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DETERMINATION OF BLOOD TYPING ON SKELETAL REMAINS FROM THE HUNGARIAN CONQUEST

A methodological study

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Abstract: *The aim of this study to review the determination and the frequency of ABO phenotypes on skeletal remains with the absorption–elution method.*

The bone samples of 77 individuals from the Hungarian Conquest were examined. In 67 cases the authors got the same results several times but in 10 cases the serologic results were uncertain. Furthermore the authors carried out the calculation of the gene frequency, too.

Key words: *ABO phenotypes; Absorption–elution method; Skeletal material; Hungarian Conquest.*

Introduction

Genetic determinations are important factors in the classification of men and their migration history. The best known is the ABO system because they are distributed throughout body tissue. Since the initial study of Boyd-Boyd (1933), several attempts have been made to identify the antigen-antibody reaction of ancient tissue.

Regarding the mummified material the serological methods are the following: agglutination–inhibition (Boyd–Boyd 1934), serological–micro (Conolly–Harrison 1969), mixed field agglutination (Coombs et al. 1956, Otten–Florey 1964), micro–elution (Badawoy 1977).

For the determination of ABO phenotypes from fresh and ancient bones tissue the numerous versions of the absorption (Ders 1940, Salazár 1951, Thieme et al. 1956, Ezra–Cohn–Cook (1961), and the fluorescent antibody methods (Coons et al. 1941, Lengyel 1975) are generally used. Regarding the absorption methods the inhibition and the elution are the best known.

The inhibition–absorption method was first used by Holzer (1931) for demonstration of erythrocyte membran antigens from the bloodstains, and later it was applied successfully also in the examination of ancient bones (Candela 1936, 1940, 1937, Boyd–Boyd 1939, Berg et al. 1983).

All the researchers who acquired experience in criminological bloodstain diagnostics with this method and all those who used it for demonstration of the group substance in bones got sometimes "false positive" results, especially when the substance examined was exposed to bacterial contamination (Springer et al. 1961, Jenkins et al. 1972, Smith et al. 1983, Hauser et al. 1984). In the examination of archeological bone finds we can always reckon with such influences, too. As the error limit of this method in the criminological examination of bloodstains may reach 5%, it is evident that in the examination of archeological bone finds the error limit can only be wider.

The absorption–elution method was first used by Kind (1960) in criminal diagnostics of the bloodstain. The method gave very good results not only in the demonstration of ABO antigens, but also of the MN and RH factors.) The method can be used successfully also in fresh bones and for demonstration of antigens in archeological bone finds (Yah 1955, Yada et al. 1966, Omoto 1968, Yada et al. 1972). Using this method we must also reckon the influences of bacterial contamination. Yet, we must agree with the statement that an unambiguous result (e.g. demonstration of the "A" or "B" blood group) gives the best information in the examination of archeological bone finds. It is more probable that just because of the higher degree of bacterial contamination we can determine the antigens belonging to the group "B" at the expense of the antigens belonging to the group "A" more often during the examination of ancient bones and in the absence of anti–H antibodies or phytagglutinins group "0" because it may also happen more often that in the course of time the proteins responsible for the specificity of the bones are destroyed.

The fluorescent antibody method (Coons et al. 1941, Lengyel–Nemeskéri 1964, Lengyel 1975, Harsányi 1976, Lengyel 1982) utilizes fluorescent labelled antisera and histological sections. This technique seems to be more sensitive and suitable to screening large numbers of bones. The bone sample is subjected to a special decalcination procedure to make the bone tissue more suitable for microscopic immunofluorescent examination. The diagnostic sera prepared from lecitines are combined with the fluorescent dye in indicated proportions with appropriate controls (Lengyel 1984, Sokal et al. 1987).

Material and Methods

At Sándorfalva–Eperjes (South-Hungary, near to Szeged) skeletal remains were discovered in 1985 and 1986. On the basis of their archeological finds they dated from the Scythian and Sarmatian periods, and the Hungarian Conquest.

A preliminary archeological study was reported by Fodor (1985), whereas the number of the graves from the Hungarian Conquest is 105, and the cemetery was fully discovered. The general anthropological elaboration of 104 skeletons has been in progress.

We could carry out the determination of ABO phenotypes of 77 skeletons with the absorption–elution and inhibition methods. These procedures were chosen because they are in every-day practice for identification of ABO group substance in recent bone samples in our serologic–criminalistic laboratory (Szent-Györgyi Albert University Medical School Szeged).

The methods were used parallelly on the bone samples. In the cases where we obtained contradictory serological reactions we repeated the determination more times. If we could not obtain agglutination with anti–A and anti–B sera, elution was performed also with anti–H phytagglutinin (with Evonymus extract) and with this we controlled the results of the examinations of the "0" group.

In our work we also used the mixed cell agglutination technique what was devised by Coombs et al. in 1956 and used by Otten & Florey (1964) in order to identify A, B, and

H antigens on skin cells of mummies. This technique is used successfully for the demonstration of red blood cell antigens in bloodstains (Harsányi–Gerencsér 1968).

The gene frequency was counted by Berstein's method (1924). For the comparison ABO phenotypes of the living populations were available in our laboratory.

Results and Conclusion

The determination of ABO phenotypes in bone samples of 77 individuals with the absorption–elution method proved more reliable than with the inhibition (titer reduction) method.

Using the mixed cell agglutination technique in the course of the demonstration of antigens in the bones we obtained no evaluable results.

On the basis of the absorption–elution method the distribution of ABO phenotypes of 77 bone samples is presented in Table 1. From 77 individuals we have 10 uncertain and 67 certain determinations. The most frequent is the phenotype "AB" and than "B" regarding the all and the certain cases, too, resp.

Table 1. **ABO phenotypes on bone finds from the Hungarian Conquest (Sándorfalva–Eperjes)**

Cases determined with certainty		Uncertain reactions	Total samples	Phenotypes counted on all cases	Phenotypes counted on certain cases
A	3	6	9	11.69%	4.54%
B	22	1	23	29.67%	32.84%
0	18	1	19	24.67%	26.80%
AB	24	2	26	33.77%	35.82%
	67	10	77	100.00%	100.00%

Gene frequency* n=77	Gene frequency* n=67	Gene frequency** n=1774
p=0.2270 q=0.3180 r=0.4550	p=0.2015 q=0.3430 r=0.4555	p=0.2589 q=0.1922 r=0.5504

*Sándorfalva–Eperjes, 10th century
**Recent population, Szeged

The result of gene frequency corresponding with the gene frequency of recent population is evident (the differences are significant, $p > 0.05$).

It seems, that the absorption–elution method is applicable for the determination of ABO phenotypes on the ancient bone samples. Of course, the results with this method can be accepted in that case if the same results would be provided by other methods (for example fluorescent antibody technique).

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