necessarily reproduced when biochemical and histochemical preparations are compared. Although the reasons for these differences are not immediately apparent, preliminary observations with the succinoxidase system suggest that some clarification may follow closer inspection of site of transfer to tetrazolium salts in the chain of electron transport.

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Role of Local Trauma in Production of Cutaneous Calcinosis by Dihydrotachysterol.* (25855)

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A cutaneous calcinosis with sclerosis has been produced in rats treated during the first few days of life with parathyroid hormone(1) or with dihydrotachysterol, "DHT"(2), a Vit. D derivative that closely imitates parathyroid hormone in its pharmacologic properties. This skin lesion resembles human scleroderma in many respects. It has also been claimed that, in clinical scleroderma, calcium content of the skin is sometimes considerably increased(3,4) and that partial parathyroidectomy may induce marked improvement(5). Studies designed to clarify the possible interrelations between these clinical and experimental diseases would be greatly facilitated if a suitable laboratory model of this type were readily available. However, the previously described cutaneous lesions produced either with parathyroid hormone or with DHT do not lend themselves well to this kind of study because they: (a) are accompanied by high mortality, (b) develop only on the scalp and neck, (c) occur exclusively during the first few days of life and even then not in all instances. In the internal organs of DHT-treated rats, topical trauma can cause local calcification(6); clinical scleroderma also tends to develop at sites of local injury(7). The object of this communication is to show that, under suitable conditions, cutaneous calcinosis with sclerosis can be produced at will in predetermined regions of the skin, even in adult rats, if the selected area is lightly traumatized at a critical time of systemic DHT treatment.

Methods. For the sake of brevity, we shall not describe the many preliminary experiments necessary to establish optimum conditions for regular production of cutaneous calcinosis by DHT + local trauma. It should be emphasized, however, that DHT is most effective when given in oil, *per os,* and that predisposition for cutaneous calcinosis is greatest after very acute DHT intoxication. In one such experiment, 80 female Sprague-Dawley rats, mean body weight of 100 g (range: 85-115 g), were given 250 μg of DHT

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TABLE I. Role of Local Trauma in Production of Cutaneous Calcinosis by DHT,* in 7 Groups.

Day of epilation	Cutaneous calcinosis (scale: 0-3)	
	Scalp	Back
0	.6 ± .17	0
1	1.0 ± .32	0
2	1.2 ± .20	0
3	2.8 ± .17	.8 ± .54
4	3.0 ±0	2.7 ± .33
5	2.8 ± .17	2.7 ± .33
7	.3 ± .20	.2 ± .17

* All groups were treated with DHT between 2nd and 5th day, as indicated in text. Readings listed here represent the condition on 11th day, when the lesions were maximally developed.

("Calcamin," Wander) in 0.5 ml of corn oil daily on 4 consecutive days. Even without local trauma, 72 of these animals manifested minor spots of cutaneous calcinosis in the scalp, during the first 2 weeks. However, the lesions were barely detectable and never occurred in any other part of the skin. If the total amount of DHT (*i.e.*, 1 mg) is given in a single dose, incidence of the lesions is approximately the same but mortality is high; if the same amount is administered over a longer period (*e.g.*, 100 μg/day during 10 days), incidence of skin lesions drops considerably. These findings furnished the basis for the technic of the experiment to be described here. We wanted to determine: first, whether the minor local trauma of epilation could increase incidence and severity of cutaneous calcinosis in the scalp; second, whether epilation could induce such lesions, even in skin regions where they normally do not result from DHT treatment; and third, whether, in the course of DHT overdosage, there is a "critical period" during which skin trauma is most effective in precipitating cutaneous calcinosis. Forty-two female Sprague-Dawley rats, mean initial body weight of 100 g (range: 90-110 g), were subdivided into 7 equal groups as indicated in Table I. The hair was removed by plucking, from the calvarium and from a circular patch approximately 2 cm in diameter on the back. This was done at different times in the various groups (Table I). DHT was given to all rats by stomach tube, 250 μg in 0.5 ml of corn oil, once daily, from the 2nd to the 5th day. Degree of cutaneous calcinosis was repeatedly appraised in terms of an arbitrary scale: 0 (no lesion), 1 (just detectable lesion), 2 (moderate lesion), and 3 (maximal lesion).

Results. The first macroscopically detectable evidence of cutaneous calcinosis appeared, on the 9th day, in animals whose hair was removed during the last 2 days of DHT treatment. It manifested itself in the form of scattered, indurated, whitish skin plaques, which gradually tended to coalesce. These changes reached a maximum in all groups on the 11th day and the means of the readings made at that time are listed in Table I, together with their standard errors. Evidently, there is a definite "critical period" during the last 2 days of DHT treatment, at which time epilation is most effective in eliciting cutaneous calcinosis.

In rats optimally sensitized by systemic

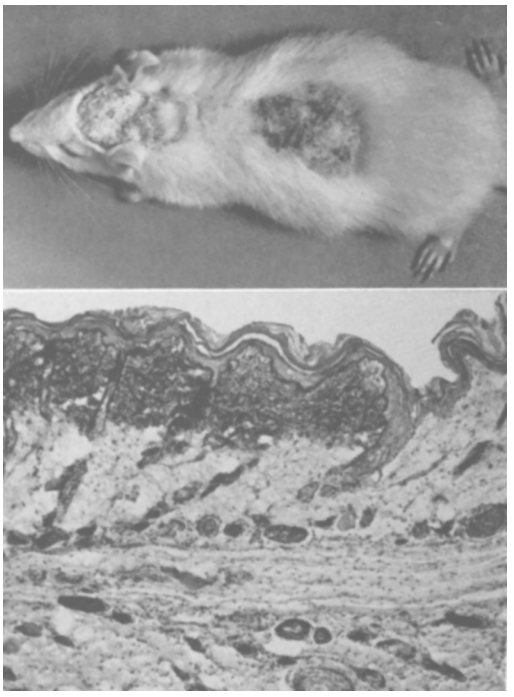

FIG. 1. *Top*, typical cutaneous calcinosis over the epilated area on scalp and back, as it appears when the lesions are maximally developed, in a rat of Group 5 photographed on 11th day. *Bottom*, typical histologic appearance of an early lesion in a rat of Group 5 killed on 8th day. Note that, initially, calcium deposition is limited to the subepithelial connective tissue fibers in a sharply circumscribed region (black granules under left two-thirds of surface). In the deeper layers, there is edema but, as yet, no sclerosis (von Kóssa × 72).

treatment with DHT, epilation greatly aggravates the otherwise inconstant and mild cutaneous calcinosis of the scalp; it can even induce lesions in an area not otherwise affected, such as the back. In both these regions, the skin is transformed into hard, heavily calcified plaques. On histologic sections (fixed in alcohol-formol and stained with von Kóssa's method), calcium deposition is seen mainly in the subepithelial connective tissue fibers and the basement membrane region (Fig. 1). Of course, on von Kóssa stained sections, it is impossible to state with certainty whether the mineral deposits are within or around the structural elements just mentioned. Yet, in following the early stages of tissue calcification, the impression is gained that the collagen fibers swell while they become gradually impregnated with calcium salts. On sections stained with Weigert's elastica stain, it is evident that simultaneously with this calcification in the collagen, the elastic fibers become fragmented. All these changes are reminiscent of human scleroderma, especially the so-called Thibierge-Weissenbach type, which is characterized by calcification.

During the 10 days following their appearance, these plaques are gradually demarcated from the underlying dermis and cast off. Then, cicatrization and sclerosis ensue. After the damaged part is shed, the skin underneath becomes re-epithelialized but remains partly or completely hairless during the following 3 weeks of observation.

Summary. In rats, a cutaneous calcinosis with sclerosis, not unlike that seen in certain types of clinical scleroderma, can be produced at will in predetermined regions of the skin. This is best accomplished if, at a critical time of systemic dihydrotachysterol overdosage, the selected cutaneous area is lightly traumatized by epilation.

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Biological Activity of Blood Insulin Complexes Examined by Rat Diaphragm Tissue Assay.* (25856)

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Antoniades *et al.*(1-3) suggested that insulin in blood and pancreas is largely bound by other proteins and, more specifically, by basic proteins. The insulin-basic protein complexes could be adsorbed on cationic exchange resin, in the sodium form, and could be extracted from the resin by elution with acid or alkali(3). The present communication describes studies on biological activity of insulin-complexes extracted from human blood with use of cationic exchange resin (Na^+ form). Two types of preparations have been examined for insulin-like activity by the rat diaphragm tissue assay as described by Vallance-Owen and Hurlock(4). The data indicated that the biological effect of insulin in its complex form examined *in vitro* by the diaphragm assay differs significantly from the biological effect of insulin in the "free" form, liberated from the complex at pH 9.8. Preliminary data suggest that, in contrast to the rat diaphragm assay, adipose tissue assay can

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