Effect of Pilocarpine on Acid-Soluble Phosphate Compounds of Rat Following Hepatectomy.* (27827)

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Diphenylhydantoin sodium (Dilantin Sodium®) is employed primarily in treatment of convulsive disease. In the past few years, however, several new actions of this drug have been described, namely a remarkably beneficial effect on wound healing and a stimulatory effect on dividing human gingival fibroblast-like cells in tissue culture. Shapiro (1) reported that administration of Dilantin to non-epileptic human subjects increased the rate of healing of experimentally induced gingival wounds through increased connective tissue activity as well as increased epithelialization. Shafer, *et al.*(2), reported that the systemic administration of Dilantin Sodium to rats produced a dramatic increase in the tensile strength of healing skin wounds. These authors have suggested that this observed action of Dilantin is due to increased fibroblastic proliferation with subsequent increased collagenization similar to the reaction which occurs in the gums of patients receiving the drug for convulsive disorders. Shafer (3,4) has also shown that Dilantin Sodium and several of its analogues have a stimulatory effect on the proliferation of human fibroblast-like cells in tissue culture and a mild stimulatory effect on HeLa cells in tissue culture, but no effect on cells isolated from a rat fibrosarcoma. No reports of the effect of Dilantin on cells of non-connective tissue origin have appeared. It is uncertain if the stimulation of proliferation of cells by Dilantin is specific for connective tissue or whether it may have this effect on cells of other types. The present experiment was designed to study the effect of Dilantin Sodium on rate of restitution of rat liver cells after partial hepatectomy, in an effort to clarify the question of the possible tissue specificity of the proliferative action of Dilantin.

Methods. Male and female albino rats of the Denver strain, weighing from 100 to 145 g were housed in solid bottom cages and maintained on a stock laboratory diet.

Eight groups of animals were employed. Six of the groups were divided equally into experimental and control animals. Experimental animals received 10 mg each of Dilantin Sodium per day, by intraperitoneal injection, for 10 days prior to hepatectomy. The drug was administered at a concentration of 100 mg/cc, dissolved in commercial diluent supplied with the drug. Control animals in each group received no drug. The seventh group was administered 0.1 cc of diluent in a similar manner. The eighth group received no Dilantin prior to hepatectomy, but was injected I.P. with 10 mg of the drug per day for each of 5 days following hepatectomy.

Hepatectomy was performed under light anesthesia by the method of Higgins and Anderson(5). With this technic, the left lateral and median lobes are removed representing 65-75% of the liver. The abdominal musculature was closed with a continuous suture of fine cotton thread and the skin with interrupted vertical mattress sutures of the same material.

The excised liver tissue was sliced, blotted dry on filter paper and weighed wet to the nearest hundredth of a gram. Sections of tissue were prepared for histologic study from animals in each group. The tissue was then dried for one week at 100°C and reweighed, and the values recorded as dry weight.

Following hepatectomy, the experimental animals were treated with 10 mg of Dilantin I.P. daily until sacrifice. Control animals received no drug. Each group of animals was allowed a different length of recovery time, ranging from 0 through 5 days. The liver was removed immediately after sacrifice, weighed wet, dried and reweighed. Sections of liver tissue were again prepared for histologic examination.

Results. Table I shows a comparison be-

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TABLE I

Days after hepatectomy	Group	No. of animals	Wet wt restituted liver (g/100 g body wt)	Ratio wet/dry wt of restituted liver	Dry wt restituted liver (g/100 g body wt)	S.D. ±	P value
0	Control	7	1.66	3.53	.47	.024	.32
	Dilantin	7	1.55	3.59	.43	.036	
1	Control	7	2.32	3.41	.68	.018	.0064
	Dilantin	8	2.73	3.55	.77	.028	
2	Control	7	2.76	3.84	.72	.028	.00006
	Dilantin	8	3.41	3.92	.87	.025	
3	Control	6	3.13	3.96	.79	.033	.0003
	Dilantin	8	4.03	3.96	1.02	.055	
4	Control	7	2.87	3.73	.77	.029	.00005
	Dilantin	7	3.51	3.74	.94	.028	
5	Control	14	3.28	3.90	.84	.027	.00005
	Dilantin	12	3.84	3.92	.98	.020	
5	Dilantin for 5 days after hepatectomy	17	3.27	3.94	.83		
	Diluent only	8	3.14	3.97	.79	.061	

tween the dry and wet weights of control and Dilantin Sodium treated animals recorded in grams of liver per 100 g of body weight at varying time intervals after hepatectomy. The amount of restitution in animals pre-treated with Dilantin Sodium is significantly greater in all groups from days 2 through 5 using 0.001 as a significant p value. At day 1 there is also a considerable difference between the 2 groups, but this is not significant by our standards.

There was no demonstrable effect of Dilantin on the liver prior to hepatectomy as evidenced by the fact that the livers of control and pre-treated experimental animals remaining immediately after hepatectomy, and the liver fragments removed at hepatectomy in all groups, control and Dilantin treated alike, were not significantly different in size nor could any histologic difference between the 2 groups be demonstrated.

The group of animals which received Dilantin Sodium, only after hepatectomy also showed no significant difference from control animals in rate of restitution.

Also recorded in Table I are ratios of wet to dry weight of restituted livers in control and experimental animals. Although there is a variance between the fluid content of restituted livers at varying intervals after hepatectomy, which correlates well with the figures of Higgins and Anderson(5), there is no significant difference in the fluid content of the livers of treated and non-treated animals on any single day.

Histologic examination of sections of liver tissue from control and Dilantin treated animals both before and after hepatectomy failed to reveal a consistent difference between the 2 groups. The livers of Dilantin treated animals prior to hepatectomy were indistinguishable from those of control animals. Following hepatectomy, both groups showed an increase in number of inflammatory and connective tissue cells present in the liver, but at no time were there significant differences between the number and kinds of cells present.

Discussion. This experiment has shown clearly the stimulatory effect of Dilantin Sodium on the restituting rat liver after partial hepatectomy. Histologic studies and wet/dry weight ratios have shown that this effect of Dilantin is due to a true increase in amount of hepatic parenchyma and not due to an increase in hepatic connective tissue, inflammatory cells or fluid retention. Pre-treatment with Dilantin is necessary to achieve an increased rate of restitution as evidenced by the fact that livers from animals which received Dilantin only after hepatectomy showed no difference in restituting liver size or histologic appearance from livers of control animals. In addition, it is of interest that pre-treatment with the drug causes no

demonstrable size or histologic changes in the liver prior to hepatectomy. Thus the mechanism of action of Dilantin in the stimulation of liver cell restitution is uncertain.

Previous attempts have been made to show the existence of serum mitotic stimulating or inhibiting substances which are modified after hepatectomy, resulting in the rapid restitution seen(6,7,8). The presently observed action of Dilantin could be related to these theories if Dilantin altered either the amount or form in which stimulating or inhibitory substances were present. Other authors, notably MacDonald(9) and Rogers, *et al.* (10) have failed to find any evidence of the presence of stimulating or inhibitory humoral factors directly governing hepatic regeneration.

In the present experiment there was no stimulatory effect of the drug on liver restitution when it was administered only after hepatectomy. Also, unpublished data from our laboratory have failed to show any increase in tensile strength of healing wounds after topical administration of Dilantin or systemic administration of the drug after experimental wound induction. Previous reports of the beneficial effects of Dilantin on wound healing have utilized preoperative treatment with the drug. It is apparent that Dilantin, under the conditions of this experiment may exert a proliferative effect on cells of tissue other than connective tissue.

Summary. Studies have been made on the effect of intraperitoneal injection of diphenylhydantoin sodium on liver restitution in the rat after partial hepatectomy. It was found that preoperative treatment of animals with 10 mg/day of the drug for 10 days followed by a continuation of therapy after hepatectomy resulted in a significantly greater rate of dry weight of liver restored per 100 g of body weight, from days 2 through 5 after hepatectomy. No effect of the drug was observed when administered only after operation. This experiment demonstrates that this proliferative effect is not limited to cells of connective tissue origin.

1. Shapiro, M., *Med. and Surg.,* 1958, v16, 41.
2. Shafer, W. G., Beatty, R. E., Davis, W. B., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 348.
3. Shafer, W. G., *ibid.,* 1960, v104, 198.
4. ———, *ibid.,* 1961, v106, 205.
5. Higgins, G. M., Anderson, R. M., *Arch. Path. and Lab. Med.,* 1931, v12, 187.
6. Christensen, B. G., Jacobsen, E., *Acta Med. Scand.,* 1949, v234, 103.
7. Stich, H. F., Florian, M. L., *Can. J. Biochem. and Physiol.,* 1958, v36, 855.
8. Kohn, R., *Exp. Cell Res.,* 1958, v14, 228.
9. MacDonald, R. A., Rogers, A. E., *Gastroenterology,* 1961, v41, 33.
10. Rogers, A. E., *et al., Am. J. Path.,* 1961, v39, 561.

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Inhibition by Glucose of the Ethionine Induced Fatty Liver.* (27828)

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Ethionine (α-amino-γ-ethylmercapto-butyric acid) has been shown to produce a fatty liver in fasted intact female rats(1,2,3) and in fasted castrated male rats, while intact male rats and testosterone treated female rats were refractory(2). Both methionine and glucose administration prevent this effect(1, 3).

According to Recknagel and Lombardi(4) the fat accumulations in liver cells injured by different agents, among them ethionine(5), are characteristically accompanied in the earliest phases by decreased triglyceride levels in the plasma.

This investigation was carried out to compare triglyceride changes in liver tissue and

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