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VIOLENCE AMONG MESOLITHIC MEN FROM MUGE BIOCHEMICAL EVIDENCE

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A. T Lopes e A. Santinho-Cunha

INTRODUCTION

Two shell-middens near Muge (ca. 7800 to 6500 BP) yielded the largest assemblage of Mesolithic human remnants in Western Europe. Skeletons from Cabeço da Arruda (CA) and Moita do Sebastião (MS) were collected under the leadership of Carlos Ribeiro between 1863 and 1880. The former was excavated again in 1884-1885 (Oliveira, 1892). All specimens obtained during the 19th century are kept at the INETI Geological Museum.

Further excavations directed by António Mendes Corrêa took place in 1930, 1931, 1933 and 1937. Later on, new excavations were carried on at Moita do Sebastião, mostly by Octávio da Veiga Ferreira with much support granted by Olga, marchioness of Cadaval and landlord of the Muge domain (Roche, 1972).

Human specimens were studied by Oliveira (idem), Corrêa (1933 and other papers), Vallois (1930), Ataíde (1940), Ferembach (1974), Lubell & Jackes (1985, 1988) and Lubell *et al*. (1989). Skull description and race were the main concern except for Lubell & Jackes, who were more interested in skeletal biology, environment, pathologies and subsistence.

Later on, a majority of Protomediterraneans was recognized alongside with a few Cro-magnoids, Alpines and hybrids (Ferembach, 1974). On the other hand, Vallois (1930) recognized but one race only, and Lubell & Jackes (1985:9) did not favour any race distinction.

After revision of the IGM skull material, Antunes & Cunha (1992--1993) concluded that there was a single Mediterranean population, whose characters may suggest endogamy. There was evidence of high violence. Modifications at death or just *post mortem* were studied through Forensic Medicine methods. Further observations also dealt with pathologies, sex and age. Conclusions about Pathology and Cultural Anthropology are quite different from previous viewpoints, especially on *ante* and *post mortem* rituals. A very high incidence of environmental hypoplasies points out to nutritional stress, which does not support the often expressed idea of plentiful food resources.

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Mortality was high. Age up to 40 to 50 years was seldom attained. The age at death spectrum is very similar for CA and MS. It shows a majority of young adult males. In general, we found *ca*. two males for each female while a more even proportion or some prevalence of women should be expected.

On the basis of MS innominates, «the group consisted of 41 individuals over 15 years of age; of these 29 were males and only 12 females» (Lubell et al., 1989: 639). This proportion of 2.4 to 1 is the same as found by us for CA (see Material and Methods) and close by the MS estimate of 1.8: 1 based on Ferembach's (l*oc. cit.*) data. According to Lubell *et al*. (1989: 638), «there are several, and perhaps irresolvable, problems with the Moita sample, viz. underrepresentation of infants, juveniles and females». The female deficit of about three men for one woman is especially obvious for the 18 to 40 year age span (Antunes & Cunha, 1992--1993).

Sex/age group's distortion is related to previously overlooked, albeit very common violence, only briefly referred to by Lubell & Jackes (1985: 1,18). High violence study has been developed by us (Antunes & Cunha, 1992-1993), and is against former viewpoints that assumed a peaceful character of the concerned populations. In most cases, there are lesions without remodelling related to traumas largely enough to be *causa mortis*.

We could recognize a succession of actions that were not always performed in the same individual:

Slaining, mostly of young adult males and sometimes of older individuals, women and children by means of skull concussion performed (eventually by several aggressors) with piercing-bruising implements as deer antler point axes;

Cuts on the frontal and less frequently on parietals, temporal and occipital that attain the underlying spongious bone and cannot be mistaken for vascular sulci;

Cuts by means of small implements as flint microlithes into Sun-like engravings/drawings on the skull, which may indicate some kind of cult;

Head crushing with cudgels with the head lying sidewards or rarely with the face downwards on the ground resulting into deformation that may account for the «brachycephaly» of some skulls;

Incomplete corpse burning in small branch fires;

Inhumation in shallow graves with deposition of shells, food and decorative adorning or utilitarian objects on the corpse or close by it;

Necrophagy by scavengers as the fox *Vulpes vulpes* or the polecat *Putorius putorius*.

General evidence and demographic distortion shown by the female deficit strongly point out to sacrifices, ritual killings, and death and burial rituals. Taking into account the high frequency of ritual practices until the inhumation, the female deficit definitely indicates male sacrificing, mostly of young men.

All this as well as deposition of shells and food, ornaments or tools on or close by the corpses show that the concerned sites were not cemeteries fully serving nearby populations. They were instead areas where rituals and sacrifices were performed.

Shells prevented decalcification and therefore the ultimate destruction of skeletons buried in otherwise acid, sandy soils. Deformation had been ascribed to the weight of overlying sediments, which could lead to deformation; even more if associated to bone decalcification (which did not occur). This interpretation is contradicted by the graves being very shallow and on the top of hills, with but a thin covering whose weight was irrelevant. Furthermore, spectacular skull deformation occurs in association with intact, not deformed yet fragile structures as cervical vertebrae.

Deformation is indeed related to concussing that explains the occurrence of a lot of linear, radiate and comminutive fractures. These result from impact of contusing implements that produced lesions, often with loss of bony matter (Antunes & Cunha, 1992-1993).

Striking with axes and cudgel-crushing produced hemorrhage. Skull bones often show blotchy, ferruginous stains — even if the enveloping sediments are not iron-rich at all. Such stains could result from blood spilling in sheets, sometimes with infiltration into the bone tissue. Hemoglobin may survive for a long time (Loy, 1994).

The main goal of the present paper consists of the biochemical study of such stains in order to blood traces recognition and its correlation to violence. It is interesting indeed to verify that some remnants of organic compounds are still preserved in more than 6000 years BP samples as the human bones under study.

MATERIAL AND METHODS

1. General data

Material is often incomplete. The number and percent of cases are therefore presented in relation to the number of specimens where a given character could be observed.

Material: Most human skull and mandible specimens are kept at the INETI Geological Museum and were collected at the Mesolithic sites near Muge: Cabeço da Arruda (CA), with 228 specimens, 125 out of 228 or 54.8% with teeth; and Moita do

Sebastião, with 66 specimens, 40 out of 66 or 60.6% with teeth. Total: 294 specimens have been studied.

The minimum number of individuals may be estimated on the basis of the number of incompatible mandibular specimens in 65 adults + 10 children for CA, and 27 adults + 5 children for MS, or respectively >75 and >32. The total number of individuals is therefore in excess of 107, including 15 children.

Age and sex: Even if sex determination is not always possible, we tried to do so according to the criteria referred to by Shipman *et al*. (1985), and Coma (1991). Antunes & Cunha (1992-1993) recognized 48 male and 20 female specimens, or 2.3: 1 for CA; as for MS (Ferembach, 1974), 16 male and 9 female ones, or 1.8: 1. The largest disproportion concerns less than about 30 years old adults: ca. 4 men for 1 woman, either for CA or for MS.

The most trustworthy, not destructive criterion for the age of death is based on dental eruption until that of the last molars at about 18 years. For later ages, the cranial sutures' synostosis, the loss of alveolar bone, cementosis and dental abrasion differences have been taken into account (Cameron & Sims, 1974; Brocheriou & Roquancourt, 1987).

Pathologies: Evidence on the following items has been recognized — intra-cranial aneurisms, palate malformations, caries, parodontoses, tartar, granulomes, osteitis, environmental dental hypoplasies, cementosis, exostosis and neoplasies.

Traumatic actions: *Ante mortem*, with remodelling; *causa mortis* or just *post mortem*; other *post mortem* actions as cuts and perhaps scalping, star-shaped cuts, hemorrhage and ferruginous stains, fire, and predation by necrophagous carnivores.

Some numerical data: Fractures (Svensson & Wendel, 1965) by increasing intensity order, on 150 (CA) and 65 (MS) specimens where they could be expected: linear, 140 or 93.3% (CA), 54 or 81.8%; *radiate*, 86 or 57.3% (CA), 35 or 53.0% (MS), *comminutive*, 68 or 45.3% (CA), 22 or 33.3% (MS).

2. Biochemical approach

The detection of blood proteins would be most interesting, even if it only can be successful if protein components had not been entirely destroyed, as it tends to occur in old samples. They also were tested in parallel.

Material: Tests were carried on three samples from the TAS 70 (our [Telles Antunes-Santinho] inventory number) CA, an incomplete occipital in association with both parietals. Samples were taken on the right parietal which shows typical ferruginous

stains. The surface areas of the skull bone sample, containing or not the presumed blood stains were scratched with a spatula, the powder being collected:

1 — Gangue, including ash and $CaCO_3$;
2 — Powdered bone from an area without stains;
3 — The same from a stained area.

The sample numbers 1, 2 and 3 are shown on the figures.

METHODS (A, B)

A. CHEMICAL REACTIONS. Attempts were made in order to verify if the gangue and bones (1, 2 and 3 samples) still reacted to the very sensitive tests for blood stain recognition:

The phenolphthalein reaction (Kastle-Meyer), in which the peroxydase in the stain causes the solution to become deep red;
The benzidine reaction on blood peroxydases, producing an intense blue (Nickols, 1956).
Conditions were favorable since no interfering contamination by metallic salts, oxidizing agents, plant enzymes or fresh cellular tissues could be detected.

B. SDS-PAGE (Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis) ANALYSIS . A suspension of powder (samples 1, 2 and 3) was made in 10 mM, Tris-HCl pH = 7.6 (1% SDS). The solutions were shaken in Vortex for several minutes, centrifuged at 10 000 rpm and the supernatant evaporated to a minimal volume (in the order of 10-50 (µl). This extract was applied to Gels that were stained for protein (Coomassie Blue and Silver Nitrate) (Laemmli, 1970; Hames & Rickwood, 1994). Low molecular mass protein markers (Pharmacia) from 94 KDa to 14 KDa were used. Hemoglobin and horse heart cytochrome c were also tested in parallel.

SDS-Page Electrophoresis on an archaeological sample/TAS 70 human left parietal from Cabeço da Arruda shell-midden, near Muge (Mesolithic)

	Sample number		
	1	2	3
Descript.....	Gangue	Bone powder outside	Bone powder from a stained
		ferruginous stains.	area.
Results.....	No band	No band	One *ca.* 14 000 Da band.

The sample 3 band may correspond to hemoglobin monomers, because hemoglobin's molecular mass is 64 500 Da. In a denaturing electrophoresis it could be expected to find a band corresponding in size lo its monomers ca. 16 000 Da.

RESULTS

The Benzidine test is the most satisfactory one. It has been performed in a huge number of cases. In less than one per thousand the positive reaction could be identified as being due to a foreign interfering substance (Nickols, 1956).

A control sample consisting of an early Neolithic skull bone from Gruta da Cesareda without evidence of presumed blood stains was tested with negative results. No reaction was obtained for Phenolphthalein and for Benzidine tests concerning gangue and non stained bone, in contrast with the *clearly positive* reactions for stained bone.

Additional SDS-PAGE analysis indicate per se that low molecular mass bands (ca. 14-16 KDa) that stained for protein were clearly detected, and are reminiscent of globin α-β subunits dissociation. The heme staining was not conclusive.

Results were very positive, even more if account is taken of the tiny quantities involved of not yet destroyed protein components.

CONCLUSIONS

1. For the first time in Portugal, Kastle-Meyer and Benzidine tests for bloodstain recognition yielded positive results, but only in the presumed blood stain areas that have formerly been acknowledged through Forensic Medicine methods.

2. SDS-PAGE analysis results point out to the presence in the same areas of blood compatible proteins and thus confirm that the stains correspond to blood.

3. Ferruginous stains on different skull bones seem therefore to result from blood shillings related to high violence.

4. All this evidence corroborates one another.

5. Blood spilling in association with spectacular traumatic lesions has now been confirmed beyond any doubt.

6. The ochre body painting hypothesis for explaining the ferruginous stains can therefore be entirely excluded.

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FIGURES

TAS 70 human right parietal from Cabeço da Arruda, near Muge (Mesolithic) with ferruginous stains that are interpreted as a result from blood spilling (Antunes & Cunha, 1992-1993: Est./Plate III, fig. 2; this specimen and other skull parts from the same individual were ascribed to a ca. 30 to 35 years old male).

Samples:

1— Earthy gangue.
2— Bone material from close by the ferruginous stains, but outside these stains.
3— Bone material from the area with ferruginous stains.

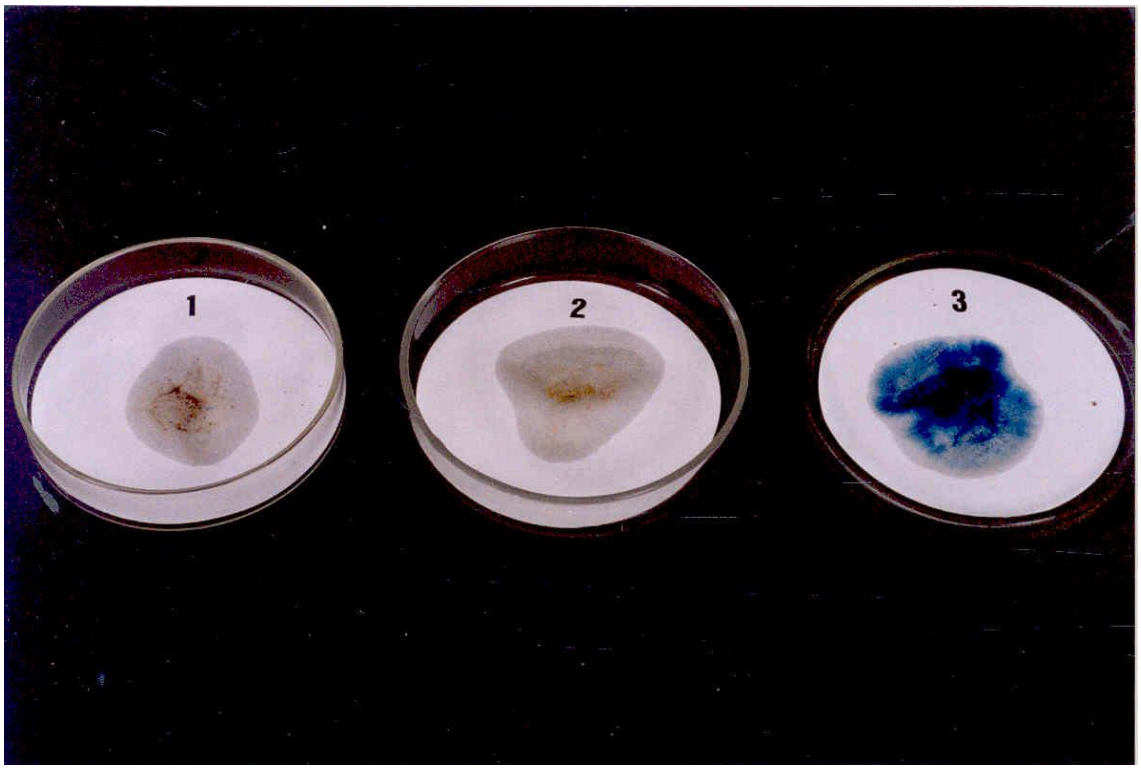

FIGURE 1
Kastle-Meyer (Phenolphthalein reaction, in which the peroxydase in the stain causes the solution to become deep red) — 1 and 2, negative; 3, positive test.

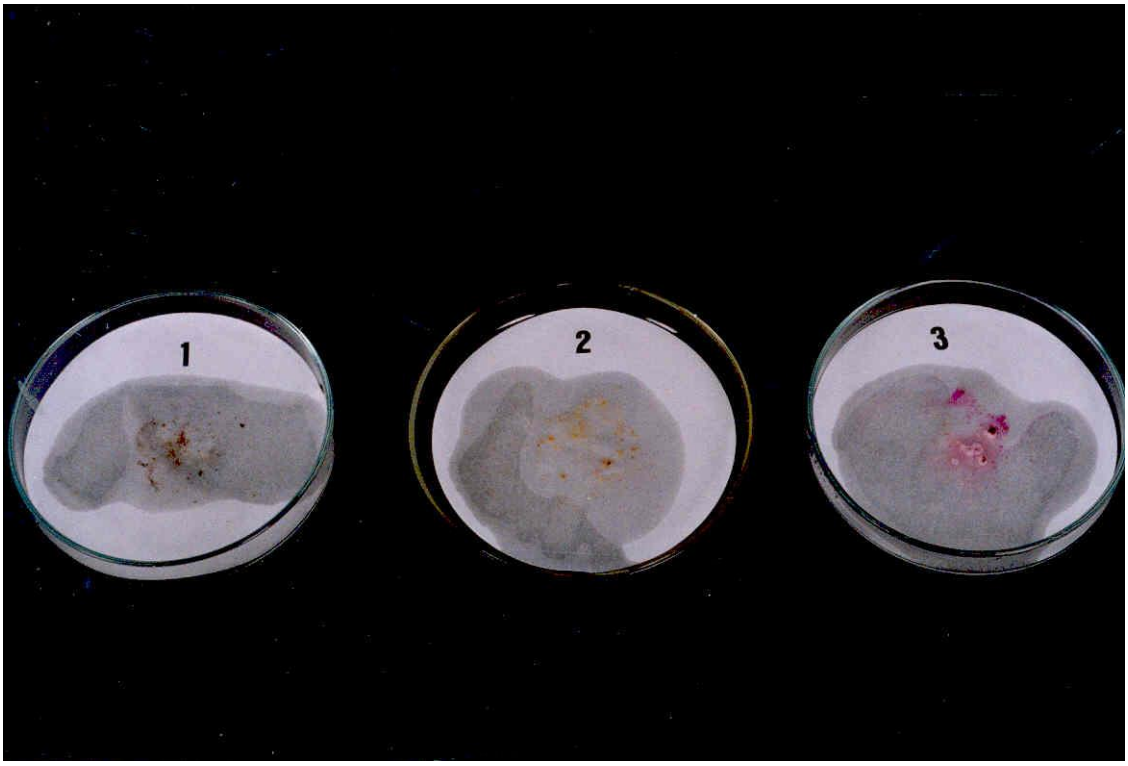

FIGURE 2
Benzidine reaction on blood peroxidases producing an intense blue — 1 and 2, negative; 3, positive test.

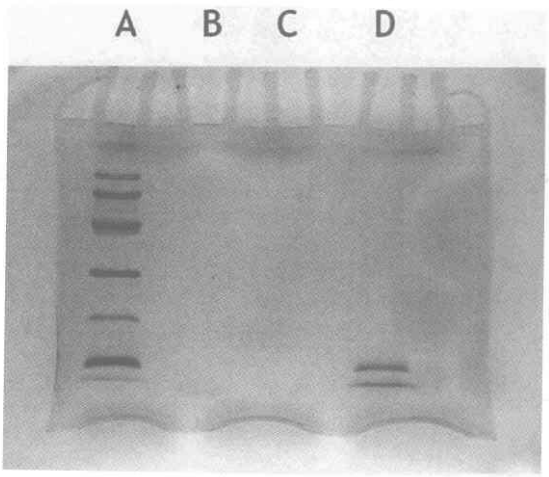

FIGURE 3
SDS-PAGE (Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis) analysis indicating low (ca. 14 KDa) molecular mass protein bands.

A. Protein Standards descending from uppermost to lowermost — 94, 67, 43, 30, 20.1 and 14.4 KDa;
B. Sample 1, negative.
C. Sample 2, negative.
D. Sample 3, positive.

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