KGS. The Cushing patient responds to methopyrapone in a qualitatively normal manner, although his base-line steroid excretion is higher than normal(8,9).

Summary. Obese subjects exhibit 2 different responses to an 11β-hydroxylase inhibitor, methopyrapone (SU-4885), depending upon the level of the base-line urinary 17-ketogenic steroid excretion. Subjects with a normal base-line 17-KGS excretion have a normal response to methopyrapone, whereas subjects with base-line elevated urinary 17-KGS give a markedly impaired response to this inhibitor.

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1. Simkin, B., *New Engl. J. Med.,* 1961, v264, 974.
2. Simkin, B., Arce, R., *ibid.,* 1962, v266, 1031.
3. Eckert, J. P., Green, O. C., Migeon, C. J., *Program of Endocrine Soc.,* 1961, p41.
4. Mlynaryk, P., Gillies, R. R., Murphy, B., Pattee, C. J., *J. Clin. Endocrinol. and Metab.,* 1962, v22, 587.
5. Green, O. C., Eckert, J. P., Migeon, C. J., *Program of Endocrine Soc.,* 1961, p62.
6. Jenkins, J. S., Meakin, J. W., Nelson, D. H., Thorn, G. W., *Science,* 1958, v128, 478.
7. Liddle G. W., Island, D., Lance, E. M., Harris, A. P., *J. Clin. Endocrinol. and Metab.,* 1958, v18, 906.
8. Liddle, G. W., Estep, H. L., Kendall, J. W., Jr., Williams, W. C., Jr., Townes, A. W., *ibid.,* 1959, v19, 875.
9. Gold, E. M., Kent, J. R., Forsham, P. H., *Ann. Int. Med.,* 1961, v54, 175.
10. Sobel, C., Golub, O. J., Henry, R. J., Jacobs, S. L., Basu, G. K., *J. Clin. Endocrinol. and Metab.,* 1958, v18, 208.
11. Cleveland, W. W., Nikezic, M., Migeon, C. J., *ibid.,* 1962, v22, 281.
12. Krieger, D. T., *ibid.,* 1962, v22, 490.
13. Simkin, B., *Clin. Res.,* 1962, v10, 90.

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Glycogen and Succinic Dehydrogenase in Wound Healing of the Oral Mucosa of the Rat. (27922)

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Differing amounts of glycogen have been found in the epithelium of healing wounds in the oral mucosa(1,2). The method of healing may be correlated with the amount of glycogen visible at a particular stage of healing(3). Epithelium moves over the connective tissue base in the healing of a superficial injury while in deeper wounds, particularly those which approach bone, a space is created which has to be filled with granulation tissue from the sides, carrying the epithelium with it.

The purpose of the present investigation is to see if the glycogen content of the epithelium in wound healing is related to the activity of the epithelium or the type of wound. To indicate the extent of oxidative activity in the epithelium, the distribution of succinic dehydrogenase during the process of wound healing will also be reported.

Methods. Wounds were made in the midline between the 2 most anterior rugae of the palate of 150 g Long-Evans rats with a trephine, 1.5 mm in diameter. Depth of the wound was regulated by a circular shield. Biopsies of the wound site were taken from different rats on successive days following the injury.

For determining the glycogen content the biopsies were fixed in alcoholic-acetic-formalin and stained by the PAS method, using diastase controls. Succinic dehydrogenase distribution was studied in fresh frozen sections cut at 40-75 μ. Sections were incubated for one hour at 37°C in the substrate of phosphate buffer, sodium succinate and nitro blue tetrazolium. After incubation permanent sec-

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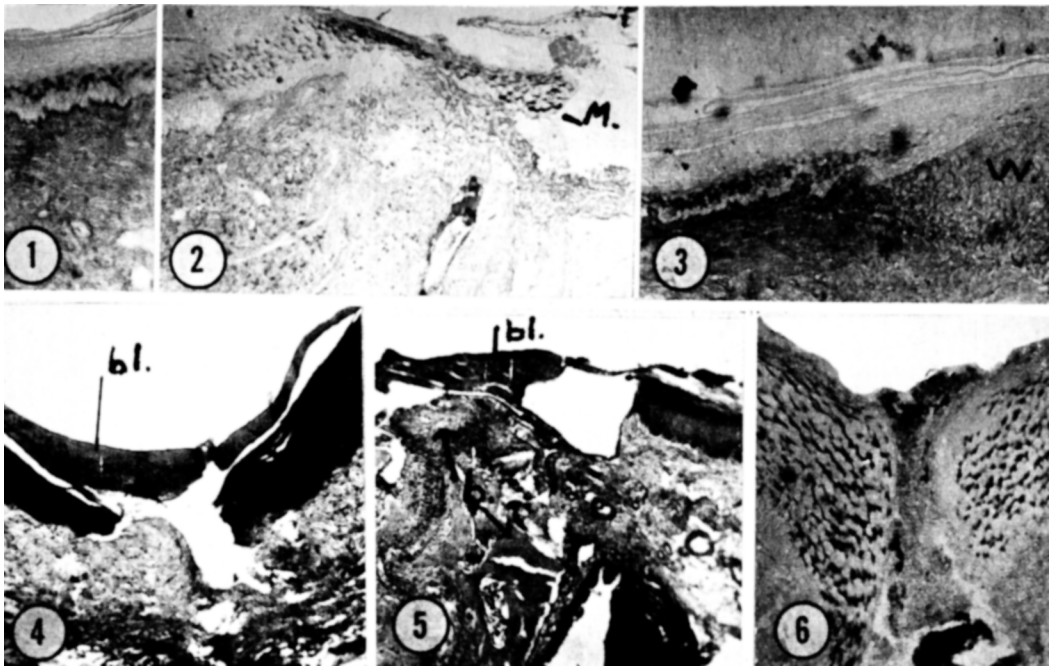

FIG. 1. Glycogen is confined to prickle cell layer in normal epithelium. Note distinct basement membrane. PAS × 75.

FIG. 2. Section taken 24 hr after injury showing a transient increase in glycogen at injured edge. PAS × 75 m., margin of wound.

FIG. 3. Wounded area 5 days after injury. Note absence of glycogen and basement membrane. PAS × 75 w., wounded area.

FIG. 4. Section of palate prepared immediately after creating a superficial wound. Epithelium and outer lamina propria are injured. H & E × 40 bl., blood.

FIG. 5. Section of half of the palate prepared immediately after creating a deep wound involving epithelium and entire lamina propria. H & E × 25 bl., blood; b., bone.

FIG. 6. Section shows epithelial margins of a deep wound approaching each other. Note abundant glycogen. PAS × 75.

tions were made by running through saline, alcohols, xylene and mounting in permount.

Superficial wounds. Fig. 1 shows the distribution of glycogen in the normal epithelium. Little glycogen is present in the basal cells possibly because it is rapidly used here for proliferative activity(4). Confirmatory evidence of this activity has been published in our studies on phosphorylase(5). Glycogen which is not used accumulates in the inactive cells of the prickle cell layer. As the cells approach the surface of skin, it has been suggested by Bradfield(6) that the metabolism is again increased and the glycogen is used to supply energy for transition of cells into cornified or partially cornified cells.

Only the epithelium and the immediately underlying connective tissue were removed in the superficial wounds (Fig. 4). Wounding interferes with the metabolism of the cells at the injured edge resulting in a transient increase in glycogen (Fig. 2). As the epithelial edge starts growth over the connective tissue, the glycogen disappears.

Five to 7 days after injury, closure of the wound appears complete (Fig. 3). However, there is neither glycogen nor basement membrane in the healed area. The normal epithelium and connective tissue relationship evidently have not yet been established. Therefore, there is probably little proliferative activity of the basal epithelial cells and no accumulation of glycogen in the prickle cells.

Deep wounds. Fig. 5 shows one side of a deep wound where the tissue has been removed down to the bone. The space will be filled with granulation tissue from the sides, the process carrying the epithelium with it. In Fig. 6 the epithelial margins have ap-

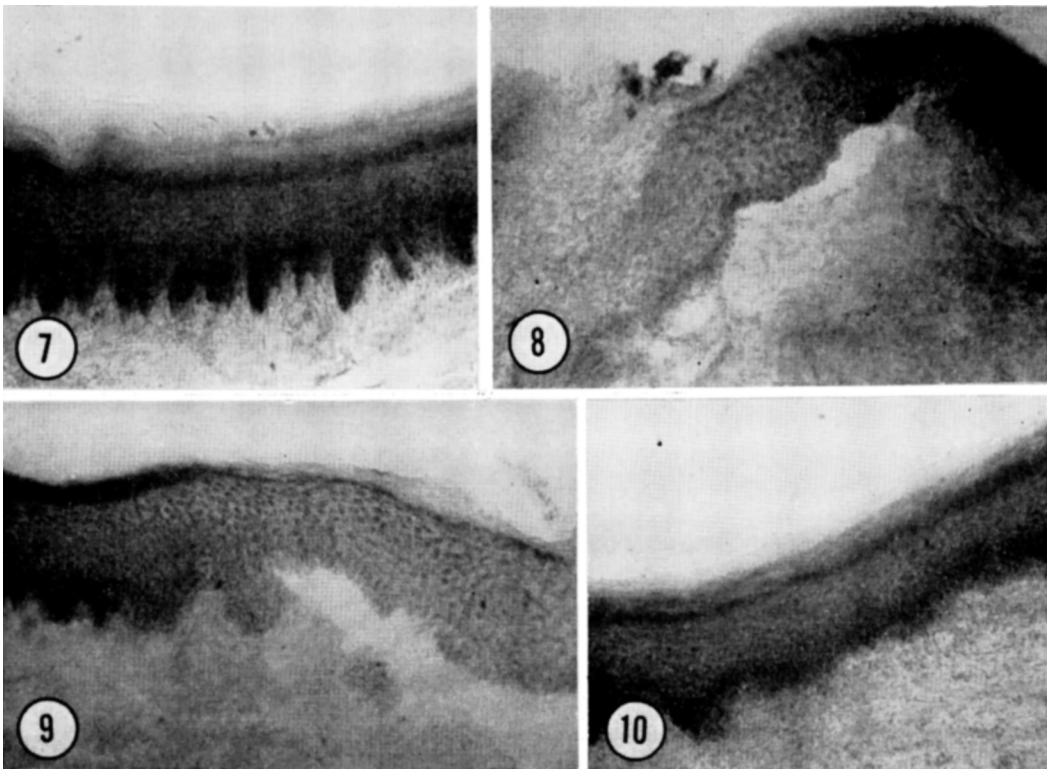

FIG. 7. Distribution of succinic dehydrogenase in normal mucosa. × 85.
FIG. 8. Margin of superficial wound shows very little succinic 2-4 days after injury. × 85.
FIG. 9. No diformazan granules are present in margins of deep wound. × 85.
FIG. 10. Succinic dehydrogenase reappears 7-9 days after the injury. × 85.

proached each other and the cells are full of glycogen. Following fusion of the epithelial edges exudate continues to form and often accumulates under the epithelium.

Succinic dehydrogenase. In sections of normal mucosa (Fig. 7), the basal cells are intensely stained and the distribution of succinic dehydrogenase appears to parallel the activity of the epithelium. The intermediate zone where glycogen was accumulated shows less diformazan granules, indicating that this is an area of low oxidative metabolism. This inactive zone exists for two reasons. In the first place, the cells are not actively participating in growth and keratin changes are not occurring. Secondly little oxygen is available since the zone is far from blood vessels and is covered by dying or partly dying cells. The little existing activity must function in anaerobic glycolysis.

The basal cells in the margin of the superficial wound (Fig. 8) show very little succinic dehydrogenase 2-4 days after making the injury. The disruption of the epithelial connective tissue continuity has eliminated the oxygen supply to the cells. In deep wounds no diformazan granules are visible in the margins of the wound, especially over exudate, (Fig. 9).

Although superficial wounds appear closed 5 days after the injury, the succinic dehydrogenase makes its appearance 7 to 9 days after the injury in the basal cells. By the tenth day after the injury succinic dehydrogenase is almost normal in its distribution (Fig. 10).

Discussion. Cells of the oral mucous membrane as the cells of the skin(4) utilize the glycogen when the metabolism is heightened. When the epithelium proliferates over connective tissue in the superficial wound the metabolism is apparently low for the glycogen was used slowly and few diformazan granules were visible. Following closure of

the superficial injury the absence of glycogen could be attributed to heightened epithelial activity which precluded an accumulation of glycogen in the prickle cell layer. An alternate reason for the absence could be that not enough glycogen was available for storage due to the discontinuity of the epithelium and connective tissue as shown by the absence of the basement membrane.

In the healing of the deep wounds, the accumulated glycogen was not used for there was no activity of the epithelium as shown by the complete lack of granules.

Summary. Morphological healing in the oral mucosa as indicated by hematoxylin and eosin preparations precedes histochemical healing. Epithelial proliferation in wound healing appears to be associated with the disappearance of glycogen. Lack of epithelial growth appears to be manifested by an accumulation of glycogen. The metabolism of the epithelial basal cells as indicated by the presence of succinic dehydrogenase appears to be related to epithelial and connective tissue continuity. Its presence in the basal cells of the epithelium confirms that metabolic activity is highest where there is no visible glycogen. Injury to the epithelium of the mucous membrane causes glycogen accumulation similar to that in skin. Succinic dehydrogenase is distributed in the mucous membrane of the anterior palate of the rat much as it is in the skin.

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1. Butcher, E. O., Klingsberg, J., *J. Dental Res. Abs.*, 1962, v41, 31.

2. Ambler, M. H., Johnson, P. L., Salman, Irving, *J. Am. Dent. Assn.*, 1960, v61, 46.

3. Butcher, E. O., Klingsberg, J., *Oral Surg., Oral Med., and Oral Path.*, in press.

4. Adachi, K., Chow, D. C., Rothman, S., *Science*, 1962, v135, 216.

5. Klingsberg, J., Butcher, E. O., *J. Histochem. and Cytochem.*, 1961, v9, 690.

6. Bradfield, J. R. G., *Nature*, 1951, v167, 40.

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Effect of Acute Pulmonary Artery Constriction on the Form of the Indicator-Dilution Curve.* (27923)

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Indicator-dilution curves are of value in detecting valvular insufficiency; however, these curves have not proven as useful in the diagnosis of valvular stenosis because such lesions do not always produce predictable changes(1,2). An opportunity recently presented itself(3) to evaluate the effects of experimental pulmonary artery constriction on the form of the indicator-dilution curve. These observations, in addition to delineating the effect of acute pulmonary artery constriction, may suggest an explanation for the inconsistent effects of stenotic lesions on the form of the indicator-dilution curve.

Method. The experimental preparation utilized has been described(3). Briefly, a ligature was placed around the base of the pulmonary artery of the anesthetized dog. This ligature was attached to a calibrated screw device so that tightening the instrument reduced the size of the loop. The size of this loop for each setting was known. In addition, these measurements were checked at the conclusion of the experiment by *in situ* measurement. Indicator-dilution curves were recorded and the ligature was loosened to permit return to control conditions. The procedure was then repeated with increasing degrees of constriction until right ventricular failure occurred as indicated by a marked

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