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Lipid Composition of Ear Wax in Hircismus

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To investigate the difference of dry ear wax and wet ear wax, the lipid composition of wet ear wax was analyzed and compared with that of dry ear wax. In dry ear wax, squalene, steryl esters, wax esters, triglycerides, free fatty acids and cholesterol were found. Squalene, triglycerides, free fatty acids and cholesterol formed the main demonstrable fractions in wet ear wax. In addition, three unidentified spots were always present in wet ear wax. Our results indicate that wet ear wax is due to the difference of quantity and composition of ear wax lipids.

Key Words: Lipid composition, ear wax, hircismus.

It is known that patients with hircismus have wet ear wax, and the basic phenomenon of wet ear wax follows the Mendelian dominant inheritance (Inaba *et al.*, 1975). In this experiment, lipid composition of wet ear wax was investigated and compared with that of dry ear wax.

MATERIALS AND METHODS

The procedures recommended by Downing (1968) were used in preparation of the plates. Glass plates (20 × 20 cm) were layered with Silica gel G, dried overnight at room temperature, and stored in an air-tight oven. Before use, plates were developed overnight in ether to remove contaminants. Plates were activated at 110°C for 30 min prior to use. The following standards were used; squalene, cetyl palmitate, cholesteryl oleate, triolein, oleic acid, cholesterol (all from Sigma Chemicals). Mouse sebum, which served as a source of wax diesters, was prepared by pipeting 10 ml of n-hexane over the back and sides of a freshly killed mouse and collecting the effluent. The ear wax was collected from 20 patients with hircismus and 20 adult volunteers without hircismus. The ear wax lipids were extracted by n-hexane and the sample was then passed through a 0.45 μm Millipore filter. The recovered lipid solutions were evaporated with a stream of nitrogen and the lipid residue redissolved in 0.2 ml of n-hexane. For analysis by thin-layer chromatography, suitable volumes of the lipid solutions (3 to 20 μl) were applied to glass plates. The plates were developed successively in hexane (to 19 cm), benzene (to 19 cm) and finally a mixture of hexane: ether: acetic acid (70:30:1, to 10 cm). After final development the plates were air dried at room temperature, sprayed with 50% sulphuric acid, and charred on cold aluminum plates lying on hot plates by heating from room temperature to 220°C in a period of 30 min. In order to resolve the nonpolar lipids, the method of Nikkari and Valavaara (1970) was used. The lipid extracts were passed through a Sep-Pak silica (Waters Co.) cartridge. The cartridge was washed with additional 12 ml of diethyl ether. The combined eluates were evaporated and the residue was dissolved in light petroleum. Aliquats of the extracts were applied on Silica gel G plates, which were developed with benzene: hexane (45:55 by vol.). The spots were detected by spraying with 50% sulphuric acid followed by charring at 220°C.

RESULTS

Fig. 1 indicates the appearance of a typical thin-layer plate after the resolved lipids have been charred. Squalene, steryl esters, wax esters, triglycerides, free fatty acids and cholesterol were found in dry ear wax. However, squalene, triglycerides, free fatty acids and cholesterol formed the main demonstrable fractions in wet ear wax. In addition, two unidentified

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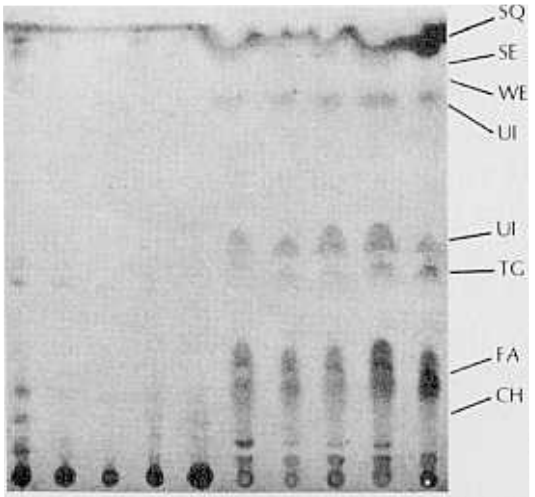
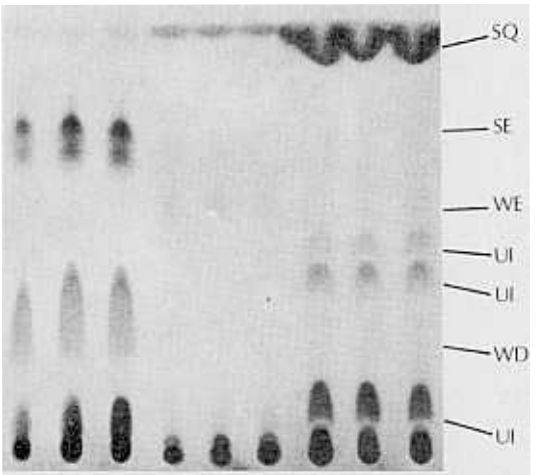


Dry ear wax | Wet ear wax

Fig. 1. Thin-layer chromatogram of lipid extracts of ear wax. SQ; squalene, SE; steryl esters, WE; wax esters, UI; unidentified, TG; triglycerides, FA; fatty acids, CH; cholesterol.



Mouse serum | Dry ear wax | Wet ear wax

Fig. 2. Thin-layer chromatogram of non-polar lipids of ear wax. SQ; squalene, SE; steryl esters, WE; wax esters, UI; unidentified, WD; wax diester.

lipids were always present in wet ear wax. The thin-layer chromatogram of non-polar lipids of ear wax was shown in Fig. 2. Wax diesters were the major lipid in mouse sebum. However, unidentified spots in wet ear wax have different patterns with wax diesters, and are further separated.

DISCUSSION

In the ear canal, hair follicles, sebaceous glands and ceruminous glands are found. The ceruminous glands are specialized apocrine glands, and produce cerumen which is main component of ear wax (Montagna *et al.*, 1963). The consistency of ear wax is soft, and the color is yellow in caucasians, while firm and white in Orientals (Matsunaga 1962). It is also known that patients with hircismus have wet ear wax (Inaba *et al.*, 1975). To investigate the difference of dry ear wax and wet ear wax, we analyzed the lipid composition of wet ear wax and compared it with that of dry ear wax. The multiple solvent system recommended by Downing (1968) and used in most of our analysis produced good separation of ear wax lipids. In wet ear wax, steryl esters and wax esters were not found as compared with dry ear wax. Instead, two unidentified spots were always present in wet ear wax. As the two unidentified lipids were less polar than triglycerides, lipid extracts of ear wax were fractionated by Sep-Pak silica cartridge. Non-polar lipid eluates were analyzed by method of Nikkari and Valavaara (1970). However, the unidentified spots in wet ear was have different patterns with wax diesters and were further separated. There are no published studies on these unidentified lipid spots of wet ear wax. We will further analyze these unidentified lipid spots by means of gas-liquid chromatography and high pressure liquid chromatography. The skin surface lipid is derived from the sebaceous glands and the epidermis. The lipid composition of skin surface was squalene, steryl esters, wax esters, triglycerides, free fatty acids, and cholesterol. The main constituents of skin surface lipids were triglycerides and wax esters. While, squalene and unidentified lipid spots were the main demonstrable fraction of wet ear wax lipids, ear wax lipids were somewhat different from skin surface lipids. The lipid extractable from the human ear wax with organic solvents is a mixture of sebum, cerumen and of lipid produced by the keratinizing epidermis. The major lipid constituents of sebum were squalene, wax esters and triglycerides (Kellum 1967). Sterols, steryl esters, glycerides and phospholipids were epidermal lipids (Nieminen *et al.*, 1967). However, lipid composition of cerumen was unknown. Thus, our results indicate that unidentified lipids in wet ear wax may be a component of cerumen lipids and wet ear

wax is due to difference of quantity and quality of ear wax lipids. Also, symptom of hircismus was more severe, the ear wax is more soft and has more amount of lipids.

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