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Amino Acid Content in Fresh Sperm and Sperm Stains

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Aminosäurengehalt von frischem Samen und Samenflecken

Summary. Seminal fluid was studied biochemically for sperm identification in stains. The qualitative and quantitative distribution of amino acids in samples of fresh sperm has been analyzed. These findings were compared to the results of a similar study of fresh samples and stains of urine, saliva, vaginal fluor, and sweat. The results obtained show a specific and relatively constant level of amino acids in each of five biological liquids. There was no significant variation in results between fresh samples and stains.

Key words: Sperm stains, amino acid content – Sperm identification

Zusammenfassung. Um Flecken als Sperma zu identifizieren, wurde Samenflüssigkeit biochemisch untersucht. Es wurde die qualitative und quantitative Verteilung der Aminosäuren in Proben von frischem Sperma analysiert. Diese Ergebnisse wurden mit denen ähnlicher Studien an frischen Proben und Flecken von Harn, Speichel, Scheidensekret und Schweiß verglichen. Die erhaltenen Ergebnisse zeigen eine spezifische und relativ konstante Menge von Aminosäuren in jeder dieser biologischen Flüssigkeiten. Es gibt keine bedeutenden Unterschiede in den Untersuchungsergebnissen von frischen Proben und Flecken.

Schlüsselwörter: Spurenuntersuchung, Sperma – Sperma, Nachweis in Flecken

The diagnosis of sperm stains in the case of a negative morphological examination has always a widely debated problem in forensic medicine, and still has not been resolved satisfactorily. The various physical, chemical, and enzymatic methods proposed have proved inadequate for a firm diagnosis, mostly because they are not specific. Even with the immunologic method, which offers higher reliability, there are some reservations regarding the effective organ specificity of the precipitation antibodies.

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In an attempt to find characterizing features of seminal fluid, we have studied its biochemical composition.

Taking Villanueva's observation [1] as point of departure, we first analyzed the quantitative distribution of the amino acids. This author reports that it is possible to identify a constant amino acid pattern in sperm stains by means of bidimensional electrophoretic paper chromatography. Despite the use of a more highly refined technique, viz., thin-layer bidimensional silica gel chromatography, we obtained results that were unsatisfactory for reproducibility and interpretation, in contrast to the results of the experiments of Keller and Pataki [2] which were limited to the identification of free amino acids. Therefore, we employed an assay method using an amino acid analyzer. This method allows for a reliable differentiation of the various amino acids and their quantitative evaluation contemporaneously. Yoshida et al. [3] used a similar technique for studying the male infertility.

Materials and Methods

The following biological materials were examined:

10 samples of fresh sperm from normospermic, oligospermic and azoospermic subjects;
10 specimens of sperm stains on cotton material, ranging in age from 1 week to 3 years;
5 samples of fresh urine from normal subjects;
5 specimens of urine stains on cotton material, ranging in age from 3 days to 1 month;
5 samples of fresh saliva;
5 specimens of saliva stains on cotton material, ranging in age from 3 days to 1 month;
5 samples of fresh sweat;
5 specimens of sweat stains on cotton material, ranging in age from 3 days to 1 month;
5 samples of vaginal fluor;
4 specimens of vaginal fluor stains on cotton material, ranging in age from 3 days to 1 month.

Urine, saliva, and sweat samples were obtained from different subjects of both sexes. Saliva samples were obtained without stimulation. Fluor samples were obtained from women without vaginal affections.

A) Preparation of Samples

For fresh samples the method is as follows:

1. Add 1 ml absolute ethyl alcohol to 0.5 ml sample;
2. Centrifuge at 4,000 g for 5';
3. Add absolute ethyl alcohol drop by drop to the supernatant until it is clear;
4. Evaporate in water bath;
5. Add 1 ml 6 N HCl;
6. Close test tube under vacuum;
7. Place in oven for 24 h;
8. Dessicate in vials.

Stains, each corresponding to 0,05 ml of fresh biological fluid, were first eluted for 24 h in distilled water and the above procedure was then followed using the eluate.

B) Apparatus

A standard procedure with a taped program for the multisample fully automated JLC-5 AH amino acid analyzer was used, wich required the use of a short column (15×0.8 cm) and a long

Table 1. Chromatographic analysis: mean values and standard deviations

Amino acids	Fresh		Stains	
	$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$ $n = 10$	$s_x = \sqrt{\frac{\sum x_i^2 - n\bar{x}^2}{n-1}}$	$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$ $n = 10$	$s_x = \sqrt{\frac{\sum x_i^2 - n\bar{x}^2}{n-1}}$
Semen				
Aspartic acid	8.92	1.33	9.70	1.76
Threonine	5.77	0.86	5.81	0.88
Serine	9.51	1.48	9.80	1.22
Glutamic acid	23.36	2.43	24.99	0.91
Proline	2.22	0.56	1.89	0.46
Glycine	5.14	0.98	4.50	1.20
Alanine	2.47	0.34	1.88	0.59
Valine	4.95	0.42	5.54	0.72
½ Cystine	—	—	—	—
Methionine	—	—	—	—
Isoleucine	4.46	0.37	4.92	0.29
Leucine	7.60	0.56	7.96	0.64
Norleucine	—	—	—	—
Tyrosine	6.07	1.33	7.38	0.84
Phenylalanine	2.56	0.67	2.65	1.23
Lysine	7.30	4.45	5.42	1.69
Histidine	6.99	1.74	7.85	0.87
Arginine	2.70	1.15	2.38	0.84
Saliva				
Aspartic acid	7.13	0.77	7.88	0.63
Threonine	1.50	0.77	1.90	1.01
Serine	4.55	0.94	5.66	0.97
Glutamic acid	20.42	2.41	21.78	1.54
Proline	28.20	6.09	30.06	6.03
Glycine	11.84	1.52	13.60	1.71
Alanine	3.25	0.83	2.86	1.19
Valine	2.47	0.74	2.83	0.93
½ Cystine	—	—	—	—
Methionine	—	—	—	—
Isoleucine	1.28	0.46	1.50	0.50
Leucine	3.52	1.06	2.90	1.58
Norleucine	—	—	—	—
Tyrosine	3.40	—	—	—
Phenylalanine	2.47	0.46	2.45	—
Lysine	7.08	0.98	6.86	1.11
Histidine	2.74	0.93	2.23	0.61
Arginine	5.03	1.01	2.78	0.97

Table 1 (continued)

Amino acids	Fresh		Stains	
	$\bar{x}=\frac{1}{n}\sum_{i=1}^{n}x_i$	$s_x=\sqrt{\frac{\sum x_i^2-n\bar{x}^2}{n-1}}$	$\bar{x}=\frac{1}{n}\sum_{i=1}^{n}x_i$	$s_x=\sqrt{\frac{\sum x_i^2-n\bar{x}^2}{n-1}}$
	$n=5$		$n=5$	
Urine				
Aspartic acid	6.26	3.22	6.80	3.63
Threonine	2.62	1.06	2.90	1.10
Serine	4.92	1.57	4.86	2.16
Glutamic acid	26.58	2.01	26.68	2.34
Proline	3.52	1.45	4.54	1.75
Glycine	28.02	7.06	26.24	3.45
Alanine	4.04	1.03	3.92	1.16
Valine	2.66	2.10	3.55	2.82
½ Cystine	3.60	—	2.17	—
Methionine	—	—	—	—
Isoleucine	0.98	0.69	0.35	—
Leucine	2.00	1.21	2.17	2.08
Norleucine	—	—	—	—
Tyrosine	2.80	1.36	2.88	1.35
Phenylalanine	3.05	—	—	—
Lysine	5.80	2.69	6.22	3.69
Histidine	10.38	1.89	8.88	4.68
Arginine	—	—	—	—
Sweat				
Aspartic acid	3.96	2.21	4.82	1.02
Threonine	6.28	2.36	3.11	0.79
Serine	7.70	1.43	6.44	1.73
Glutamic acid	25.83	2.61	31.63	3.62
Proline	4.36	0.61	3.52	0.95
Glycine	15.11	2.15	16.18	1.67
Alanine	10.58	2.66	10.57	2.14
Valine	4.11	0.69	3.94	1.12
½ Cystine	—	—	—	—
Methionine	1.17	0.53	—	—
Isoleucine	2.26	0.81	1.98	0.60
Leucine	1.88	0.66	3.09	0.91
Norleucine	—	—	—	—
Tyrosine	2.42	1.25	1.18	0.36
Phenylalanine	2.22	0.48	2.18	1.02
Lysine	10.30	1.40	10.96	2.07
Histidine	2.35	1.13	2.99	1.95
Arginine	1.87	—	—	—

Table 1 (continued)

Amino acids	Fresh		Stains	
	$\bar{x}=\frac{1}{n}\sum_{i=1}^{n} x_i$	$s_x=\sqrt{\frac{\sum x_i^2 - n\bar{x}^2}{n-1}}$	$\bar{x}=\frac{1}{n}\sum_{i=1}^{n} x_i$	$s_x=\sqrt{\frac{\sum x_i^2 - n\bar{x}^2}{n-1}}$
	$n=5$		$n=4$	
Fluor				
Aspartic acid	7.96	0.66	10.94	0.36
Threonine	5.24	0.37	4.73	0.57
Serine	5.56	0.27	6.75	0.44
Glutamic acid	19.71	0.88	21.81	0.66
Proline	4.95	0.20	8.58	0.82
Glycine	6.76	0.32	8.68	0.60
Alanine	6.18	0.53	8.31	0.25
Valine	5.03	0.54	6.31	0.49
½ Cystine	1.35	0.33	—	—
Methionine	1.34	0.51	0.85	—
Isoleucine	3.76	0.26	3.60	0.53
Leucine	8.03	0.36	5.85	0.44
Norleucine	—	—	—	—
Tyrosine	3.01	0.17	1.25	—
Phenylalanine	3.79	0.23	2.04	1.14
Lysine	9.23	0.98	7.93	0.67
Histidine	2.98	0.10	1.58	0.61
Arginine	4.96	1.02	1.84	0.59

column (70 × 0.8 cm), both packed with JLC-R-2 resin. The basic amino acids were resolved on the short column by using 0.35 N sodium citrate buffer at pH 5.28 (171.5 g of $Na_3C_6H_5O_7$. $2\,H_2O$, 32.5 ml of concentrated HCl, 0.5 ml of n-caprylic acid and water to 5000 ml). The acidic and neutral amino acids were separated on the long column by using a stepwise buffer gradient starting with 0.2 N sodium citrate at pH 3.30 (98.5 g of $Na_3C_6H_5O_7$. $2\,H_2O$, 61.5 ml of concentrated HCl, 0.5 ml of n-caprylic acid, 25 ml of thiodiglycol, 400 ml of methyl alcohol and water to 5000 ml) and changing to 0.2 N sodium citrate at pH 4.25 (98.5 of $Na_3C_6H_5O_7$. $2\,H_2O$, 42 ml of concentrated HCl, 0.5 ml of n-caprylic acid, 25 ml of thiodiglycol and water to 5000 ml) after 230 min.

Results and Discussion

The data obtained (the mean values and standard deviations) by chromatographic analysis of sperm, saliva, urine, sweat, and vaginal fluor are reported in Table 1 and illustrated in Figs. 1—5.

In the graphs, amino acid succession was held constant on the base decreasing values of $\bar{x}$ obtained on fresh sperm samples in order to better demonstrate the different amino acid contents in the other biological liquids analyzed.

Regarding *urinary amino acids content* our result are consistent with other authors data [4—6].

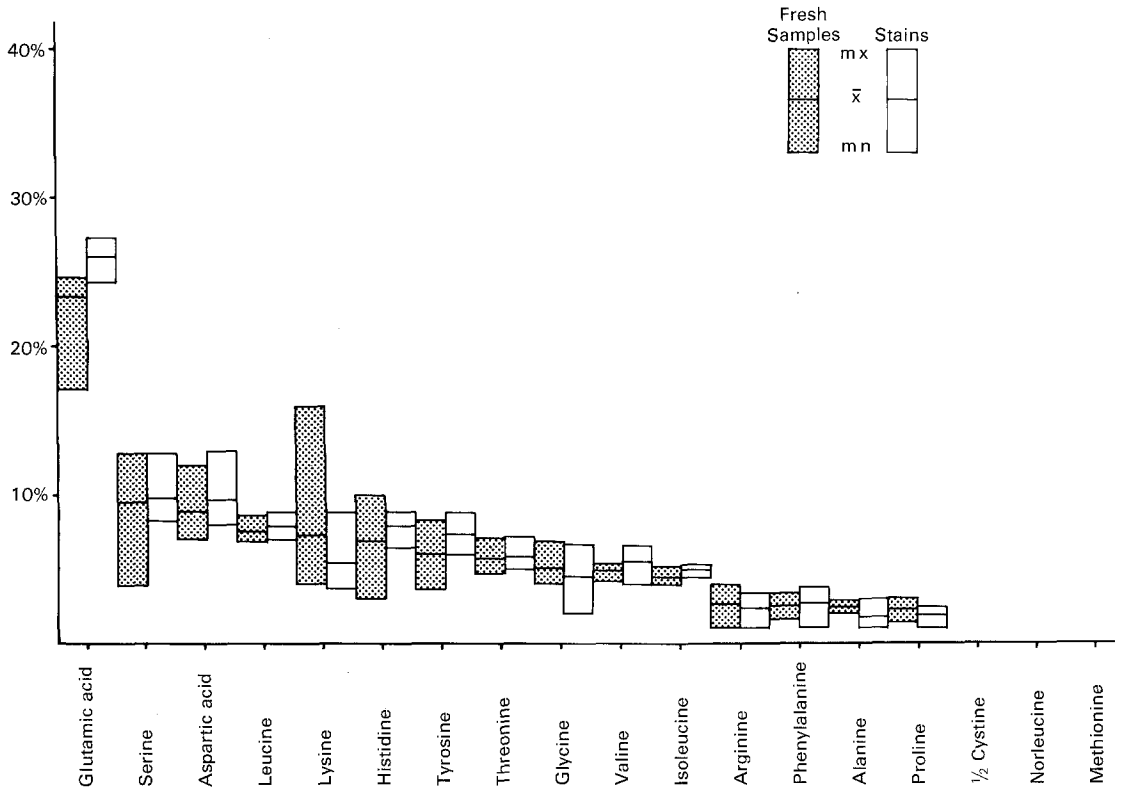

Fig. 1. Semen

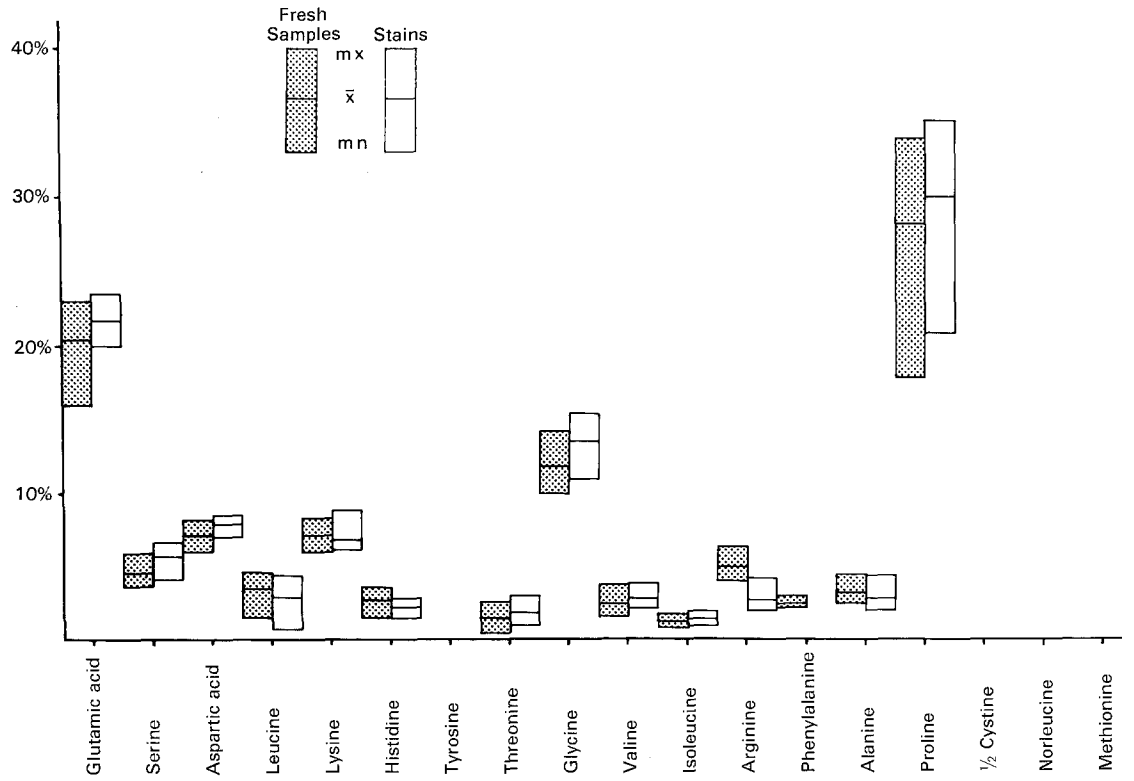

Fig. 2. Saliva

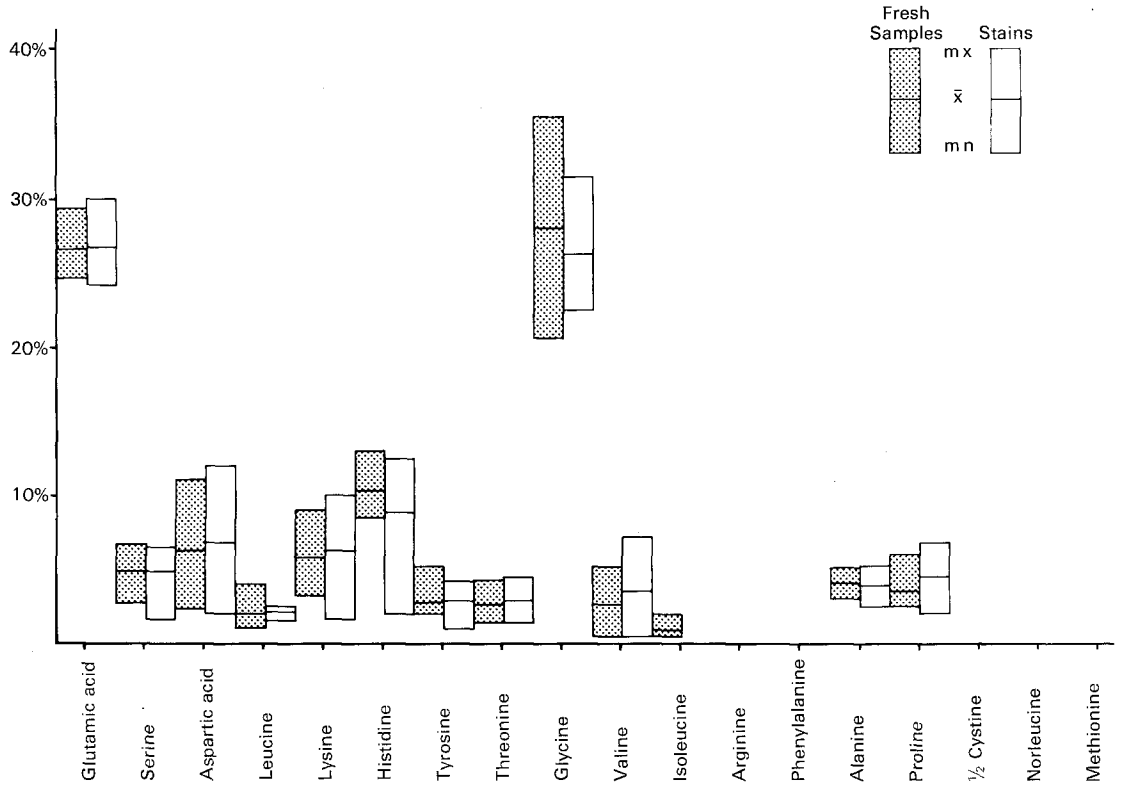

Fig. 3. Urine

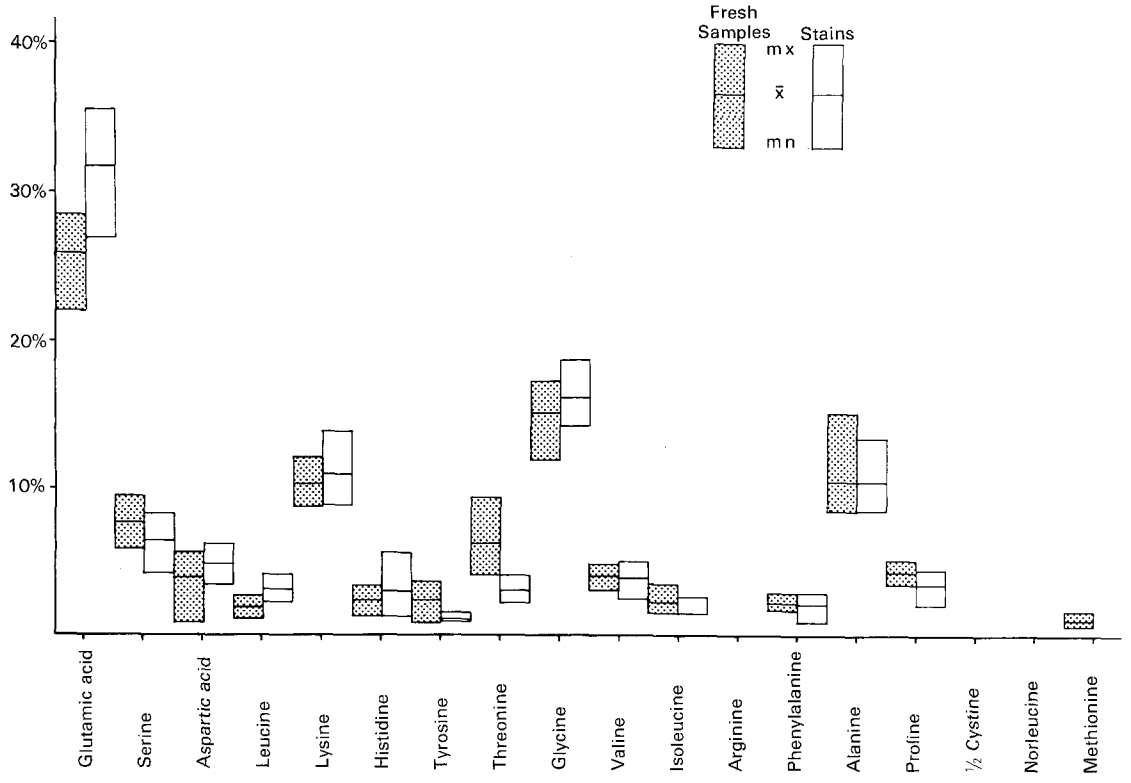

Fig. 4. Sweat

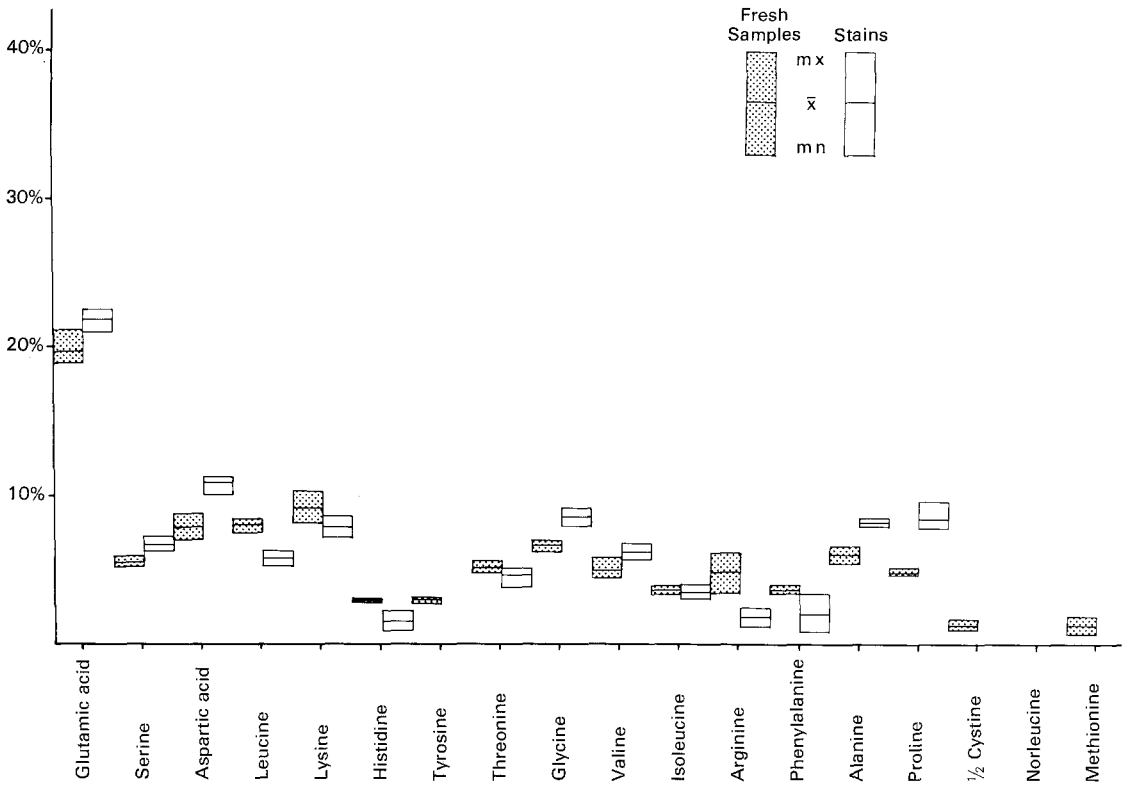

Fig. 5. Fluor

Salivary amino acid content, to our knowledge, has been reported only once [4], and the values we observed are in apparent disagreement with those referred. However, given the diversity of the two determination methods used, a valid explanation is difficult.

No reference can be found on the *amino acid quantitative composition of sweat and vaginal fluor* with which to compare our findings.

As far as the *amino acid content of the sperm* is concerned, the values we obtained are in good agreement with the values reported by Krampitz et al. [7] (Table 2). In particular, it can be observed a very good correspondence between the following amino acids; aspartic acid, serine, proline, glycine, alanine, valine, leucine, phenylalanine. The values of the arginine and the glutamic acid we found are different from those obtained by Krampitz, even through the difference is not so big. All the other amino acids found by Krampitz fall in the range of the values we obtained. In addition, the cystine and the methionine are represented in very small amount, as demonstrated by Krampitz and ourself. Concurring with Yoshida et al. [3] we found no significant differences between normo-, oligo-, and azoospermia in human seminal plasma.

From the analytical data we reported, it can be seen that the biological liquids examined, either fresh or stains, show an almost constant relative percentage of

Table 2

Amino acids	A	B	C
Aspartic acid	8.92	8.38	7.00—12.00
Threonine	5.77	4.00	4.70— 7.10
Serine	9.51	9.38	3.90—12.80
Glutamic acid	23.36	15.00	17.20—24.60
Proline	2.22	2.26	1.40— 3.10
Glycine	5.14	4.95	4.00— 6.90
Alanine	2.47	2.44	2.00— 2.90
Valine	4.95	4.18	4.20— 5.40
½ Cystine	—	0.28	— —
Methionine	—	0.32	— —
Isoleucine	4.46	5.24	3.90— 5.20
Leucine	7.60	8.12	6.90— 8.60
Norleucine	—	—	— —
Tyrosine	6.07	4.32	3.60— 8.30
Phenylalanine	2.56	2.37	1.60— 3.40
Lysine	7.30	12.78	4.00—16.00
Histidine	6.99	9.17	3.00—10.00
Arginine	2.70	6.64	1.00— 4.00

A = Mean values of the amino acids concentration (μg%) from Table 1 (Fresh Samples)
B = Concentration values (μg%) of the amino acids obtained by G. Krampitz and R. Doepfmer in a reported case of normal semen
C = Variability range obtained on the basis of 10 amino acid determinations as reported in Table 1 (Fresh Samples)

Table 3

Biological fluid	Amino acids	
	Glycine	Proline
Semen	< 7	< 3
Urine	> 21	< 7
Saliva	> 10	> 18
Sweat	> 12	< 5
Fluor	< 9	< 10

The concentration limits (μg%) of the two amino acids, as proposed in the table, have been obtained by the round values reported in Table 1

the various amino acids. Such a characteristic amino acids composition seems to make possible the differentiation of the various liquids. The glycine and proline content allows the differentiation of the sperm from the other liquids, as proposed in Table 2. The sperm, in fact, can be differentiated from urine, saliva, and sweat on the basis of the different relative percentual quantity of these two

amino acids. Such a possibility is less notable with regards to vaginal fluor. Of course, the evaluation of the percentual relative amount of other amino acids can further confirm our proposal.

The differentiation of small biological stains can be difficult expecially in order to detect those amino acids wich are present in stains. On the other hand, it must be pointed out that with the chromatographic method we used it is possible to detect very small quantity of amino acids (0.1—0.2 μg).

From our experiments it is almost impossible to obtain the differentiation when the stains are composed of a mixture of different biological materials.

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