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ORGANIC RESIDUE ANALYSIS OF LITHIC ARTIFACTS FROM LE TROU MAGRITE

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Recent studies have demonstrated that lithic artifacts often retain traces of organic residue resulting from their original use (Briuer 1976; Broderick 1979; Downs 1985; Hyland *et al.* 1990; Kooyman *et al.* 1991; Newman 1990; Newman and Julig 1989; Shafer and Holloway 1979; Yohe *et al.* 1991). Through the use of immunological and biochemical techniques the animal of origin can be identified to at least the family level of identity. This information can be used in the reconstruction of prehistoric subsistence patterns and possibly in identifying artifacts used for specific tasks.

Immunological tests have been used for many years to characterize bloodstains in medico-legal work. Since the introduction of the precipitin test for the medico-legal identification of bloodstains at the turn of the century (Culliford 1964; Gaensslen 1983), several new techniques have been introduced. However, the basis of all subsequent tests is the antigen-antibody reaction first observed in the classic precipitin test (Gaensslen 1983:53). The successful identification of such residues is dependent on the amount and condition of antigen retained in the stain. However, forensic studies have demonstrated that blood proteins can generally withstand harsh treatment and still be identified (Gaensslen 1983; Macey 1979; Sensabaugh *et al.* 1971, among others). The sensitivity and specificity of precipitin reactions makes them an extremely effective method for the detection of trace amounts of protein (Kabat and Meyer 1967:22).

MATERIALS AND METHODS

The method of analysis used in this analysis is cross-over immunoelectrophoresis (CIEP). This is based on the work of Culliford (1964) with minor changes made following the methods of the Royal Canadian Mounted Police (RCM Police) Serology Laboratory (Ottawa) and the Centre of Forensic Sciences (Toronto). The test is extremely sensitive and can detect 10^{-8}g of protein (Culliford 1964:1092). The procedure is discussed fully in Newman and Julig (1989).

Eighteen lithic artifacts from le Trou Magrite Cave, a Palaeolithic site in southern Belgium, were submitted for immunological analysis. Two control soil samples from the site were also sent for analysis. It is important that site soil samples are tested as contaminants in the soil, such as bacteria, tannic acid and iron chlorates, may result in nonspecific precipitation of antisera thus giving false positive results (Gaensslen 1983).

Possible residues were removed from the artifacts by the use of 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 0.5 cc of the 5% ammonia solution applied with a syringe and needle. Initial disaggregation of residue is carried out by floating the plastic dish and its contents in an ultrasonic cleaning bath for two to three minutes. Extraction is continued by placing the boat and contents on a rotating mixer for thirty minutes. The resulting ammonia solution is removed with a pipette and placed in a numbered plastic vial and refrigerated prior to further testing. Approximately 1 ml of Tris buffer (pH 8.0) was added to each of the soil samples. Samples were mixed well then allowed to extract for 24 hours at 4^0C to prevent bacterial contamination. The resulting supernatant fluids were removed and tested against pre-immune serum.

Artifact and soil samples were first tested 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. All artifact extracts were then tested against the antisera shown in Table 10.1. Duplicate testing is carried out on all positive reacting specimens.

Except where noted, the animal anti-sera used in this analysis are primarily obtained from commercial sources and are developed specifically for use in Forensic Medicine. These anti-sera are polyclonal, that is they recognize epitopes shared by closely related species. For example, anti-deer will give positive results with other members of the Cervidae family such as deer, moose, elk and caribou as well as with pronghorn (Antilocapridae family). Three additional antisera, bison, elephant and elk, were raised at the University of Calgary. The bison antiserum was raised against modern species (*Bison bison bison*), however, the immunological relationship between extinct and extant forms is very close so that all will be detected. Similarly, the elephant antiserum was raised against modern African elephant but will elicit a positive reaction with extinct forms of the Order Proboscidea such as mastodon and mammoth (Lowenstein 1986). The elk antiserum was raised against modern elk (*Cervus elaphus*) and is species-specific. Immunological relationships do not necessarily bear any relationship to the Linnaean classification scheme although they usually do (Gaensslen 1983).

Table 10.1 : Antisera used in analysis

ANTISERA	SOURCE
anti-bear	Organon\Teknika
anti-bovine	Forensic Medicine
anti-cat	"
anti-chicken	"
anti-deer	"
anti-dog	"
anti-human	"
anti-rabbit	"
anti-sheep	"
anti-guinea-pig	Sigma Scientific Co.
anti-horse	"
anti-mouse	"
anti-rat	"
anti-swine	"
anti-bison	University of Calgary
anti-elephant	"
anti-elk	"

RESULTS

The results obtained in CIEP analysis are presented in Table 10.2 and discussed below.

Positive results to bovine anti-serum were obtained on two artifacts, a retouched flake and a keeled endscraper, from Trou Magrite. Positive results to this anti-serum occur with members of the Bovini and Ovibini tribes of the Bovidae family, such as bison (extinct and extant forms), cattle and musk-ox. Cross-reactions with other orders do not generally occur.

A positive reaction to rabbit anti-serum was also obtained on the keeled endscraper. Other members of the order Lagomorpha (rabbits, hares, pikas) may be represented by this result but cross-reactions with other orders are not known to

occur. This result implies the processing of lagomorphs or that rabbit sinew or blood was used in a hafting process.

Positive results to human antiserum were obtained on two artifacts from Le Trou Magrite (152 and 71). A positive result to guinea-pig antiserum was also obtained on artifact # 71. Positive reactions to human antiserum occur only with humans and apes. Unless these results indicate prehistoric crime, the most likely explanation is that they represent accidental cuts incurred during use and/or manufacture of the artifacts. It is also possible that skin oils or perspiration from recent handling are responsible for these results, however, if this were true then more positive results would be expected. Strong positive results to porcupine (Erethizontidae) are known to occur with this antiserum while weak reactions to beaver (Castoridae) and squirrel (Sciuridae) also occur.

The absence of identifiable proteins on other artifacts may be due to poor preservation of protein or that artifacts were used on species other than those covered by the anti-sera used. It is also possible that the artifacts were not utilized.

Table 10.2 : Results of CIEP Analysis

Artifact #	Stratum	Artifact type	Result
TM-I7-33	2	Retouched flake	Bovine
TM-I8-23	3	Keeled endscraper	Bovine, rabbit
TM-J7B-79.1	5	Sidescraper	Negative
TM-J8C-110	5	Flint chunk	Negative
138	2	Endscraper	Negative
145.1	2	Sidescraper	Negative
152	2	Endscraper	Human
114	3	Bec	Negative
89	3	Truncation	Negative
71	3	Endscraper	Human, guinea-pig
102	3	Endscraper	Negative
317	5	Denticulate	Negative

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