Human Sweat Components - Attractancy and Repellency to Mosquitoes

Recently we reported that whole sweat samples collected from human subjects were attractive to female *Aedes aegypti* mosquitoes[1]. We find, however, on comparison of sweat from large numbers of individuals, that the attractiveness as measured in a dual-port olfactometer varies greatly and is sometimes absent in sweat from some individuals (Table I). Our efforts have thus been aimed at the isolation and identification of endogenous substances in sweat and other body emanations such as skin lipids[2] that may contribute in mutually antagonistic fashions to the attractiveness of humans to mosquitoes. Variations in the secretion of such substances may be responsible for demonstrated differences in individual attractiveness[3] and might be dependent upon dietary, metabolic, emotional or environmental factors.

Liquid-liquid extraction with diethyl ether of whole sweat adjusted to pH 1 yielded a fraction with a high degree of repellency. Such a fraction has been isolated from all samples of sweat thus far examined including those with initially high degrees of attractive activity (Table II). Extraction at pH 7 or 8 yielded much smaller amounts of material though such fractions also exhibited some repellency (Table II). Removal of the repellent fraction did not greatly affect the attractiveness of the residual whole sweat.

The nature of the repellent fraction was examined by a variety of techniques. The much higher extraction efficiency at low pH is consistent with the highly acidic nature of the material (dilute aqueous solution, pH 1). No residual hydrochloric acid could be detected after removal of the diethyl ether in vacuo. The majority of the repellent could

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Table I. Incidence and degree of attractiveness of sweat samples from various individuals

Subject No.[a]	Average indices of attraction (IA) of individual samples[b]	% of samples showing significant attraction[c]
1	50, 79, 3, 9	50
2	61, 5, 0, 38, 12, 13, 21, 28, 4	33
3	29, 6, 71	67
4	0, 44, 32, 26, 18, 35, 0, 39	63
5	39, 26, 36, 53, 39, 24, 55, 15	75
6	15, 6, 7, 0	0
7	38, 26, 11, 40, 32, 46, 36, 14	75
8	71, 27, 10, 1, 2, 0, 11, 20, 0, 84	30
9	59, 61, 35, 0, 34, 30, 26, 0, 13	67
10	35, 22, 23, 7, 32, 0, 7, 0	25

[a] Normal, healthy males. [b] Values are averages of 2–5 determinations; for testing methodology see [1]. Samples collected on different days over a period of 1–2 years. IA = (No. of mosquitoes on sample side — No. of mosquitoes on control side/Total No. of mosquitoes) × 100. [c] Index of attraction of 26 or greater. Negative IA values (repellent samples) are listed as '0'; in no cases were such samples significantly repellent.

Table II. Liquid-liquid extraction of sweat with diethyl ether

pH	Time (h)	IA[a] sweat	IR[b] ether extract	Weight mg. ether extract	Vol. of sweat	IA sweat residue pH 7
7	24	16	36	6	300	28
7	24	16	24	9	300	23
8	24	16	25	9	300	30
1	24	16	59	147	300	20
1	24	44	50	225	450	43
1	24	44	55	235	450	45
1	24	32	80	176	450	27
1	48	39	70	–	500	3
1	90	33	47	412	500	5
1	90	32	78	413	450	17
1	90	60	44	348	400	16
1	120	60	77	389	400	31
1	120	60	82	316	400	8

[a] Normal, healthy males. [b] Index of Repellency, IR = (No. of mosquitoes on control side — No. on sample side/Total No. of mosquitoes) × 100.

Table III. Characteristics of thin-layer chromatographic fractions of ether extract of whole sweat[a]

	Lactic acid	Fraction I	Fraction II	Fraction III
Thin-layer chromatography Rf[b]	0.1	0.1	0.5	0.8
VPC retention time (min)[c]	3.5; 18.8	3.6; 19.0	–	–
VPC retention time[d]	–	–	216°/12 min 240°/14 min 240°/15 min	155°/ 6.5 min 218°/12.5 min 236°/14 min
IR, λ_{max}^{NaCl} cm^{-1}; OH	3330	3360	3330	3350
bonded OH	2700–2500	2700–2500	–	–
C–O	1125	1125	–	–
C=O	1725	1725	1740	1740
Index of repellency	38	30	41	49

[a] Fractions from continuous liquid-liquid extraction of whole sweat adjusted to pH 1 were vacuum distilled (50°C/0.1 mm; 4 h). [b] Silica gel G; petroleum ether-ether-acetic acid, 90:10:7.5. [c] Varian Aerograph Model 204, 3 feet × 1/8 inch SS column, Porapak Q, 150/200 mesh, 250°C, He 25 ml min^{-1}, FID. [d] Varian Aerograph Model 204, 5 feet × 1/8 inch SS column, 20% FFAP, 70/80 mesh, chromasorb W-DMCS, 100–240°, He 21.4 ml min^{-1}, FID.

be separated from the crude extract by short-path vacuum distillation at 50 °C and 0.1 mm for 4 h. Thin-layer chromatography indicated the presence of at least 3 major components in the distillate (Table III). The component present in largest quantity (fraction I) has been identified as lactic acid which is a known major component of sweat and has been shown to be repellent to mosquitoes when tested on the skin[4]. This component was isolated by preparative thin-layer chromatography and identified by comparison of its thin layer and vapor phase chromatographic behavior and IR-spectral characteristics with those of an authentic aqueous solution of lactic acid subjected to similar evaporative drying in vacuo. Such samples exhibit 2 major peaks on vapor phase chromatography – presumably due to lactic acid and lactic anhydride. The other 2 major repellent components of sweat (II and III) have not yet been identified but appear to be less polar hydroxy carbonyl compounds from their IR-absorption spectra. Vapor phase chromatography indicates that these fractions are each composed of a major (216°/12 min and 155°/6.5 min) and 2 minor components.

Addition of water to the ether-extracted lactic acid (fraction I) or to authentic acid reduced the repellency in the olfactometer to marginal levels. Therefore, the relative significance of lactic acid as a nullifying factor in the attractiveness of bulk sweat samples is uncertain. However, the presence of varying amounts of lactic acid on the skin surface may affect the degree of attractiveness of individuals – particularly in combination with other factors such as the amount of moisture on the skin. For instance, anhydrotic humans have greatly decreased attraction to *Aedes* mosquitoes. On addition of water to their skin, normal levels of attractancy return[5]. It is becoming increasingly apparent that the attractiveness of humans to mosquitoes is governed by a complex interaction of numerous endogenous factors including heat, moisture, carbon dioxide, lipids, acids and possibly unknown substances in trace amounts[2,6,7].

Zusammenfassung. Es wird gezeigt, dass menschliche Schweissabsonderungen verschiedener Versuchspersonen auf weibliche Moskitos (*Aedes aegypti*) verschieden attraktiv sind. Mit Diethyläther (pH 1) lassen sich im Schweiss sowohl «anlockende» wie auch «abschreckende» Bestandteile extrahieren. Für den «repellent effect» sind teilweise gleiche Mischungen von Milchsäure und Milchsäureanhydrid verantwortlich. Zwei weitere, noch nicht identifizierte Substanzen zeigten im Zwei-Kammer-Olfaktometer identische «repellent» Wirkungen.

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Effect of Erythropoietin on Nucleic Acid Metabolism from Polycythemic Rat Bone Marrow

Many facts appear to be in favour of the control activity of the erythropoietin on erythropoiesis by means of the regulation of the metabolism of RNA[1–4]. However, the lack of uniformity in the erythropoietic tissues, which consists of cells with markedly different functional properties, makes the problem very difficult to investigate at the molecular level. The difficulties of interpreting the experimental data prompted us to search for conditions more suitable for understanding the biochemical mechanisms involved in the erythropoietic process. One of these experimental conditions is the establishment of metabolic patterns of the nucleic acids from hematopoietic tissues under the effect of erythropoietin. In this way, it is possible to obtain valuable biochemical information in order to know more about the mode of action of the hormone. The metabolic patterns are determined by measuring nucleic acid metabolism with the criterion of the incorporation of some labelled precursors into the macromolecules. Formate is a suitable precursor for the biosynthesis of nucleic acid because it can be incorporated to a good extent by RNA and DNA[5].

This communication describes experiments in which different metabolic patterns of bone marrow from normal, polycythemic (hypertransfusion) and polycythemic rats treated with erythropoietin were studied.

The materials and methods were as previously described[1]. Other conditions are given in the Table.

The (^{14}C)formate incorporation patterns are shown in the Table. It is clear that different patterns are obtained under the 3 experimental conditions. The metabolism of nucleic acid from normal marrow follows a pattern already demonstrated[1]. In polycythemic rats the nucleic acid metabolism decreased 2- to 4-fold, especially RNA adenine, while the specific activity of thymine was not changed to such an extent. The injection of erythropoietin produced an increase of 2- to 3-fold in acid-soluble adenine and RNA, whereas no observable effects are produced in DNA. These observations corroborate our previous findings in spleen and bone marrow from polycythemic mice

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