terone acetate for 4 to 10 days. 3. In one digitalized patient, digitalis toxicity was precipitated by intravenous injection of insulin and glucose, which was followed by the development of hypokalemia. 4. It is suggested that this increased myocardial sensitivity to digitalis probably results from alterations in intracellular potassium distribution since no significant changes in serum (or extracellular) potassium were observed in 8 of 10 patients.

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## Hair Loss from Squalene.* (18637)

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In previous reports the reversible local depilatory action of the intermediary polymers (cyclic dimers) of chloroprene was described (1,2). These compounds, when applied to the skin of laboratory animals, caused localized temporary cessation of hair growth with marked thickening of the epidermis and anomalous keratinization(3). *In vitro* the dimers inactivated the free sulfhydryl groups of glutathione and of tissue homogenates in amounts which corresponded well with the *in vivo* depilatory concentrations. We have attributed the sulfhydryl inactivating action of the dimers to the unsaturated bonds in the molecule which alkylate the –SH group according to the equation: $-CH=CH- + RSH \longrightarrow RS-\underset{|}{CH}-CH_2-$. The theory was advanced that naturally occurring unsaturated compounds may owe their effect upon keratinization to a similar chemical reaction(4). Such a compound is vit. A, a polymer of isoprene; isoprene differs from chloroprene in having a $CH_3$ group in place of the Cl atom of chloroprene. We have confirmed previous findings that vit. A, in addition to causing generalized hair loss in animals(5-7) and human beings(8-10), has a distinct local depilatory effect(4,11). However, vit. A had no demonstrable effect on free sulfhydryl compounds *in vitro* and it is possible that its depilatory action is due to a metabolic breakdown product.

The present paper deals with the localized reversible depilatory effect of another naturally occurring isoprene polymer, squalene. Squalene, an unsaturated hydrocarbon, is a normal component of human sebum(12) and therefore it may have a direct effect on normal, as well as pathologic, hair formation in man.

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*Experimental.* Squalene† was applied without rubbing to the skin of 10 rabbits, 4 guinea pigs (1 ml each) and 16 mice (0.2 ml each). The area between the ears and on top of the head was chosen, as being inaccessible to scratching and licking. In preliminary experiments squalene was mixed with 1% ascaridole (final concentration), since it has been shown that the addition of –SH groups to the unsaturated double bond is catalyzed by organic peroxides(13). In subsequent experiments pure squalene was used with the same results. Sulfhydryl determinations *in vitro* were made with buffered (pH 7.4) aqueous solutions of glutathione‡ (70 μg in 0.2 ml) and in 10% aqueous homogenates of mouse liver and of heat separated human epidermis(14) by using Bennett's reagent(15) and Anson's method(16). The sulfhydryl compounds were incubated with 0.006-0.1 ml squalene at room temperature for 15 minutes with frequent shaking. Measurement of the activity of a sulfhydryl enzyme, succinic dehydrogenase, was carried out with the method of Kun and Abood(17) after incubating 1 ml of 10% aqueous mouse liver homogenate with 0.2-1.0 ml squalene at room temperature for 15 minutes.

*Results.* After a single application of squalene the hair of all rabbits treated began to fall out in 1 week; complete baldness resulted in 10-12 days. Three out of 4 guinea pigs lost their hair after 10 days. The hair loss was limited to the area of application (Fig. 1). The hair came out by the root in bundles consisting of 3 to 6 hairs, embedded in small white scales. The underlying skin was smooth and finely scaling. In contrast to the depilatory effect of the chloroprene dimers and of the allyl esters(4), depilation by squalene was not accompanied by inflammation and crusting. The first signs of hair regeneration were visible at the beginning of the third week and within a few weeks the fur resumed its normal appearance in all animals. No depilation could be elicited in mice in spite of repeated applications. None of the animals displayed any toxic symptoms.

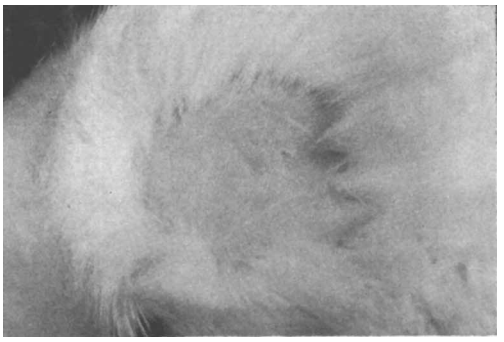

FIG. 1.
Local hair loss in rabbit 14 days after single topical application of 1 ml squalene.

Hairs immersed in squalene for several weeks showed no macroscopic or microscopic signs of disintegration. The action of squalene is therefore similar to that of the other unsaturated depilatory compounds which have no keratolytic effect.

Biopsy specimens of rabbit skin taken 12 days after application of squalene revealed a hyperplasia of the cutaneous epithelium. This was especially pronounced in the more active cells, namely the bulbs of the hair follicles; here it was of adenomatoid proportions. The hair shafts had either disappeared completely or only keratinous shreds remained in the upper parts of the follicles. Many of the deeper follicular cells developed eosinophilic (keratohyalin?) granules. There was no inflammatory reaction of significance, at the most only scattered inflammatory cells under the epidermis without edema.

Avulsed hairs, examined *in toto* under the microscope, showed the features of normal exfoliative hairs with the roots embedded in keratotic (not exudative) scales.§

*In vitro*, squalene inactivated the free

† Obtained through courtesy of Dr. M. Hunt, DuPont and Co.

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§ The author is greatly indebted to Dr. Fred D. Weidman for the microscopic studies.

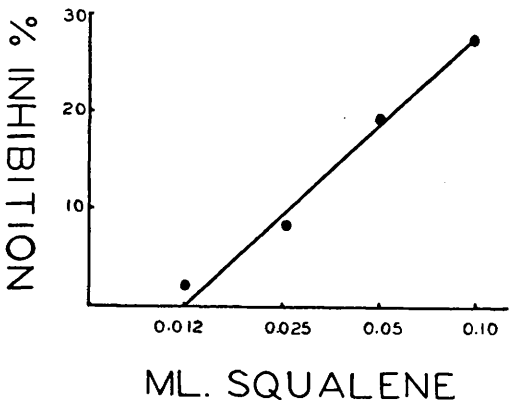


FIG. 2.
Inactivation of free sulfhydryl groups of glutathione by squalene.

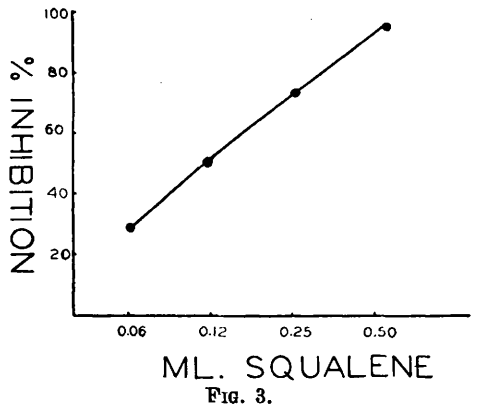


FIG. 3.
Inhibition of succinic dehydrogenase activity of 100 mg of mouse liver homogenate by squalene.

sulfhydryl groups of glutathione, human epidermis and mouse liver homogenates. The percentage inactivation gave a linear relationship when plotted against milliliters of squalene on semilogarithmic paper (Fig. 2). Inactivation of succinic dehydrogenase followed a similar curve. (Fig. 3).

*Discussion.* The finding that squalene causes local depilation in rabbits and inactivates sulfhydryl compounds *in vitro,* further supports the theory that hair growth can be inhibited by unsaturated compounds which alkylate the free sulfhydryl groups. This reaction between squalene and sulfhydryl compounds apparently occurs under milder conditions than has been described previously (18). In view of the non-irritating nature of squalene, the hair loss cannot be ascribed to the secondary effect of inflammation. At present no explanation can be offered for the lack of depilatory activity in mice. Species differences have been found previously with the allyl esters which depilate mice, but have no effect on rabbits(19).

The question whether or not squalene or a related unsaturated compound in sebum may cause disturbances in human hair growth, cannot be answered at present. Such a possibility is supported by our previous observation that application of the chloroprene dimers in mice for several months may result in irreversible thinning of the hair(19). Theories concerning the relationship of common male baldness with sebaceous dysfunction have been advanced previously(20,21). In view of the present findings, these theories should be reevaluated by quantitative measurements of unsaturated compounds in the sebum of normal children and adults and of persons with incipient baldness. Studies of this nature are in progress. Clinical trials with squalene in the treatment of fungous infections of the scalp have been started.

*Summary.* Squalene, an isoprene polymer occurring in normal human sebum, caused complete reversible local depilation in rabbits and guinea pigs after a single topical application. No hair loss was observed in mice. *In vitro,* squalene inactivated the free sulfhydryl groups of glutathione, human epidermis and mouse liver and inhibited succinic dehydrogenase activity of mouse liver. The depilatory and sulfhydryl inactivating effects of squalene are believed to be due to alkylation of the sulfhydryl group by the unsaturated double bonds in the molecule. The possibility is raised that squalene or a related unsaturated compound in sebum may influence human hair growth.

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