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Effect of Sodium Diphenylhydantoinate on Oral Mucosa of Rats.* (28436)

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The gingival hyperplasia resulting from dilantin therapy has been reported by various investigators(1-5). Shafer *et al.*(6) observed an increase in the tensile strength of healing skin wounds in dilantin treated rats. They suggested that this may be due to an increase in collagen synthesis accompanying the proliferative response of fibroblasts to dilantin therapy. No effect on the proliferation of epithelial cells could, however, be noted. Houck *et al.*(7) have shown that dilantin administration to rats resulted in accumulation of super-normal amounts of collagen within the dermis. Shapiro(8) reported acanthosis of the epithelium in human gingiva but failed to observe any alteration in keratinization of this tissue. The purpose of this investigation was to demonstrate the effect of systemic administration of sodium dilantin on the oral mucosa of rats.

Materials and methods. Five litters, each of 10 newborn albino rats, were used in this study. Beginning at 3 days of age, 8 rats in each litter were subjected every 3 days to intraperitoneal injections of 0.04 ml suspension of 100 mg/kg body weight of dilantin sodium in isotonic saline. The remaining 2 animals in each group received 0.04 ml of isotonic saline and were used as controls. The experimental rats in each litter received from 1-5 doses of dilantin sodium.† Two dilantin treated animals from each litter were sacrificed by decapitation 6 days after administration of the last dose. The controls were sacrificed at corresponding intervals. The oral mucosa from the cheek region was removed and fixed in 10% alcoholic formalin. The tissues were processed by routine histological procedure and embedded in paraffin. Serial sections were cut at 6 μ thickness and stained with Hematoxylin and Eosin, Masson Trichrome, Periodic Acid Schiff, and Mowry's procedure for acid mucopolysaccharides.

Results. No visible clinical changes were noted in the mucous membrane of the cheek of experimental animals. The initial *histological* changes in the mucosa were observed in animals which had received only one dose of sodium dilantin (Fig. 1-2). An increased mitotic activity in the basal layer of the epithelium was noted. Intercellular edema within the epithelium appeared to have caused prominent spongiosis and acanthosis. There was an apparent disturbance in cornification resulting in parakeratosis. In animals which had received 4 doses of sodium dilantin (Fig. 3 & 4), hyperplasia of epithelium was a common feature. The new cells produced were devoid of nuclear detail. The rete pegs showed elongation and splitting. Occasional intertwining of the rete pegs produced isolated areas of connective tissue stroma among the epithelium. Unusually large numbers of discrete basophilic keratohyaline granules were diffusely distributed throughout the epithelial cell layers. This was always associated with hyperkeratosis. Increased vascularity and scattered areas of chronic inflammation were sometimes observed in the connective tissue. Elevated fibroblastic activity

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† Parke-Davis (Sodium 5,5 diphenylhydantoinate).

(Fig. 5) was accompanied by increased collagen fiber formation.

The initial alterations in the *histochemical* staining reactions of the oral mucosa were observed in animals which had received 2-3 doses of sodium dilantin. The acanthotic epithelial eccentrically located aggregates of PAS positive material (Fig. 6 & 7). The underlying connective tissue showed a greater intensity of staining reaction. When these

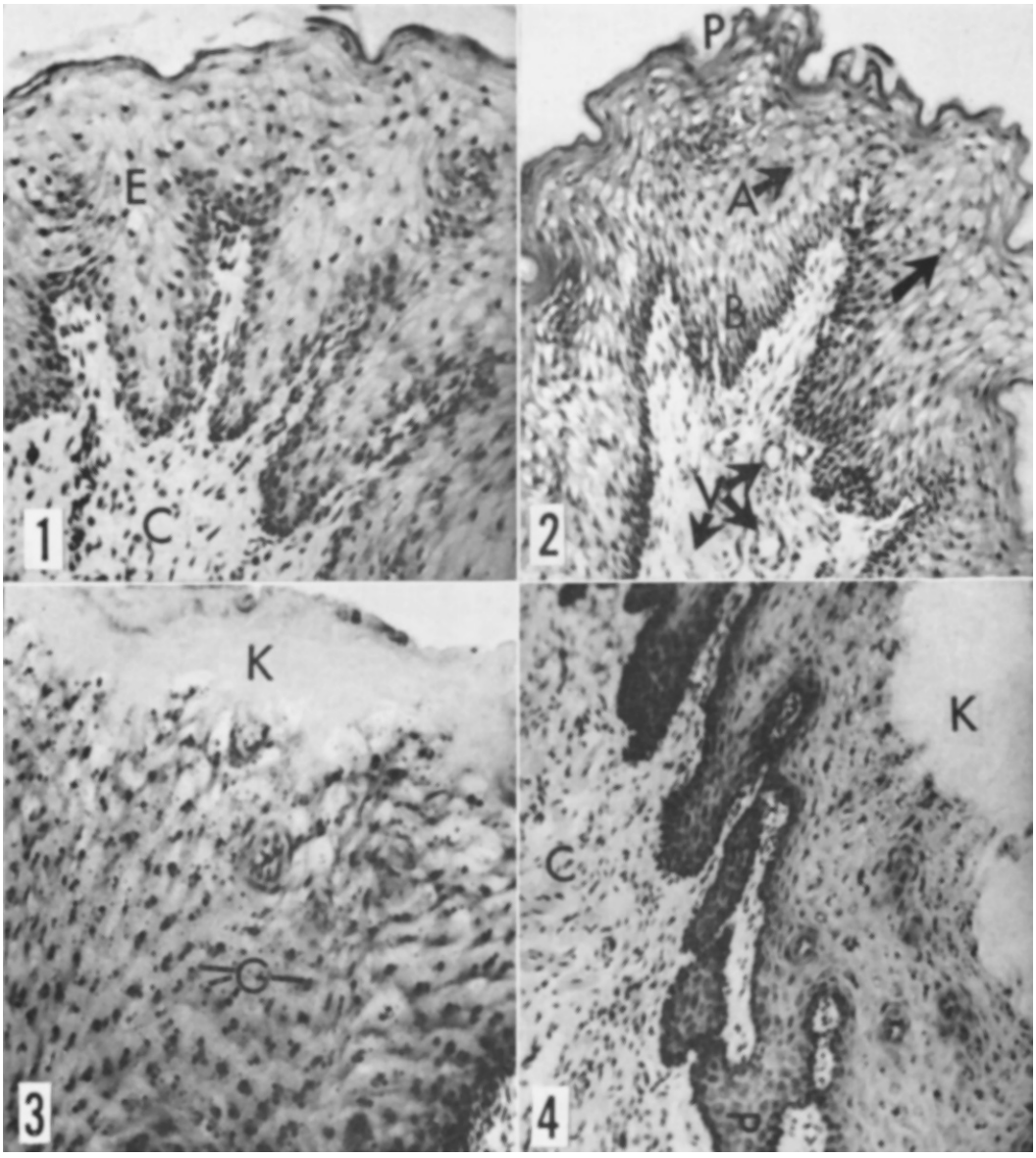

FIG. 1. Section of cheek mucosa of control rat 9 days of age stained with H & E (× 250).

FIG. 2. Oral mucous membrane of 9-day-old rat cheek after one dose of 0.04 ml dilantin sodium (× 250). Arrows in epithelium point to acanthosis of cells. P—Parakeratosis, B—increased mitosis in basal epithelium. Arrows in connective tissue show increased vascularity.

FIG. 3. Cheek mucosa of rat 12 days of age after 2 doses 0.04 ml dilantin, H & E stain (× 400). K—Hyperkeratosis, G—Basophilic granules.

FIG. 4. Oral mucous membrane of rat 21 days of age after 5 doses 0.04 ml sodium dilantin, H & E stain (× 250). Note splitting of rete pegs (p).

sections were stained with Mowry's procedure for acid mucopolysaccharides (Fig. 8, 9, & 10), the cells and intercellular bridges which normally appear distinct and stain Prussian Blue, showed a PAS positive stain. The intensity of this reaction appeared to be directly related to the amount of dilantin received by the animal. Epithelial pearls, occasionally present, were PAS positive. The diffusely distributed basophilic granules (Fig. 3) were stained Prussian Blue. When areas of hypertrophied epithelium in these sections were examined under higher magnification (Fig. 10), the staining ranged from light

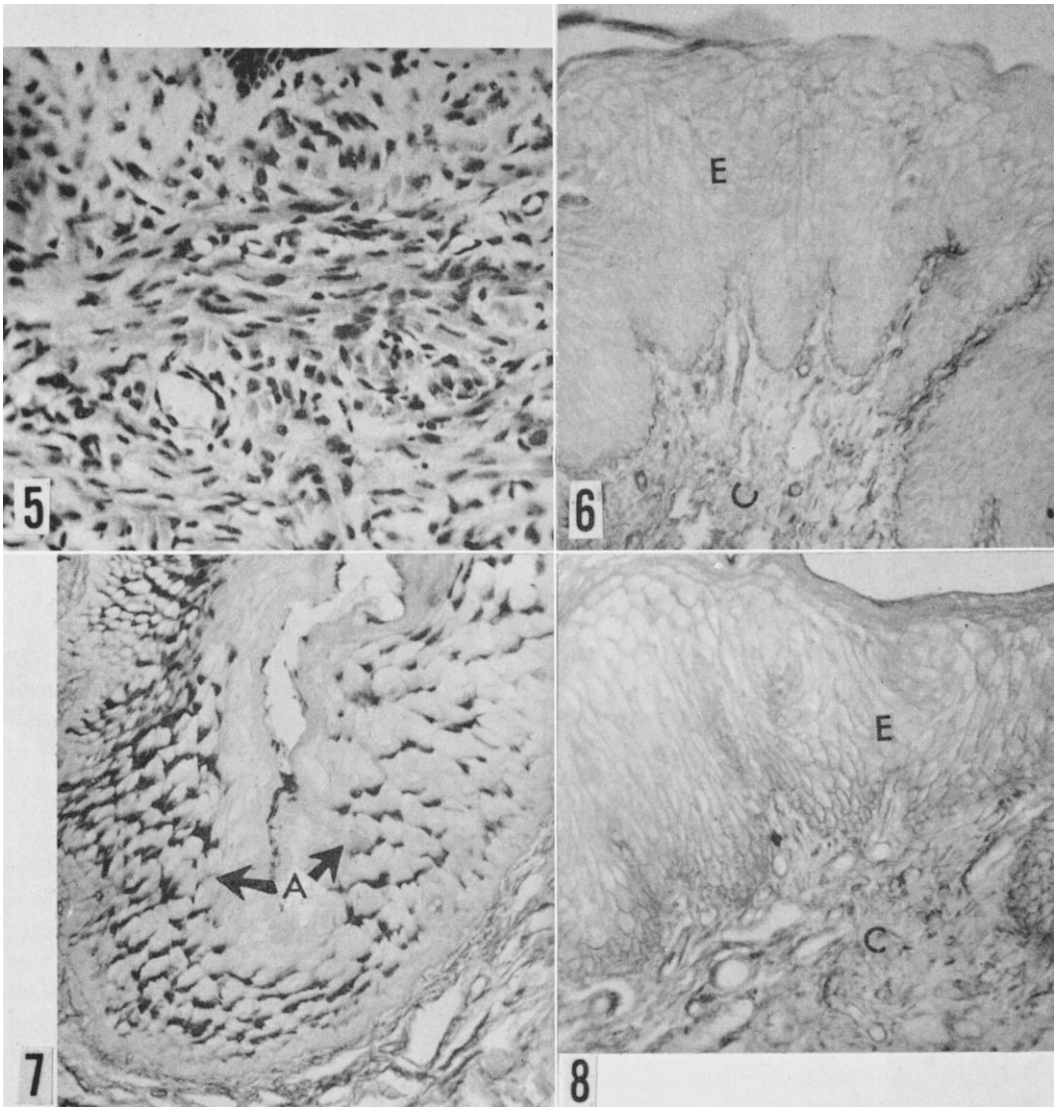

FIG. 5. High magnification of connective tissue in Fig. 5 to demonstrate elevated fibroblastic activity (× 400).

FIG. 6. Cheek mucosa of 12-day-old control rat stained with Periodic Acid Schiff (× 250). The epithelium does not stain well. Basement membrane is distinct.

FIG. 7. Oral mucosa of rat 12 days of age after 2 doses of dilantin, PAS stain (× 400). Note aggregates of PAS positive material in epithelium.

FIG. 8. Section of cheek mucosa of control rat 15 days old, stained with Mowry's procedure (× 250). This demonstrates Prussian Blue reaction in intercellular bridges of epithelium.

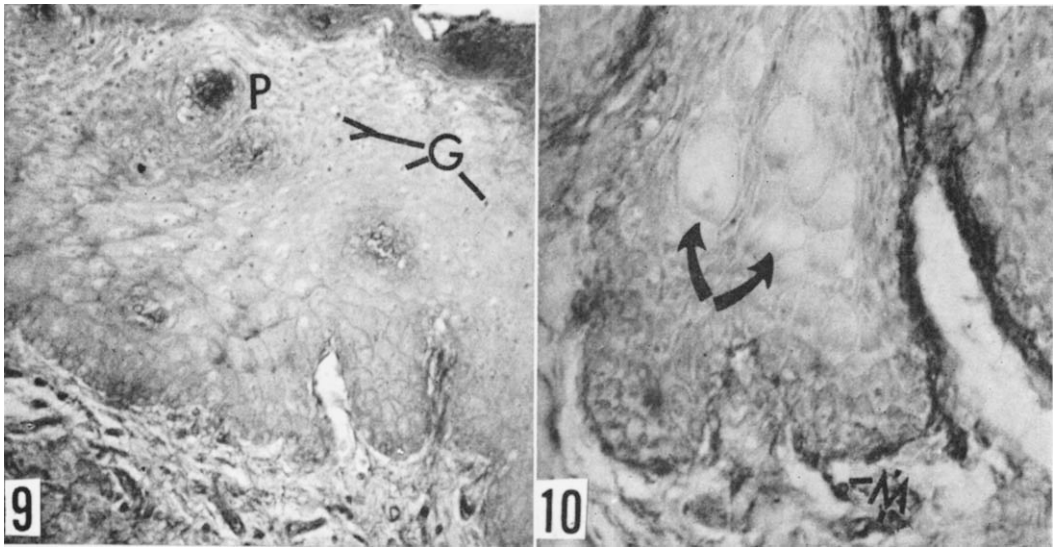

FIG. 9. Section from cheek mucosa of 15-day-old dilantin-treated animal. Epithelial layers show a generalized magenta color instead of the Prussian Blue reaction. Keratohyaline granules and epithelial pearls are PAS positive. Connective tissue fibers show an increased PAS reaction.

FIG. 10. High magnification (× 400) of area (A) in Fig. 9 to demonstrate enlargement and swelling of some epithelial cells.

Prussian Blue to magenta red color suggesting a substitution of glycoproteins for mucopolysaccharides. The cornified layer always stained PAS positively. The connective tissue fibers demonstrated an increased PAS reaction and a larger number of mast cells than normal were evident. The Prussian Blue staining reaction in the ground substance was of greater intensity than seen in the controls.

It became obvious from this study that the histological and histochemical alterations in epithelium and connective tissue of the oral mucosa became more severe with increasing doses of sodium dilantin to the animals.

Discussion. Our results suggest that changes in both the epithelium and connective tissue of the oral mucosa of animals occur following systemic dilantin administration. Houck(9) has shown that the aged rat was refractory to dilantin in terms of the chemistry of the connective tissue. The possibility that the young actively growing animals used in this experiment were uniquely susceptible to dilantin can be dismissed, since similar changes were also observed in adult rats receiving the same treatment(10).

Alterations in connective tissue consisted of elevated fibroblastic activity with pronounced collagen fiber formation. These observations are similar to those reported by other investigators(6,7,8).

The most significant observation on the oral mucosa of dilantin treated animals was the proliferation and increased keratinization of the oral epithelium. This appeared to be related to the unusually wide distribution of basophilic granules in the epithelium. Although Shapiro(8) reported acanthosis of epithelial cells in human gingiva, he did not demonstrate any alteration in the formation of keratin in this tissue. Changes in the histochemical staining reactions of epithelial cells and intercellular bridges indicate an apparent substitution of glycoproteins for mucopolysaccharides. The observation of increased mucopolysaccharide and glycoprotein staining material in the connective tissue of the oral mucosa is similar to Houck's(7) finding of an increase in the collagen fiber bound hexosamine in the dermis of animals receiving dilantin. Further, his observation (7,9) of an increased concentration of an insoluble non-collagenous protein in rat dermis after dilantin administration is consonant with our findings of increased amounts of keratin in the mucosa of experimental ani-

mals. This latter material would be both insoluble and free of hydroxyproline and hence would correspond to the insoluble noncollagenous material found by Houck in rat skin(7,9).

Summary and conclusions. Intraperitoneal injections of 100 mg/kg body weight of dilantin sodium to young rats resulted in significant increase in keratinization of the oral epithelium. Connective tissue of oral mucosa showed elevated fibroblastic activity with pronounced collagen fiber formation. Histochemical staining reactions suggested a substitution of glycoproteins for mucopolysaccharides in the epithelial cell layers. Increase in the glycoprotein and mucopolysaccharide staining material in the connective tissue was noted. This investigation suggested a relation between the severity of microscopic changes in the oral mucosa and dose and duration of dilantin therapy.

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Technique of Permanent Cannulation of the Right Ventricle in Rats and Ground Squirrels.* (28437)

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Permanent cannulation of the aorta and vena cava or right atrium(1) permits simultaneous blood sampling and circulatory studies in unanesthetized and undisturbed small animals(2,3). This method is, however, unsuitable for cardiac output determinations based on the Fick principle, because venous blood is not mixed sufficiently in the right atrium(4). The purpose of this paper is to describe a new technique for permanently implanting a cannula in the right ventricle where blood mixing is adequate.

Methods. Preparation of cannulas. For optimal results the ventricular cannulas (polyethylene tubes #PE 10, Clay Adams, diameter .28 × .61 mm) are prepared at least 48 hours prior to implantation. A ventricular cannula 15 cm long and having the shape of a hook (Fig. 1) is manually shaped

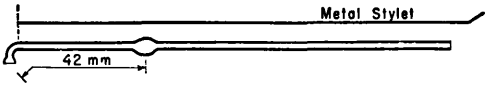

FIG. 1. Schematic presentation of a ventricular cannula made of a polyethylene tube with its stainless steel stylet.

in 90°C water. A small bulge is made 4.2 cm from the internal tip of the cannula by intermittent heating with a nickel wire having a resistance of 2 ohms, through which 3 volts are supplied. A 17 cm long, 0.2 mm wide stainless steel stylet is then inserted into the cannula up to the curvature. The cannula is filled with heparinized saline (4 mg/ml) and its external tip is flame sealed around the stylet. For young rats and ground squirrels the cannulas should be made shorter.

Insertion of ventricular cannula. The anesthetized animals (ether or Nembutal) are placed in supine position. The right internal jugular vein in ground squirrels or external jugular vein in rats is dissected free for one

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