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JOINT COMMITTEE ON AVIATION PATHOLOGY: IX

Laboratory Examination of Unidentified Suspected Tissue Fragments Found at Aircraft Sites

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The identification of victims of an aircraft accident may be very difficult because of the degree of fragmentation associated with the accident. Periodically, the Divisions of Aerospace Pathology and Toxicology have been asked to identify tissue, bone, or bloodstains of undetermined origin. Usually this request has been precipitated by situations in which a) it is questionable whether an aircraft has sustained a bird strike, b) unidentified pieces of tissue are found floating at sea, or c) fragments of bone, tissue, or blood-stained flight apparel are found near a crash site. Preliminary studies have shown that gross examination and the methods and procedures used in forensic serology may also be applied in aircraft investigation with very good results. These methods are used as an aid to confirm the identity of the victims involved.

THE PURPOSE of this paper is a) to describe some specific types of problems in determining whether materials found at the aircraft accident site are of human or nonhuman origin, and b) to describe basic techniques that may be used by local laboratories to assist in identifying unknown materials.

Although many factors must be considered during investigation of a fatal aircraft accident, two particularly difficult situations have posed unusual problems. With severely mutilated and fragmented bodies, it is necessary to verify that the material found is, in fact, of human origin and, if so, to determine the identity of the person. This is especially difficult when an aircraft is lost at sea or when the body and aircraft wreckage are so severely fragmented that no tissue is readily identifiable as being human. Another problem is presented by bird strikes (11,12), though it is not difficult to identify avian tissue when feathers or other recognizable fragments of birds are found, it is much more difficult when only a single small fragment of tissue is found, that may or may not be from a bird. These problems are amenable to study by laboratory methods, but the special techniques necessary to make these determinations are usually not readily available to the field investigator.

TECHNIQUES

A. *Gross examination.*

1. *Of appearance*: The unidentified specimen must first be examined grossly. Visual examination may reveal the organ from which it came. Skin, muscle, and fat are tissues most frequently recovered when there has been massive tissue destruction. When an aircraft is lost at sea, tissues containing large amounts of fat are the most frequently recovered because of the greater buoyancy of adipose tissues. The characteristic consistency and color of brain tissue render it relatively easy to identify, particularly when the victim sustained a severe head injury. Kidney, intestines, and lung are also relatively easily identified on the basis of characteristic appearance.

2. *Of consistency*: Consistency may be of value, but the risk of further tissue destruction must be evaluated before subjecting the material to vigorous palpation. It is generally possible to determine whether the material is fibrous (such as muscle, fascia, nerve, or skin), gelatinous (as brain), or firm (as kidney or liver).

3. *Of odor*: The odor of a specimen is useful in determining whether material found at the accident site is tissue or other artifactual material. The onset of putrefaction of fragmented tissues is sufficiently rapid that, except in the coldest geographic regions, the characteristic odor is readily identified by even the inexperienced. Even in situations where the material is saturated with the odor of aviation fuel, smelling it carefully should reveal the odor of decomposing tissue. The characteristic odor of fish may be detected in materials recovered from aircraft lost at sea.

B. *Serologic examination*: Methods and procedures used in forensic serology may also be applied to air-

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TABLE I. SOURCES GIVING POSITIVE REACTIONS TO THE BENZIDINE, ORTHOTOLIDINE, OR PHENOPHTHALEIN TESTS FOR BLOOD.

Animal	Plant	Chemical
Bone marrow	Apples	Rust
Brain tissue	Asparagus	Soil
Spinal fluid	Beans	Copper sulfate
Intestine	Beets	Potassium dichromate
Liver	Horseradish	Some bleaches
Lung	Potatoes	
Saliva	Turnips	
Mucus		

craft-accident investigation. Forensic serology, simply stated, is that scientific discipline concerned with the use of antigens and antibodies and the study of their properties as they pertain to criminal justice. For our purposes this definition should be altered to read, "as they pertain to aircraft accident investigation."

1. *Blood*: Determination of whether blood is present in the material being examined is a useful initial screening procedure Many substances, when viewed with the naked eye, or even when subjected to analysis, appear indistinguishable from blood (Table I). Additional analytical procedures must be utilized to distinguish these substances.

The three classical tests used to indicate the presence of blood are the benzidine, the orthotolidine, and the phenophthalein tests (5). All three are based on the ability of the enzyme peroxidase, found in the heme portion of the hemoglobin molecule, to catalyze decomposition of organic peroxides, which oxidize the reagent used to produce a color reaction.

The most popular and widely used screening test is the benzidine test (Alder reaction). It is a convenient presumptive test, is very sensitive, and can detect hemoglobin at levels as low as 2 or 3 ppm. Unfortunately, benzidine has been found to have carcinogenic properties and, for this reason, the phenophthalein test is the screening procedure used in our laboratory.

The orthotolidine test is closely related to the benzidine test and the chemistry of the test is essentially the same. Although it is not widely used, it has the same advantages and disadvantages as benzidine and may gain in popularity with the decreasing availability of benzidine.

The phenophthalein test has many of the same disadvantages as the benzidine test and is more difficult to prepare. It has been found to be just as sensitive, however, and is not reported to be carcinogenic.

The most important limitation of these tests is that plant, chemical, and other animal sources possess similar peroxidase activity and will yield false-positive results. The disadvantage of nonspecificity is not considered important when the test is used only as a preliminary screening test to decide whether the stain under investigation may contain blood. The tests, when positive, strongly suggest that blood is present, but the high incidence of false-positive reactions requires that confirmatory tests be performed. When negative, the tests are good indicators that no blood is present.

Fig. 1. Hemochromogen crystals observed in Takayama reaction. (X440; AFIP Neg. 76-12451-4.)

The Takayama test (2,4,6) has long been a preferred confirmatory method, and experience indicates that it is a very satisfactory test to be performed in a general laboratory. The presence of blood in suspected bloodstains is confirmed by the formation of characteristic hemochromogen crystals.

Hemochromogen crystals, microscopically, are orange-red in color and are needle- or rhomboid-shaped. The hemochromogen test uses an alkaline medium for hydrolysis and pyridine for crystal development (Fig. 1) It is simple to perform and more sensitive than other methods tried. Numerous workers have reported various modifications of the reagent, but none has shown improvement on the original Takayama test. The time taken for crystals to develop varies with the age of the stain, which could be from a few minutes for fresh stains to a few hours for older stains.

The hemin (Teichmann) test may be used to demonstrate hemin crystals, which are brown in color, rhomboid in shape, and 1 to 20 microns in size when viewed microscopically. It is older but is considered less sensitive than the Takayama test. It utilizes an acid medium and an inorganic halogen for crystal development.

2. *Species*: The next question to be answered is whether the material is of human or of nonhuman origin Unusual materials found at accident sites and submitted to the AFIP for examination have included

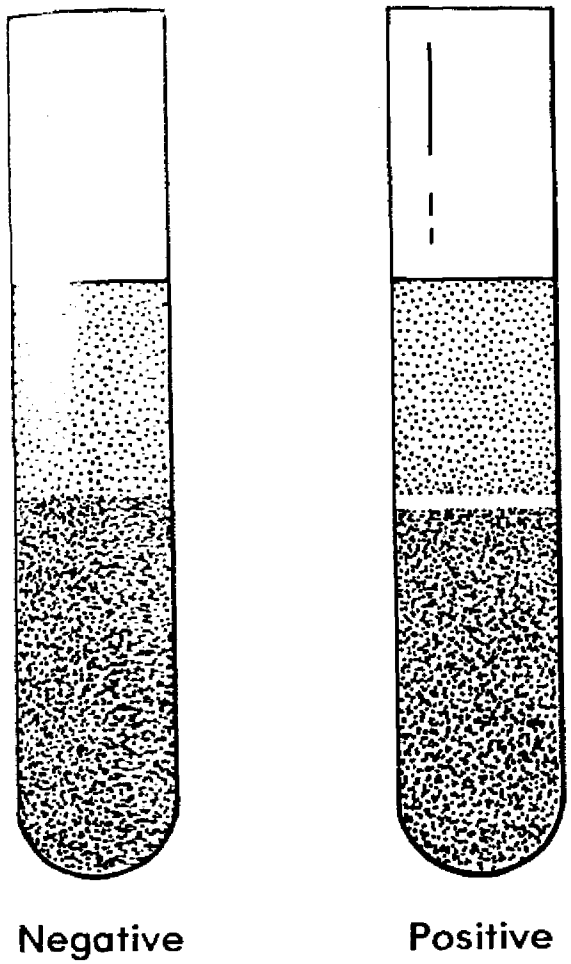

Fig. 2. Interfacial ring-precipitin test. A. Negative control. B. Positive reaction. (AFIP Neg. 77-665.)

bird, cow, pig, fish, bear, and certain human tissues, as well as various plant materials and other artifactual substances suspected of being human.

The forensic serologist has several techniques to choose from for finding the species of origin of a bloodstain or tissue. Most of these methods involve the use of species-specific antisera. Although the procedures are not difficult, they are sufficiently different from standard serological tests used in blood banks that special care should be taken to insure that adequate controls are prepared. The difficulty of interpretation and unavailability of fresh antisera in most laboratories make these species identification procedures a problem properly referred to a reference laboratory.

The interfacial ring-precipitin test, Ouchterlony gel double-diffusion test, and the antiglobulin-inhibition technique are the three methods most commonly used. All three are antigen-antibody reactions that identify proteins present in the blood as being of human or other origin. Because of their simplicity and speed, the ring-precipitin and Ouchterlony double-diffusion are the methods most easily adapted to general laboratory use. The inhibition technique is especially recommended to differentiate between man and closely related subhuman primates. This does not present a problem in the United States.

Reliability of the tests relies on the presence of a sufficient amount of protein to react with specific antibody. Many factors may be present, especially in aircraft accident investigation, that may contribute to the denaturation of protein and thereby affect the outcome of the test. Some of these factors the investigators should be aware of is the age of the specimen submitted, its exposure to air, sunlight, humidity, and high temperatures, and possible contamination by chemical substances.

The ring-precipitin test is run with antisera under varying dilutions of stain extract or serum in a series of precipitin tubes. The extract of the stains should be at a pH near neutral and the test carried out at room temperature. A positive reaction is demonstrated by the formation of a precipitate at the interphase of the two fluids (Fig. 2). All the fluids must be clear, since cloudiness interferes with interpretation. The reaction should be read in a good light with a dark background behind the tubes. Extracts should be made in the smallest possible volume, and extraction time may vary depending on the age of the stain and other influencing factors.

The Ouchterlony technique, which is carried out in a gel medium, offers an advantage over the precipitin test in that the extracts need not be clear in order for the reaction to be interpreted. The disadvantage is that it takes a few hours to perform. The precipitin test is generally used on clear extracts because of its simplicity and speed, and the Ouchterlony method for opaque extracts. Electrophoresis utilizes the advantage of both techniques and is considered even more sensitive.

If the antihuman serum reaction indicates that the material is of human origin, the next step is to attempt to identify the victim. There are three classes of blood constituents available to the forensic serologist for individualizing blood (13): a) The blood-grouping and blood-typing antigens. Three systems have been found acceptable and are generally used in the forensic laboratory. These are the ABO, the MN, and the Rh systems; b) The polymorphic enzymes. The three mainly used in the United States are the phosphoglucomutase (PGM), adenylate kinase (AK), and erythrocyte acid phosphatase (EAP). Although there are more, these three are usually run, mainly because of their relative stability, ease of handling, or differential frequency of genetic markers; and c) The polymorphic proteins haptoglobin and hemoglobin are used for genetic variants and as an anthropological marker, respectively. Electrophoretic techniques are used to analyze the enzymes and proteins. Research, utilizing such techniques as isoelectric focusing, isotachophoresis, and cross-immunoelectrophoresis, is continually being done toward individualizing bloodstains (9). The use of radio-immunoassay (RIA) techniques for determinations of sexual origin are also being investigated. The blood-grouping and blood-typing antigens are the only constituents used in our laboratory at the present time.

The more familiar techniques utilized in blood banks for antigen-antibody detection are no longer applicable

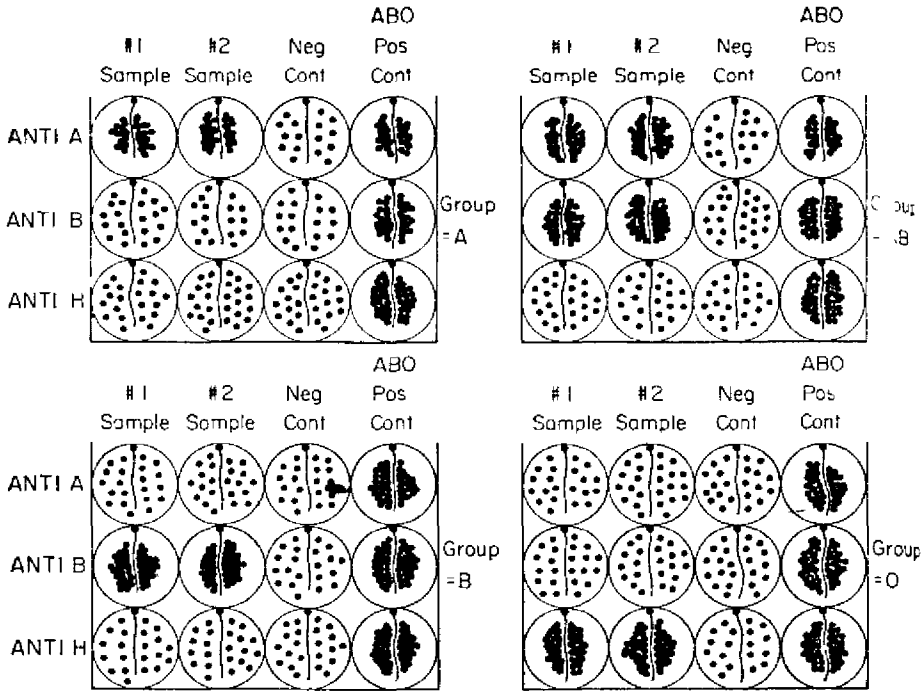

Fig 3. Typing of bloodstains. "Quick" absorption-elution technique. (AFIP Neg 76-12451-2.)

to detection of these in bloodstains. All the red cells in a dried bloodstain have been *damaged*, and direct agglutination methods with known antisera to detect red cell antigens can no longer be used. The red cell antigen has not been destroyed in this process, however, and maintains its capability to combine with specific antibodies. There are several ways to demonstrate agglutination and subsequently the presence or absence of antigen-antibody complexes. It should be kept in mind that various antibodies differ in their ability to agglutinate antigens, and this is one of the problems associated with this examination.

Some of the methods used to type bloodstains are absorption-inhibition, absorption-elution, the Lattes crust test, and the Howard-Martin cellulose acetate sheet test (2,4,6). We have found the *absorption-elution* (Fig 3) and the *Lattes crust* tests (Fig. 4) satisfactory for those samples submitted to this laboratory. The absorption-inhibition test relies on the ability of antigenic material associated with red cell membranes and water-soluble antibody molecules to form antigen-antibody complexes. Specific antigens in damaged red cells are capable of neutralizing specifically the antigen's homologous antibody. Absorption is allowed to proceed between the antigen and antibody, which renders the anti-

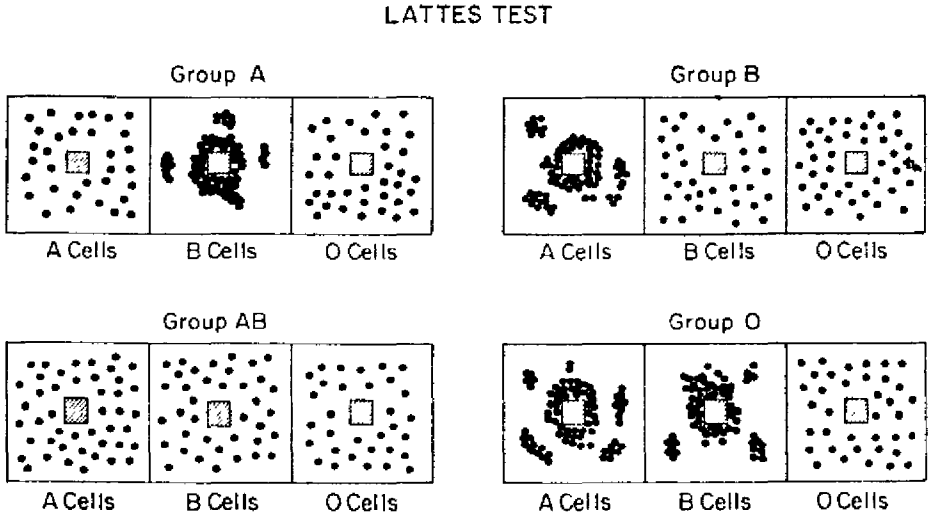

Fig. 4. Typing of bloodstains Lattes test (AFIP Neg. 76-12451-3.)