a support is not coated uniformly, the particles with more stationary phase may accumulate in a particular part of the column due to the increased weight. A non-uniform distribution of the stationary phase causes a non-uniform solute band velocity.

The rate of heat transfer to the center of a column decreases with larger column diameters. A temperature gradient between the center and the wall creates a solute band velocity gradient. Both gas velocity and solute partition coefficient vary with temperature. The two effects oppose, but not necessarily cancel, each other. A radial temperature gradient occurs when a large diameter column is temperature-programmed. With isothermal conditions, an axial temperature gradient along a column will produce a radial temperature gradient. If the carrier gas enters the column at a temperature above or below the column temperature, a radial temperature gradient will be set up in the first part of the column.

Another factor which is important in large diameter columns and with the use of large sample sizes is transient temperature changes caused by the solutes themselves passing through the column. Such temperature changes are due to latent heats of vaporization of the solutes. As a solute band enters a plate or section of a column, a net condensation process occurs accompanied by the evolution of heat. After the band maximim passes, the net process is that of vaporization, and heat is absorbed by the solute. If a column were adiabatic with no heat loss to or gain from the surroundings, heat would be transferred to the packing as the solute band condensed in a section, and the packing temperature would rise. As the solute band evaporates from the section, the same amount of heat is transferred from the packing and its temperature returns to the base level. Assuming a symmetrical solute band, a temperature curve of the type shown in Figure 9A is expected. In an actual column, with finite rates of heat loss and gain, as the temperature rises, some of the heat evolved upon solute condensation is lost to the carrier gas and to the surroundings. As the solute is revaporized from the packing, all of the heat originally evolved is not available in the packing and the temperature falls below its base value before returning to steady state as shown in Figure 9B. Scott (19) has calculated, from theory, temperature curves of the types shown in Figure 9 and has experimentally measured temperature changes of the order of one degree in an analytical column. The experimental curves were of the type shown in Figure 9B.

These temperature changes due to the solutes themselves cause the band maximum to move faster than the rear of the band, thereby producing a tailing effect and band spreading.

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Isolation and Characterization of Insect Attractant Lipids

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Abstract

Isolation and characterization of the naturally occurring sex attractants of the silkworm moth (*Bombyx mori*), gypsy moth (*Porthetria dispar*), cotton leafworm (*Prodenia litura*), American cockroach (*Periplaneta americana*), an introduced pine sawfly (*Diprion similis*), and mating attractants of the honey bee (*Apis mellifera*) and bumblebee (*Bombus terrestris*) are discussed.

MOST INVESTIGATIONS of insect attractant lipids have been concerned with insect sex attractants. Other insect attractant lipids will therefore be treated in this presentation in only a minor way. The sex attractants of only two insect species have thus far been identified, namely those of the silkworm moth and gypsy moth. The sex attractant of the American cockroach has not as yet been prepared synthetically.

The Sex Attractants

Silkworm Moth (Bombyx mori)

Isolation. Isolation of the silkworm moth sex attractant was begun by Butenandt (7) in 1941 and completed 19 years later (18,9). The early work was done with a benzene extract of the last two abdominal segments from 7,000 virgin female moths; the 1.5 g of extract obtained was freed of acidic and basic substances, esterified with succinic acid, and the resulting esters saponified. Sublimation at 60–70C of the neutral product obtained gave 100 mg of an impure, waxy, crystalline substance reported to be a diol of approx composition $C_{16}H_{30}O_2$.

In 1956, Makino et al. (30) described a purification procedure, including column and paper chromatography, that gave a substance designated as "bombixin." An ethanolic extract of the abdomens of 60,000 females that had previously mated was evaporated to a small volume, treated with sodium hydroxide at below 40C, freed of solvent, extracted with

ether, and the extract dissolved in benzene and passed through a column of alumina. Evaporation of the eluate at below 60C gave 4.5 g of an orange wax that was freed of sterols ("bombicesterol") by repeated treatment with methanol; the methanol-soluble brown syrup was distilled at 100–110C (0.06 mm) to a thick, faintly yellow oil. Paper chromatography of the latter showed an active spot (bombixin) with R_f 1.0, 0.98, and 0.82 when the chromatogram was developed with n-butanol-acetic acid-water, 85% phenol, and benzene-methanol (6:4), respectively. Although bombixin contained 86.41% carbon and 12.05% hydrogen, showed no UV absorption, and appeared to contain a primary alcohol group according to its IR spectrum, it was later shown to contain less than 0.1% of the pure attractant (29).

Although Anders and Bayer (2,3), as a result of an independent investigation of the gas chromatography of the female silkworm extract, reported the presence of three materials attractive to males, only one of these was highly active. Schneider and Hecker (35) had previously shown that several unsaturated alcohols were attractive to males in varying degree, but the most potent of these was only $1/10^6$ times as strong as the natural attractant.

Isolation of the pure sex attractant, designated "bombykol," was successfully accomplished by the use of an ethanol-ether (3:1) extract of the abdominal glands of 500,000 virgin female moths (19,9). The crude extract (280 g) was separated into 42 g of free acids and 125 g of neutral portion; saponification of the latter with methanolic KOH gave 73 g of combined acids and 33.6 g of unsaponifiables. The unsaponifiable fraction was freed of 12 g of crude cholesterol by recrystallization from methanol and the methanol-soluble portion was treated with succinic anhydride. Saponification of the resulting acid succinate gave 3.4 g of an alcohol that was then esterified with 4'-nitroazobenzene-carboxylic acid chloride; digestion of the resulting ester with petroleum ether gave 650 mg of fraction A, 500 mg of fraction B, and 200 mg of fraction C. Thrice-repeated partition chromatography of these fractions on hydrophobic silica gel, with paraffin oil as the stationary phase and 85 and 90% acetone as mobile phases, gave 12 mg of active derivative, mp 95–96C, $\lambda_{max.}$ 230, 331 mμ (11).

Characterization. The molecular formula ($C_{29}H_{37}N_3O_4$) and mol wt (491.6) of the attractant derivative indicated that the pure attractant was a doubly unsaturated alcohol having the formula $C_{16}H_{30}O$; its UV absorption at 230 mμ showed these double bonds to be conjugated. The IR spectrum of the free alcohol (obtained by saponification of the derivative with alkaline glycol monomethyl ether in benzene) showed it to possess a primary hydroxyl group (9.50–9.75 μ) and two conjugated double bonds indicative of *cis, trans* unsaturation. Catalytic hydrogenation of 2 mg of bombykol with platinum oxide gave palmityl alcohol, and permanganate oxidation of 1 mg of the bombykol derivative gave acid moieties which were identified by paper chromatography of their methyl esters (9,10). By means of this brilliant investigation, bombykol was shown to be 10,12-hexadecadien-1-ol (structure I). Although the configuration of the conjugation in bombykol was at first thought to be *cis, trans* (11), subsequent synthesis of the four possible geometrical isomers of structure I showed it to possess the *trans*-10,*cis*-12 form (19,13,40).

It had been observed that the sexual attraction of the neutral fraction, separated from free acids, increased following alkaline saponification, leading to the hypothesis that bombykol, or a portion of it, occurred in the insect in the form of a waxy ester. This belief was strengthened when it was found that lithium aluminum hydride reduction of the free and combined acids to the corresponding alcohols gave a mixture attractive to male silkworm moths (8). An attempt was therefore made to isolate and identify the attractive acid (14). The fatty acid mixture was separated into saturated acids (lauric, myristic, palmitic, and stearic) and unsaturated acids (oleic, linoleic, and 14-methyl-9,12-pentadecadienoic) by low-temp acetone crystallization, followed by partition chromatography on hydrophobic silicic acid and by paper chromatography. The 14-methyl-9,12-pentadecadienoic acid showed only slight sex attractant activity, attributed to traces of bombykol present as impurities, and the other acids were inactive.

$$CH_3(CH_2)_2CH{=}CHCH{=}CH(CH_2)_8CH_2OH \quad (I)$$

The four geometrical isomers of I (19) showed the following attractiveness to male silkworm moths (μg/ml) in laboratory tests (12): *cis*-10,*cis*-12, 1; *cis*-10,*trans*-12, 10^{-3}; *trans*-19,*cis*-12 (*bombykol*), 10^{-12}; *trans*-10,*trans*-12, 10.

Gypsy Moth (Porthetria dispar)

Isolation. The pioneering detailed chemical studies on this problem were conducted by Haller and Acree (18,1), who soon determined that the active material was a lipid residing in the unsaponifiable neutral fraction of a benzene extract of the virgin female abdominal segments. It was partially volatile with steam, reacted with phthalic anhydride, and could be recovered from the phthalic acid ester upon saponification. Considerable concn of the attractant was obtained by chromatography of the neutral fraction in sequence on columns of magnesium carbonate and magnesium oxide. These fractions gave definite indications of the presence of more than one substance attractive to males. From the abdominal glands of 100,000 female moths, Acree obtained about 12 mg of an active impure fraction which he designated "gyptol." Saponification of "gyptol" with potassium hydroxide in diethylene glycol at 120–130C gave a few miligrams of palmitic acid (25) and an unidentified alcohol fraction.

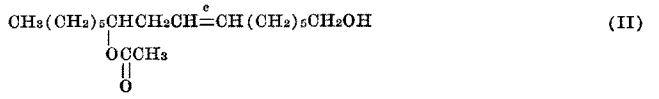
Stefanovic (36,37), working with a hydrogenated benzene extract of the female abdominal tips, obtained by countercurrent extraction a bright yellow oil that was highly attractive to males. A crystalline solid, mp 61.0–61.5C, isolated in large amt from the crude extract, was identified as a triglyceride α-palmitodistearate.

The sex attractants of this insect were isolated in pure form in 1960 by Jacobson, et al. (24). Chromatography of the unsaponifiable fraction from 200,000 female moth abdomens on successive columns of magnesium carbonate and magnesium oxide by Acree's method (1), with absorbancy ratios at 249 and 285 mμ, gave a yellow oil which was highly attractive to males. Subsequent investigations showed that these time-consuming operations (at least 3 days per column per 20,000 tips) could be avoided and approx equal fractionation obtained by repeatedly dissolving the crude, unsaponifiable fraction from 300,000 tips in acetone and filtering off the large amt of inactive solid (pigments, cholesterol, heptacosane, nonacosane, and hentriacontane) that separated at room temp, 5C, and −5C. In this way a total of 75 mg of attractive oil was obtained from 500,000 female moths. Reversed-phase chromatography of this oil by the ascending

technique on silicone- or polyethylene-impregnated filter paper sheets, with methanol-benzene-water (5:1:1) as developing solvent, gave five spots (R_f 0.0, 0.2, 0.34, 0.73, and 1.0), only one of which (R_f 0.0) was attractive to male moths. Successive extraction of this zone with cold ethanol and with Skellysolve B gave 3.4 mg of white, waxy crystals, mp 37.0–37.5C, soluble in ethanol, and 20 mg of colorless, hydrocarbon-soluble liquid that solidified in the cold but remelted at room temp. The crystals were attractive to male moths in the field at 10^{-2} μg, where as the liquid attracted males at 10^{-7} μg/trap.

Characterization. Gas chromatography of the major attractant showed it to be a pure material, and its analysis indicated the formula $C_{18}H_{34}O_3$. Its IR spectrum showed the presence of a primary hydroxyl and ester carbonyl groups, a *cis* double bond, and an unbroken chain of at least four methylene groups. Microhydrogenation of 2 mg of the attractant showed the presence of only one double bond and a mol wt of 298. Saponification of the hydrogenated material with potassium hydroxide in diethylene glycol at 120–125C (failure to saponify this material with alcoholic KOH indicated that the ester group was secondary) gave acetic acid and a diol in excellent yields. Lack of absorption in the UV precluded the 2-position for the attractant double bond, and its optical activity showed that the acetoxyl group did not lie on an unsaturated carbon atom, since an asymmetric carbon atom would not then exist. Stability of the attractant to high-temp vapor phase chromatography on Craig polyester succinate indicated the probability that at least one methylene group separated the acetoxyl group from the double bond, since Stoffel et al. (39) have shown that the common methylene-interrupted polyunsaturated esters may be safely analyzed by gas chromatography. (This has since been substantiated for unsaturated hydroxy acetates by Morris et al. (31), who found that hydroxy esters not vicinally unsaturated are stable under gas chromatography on polyester resins at high temp whereas vicinally unsaturated hydroxy esters are dehydrated under these conditions).

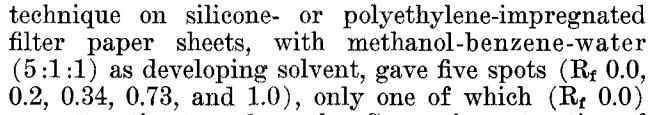
Oxidation of 4 mg of the attractant with periodate-permanganate reagent cleaved the double bond only, leaving the hydroxyl and acetoxyl groups intact. A 92% yield of 3-acetoxy-1-nonanoic acid was obtained together with an ω-hydroxy acid which was further oxidized in 71% yield with alkaline permanganate to pimelic acid. The only structure for the attractant consistent with the data was (+)-10-acetoxy-*cis*-7-hexadecen-1-ol (II).



(II)

The *dl*-form of II, identical in all respects save optical activity with the natural attractant, was synthesized in 0.2% over-all yield (25); this form was successfully resolved by treating its acid succinate with L-brucine, separating the brucine salts by fractional crystallization from acetone, decomposing the salts, and saponifying the acid succinates with ethanolic alkali (21).

Characterization of the natural attractant resulted in the synthesis of an active homolog, (+)-12-acetoxy-*cis*-9-octadecen-1-ol (III), which has been designated "gyplure" (20,22,27).



(III)

The comparative attractancy of the natural and synthetic materials to male gypsy moths is shown in Table I (27).

It was found that the activity of *cis*-gyplure could be masked by the presence of varying amts of structurally related compounds, and adsorption and gas chromatographic methods were developed for determining the *cis*-gyplure content of its mixtures, as well as for separating the *cis* isomer from its contaminants (28).

Cotton Leafworm (Prodenia litura)

Although the sex attractant produced by the lateral abdominal segments of the female leafworm has not yet been obtained in pure form, a highly active fraction has been isolated by the following procedure (44). A homogenized and lyophilized ethanol-ethyl ether (3:1) extract of 23,000 female abdomens was separated into free and combined acids and 2.7 g of an active neutral fraction; the latter was freed of carbonyl-containing substances by treatment with Girard P-reagent, freed of sterols by methanol treatment, and esterified with succinic anhydride. The half-ester formed was saponified, the alcohol obtained was esterified with 4′-nitroazobenzene-4-carboxylic acid chloride, and the derivative (100 mg) was saponified. The hydroxylic material thus obtained elicited a sexual response in males at a concn of 10^{-5} μg/ml. The attractant appears to be an alcohol.

American Cockroach (Periplaneta americana)

Isolation. Roth and Willis (34) had shown that, although the female roach produces a highly potent attractant for males, active extracts could not be obtained from any part of the female's body with organic solvents. However, the attractant adheres to paper with which the virgin female comes in contact, and this was the basis of a collection procedure described by Wharton et al. (41) which utilizes a petroleum ether extract of filter papers exposed to such females. Nevertheless, aqueous extracts of such papers were used by these investigators to isolate an attractant which elicits a sexual response in the male at 10^{-11} μg or less (42). These extracts were made 0.1 *N* with NaOH, acidified after 15 min to pH 5.0–5.5 with H_2SO_4, and 60% of the solution was distilled. The distillate was treated with lead nitrate to remove fatty acids, redistilled, and the process repeated several times to obtain a small volume of active distillate. The attractant was extracted with isopentane, and the extract was subjected either to successive chromatography on columns of alumina, Florisil, silicic-acid-Celite, Florisil, and alumina (developing with increasing amts of ether in isopentane) and then to preparative gas chromatography, or to successive chromatography on the first two columns and then to preparative gas chromatography; the column packing

TABLE I

Comparative Attractancy of Gyplure and Its Homologs to Male Gypsy Moths

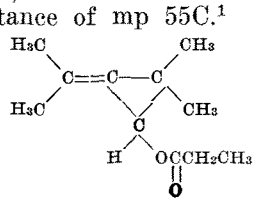
Compound	Attractancy, μg	
	Laboratory	Field (28)
d-I (natural)	10^{-12}	10^{-7}
d-I (synthetic)	10^{-12}	10^{-7}
l-I (synthetic)	10^{-12}	10^{-6}
dl-I (synthetic)	10^{-12}	10^{-6}
d-II (*cis*-gyplure)	10^{-12}	10^{-5}
Trans-gyplure	10^{4}	2.5×10^{5}

used for gas chromatography was 5% Apiezon M on Chromosorb-W (4 ft) at 130C, argon flow rate 110 ml/min. In this way a total yield of 28 μg of pure attractant was reported having retention times of 100–110 or 140–160 min. With this small amt of material the investigators could determine only that it was an aliphatic compound containing ester carbonyl.

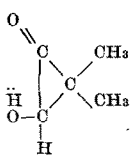
The pure attractant was obtained in much higher yield by Jacobson et al. (26) utilizing a method of condensing the attractant from air passed over female roaches confined in a large container (43). The vapors were condensed in a Dry Ice-cooled flask containing a little 0.1% HCl, the condensate was extracted with hexane, and the extract was chromatographed on a column of silicic acid, eluting successively with hexane, 3% ether in hexane, and 10% ether in hexane. The latter solvent removed the attractant completely and it was obtained in pure form by steam distillation. A total of 12.2 mg of liquid attractant was obtained by continuous collection from 10,000 females over a 9-month period; it elicited a sexual response from males at levels below 10^{-14} μg. In contrast to the retention times reported by Wharton, this attractant showed a retention time of 6 min under the same conditions. Gas chromatography on several other column packings verified the purity of the material (23).

Characterization

The attractant analyzed for $C_{11}H_{18}O_2$ and its IR spectrum showed the presence of ester carbonyl (26). Catalytic hydrogenation of 2.2 mg showed it to have one double bond, and alkaline saponification of the saturated compound gave 1.5 mg of a secondary alcohol and 0.85 mg of propionic acid. Oxidation of 4 mg of the attractant with HIO_4-$KMnO_4$ reagent gave propionic acid, acetone, and 2.2 mg of a neutral substance, mp 55C, that yielded dimethylmalonic acid on further oxidation with HIO_4. The only structure for the attractant consistent with the foregoing data was 2,2-dimethyl-3-isopropylidenecyclopropyl propionate (IV); the structure V was proposed for the neutral substance of mp 55C.[1]



(IV)



(V)

Introduced Pine Sawfly (Diprion similis)

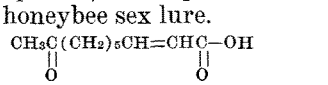
In 1960, Coppel et al. (16) reported that virgin females in the field were capable of attracting exceptionally large numbers of males; one caged female attracted well over 11,000 males. An attempt to trap the attractant by passing air rapidly over virgin females was unsuccessful, but the crude attractant was obtained by extracting crushed whole females with acetone or benzene and by rinsing, with ether, glassware that had contained the live or dead females. Column chromatography of the extracts on Florisil yielded a substance which, though impure, in amts as small as 0.004 μg. attracted 500–1000 males over a distance of 100–200 feet within 5 min (15). Although the activity was sometimes masked in some types of solvent extracts, chromatography resulted in active fractions.

1 Structure IV has recently been shown [Day, A. C., and M. C. Whiting, Proc. Chem. Soc. *1964*, 368; Jacobson, M., and M. Beroza, Science *147*, 748 (1965)] to be incorrect for the natural attractant, and the structure remains to be determined.

Other Insect Attractants

Honey Bee (Apis mellifera)

Gary (17) has demonstrated the existence of volatile chemicals functioning as mating attractants in queen honey bees, and this has recently been substantiated by Pain and Ruttner (33). Drones in flight are attracted to the flying queen by substances present on or in her mandibular glands. Fractionation of the gland lipids, obtained by extraction with ether, on silicic acid yielded "queen substance" (*trans*-9-oxodec-2-enoic acid) (VI), attractive to drones at 0.1 mg per assay tube, and at least two other substances (phospholipid fraction) with some attractiveness. However, reconstitution of the lipid complex resulted in considerably more attractiveness than was shown by individual fractions. Since sex lures are usually much more potent, the "queen substance" may not be the true honeybee sex lure.



(VI)

Attraction of worker bees to queens in the hive is also due to a mixture of volatile acids (containing "queen substance") extracted from queen mandibular glands; attractivity is lost by chromatography and can be recovered by remixing fractions (32).

The Nassanoff gland of honey bees produces a scent which attracts large numbers of these insects. Boch and Shearer (5) obtained the crude pheromone by wiping the exposed glands with small pieces of filter paper and then extracting these papers with ether. Geraniol isolated from this extract, although attractive, was much less attractive to bees than was the complete scent (6). The acidic portion of the ether extract yielded nerolic and geranic acids, identified by gas chromatography of their methyl esters; this acid mixture was slightly more attractive to bees than geraniol. (It may be that geranic acid is not produced by the bee but is formed by isomerization of nerolic acid during extraction and methylation.)

Bumble Bee (Bombus terrestris)

The swarming of male bumble bees in the summer is due to an odorous material; during this swarming mating with the females occurs. The attractant is found in the male mandibular glands and may be obtained by extracting the male heads with pentane (38). The extract was subjected to thin-layer chromatography on silica gel, with petroleum ether-ethyl ether (1:1) as developing solvent; plates sprayed with antimony pentachloride solution showed the attractant as a brown spot of R_f 0.45. It was identified as farnesol by its R_f and IR spectrum. (Farnesol is present in the flower oils of many plants, and it is possible that the males take up this compound with their food and feed it into their mandibular glands.)

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Skin Lipids. I. Sampling Problems of the Skin and Its Appendages

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Abstract

A review of the problems of sampling various skin tissues for study of lipids is presented following a survey of the anatomy and physiology of skin and its appendages.

Anatomy and Physiology of the Skin (1,2)

THE SKIN SERVES a variety of functions which include regulation of body temperature, sensory contact to the external environment, and production of Vitamin D. The primary function of the skin, however, is that of the protection of the body from the external physical and chemical environment. This is accomplished by the continuous proliferation of living epidermal cells whose ultimate fate is to die and become the outermost horny layer (stratum corneum, keratin) (3).

The skin has three layers: the epidermis, the dermis, and the subcutaneous or adipose tissues (Fig. 1). Overlying the subcutaneous tissue is the dermis (corium) which functions as the physical and nutritional support of the epidermis. The dermis consists mainly of connective tissue fibers (collagen, reticulum and elastin) between which pass the blood vessels and nerves of the skin. The ground substance, an amorphous, viscous, semisolid gel, fills the remaining dermal spaces between the fibers, vessels and nerves. Fibroblasts, the precursors of collagen fibers, are present as well as wandering cells of the blood and reticuloendothelial system.

The epidermis is composed of four layers: the basal or germinative layer, the prickle or Malpighian layer, the granular layer and the outermost horny layer (Fig. 2). Fine nerve fibers terminate in the lower epidermis. No blood vessels, however, are found in epidermis which is nourished by diffusion of nutrients from the underlying dermis. The epidermis varies in thickness from 0.1 to 0.3 mm. That portion of the epidermis which extends from the basal layer through the granular layer is considered as the *living epidermis*, and the outermost horny layer of keratin, the *dead epidermis*.

As the epidermal cells move upward in a pattern of continuous regeneration and enter the upper Malpighian layer, they undergo a process of enzymatic dissolution, become dehydrated and flattened to form the dead, horny layer. The lower horny layer is compact and tightly adherent, while in the upper horny layer, the dead cells lose this adherence and slough as microscopically fine scales. In normal human skin, 27 days are required for the transition from the basal layer to shedding stratum corneum.

The epidermal appendages are three in number: the pilosebaceous apparatus, the eccrine sweat gland, and the apocrine sweat gland. The pilosebaceous apparatus consists of the hair follicle and the multilobulated sebaceous gland (4). The sebaceous gland is connected by a short duct to the follicular canal about midway along the follicle (Figs. 1 and 3). The sebaceous gland cells mature as they grow from the periphery toward the center of the gland, become fat-laden, and disintegrate in the central portion of the gland as is characteristic of holocrine excretion. This is not a secretory function. This mass of lipid and cellular debris discharges into the sebaceous duct and is called sebum. Sebaceous glands have a variable distribution over the surface of the body. In the human being, they are most numerous on the scalp and forehead, and occur with decreasing frequency on the chest, back, axillae, genitocrural area, abdomen, arm and leg. They are not found on the palms or soles.

Variants of the sebaceous gland exist in man. Large sebaceous glands (often on the nose and cheeks) may be associated with tiny vellus hairs, the size of the gland being inversely proportional to the size of the associated hair structure. These glands, with large, dilated, patulous ducts and rudimentary hairs, have been called sebaceous follicles (5). Sebaceous glands may occur independent of hairs and excrete directly onto the skin surface. In the human eyelid, Meibomian glands, a modified sebaceous gland, empty their contents at the margin of the eyelids. The cerumenous glands of the human external ear canal are also modified sebaceous glands. Typical sebaceous glands are also found in the external ear canal as well as eccrine and apocrine sweat glands. Thus, cerumen, or ear

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