laboratory(1,2) have clearly shown the dramatic stimulatory effects of sodium 5,5 diphenylhydantoin (Dilantin Sodium) and certain related analogues on the growth of a human gingival fibroblast-like cell in tissue culture. This property is consistent with the Dilantin-induced stimulation of wound healing in rats as judged by increased tensile strength also reported by this laboratory(3) and with well-known gingival hyperplasia occurring in epileptic patients under treatment with this drug. During these studies, it was found that the HeLa cell in tissue culture responded to Dilantin by a very limited stimulation of proliferation but there was no significant stimulation of cells isolated from rat fibrosarcoma. This variability of cell response suggested that this stimulatory effect of Dilantin might be quite cell-specific and, for this reason, a series of different cell lines carried in this laboratory have been tested.

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Materials and Methods. Two general types of cell lines have been tested: those isolated from 1) normal tissue and 2) tumor tissue (Table

I†). In these studies, actively growing serial transfer cultures of cells were trypsinized, washed, suspended in their appropriate growth media and dispensed into culture tubes. The growth medium for each cell line was not identical but usually differed only in type and concentration of blood serum (calf or horse) and the variable presence or absence of such constituents as lactalbumin hydrolysate and egg ultrafiltrate. The medium in the experimental tubes was identical with that in control tubes for each cell line in all cases except that, in addition, the experimental medium contained 200 μg/ml of Dilantin.**

After incubation at 37°C in a stationary position, either 2 or 3 tubes were picked at random from each group at the varying time intervals shown and cell population determined by counting viable resuspended cells, stained with trypan blue, in a hemacytometer. The complete technique for these various procedures has been reported(1,2).

Results and Discussion. The results of these studies are shown in Table I. A single test series on the human gingival fibroblast-like cell and on the HeLa cell, both previously reported (1), are shown for comparative purposes. Only data for a single test series are shown for each cell line. However, in every case the findings in each series were consistent with respect to the magnitude of cell response to the drug. The individual data shown were carefully selected to be respresentative of the various series of studies carried out on each cell line.

The data indicate that a distinctive selectivity exists in the stimulatory effect of Dilantin on different cells in tissue culture. Of these cells tested derived from normal tissues, only fibroblasts (the human gingival fibroblast isolated in this laboratory and Earle's L929 mouse fibroblast) responded to the drug by a dramatic growth stimulation. Epithelial cells or cells derived from ectodermal organs demonstrated no remarkable response to Dilantin. Cells derived from adult mouse heart demonstrated a moderate stimulation in response to Dilantin at a 7 day period. However, this culture, even after 125 transfers appears to consist of 2 types of cells, and the stimulated cell bears a great resemblance to a fibroblast.

Of cell lines derived from tumor tissues tested, only the BW1081 mouse salivary gland tumor showed a similar dramatic stimulatory response. This is somewhat surprising, as this tumor was originally presumed to be of epithelial origin. However, evidence reported by Beck suggests that this tumor may be related to the polyoma virus tumor(4). Furthermore, reintroduction of these tumor cells grown in tissue culture for over 155 serial transfers into the BALB/c mouse in this laboratory has resulted in tumor formation but, interestingly, of a somewhat different microscopic pattern, more suggestive of a fibrosarcoma than the original epithelial neoplasm. The significance of this finding is not apparent although sarcomatous transformation of epithelial cells is rather common in tissue culture and has been discussed by Sandford et al(5).

The HeLa cell, as previously reported, continued to show a moderate stimulation of cell proliferation but of far less magnitude than either normal fibroblasts or the BW1081 tumor. Upon further testing the rat fibrosarcoma also showed a stimulation of the same approximate magnitude as the HeLa cell. Furthermore, the H-P melanoma line showed a response quite comparable to the normal adult mouse heart, the rat fibrosarcoma and HeLa cell. Despite the fact that these latter cell lines showed a statistically significant ($p = <0.01$) stimulation of prolifieration in response to Dilantin, this cannot be considered of biologic significance equal to the dramatic response shown by the 2 lines of normal fibroblasts and the BW1081 tumor.

The data definitely suggest that the stimulatory effects of Dilantin are specific for fibroblasts. These findings are consistent with the report of Houck *et al*(6) that administration of Dilantin to rats resulted in changes in skin chemistry consisting of a marked increase in insoluble collegen and insoluble hexosamine, and the appearance of a unique insoluble noncollagenous protein, all implying a marked al-

† The human squamous epithelium utilized in these studies was courteously supplied by Dr. Irving S. Johnson, Eli Lilly Co., Indianapolis, Ind. The Earle's L929 fibroblast was purchased from Microbiological Associates, Bethesda, Md. The Henle's epithelial cell was courteously supplied by Dr. J. Donald Hubbard, Indiana Univ. School of Med., Dept. of Pathology.

** Generously supplied by Parke, Davis & Co., Detroit, Mich.

TABLE I. Effect of Dilantin Sodium on Growth of Various Cell Lines.

Cell Line	Mean Cell Count (per ml of medium) x 10^5 Interval (days) 0	1	4 or 5	7 or 8	% stimulation of cell proliferation (4 or 5 days)
Normal tissues					
1) Adult mouse heart(3) *					
Control	.78	.83	2.00	3.83	18
Dilantin	.81	.83	2.36	4.66	—
2) Adult mouse kidney(3)					
Control	1.01	1.16	3.85	5.19	0
Dilantin	1.01	1.15	3.67	5.08	—
3) Adult mouse liver(2)					
Control	.97	1.04	2.68	4.04	1
Dilantin	.97	1.05	2.71	4.03	—
4) Newborn mouse lung(2)					
Control	.98	1.03	2.85	4.06	—
Dilantin	.97	1.04	3.03	4.08	7
5) Henle's intestinal epithelium(2)					
Control	.70	.71	2.11	3.49	—
Dilantin	.71	.70	2.15	3.49	2
6) Human squamous epithelium(2)					
Control	.74	.78	1.99	3.40	—
Dilantin	.78	.81	2.00	3.48	0
7) Earle's L929 mouse fibroblast(2)					
Control	.74	.79	1.99	3.39	—
Dilantin	.74	.81	2.99	4.28	50
8) Human gingival fibroblast-like cell(18) †					
Control	1.01	1.18	2.23	4.00	—
Dilantin	1.01	1.20	4.26	6.04	91
Tumor Tissues					
1) Rat fibrosarcoma(3)					
Control	.94	1.13	4.02	5.36	—
Dilantin	.94	1.13	4.69	5.99	17
2) Rat rhabdomyosarcoma(2)					
Control	1.01	1.14	4.09	—	—
Dilantin	1.01	1.14	4.04	—	0
3) H-P melanoma(2)					
Control	.68	.88	2.13	2.71	—
Dilantin	.70	.86	2.46	3.08	16
4) HeLa Cell(3) †					
Control	1.02	1.16	1.78	6.05	—
Dilantin	1.00	1.26	1.99	6.86	12
5) BW1081 mouse salivary gland tumor(5)					
Control	.92	1.05	3.36	5.80	—
Dilantin	.90	1.05	6.86	7.35	104

* Figures in parentheses indicate minimum number of times cell line was tested.
† From previously reported studies(1,2), for comparative purposes.

teration in fibroblast metabolism.

The significance of these findings is manifold. They suggest not only a technical method for separating fibroblasts from other contaminating cells in tissue culture, but also a possible means for better understanding of the metabolism of the fibroblast.

Summary. A variety of cell lines, derived from both normal and tumor tissues, has been tested for possible stimulatory effects induced by sodium 5,5 diphenylhydantoin (Dilantin Sodium). Of these cells utilized, only fibroblasts responded by a stimulation of proliferation with the exception of the BW1081 mouse salivary gland tumor. The significance of these findings may be of considerable importance in our ultimate understanding of fibroblast function.

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