Volatile Acids in the Secretion Products (Hair Grease) of the Skin

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The secretory products of the sweat glands and the sebaceous glands are in several respects physiologically important. The function of the sweat in the excretion of waste products and in the regulation of body temperature is well known. The sebum from the sebaceous glands doubtless aids in preventing the penetration of harmful substances into the skin, it affords protection against the disintegrating influence of external moisture, it gives pliability to the skin, and it contains provitamin D (Dukes, 1947). In healthy animals it confers on the hair its glossy appearance, whilst in illness the fur may be dry and the hair brittle perhaps due to lack of sebum. Moreover, the secretion products of the skin are said to have antimicrobial properties. Finally, the odorous substances are of considerable importance in the relations between male and female animals and between mother and young.

Very little is known with certainty about the odorous substances in the skin secretions. Nevertheless, it is usually assumed that the repugnant smell of male goats originates from skin secretions and is due to caproic and caprylic acid (Zwaardemaker, 1895). However, in scrutinizing the literature, we did not find reliable chemical analyses corroborating this assumption. It seemed, therefore, worth while to determine the volatile acids in extracts of hair of male goats in comparison with other animals with the aid of the newer methods of chromatography. We found, however, that the C_6 , C_8 and C_{10} acids were by no means abundant. Nevertheless, the volatile acids in the hair extracts of different species of animals presented several interesting features. Moreover, the study of the skin secretions, as compared with the milk secretion, is of fundamental importance, because the udder can be considered to be a huge complex of modified skin glands.

METHODS

The chromatographic method used was the one initiated by Ramsey & Patterson (1948) as improved upon by Nijkamp (1951). As far as the extraction of the hair is concerned, the following procedure was adopted. The hair was collected from the back and sides of the animals and stored at -20° if not used immediately. After clipping and, if necessary,

drying over blue silica gel, about 20 g. of hair was extracted for several hours with peroxide-free diethyl ether. The ether was washed with a dilute aqueous solution of K_2CO_3 . After evaporation of the ether, a residue of fatty material was left behind and has been named by us the 'lipid fraction'. It includes the triglycerides, the waxes, etc. but not, of course, the free fatty acids. After saponification with a solution of NaOH in acid-free ethanol, the volatile acids were collected by steam distillation and separated on a silica gel column as described (Nijkamp, 1951).

The watery layer separated from the lipid fraction will be termed the ' K_2CO_3 fraction'. It also contained some volatile acid and was therefore treated separately. After evaporation to small volume and acidification, the volatile acids were collected by steam distillation and separated on a silica gel column.

Finally, the hair, as left after the ether extraction, was (at $35-40^\circ$) extracted once more, this time with a dilute aqueous solution of $KHCO_3$ containing enough K_2CO_3 so that the reaction remained faintly alkaline to phenolphthalein after the solution had been added to the hair. The extract plus washings was called the 'alkaline hair extract'. It was evaporated down to small volume and treated in the same way as the watery layer separated from the ether extract mentioned above.

All processes were shown by special experiments to be such that no breakdown of glycerides or waxes could occur during the extractions. The volatile acids obtained after saponification of the lipid fraction therefore originated from glycerides, and perhaps waxes and allied compounds. On the other hand, the volatile acids in the K_2CO_3 fraction and in the alkaline hair extract must have been present in the hair as such or in soaps; minor quantities may also have been produced in these watery extracts during evaporation and steam distillation as a result of decomposition of non-lipid material present in small amounts. There would, perhaps, have been some reason to combine the volatile acids from these two watery extracts. Nevertheless, in Table 1 they have been kept apart. Presumably, most of the free acids are found in the K_2CO_3 fraction and most of the soaps in the alkaline hair extract.

Although it was our intention to study in the first place the acids C_4-C_{10} , the figures for C_2 and C_3 are also given. The latter two acids must, however, be considered as minimum values because C_2 and C_3 acids are taken up by the solvent (*iso*-octane) with more difficulty than the acids C_4-C_{10} .

In Table 1, data are set out on C_2 , C_3 , C_4 , C_6 , C_8 and C_{10} acids. In some instances, however, still other volatile acids must have been present, presumably C_7 and C_9 acids and an unknown volatile acid moving on the column between C_3 and C_4 .

RESULTS

Lipid fraction

Although precautions had been taken to prevent any contamination whatever with acetic and propionic acids, many samples (especially those from ruminants) did contain measurable quantities of those compounds. It must, therefore, be concluded that the two acids were present (as esters) in the lipid fraction. The biggest quantity was only about 0.1 %, but this represents a minimum. As a matter of fact, French, Hunter, Martin & Popják (1951) have recently isolated and identified acetic acid from goat milk fat. In addition, traces of propionic acid and valeric acid have also been identified by these authors.

The proportions of the volatile acids in the various lipid fractions presented interesting features. In the cases of the horse, pig, rabbit, cat, guinea pig, rat and man all volatile acids up to C_8 were only present in minor or even negligible quantities. In most instances, however, the quantity of C_{10} acid was somewhat higher. Up to now only one sample from each of these species has been examined.

In the lipid fraction of three samples of goat hair all members (C_2 , C_3 , C_4 , C_5 , C_6 , C_8 , C_{10} acids) were present in appreciable quantities, the aggregate amounting to about 3 % of the lipid fraction. This great variety of volatile acids seems characteristic of ruminants, for it was also found with hair of cattle and of sheep. Nevertheless, with the latter two species, the results differed somewhat from those associated with goats. With cows (either in milk or not) a shift was shown to C_8 and C_{10} acids. The quantities of C_2 , C_3 , C_4 and C_5 acids were smaller, the quantities of C_6 acid about equal and of C_8 and C_{10} acids larger than with male goats. With the bull, the distribution of the acids was about the same as with cows. Thus, with male goats, contrary to what was anticipated, the quantities of C_6 , C_8 and C_{10} were by no means larger than with cattle.

With sheep, the distribution of the volatile acids was different again, with C_4 and C_6 acids predominating. The other volatile acids, though not absent, were present in so much smaller quantities that the aggregate of volatile acids amounted to only half the quantity found in the lipid fractions of goats and cattle.

Still another type of distribution prevailed with the dogs. The total quantity of volatile acids amounted to about 5 %, and with five animals consisted almost exclusively of valeric acid. With three other dogs, some other volatile acids were also present, but only in small quantities, most of them being C_6 , C_8 and C_{10} acids. Whether the C_6 is *n*-valeric acid or *isovaleric* acid has not yet been established. It is of interest that *isovaleric* acid has been recognized as an important constituent of

the fat of the dolphin and the porpoise. The percentages (by weight in the fatty acids) in the depot fat of the body, head and jaw of the porpoise (*Phocaena communis*) were respectively 13.6, 20.8 and 25.3 (Lovern, 1934).

K₂CO₃ fraction and alkaline hair extract

As a rule, the total quantities of volatile acids in these fractions were not very different. Except with the sheep, they amounted to only a few mg./100 g. of hair. Nevertheless, with the horse, pig, rabbit, guinea pig, rat and man, the totals were somewhat higher than those in the lipid fractions, which were almost devoid of volatile acids. With the cat they were a little lower. The question whether the small quantities of volatile acids in the K_2CO_3 fraction and in the alkaline hair extract have been secreted as such or as esters or whether they might partly be decomposition products, cannot be settled from our experiments. In most cases C_2 and C_3 acids were preponderant. In many instances, however, the amount of C_{10} acid was also somewhat higher than that of the intermediate volatile acids.

Nearly the same result (C_4 , C_5 , C_6 low, and C_2 , C_3 , C_{10} as a rule somewhat higher) was arrived at with the cows and the goats. Again with the male goats, C_6 , C_8 and C_{10} acids were by no means higher than with the cows and the bull. On the contrary, with the bull, C_6 was distinctly higher. Thus, with the goats, neither the lipid fraction nor the other fractions showed high contents of C_6 , C_8 and C_{10} acids. Still it was quite striking that these fractions often displayed a strongly offensive goat smell. For this reason, we are inclined to believe that the smell should not be attributed to C_6 - C_{10} acids but rather to other, still unknown, odorous substances.

With the dogs the quantity of C_6 acid was again conspicuously higher than with the other species of animals. With the dogs it was only second to C_2 acid.

With the sheep, the volatile acids in the two fractions under consideration were distinctly larger than with the other animals, the totals being in two instances as big as in the lipid fraction. The amounts of the individual acids varied considerably. The averages of the individual acids, however (including C_2), did not show big variations. More than with the other species of animals we got the impression that, with sheep, some decomposition of the esters had taken place. The fact that the wool protects the grease from being continuously rubbed off by the action of the animal may contribute to this phenomenon.

SUMMARY

1. The volatile fatty acids C_2 , C_3 , C_4 , C_5 , C_6 , C_8 and C_{10} (free and as esters) of the skin secretions (hair grease) of man and of several species of animals have been studied. The quantities of these acids

Table 1. *Distribution of volatile fatty acids in various fractions obtained from hair grease of various species of animals*

Exp.	Lipid fraction of hair (g./100 g.)	Volatile acids (g./100 g. of lipid fraction)										Volatile acids (mg. in the lipid fraction of 100 g. of hair)										Total																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
		C ₂	C ₃	C ₄	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	Total	C ₂	C ₃	C ₄	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	Total																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
Goat (M.)	3	2.14	0.08	0.04	0.26	0.14	0.16	0.80	2.32	3.80	1.8	0.9	5.4	3.0	3.4	17.0	49.6	81.1																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															

Exp.	Lipid fraction of hair (g./100 g.)	K ₂ CO ₃ fraction										Alkaline hair extract									
		Volatile acids (mg./100 g. of hair)										Volatile acids (mg./100 g. of hair)									
		C ₂	C ₃	C ₄	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	Total	C ₂	C ₃	C ₄	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	Total
Goat (M.)	3	1.6	0.0	2.8	0.0	0.0	0.4	0.4	1.3	6.1		6.6	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.2
	23	0.6	0.0	0.0	0.1	0.3	0.4	1.2	2.6	7.0		7.6	0.0	5.3	0.2	0.6	0.6	0.4	2.4	1.0	18.5
	24	2.5	0.8	0.6	0.4	0.7	0.8	1.2	7.0			4.3	0.5	1.2	0.0	0.6	0.6	1.0	1.0	1.0	8.2
Cow D, no milk	0	3.0	1.5	0.2	0.0	0.4	0.6	2.4	8.1			0.9	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Heifer, no milk	6	0.2	0.4	0.0	0.0	0.2	0.4	1.2	2.4			4.0	0.8	0.0	0.0	0.0	0.4	1.3	6.5		6.5
Cow, 8 kg. milk	1	1.0	0.6	0.3	0.0	0.2	0.2	1.0	3.3			1.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
Cow, 6 kg. milk	2	2.0	0.8	0.0	0.0	0.4	0.2	0.7	4.1			0.8	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Cow 13 kg. milk	5	3.2	?	0.0	0.0	1.7	0.4	1.7	7.0			2.2	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1
Bull	4	8.9	11.8	>82.0	4.5	13.2	1.6	3.1	>125.1			3.8	1.7	17.7	0.7	3.8	0.0	0.0	0.0	0.0	27.7
Sheep (F.)	26	1.4	2.4	3.8	1.6	0.0	2.0	2.8	14.0			8.0	4.8	0.4	1.0	0.2	0.6	0.8	0.8	0.8	15.8
	27	1.8	3.5	1.6	5.3	1.4	2.5	0.6	16.7			15.0	11.7	3.1	0.7	0.0	0.8	0.0	0.8	0.0	31.3
	28	2.0	0.1	0.8	0.5	1.3	0.6	0.4	5.7			8.0	0.0	19.4	2.4	1.0	0.4	1.0	0.4	1.0	32.2
Fat-tailed sheep*	22	2.4	0.0	1.6	2.6	1.6	0.8	1.6	10.6			2.4	0.0	1.2	2.2	0.4	0.5	0.4	0.4	0.4	7.1
Horse (F.)	12	2.4	0.0	0.7	0.2	0.1	0.4	±0.6	±4.4			2.2	0.2	0.4	0.0	0.0	±0.3	±0.4	±0.3	±0.4	±3.5
Pigs (F. and M.)	10	2.2	0.5	0.1	0.3	0.2	0.4	0.3	4.0			1.8	0.8	0.4	0.4	tr.?	0.0	0.0	0.0	0.0	3.4
Rabbit (M.)	11	3.0	0.4	0.2	0.1	tr.	0.4	0.2	4.3			4.3	tr.	0.0	0.2	0.0	0.0	0.0	0.0	0.0	4.5
Dog (M.)	8	1.3	0.2	0.0	0.8	0.0	0.0	0.0	2.3			1.8	0.4	0.0	1.2	0.0	0.0	0.0	0.0	0.0	3.4
	13	1.6	0.4	0.3	0.4	0.0	1.1	1.4	5.2			1.6	0.2	0.0	1.4	0.0	0.0	0.0	0.0	0.0	3.2
	16	1.1	0.4	0.6	0.2	1.8	2.2	10.2	16.5			1.4	0.3	0.0	1.0	0.0	0.0	0.0	0.0	0.0	2.7
	17	2.63	0.0	0.0	0.2	0.3	0.4	1.9	2.8			0.4	tr.	0.0	0.8	0.0	0.0	0.0	0.0	0.0	1.2
	18	3.04																			
	19	3.55																			
	20	4.38																			
	21	1.67																			
Cat (M.)	15	1.0	0.2	tr.	0.1	0.1	0.4	0.9	2.7			1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	1.8
Guinea pig (M.)	7	3.4	3.6	0.0	0.0	0.0	0.0	0.0	7.0			1.8	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.4
Rats (F. and M.)	25	1.6	0.9	1.0	0.8	1.0	0.7	0.7	6.7			2.6	1.1	1.1	0.5	0.4	0.1	0.0	0.0	0.0	5.8
Mart (M.)	14	1.3	1.2	0.1	0.4	0.0	3.2	6.1	12.3			2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9

* Only hair from the head.

† Hair from the head; the hair was not washed during the period of gathering.

tr. = trace.

found in the secretions of man, horse, rabbit, cat, guinea pig and rat were very small.

2. With male goats, cattle, sheep and dogs, appreciable quantities were found, mainly in the lipid fraction of the hair, i.e. the ether extract from which the free acids had been removed. The acids (including some C_2 and C_3 acids), were therefore present in this fraction in combined form. The aggregates of volatile fatty acids C_2 – C_{10} in the lipid fractions amounted for the four species of animals mentioned respectively to about 3, 3, 1.5 and 5 %.

3. In the lipid fractions relating to the three species of ruminants all seven volatile acids were present. However in cattle, C_8 and C_{10} acids were preponderant, whilst in sheep C_4 and C_6 acids predominated. In male goats the seven volatile acids were more evenly distributed, but C_8 and C_{10} acids predominated. The lipid fractions from eight dogs contained virtually C_6 acid only.

5. It is questionable whether the offensive smell of male goats is really due to C_6 and C_8 as is generally assumed.

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The Occurrence of Growth Factors for *Lactobacillus leichmannii*, *Streptococcus faecalis* and *Leuconostoc citrovorum* in the Tissues of Pernicious Anaemia Patients and Controls

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The chief therapeutic substances that have been shown to be of value in the treatment of pernicious anaemia in man are: vitamin B_{12} (cyanocobalamin) which is a growth factor for *Lactobacillus leichmannii*; pteroylglutamic acid (probably folic acid) which is a growth factor for *Streptococcus faecalis*; 5-formyl-5:6:7:8-tetrahydropteroylglutamic acid (the citrovorum factor or probably folinic acid) which is a growth factor for *Strep. faecalis* and for *Leuconostoc citrovorum*. The metabolic interrelationships of these factors are uncertain and have already been discussed (Girdwood, 1952*a, b*).

The present investigation commenced with a study by microbiological assay methods of the content of vitamin B_{12} and of growth factors for *Strep. faecalis* in the tissues of a patient who died of untreated pernicious anaemia. Later, published work suggested that the citrovorum factor is of importance in relation to haemopoiesis (Nichol & Welch, 1950), and accordingly we commenced studies of the amounts of citrovorum factor in human tissues. Swendseid, Bethell & Ackermann (1951) have introduced a differential microbiological assay procedure for measuring the extent to which the apparent folic acid content of tissues is, in fact, due to citrovorum factor. By a similar approach, and using autopsy material, we have found that in

certain human tissues the whole of the material with folic acid activity is citrovorum factor. This is in keeping with the findings of Swendseid *et al.* (1951) who carried out their investigations on fresh mouse livers.

EXPERIMENTAL

Various tissues and organs were obtained at autopsy from the following patients.

Case I. A man aged 65, was admitted to hospital with severe untreated pernicious anaemia diagnosed on the clinical and haematological findings which included sternal puncture. The haemoglobin level was 5.7 g./100 ml., R.B.C. 1.95 millions/cu.mm. Before treatment commenced the patient died suddenly of a coronary thrombosis.

Case II. A woman aged 79, was admitted to hospital with untreated pernicious anaemia (Hb 5.3 g./100 ml., R.B.C. 1.35 millions/cu.mm.). She was given 2 ml. of liver extract on admission (containing about 6 μ g. of vitamin B_{12} /ml.) and the following day 100 μ g. of vitamin B_{12} together with a transfusion of a pint of blood. She died on the third day in hospital through having aspirated her vomitus into the trachea and bronchi. At the time of death the marrow was not completely converted to the normoblastic state.

Case III. A woman aged 57 was admitted to hospital with hypertensive cardiac failure. There was collapse of the left lung and at autopsy a bronchial carcinoma was found to be present. No visible tumour deposits were present in the tissues that were used for assay. The patient was not anaemic.