Journal of Archaeological Science (1996) 23, 289–296

Protein Identification of Blood Residues on Experimental Stone Tools

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(*Received 26 October 1994, revised manuscript accepted 16 February 1995*)

An experimental butchery of a goat was performed at the site of Olorgesailie, Kenya, utilizing tools made from local source rock. The presence of blood on the experimental tools was tested with a series of commercially available polyclonal antisera. Blood could be identified immunologically on the experimental tools, and laboratory exposure to UV irradiation effectively destroyed all immunoreactivity of the blood on the lithics. Verification of the source of the blood as goat was observed in the relative reactivity of these antisera through a small range of dilution of the rock extract. Albumin was identified as part of the protein fraction remaining on the tools; IgG was not preserved at nanogram levels of detection.

These experiments illustrate the importance of (1) antiserum concentration, (2) antigen (tool extract) concentration and (3) environmental impacts such as sunlight in the interpretations of residues extracted from stone tools.

Keywords: TOOLS, BLOOD, LITHICS, IMMUNOLOGY, ALBUMIN, IgG.

Introduction

The study of blood residues on lithic artefacts derived from archaeological contexts has been a popular focus over the last decade (Loy, 1983; Loy & Wood, 1989; Newman & Julig, 1989; Herr, Benjamin & Woodward, 1989; Hyland *et al*., 1990; Loy & Hardy, 1992; Kooyman, Newman & Ceri, 1992; Newman *et al*., 1993; Tuross & Dillehay, 1995). This line of experimentation has been met with a fair amount of scepticism (Gurfinkel & Franklin, 1988; Smith & Wilson, 1992; Cattaneo *et al*., 1993; Manning, 1994; Remington, 1994). The techniques employed in blood residue analysis have ranged from Hemastix testing, a colorimetric determination of occult blood designed primarily for faecal samples (Hyland *et al*., 1990; Loy & Hardy, 1992) to cross-over electrophoresis (Kooyman *et al*., 1992) and high pressure liquid chromatography (Loy & Hardy, 1992). The technique of haemoglobin crystallization (Loy, 1983; Bahn, 1987; Gurfinkel & Franklin, 1987; Remington, 1994) has been challenged, and five specific criteria have been suggested to impose analytical rigour on the application of crystal testing to lithic residue analyses (Smith & Wilson, 1992).

From the long history and large literature in forensic science it is clear that the identification of degraded molecules is best done with techniques that optimize sensitivity and selectivity (e.g. Sensabaugh, Wilson & Kirk, 1971). By the 1950s, most of the chemical tests for haemoglobin were found wanting for analysing old, dried blood because of both false positive and negative results (Olbrycht, 1950). The interference by both other heme-proteins and microorganisms in a variety of these chemical tests, including haemoglobin crystal formation, compromised their applicability in many forensic cases (Blake & Dillon, 1973). Crystal tests were also found to be less than optimal in the study of dried blood due to the large amount of material needed to precipitate pure protein crystals, and the general qualitative nature of the test (Olbrycht, 1950). While simple, less expensive tests have their place, it is not in the study of ancient blood residues on lithic material.

A variety of immunological approaches have also been used in the study of stone tool residues (Loy, 1987; Lowenstein, 1988; Newman & Julig, 1989; Hyland *et al*., 1990; Tuross & Dillehay, 1995). The cross-over immunoelectrophoresis (CIEP) approach (Newman & Julig, 1989) is a qualitative determination of antigen/antibody binding based on precipitation of

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the antigen/antibody complex; CIEP is in the range of 100 to 1000 times less sensitive than the alternative, enzyme-linked immunosorbant assay (ELISA) or radioimmune assay (RIA). In addition, cross-over electrophoresis requires a rather concentrated antibody solution (Newman & Julig, 1989) which contributes to a large number of false positives.

The enzyme-linked immunosorbant assay (ELISA), as well as RIA, have the advantage of sensitivity and quantitation. The technique lends itself to studies where the putative reaction is ''competed away'' by preincubating the antisera with the antigen (e.g. anti-haemoglobin is preincubated with haemoglobin in order to assess whether the reactivity of the antisera to the tool is obliterated). As with every other technique mentioned, ELISA may exhibit a non-specific reactivity with the soil (Hyland *et al*., 1990; Tuross & Dillehay, 1995), however, because the final data are quantitative, it is possible to establish criteria for scoring positive results. In the analysis of the Monte Verde tool extracts only one of the samples was sufficiently reactive (nine times) above the soil blank to warrant further investigation while the other eight tools reacted with the antibody at less than twice the soil control value (Tuross & Dillehay, 1995). In addition, ELISA assays tend to use dilute (1/1000–1/5000) polyclonal antisera which leads to a lower background in the analysis. Most importantly, the sensitivity of the ELISA may leave the analyst with sufficient material from an ancient tool extract to perform more definitive assays such as the Western blot or even, microsequencing of the amino acid residues in the protein.

Choosing the appropriate technique for blood residue analysis is difficult because there is no generalized view of ancient biomolecule preservation on lithics, little evidence that addresses the impact of molecular degradation on chemical or immunological analyses, and meagre opportunities for replicative experimentation between laboratories. A recent report of experimental stone tools that had been buried for up to 2 years, yielded only one positive result with a monoclonal albumin antibody (Cattaneo *et al*., 1993).

The recent history of blood residue analysis lacks not only a methodological uniformity, but also data pertaining to the macromolecular state of proteins on lithic material. Rather, a wide range of approaches have been tried, some only once by a single investigator, and there is little information regarding the impact of degradation on residue existence or analyses.

A three-way blind study (Downs, Lowenstein & Newman, 1992; Downs & Lowenstein, 1995) of immunological techniques (1) cross-over electrophoresis, (2) a similar gel-based approach called Ouchterlony diffusion and (3) radioimmune assay (RIA—a technique that is similar to ELISA) reported major disagreements in the residue analyses of the archaeological samples: human mummy skin was identified as rabbit by cross-over electrophoresis, and one stain on a lithic tool was identified as possible bovine or deer by Ouchterlony diffusion, unidentified by cross-over electrophoresis and designated human by radioimmune assay (Downs *et al*., 1992). Another comparison between CIEP and RIA indicated that ''none of the positive identifications . . . on archeological specimens agreed with one another'' (Downs & Lowenstein, 1995). One explanation for the discordance in these results derives from differences in selectivity and sensitivity of the methods, and also from the use of different antisera.

In this study we chose an experimental approach in which the archaeologist, as proxy for ancient man, used freshly hafted tools in the butchery of a goat. Two issues tested here are whether sufficient blood was transferred to stone tools in cutting animal flesh, and whether the tools used in a butchery could be distinguished from unused implements. In addition, the ability to transport the tools from an archaeological site, and subsequently extract the adhering residue in a laboratory setting was examined. The tool extracts were analysed using commercially available, relatively inexpensive antisera in order to test the following hypotheses.

(1) Polyclonal antisera to whole serum will allow identification of whole blood, with some selectivity as to the source blood.
(2) Tools used in the butchery experiment will show immunological evidence of blood, and a control tool will be negative.
(3) The animal used in the butchery experiment can be identified immunologically to the level of genus.
(4) Exposure to light of known energy and UV content will affect molecular preservation of blood residues on stone tools.
(5) The types of protein in the blood residue can be identified and quantified.

With a better understanding of which proteins (or protein remnants) are preserved in blood residues on lithics, an estimate of the amount of protein on stone tools, and an assessment of the utility of blood residues in the archaeological context, we can better chart the course of experimental residue analyses. The vagaries and vicissitudes of molecular preservation in the fossil record (Eglinton & Logan, 1991; Tuross & Stathoplos, 1993) require a careful, systematic approach to applications of potentially far-reaching archaeological import. The experimental archaeology and subsequent laboratory analyses reported here represent one ''best case'' scenario for the preservation of blood products on lithic artefacts.

Materials and Methods

Seven sharp stone flakes were manufactured prior to an experimental butchery of an adult male goat. The flakes were produced from tyachyte boulder obtained in the foothills of Mount Olorgesailie, Kenya, close to

Table 1. Tool use and treatment

Tool number	Use	Irradiation treatment
1	Cut skin	Exposed to 500 kJ quartz filter (230 nm cutoff)
2	Cut fatty muscle tissue	Unexposed
3	Cut tendon and joint capsule	Exposed to 283 kJ quartz filter (230 nm cutoff)
4	Dipped in blood	Unexposed
5 a,b,c	Unused	Unexposed

where the butchery was carried out. The boulders and hammerstone were handled with sterile gloves and the flake by-products stored in sterile bags. Butchery was also conducted with sterile gloves. Contact by human hands was thus eliminated.

Four of the seven flakes had direct contact with specific goat tissues during the butchery-skin, fatty muscle, joint capsule and blood (Table 1). Contact between flake and tissue occurred within 20 min of dispatching the goat. The period of contact in each case was approximately 1–2 min. The four flakes were then dried on the ground for one day. Upon collection of the artefacts, a small amount of dirt underlying each tool was also collected.

Laboratory handling and UV irradiation of tools

Although the tools were supplied to the laboratory uncoded, the lithics that had been used in the goat butchery could be easily identified visually by hair adhering to the edge and by fatty residue. It is interesting to note that little in the way of typical dried blood staining was observed on any of the tools.

Of the four tools that were thought to be butchery tools based on visual clues, two were chosen at random and subjected to irradiation in a Weatherometer as described in Table 1. This brief exposure to incident energy in the form of light was designed to test the sensitivity of the adhering butchery residue to irradiation. The UV cutoff on the light source was much lower than an object would normally experience during outdoor surface exposure, however the total incident energy was quite modest and well within the range of ''reasonable'' outdoor exposures (weeks to months, depending on atmospheric conditions).

Enzyme-linked immunosorbant assay (ELISA)

Antibodies used in these experiments were polyclonal antisera to whole blood from goat, pig, turkey, cow and horse (Sigma) made in rabbits. These experiments utilized approximately 10% of the smallest aliquot of commercially available antibody product. All extracts, antisera and solutions were filtered through 0·45 µM filters (Nalgene) in order to remove aggregated material that can affect the background and precision of the experiments. The goat, pig, turkey, cow, and horse blood (Sigma), as well as freshly collected human blood were used to test the relative antibody reactivities and initially diluted at a concentration of 1 to 2000 for the ELISA assays.

The blood or tool extract was applied to the ELISA wells in constant 100 µl volumes, minimally in triplicate. Serial dilutions, one to two, in 0·2 M carbonate/bicarbonate buffer were done in some experiments; in other experiments denaturing conditions in 4 M guanidine HCl were utilized in binding the most concentrated antigen to the ELISA plate. The blood or tool extract was incubated for 2 h at 23°C to bind the antigen to the ELISA wells. After washing with tris buffered saline (TBS), blocking excess binding sites in the ELISA wells was achieved with 200 µl of 1% nonfat dried milk (NFDM) in phosphate buffered saline (PBS) for 18 h (1 h, 23°C and the remaining time at 4°C). The blocking agent was removed, and 100 µl of test antibody (most commonly 1/1000 dilution; see individual figures) in NFDM-PBS was added for 18 h at 4°C. The ELISA plates were washed twice with TBS-tween, and once with TBS, and 100 µl of the affinity purified horseradish-peroxidase (HRP) goat antirabbit second antibody (Kirkegaard and Perry) in NFDM was added for 1 h at 23°C. After washing in TBS-tween and TBS, the bound antibody was visualized by adding 100 µl 0·4 mg ml^{-1} o-phenylenediamine (OPD) in 0·05 M citrate buffer, pH 5, with 0·035% H_2O_2. The reaction was stopped after 60 s with 100 µl of H_2SO_4. The absorbance at 490 nm was determined immediately in an ELISA plate reader (Molecular Devices).

Extraction of blood residues from tools

The stone tools were placed in individual weigh boats and 50 ml of 4 M guanidine HCl, 0·5 M Tris, pH 7·4 were added to cover all edges of each sample. The tools were left to shake on a vertical vibrator for 18 h, and the extracting solution was concentrated by Amicon filtration (YM10) to a volume of approximately 2 ml. This concentrated solution was stored at −80°C. Prior to concentrating each tool extract, 50 ml of 4 M guanidine HCl was reduced in volume in the stirred-cell Amicon filtration unit, and used as a reagent blank in all immunological analyses. The stirred-cell unit is used only for lithic extracts, and thus, is not exposed to high levels of protein.

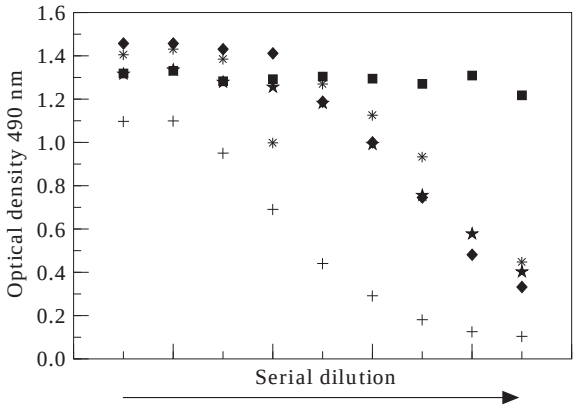

Figure 1. Whole goat blood at a constant concentration of 1/2000 (volume/volume) and the reactivities of serially diluted (1/2) antisera made to goat, horse, pig, cow and turkey blood in rabbits. The initial concentration of each antiserum in the ELISA assay was 1/100. ■: Goat; ◆: horse; ★: pig; *: cow; +: turkey.

Results and Discussion

Fresh blood and antibody analyses

The first experiments examined the relative selectivity and reactivity to whole goat blood of a group of polyclonal antisera made against the serum of several animals (goat, pig, horse, cow and turkey) and purchased from Sigma. In the first experiment a solution (1/2000) of goat blood in binding buffer (see Materials and Methods) was tested with the five test antisera. The antisera were serially diluted from a starting concentration of 1/100 (Figure 1). The antisera made to goat, pig, horse and cow bloods were all nearly equally reactive up to a concentration of 1/1600. Upon further dilution, only goat antiserum remained maximally reactive. The antiserum made to turkey blood was substantially less reactive at all tested dilutions. The data in Figure 1 illustrate the importance of antibody concentration in immunological reactions, and the widespread crossreactivity that can be expected with many blood derived proteins.

Equally important in immunological assays is the concentration of the antigen, and in these experiments, two chemical conditions, nondenaturing and denaturing, were tested. Denaturation is the removal of some or all of the three-dimensional configuration that defines native proteins. The effect of denaturation on immunological reactivity is important because 4 M guanidine HCl (a denaturing agent) was used to remove blood from the tools in this experiment, and, also, because denaturation is a likely diagenetic effect in any aged and oxidized protein product. The questions posed were, (1) is the antiserum made against goat serum more reactive to goat blood than other (pig, horse, cow and turkey) test antisera, and (2) does the chemical environment (e.g. 4 M guanidine HCl) affect relative reactivities of these antibodies? The concentration of antisera chosen for these experiments, based on the results shown in Figure 1 was 1/1000.

In serial dilutions of the antigen (goat blood), the antiserum made against goat serum was more reactive to goat blood than the other antisera tested in both nondenaturing and denaturing conditions (Figure 2(a) and (b)). The curves shown in Figure 2(a) and (b) illustrate several immunological properties of the antigen/antibody reaction. As shown in Figure 1, the antisera to horse, pig and cow serum cross-react with goat blood. At goat blood concentrations greater than 1/8000, the ability to immunologically distinguish goat from horse, pig or cow on the basis of relative reactivity is severely diminished. This cross-reactivity results from shared epitopes, portions of molecules that bind to the antibody. At very low concentrations of blood, represented in the sloping lines in Figure 2(a) and (b), the antisera made to the goat proteins reacts, relatively, much more than the horse, pig or bovine antisera. In this range relative reactivities to the suite of antisera

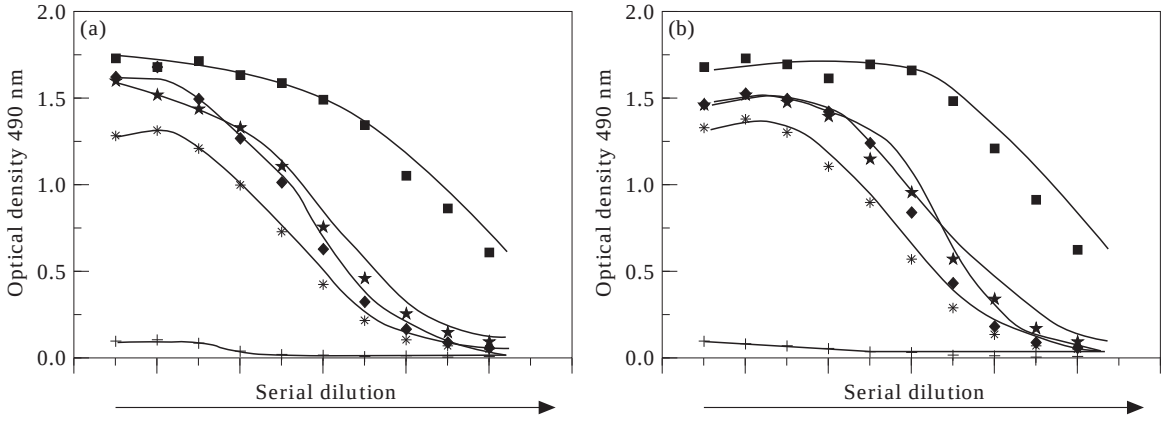

Figure 2. Serial (1/2) dilution of goat blood at an initial concentration of 1/1000 shown in an ELISA assay in which polyclonal antisera to whole serum from goat, horse, pig, cow and turkey were used at a concentration of 1/1000. (a) Illustration of the reactivity and crossreactivity of these antisera to goat blood under nondenaturing conditions, (b) Representation of the same experiments under denaturing conditions. Key as in Figure 1.

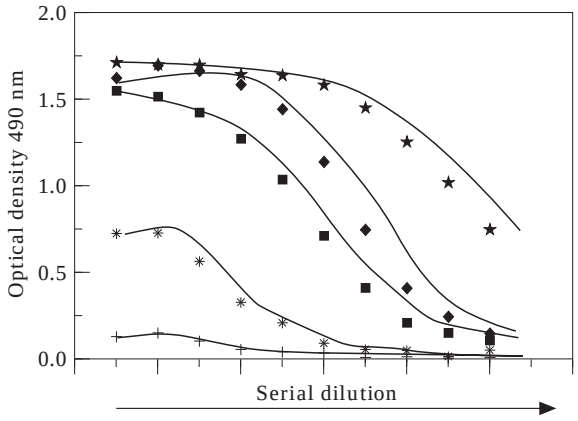

Figure 3. Analogous experiment to that shown in Figure 2, with pig blood substituted for goat blood. Key as in Figure 1.

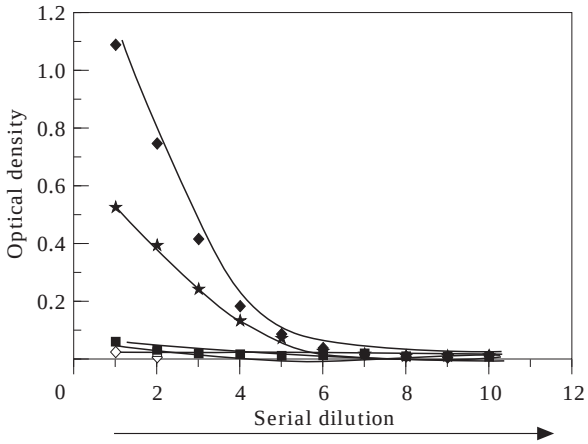

Figure 4. Relative reactivities of test antisera against human blood. ■: Goat; ◆: horse; ★: pig; □: cow; ◇: trout.

could be used to assess the generic origin of blood. Under denaturing conditions in 4 M guanidine HCl (Figure 2(b)), the same general order and profile of reactivity is observed.

The relative reactivities of four of the polyclonal antisera (goat, pig, horse, and cow) to goat blood do not exhibit phylogenetic preference: goats are more closely related to cows phylogenetically; both belong to the family Bovidae. A meaningful concordance between phylogenetic relatedness and relative immunological reactivity, ''immunological distance'', is observed in some studies of serum albumins (Wilson, Carlson & White, 1977; Wyles & Gorman, 1980; Maxson, 1992; Hass *et al*., 1992), however, the impact of diagenetic alterations on relative immunological reactivity has not been studied.

Two additional experiments were done in order to determine the suitability of these antisera for use in these experiments. Goat blood was replaced with pig blood as the test antigen in the ELISA assay (Figure 3), and relative reactivities of the five test antisera were determined. Again, at high dilutions, the antisera made against pig serum was more reactive to the pig blood, demonstrating that the reactivities observed in Figure 2(a) and (b) were not the result of a ''superior'' goat antiserum.

The relative reactivity of the test antisera to human blood was determined (Figure 4), and the goat antisera were found to be unreactive across the concentrations tested. This determination, the exclusion of human source blood, is of critical importance, because post-excavation contamination by human handling is the most likely source of immunologically true, archaeologically false results (Manning, 1994; Tuross & Dillehay, 1995). The entire suite of antibodies tested (excluding turkey) was significantly less reactive to human blood than to goat or pig blood. This overall diminution in reactivity may be due to the method of sample collection or the freeze/thaw cycles to which this sample of human blood had been subjected. In cases where the tool residue is thought to derive from ancient human blood, additional excavation control would be necessary in order to assure analytical accuracy.

In summary, a set of five commercially available polyclonal antisera made by injecting rabbits with the sera from goat, pig, horse, cow and turkey bloods, were tested for their ability in identifying goat blood. Under defined conditions of blood and antisera dilution, goat antisera did exhibit greater reactivity of goat blood than did the antisera to pig, horse, cow or turkey blood. The major potential for this approach, however, is the baseline determination of the *presence or absence* of blood products at the very low levels likely to be found on archaeological artefacts.

Experimental tools

The experiments designed to test the relative reactivities of antisera to a specific modern blood type illustrate the importance of knowing the concentration of the antigen, that is, how much blood is in the tool extract. Because the amount of blood removed from the tool is an unknown, at least in the initial extract, the first test was a direct (not a serially diluted) ELISA assay utilizing the antiserum to goat serum (Figure 5). The concentration of the extract was that obtained after reduction as described in Materials and Methods.

Two of the tools (2 and 4) exhibited a positive reaction to rabbit antigoat antibodies (Figure 5) that was 5–8 times above the chemical and soil extract controls. In contrast, the two tools that were used in the butchery experiment (1 and 3) and subsequently irradiated with UV light did not react to the goat antibody, suggesting that the incident radiation had destroyed the epitopes of any adhering blood residue. Both the chemical and soil matrix controls were approximately equal in background contribution in this experiment, and the extracts from two tools that

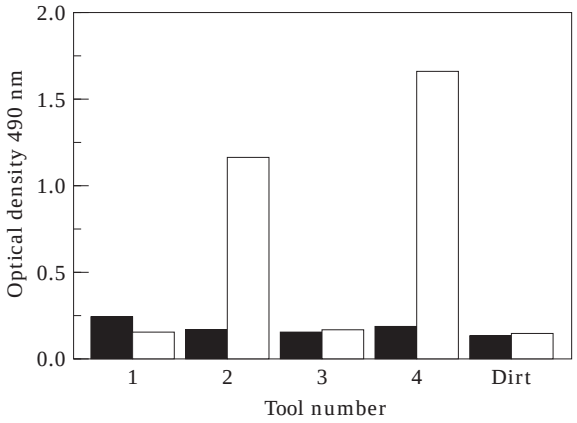

Figure 5. Amount of blood antigoat antibody bound to concentrated tool and soil extracts from the experimental butchery. Tools 1 and 3 were exposed to UV light as described in Table 1. ■: Blank; □: tool.

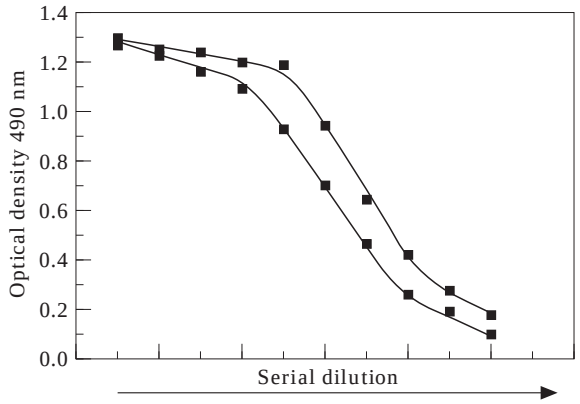

Figure 6. Concentrated tool extracts (2 and 4), serially diluted (1/2), and reacted with goat antisera (1/1000). Both tool extracts exhibit a diminution of immunological reactivity with dilution.

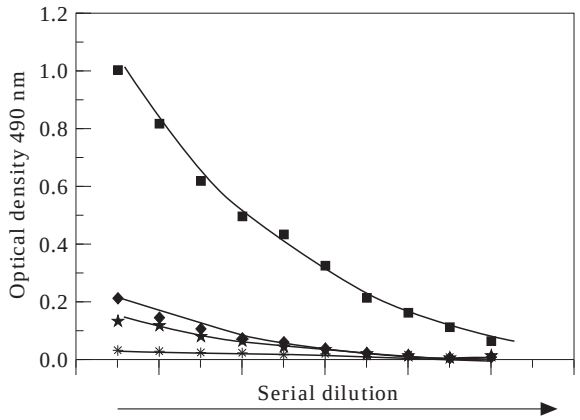

Figure 7. Experimental tool (2) extract reactivity in a serially diluted (1/2) ELISA assay reacted with the four antisera described above. Key as in Figure 1.

had not been used in the goat butchery (5a and 5b) were at or below the background readings of the surrounding dirt extract.

This experiment has important implications for a taphonomic bias in the preservation of immunological reactivity of blood residues on lithics. All else being equal, stone tools that are buried quickly or are otherwise protected from sunlight, are more likely to have adhering blood product preserved. The burial environment of the tools is also critical to molecular preservation as was documented in the work of Cattaneo *et al.* (1993).

In order to determine whether the antibody was reacting to goat blood, a competitive ELISA was performed. The antigoat antibody was preincubated with goat blood, and this antibody was then used in a repeat of the experiment shown in Figure 5. No reactivity was observed in any of the tool extracts or controls, suggesting that the binding of antibody to tool extracts 2 and 4 was the result of preserved blood proteins, and not other unidentified high affinity molecules. The extracts from tools 2 and 4 (Figure 6) did decline in reactivity to the goat antibody when serially diluted one to two. These combinations of experiments, direct ELISA, competitive ELISA and dilution assays, confirm the presence of blood proteins on the experimental tools 2 and 4. These series of experiments also depleted the tool extract from 4.

A more dilute extract from tool 2 exhibited the same relative reactivities, albeit greatly reduced, to the suite of antisera used in the experiments with modern goat blood (Figure 7). That is, based on immunological data, the most likely source of blood on this tool, among the four types tested, was goat (turkey antisera were not used in order to conserve tool extract). It is important to note, however, that if another type of animal, such as impala, had been used as the source blood, these experiments would not identify an ''unknown''. Conversely, if the animal origin of the blood had been truly unknown, and goat antisera had been omitted from the suite tested, the tool extract could have been easily misidentified as cow, pig or horse.

By comparing the amount of antibody bound to the tool extract with the known dilution of whole goat blood, an ''apparent'' concentration of antigen in the tool extract can be roughly estimated as the equivalent of 2 μl of blood. There was very little blood recovered from these experimental tools, even under denaturing conditions.

Protein identification in tool extracts

In the final phase of these experiments, two proteins, albumin and IgG, that exist in high concentrations in serum were tested in tool extract 2 by using antisera that recognize these specific protein targets. Although the presence of immunoglobulins of the IgG class has been previously reported in ancient tool extracts (Loy,

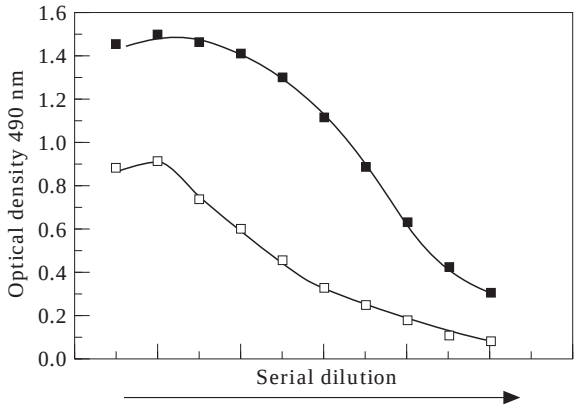

Figure 8. Reactivity of the tool extract (2) to a goat antialbumin antibody (□) compared to a dilution of goat blood (■).

1987; Loy & Wood, 1989), we observed no reactivity to an affinity purified antigoat IgG antibody in tool extract 2.

Albumin reactivity was observed in a serial dilution ELISA utilizing an antibody made against goat albumin in rabbit (Sigma) (Figure 8). The tool extract (2) dilution curve was offset from the goat blood control. This decline in overall reactivity of the tool extract to the albumin antibody likely derives from destruction of labile epitopes in the albumin molecule during the drying process and storage prior to extraction of blood from the tools (Sensabaugh *et al*., 1971).

Conclusions

The single most important concept that emerges from these experiments is the significance of protein concentration in the tool extract as a dependent variable in residue analysis and source determination. Too concentrated an extract in terms of protein content will disallow any discrimination as to the source of the blood, while dilute extracts require the sensitivity of techniques such as ELISA or radioimmune assays (RIA).

The evidence from these experimental tools indicates that it is prudent to assume that very little blood, or at least immunologically reactive product, will exist on any tool. While it can be argued that tools recovered from some archaeological contexts may, through intensive use, have a large amount of blood on them, diagenetic alterations may mitigate this factor.

The lack of immunoglobulins of the IgG class, and the preservation of albumin in the experimental tool extract demonstrates that differential destruction of protein occurs quickly in this type of setting, and is in agreement with the work of Cattaneo *et al*. (1993). A better understanding of which molecules are preserved in blood residues greatly increases our ability to devise targeted immunological experiments. Anticipating that little blood may go to the tool originally; that even fewer molecules survive to the time of analysis, and that extraction procedures may not remove all of the adhering residue, laboratory approaches need to be direct, circumscribed and as sensitive as possible.

The approach described here would allow archaeologists to test for the preservation of blood products on archaeologically recovered materials, and armed with this knowledge make informed choices regarding research designs, curation and conservation. In addition, excess extracted material could be frozen for future analysis. Conversely, lithic material that had no adhering blood residues based on reactivity to a range of polyclonal antisera, could be washed, subjected to edge wear analysis (including the making of moulds), and stored without regard to biomolecule preservation.

For the archaeologist, it is important to define the minimal information that is meaningful in this type of analysis. For instance, is it sufficient to determine that some subset of tools were used on animal products? Is ruling out humans as a source of blood helpful in an archaeological interpretation? Based on faunal evidence, does distinguishing between a small number of source possibilities provide useful information?

There is a wide gap between what is theoretically possible in terms of immunological identification with well characterized, intact blood, and what is realistic when faced with a small and degraded sample of unknown origin. To address the problem of crossreactivity of polyclonal antibodies, the use of monoclonal antibodies has been suggested (Herr *et al*., 1989), and applied (Cattaneo *et al*., 1993). Monoclonal antibodies recognize specific regions of a molecule. With sufficient screening of clones, products that recognize phylogenetically narrow, even sole, sources may be produced. With the exception of a few human and bovine specific monoclonal antibodies, there are precious few monoclonal antibodies that are commercially available *and* of archaeological interest. In addition, many monoclonal antibodies recognize higher order (conformational determinants) in proteins, as opposed to the primary structure (sequential determinants) (Benjamin *et al*., 1984), and the preservation of native protein structure in the archaeological record is untested, and, theoretically, unlikely. The use of monoclonal antibodies is a powerful immunological approach that can be of use, especially in the exploration of the differential preservation of epitopes. With the exception of the possible identification of human blood, monoclonal antibodies will not, for the foreseeable future, provide the ''magic bullet'' to blood residue analysis aimed at determining unknown species.

Perhaps the most unexpected result of these experiments was the amount of blood extracted from the experimental tools. The extracted material was completely exhausted in the analysis, and there was insufficient material for a Western blot (a technique that can provide information as to the molecular weight of the immunoreactive material).

The custom design of experimental approaches to study molecular information found in association with the fossil record may, at times, seem tedious to the archaeologist. If archaeological artefacts presented plentiful, undegraded blood left on unwashed edges, the temporal gap between the experimental design and the interpretive application could be shortened. The impact of time and environment on molecular structure, and effect of that change on fine immunological interpretations is, at present, largely unknown.

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