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Blood and Tissue Identification of Selected Birds and Mammals, Part 5

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APPENDIX

ELECTROPHORESIS TECHNIQUES

U.S. SURVEY RESULTS

The discussion, advantages, and disadvantages are the views of the province or state under which they appear. Information was obtained from questionnaires sent to the individual states on August 3, 1973. The following is a summary of the responses to this questionnaire and does not take into consideration any progress achieved since then.

Alabama: No methods of sample identification are currently in use.

Alaska: No implementation of electrophoresis as yet though equipment of this nature is hoped to be available to field personnel in the near future.

Arizona: Using precipitin antisera with tubes and/or immunodiffusion.

DISCUSSION - The techniques are applied to tissue, blood, and serum of big game animals only. Approximately 10 identifications are performed each year and are occasionally used in court cases. The results are accepted by courts when used in prosecution. Identification cannot be made between closely related species or determination of sex or age using tissue, dried blood, or serum. Components in processed meats such as salami, sausage, or cooked meats cannot be identified.

Arkansas: Use anti-deer and anti-bovine precipitin tests. Tests are also run on small mammals and furbearers.

DISCUSSION - Approximately 10 identifications are performed each year. The results are accepted in legal cases but few are used. Identification cannot be made between closely related species using tissue, dried blood, or serum but report that components of processed or cooked meats can be determined.

California: Using starch and acrylamide gel electrophoresis, paper and thin-layer chromatography, micrography, histology, and serology. Gel electrophoresis has been in use for two years.

DISCUSSION - Earle W. Cummings, a junior wildlife manager-biologist at Wildlife Investigations Laboratory, 987 Jedsmith Drive, Sacramento, Calif., 95819, wrote a thesis entitled "Techniques of Game Law Enforcement Using Starch Gel Electrophoresis." Private communication with Mr. Cummings provided the following information.

A combination of many of the techniques may be applied to most problems in identification. Tissue, dried blood, and serum are used in identifying big game animals, small mammals, furbearers, game birds,
and protected avian species. Starch gel electrophoresis is used for identification of uncooked meat, fish, fowl, shellfish and blood.

The use of electrophoresis for furbearers has been pretty cursory. They have found a number of esterase and protein variations in black bear and mountain lion--enough to be able to say that individual animals could probably be distinguished. Birds are more difficult to deal with. Attempts to distinguish two subspecies of white-fronted goose electrophoretically were unsuccessful even though differences between individuals existed. Segregation is possible between closely related species using tissue, dried blood, and serum (subject to storage conditions). Subspecies or races may be more difficult or impossible. There is no way at present to detect sex from a blood sample although it might be possible if a sufficiently sensitive assay for sex hormone ratios exists. It is also believed that meat components can be identified in cooked and processed meats.

Regarding deer, they are dealing with several recognized subspecies rather than species in California. Serum protein pattern differences exist but are partially obscured by hemoglobin, myoglobin or hemoglobin-haptoglobin complexes when meat, blood, or dried blood are run. There are, however, consistent differences which are not obscured which allow an unknown blood or meat to be assigned to a subspecies or regional population. They can approximate the age of a dried blood sample by the rate at which particular proteins are denatured. Of the 20 or so bands which may appear in a fresh blood sample, some represent proteins with a less stable structure that rapidly become indistinct. Others, present in higher concentrations or more stable molecular structure, remain clear for longer periods. Approximately 600 identifications are performed each year and 10 per year may be used in court.

Colorado: Using gel diffusion.

DISCUSSION - Tissue and dried blood are used to identify big game which result in 15 to 20 identifications per year. All are used in legal proceedings, although court appearance is not necessary in many cases, since they end with penalty assessment. No effort has been made to segregate closely related species other than deer-elk or to differentiate sex or age.

Connecticut: Use tube agglutination method.

DISCUSSION - Serum is used to identify big game animals, game birds, and protected avian species. These tests were applied to buffalo as a brucellosis test and game bird cases as a pullorum indicator. The method has never been tested in court. Components in processed meats such as salami, sausage and cooked meats can be identified.

Delaware: No tests are performed by the Division of Fish and Wildlife.
DISCUSSION - The Department of Agriculture employs the tube precipitin test for the serological determination of animals using domestic blood. They are now in the process of adding deer to their list.

Florida: Does not have the necessary equipment, the work is done by the Florida Department of Law Enforcement (Crime Lab) free of charge.

DISCUSSION - Meat and blood identification is done on request, but initial evidence gathering is done by game management personnel. Tissue, dried blood, and serum of big game animals, small mammals, furbearers, game birds and protected avian species are used, and 50-75 of these identifications are performed each year with about half used in court. Closely related species and sexes cannot be differentiated but components of processed and cooked meat can be identified if comparative suspect samples are available.

Georgia: Work in this area is contracted to the Southeastern Cooperative Wildlife Disease Study in Athens.

DISCUSSION - The division has become aware through contact with the above mentioned that certain types of identification are extremely difficult, particularly goat and deer. Georgia is presently without the technical expertise needed to make solid convictions in questionable cases.

Hawaii: Not employing any method for meat or blood identification.

Idaho: Using paper chromatography, anti-sera precipitin test and starch gel electrophoresis.

DISCUSSION - Tissue, dried blood, and serum are used to identify big game animals, small mammals, furbearers, game birds, and protected avian species. Approximately 12 identifications are performed each year with one-half used and accepted in court when introduced by a fully qualified individual. This division is working with Oscar Brunetti of the California Fish and Game Division and/or Dr. Charles H. Drake of Washington State University in differentiation of closely related species and sexes. Components of processed and cooked meats can be identified to some degree using paper chromatography.

Illinois: No program at present.

Indiana: Use Indiana State Police Laboratory for meat and blood identification.

DISCUSSION - Tissue, dried blood, and serum are sent to the Police Lab when the suspected offense concerns big game. Approximately one such case occurs per year and there is no problem having the methods accepted in court when presented by an expert witness from either of the departments. Closely related species, sex and age differences, and components in cooked or processed meats cannot be identified.
Iowa: Use spot tests for blood (Benzidine and Phenolphthalin) and precipitin using anti-sera (tube method).

DISCUSSION - Tissue and dried blood are used in the identification of big game animals, small mammals, fur-bearers, game birds, and protected avian species. Approximately 50 identifications are made each year with all being brought before court. Many are not contested in view of the evidence and a guilty plea is entered. In Iowa the crime lab report stands on its own merit in lower courts. Plans are being made to use electrophoresis in identifying closely related species and work is being done in determining sex.

Kansas: No employment of electrophoresis in the enforcement of game laws.

Kentucky: Using sensitized blood serum for blood; microscopy for meat.

Louisiana: No method for species identification in practice.

Maine: Use precipitin test, immunodiffusion in agar gel and will be attempting fluorescent antibody next.

DISCUSSION - Tissue, dried blood, and serum are used in identifications of 20-25 big game species annually. Of these approximately 95 percent are used in court. Bryan C. Buchanon, warden specialist, has qualified as an expert witness in each case in which he has been requested to testify. He indicates he cannot determine sex or age through these methods nor can the components of processed or cooked meats be identified. However, raw hamburger can be identified as to component materials.

Maryland: Use the equipment belonging to the Federal Bureau of Investigation in Washington, D.C.

DISCUSSION - The services provided by the FBI Lab have been confined to the identification of deer meat, deer blood, and/or deer hair. The expert testimony of the special agent conducting the test has always been accepted in court.

Massachusetts: Not implementing any method for species identification.

Michigan: Use precipitin test.

DISCUSSION - Tissue and dried blood are utilized to identify big game animals only. About a dozen identifications are performed each year with about half used in court cases. Most identifications involve arrests for game law violations. However, segregation of closely related species and sex and age differences cannot be determined, nor can components of processed and cooked meats.

Minnesota: Using the Beckman Microzone Electrophoresis System.

DISCUSSION - Tissue and serum are used to identify big game animals, small mammals, fur-bearers, game birds, and protected avian species.
Dried blood methodology and trials are being explored. Approximately 75 identifications are made annually, and on occasion a case is prosecuted in court. Using this method with serum, it is possible to differentiate between closely related species. However, sex and age differences cannot be determined and components in processed meats and cooked meats cannot be identified.

**Mississippi:** Using Takayama test for blood identification, precipitin test, and absorption elution; Latess.

**DISCUSSION** - Dried blood is used in the identification of big game animals, small mammals, furbearers, game birds, and protected avian species. These techniques are used mainly to distinguish between animal and human blood, human blood type, and to determine origin of animal blood (deer, etc.). Over 30 identifications are performed annually and 10-15 of these are prosecuted in court. Both the precipitin test and Takayama tests are readily accepted as evidence. Closely related species as well as sex and age differences are not determined. Components in processed meats or cooked meats cannot be identified.

**Missouri:** Use precipitin test.

**DISCUSSION** - Tissue, dried blood, and serum are used to identify big game species only. Approximately 25 identifications are performed each year with about 10 of these being taken to court. The technique is not questioned by counsel, presumably because they are unfamiliar with the test and do not know what questions to ask. No work has been done to distinguish between closely related species and sexes but identification of components in processed and cooked meats has been accomplished.

**Montana:** Use Ochterlony diffusion method.

**DISCUSSION** - Tissue, dried blood, and serum are used to identify big game and domestic species. The tests are applied approximately 25 times per year with usually one of these taken to court for prosecution. Closely related species and sexes of big game cannot be separated nor can the components of processed meats be identified.

**Nebraska:** Use agar-gel immunodiffusion and immunoelectrophoresis. Soon to employ Beckman Microzone used in conjunction with acrylamide gels.

**DISCUSSION** - Tissue, dried blood and serum are used to identify big game animals, small mammals, furbearers, game birds and protected avian species. Approximately 25 identifications are performed each year with 4-5 used in court. Segregation of closely related species is difficult. Sex identification through observation of chromatin bodies in nerve, bone, and muscle tissue is being attempted for deer. Components of mixed uncooked meat can be identified to a certain extent, depending on species involved.
Nevada: Does not currently have the necessary lab facilities or qualified personnel to make electrophoresis determinations. Blood analysis is done by the California Department of Fish and Game.


New Jersey: The technique was not mentioned for blood and meat identification.

DISCUSSION - Tissue, dried blood, and serum are employed to identify big game animals, small mammals and furbearers. Two to three identifications are performed each year but are seldom used in legal cases. The technique has been accepted in court. Tissue, dried blood, and serum can be utilized to segregate closely related species. Sex and age differences and identification of components in processed and cooked meats has not been attempted.

New Mexico: Uses the facilities of the FBI Crime Lab in Santa Fe.

DISCUSSION - Tissue, dried blood, and serum are analyzed only for big game as a rule. However, small mammals and birds are analyzed where poisoning is suspected. Approximately 15 identifications are performed each year, and most are used in court. This type of evidence has rarely if ever been refused or suppressed when used. Closely related species cannot be differentiated nor can age or sex be determined. The components in mixed uncooked meats can be identified but not in cooked meats.

North Carolina: Use skeletal structure primarily, on exceptional cases precipitin tests are conducted (expensive).

DISCUSSION - Tissue is used to identify big game animals, small mammals, furbearers, game birds, and protected avian species. Approximately 5 to 10 identifications are performed each year and all are used in court. Dr. F. S. Barkalow, Jr., professor of zoology and forestry, North Carolina State, Raleigh, N.C., 27607, is a qualified expert witness in this area. Using tissue, it is possible to identify closely related species and determine sex and age. Components of processed or cooked meats cannot be identified.

North Dakota: Work in the area of identification is done for the Game and Fish Department by Dr. Gary Pearson of the Laboratory of Northern Prairie Research Station, Jamestown, N.D.

DISCUSSION - There are not too many instances where methods of this nature are required in bringing a case to conclusion, possibly two or three per year. The testimony and reports of Dr. Pearson have been successful in the prosecutions.

Ohio: Blood tests are conducted for the Division of Wildlife by the Bureau of Criminal Investigation using the precipitin method. Meat and bone identification is conducted by a professor from Ohio State University.
DISCUSSION - Tissue, dried blood, and serum are used to identify big game animals, small mammals, furbearers, game birds, and protected avian species. Approximately 25 identifications are made annually with 15-20 used in court. Using serum, it is possible to distinguish between closely related species, but sex and age cannot be determined. Also the components in processed meats or cooked meats can be identified.

Oklahoma: The Department of Wildlife Conservation does not have the necessary equipment, but some work in this area is done for the department by the State Crime Bureau.

Oregon: Using immuno gel diffusion - Ochterlony technique and agar gel electrophoresis on hemoglobin and metaglobin.

DISCUSSION - Tissue and dried blood are used in the identification of big game animals only. Approximately 100 identifications are performed each year with about 25 of these used in court. Separation of closely related species, determination of sex or age, and identification of components in processed or cooked meats is not done.

Pennsylvania: Technique was not identified.

DISCUSSION - Tissue, dried blood, and serum are used in the identification of big game species. The Pennsylvania State Police Crime Lab conducts all tests for the Game Commission in 15-20 cases per year. Practically all identifications are made in preparation for a hearing or court case. Occasionally the defendant will plead guilty to the charges without testimony and evidence presentation being necessary. Segregation between closely related species and determination of sex and age is not done. Identification of components in processed meats is sometimes possible depending on the amount of questionable meat involved, seasoning, amount of cooking, etc.

Rhode Island: Using precipitin technique.

DISCUSSION - Dried blood is used in the identification of Virginia white-tailed deer. Anywhere from 1-7 identifications are made per year with the results used in court. The tests are conducted at the University of Rhode Island by a serologist who is qualified as an expert witness. The need to use the test to identify closely related species and to determine age and sex has never arisen.

South Carolina: No work is being done in blood or meat identification as yet but the value of the technique is apparent.

South Dakota: The Department of Game, Fish and Parks does not have the necessary equipment, but a few samples have been taken to the lab at the University of South Dakota at Vermillion for testing. They plan to obtain the necessary equipment in the near future.
Tennessee: Samples of suspect meat are submitted to the FBI Lab in Washington, D.C., for analysis.

DISCUSSION - Dried blood is used in the identification of big game species in approximately 30 cases per year. Of these, about 15 are used in court. Components in processed meats such as salami or sausage and cooked meats cannot be identified.

Texas: Using commercially prepared species-specific precipitin antisera for the past year.

DISCUSSION - Tissue and dried blood are used in the identification of big game animals, small mammals, furbearers, game birds, and protected avian species. Closely related species, sex and age differences, and components in processed and cooked meats cannot be identified. The major drawback is that the anti-sera is available for very few species. Earlier investigation into paper chromatography showed the procedure to have merit but it never produced results that could be entered as evidence with confidence in court. The precipitin technique has been accepted in legal cases. However, the frequency that these identifications are used in court is unknown, since they have been involved with forensic identification for only six months.

Utah: No technique reported.

Virginia: Blood and meat samples are sent to the USFWS Lab in Missouri and hair samples are sent to the FBI Lab in Washington, D.C.

DISCUSSION - Tissue, dried blood, and hair are used in the identification of big game animals in approximately 10 cases per year, all of which are used in court. Identification of closely related species and determination of sex and age are not done, nor is the identification of components in processed or cooked meats.

Vermont: Work in the area of species identification is done for the Department of Fish and Game by the State Police Lab.

DISCUSSION - Tissue and dried blood identification on big game animals is performed 10-15 times per year. It is seldom necessary to bring these cases to court as positive results will very often bring a guilty plea. Experimentation is presently under way in an effort to find means of accurate segregation of closely related species and determining the reliability of the technique in the area of sex and age determination.

Washington: Using paper chromatography for meat and blood; melting and solidifying temperatures for fat identification.

DISCUSSION - The state has been using paper chromatography for the past nine years to identify species as well as proving that meat marked "pork" or "veal" is in fact contraband game. The precipitin test supplies the investigator with general families only while chromatography provides entire files of information which would
enable a researcher to identify big game to the level of the area in which they were grown. There have also been a number of calls for fish identification where steelhead may be passed for salmon. Approximately 30-50 identifications are made annually with 5-10 of these being brought to court. The real value of the technique lies in its availability to the officers and the psychological tool it supplies. Using tissue and dried blood, it is possible to segregate closely related species but there is no real need to identify species on the basis of sex or age. The identification of components in processed and cooked meats has proven very difficult and time-consuming.

West Virginia: No methods of meat and blood identification employed.

Wisconsin: Using ring precipitin test and immunodiffusion.

DISCUSSION - Tissue, dried blood, and serum are used in the identification of big game animals, small mammals, furbearers, game birds, and protected avian species. Approximately 30 identifications are made per year, and the results are used very often in court without question. Segregation of closely related species and the determination of sex and age have been either unsuccessful or untried. Components of cooked meats can be identified if not too fully cooked. Good success has been experienced with salami and summer sausage, some success with bologna.

Wyoming: Using micro-immunodiffusion tests which are an application of antigen-antibody precipitin reactions.

DISCUSSION - Tissue, dried blood, and serum are used in the identification of big game animals. In 1973, 64 tests were conducted; 32 meat and blood samples for 9 different cases. Since 1965, results from 10 such tests have been used in court cases. Only in the first case was evidence of this nature contested because the technician performed the test only once prior to that case. Closely related species, sex and age differences and components of processed or cooked meats cannot be determined.

Soon to be published for hair identification, "Identification of the Dorsal Guard Hairs of Mammals in Wyoming," by Tom D. Moore, biologist, Wyoming Game and Fish Department. A Federal Aid in Fish and Wildlife Restoration research project entitled "Improved Methods for Serological Identification of Native Wyoming Ungulates" is proposed for 1974-75. The purpose of this study is to further develop and test procedures, so the laboratory can reliably identify uncooked and cooked pure or mixed meat and blood samples including sausage and salami.
SAMPLE U.S. QUESTIONNAIRE

We are planning to prepare a handbook for meat and blood identification and would like to determine what methods are being utilized by other states. Information on the following questions would be appreciated.

1. What technique or techniques do you employ in blood and meat identification?

2. To what extent do you employ these techniques? (check appropriate boxes)

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<th>Tissue</th>
<th>Dried Blood</th>
<th>Serum</th>
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<td>Yes</td>
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   (a) Big game animals
   (b) Small mammals and furbearers
   (c) Game birds and protected avian species

Comments:

3. Approximately how many identifications are performed each year? _____

4. How often are these identifications used in court cases? __________

5. Has your technique been accepted in legal cases? __________________

   Comments:

6. Can you segregate closely related species; i.e. mule deer and white-tail deer or pintail and mallard?

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<td>Yes</td>
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7. Can you determine sex and age differences in big game species?

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dried Blood</th>
<th>Serum</th>
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<tr>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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8. Can you identify components in processed meats such as salami, sausage, or cooked meats? _____

9. Would you be interested in a copy of this handbook upon its completion? _____

Any literature pertaining to the techniques employed by your agency would also be appreciated. Thank you.
The following information concerning the Canadian provinces was supplied by Stan Webb of Alberta for the Alberta Fish and Wildlife Division, Department of Lands and Forests in Edmonton. Mr. Webb prepared a survey in the provinces similar to our survey in the United States in preparation of a report entitled "Positive Identification of Big Game Meat Through Electrophoresis and Related Techniques." We would like to extend our appreciation to Mr. Webb for giving us access to the information gathered.

SAMPLE CANADIAN QUESTIONNAIRE

POSITIVE IDENTIFICATION OF BIG GAME MEAT THROUGH ELECTROPHORESIS

1. Are you aware of the electrophoresis method for species determination?  
   Yes____ No____

2. Are you presently employing electrophoresis in the enforcement of game limits?  
   Yes____ No____
   If yes:
   a. Method used:  Paper____ Gel____
   b. Length of time this technique has been employed:  ______________
   c. Do personnel using the electrophoresis technique require any specialized training?  Yes____ No____

Advantages to method:  ______________________________________________________

Disadvantages to method:  ____________________________________________________

3. Do you know of any other provinces or states that are employing this technique?  Yes____ No____
   If yes, could you possible list their addresses:

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
ELECTROPHORESIS TECHNIQUES
Canadian Survey Results

The discussion, advantages, and disadvantages are the views of province or state under which they appear.

Alberta: Aware of technique - Yes Using technique - Yes Type used - Gel

DISCUSSION - Work on acrylamide gel electrophoresis was started in Alberta in 1973 by wildlife research technician Sandy Drummond. Research at present is being continued by Bob McCymont, wildlife research technician, who is experimenting and building a species comparison index. This material has not yet been used in Alberta courts.

British Columbia: No reply to questionnaire; apparently not as yet using electrophoresis in species determination.

Manitoba: Aware of technique - Yes Using technique - No

DISCUSSION - The R.C.M.P. Crime Detection Lab in Winnipeg is currently investigating gel electrophoresis technique and will possibly be using it within a year. Presumably other R.C.M.P. labs are taking similar action. Advantages to method - Identification of bison meat from that of domestic cattle. Differentiation of meat from all members of the deer family.

New Brunswick: Aware of technique - Yes Using technique - Yes Type - Gel

DISCUSSION - T. G. Dilworth, assistant professor at the university of New Brunswick, has been doing the electrophoresis work for the Fish and Wildlife Branch since 1969. The method has been accepted in the provincial courts since 1971. At present he is concerned only with moose and deer for enforcement purposes but has looked into other species for comparison purposes. Advantages to method - Accurate if carefully employed. Disadvantages to method - No faster than the precipitin test if the antisera are available for the precipitin test.

Newfoundland: Aware of technique - Yes Using technique - No

Nova Scotia: Aware of technique - Yes Using technique - No

Northwest Territories: Aware of technique - No

Ontario: Aware of technique - Yes Using technique - No

DISCUSSION - It is the opinion of the Ministry of Natural Resources for the province that hair identification, which is already accepted in court where evidence of this nature is required, is sufficient as it can usually be found on suspect meat. In addition, a booklet entitled "A Manual for the Identification of Hairs of Selected Ontario Mammals" by A. S. Adorjan and G. B. Kolenosky has been published. In this manual can be found examples of hair features of 53 animals of the province.

A-12
Prince Edward Island: Aware of technique - Yes Using technique - No
Quebec: Aware of technique - No
Saskatchewan: Aware of technique - No
Yukon Territory: Aware of technique - Yes Using technique - No

NOTE: It is possible that the R.C.M.P. crime labs in the majority of the provinces have such equipment or access to it. Universities also may have electrophoresis or related units on the campus for use in the medical sciences. These could possibly be used where evidence of this nature is required by game management divisions that do not have the necessary equipment. A comparison index must be available also in cases where this is done.