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FREE AMINO ACIDS AND WATER SOLUBLE PEPTIDES IN STRATUM CORNEUM AND SKIN SURFACE FILM IN HUMAN BEINGS§

In recent years, increasing interest has been centered on the biochemistry and physiology of the skin surface, in particular, its potential for resisting physical, chemical, and microbiologic challenges. Morphologically, the skin surface is composed of the stratum corneum, sweat and sebaceous gland ducts, and hair follicles that open onto the surface through pores. The stratum corneum consists of dead, keratinized cells which act like a sponge in holding the surface film material. Surface film is derived primarily from sebaceous glands, sweat glands, and keratinizing epidermis. It is an extremely complex substance. Partly aqueous and partly lipid in nature, homogenized by cholesterol and wax alcohols, it contains proteins, carbohydrates, lipids, acids, salts and their derivatives. Surface film is the skin's first defense. It helps prevent overdrying and overwetting; it slows absorption of foreign chemicals from the skin surface; and it protects against some infections and infestations.[1] It supports the growth of some normal and some pathogenic skin organisms.[2] The stratum corneum and its surface film are interdependent and are treated as one unit in this study.

Literature on the origin, action, and composition of the stratum corneum and skin surface film has been increasing steadily, especially since Rothman's[1] monumental work on the physiology and biochemistry of the skin. We[2] have been concerned with several aspects of this work and, more recently, with the water soluble organic components of the stratum corneum-surface film complex. Knowledge of the water soluble components is basic to the study of normal and pathogenic skin organisms and patterns

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of susceptibility and immunity to superficial infections. It is also basic to a clearer understanding of changes wrought by pathologic processes, to studies of the role of the skin in applied physiology, and to the study of the biochemistry of keratinization.

A number of papers deal with water soluble organic components and, in particular, with the amino acids of human epidermis and stratum corneum.[2-22] Most of these reports are either the result of qualitative studies or are derived from pathologic material or specimens hydrolyzed before

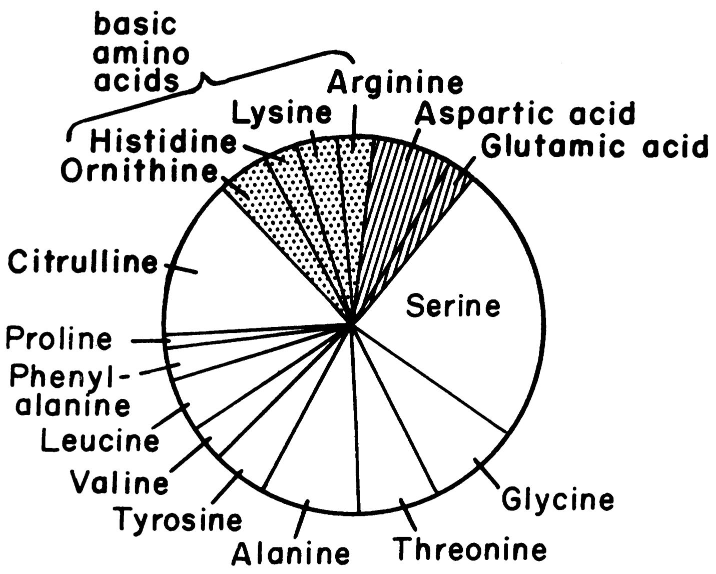

FIG. 1. From Spier and Pascher[7]: "Division by percentage of the free amino acids in the scrapable stratum disjunctum (average values)."

analysis. In addition, some studies deal with material collected from inadequately described anatomical sites and are without specification of length of time since bath. The most definitive study is that of Spier and Pascher[7] who, using paper electrophoresis and two-dimensional paper chromatography, showed 16 amino acids to be present in samples collected from dorsal surfaces (Fig. 1).

The following study is concerned with the establishment of baseline values of the free amino acid composition of the stratum corneum and skin surface film in white adult males and females and prepubertal children. Several problems were investigated:

1. Free amino acids and water soluble peptides present in the stratum corneum and skin surface film of the upper trunk (sternal, upper thoracic, and shoulder areas) in adult males, females, and prepubertal children.
2. Free amino acids and water soluble peptides of the plantar surfaces in adult males, females, and prepubertal children.
3. Comparison of the free amino acid and water soluble peptide patterns from four areas of one subject.
4. Effect of time after bathing on the free amino acids and water soluble peptides present in stratum corneum and skin surface film of upper trunk and plantar surfaces.
5. Comparison of the free amino acids and water soluble peptides present in stratum corneum and skin surface film of upper trunk in aliquot samples before and after acid hydrolysis.

METHODS

The upper trunk (sternal, upper thoracic, and shoulder areas), lower leg, and inner aspect of the upper thigh were washed with distilled water and rubbed with gauze pads. The gauze pads had been washed repeatedly in alcohol and water; washings from these cleansed pads were negative by ninhydrin reaction for amino acids or water soluble peptides. Rubber gloves were worn by the individual collecting the sample. A strong rubbing action resulted in the removal of a considerable amount of stratum corneum, the washings becoming milky during the process. Plantar surfaces were extracted by immersion of the sole of the foot in distilled water for 10 minutes together with rubbing and/or scraping to collect stratum corneum. After storage in the cold overnight to allow extraction of the water soluble components, the washings were filtered through glass wool to remove gross material, then through sintered glass filters and finally reduced in volume to about 10 ml. on a flash evaporator at 56°C. The washings were assayed using a modified ninhydrin reagent[23] and samples containing 20 μmoles amino nitrogen were chromatographed on ion-exchange columns according to the procedure of Moore, Spackman, and Stein.[24] In addition, bound amino acids and peptides were determined in hydrolyzed aliquots of the same specimens: samples containing 20 μmoles amino nitrogen were dried, then hydrolyzed in 6N HCl *in vacuo* in sealed tubes at 105-110°C. for 24 hours. The hydrolyzed specimens were analyzed, using the same procedure of column chromatography. A series of samples were also assayed in the laboratory of Dr. Frederic M. Richards, using automated equipment and the procedure of Spackman, Stein, and Moore.[25] These results served as duplicate determinations and are essentially similar to the first analysis.

RESULTS

1. *Free amino acids and water soluble peptides of the stratum corneum and surface film of the upper trunk (sternal, upper thoracic and shoulder areas) in adult males, adult females, and prepubertal children 20-30 hours after bath.*

Table 1 presents the results of the analyses of water washings of the upper trunk in 4 adult males, 4 adult females and 2 children. The presence of 20 amino acids, urea, NH_3 and several unidentified ninhydrin-reactive substances were recorded.

The amino acids present in greatest molecular concentration were serine, glycine, citrulline, alanine, histidine, and threonine, in that order; occasionally threonine is present in greater concentration than histidine. These six amino acids may account for as much as 80 per cent of the ninhydrin positive substances in the horny layer. Spier and Pascher[7] note serine, citrulline, alanine, glycine, threonine, and aspartic acid, in that order, to be present in greatest amount (Fig. 1). Their results for histidine are much lower than those obtained here for upper trunk; this difference may be due to choice of site of collection, which is noted in their studies to be "dorsal surfaces."

Serine, the amino acid present in greatest molecular concentration, ranges from 25-31 per cent in the 10 samples presented in Table 1. Similar findings were indicated in three samples of stratum corneum scraped from the upper trunk, dried, weighed, extracted with distilled water, and chromatographed. Spier and Pascher[7] report 31 per cent of the free amino acids to be serine and about 2-2.5 per cent of the water soluble ingredients from dry stratum corneum to be serine.

Methionine, cystine, and tryptophan are present in smallest concentration; proline is obscured because of the large amount of citrulline masking its presence. That proline is present in these samples is evident from preliminary 2-dimensional paper chromatographic studies using butanol: acetic acid: water and cresol: phenol: borate solvent systems.[26] Rothman and Sullivan[5] also demonstrated the presence of proline in water wipings of the skin by using paper chromatography; and Spier and Pascher[7] noted proline to be present in trace amounts in their study of dorsal surfaces. The arginine and citrulline values are variable but the sum of the values for these two amino acids remains fairly constant (about 13 per cent) in the 10 subjects. It is quite possible that some of the citrulline is formed by the conversion of arginine.

No outstanding differences among the individual known amino acids can be seen in the results (Table 1) of the analyses of the stratum corneum

TABLE 1. FREE AMINO ACIDS AND WATER SOLUBLE PEPTIDES PRESENT IN STRATUM CORNEUM AND SKIN SURFACE FILM IN UPPER TRUNK IN ADULT MALES AND FEMALES AND PREPUBERTAL CHILDREN 20-24 HOURS AFTER BATH. (Determined as leucine equivalents and presented as μmoles/100 μmoles recovered. Adult males and females in fourth decade of life.)

	Adult male	*Adult male*	*Adult male*	*Adult male*	*Average, male*	*Adult female*	*Adult female*	*Adult female*	*Adult female*	*Average, female*	*10-year male*	*8-year female*	*Range*
$CySO_3H$	0.21		0.20	0.57	0.25	1.20	1.00	1.86	1.00	1.29	2.44	0.20	0– 2.44
ASP	3.15	1.37	3.07	3.00	2.65	5.35	3.07	3.06	5.56	4.26	2.83	2.51	1.37– 5.56
THR	5.63	6.79	5.66	4.72	5.70	6.12	5.50	5.30	6.99	5.91	6.32	6.07	5.30– 6.99
SER	28.13	30.08	28.79	25.77	28.19	27.78	27.47	27.94	28.85	28.01	27.98	30.71	25.77–30.71
GLU	2.68	0.87	4.00	1.99	2.38	2.73	2.61	3.74	2.62	2.93	2.96	1.67	0.87– 4.00
CIT	8.55	11.12	11.26	9.59	10.13	8.73	8.42	11.53	9.58	9.57	9.41	6.98	8.42–11.53
PRO													
GLY	14.38	16.11	16.27	14.86	15.40	13.91	14.04	15.82	16.20	14.99	14.87	16.75	13.91–16.75
ALA	9.83	10.91	9.47	11.09	10.28	8.52	9.59	8.96	6.99	8.52	10.64	11.17	6.99–11.17
½CYS	0.59	0.52	0.65	0.53	0.57		0.57	0.61	0.43	0.40			0– 0.65
VAL	2.98	3.04	2.69	2.24	2.76	3.41	3.11	2.52	2.56	2.90	2.97	2.51	2.51– 3.41
MET	0.02		0.33	0.60		0.18	0.20		0.20		0.13	0.21	0– 0.60
ILEU	1.44	1.28	0.95	1.79	1.36	1.40	1.39	0.88	0.60	1.07	1.23	0.31	0.31– 1.79
LEU	1.90	1.12	1.59	1.85	1.61	2.03	1.85	1.49	1.05	1.61	1.48	1.19	1.05– 1.85
TYR	1.91	1.74	1.22	0.77	1.41	1.54	1.86	1.15	1.00	1.39	1.59	1.19	0.77– 1.91
PHE	1.25	1.02	1.44	0.80	1.13	1.10	1.20	2.48	2.80	1.90	0.72	0.77	0.72– 2.80
TRY	0.85	0.99	0.06	1.21	0.78	0.78	0.82	0.06		0.42	0.90	0.37	0– 1.21
LYS-ORN	4.22	4.26	3.23	3.68	3.85	4.53	4.11	3.02	3.05	3.68	2.71	2.79	2.71– 4.53
HIS	7.63	7.36	7.05	8.32	7.59	7.02	7.39	6.60	6.60	6.90	6.79	6.72	6.60– 8.32
ARG	4.65	1.42	1.88	5.12	3.26	3.32	4.53	1.75	3.56	3.29	3.86	4.82	1.42– 5.12
Unknowns			0.19	1.50		0.35		1.23	0.36	0.36	0.17	3.06	0– 3.06
Urea	+	+	+	+	+	+	+	+	+	+	+	+	+ +
NH_3	+	+	+	+	+	+	+	+	+	+	+	+	+ +
Total	100.00	100.00	100.00	100.00		100.00	100.00	100.00	100.00		100.00	100.00	

and surface film of the upper trunk in adult males and females reported in Table 1. The results for the small sampling of children suggest no outstanding difference in them in free amino acid pattern as compared with adults. Most evident variation in the 10 chromatograms is in the presence or absence of several unknown substances. A number of substances may occur prior to the elution of aspartic acid: cysteic acid and urea have been identified among this complex; a third substance occasionally occurs just prior to urea; a fourth substance just prior and a fifth substance, just after aspartic acid. Moore, *et al.*[24] note the occurrence of methionine sulfoxide and methionine sulfone at these latter two loci. The amount of ammonia varies considerably from specimen to specimen; the values obtained represent not only NH_3 present in the washings but also NH_3 accumulated during processing. Values of from 4 to 12 per cent of the total ninhydrin reactive substances were found in those specimens for which NH_3 was calculated.

2. Free amino acid and water soluble peptide composition of the plantar surfaces in adult males and females and prepubertal children

The quantitative results of the free amino acids and unknown peptides present in the water washings of plantar surfaces in 2 adult males, 2 adult females, and 2 children (1 male and 1 female) are presented in Table 2. All of these were taken 18-30 hours after bath; the A-1 and A-2 results are from the same female at 24 and 20 hours after bath respectively, with collection being several months apart.

The qualitative pattern of the free amino acids is similar in all subjects with one difference, the absence of arginine in the two adult males. The quantitative patterns vary from subject to subject. There is indication of some possible quantitative difference in the arginine-citrulline values for adult males and females in that the average value for the 3 female samples is from 2 to 6 times that for the 2 males and 2 prepubertal children. The three most prevalent amino acids are the same in each sample but do not necessarily occur in simimlar order: serine and glycine occur first and second in most cases with the lysine-ornithine mixture usually third; alanine is usually fourth, and threonine and histidine are fifth and sixth in order of prevalence. As in the upper trunk, the six most prevalent amino acids may account for seventy to eighty per cent of the free amino acids and water soluble peptides (excluding urea and NH_3) present in water washings of stratum corneum of plantar surfaces.

Cysteic acid, urea, NH_3 and several unidentified substances are present in some of the chromatograms. Most of the unidentified components are acidic substances eluted prior to aspartic acid.

In comparing the chromatographic results obtained in plantar surfaces with those in upper trunk, the most obvious changes are the dramatic decreases in amount of citrulline and glutamic acid, the former being one of the six most prevalent amino acids in stratum corneum of the upper trunk. In contrast to the decrease of citrulline and glutamic acid in plantar areas, there is a strong increase in the lysine-ornithine mixture and a considerable increase in valine, isoleucine, leucine, tyrosine, and phenylalanine. In general, the histidine values are lower in the plantar areas of adults than in the upper trunk. The decrease of citrulline in plantar surfaces makes possible determination of proline which is obscured in upper trunk and other areas (Section 3). The results obtained here for proline are

TABLE 2. FREE AMINO ACIDS AND WATER SOLUBLE PEPTIDES PRESENT IN STRATUM CORNEUM AND SKIN SURFACE FILM OF PLANTAR SURFACES. (Collected 20-30 hours after bath. Figures as leucine equivalents and expressed as μmoles/100 μmoles recovered. Adult males and females in fourth decade of life.)

	Adult male	*Adult male*	*Adult female*	*Adult-A1 female*	*Adult-A2 female*	*10-yr. male*	*8-yr. female*	*Range*
$CySO_3H$	0.10		0.11	1.93	0.48	0.13	0.18	0– 1.93
ASP	4.83	3.76	4.23	4.41	5.17	3.71	5.16	3.71– 5.17
THR	5.02	5.45	6.00	4.95	5.89	4.35	5.68	4.35– 6.00
SER	26.50	25.74	15.91	16.11	22.01	24.33	23.50	15.91–26.50
GLU	0.73	0.94	2.78	0.60	0.75	0.64	1.08	0.60– 2.78
CIT	0.21	1.27	2.90	0.97	0.57	0.51	0.36	0.21– 2.90
PRO	3.00	3.00	1.01	3.01	2.25	5.12	2.03	1.01– 5.12
GLY	20.25	18.41	15.03	16.45	17.00	15.36	17.01	15.03–20.25
ALA	7.30	11.11	11.71	8.92	7.48	8.83	9.54	7.48–11.71
½CYS	0.60							0– 0.60
VAL	3.70	2.98	3.34	3.65	4.03	2.56	4.12	2.56– 4.12
MET	0.37	0.10	0.59	0.43	0.52	0.25	1.08	0.10– 1.08
ILEU	2.02	1.33	3.40	2.36	2.30	1.66	3.09	1.33– 3.40
LEU	2.93	2.46	3.98	3.55	3.16	2.18	3.35	2.18– 3.98
TYR	4.31	3.07	4.70	1.93	2.01	2.30	2.58	1.93– 4.70
PHE	2.51	2.07	3.00	1.61	1.73	1.53	1.99	1.53– 3.00
TRY	0.26	1.16	3.98	0.75	3.85		0.44	0– 3.98
LYS-ORN	13.31	13.00	13.83	14.62	14.00	18.30	15.47	13.00–18.30
HIS	2.05	4.15	3.37	5.48	3.45	6.53	2.42	2.05– 6.53
ARG			0.03	4.62	1.93	0.06	0.28	0– 4.62
Unknowns			0.10	3.65	1.42	1.65	0.64	
Urea	+	+	+	+	+	+	+	
NH_3	+	+	+	+	+	+	+	
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	

somewhat higher than those noted by Spier and Pascher[7] for dorsal surfaces and may be characteristic for plantar stratum corneum or may be due to the more sensitive column chromatographic method.

3. *Free amino acids and water soluble peptide composition of water washings of four areas of the body 24 hours after bath*

Table 3 presents the results of the analyses of the free amino acids and water soluble peptides in the stratum corneum and skin surface film of four areas of the body in one subject, a 39-year-old female, 24 hours

TABLE 3. FREE AMINO ACIDS AND WATER SOLUBLE PEPTIDES IN STRATUM CORNEUM AND SKIN SURFACE FILM IN VARIOUS AREAS OF THE BODY 24 HOURS AFTER BATH. (Determined as leucine equivalents and expressed as µmoles/100 µmoles recovered. Female subject in fourth decade of life.)

	Upper trunk	*Upper thigh*	*Lower leg*	*Plantar surface*		*Upper trunk*	*Upper thigh*	*Lower leg*	*Plantar surface*
$CySO_3H$	1.04	0.56		1.93	ILEU	1.22	1.52	1.61	2.36
ASP	5.39	4.89	5.11	4.41	LEU	1.22	1.20	1.61	3.55
THR	5.91	5.25	4.82	4.95	TYR	1.22	1.28	0.07	1.93
SER	29.56	30.56	26.34	16.11	PHE	0.69	1.44	0.50	1.61
GLU	1.74	2.16	0.94	0.60	TRY	0.43	0.73	0.18	0.75
CIT	10.61	10.78	10.46	0.97	LYS-ORN	4.17	2.70	6.26	14.62
PRO	+	+	+	3.01	HIS	6.61	7.34	8.41	5.48
GLY	12.00	13.80	15.86	16.45	ARG	4.17	1.43	1.53	4.62
ALA	10.61	8.58	10.47	8.92	Unknowns	0.75	3.25	2.87	3.65
VAL	2.61	2.48	2.71	3.65	Urea	+	+	+	+
MET	0.05	0.05	0.25	0.43	NH_3	+	+	+	+
					Total	100.00	100.00	100.00	100.00

after bath. The four areas studied were upper trunk, upper thigh, lower leg, and plantar surfaces.

Serine makes up about 30 per cent of the free amino acids and other ninhydrin–reactive compounds (excluding urea and NH_3) of the upper trunk, thigh, and lower leg specimens but only about 16 per cent of the plantar surface specimens. It occurs in greatest concentration in the three former areas and second in plantar surfaces. Glycine, which is the second most concentrated amino acid in upper trunk, thigh, and lower leg, is most prevalent in plantar surfaces. However, the values of glycine and serine do not differ greatly and it was shown in Section 2 that these two acids occurred in similar concentration in plantar surfaces on the six subjects tested. Alanine and citrulline are the third and fourth most prevalent amino acids and are present in comparable amounts except in plantar surfaces where citrulline is present in only minute quantity. In contrast to the modest amount of lysine-ornithine observed in upper trunk and thigh specimens, the concentration of these two acids is increased to the seventh most prevalent amino acid in lower leg skin washings and to third most prevalent acid in plantar surface washings.

Methionine and tryptophan are present only in very small quantities; glutamic acid, phenylalanine, tyrosine, leucine, isoleucine, and valine also are present in small amounts in most specimens. The leucine, valine, and isoleucine values are slightly higher in plantar surfaces than in specimens from other areas of the body.

Glutamic acid, which is present in lower leg skin surface film in about one half the amount measured for upper trunk and upper thigh, likewise shows further decreases in plantar surfaces to about one third that of upper trunk and thigh. Histidine is present in lower concentration in plantar surfaces than in the other three test areas. Proline, not determined in upper trunk, thigh, and lower leg because of the masking effect of citrulline, is easily demonstrated in plantar surfaces because of the dramatic decrease of citrulline in this area. The low concentration of citrulline appears to be an inherent characteristic of plantar surfaces and is not necessarily due to conversion of citrulline to arginine by micro-organisms (Tables 2 and 3).

4. *Effect of time since bathing on the free amino acid and water soluble peptide composition of the stratum corneum and skin surface film of upper trunk and plantar areas*

The results of the effect of time since bathing on the free amino acid and peptide composition of water washings from upper trunk and plantar surfaces in one subject, a 39-year-old female, are presented in Table 4. All specimens were collected during winter except for the 20-hour specimen, collected in the summer.

The results obtained for many of the free amino acids for the 20 to 24-hour periods after bath are remarkably similar both in upper trunk and in plantar surfaces; the experimental error is about $\pm$ 5 per cent. Results for threonine, serine, glutamic acid, citrulline, alanine, valine, isoleucine, leucine, tryptophan, phenylalanine, and lysine-ornithine are fairly similar in plantar surfaces. For a number of amino acids there is some increase or decrease during the first 48 hours, followed by a tendency at 96 hours toward the 20 to 24-hour results.

The order of occurrence of the six most concentrated amino acids in the upper trunk skin washings at 20 and 24 hours is the same as noted in Section 1: serine, glycine, citrulline, alanine, histidine, and threonine. At 48 hours, glycine becomes the most concentrated acid, followed by citrulline and serine; at 96 hours, serine is first, glycine is second, and threonine is third. These changes are in part mirrored by similar changes in plantar surfaces. The order of occurrence in plantar surfaces at the 20 to 24-hour periods is the same as noted in Section 2: glycine, serine, lysine-ornithine, alanine, histidine, and threonine. At 48 hours, glycine becomes the most concentrated acid followed by lysine-ornithine, serine, alanine, histidine, and aspartic acid; at 96 hours, serine is again first, followed by glycine, lysine-ornithine, alanine, threonine, and aspartic acid.

TABLE 4. FREE AMINO ACIDS AND WATER SOLUBLE PEPTIDES PRESENT IN THE SKIN SURFACE FILM AND STRATUM CORNEUM IN ONE SUBJECT AT FOUR TIME INTERVALS AFTER BATHING. (Determined as leucine equivalents and expressed as µmoles/100 µmoles recovered. Subject in fourth decade of life.)

	Upper trunk				*Plantar surfaces*			
Hours after bath	*20*	*24*	*48*	*96*	*20*	*24*	*48*	*96*
$CySO_3H$	0.73	1.04			0.48	1.93		
ASP	3.59	5.39	6.84	3.41	5.17	4.41	6.07	5.18
THR	6.03	5.91	8.01	12.72	5.89	4.95	5.63	5.44
SER	30.99	29.56	13.03	25.20	22.01	16.11	10.14	23.00
GLU	1.63	1.74	2.17	2.87	0.75	0.60	0.75	1.67
CIT	10.06	10.61	16.20	11.53	0.57	0.97	0.75	3.29
PRO	+	+	+	+	2.25	3.01	3.25	3.00
GLY	16.96	12.00	20.04	15.65	17.00	16.45	19.99	16.34
ALA	11.42	10.61	12.36	10.80	7.48	8.92	9.26	9.01
VAL	2.61	2.61	3.17	2.55	4.03	3.65	3.75	3.84
MET		0.05	0.33		0.52	0.43	0.50	0.20
ILEU	1.32	1.22	1.34	0.91	2.30	2.36	2.50	2.37
LEU	1.11	1.22	0.33	0.81	3.16	3.55	3.50	3.11
TYR	1.13	1.22	1.50	3.02	2.01	1.93	2.38	3.38
PHE	0.57	0.69	0.67	0.53	1.73	1.61	1.88	1.29
TRY	0.39	0.43	0.33	0.56	3.85	0.75	0.87	0.28
LYS-ORN	3.42	4.17	1.83	2.41	14.00	14.62	17.90	12.57
HIS	4.73	6.61	6.01	4.98	3.45	5.48	6.26	2.91
ARG	2.61	4.17	2.84	2.06	1.93	4.62	3.50	2.36
Unknowns	0.70	0.75	3.00	tr.	1.41	3.65	1.12	0.76
Urea	+	+	+	+	+	+	+	+
NH_3	+	+	+	+	+	+	+	+
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

5. *Comparison of ninhydrin-positive components in aliquots of skin washings before and after hydrolysis*

Table 5 presents the results obtained in four specimens of skin washings from upper trunk. Nineteen amino acids, urea, NH_3 and several unknown substances are present. The amino nitrogen recovery in hydrolyzed aliquots exceeds that of the unhydrolyzed specimens. This is probably because of increased color yield of hydrolyzed peptides and proteins. The results have been normalized to 100 per cent to allow percentage comparison

TABLE 5. COMPARISON OF FREE AMINO ACIDS AND WATER SOLUBLE PEPTIDES PRESENT IN STRATUM CORNEUM AND SKIN SURFACE FILM (UPPER TRUNK) WITH AMINO ACIDS AND PEPTIDES IN HYDROLYZED ALIQUOTS OF SAME SAMPLE. (Figured as leucine equivalents and expressed as μmoles recovered in sample 1, and as μmoles/100 μmoles recovered in samples 1-4.)

	1. Wash μmoles	*1. Hydro. μmoles*	*1. Wash % μmoles*	*1. Hydro. % μmoles*	*2. Wash μmoles*	*2. Hydro. % μmoles*	*3. Wash μmoles*	*3. Hydro % μmoles*	*4. Wash μmoles*	*4. Hydro. % μmoles*
$CySO_3H$		0.081		0.37	0.11	0.82			tr	tr
ASP	0.571	1.019	2.83	4.65	5.33	7.28	1.36	4.05	3.15	7.43
THR	1.276	1.207	6.32	5.51	6.09	4.72	6.26	5.51	5.63	5.28
SER	5.636	3.893	27.92	17.78	27.67	17.12	29.92	22.81	28.04	17.36
GLU	0.599	3.815	2.97	17.43	2.71	8.15	0.87	16.66	2.78	14.80
CIT	1.898	0.935	9.40	4.27	8.69	5.91	11.06	5.92	8.55	3.36
GLY	2.998	3.606	14.85	16.47	13.85	13.72	16.03	16.12	14.28	14.77
ALA	2.146	2.212	10.63	10.11	8.48	6.15	10.85	9.74	9.83	8.98
½CYS						0.52	0.51	0.47	0.59	0.99
VAL	0.598	0.806	2.96	3.69	3.48	3.50	3.02	2.50	3.19	2.98
MET							0.23	0.14		
ILEU	0.247	0.287	1.22	1.31	1.39	1.81	1.27	1.31	1.44	1.21
LEU	0.298	0.698	1.48	3.18	2.02	1.30	1.11	0.99	1.90	1.18
TYR	0.321	0.171	1.59	0.78	1.53	1.48	2.30	0.91	1.91	1.68
PHE	0.146	0.158	0.72	0.72	1.09	1.79	0.90	0.76	1.25	1.38
TRY	0.126		0.62		0.78		0.99		0.85	
LYS-ORN	0.486	0.690	2.41	3.15	4.52	8.13	4.24	4.50	4.22	8.20
HIS	1.359	0.657	6.73	3.00	6.99	8.81	6.84	5.58	7.63	6.66
ARG	0.777	0.308	3.85	1.41	3.31	4.75	1.41	1.49	4.76	3.74
Unknowns	0.704	1.350	3.50	6.17	1.96	4.04	0.83	0.54	tr	tr
Urea	+	+	+	+	+	+	+	+	+	+
NH_3	+	+	+	+	+	+	+	+	+	+
Total	20.186	21.893	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Total with NH_3	21.130	29.076								

of the four specimens; the actual μmoles recovered (in leucine equivalents) are included for Specimen 1 to permit comparison with percentage results.

For many of the amino acids there is no outstanding change in relative concentration before and after hydrolysis. In contrast, serine and citrulline show considerable decreases and aspartic and glutamic acids, considerable increases in concentration in hydrolyzed material. The lysine-ornithine mixture is increased in two hydrolyzed specimens and remains about the same in two others. In the case of aspartic and glutamic acids, these increases probably result from hydrolysis of ninhydrin-negative compounds and from asparagine and glutamine. Glutamine was found to be present in several of the preliminary two-dimensional paper chromatograms. The observed increase in the lysine-ornithine mixture may result from the conversion of citrulline to ornithine on hydrolysis. In two of the four specimens, decrease in amount of citrulline in hydrolyzed specimens is not accompanied by an increase in the amount of the lysine-ornithine mixture, suggesting that the peak taken as citrulline on the chromatograms may contain other substances as well.

Serine, threonine, histidine, tyrosine, methionine, and cysteine are destroyed to some extent during acid hydrolysis, as expected. In one specimen the histidine value increased, suggesting, as in the case of aspartic and glutamic acids, the presence of ninhydrin-negative bound forms in washings that yield histidine on hydrolysis. Tryptophan, and possibly proline, are completely decomposed during hydrolysis. Proline, present but not demonstrable by the method employed in unhydrolyzed specimens because of the large amounts of citrulline (Sect. 1), cannot be demonstrated in hydrolyzed material, although the amount of citrulline is decreased in the latter to the point where proline, if present, should be evident.

NH_3 increased greatly in hydrolyzed specimens because of the breakdown of amino acids and other substances; methionine yields methionine sulfoxide and methionine sulfone.

DISCUSSION

The skin surface consists primarily of the stratum corneum, epidermal invaginations, nails, and skin surface film. The stratum corneum is a morphologic layer of fully keratinized, desquamating epidermal fragments; the surface film is a complex substance, derived chiefly from sweat and sebaceous glands and the keratinizing epidermis. Surface film covers the skin surface and hair; it surrounds the desquamating horny cells of the stratum corneum and may extract any non-keratinous substances still present.

Many investigators undertaking physiologic and biochemical studies of the skin surface have termed their substrate "stratum corneum" (or "horny layer") when they were actually referring to "stratum corneum and its surface film." We have maintained the term "stratum corneum" or "horny layer" as used in these earlier studies in the following discussion.

An astonishingly high proportion of the stratum corneum is composed of free amino acids. Spier and Pascher[7] noted averages of 12 per cent of the dry weight of the horny layer and 40 per cent of the water soluble components to be free amino acids (Fig. 2). Dowling and Naylor[10] reported

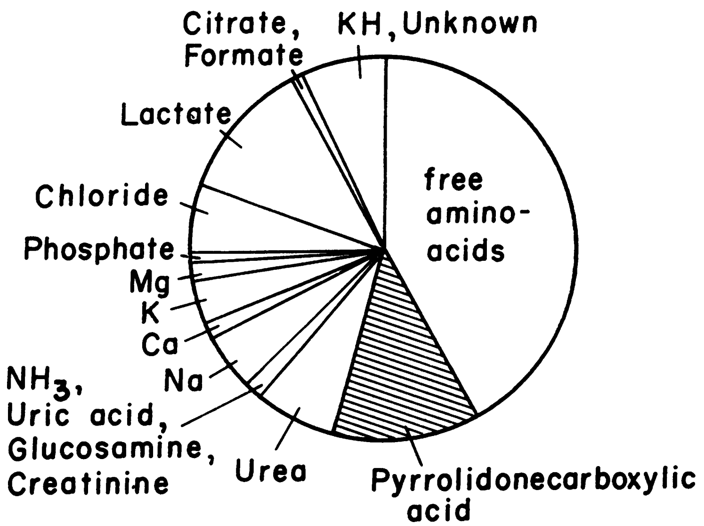

FIG. 2. From Spier and Pascher[7]: "Division by percentage of the water soluble ingredients of the scrapable stratum disjunctum (average values)."

an average of 15 per cent of the dry weight of the stratum corneum to be free amino acids. They found that the amount of free amino acids increases with depth of the stratum corneum and depends, in part, on time since bathing.[11] Burke[2] similarly noted that the total free amino acids in stratum corneum and surface film ranged in upper trunk from 7 per cent 24 hours after bath to 22 per cent 4 days after bath. Early authors[3,5,27,28] considered the main source of these amino acids to be sweat glands. However, since the work of Bollinger,[29] who extracted from hair a large number of free amino acids, evidence has accumulated to indicate that the free amino acids are of epidermal origin.[1,2,11-13,17,19,21,22] To account for their presence in stratum corneum Rothman[1] proposed a theory to the effect that before or during the keratinization process hydrolysis takes place and the sulfur-containing amino acids are incorporated into keratins while other amino acids remain free in the cell wall or cellular debris. A small amount of sulfur-containing amino acids and their products may be found free in the stratum corneum (Table 1).

The free amino acids participate in buffering the skin against alkaline substances and contribute to its low pH. Dowling and Naylor,[10] in studying the buffering capacity of the skin surface by the Burckhardt[30] alkali neutralization method, and Vermeer[31] noted that buffering is greater on the plantar and palmar surfaces than on any other area. They relate this to the greater thickness of the stratum corneum and show that water extracts possess buffering ability proportional to the free amino acid concentration. Other buffering systems also exist. Keratin, being an amphoteric protein, has the ability to neutralize alkalies and acids, though more slowly than the immediately available free amino acids;[10,32,33] CO_2, diffusing from the skin, is said to neutralize alkali,[34] and the lactic acid-lactate system of sweat has been suggested as being a powerful buffer at pH 4-4.45.[35] However, recent studies[7,10,11] indicate that the free amino acids, and in particular the carboxylic groups of amino acids, are the more important buffers on the skin surfaces.

It is popularly held that the free amino acids of the skin surface play an important role in determining the pH of the skin surface film. This pH has been variously reported in recent years to range from about pH 4.2-5.6.[36-38] Spier and Pascher,[7] however, conclude from their studies of the free amino acids of the skin surface that ". . . das pH der Summe der freien AS als um den Neutralpunke liegend Kennzeichnen (pH um 7.2)" (the sum of these acids is more nearly pH 7.2). In contrast, we find that a mixture prepared according to the results in Table 1 and Table 2, at a total amino acid concentration of 100 μmoles per liter, shows a range from pH 4.0-4.8, depending upon site. The pH of the solution of amino acids in plantar surfaces is higher than in upper trunk; this may be attributed to the greater concentration of basic amino acids on plantar surfaces (Tables 1 and 2). Potentiometric readings, made with the Beckman #39182 electrode adapted for flat surfaces, give more acid pH values for trunk than for plantar surfaces. Dowling and Naylor,[10] after extraction of 100 mg. dried stratum corneum in 15 ml. distilled water, obtained solutions with pH below pH 5. Their extracts, however, contained water soluble substances other than free amino acids that may influence the pH.

The results for upper trunk surfaces show quite comparable free amino acid patterns in the group of subjects tested; 10 such patterns representing the results for 4 adult males, 4 adult females and 2 prepubertal children appear in Table 1. Similarly, comparable free amino acid patterns are obtained for plantar surfaces (Table 2). However, the two patterns are quite distinct from one another. A gradient from upper trunk pattern to plantar surface pattern is evident in the changes noted in the study of four areas in one subject (Table 3). Upper trunk and upper thigh yield rather similar results but with indication in upper thigh of the increase or decrease in concentration of certain amino acids which make the plantar surface pattern so distinct from that in upper trunk; these changes are heightened even more in lower leg and, as indicated, increase in plantar surfaces. Notable changes in plantar surfaces as compared with upper trunk are an increase in the lysine–ornithine mixture from about seventh or eighth to third place, increase in amount of more neutral amino acids, a strong decrease in citrulline and a smaller decrease in histidine; four of the seven analyses of plantar surfaces showed no or only trace amounts of arginine (Table 2). The observed increase in ornithine may result in part from conversion of citrulline to ornithine by micro-organisms. Ninhydrin-positive substances including cysteic acid, urea, NH_3, possible taurine, and methionine sulfoxide and several unknown substances are present in many of the analyses. With the exception of NH_3 and urea, these compounds account for only a small percentage of the recovered material. The value of NH_3 varies considerably from sample to sample and is, in part, artificial, because of accumulation during processing: values of from 4 to 12 per cent of the total ninhydrin-positive substances were obtained in those specimens calculated. The color yield of urea in the ninhydrin procedure is poor, only about 3.14 per cent, necessitating multiplying the resultant peak by about 30 to place urea on a molar scale comparable to that of the amino acids.[28] The values of urea vary considerably from specimen to specimen. Spier and Pascher[7] noted urea to constitute approximately 7 per cent of the water soluble substances in skin washings (Fig. 2).

Hydrolysis of the water soluble components of water washings of stratum corneum and surface film yields an amino acid pattern quite different from the free amino acid pattern of unhydrolyzed washings (Table 5). Serine still leads in hydrolyzed material; glutamic acid, a minor constituent in the free amino acid pattern, is increased dramatically almost to supplant serine. Spier and Pascher[7] similarly note this increase in glutamic acid and suggest that it is derived from a thermostable, ninhydrin-negative, acid substance found on the cutaneous surface in large quantities. They identify the substance in part as α-pyrrolidonecarboxylic acid (an inner anhydride of glutamic acid) that could originate from glutamic acid or from the oxidation of proline.

Also in hydrolyzed material, aspartic acid shows a large increase and the lysine-ornithine mixture a smaller increase. In contrast, citrulline decreases markedly and serine slightly. The increased concentration of glutamic and aspartic acids may result from the hydrolysis of ninhydrin-negative compounds and that of ornithine from the hydrolysis of citrulline. Frankel and Reiches,[12] in two-dimensional paper chromatographic studies in human epidermis, similarly note large increases in aspartic and glutamic acids in hydrolyzed epidermal scrapings. Large increases of serine and arginine, evident in their chromatograms, do not occur in our hydrolyzed specimens. This may be due both to their use of epidermis and not just stratum corneum, and to their extraction method, which frees more proteins than one obtains in a study of water soluble components.

Müting,[6] in his study of hydrolyzed epidermis, demonstrated the presence of hydroxy-proline; this amino acid does not occur in stratum corneum.

The free amino acid patterns observed in the subjects studied as well as in samples collected several months apart from the same subject (Tables 1 and 2), indicate that the patterns for the same area of the body are fairly characteristic from subject to subject. In addition, they indicate that, as far as the free amino acids and ninhydrin-positive peptides and proteins are concerned, there are differences in the chemical composition of the stratum corneum and surface film in various areas of the body. Other authors have pointed out differences in the free amino acid patterns of tissues in various species: Awapara, *et al.*,[39] in their studies of rat tissues, found characteristic patterns of free amino acids in each tissue. Similarly, Stein and Moore[40] found the free amino acid pattern of human plasma quite similar from subject to subject and quite different from the free amino acid pattern of human urine. Neither the pattern of human urine nor the pattern of human plasma bears resemblance to that of washings of the stratum corneum and surface film in the four areas tested in this study.

The difference in composition of the stratum corneum and skin surface film in various areas of the body may account for the predilection of certain areas to infections by micro-organisms, to reactions to possibly injurious substances, and to eruptions of unknown etiology.

The effect of time after bathing is not clear in the experiment cited in this paper (Table 4). Certainly more extensive studies should be undertaken before concluding that considerable change can take place in the free amino acid pattern in unbathed skin and that this pattern may become more normal later. Yet such a possibility is not far-fetched. The very clothing worn absorbs surface film and may possibly alter composition by virtue of chemicals inadequately removed during laundering. In addition, the skin is host to numerous microbes that use and modify surface film material for their vital processes. The epidermis continually produces stratum corneum; keratinization continually takes place and free amino acids are released into the cell or cellular debris. The horny cells and surface film with it are constantly shed and new cells added underneath. It would not be surprising, therefore, if an "abnormal" pattern due to chemical and/or microbial activity would be supplanted by a more "normal" pattern on shedding of the surface film and stratum corneum so affected.

The variation in sample taken 48 hours after bath (Table 4) suggests the need for controlled conditions in studies of the free amino acids of the stratum corneum. Furthermore, in view of the differences in composition observed in upper trunk, thigh, and lower leg, as well as plantar surfaces, description of site should be carefully detailed.

SUMMARY

1. Ion exchange chromatographic analysis of water soluble, ninhydrin-reactive substances in stratum corneum and skin surface film of white adult males and females and prepubertal children revealed the presence of 20 free amino acids, together with urea, NH_3, and several unknown ninhydrin-reactive substances.

2. The quantitative and qualitative free amino acid and water soluble peptide patterns of stratum corneum and surface film in upper trunk are similar from subject to subject, with no outstanding differences noted between adult males and females and prepubertal children.

3. Similarly, the free amino acid and water soluble peptide patterns of plantar surfaces are similar from subject to subject with possible exception of low arginine-citrulline values in males.

4. The free amino acid and water soluble peptide patterns for upper trunk and for plantar surfaces are quite distinct from one another and are characteristic for each area of the body studied.

5. There appears to be a gradient of concentration in several amino acids from upper trunk to upper thigh to lower leg to plantar surface.

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