

ment of Bridgeport, Connecticut, it was possible to obtain material from a number of cases of variola. This was studied as indicated above in the complement fixation reaction. The results obtained are summarized in Table I. The data show that material from all except 2 cases of variola gave complete fixation. In those not fixing complement, the material was obtained on the twelfth and fourteenth days of the eruption, when only a few pustules remained. There was no fixation by any of the material derived from a number of different conditions, and in no instance was the antigen anticomplementary.

On the basis of these observations it is suggested, therefore, that complement fixation may be of value in the early diagnosis of variola.

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Relation of the Lipids to Physiological Activity. Changes in Lipids of the Epidermis During Keratinization.*

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Bloor¹ has shown relationships between physiological activity and the nature of the lipids in various tissues of the animal body. A high degree of activity is paralleled by a high phospholipid to cholesterol ratio. Since cells of the epidermis undergo a marked change in physiological activity during keratinization, we determined some of the lipids in epidermal cells before and after this process. The epidermis was separated from the cutis by digesting the skin, from which all subcutaneous fat had been removed, in cold 1% acetic acid. The completeness of the separation was confirmed by microscopic examination of sections of the skin. Several samples of epidermis were analyzed separately. Since it was impossible to obtain sufficiently large samples composed entirely of *stratum germinativum*, material containing considerable but varying quantities of this layer of active cells was scraped from the proximal surface of the epidermis and analyzed. Samples composed entirely of *stratum corneum*, trimmed from the soles of feet,

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¹ Bloor, W. R., Okey, R., and Corner, G. W., *J. Biol. Chem.*, 1930, **86**, 291.

were obtained from chiropodists and the lipids in keratinized cells determined. Skin from palms and soles of human cadavers was used because of the absence of hair and sebaceous glands and the greater thickness of the epidermis on these parts.

The material for analysis was cut into fine bits with scissors, or by passing it through a meat chopper, and extracted with hot alcohol vapors in a continuous extractor for 12 hours, changing the alcohol every 3 hours. The combined alcohol extracts were evaporated under diminished pressure and the residue extracted with ethyl ether. The ether extract was made up to volume in a volumetric flask and aliquots were used for the analyses.

The completeness of this extraction method was tested by saponifying several samples of extracted material with concentrated sodium hydroxide and extracting the remaining lipids with petroleum ether after acidification. In no case did the petroleum ether extract contain more than 4% of the total amount of lipid extracted from the material.

The phospholipid content was determined indirectly by the ether-soluble phosphorus, by the titration method of Widmark and Vahlquist,² and multiplying by a theoretical factor for lecithin. Bloor's oxidation method³ was used for the determination of total fatty acids, and Okey's application of this to the digitonin method for cholesterol.⁴

The results in Table I are expressed as percent of the dry extracted skin.

TABLE I.

Material	No. of Samples	Phospho-lipid	Chol-esterol	Fatty Acids	Phos. Chol.
Epidermis	8	Mean 0.62	0.90	1.71	0.70
Basal Layers	5	Mean 2.62	1.56	3.94	1.8
Str. Corneum	3	Mean 0.14	0.73	2.87	0.20

The basal layers of the epidermis have a lipid and phospholipid content comparable with that of the more active glandular tissues of the body. During the process of keratinization there is a diminution in all of the lipids, but the most striking loss occurs in the phospholipids, thus making the ratio of phospholipid to cholesterol much lower for the inactive keratinized cells than for the active cells of the basal layers. These results fit in very well with the concepts of Bloor concerning the lipids and physiological activity.

² Widmark, G., and Vahlquist, B., *Biochem. Z.*, 1931, **230**, 245.

³ Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

⁴ Okey, Ruth, *J. Biol. Chem.*, 1930, **88**, 367.