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IMMUNOLOGICAL ANALYSIS OF LITHIC ARTIFACTS

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In recent years there has been an increased use of molecular, biomolecular and biochemical techniques in the analysis of archaeological materials. Immunological methods have been used to identify plant and animal residues on flaked and groundstone lithic artifacts (Downs, 1985; Hyland *et al.*, 1990; Kooyman *et al.*, 1992; Newman, 1990; Newman and Julig, 1989; Yohe *et al.*, 1991). Plant and animal residues on ceramic artifacts have been identified by their amino acid sequences (Broderick, 1979) and by analysis of lipid and fatty acids (Fredericksen, 1988; Heron *et al.*,1991; Hill *et al.*, 1985) while serological methods have been used to determine blood groups in skeletal and soft tissue remains (Heglar, 1972; Lee *et al.*, 1989) and in the detection of hemoglobin from 4,500-year-old bones (Ascenzi *et al.*, 1985). Human leukocyte antigen (HLA) and deoxyribonucleic acid (DNA) determinations made on human and animal skeletal and soft tissue remains have demonstrated genetic relationships and molecular evolutionary distances (Hansen and Gurtler, 1983; Lowenstein, 1986; Pääbo, 1985, 1986, 1989; Pääbo *et al.*,1989). It has become evident that data obtained from these analyses can contribute valuable information to archaeologists - information that cannot be obtained by other means.

Several immunological methods have been utilized in the analysis of archaeological materials including Ouchterlony (Downs, 1985), cross-over immunoelectrophoresis (CIEP) (Barr, 1989; Newman, 1990), radioimmunoassay (Lowenstein, 1980, 1986) and enzyme immunoassay (Hyland and Anderson, 1990, Hyland *et al.*, 1990). These methods differ only in degrees of sensitivity with Ouchterlony being the least and RIA as the most sensitive. However the use of RIA is limited to a facility and person(s) licensed for nuclear medicine. Immunological techniques were first used in medico-legal work in the early 1900s and despite some dissenters at this time (Gaensslen, 1983, p.223) have continued to play an integral role in forensic medicine. Although the application of these techniques to archaeological materials has been questioned, literature reviews of forensic studies (Arquembourg, 1975; Haber, 1964; Gaensslen, 1983; Lee and DeForest, 1976; Macey, 1979; Sensabaugh *et al.*, 1971, among others), demonstrate that old and denatured bloodstains will still result in a positive precipitin test (Gaensslen, 1983, p.225). While these studies generally deal with relatively recent stains, at least in comparison to the age of most archaeological materials, it has been shown that various efforts to remove bloodstains from clothing or other materials, using solutions such as bleach, harsh detergents or boiling, are generally unsuccessful (Gaensslen, 1983, p.225; Lee and DeForest, 1976). Species identification has also been made on tissues recovered from a sewer (Milgrom and Campbell, 1970) and on body tissues (Bjorklund, 1952; Milgrom *et al.*, 1964). Chemicals present in soils such as tannic acid, aluminum chromate or organic solvents may result in non-specific precipitation of antiserum (*i.e.*, false positive). However, routine testing of site soils indicates the presence of substances that may interfere with artifact analysis, thus validating these tests.

One of the pioneers in the field of forensic medicine was George Nuttall. During the course of his studies he carried out the most extensive testing of antisera in order to determine the

relatedness of animals (Nuttall, 1901a, 1901b, 1904). In this work, more than 16,000 precipitin tests were carried out on over 500 animal species, which included mammals, birds, reptiles, and fish. When one considers that these experiments were carried out nearly 90 years ago, it is a truly remarkable piece of work and, moreover, has been substantiated to a great extent by recent work in molecular evolution. It is interesting to note that many of the problems and sources of error experienced by Nuttall and other researchers are still applicable today. Such problems as the strength and reliability of anti-sera, the pH of the medium, bacterial contamination, the difficulty of re-solubilizing dried blood, and the fact that blood heated over 100°C will not give a positive reaction often occur today as they did in the past (Nuttall, 1904). However, he also noted : «The fact that dried bloods give reactions after the lapse of a considerable time, months, or even years has been fully established by Uhlenhuth and confirmed by others» (Nuttall, 1904, p.120).

Materials and Methods

The method of analysis used in this laboratory is cross-over electrophoresis (CIEP). Minor adaptations to the original method were made following procedures used by the Royal Canadian Mounted Police Serology Laboratory, Ottawa (1983) and the Centre of Forensic Sciences (Toronto). Although this test is not as sensitive as RIA, it has a long history of use in forensic laboratories, does not require expensive equipment, is reasonably rapid and lends itself to the processing of multiple samples (Culliford, 1964). In this test the antigen and antibody are driven together by an electrophoretic force instead of simple diffusion as in the Ouchterlony test. The test is performed in agarose gels with a pH of 8.5, by this the antigen is positively charged and the antibody is negatively charged. Paired wells, roughly 1.5 mm. in diameter are punched in the agarose gel approximately 5 mm. apart. The antigen (unknown extract) is placed in the cathodic well of the pair and the anti-serum in the anodic one. The gel is placed in an electrophoresis tank containing a barbital buffer, pH 8.6, and triple thicknesses of filter paper are used as wicks to connect the ends of the slides with the buffer. The application of an electrical current, set at a constant 100v, moves the two towards each other. If the unknown sample contains protein corresponding to the species antiserum against which it is being tested, an extended lattice forms as a result of cross-linking, and a precipitate forms where they reach equivalence concentrations. Weak positive reactions, common in archaeological samples, are more readily observed if the gel is dried and stained with a protein stain, such as Coomassie Blue. Appropriate positive and negative controls, prepared in 5% ammonia solution, are run with each gel. These are: (a) *positive* - blood of species being tested for *e.g.*, deer blood for deer antiserum and (b) *negative* - blood of species in which antiserum is raised *e.g.*, rabbit if raised in that animal. Duplicate testing is carried out on all positive results.

The specific substances that are tested for in CIEP are immunoglobulins, or antibodies, a group of glycoproteins present in the serum and tissue fluids of all mammals (Roitt *et al.*, 1985). There are five known immunoglobulin groups in normal human serum, IgG (70-75%), IgM (10%), IgA (15-20%), IgD (<1%) and IgE (in trace amounts). IgA is the predominant immunoglobulin in serosecretions such as saliva, tracheobronchial secretions, colostrum, milk, and genito-urinary secretions (Roitt *et al.*, *ibid.*). These are present in varying amounts in all vertebrates, but are absent in invertebrates (Roitt *et al.*, *ibid.*).

Twelve flaked lithic artifacts recovered from the Grotte de Bois Laiterie, Burnot, Belgium, were submitted for potential identification of animal residues by immunological analysis. A control soil sample from the site was also sent for analysis. Possible residues were removed from the artifacts using a 5% ammonium hydroxide solution. This has been shown to be the most effective extractant for old and denatured bloodstains and does not interfere with subsequent testing (Dorrill and Whitehead, 1979; Kind and Cleevely, 1969). Artifacts were placed in shallow plastic dishes and 0.5mL of the 5% ammonia solution applied directly to each. Initial disaggregation was carried out by floating the dish and contents in an ultrasonic cleaning bath for two to three minutes. Extraction was continued by placing the boat and contents on a rotating mixer for thirty minutes. The resulting ammonia solutions were removed with a pipette, placed in individual numbered plastic vials and refrigerated prior to further testing. One milliliter (1 mL) of Tris buffer (pH 8.0) was added to approximately 1g of soil, mixed well and allowed to extract for 24 hours at 4°C to prevent bacterial contamination. The resulting supernatant fluid was removed and tested against pre-immune serum only. Initial testing of all samples was carried out against pre-immune serum (*i.e.*, serum from a non-immunized animal). A positive result against pre-immune serum could arise from non-specific protein interaction not based on the immunological specificity of the antibody (*i.e.*, nonspecific precipitation). No positive results were obtained and testing of artifacts was continued against the antisera shown in Tab.1.

TABLE 1: Antisera used in analysis.

ANTISERA	SOURCE
anti-bear	Organon Teknika
anti-bovine	"
anti-cat	"
anti-chicken	"
anti-deer	"
anti-dog	"
anti-guinea-pig	"
anti-horse	"
anti-human	"
anti-pig	"
anti-rabbit	"
anti-rat	"
anti-sheep	"
anti-elk	University of Calgary

Antisera obtained from commercial sources are developed specifically for use in forensic medicine and, when necessary, these sera are solid phase absorbed to eliminate species cross-reactivity. However, these antisera are polyclonal, that is they recognize epitopes shared by closely related species. The relationship of animal antisera used to potential prey species identified is shown in Tab.2. The antiserum to elk, raised against modern species (*Cervus canadensis*) is species-specific.

TABLE 2: Relationship of animals to antisera used in analysis.

ANTISERA	MOST PROBABLE SPECIES
Bear	Grizzly, brown or black bear
Bovine	Cow, bison, musk-ox.
Cat	Bobcat, lynx, mountain lion, cat.
Chicken	Chicken, turkey, quail, grouse, pheasant.
Deer	Deer (all species), elk, moose, caribou, pronghorn.
Dog	Coyote, wolf, fox, dog.
Guinea-pig	Porcupine, squirrel, beaver.
Horse	Equids
Pig	Pig
Rabbit	Rabbit, hare, pika.
Rat	Rat (all species), mouse (all species).
Sheep	Sheep, goat.

Results

The results of CIEP analysis are shown in Tab.3 and discussed below.

A positive result to bovine antiserum was obtained on one artifact, a simple endscraper (T5-55). As shown in Tab.2, a positive result to bovine antiserum is obtained with bison, cow and musk-ox of the family Bovidae. Cross-reactions with other members of the family Bovidae or other unrelated families do not occur with this antiserum.

One artifact, an endscraper, (U7-10), tested positive to rabbit antiserum. As shown in Tab.2, other members of the Order Lagomorpha (rabbits, hares and pikas) could be represented by this result.

A positive result to pig antiserum was obtained on one artifact, an atypical perforator (U7-29.1). This antiserum has not been tested against Old World members of the Suidea family so species identification is not possible at this time.

No other positive results were obtained in this analysis. The absence of identifiable proteins on artifacts may be due to poor preservation of protein or that they were used on species other than those encompassed by the antisera. It is also possible that the artifacts were not utilized.

TABLE 3: Results of CIEP analysis.

Artifact #	Artifact type	Result
T5-22	Burin	Negative
T5-30	Burin	Negative
T5-55	Endscraper	Negative
T7-16.1	Endscraper	Bovine
U6-127	Burin	Negative
U6-132	Perforator	Negative
U6-149	Burin	Negative
U6-179	Azilian point	Negative
U7-10	Endscraper	Rabbit
U7-29.1	Perforator	Pig
U7-68	Burin	Negative
U10-6	Endscraper	Negative

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