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PALEOSEROLOGY OF THE SAYALA CEMETERIES: COMPARISONS AMONG TECHNIQUES, LABORATORIES AND SUBSTRATA

Preliminary Results

ABSTRACT: A paleoserological study of the human remains coming from the three Christian cemeteries of Sayala (Egyptian Nubia), dating back to the 6th – 11th centuries A. D., was carried out applying different techniques (haemagglutination – inhibition and absorption – elution) on different substrata (bones and hair) in two laboratories (Pisa and Prague). The research aimed at evaluating the degree of reliability of paleoserological diagnoses. The results show differences in the sensitivity of techniques and in the reactivity of substrata, while the prevalence of discordant diagnoses in the two laboratories is reasonably low.

KEY WORDS: Paleoserology – Absorption – elution – Haemagglutination – inhibition – Parallel blind test – Christian cemeteries – Egyptian Nubia.

INTRODUCTION

As a part of the Nubian safeguarding action of the UNESCO a common research project of the laboratories in Pisa and in Prague was carried out, concerning paleoserological study of the human remains coming from the Christian fortified settlement near the modern village of Sayala in Egyptian Nubia, dating to the 6th – 11th centuries A. D. (Strouhal 1987).

This research has been intended at comparing the results obtained by two techniques (haemagglutination – – inhibition and absorption – elution) on two different substrata (bone and hair samples from the same subjects). Moreover, a parallel blind test by the same technique (absorption – elution) between two laboratories (Pisa and Prague) has been performed. The aim of the above has been to evaluate the repeatability of the paleoserological results and the reliability of the final diagnoses.

MATERIALS AND METHODS

A total of 98 bone samples were tested in the two laboratories. Further 47 hair samples were tested only in Pisa.

Bone samples, consisting of ribs, carpal and tarsal bone fragments, were generally well preserved. On the contrary, the state of preservation of the hair samples was not very good. The morphological analysis (performed by WILD M5A binocular microscope) showed, in most of the samples observed, five different patterns of incrustation (Tofanelli 1987 – 88) and a reddish-brown colour, as the final result of the partial oxidization of both melanine and keratine (Brothwell and Spearman 1963). The ultrastructural analysis (performed by a Philips SEM) showed a high degree of superficial erosion obtained through the progressive defoliation of the cuticular scales and large amounts of deposits (Tofanelli 1987 – 88) (*Figure 1*).

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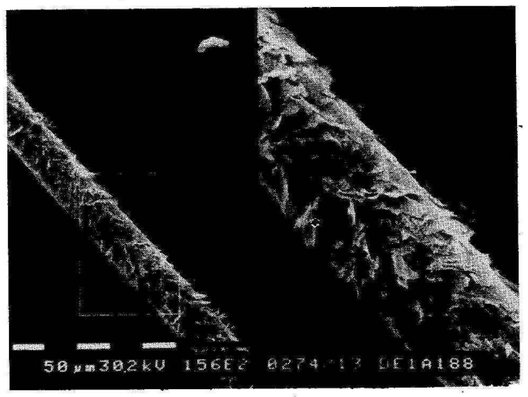

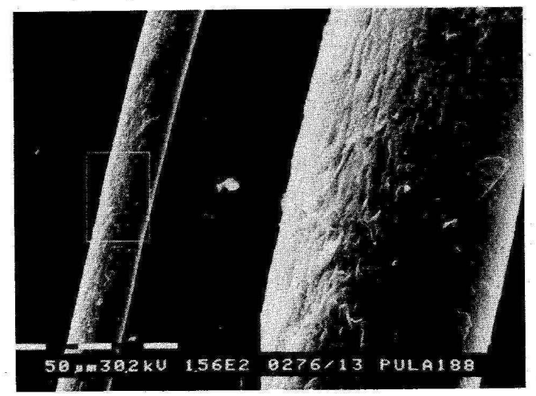

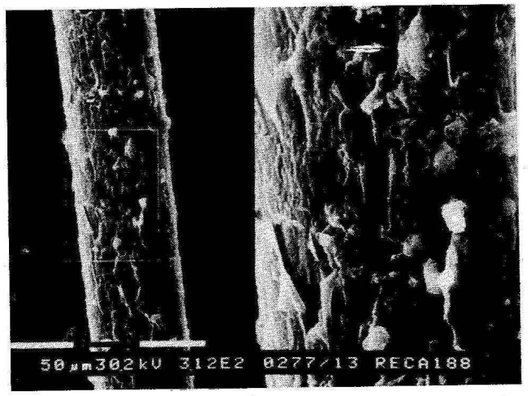

Figure 1. SEM photos of hair samples showing the high degree of cuticular erosion and the presence of scaling and of deposits (top: defoliation, center: erosion, bottom: deposits).

Haemagglutination – inhibition (HI) as described in Paoli et al. 1978, and absorption – elution (AE), as described in Paoli et al. 1986, were applied in Pisa to bone samples. The same AE technique, previously calibrated on a sample of 102 recent hair samples of a known blood group which gave 97.1% concordant positive results, was applied in Pisa to the Nubian hair samples. The AE technique with non-systematic use of anti-H serum and of monoclonal antibodies was applied to bone samples in the Prague laboratory .

RESULTS AND DISCUSSION

Repeated tests with the HI and AE methods were performed in Pisa laboratory on a sample of 97 subjects, 73 samples were tested by the AE technique in Pisa and Prague laboratories and 47 subjects were tested on both bone and hair samples in Pisa laboratory.

Table 1 shows the results obtained for each comparison (between methods, laboratories and substrata respectively) on the level of the final reaction. Overall comparisons between distributions show increasing differences between laboratories, substrata and methods (from columns 1–2 to columns 5–6). A more detailed analysis shows that the frequency of definite positive reactions obtained is significantly higher with the AE than with the HI technique (m^2 test for paired samples = 3.93, d. f. = 1, $P<0.05$). The AE method shows significant differences in the frequency of non-specific and mixed reactions as compared to all the other reactions ($m^2 = 4.50$, d. f. = 1, $P<0.05$) and in the frequency of non diagnosable cases versus all the other cases ($m^2 = 5.44$, d. f. = 1, $P<0.02$). This finding is partly due to the different sensitivity of the two methods. In fact, the HI is considered to be less sensitive because the purification procedure can determine a certain loss of blood-group reacting substances.

The frequency of positive vs negative reactions is significantly higher in the Prague laboratory than in that of Pisa ($m^2=5.44$, d. f.=1, $P<0.02$). The non-systematic use of anti-H serum in Prague could be at the basis of the high frequency of positive diagnoses obtained.

No differences were found in the comparison between substrata in terms of positive vs negative or of non-specific and mixed vs other reactions. Haemolysis was found to affect only the results of the HI method applied to bone samples (9.3%) and of the AE method applied to hair samples (14.8%). In the first case, haemolysis could be due to the great amount of substratum utilized (ten times higher than that required by the AE), in the second case, the phenomenon could be related to the presence of encrusting substances on the hair surface (as described above, see *Figure 1*) which, melting during the elution phase, could modify the ionic equilibrium of the erythrocyte suspension used in the final stage of the reaction (Tofanelli 1987 – 88).

On the whole, independently from the method used, positive diagnoses are less frequent than those obtained in previous paleoserological studies performed in Pisa and in other laboratories (Borgognini Tarli et al. 1986, Lengyel 1975). As a matter of fact, the bone samples we were obliged to use do not represent the most suitable material for paleoserological studies (Borgognini Tarli et al. 1986).

Table 2 shows the results related to the comparisons between methods, laboratories and substrata on the level of final diagnoses. As regards repeated tests between the AE and HI techniques, concordant results (same final diagnoses by the two methods) were obtained in 49.5%, non-discordant results (a positive reaction by one method and a negative, non-specific or other reaction by the other method) in 44.3%, and discordant results (when two different phenotypes are obtained) in 6.2% of cases. A

Table 1. Comparisons between final diagnoses obtained in repeated tests by two different methods (AE and HI), in two laboratories (Pisa and Prague) and on two substrata (bones and hair): absolute and percent frequencies of positive (A, B, H, AB), negative, non-specific (ABH), mixed (AH, BH), haemolysis and indeterminable reactions on samples drawn from the same subject.

	PISA AE bones		PISA HI bones		PISA AE bones		PRAGUE AE bones		PISA AE bones		PISA AE hair	
Reactions	N	%	N	%	N	%	N	%	N	%	N	%
POSITIVE	49	(50.5)	40	(41.3)	29	(39.7)	43	(58.9)	22	(46.8)	20	(42.6)
NEGATIVE	32	(32.9)	45	(46.4)	40	(54.8)	29	(39.7)	22	(46.8)	20	(42.6)
NON SPECIFIC	5	(5.2)	1	(1.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
MIXED	3	(3.1)	1	(1.0)	1	(1.4)	0	(0.0)	1	(2.1)	0	(0.0)
HAEMOLYSIS	0	(0.0)	9	(9.3)	0	(0.0)	0	(0.0)	0	(0.0)	7	(14.8)
INDETERMINABLE	8	(8.3)	1	(1.0)	3	(4.1)	1	(1.4)	2	(4.3)	0	(0.0)
TOTAL	97	(100.0)	97	(100.0)	73	(100.0)	73	(100.0)	47	(100.0)	47	(100.0)

Table 2. Comparisons between final diagnoses obtained by two different methods (AE and HI), in two different laboratories (Pisa and Prague) and on two different substrata (bones and hair): absolute and percentual frequencies of concordant (both positive or both negative), non-discordant (a positive reaction in one case and a negative, non-specific or other reaction in the other case) and discordant (two different phenotypes) results.

	AE bones PISA/ HI bones PISA		AE bones PISA/ AE bones PRAGUE		AE bones PISA/ AE hair PISA	
Results	N	%	N	%	N	%
CONCORDANT	48	(49.5)	29	(39.7)	16	(34.0)
NON DISCORDANT	43	(44.3)	36	(49.3)	26	(55.3)
DISCORDANT	6	(6.2)	8	(11.0)	5	(10.7)
TOTAL	97	(100.0)	73	(100.0)	47	(100.0)

Table 3. Individual cases of discordant results obtained testing repeatedly samples from the same subject by two different methods (AE and HI), in two different laboratories (Pisa and Prague) and on two different substrata (bones and hair).

AE bones PISA/ HI bones PISA		AE bones PISA/ AE bones PRAGUE		AE bones PISA/ AE hair PISA/	
AE	HI	PISA	PRAGUE	BONES	HAIR
A	0	B	A	B	A
A	0	A	0	0	AB
0	A	0	A	A	0
0	AB	0	A	0	A
B	A	A	AB	0	A
AB	A	0	A		
		0	A		
		0	A		
TOT=6		TOT=8		TOT=5	

parallel blind test between Pisa and Prague laboratories by the AE technique gave 39.7% concordant, 49.3% non-discordant and 11.0% discordant results. In the comparison between bone and hair samples belonging to the same subject, concordant results were obtained in 34.0%, non-discordant in 55.3% and discordant in 10.7% of cases.

The frequency of concordant results is low and decreases from different methods to different laboratories to different substrata. Moreover, the frequency of discordant results shows an opposite trend, decreasing from different laboratories and substrata to different methods. The last finding could be anticipated, as the whole differences between laboratories include the use of different substrata and reagents by different research workers, a greater amount of changes as compared to that between two different techniques applied in the same laboratory. Anyway, the frequency of discordant results obtained in the three comparisons is not so far from the prevalence of experimental error (7.3%) evaluated in the Pisa laboratory on recent bones of a known blood-group by the HI technique (Borgognini Tarli et al. 1986).

A more detailed examination of discordant results, as reported in *Table 3*, shows that, in the case of the comparison between laboratories, the frequency of phenotypes diagnosed as H(O) in Pisa and as A in Prague is higher than expected under the condition of random distribution (62.5% of total discordant diagnoses). On the contrary, no preferential distribution is observable in the comparison between substrata and between methods. It has to be noted, however, that the high frequency of O/A discordant results refers mainly to tests in which anti-H serum was not used in the Prague laboratory (80.0% of cases), thus rendering the distinction between possible non discordant (H(O) in Pisa and mixed A–H in Prague) and actually discordant results rather problematic.

More puzzling is the high frequency of non-discordant results, which corresponds to about half of the repeated tests in all the three comparisons reported in *Table 2*. A more detailed analysis (*Table 4*) shows that, at least in the case of the comparison between methods and between laboratories, this finding is related, as already mentioned above, to the

Table 4. Individual cases of non-discordant results obtained testing repeatedly samples from the same subjects by two different methods (AE and HI), in two different laboratories (Pisa and Prague) and on two different substrata (bones and hair).

AE bones PISA/ HI bones PISA			AE bones PISA/ AE bones PRAGUE			AE bones PISA/ AE hair PISA		
+AE −HI	21	(48.8)	+PISA −PRAGUE	10	(27.8)	+BONES −HAIR	10	(38.5)
+AE	7	(16.3)	+PISA	1	(2.8)	+BONES	4	(15.3)
nd HI			nd PRAGUE			nd HAIR		
tot	28	(65.1)	tot	11	(30.6)	tot	14	(53.9)
+HI −AE	10	(23.3)	+PRAGUE −PISA	23	(63.9)	+HAIR −BONES	10	(38.5)
+HI	5	(11.6)	+PRAGUE	2	(5.5)	+HAIR	2	(7.7)
nd AE			nd PISA			nd BONES		
tot	15	(34.9)	tot	25	(69.4)	tot	12	(46.2)
TOTAL	43	(100.0)		36	(100.0)		26	(100.0)

Table 5. Comparisons between absolute and percentual frequencies of positive concordant, negative concordant, non-discordant (positive by the AE and negative or other non-diagnosable reaction by the HI), non-discordant (positive by the HI and negative or other non-diagnosable reaction by the AE) and discordant results (two different phenotypes) obtained in repeated tests by the AE and HI methods in the remains from Sayala and in those from St. Laurent (Aosta, Paoli et al.1986).

	ST. LAURENT (Paoli et al. 1986) AE bones/ HI bones		SAYALA (present paper) AE bones/ HI bones	
Results	N	%	N	%
POSITIVE CONCORDANT	12	(22.6)	17	(17.5)
NEGATIVE CONCORDANT	11	(20.8)	31	(32.0)
NON-DISCORDANT (+ AE)	15	(28.3)	26	(26.8)
NON-DISCORDANT (+ HI)	13	(24.5)	17	(17.5)
DISCORDANT	2	(3.8)	6	(6.2)
TOTAL	53	(100.0)	97	(100.0)

significantly higher frequency of positive results obtained by the AE method and in the Prague laboratory.

In a previous research performed in Pisa by Paoli et al. (1986) on Italian medieval bone samples, the distribution of concordant, non-discordant and discordant results obtained with repeated tests by the HI and AE techniques (*Table 5*) is surprisingly similar to the one obtained in the present research ($m^2 = 3.24$, d. f. = 4, $P > 0.50$), even though the two series are different as regards their state of preservation, chronology and skeletal elements tested (mostly femora in the case of the medieval series). This finding could be

Table 6. Gene frequencies, σq and D/σ values obtained from the subsamples of positive results for each comparison considered.

	N	I^A	σq	I^B	σq	I^O	σq	D/σ
AE bones PISA	49	21.69	4.43	7.40	2.69	70.92	4.86	−0.40
HI bones PISA	40	29.33	5.59	9.18	3.31	61.48	5.96	+0.13
AE bones PISA	29	30.35	6.63	8.98	3.83	60.68	7.02	−0.42
AE bones PRAGUE	43	39.18	6.05	7.27	2.86	53.55	6.18	+0.39
AE bones PISA	22	23.02	6.78	7.04	3.92	69.94	7.35	-0.40
AE hair PISA	20	40.07	8.86	5.05	3.48	54.87	8.99	-0.25

due to random convergence, or to the fact that the results might be more influenced by the methods used than by the characteristic of the samples analyzed. In this regard, the following considerations can be made:

- positive concordant results could be related to samples still containing a fair amount of well preserved ABO blood-group substances, so that any method, whatever its sensitivity, would be able to detect them;
- negative concordant results could be related to samples having an amount of antigens so low that any method is unable to detect them;
- the high frequency of non-discordant results might reflect the different sensitivity of the two methods (see above);
- discordant results might reflect the incidence of experimental error (predictably a random varying low frequency).

The percentual gene frequencies calculated by the phenotype distributions obtained from the same subjects tested by different techniques, in different laboratories and on different substrata are shown in *Table 6*.

The sampling error σ_q and the D/σ parameter, calculated in order to control the presence of the genetic equilibrium according to the Hardy-Weinberg rule, are also shown in *Table 6*. The genetic equilibrium is always largely verified ($P > 0.50$). Considerable

differences between the gene frequencies obtained with the two techniques, in the two laboratories and on the two substrata can be observed. However, the high values of the sampling error, attributable to the low number of individuals so far typed, must be taken into account in the interpretation of these results. The 1.96 σ values vary, in the three comparisons, between 43% and 67% of the estimated gene frequencies, thus lowering their significance as representative of the genetic structure of the population. Moreover, it has to be noted that the AE technique, as applied in Pisa laboratory, systematically gives higher values of I^O frequencies and lower values of I^A frequencies than the HI technique. A higher frequency of H(O) phenotype by the AE method had already been obtained in previous paleoserological research (St. Laurent, Paoli et al. 1986, Abusir, Pisa laboratory unpub. results). This result could be due to the different behaviour of lectines (−H) as compared to non-immune human sera (−A, −B) when they get in contact with the bone powder. Actually, lectines could be more easily susceptible to be linked by short oligosaccharide sequencies with terminal L-fucose, while human antisera would require longer and more specific sequencies (Borgognini 1966, 1968).

A deeper discussion on the results of the repeated tests on different substrata and between different laboratories would require comparison with analogous data, at present not available in the literature. Moreover, the research is still in progress, increasing the number of the samples tested in both laboratories, while the haemagglutination – inhibition technique will be applied also in Prague laboratory. The discussion of the results presented in this preliminary report will therefore be extended and reconsidered in light of the final results.

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