Reports

Prehistoric Blood Residues: Detection on Tool Surfaces and Identification of Species of Origin

Abstract. *Blood residues from several animal species have been discovered on the surfaces of chert, basalt, and obsidian prehistoric tools (1000 to 6000 years old) from open-air sites along the western coast and in the northern boreal forest of Canada. A screening test has been developed to detect residual blood. Hemoglobin has been crystallized from the residues, and the species of origin determined.*

A major concern of archeological lithic analysts is the determination of the material upon which prehistoric tools were used (*1*). A partial solution to this problem can be obtained from detailed microscopic and chemical examination of tool surfaces. I have examined 104 lithic tools of varying types and materials from four archeological sites in widely differing environments; 90 of them (86 percent) show definite surface blood deposits (Fig. 1). The artifacts examined are between 1000 and 6000 years old (*2*). Using a variety of tests, I have confirmed the presence of amino acids, hemoglobin (Hb), and red blood cells. I have developed inexpensive and reliable techniques to both detect the blood residues and identify the species of origin. Animal and plant tissue, feather barbules, and hair fragments have also been observed. In all cases, some level of identification is possible. I have identified the species of origin of the blood residues by crystallizing the Hb fraction of the residue and comparing it with crystals prepared by the same technique from modern control blood smears of known species.

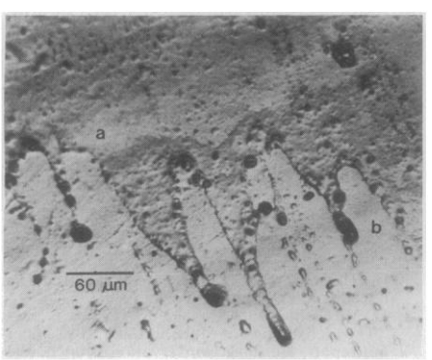

Fig. 1. Residual blood film (a) on the surface of an obsidian flake knife (b) (Photographed with incident light and Nomarski contrast apparatus). This artifact (sample IgSk 7:34) was excavated from a site in northern British Columbia. It is firmly associated with, and probably older than, a radiocarbon date of 2830 ± 210 years.

I have identified blood residues of the following species on prehistoric tools: human, caribou (*Rangifer tarandus osborni*), Columbian black-tailed deer (*Odocoileus hemionus*), Stone Mountain sheep (*Ovis dalli stonei*), moose (*Alces alces*), grizzly bear (*Ursus arctos horribilis*), snowshoe rabbit (*Lepus americanus*), California sea lion (*Zalophus californianus*), and genus *Sciuridae*. The oldest absolutely dated specimen (sample IgSk 7:34, directly associated with radiocarbon samples SFU150 dated at 2830 ± 210 years) indicates the presence of caribou Hb (Fig. 2) and grizzly bear Hb (Fig. 3).

Suspected blood residues have been confirmed as proteinaceous on the basis of the following evidence: (i) their positive reaction to the ninhydrin-Schiff test for amino acids (*3*); (ii) their fluorescence when treated with phthaldialdehyde (*4*); (iii) colorimetric determination of total protein (*5*); (iv) independent amino acid analysis (*6*); and (v) a measured refractive index of greater than 1.55 (*7*). Red blood cells were confirmed as such on the basis of their morphology and staining characteristics (*8*) (Fig. 4). Where relatively thick, the films have a reddish-brown color indicative of reduced Hb; spectrophotometric analysis revealed absorption bands characteristic of reduced Hb (*9*).

My routine examination of the artifacts began with low-power (12 to 30 diameters) microscopic examination of the surfaces; to the experienced eye, many blood films are readily apparent. Suspected blood deposits were then tested with a test strip, used medically in urinalysis (*10*), that is sensitive to small amounts of serum albumin and Hb. The test-strip reagent reacts to porphyrin ring structures and will give a positive reaction to compounds other than Hb, such as myoglobin and chlorophyll (*11, 12*). The presence of chlorophyll-containing algae could prove misleading. Nevertheless, for the artifacts examined to date no evidence of algal contamination has been encountered. Control experiments show that typical midden soils adhering to the tool surface do not interfere with the test-strip reaction; blind tests have consistently discriminated between pristine and blood-smeared controls. Visually suspected and Hb-positive tools were then examined at high magnification (200 to 500 diameters) to observe the morphology of the deposit and the presence of red blood cells and other possible residues such as collagen, tissue, and feather or hair fragments. During the high-power examination, plant residues from prehistoric use were also found (*13*).

The method for species-of-origin determination presented here relies on Hb crystallization by salting-out. Precipitation of Hb in its crystalline form is deter-

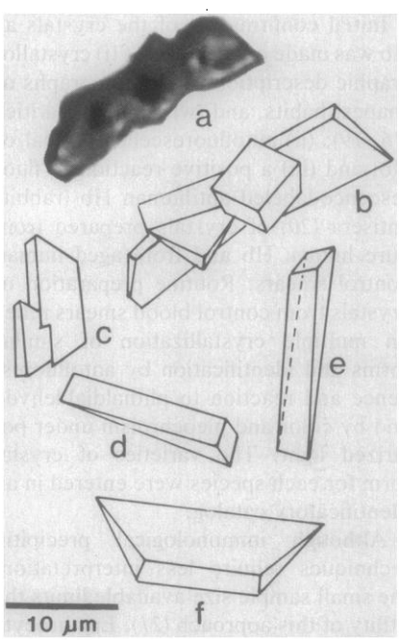

Fig. 2. Hemoglobin crystals of caribou (*Rangifer tarandus*) grown from the blood film on the flake knife illustrated in Fig. 1: (a) photograph of a crystalline aggregate; (b) line drawing of the crystalline aggregate; (c and d) crystal forms grown from a modern control blood smear by the same technique; (e and f) crystal forms illustrated in Washino (*16*).

mined by a number of interrelated variables: temperature, solution *p*H, concentration of the salt solution, choice of salts, and the concentration and solubility characteristics of the protein itself (*14, 15*). A salting-out procedure that has been successfully applied to the identification of mosquito blood-meal hosts has been modifed to permit the successful crystallization of the blood-residue Hb fraction of eluted solutions having Hb concentrations in the nanomole range (*16*). This procedure has been used to crystallize Hb from mammalian and avian species eluted from control blood smears, use-replicated tools aged for 6 months to 4 years, and archeological specimens. Blind testing has demonstrated the reliability of this procedure (*17*).

The variety and diagnostic specificity of Hb crystals derive from the ionic nature of the crystal and variations in the amino acid sequence from one species to another, which results in distinct and slightly different shapes and surface-charge distributions around the Hb molecule. This variation also influences the solubility and precipitation properties of the Hb crystal, permitting the identification of more than one species of origin from the same blood-smeared tool (*12, 15, 18*). This variation also gives rise to differences in the rates of crystal growth, crystal size, twinning regularities, and number of crystals, all of which assist in the identification of the species of origin.

Initial confirmation of the crystals as Hb was made on the basis of (i) crystallographic descriptions and photographs of shapes, habits, and twinning regularities (*16, 19*); (ii) autofluorescence typical of Hb; and (iii) a positive reaction to fluorescence-labeled antihuman Hb (rabbit) antisera (*20*) of crystals prepared from pure human Hb and from aged human control smears. Routine preparation of crystals from control blood smears relies on multiple crystallization of similar forms and identification by autofluorescence and reaction to phthaldialdehyde and by color and pleochroism under polarized light. The varieties of crystal form for each species were entered in an identificatory catalog.

Although immunological precipitin techniques require less interpretation, the small sample size available limits the utility of this approach (*21*). Erythrocyte morphology and size can in some instances assist one in distinguishing between mammal and avian or reptilian bloods; generally, there are too few red blood cells present to permit the reliable assignment of taxonomic identity (*8*).

The changes in these residual blood films over time may be summarized as follows. The exposure of a blood-covered tool to ultraviolet light from the sun and to air, which dries the blood, lyses most of the red blood cells and affects the tertiary denaturation of the serum proteins (*22, 23*). Burial of the tool provides a stable heat and moisture environment; the denaturation of the serum proteins protects the Hb from degradation by soil organisms (*24*) and moisture. Typical archeological soils vary between *p*H 6 and 8, well within the range of stability of most blood proteins. Prolonged exposure to air changes the Hb from its oxygenated form to a more stable, reduced form (*23*). To my knowledge, no upper time limit on the presence or reactivity of blood residues has been ascertained. The longevity of proteins, frozen blood, and dried tissue is well documented (*25*).

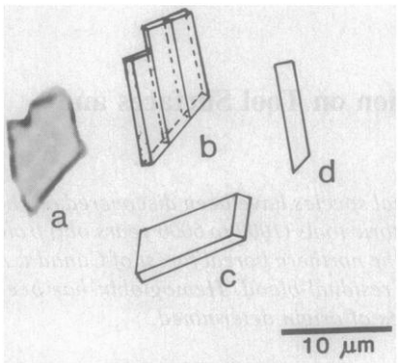

Fig. 3. Hemoglobin crystals of grizzly bear (*Ursus arctos horribilis*) grown from the blood film on the flake knife illustrated in Fig. 1: (a) photograph of a crystalline aggregate; (b) line drawing of the crystalline aggregate; (c and d) crystal forms grown from a modern control blood smear by the same technique (*16*).

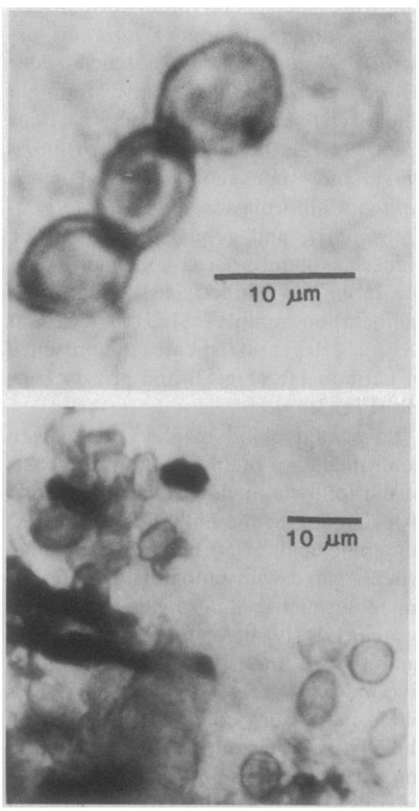

Fig. 4. Red blood cells in a plaque of dried blood removed from the surface of a flake knife (sample IgSk 8:85) excavated from a site in northern British Columbia (stained with *o*-phthaldialdehyde and illuminated in transmitted light with a quartz-halogen lamp and a Schott blue-violet filter to increase contrast).

The likelihood of finding blood residues in many localities and the development of techniques that permit detection and identification to species of origin have major implications. For the first time lithic analysts can positively identify worked material and thus more certainly assign functional tasks to prehistoric tools. In addition, zooarcheological studies can be carried out in regions where acid soils interfere with the preservation of bone. Human blood residues can be used to extend the paleoanthropological knowledge of blood types without the need to rely upon mummies to provide samples. The finding of a wide variety of ancient blood proteins from dated contexts will assist in paleozoological and protein-evolutionary studies. The results of this research make possible a better understanding of past animal distributions and man's use of those animal resources.

Note added in proof: Recent experiments I have done indicate that the residues are protected from microbial attack and ground-water removal while buried by electrostatic interactions with soil clay particles. In some cases, up to 50 percent of the original blood residue may be sequestered in the first 0.1 mm of soil; thus, much of the residue may in fact be lost by extensive cleaning of artifact surfaces (*26*).

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Middle Holocene Age of the Sunnyvale Human Skeleton

Abstract. *A morphologically modern human skeleton from Sunnyvale, California, previously dated by aspartic acid racemization to be approximately 70,000 years old and by uranium series isotopic ratios to be 8300 and 9000 years old, appears to be younger when dated by the carbon-14 method. Four carbon-14 determinations made by both decay and direct counting on three organic fractions of postcranial bone support a middle Holocene age assignment for the skeleton, probably in the range of 3500 to 5000 carbon-14 years before the present. This dating evidence is consistent with the geologic, archeological, and anthropometric relationships of the burial as well as previously determined carbon-14 determinations on associated materials.*

Bada and Helfman in 1975 (*1*) assigned an age of approximately 70,000 years, based on aspartic acid racemization measurements, to a morphologically fully modern, nearly complete female human skeleton excavated in 1972 from the Sunnyvale East Drainage Channel, located in the southern portion of San Francisco Bay, California. If correctly dated, the Sunnyvale hominid would be one of the oldest directly dated *Homo sapiens sapiens* skeletons in the world. It would predate by approximately 30,000 years the generally accepted age for the earliest appearance of anatomically modern human populations in the Old World (*2, 3*). Several investigators have accepted the essential accuracy of the 70,000 years age assignment for the Sunnyvale skeleton (*4–6*) and have offered it as evidence to argue against the view that the earliest human populations reached the New World relatively late in the Pleistocene (*7–9*).

The validity of the age derived from aspartic acid racemization dating was challenged by Gerow, Lajoie, and Peterson on geologic, archeological, and anthropometric grounds supported by several ^{14}C determinations (*9, 10*). The skeleton had been interred in a well-defined grave pit, 53 cm in diameter, excavated to a depth of 2.7 m below the present ground surface [figure 6 in (*10*)]. The pit had been excavated into a buried soil of terminal Pleistocene age that contained Rancholabrean fauna and freshwater shells which yielded two ^{14}C determinations of 10,110 ± 260 (I-8084) and 10,430 ± 150 (I-6476) ^{14}C years before present (B.P.). An artifact fashioned from antler (possibly elk) was recovered from a feature (a possible trash pit) that is 400 m north of the burial locality and intrudes into sediments similar to those surrounding the burial pit. Charcoal directly associated with the antler artifact yielded an age of 4460 ± 95 ^{14}C years B.P. (I-6977). The antler tool and human burial exhibit similar aspartic acid racemization measurements, and neither show evidence of heating by fire. Physiographically the location of the burial site resembles a common late Holocene prehistoric settlement pattern found along the shores of the southern portion of the San Francisco Bay (*11*). Finally, the reconstructed Sunnyvale female is statistically indistinguishable in 32 standard measurements and indices from a selected local population of female skeletons dated by radiocarbon and cultural associations to between 400 and 1600 ^{14}C years ago [table 5 in (*10*)]. On the basis of these lines of evidence, it has been argued that the age of the Sunnyvale skeleton was less than 10,000 years (Holocene) and possibly less than 4500 ^{14}C years (*9, 10*). Ages of 8300 and 9000 years old were obtained from uranium series analyses of bone samples from the Sunnyvale skeleton by Bischoff and Rosenbauer (*12*). Bada and Finkel (*13*) suggested that the uranium values should be considered minimum estimates, but Bischoff and Rosenbauer (*14*) said that the validity of the various age estimates should be tested by comparison with an independent radiometric technique such as ^{14}C.

We have obtained both decay and direct counting ^{14}C determinations on three organic fractions of postcranial bone from the Sunnyvale skeleton. To eliminate the inorganic carbonate fraction, the Sunnyvale bone, after mechanical and ultrasonic cleaning, was ground to pass through 0.104-mm mesh and

Table 1. Radiocarbon counting data for calibration samples.

Sample	R^* expected	R^* measured
ANU sucrose	1.000	1.003
ANU sucrose		±.006
New NBS oxalic acid†	1.369	1.395
Tree ring, A.D. 1890	±.010	±.017
Tree ring, 5050 B.C.	0.48‡	0.50
Tree ring, A.D. 1890		±.03
Tree ring, 1000 B.C.	0.71‡	0.74
Tree ring, A.D. 1890		±.04

*R = the expected and measured ratios of the $^{14}C/^{13}C$ values for the indicated materials. †National Bureau of Standards reference material RM-49. ‡Based on data from Klein *et al.* (*21*).