Use of museum specimens in toxic chemical research

by Anne Meachem Rick

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Abstract

Biological samples provide valuable data on contamination of the environment by toxic chemicals, and much knowledge of current environmental toxic chemical distribution has been derived from chemical analyses of plants and animals. Museum specimens, collected at various times in the past, can provide historical information concerning environmental toxic chemical incidence, thus adding another dimension to environmental contamination studies.

The nature of biological specimens in various types of museum collections is described. Possible sources of tissue contamination due to museum collecting and preserving techniques are discussed to help the toxic chemical researcher evaluate the occurrence of toxic chemical residues in museum specimens. Suggestions are given on using museum material in toxic chemical research without destroying the biological value of specimens to the museum.

Résumé

Les échantillons biologiques fournissent de précieuses données sur la contamination de l'environnement par les produits chimiques toxiques. Beaucoup de nos connaissances sur la répartition actuelle de ces toxiques dans l'environnement proviennent d'analyses chimiques de plantes et d'animaux. Les spécimens de musée, recueillis à diverses époques, peuvent fournir des renseignements d'ordre chronologique sur l'incidence des toxiques dans l'environnement, permettant ainsi d'ajouter une nouvelle dimension aux études sur la contamination de l'environnement.

La publication décrit la nature des spécimens biologiques de collections de musée de types variés. On y traite des sources possibles de contamination des tissus causée par les techniques de cueillette et de préservation afin d'aider le chercheur dans son évaluation des résidus de produits chimiques toxiques se trouvant dans les spécimens. On y fait aussi des suggestions sur la façon d'utiliser ces spécimens pour fins d'analyses chimiques sans en altérer la valeur biologique.

Introduction

Over the last few years, several problems concerning persistent chemical residues in organisms have been clarified or solved through the utilization of animal samples from the past. Investigators have used mainly feathers or eggshells of birds and either whole bodies or muscle tissue from fish in studies of heavy metal and persistent organochlorine compound residues. As museums are the major repositories for dated biological specimens collected from known localities, they have been the primary source of such materials.

Specimens from museum and other collections have been used in a number of recent toxic chemical studies. Ratcliffe (1967) was the first of several investigators to examine eggshell thickness trends over a long period. He studied museum collections of eggs collected from 1902-1967 of the Peregrine (*Falco peregrinus*), Sparrow Hawk (*Falco sparverius*) and Golden Eagle (*Aquila chrysaetos*) in Britain. Ratcliffe (1967) discovered a time correlation between a decrease in eggshell weight (and therefore thickness) in these species and the extensive introduction of persistent organochlorine pesticides into the environment. In the United States, Hickey and Anderson (1968) examined eggs from both museum and private collections. They demonstrated a decline in eggshell weight for Peregrines, Bald Eagles (*Haliaeetus leucocephalus*) and Ospreys (*Pandion haliaetus*) coincident with population declines of those species. Shell weight remained the same in eggs of Golden Eagles, Red-tailed Hawks (*Buteo jamaicensis*) and Great Horned Owls (*Bubo virginianus*), species whose populations had remained stationary. Hickey and Anderson (1968) also noted that population declines of the three raptor species with thin eggshells were coincident with the use of DDT. Eggshell collections were extensively used in a later study of population decline in Brown Pelicans (*Pelecanus occidentalis*) by Anderson and Hickey (1970). Peakall (1974) used DDE concentrations in dried membranes of Peregrine eggshells collected between 1894 and 1968 to trace the occurrence of DDT in this species.

Whereas eggshells often can be studied without destroying them, most other toxic chemical studies using museum specimens have required chemical analyses of portions of the animals. Jensen (Anonymous, 1966) analyzed eagle feathers for polychlorinated biphenyl (PCB) residues; although he analyzed feathers from as early as 1880, he first found these industrial compounds in feathers from a bird collected in 1944, suggesting environmental contamination with PCBs by that date. Feathers have also been used in studies on mercury; analyses of feathers from fresh and museum specimens of ten Swedish species, spanning the last 100 years (Berg *et al.*, 1966), showed a large increase in mercury concentration during the 1940's and 1950's when alkyl-mercury compounds were extensively used as seed dressings in Sweden.

Recent interest in heavy metal residues in fish has stimulated studies in which muscle tissue from museum fish has been analyzed for mercury content. Although results have not been published, several Canadian federal and provincial agencies have analyzed museum fish caught in the last few years in order to document mercury contamination of certain Canadian lakes and rivers. Zitko *et al.* (1971) analyzed muscle tissue from two museum specimens caught in 1924, an American eel (*Anguilla rostrata*) and a lake trout (*Salvelinus namaycush*), in a study on methylmercury residues in freshwater fish from New Brunswick and Nova Scotia. The methylmercury level in the museum eel did not differ significantly from concentrations in modern eels. Evans and Bails (1972) made more extensive use of museum fish in their report on total mercury levels in muscle of fish from the Lake Erie–Lake

St. Clair region of Ontario. Residues in fish collected between 1920 and 1965 were compared with those in fish collected in 1970 and 1971; results showed trends toward higher mercury levels in more recent specimens. Miller *et al.* (1972) looked at mercury residues in tuna and swordfish. Results of their analyses indicated no differences in residue magnitude between museum specimens collected from 1878 to 1946 and modern fish, providing evidence that mercury levels in these fish today may be of natural origin rather than the result of pollution of the oceans by industrially-derived mercury. A study on recent and 90-year-old benthopelagic fish by Barber *et al.* (1972) suggested that mercury levels in two species had not changed with time despite increasing concentrations of mercury in the environment.

Although the studies cited above seem to demonstrate the usefulness of the historical perspective in solving problems of toxic chemicals in fish and wildlife, there are several difficulties in this approach. First, many toxic chemical researchers are not familiar with traditional museum collecting practices and may not know what specimens are available. Among vertebrate classes, for example, tradition in one class may have dictated saving the entire body preserved in fluid but in another class only the skin and skull of the animal have been retained.

Secondly, museum workers have developed collecting and preserving techniques which although suited to their own needs can be a source of contamination when chemical analyses are made. Organochlorine pesticides have been used to kill fish destined for museum collections, and mercury and other metals were often used in the past in preparation of both vertebrate and invertebrate museum specimens. Numerous other chemicals are also used in preparing specimens for museum storage. As accounts of the methods used in preparing individual specimens are rarely kept, the analyst may be faced with unknown contaminants in the sample he is studying. Gibbs *et al.* (1974) investigated possible specimen contamination from preservation techniques. Heavy metal analyses on museum lantern fish (*Myctophidae*) collected between 1885 and 1969, and on recent lantern fish specimens preserved by three methods suggested many factors interacted to alter the heavy metal concentrations of the preserved fish.

A third difficulty arises from the increasing popularity of the historical approach in toxic chemical research. A museum contains many animal and plant specimens, which are unique in the sense that they can never again be collected at the same locality at the same time. These specimens must suffice for the use of present and future scientists. Lately some curators have become alarmed at the number of specimens which are either being damaged or destroyed when tissue samples are taken. Museums are now placing some restrictions on the type and amount of material which can be removed for toxic chemical studies.

None of these problems are insurmountable although expanding research into environmental distribution of persistent toxic chemicals may make increased demands on museums. Continual refining of analytical techniques together with a better knowledge of chemical storage in the animal body should permit taking smaller tissue samples as well as using such parts as bone, teeth, fur, and fish scales which can often be removed without destroying the specimen's museum value. There is also the possibility that the importance of chemical analysis in relation to other methods of detecting toxic chemical effects will decline in future. If the effect of toxic compounds on animals proves to be readily detectable through studying the structure of body tissues, then gross

Mammals

examination, radiographs and other techniques for detecting chemical loads will become feasible. Thus greater use of museum specimens for toxic chemicals research need not inevitably lead to their increased destruction. And if the toxic chemicals researcher learns about museum methods and practices, becoming aware of the material available and the contamination problems, he will be more able to choose intelligently the tissue suitable to his research project.

This report informs toxic chemicals researchers about the types of plant and animal material in museum collections, and museum techniques that may cause chemical contamination of specimens. The report also points out some possibilities for using various types of museum material in toxic chemical studies and presents some curators' views on the subject.

Most types of collections discussed are in national, provincial or university museums and within university science departments. Other collections emphasizing economically-important plants and animals will be found in fishery, forestry, agricultural and health agencies. There are also private collections.

Canadian information sources have been emphasized and the techniques discussed are mostly those used in Canadian museums. However, many techniques originating in British, American or other institutions are now, or have been, used in Canadian museums and are thus treated in this report. For those interested in more detailed information about collecting and preserving techniques, selected references have been included in the Literature cited section.

The traditional museum mammal specimen usually consists of skin and skull. Only in the last two decades have some collectors begun systematically to save other parts of the specimen. Thus it is uncommon to find postcranial skeletal elements or fluid-preserved whole animals or organs among the material collected in the 19th century and the first half of the 20th century.

1. Material in collections

1.1. Skins

Small and medium-sized mammal pelts (up to approximately raccoon size) are usually made into round skins. The cleaned skin is filled with cotton batten or similar material, and the skin sewed up so that the final product slightly resembles a stretched-out live animal. Feet are left attached to the skin and most or all of the lower leg bones (stripped of flesh) are retained and wired to add rigidity. Only the skin of the tail is kept, and is strengthened with wire.

Cased skins are sometimes made from small or medium-sized mammals; the body is removed through a hole cut between the hind legs and the skin is not stuffed or sewn. Foot bones are usually left with the skin. Most large skins are dried flat with feet attached but some or most of the foot bones skinned out. Skins purchased from the fur trade often have feet and tails trimmed off.

1.2. Skeletons

The skull of every mammal specimen is saved whenever possible and skulls picked up in the field often are added to collections as well. Partial skeletons such as skull and long bones or skull and bones that were not retained in the skin, are more common in museum collections than complete skeletons. Curators particularly interested in osteology may have in their collec-

tions carefully prepared skeletons containing hyoids, small toe bones and other delicate bones. Bones of large mammals such as sea elephants and whales are rare in collections.

Some museums have collections of antlers, with or without portions of the skull attached, and horn sheaths from bovines and pronghorns.

Specialized collections of bones useful in taxonomic separation, such as calcanea (heel bones) or bacula (penis bones) may exist in some museums.

1.3. Whole specimens, soft parts

Occasionally reproductive or digestive tracts or embryos were saved in the past, but seldom whole animals. Within the last 10 or 15 years, however, curators have begun to preserve large numbers of whole animals in fluid, especially small forms like bats and rodents. Sometimes skin and skull of a carcass are prepared in traditional fashion and the internal organs are preserved in fluid. Temporary study collections of internal parts may occur in museums, usually separate from the main collection.

There is little permanent storage of frozen mammal material. Yet whole specimens may be kept in freezers for several years, awaiting preparation into standard study skins and skeletons. Some museums also keep large mammal skins in cold storage until they can be sent to the tannery. Space limitations usually ensure fairly rapid turnover of frozen material so that it is unusual for carcasses to be kept for more than a year or two.

Whole small mammals can now be prepared by freeze-drying, but the technique is new as well as costly and few museums have many specimens preserved in this way.

Mammal tissues for pathological studies which have been fixed, stained and mounted on slides, teeth sectioned for age estimation and other material from particular research projects may be found in museums. These specimens are usually not part of the catalogued collections and comprise only a small amount of material.

2. Preservation techniques

2.1. Killing agents

Most mammals in museum collections have been trapped or shot, but some of the larger predators may have been poisoned. Consequently, it is possible for a specimen to be contaminated by lead from shot or poisons such as arsenic, sodium fluoroacetate, strychnine or thallium sulphate. However, preservation techniques are more likely sources of contamination than killing agents, and they are discussed in detail below.

2.2. Skins

The collector may apply various chemicals to a museum study skin in an attempt to cure it and to prevent insect damage. Alum, borax, magnesium carbonate, salt and saltpetre have been used as preservatives, and arsenic, DDT and mercuric chloride as insecticides. In the past, a mixture of powdered arsenic, potassium bicarbonate, camphor, alcohol and white soap was used to preserve skins and skeletons. Most museums no longer apply insecticides directly to skins, although arsenic is still put on skins collected in the tropics. Various commercial hide poisons were used by early collectors.

Sawdust, cornmeal or similar moisture absorbing materials are rubbed on the skin to blot up blood and other fluids during skinning. Fatty skins may be rinsed in solvents such as carbon tetrachloride, benzene, kerosene, turpentine, acetone, gasoline, or naphtha gas and washed in detergent. The fleshy hoofs or footpads of large animals are sometimes injected with formalin. Small mammal skins dry quickly and need only

minimal chemical treatment to make good study skins, whereas large skins are more difficult to preserve and are often sent to a tannery.

Cased skins of small or medium-sized mammals may have been mounted on cardboard stretchers cut to fit inside the skin. There is a possibility of specimen contamination from the cardboard.

2.3. Skeletons

Museum skeletons are prepared in four ways: (1) burying the animal until the flesh disappears; (2) macerating in water until the flesh rots; (3) boiling; and (4) allowing carnivorous insects such as dermestid beetles to clean the skeleton. Detergents, enzyme formulations, sodium sulphide, sodium perborate, ammonia or crescylic acid are sometimes used in techniques (2) and (3) to hasten flesh removal. Some rough-fleshed skeletons are treated with arsenical formulations in the field to repel insects.

The cleaned skeleton may receive no further treatment or may be degreased and bleached. Degreasing agents include ammonia, benzene, gasoline, carbon tetrachloride, varsol, trichloroethylene and sodium perborate. Bleaching solutions usually contain hydrogen peroxide or chlorine.

Plastic compounds such as polyvinyl acetate and acrylics are currently used for impregnating and coating bones, both modern skeletons and paleontological or archaeological specimens. Elmer's glue, Bulldog glue, alvar and Gelva V-15 are all polyvinyl acetate formulations in common use. When thinned with a solvent such as acetone they penetrate and strengthen fragile bone; applied as a thicker solution they produce a protective, elastic surface coating. Both natural animal glues and plastic glues are used to repair broken bones. Shellack was used on bones, especially skulls, but is no longer popular.

2.4. Fluid preservation

Whole or partial mammal specimens for wet storage are preserved in formalin, ethyl alcohol or isopropyl alcohol. Various chemicals such as acetic acid, picric acid, phenol (carbolic acid) and glycerine may be added to the formalin or alcohol to improve its preservation qualities.

2.5. Freezing

Frozen specimens are usually kept in plastic bags, but some may be put in the freezer without any covering. Freezing is only temporary storage until the specimen can be processed further.

2.6. Fumigants

Naphthalene, paradichlorobenzene and other mothball-type crystals are used in many museums as insect deterrents inside specimen cases. If severe insect infestations occur, it may be necessary to fumigate the entire storage area. Fumigants such as carbon disulphide, carbon tetrachloride, ethylene dichloride and methyl bromide are used. Heavy fumigation can leave a residue coating on the specimens which is sometimes visible as crystals.

3. Remarks

Skins and skulls, abundant in collections for more than a hundred years, should be good sources of material for toxic chemical research, whereas other mammal parts are less common in museums. Skulls receive little chemical treatment for preparation as museum specimens. Small amounts of bone could be taken from a skull for chemical analysis without damaging the skull's museum value or a portion of a tooth could be removed instead of an entire tooth. Postcranial skeletons could be valuable both for measuring or weighing to detect contaminant-induced changes in bone, and for chemical analysis. Analyzing pieces of long bones or foot bones

Birds

left in skins could partly solve the problem of skeleton rarity.

Fur and skin will often be difficult to get from museums because sample removal may disfigure the specimen, but fur analysis could be rewarding in studies on mercury and other toxic compounds which are stored in keratinized tissue which also includes hoofs, claws and horns.

Parts of internal organs from whole mammals preserved in fluid should be easier to obtain than preserved muscle tissue since samples can be taken without much specimen damage.

Although major museum sources of muscle, fat, liver and kidney tissue are fluid-preserved whole specimens, some dried muscle and fat may adhere to the accessible inner surfaces of cased and flat skins and could be removed without harming the skins themselves.

Museum bird collections are composed mostly of skins, although there may also be substantial numbers of eggs, nests and wings. Bird skulls are less useful taxonomically than mammal skulls, and have not ordinarily been saved as separate study items but instead are left inside the skin to produce more realistic specimens. Many curators of ornithology have begun to save skulls separately, and to build up their skeletal collections. Few birds are preserved in fluid.

1. Material in collections

1.1. Skins

Birds are prepared as round skins. The tibiotarsus and more distal bones of each leg usually remain with the skin, as do the humerus and other wing bones. The occipital region of the skull is cut away so that the brain and tongue can be removed, and the forward portion of the skull as well as the beak are left in the skin. Fleshy areas in large birds such as the wing and lower leg muscles and the fatty uropygial gland are removed to prevent rotting and staining of the skin. The cleaned skin is stuffed with cotton batten or other filler which has been wound around a wooden stick. Some bird skins are now prepared according to the Power method in which the skull and a complete leg and wing from one side of the body are removed and skeletonized, the other leg and wing remaining with the skin.

1.2. Skeletons

Skulls and skeletons are poorly represented in collections made prior to the last 10 years. Some museums are now gathering representative skeletal collections, but these often include only a few individual skeletons from each order of birds.

1.3. Eggs and nests

Most egg and nest collections date from the late 19th and early 20th centuries when egg

collecting was popular among amateur and professional ornithologists. The Migratory Birds Convention Act of 1916 prohibited the taking of eggs and nests of many species without a special permit which limited subsequent egg and nest collecting.

1.4. Wings

Some museums may have reference collections of dried, spread-out waterfowl wings which they use for teaching and identification purposes.

1.5. Whole specimens, soft parts

Whole birds and various soft parts, preserved in fluid, are rare in collections made before the 1960's, and usually consist of a few embryos or stomachs. Since that time some museums have built up collections of pickled birds, especially the smaller forms.

Frozen whole birds are often kept for as much as several years while awaiting processing into study skins, but museums do not have catalogued collections of frozen birds.

2. Preservation techniques

2.1. Killing agents

Birds are usually shot, netted or trapped but large numbers are sometimes collected after aerial collisions with high structures. Some waterfowl may have been victims of lead shot poisoning, raptors are sometimes poisoned by mammal baits, and pesticides kill both small and large birds. The organophosphate insecticide, fenthion, is sometimes used as a bird toxicant.

2.2. Skins

Bird skins receive similar preparatory treatments as most mammal skins and many of the same compounds are used. Substances which may be applied to the skin or feathers are alum, arsenic, mercuric chloride, borax, phenol, DDT, magnesium carbonate, magnesium oxide, salt or saltpetre. Skins may be washed in a solvent or a detergent, to which ammonia or other chemicals may be added. Museums now frequently use only salt or borax on their bird skins prior to washing; salted skins may be kept frozen for some time until they can be washed and stuffed. Absorbants such as cornmeal, sawdust or plaster of Paris are used during skinning.

Large bird skins are not commercially tanned as are many medium and large-sized mammal skins. However, feet of larger birds are sometimes treated with insecticide to prevent insect attack. Arsenic and DDT have been used and are either painted or sprayed on the foot surface and bare leg.

2.3 Skeletons

The techniques for preparing bird skeletons are similar to those used for mammals.

2.4. Fluid preservation

Pickled birds are treated in the same way as mammals preserved in fluid.

2.5. Eggs and nests

Egg contents are blown out through a small hole and the interior sometimes rinsed with water; the egg is then air-dried. Enzymes can be used to help clean the inside of the shell. No further treatment is needed.

Nests lined with feathers or hair may attract insect pests, and such nests must be fumigated in the same way as mammal skins.

2.6. Freezing

Frozen whole birds or bird skins are stored in plastic bags until prepared for the collection.

2.7. Fumigants

Refer to the section on fumigants in mammal collections.

3. Remarks

Feathers have been used as indicators of mercury and PCB contamination in birds and should prove to be useful for studies of other compounds. Curators of ornithology may object to feather removal from specimens unless the feathers wanted were small ones, which could be removed without damaging the bird skin. Bones could also be valuable for chemical analysis but are scarce in bird collections. Bone samples might be obtained from round skins if a method for removing samples without tearing the specimen could be developed. Many curators would be willing to provide small tissue samples from internal organs of birds preserved in fluid, and perhaps muscle tissue also if they were removed carefully.

Eggs might prove valuable not only for studies on eggshell thinning, but also as material for chemical analysis.

Twigs and other plant material in nests could be sources of known-age matter for chemical analysis. Feathers or fur lining bird nests could serve as vertebrate analysis samples if they could be properly identified and dated.

Fish, amphibians and reptiles

1. Material in collections

Most fish, amphibian and reptile material in museums consists of entire bodies, including internal organs, preserved in formalin or alcohol. Fish and reptile collections sometimes contain eggs, and amphibian collections may also include larvae and eggs. Only the head and perhaps the tails from some large fish specimens may be preserved.

Occasionally museum collections have skins of reptiles (including shed snake skins), frogs and fish. The skins may be dried or preserved in fluid.

Many museums are now expanding their skeletal collections, but few skulls or skeletons from these classes are to be found among the older material in museum collections. Most museums have a few turtle shells and may even have turtle skeletons. There may also be collections of fish scales or otoliths (bony structures in the fish inner ear) and some material from pathologic studies. As yet few specimens are freeze-dried.

2. Preservation techniques

2.1. Killing agents

Most amphibians and reptiles are caught by hand or in dip nets, but some are taken by shooting or other techniques. Fish are usually collected with nets or traps, or by use of electric shockers or poisons. Reptiles and amphibians are killed with chloroform, ether, or carbon tetrachloride, by immersing in alcohol, formalin or chloretone or by nembutal injection. Fish may be killed with rotenone, toxaphene, antimycin (an antibiotic) or experimental fish poison formulations, anaesthetized by agents such as quinaldine, or immersed in strong formalin or alcohol. Some fish in collections may have been killed as the result of sea lamprey larviciding programs with Bayluscide (2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide) and TFM

(α,α,α-trifluoro-4-nitro-*m*-cresol). Some of the compounds discussed above, especially the persistent organochlorine, toxaphene, may contribute to contamination of an amphibian, reptile or fish specimen.

2.2. Skins

Skins are dried with no chemical treatment or may be impregnated with salt, arsenic, borax or other chemicals used in preparing mammal and bird skins. Frog skins have occasionally been mounted on cardboard to preserve their color.

2.3. Skeletons

Skeletons are prepared according to the techniques used for mammal and bird skeletons. A clearing and staining method is sometimes used on fish to make the skeleton visible through the tissue; sodium hydroxide or trypsin is used to clear the specimen, after which it is stained and stored in glycerine.

2.4. Fluid preservation

Formalin or alcohol (ethyl or isopropyl) is used to preserve cold-blooded vertebrates. In the past formalin was widely used both to fix the tissues and to store the specimen permanently. Specimens are now usually fixed initially in formalin, rinsed in water to remove the formalin and stored permanently in alcohol, or preserved directly in alcohol. Brine and even whisky or other spirits have occasionally been used for initial preservation when alternatives were not available.

The preserving solution may contain additives such as borax or sodium phosphate (used as buffers in formalin), or antioxidants like Ionox 330 (1,3,5-trimethyl-1-2,4,6-tris[3,5-di-*tert*-butyl-4-hydroxybenzyl]benzene) or Ionol (butylated hydroxytoluene) which are used to help retain natural colors of fish. Glycerine is sometimes added to the preservative to keep the specimen from hardening and shrinking excessively.

A specimen preserved in fluid is exposed to contamination from various sources including impurities in the water, formalin, alcohol or other preserving chemicals, as well as compounds derived from the jar, its lid, jar sealants, or tags inside the jar. An individual specimen may have been transferred from one type of preservative to another during its storage history, and is nearly certain to have been subjected to routine addition of fluid to replace loss by evaporation.

2.5 Fumigants

Amphibian, reptile and fish collections seldom require fumigation because most specimens are preserved in fluid. Dried specimens can be fumigated in the same way as mammals and birds.

3. Remarks

Muscle tissue, fat and internal organs, good sites of pesticide accumulation, are common in collections of cold-blooded vertebrates because most specimens are preserved as whole animals in fluid. Although few reptiles, amphibians or fish are made into skeletons, bone samples could be taken from the preserved whole specimens.

Cold-blooded vertebrates in their various life stages are often collected in much larger numbers than are mammals and birds; thus a few individuals could be removed from large series without seriously affecting the collection.

Molluscs

1. Material in collections

Mollusc collections contain mostly dried shells (or plates in the case of chitons) as the taxonomy of many species was determined on shell characteristics. Only species without external shells such as slugs, squids and marine nudibranchs have traditionally been preserved in fluid. In the past 15 years some museums have tried to expand their small collections of complete specimens.

Other types of mollusc material which may occur in collections are snail radulae (the toothed tongue of molluscs), internal shells and some fossil or subrecent shells.

2. Preservation techniques

2.1. Killing agents

Most molluscs are collected by hand, dredging or netting; some are taken from fish stomachs. Specimens to be preserved whole are usually killed by immersion in alcohol, formalin or chloretone, although narcotizing and killing agents such as menthol, mercuric chloride and tobacco solutions have also been used. If only the shell is to be kept the specimens are often boiled or left to rot.

2.2. Shells

Many molluscs are dead when collected so the shell needs no treatment other than washing. Specimens collected live are usually boiled to remove the bodies, although enzymes may be used to clean the flesh from tiny shells. Shells are occasionally cleaned with laundry bleach, caustic potash or oxalic acid. Wax or vaseline is sometimes applied to preserve the periostracum (outer horny covering of the shell) in some species. Glue is used to repair shells or to affix labels.

2.3. Fluid preservation

Whole molluscs are preserved in alcohol (ethyl or isopropyl) or buffered formalin. Storage in unbuffered formalin will eventually cause the calcium carbonate of the shells to dissolve, and old specimens preserved in this way will often have thin shells or only fragments of the shells remaining. Borax or seawater are commonly used as buffers. Some fixative and preserving mixtures used in the past may have contained both alcohol and formalin as well as acetic and/or picric acid. Glycerine is sometimes added to the preservative to keep the bodies flexible.

2.4. Fumigants

Although fumigants are seldom needed in mollusc collections, naphthalene crystals or similar materials are occasionally used to protect dried specimens.

3. Remarks

Many molluscs are collected in large lots from a single locality, so that removal of a few individuals would not destroy the collection's value. If toxic compounds (perhaps heavy metals?) are found to accumulate in shells as well as soft parts of molluscs, then dried shells could be used.

Molluscs add to their shells for most of their lives, and these additions are often readily visible as growth lines and shape changes. In species which attain large size and live for several years, one specimen could yield samples for analysis representing several different time periods, thus reducing the number of specimens needed from the collection.

Invertebrates (except molluscs)

1. Material in collections

The numerous invertebrate classes are extremely varied in their form, size and type of skeleton so that the actual portion of the animal preserved in museums depends on its class. Many invertebrates are preserved entire, although with some forms only the endo- or exoskeleton has traditionally been saved. As with vertebrates and molluscs, there has been a recent trend in museums toward keeping the whole individual instead of only a part of it. Sponges, corals, sea fans, starfish, sea urchins, sand dollars, some crustaceans and insects are groups in which only hard parts of many specimens may be preserved.

2. Preservation techniques

Only a brief discussion of techniques is given here, as many different and sometimes complex preservation methods are in use. Detailed information on preserving invertebrates can be found in books listed in the bibliography.

2.1. Killing agents

Many invertebrates are killed by immersion in formalin or alcohol; those in which only hard parts are to be saved are often cooked or left to rot and dry. Insects may be killed by potassium cyanide fumes or sometimes by fumes from other compounds such as ethyl acetate, tetrachlorethane, carbon tetrachloride, ether, chloroform, benzene or ammonia. Other anaesthetizing and killing agents which have been used for various invertebrate groups are menthol, magnesium sulphate (Epsoms salts), chloral hydrate, stovaine (amyl chlorohydrin), β-eucaine hydrochloride, carbon dioxide gas and acetic acid.

2.2. Skeletons

A certain amount of washing and bleaching may be done to calcareous invertebrate skeletons, as for mollusc shells, but some skeletons in collections were probably picked up from dead animals and have had little or no treatment. Many insects, especially hard-bodied ones, are kept as dried specimens mounted on pins or paper; some of the internal soft parts may have been treated with preservative fluids prior to drying.

2.3. Fluid preservation

Many invertebrates (like some vertebrates) must be fixed to harden the tissues to prevent distortion and shrinkage. While alcohol or formalin can fix specimens and also be used for permanent storage, other compounds have been used as fixatives for various invertebrates. Mercuric chloride, acetic acid, picric acid, potassium bichromate, sodium sulphate, platinum chloride, chromic acid, osmic acid and alum are ingredients of various fixing fluids.

Ethyl or isopropyl alcohol and formalin are the usual pickling fluids for long-term specimen storage. Glycerine or acetic acid, iodine and various buffers are sometimes added to the preservative.

2.4. Fumigants

Naphthalene or dichlorobenzene crystals are sometimes used in the cabinets or boxes containing dried specimens. Other fumigants may occasionally be used; see the section on fumigants under Mammals.

3. Remarks

Several or many individuals would be needed to make up an adequate sample of small forms for chemical analysis, but as many invertebrates are collected in large quantities from each locality the need for several in a sample should not be a problem. Internal tissues for analysis could be dissected from large invertebrates depending on the type of material wanted and the curator's willingness to provide it.

Plants

1. Material in collections

Plants of small to medium size are usually collected whole. Often only leaves, twigs and fruit or flowers are taken from shrubs and trees, although bark, stem cross-sections and other parts may be found in some collections. Many delicate plants such as mosses, liverworts and algae are commonly collected with some of the substrate to which they were attached, so that bits of wood, rock, soil, etc. may be included in the specimen packet. Special seed, twig or wood reference collections may be found in some museums.

2. Preservation techniques

Most plants are preserved by air-drying, but variations on this method are discussed.

Fungi, mosses and lichens are air-dried and often partially flattened so that they will fit more easily into envelopes or boxes. Some lichens are glued onto a paper or cardboard base before being put in envelopes. Preservation chemicals are seldom used on those plants.

Vascular plants, which include the ferns, conifers and flowering plants, may receive more complex treatment. Many plants are arranged between sheets of paper after which they are placed in a press until dry. They are then mounted on a sheet of stiff paper using paste, paper tape or a liquid mounting plastic material composed of toluene, methanol, Dow resin and ethocel. After the adhesive has dried the specimen may be sprayed with a fixative similar to those used by artists, but this is not common technique. Some plants may have been dipped in an alcohol or formalin solution after collection to keep them from deteriorating prior to drying, or lightly boiled to kill the outer cells and permit rapid water loss during drying. Large seeds such as pine cones may be stored in boxes rather than on sheets of paper, although heavy plant parts will stay attached to paper if sewn instead of glued.

Pesticides were applied to plant specimens in former years but are seldom used today. Some plants were dipped in lauryl pentachlorophenate which acted both as an insecticide and fungicide; other insecticides used on specimens were mercuric chloride, phenol, ammonium chloride, silver chloride and DDT.

Algae are often put into a formalin solution when collected and may later be transferred to a formalin and ethyl alcohol solution with glycerine and a buffer such as sodium borate added. Most macroscopic specimens are removed from the preserving fluid for identification and are ther dried and mounted in the same manner as higher plants. The minute phytoplankton are often mounted on slides.

Higher plants are seldom preserved in fluid, as drying is efficient and quite satisfactory as a preservation technique. Some museums may keep certain specimens in formalin or alcohol, with glycerine added to keep the tissues soft.

Many dried plants are fumigated in a special compartment before going into the collection. In the past carbon disulphide, carbon tetrachloride and methyl bromide were used, but today paradichlorobenzene is probably the most common fumigant. Paradichlorobenzene and naphthalene crystals are also used in specimen cases as fumigants.

3. Remarks

Individual seeds, leaves or other parts could be removed without seriously damaging plant specimens. Tree trunk cross-sections might be useful long-term indicators of chemical contamination; chemical residues in different growth rings could be compared to determine their concentration at different stages in the growth of a single tree. Analysts might also be interested in the soil or other substrate often collected with mosses, liverworts and algae.

Archaeological collections

1. Material in collections

Most archaeological material is nonbiological in origin or is biological material so altered by human manufacturing techniques that the species from which it came cannot be determined. Such material will be of little interest to the toxic chemical researcher. However, quantities of unmodified bone and shell may be found in archaeological collections as well as some plant remains and even soil samples.

Most archaeological collections contain human skeletons, some so well-preserved that even hair and bits of body tissue remain. There may also be quantities of bone and shell refuse from archaeological sites which have been saved for faunal analysis. If the animal material from a site has not been studied it will probably be found stored in bags according to the part of the site in which it was found, but if an analysis has been done, the bone and shell will also be identified, often to genus or species.

Plant remains decompose rapidly and are not usually found in abundance at archaeological sites unless special conditions of preservation prevail. Seeds may occasionally be found, or fibrous plant parts such as corncobs. The most abundant plant material in collections is usually wood, and may comprise anything from small charred fragments to posts from Indian houses or even totem poles. Other materials potentially available are reference soil samples taken during excavations and artifacts of bone, horn, shell or wood.

2. Preservation techniques

Archaeological bone and shell are washed or cleaned with a dry brush and then stored in bags or boxes. Crumbling bone and shell may be treated with a strengthening compound that penetrates to a greater or lesser extent: Gelva V-15, Elmer's Glue, alvar and other polyvinyl acetate formulations are often used for this purpose (see Mammals section).

Plant remains other than wood are often left untreated but are kept dry so that they will not decompose further. Wood is preserved in several ways. Water-saturated samples can be kept immersed in water for some years without further treatment. Dry wood is often just cleaned off before going into the collection. Wood in poor condition and wood which is being dried out may be impregnated with polyethylene glycol (Carbowax) or polyvinyl acetate to prevent cell wall collapse. Some wood may be treated with a fungicide. Soil samples from archaeological sites are not chemically treated and are stored in glass or plastic jars or in plastic bags. Fumigation is seldom needed in these archaeological collections.

3. Remarks

For the toxic chemical researcher, biological material in archaeological collections could be an important supplement to similar material in biological collections, assuming the archaeological specimens have been properly identified to genus or species. While this material usually is limited to fragments of bone, shell and wood, the time span represented by these specimens will be much greater than that of material in biological collections. In Canada, such material can range from Indian refuse thousands of years old to bones from French and English forts and from 19th or early 20th century buildings. Curators of archaeology keep more complete records of specimen preservation techniques than do curators of biological collections, so that the chemicals used to treat a particular specimen are often known. But archaeologists often give study of biological material from an archaeological site very low priority compared to artifacts, so much bone and shell is never identified and formally

placed in archaeological collections. Specimens which have been modified by man to form tools, ornaments, etc., will all be catalogued, but curators usually consider such artifacts too valuable to give away for toxic chemicals analysis.

In addition to the plant and animal collections already discussed, there may be other sources of biological material within museums. Paleontology curators may have small, informal collections of bones and other hard parts from vertebrates and invertebrates to compare with extinct forms, but these reference specimens may lack adequate collection data. Palynologists may have pollen reference collections and samples of pollen-containing sediment. Freeze-dried cores from lake beds could serve as sources of sediment, pollen, other plant fragments, and animal remains. Even specimens from old museum displays could have value for the toxic chemical analyst.

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