resistance to infection observed in stressed mice deserves further study.

The increased retention of virus in the tissue of stressed mice suggests impairment of host defense mechanisms for destruction of virus. Teodoru(10) found retention of polio virus in interscapular brown fat of hamsters subjected to stress and/or cortisone injections, and suggests that the stress-response is a reflection of hypercortisonemia initiated by stress. Our results also show a greater retention of virus in the tissues of stressed animals, but the experiments using adrenalectomized mice, in which similar retention was found, indicate no correlation between adrenal secretions and this retention. The generally held concept that increased adrenal cortical secretions during stress universally explain the diminished resistance to infectious diseases in stressed animals is not supported by our present data nor by data from our earlier studies with sound stress(3).

Summary. Mice subjected to avoidance-learning stress for 2 weeks were inoculated intramuscularly with vesicular stomatitis virus. By measuring serum neutralizing antibodies, no differences in immune response between stressed and control mice were noted. Virus disappearance from the site of inoculation was significantly retarded in stressed mice. Analogous results were obtained with adrenalectomized mice.

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An *in vitro* Effect of Insulin on the Preputial Gland.* (29343)

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Although insulin has been shown to act on the carbohydrate metabolism of a variety of tissues *in vivo* and *in vitro,* documentation of its effects on skin have been sparse. O'Connor demonstrated temperature conditioned changes in oxygen consumption of mouse tail skin following *in vivo* administration of insulin(1). Gaylor documented enhanced incorporation of acetate-C^{14} into squalene in isolated rat skin incubated with insulin and glucose in an atmosphere of nitrogen(2). Adachi reported increased glycogen C^{14} in the dog skin flap perfused with glucose-U-C^{14} and insulin(3).

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While the physiological relevance of the first two observations remains doubtful, that insulin effects glucose metabolism in whole skin seems probable from the findings in the dog skin. However, in all these reports insulin effects have been examined in whole skin and no distinction drawn between effects on its two distinct components, *i.e.,* the dermis and the epidermis with its appendages. This distinction assumes some importance since the clinical manifestations of insulin lack (*i.e.,* diabetes) appear largely confined to alterations in the collagen and elastic tissue as well as the vasculature of the dermis, *e.g.,* necrobiosis lipoidica diabeticorum, poor wound healing and other less obvious changes

in collagen(4). Alterations in epidermal components have not been described in diabetes.

The present studies have been designed to observe the effects of insulin on an epidermally derived structure free of dermal components. For this purpose the influence of insulin on the metabolism of glucose C^{14} *in vitro* was examined in a modified sebaceous gland, the preputial gland of the rat.

Methods and materials. Paired preputial glands were excised from fed male albino rats (Sprague-Dawley derivatives) weighing 150-200 g. The individual glands weighing from 30-50 mg were freed of fat and fascia and bisected longitudinally. Each gland was individually incubated in 1 ml Krebs-Ringer bicarbonate buffer (KRB) containing 50 μg penicillin and 100 μg streptomycin and 2 mg glucose U-C^{14} with a specific activity of 0.5 μc per mg. One gland of each pair served as a control. To the other was added "glucagon-free" insulin† in concentrations of 0.2 to 1.0 unit/ml. Incubations were performed for 90 minutes in a Dubnoff metabolic shaker in an atmosphere of 95% O_2/5% CO_2 at 37°C. Experiments were terminated by addition of 1 ml of 0.1 N HCl to the closed vessel. Evolved $C^{14}O_2$ and concentration of glucose in the medium were assessed by methods detailed elsewhere(5). To extract total lipids, tissues were homogenized in chloroform-methanol (2:1) and the supernatant was removed after centrifugation. The chloroform-methanol extract was treated twice with a 20% volume of .85% saline to remove water soluble components. An aliquot of the resulting lipid extract was assessed for radioactivity. In a limited number of experiments the total lipids were fractionated further and radioactivity assessed in the squalene and fatty acid fractions by methods discussed elsewhere(5). These lipids were chosen for examination because they constitute the major portions of preputial gland lipids: squalene comprising 40-50% and fatty acids contributing 4 to 10%(5). All radioactive assays were done in a Tri-Carb scintillation counter.

† Kindly supplied by the Eli Lilly Co.

TABLE I. Effect of Insulin on Metabolism of Glucose U-C^{14} in Rat Preputial Gland *in vitro*.

	No.	Effect of insulin treated/untreated × 100 ± SEM	P
Glucose assimilation	12	115 ± 5.8	<.02
$C^{14}O_2$	12	118 ± 7.4	<.05
Total lipid-C^{14}	12	121 ± 6.7	<.01

Paired glands were incubated in KRB and glucose U-C^{14} (2 mg/ml) with and without insulin (1 u/ml). Results in μg glucose or counts per minute per mg tissue for insulin treated glands are expressed as percent of results for untreated gland. Mean % ± SEM for 12 pairs of glands.

Values for each gland incubated with insulin are expressed as a percent of the paired control gland; by this convention 100% denotes no effect. Mean percentages were analyzed for statistical significance by "Student" t test.

Results. Addition of 1 unit of insulin (Table I) effected a mean increase of 15% ($p<.02$) in the assimilation of stable glucose from the incubating medium. The enhanced glucose assimilation was accompanied by an 18% increase in the evolution of $C^{14}O_2$ ($p<.05$) and by a 21% increase in incorporation of glucose C^{14} into total tissue lipids ($p<.01$). Fractionation of tissue lipids, in 8 experiments, into squalene C^{14} and fatty acids C^{14} disclosed increases of 11% and 26% respectively. However, because of the limited number of observations and substantial scatter, these values were not statistically significant.

To assess whether these phenomena represented a maximal response and whether they were influenced by the protein added (*i.e.*, 40 μg of insulin) added studies were performed with 0.2 and 0.5 units of insulin. Augmentations of comparable magnitude from 18 to 46% were observed. Thus, the increases observed with 1 unit of insulin were apparently at the plateau region of the dose response curve, since they could also be elicited with one-fifth of the concentration of added hormone.

Discussion. The present observations provide direct evidence of an action of insulin on glucose metabolism of an epidermally derived structure. The preputial gland is

embryologically derived from the epidermis and its response to sex hormones, in its morphology, as well as its metabolism retains the characteristics of a sebaceous gland (6,7). The epidermis, hair follicles and sebaceous and sweat glands represent modifications of a common pluri-potential cell type. Indeed, under certain conditions, the lipid producing cells of sebaceous glands are transformed into cells resembling keratinizing epidermal cells. It, therefore, seems likely that the response of sebaceous glands to insulin may be extrapolated to other epidermal tissues.

The response of this sebaceous structure to a maximal dose of insulin is small in comparison to values reported for other insulin sensitive tissues(8). The small size of the response, however, does not necessarily mean it is not of physiological significance. It is possible that the gland is normally near maximal stimulation and cannot be much further stimulated. This possibility is supported by the deterioration observed in glucose metabolism of the gland when relative insulin lack is induced by starvation(5). Moreover, epidermal structures, which normally have a high rate of aerobic glycolysis (9) are subjected to enormous variation in blood flow; under these circumstances insulin may provide a needed increase in the extraction ratio of glucose when substrate delivery is limited.

The fact that the response to insulin is small may account for the difficulty which the author (and possible other workers) have encountered in attempting to elicit such an effect in either animal or human skin. Lack of homogeneity in adjacent samples of rat skin results in variability of metabolic activity up to 30% (Table II). Similar discrepancies have been reported in human skin(10). In contrast the more homogeneous preputial gland has a biological variability of less than 5%. Thus, this tissue provides a sensitive system for studies of quantitatively minor but possibly physiologically major hormonal actions, particularly on lipid metabolism.

TABLE II. Variation in Metabolic Activity in Rat Skin vs Preputial Gland.

		Evolved $C^{14}O_2$		
Tissue	No.	Mean counts/min/mg tissue	±S.D.	S.D./Mean ×100
Skin	3	177	± 56	31
	4	164	± 29	18
Preputial gland	2	843	± 33	4
	2	366	± 8	2
	2	345	± 10	3

Adjacent discs of rat skin or paired preputial glands were incubated in KRB and glucose U-C^{14} (2 mg/ml).

Summary. The present studies have shown that the glucose metabolism of the rat preputial gland is augmented by *in vitro* addition of insulin. Thus this modified sebaceous gland must be added to the list of insulin responsive tissues. The findings constitute the first direct evidence that the carbohydrate metabolism of epidermal tissues *per se,* may be regulated by insulin.

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