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Connective Tissue VI: Dermal Lipid Response to Diphenylhydantoin.* (27234)

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Diphenylhydantoin (Dilantin) is known to produce changes in the chemistry of the connective tissue, particularly with regard to dermal concentration of collagen, scleroprotein and hexosamine(1,2). One of us (JCH) has suggested that from the increases in dermal collagen produced by diphenylhydantoin administration there might be a decrease in dermal lipid as well as dermal water concentration(1). This paper describes the results of an analysis of lipid concentration of rat skin from animals which had received diphenylhydantoin, and compares these results with 3 different groups of control animals.

Materials and methods. Twenty-four male Sprague-Dawley rats (220-250 g) were grouped into 4 groups of 6 animals each. One group was subjected to daily subcutaneous injections of 0.65 mg of cortisol (Cortil, C. Pfizer Co.), another to daily intraperitoneal injections of one ml of 0.15 M NaCl in which was suspended 25 mg of Dilantin sodium (Parke, Davis & Co.). The third group was similarly injected with 25 mg of α,α-diphenyl α-aminoacetic acid. (Parke, Davis & Co.), an inactive analogue of Dilantin, and the last group served as a normal control. After 10 daily doses of Dilantin, cortisol or diphenyl aminoacetic acid, all animals were sacrificed and about 1 g of abdominal skin from each animal was removed, shaven and dissected free of adhering fat, fascia and muscle. The cleaned skin from each rat was minced, pooled with the tissue from the other members of each experimental group and lyophilized. One g of pooled, lyophilized tissue from each group was further minced, allowed to stand in 10 ml of 0.15 M NaCl for 2 hours and then extracted in a Virtis homogenizer for one hour. The aqueous suspension of macerated tissue from each group was then extracted with 250 ml of a mixture of chloroform and methanol (2:1, v/v) in a Waring blendor according to Folch (3). The chloroform layer of the extract was analyzed with respect to total and esterified cholesterol(4), lipid phosphorus(5) and neutral fats (glycerides) according to Van Handel and Zilversmitt(6). Total lipids were determined gravimetrically by drying an aliquot *in vacuo,* redissolving the residue in petroleum ether (B.P. 40-60°C), filtering, redrying and determining the weight of the residues in a semi-micro balance. The isolation of neutral glycerides(7) and their separation into tri-, di-, and mono-glycerides was accomplished by silicic acid column chromatography(8).

Results. The results of dermal lipid analysis are shown in Table I. None of the 3 control groups demonstrated any meaningful differences in dermal lipids. These results are in very close agreement with those reported by Kao *et al.*(9) for normal male rats of this weight range when expressed in terms of wet weight (50-55% water).

Mean concentrations of phospholipid, neutral and total fat in the pooled tissues obtained from 3 groups of control animals were 13.08, 205 and 240 mg/g respectively. The standard deviations of each of these means, representing 3 pools of 6 rats each, were ±

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0.9, ± 1.0 and ± 1.0 respectively. Dilantin administration resulted in dermal concentrations of these 3 lipid fractions which differed by more than 3 standard deviations from the mean concentrations of control animals. These differences between the lipid composition of rat skin from Dilantin treated rats and mean dermal lipid composition of control animals were therefore significant statistically ($p<.03$).

The effect of Dilantin administration upon dermal concentration of lipids therefore involved primarily a profound decrease of neutral glycerides, and hence total lipids, and a meaningful decrease in phospholipid concentration.

Since the primary reduction in dermal lipid concentration associated with Dilantin administration was found in the neutral glyceride fraction, the distribution of total glycerol in the tri-, di- and mono-glyceride fractions was determined in terms of percent of total glycerol involved in each form of glyceride. These results presented in Table II indicate, according to a statistical analysis similar to that described above, that with Dilantin the percent of total dermal glycerol found in the form of either tri- or di-glycerides differed by more than 3 standard deviations from the mean of all 3 controls. Dilantin most markedly decreased the di-glyceride fraction.

TABLE I. Variation in mg of Lipid per g of Rat Skin Induced by Diphenylhydantoin.

Lipid	Agents: Normal	Cortisol*	D.A.A.†	Dilantin‡
Cholesterol:				
Total	6.64	7.43	6.13	6.54
Ester	2.82	2.40	2.37	2.23
Free	3.82	5.03	3.76	4.31
Phospholipids	14.68	13.60	10.80	8.98§
Neutral fat	216	194.6	205	131 §
Total lipid	243	239.9	238	160.4 §

* .65 mg of cortisol daily (subcut.) for 10 days.
† D.A.A. = α-diphenyl α-aminoacetic acid, 25 mg /ml (intraper. daily for 10 days).
‡ 25 mg/ml of Dilantin (intraper.) per day for 10 days.
§ Significantly different from the mean of all 3 control values ($p<.03$).

TABLE II. Percent Distribution of Glycerol in Tri-, Di- and Monoglyceride Content of Dermal Lipids after Administration of Diphenylhydantoin.

Agent	% of total glycerol found in: Triglyceride	Diglyceride	Monoglyceride
Normal	80.5	13.7	5.8
Cortisol*	78.5	20.3	1.2
D.A.A.†	80.5	13.5	6.0
Dilantin‡	87.6§	9.0§	3.4

* .65 mg of cortisol daily (subcut.) for 10 days.
† α,α-diphenyl α-aminoacetic acid, 25 mg/ml (intraper.) daily for 10 days.
‡ 25 mg/ml of Dilantin (intraper.) per day for 10 days.
§ Significantly different from the mean of all 3 control values ($p<.03$).

Summary and conclusions. Dilantin decreased significantly the dermal concentration of neutral glycerides, total lipids and phospholipids in rats. This loss of neutral glycerides from dermal tissue was associated with a decrease in the percentage of di-glycerides found in the neutral glyceride fraction of the skin.

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