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The Preparation of Laboratory Specimens as an Aid to the Diagnosis of Animal Diseases

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Circular 16

June, 1922
Revised June, 1926

**The Preparation of Laboratory Specimens
as an Aid to the Diagnosis
of Animal Diseases**

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LINCOLN

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DEPARTMENT OF ANIMAL PATHOLOGY AND HYGIENE

INTRODUCTION

Many problems in connection with animal diseases require for their solution the application of methods which can only be undertaken in properly equipped laboratories. This ordinarily involves the collection, packing, and shipping of samples of specimens. The manner in which this is done is a most important factor and to a very large extent is responsible either for the success or for the failure of the undertaking.

The discussion of the subject to which the following pages are devoted is taken in hand in the hope that it may provide guidance to the veterinarians and others who may have occasion to require the assistance of a laboratory in the solution of disease problems.

GENERAL DIRECTIONS

Before consideration is given to more specific methods, it seems advisable to make mention of certain details of general importance. Among these, emphasis must be placed on the need of having the specimen accompanied by as complete a description of the problem as possible.

LABORATORY TO BE ADVISED

Not only should the laboratory be notified that certain material is being sent, but the nature of the latter should be stated. The kind of animal and the part of the body from which the specimen is taken should be mentioned. A brief description of the symptoms of the disease involved will be useful, while the suspected nature of the disease or its tentative diagnosis should be given if this is at all possible. When the material pertains to an outbreak of disease, knowledge as to its course and behavior in the herd or flock will be as useful to the laboratory worker as to the practitioner or owner.

THE IMPORTANCE OF CLEANLINESS

Another important detail is the need of collecting the specimens with regard to a maximum degree of cleanliness. Whether they be taken from the living or from the dead, absolute cleanliness must be observed, and this should be persisted

in in all further handling of the material, no matter what type of examination be required. In the case of autopsies, this cleanliness can best be promoted by a systematic and orderly method of procedure; while, when material is collected from the living animal, the same aseptic technique which a good surgeon follows should find application.

IDENTIFICATION OF SPECIMENS

When material in one shipment is taken from more than one animal it should be provided with proper identification marks. All parcels shipped should be plainly addressed and should show the name and address of the shipper. It should be remembered that prepaid shipments as a rule are more promptly delivered.

In the collection and packing of material sent for more definite purposes, close attention should be given to the more specific directions furnished when dealing with those special cases. Shipment should be made at a time when there is the least possibility of the specimens being kept in warm express offices or post offices over Sundays or holidays.

LABORATORY NOT AN ORACLE

Before engaging in the discussion of special features one more thing remains to be said, and that is, that many problems cannot be solved by laboratory methods and in order to avoid disappointment the patron of a laboratory should know what it can do and what it cannot do. It is a mistaken notion that by a peep into a microscope a laboratory worker can solve almost any problem. There are many which he cannot as yet solve at all, while there are others in which the solution involves tedious cultivation methods and animal inoculations.

NEED OF DISCRIMINATION

Certain diseases can be diagnosed with a fair degree of accuracy by blood examinations, but in many this is as yet impossible. Neither is it always possible to tell by the examination of a given organ what may be the cause of disease. For instance, laboratories frequently receive samples of blood and are asked to determine whether or not a given animal died of blackleg, when the sender of such a specimen was not aware of the fact that the germs of blackleg are usually absent from the blood, while they always can be found in the affected muscles. It is necessary therefore that a measure of discrimination be used in the sending of specimens for the solution of the various problems.

FOUR TYPES OF EXAMINATION

The material received at the laboratories for diagnostic purposes can usually be roughly divided into four groups in accordance with the type of examination required. They are the specimens sent for anatomic (pathologic), bacteriologic, serologic, and parasitologic examinations. Their treatment as to collection and packing somewhat varies and hence this must be mentioned separately. In a few cases the diagnosis may require that the material submitted be looked into from all angles, and then it is sometimes advisable that it be prepared in more than one way.

PATHO-ANATOMIC EXAMINATIONS

The anatomic examination of diseased animals or their organs has as its purpose the recognition of the disease involved by the changes it produces in the organs. Such diseases as tuberculosis, glanders, necrobacillosis, hog cholera, and many others leave their distinctive marks upon one or several organs by which they can be recognized.

Material requiring this kind of examination should be collected soon after the death of the animal and in as clean a manner as possible. Organs or tissues sent to a laboratory should never be washed, as the water used is apt to change the original color. If weather conditions permit, the material should be sent in the fresh state, that is, without the use of preservatives. This is important, as the latter are apt to change the original color and may make it impossible to use the material also for bacteriologic examination. Large pieces of tissue may, however, be packed in powdered borax. This does not penetrate very deeply and will certainly retard surface decomposition during warm weather. As a rule, smaller pieces decompose less rapidly than large ones during transit. When pieces of organs are sent in the fresh state, they should be removed from the body in the cleanest manner possible and not be permitted to come in contact with any other object or substance. They should be wrapped preferably in a few layers of cheesecloth or muslin. When those fabrics are not procurable, a loose wrapping of a porous kind of paper (paper toweling or news paper) may be the next choice. Each piece should be wrapped separately and the different packages should not be piled on top of one another, the object being to permit a rather free access and circulation of air around them.

After their collection they can be placed in the box to be used for shipping. This box should never be made air-tight;

it must be of rather open construction, so that the moisture given off by the material will not be retained within the package. This is of special importance when a considerable bulk is being included in the shipment. When small pieces are being sent, this feature is not so important provided the container is quite roomy. There should be an abundance of excelsior, hay, sawdust, or chaff between the specimens and the sides, bottom, and top of the box as well as between the various parcels comprising the shipment. In the case of poultry and the smaller farm animals, the whole carcass can be shipped after being prepared like the larger pieces. The shipping of carcasses during the warmer weather can not, however, always be advised. When it is done nevertheless, the wrapping of the carcass should be sprinkled with formaldehyde before being placed in the box.

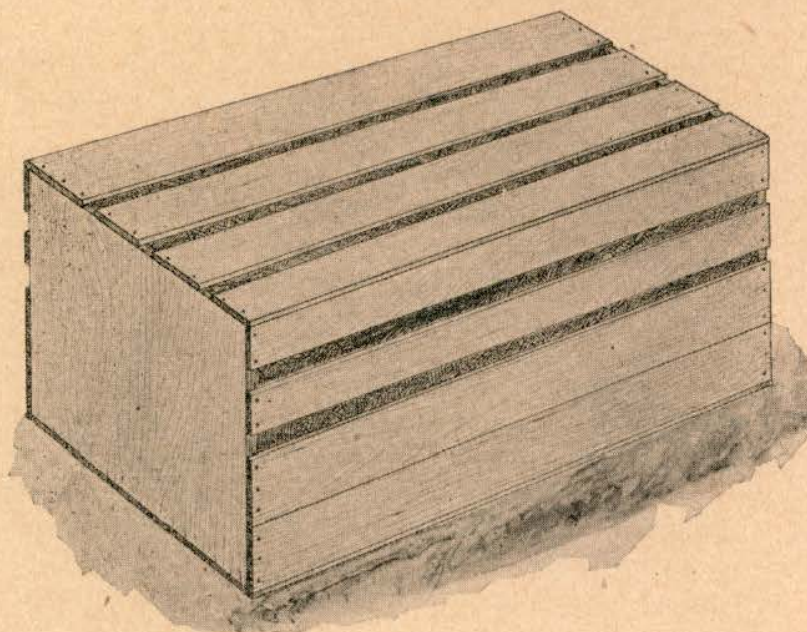


FIG. 1.—A specimen box ready for shipment. Note the open sides and closed bottom.

While it is preferable that tissues for pathologic examination be sent in the fresh state, this cannot always be done, owing to the long periods involved in transportation. In such cases small pieces may be placed in a 10 per cent solution of formalin.¹ This is even preferable in the case of tumors sent for microscopic examination. Care must, however, be taken

¹ The formaldehyde solution of commerce is supposed to contain 40 per cent of formaldehyde gas. A good solution for preserving and fixing tissues should contain 4 per cent of the gas, or, in other words, one part of the strong commercial solution to nine parts of water.

that the liquid has about ten times the volume of the tissues sent, in order to bring about the desirable degree of fixation.

Of tumors, the whole may be sent; but when this is not possible, small pieces may be taken, preferably from the growing margin, in such a way that a small bit of the invaded normal tissue remains attached.

Material taken from organs or tissues of a more or less bulky nature should never be sent for any purpose whatever in sealed containers unless it be frozen or packed in ice. Nothing is more inducing to rapid putrefaction than the packing of a juicy organ in a tightly sealed jar or can. When sections of intestine are sent, they can be ligated on both ends and preferably be packed in such a manner that they cannot come in contact with the other materials in the shipment.

BACTERIOLOGIC EXAMINATIONS

In many problems connected with disease of animals, the anatomic examination is not sufficient, as characteristic lesions are frequently absent. In quite a large number of such cases it is only possible to arrive at a definite diagnosis by the finding and identification of the causative microörganism. In some cases this can be done by a simple microscopic examination, but the great majority require animal inoculations or the use of culture methods. It is thus necessary not only that the organisms presumed to be present in the material be in a living or viable state but that they be not accompanied by a great assortment of bacteria of putrefaction. If it is thus essential to have material taken, packed, and shipped under the best possible condition of freshness and cleanliness, as was pointed out above, it is doubly so when a bacteriologic examination is in prospect. Furthermore, samples which are to serve for this purpose cannot be submerged in preservatives or antiseptics, as those substances will also kill all organisms of disease. At the most the packing of large pieces of material in powdered borax can be allowed.

The use of ice is often indicated in cases of this kind. On the whole, the specimens sent for bacteriologic examination need not be very large. They can be wrapped in clean gauze, placed in a clean, closed fruit jar, and thus packed in a candy pail full of crushed ice or snow. Pieces of organs may also be permitted to freeze and be shipped in a frozen condition, provided that enough wrapping be supplied to prevent thawing during transportation. In the case of whole carcasses the freezing should not be carried too far, as the thawing process would require too long a period for practical purposes.

Small samples of milk, pus, or blood can be sent in crushed ice in the manner already indicated, or they may be placed in a large, thick walled, sterile test tube, closed with a sterile rubber stopper and placed in a hole drilled in a block of ice by means of a brace and large bit. The hole is then closed by means of a plug of cotton and the block is wrapped in burlap and placed in an abundance of hay or excelsior.

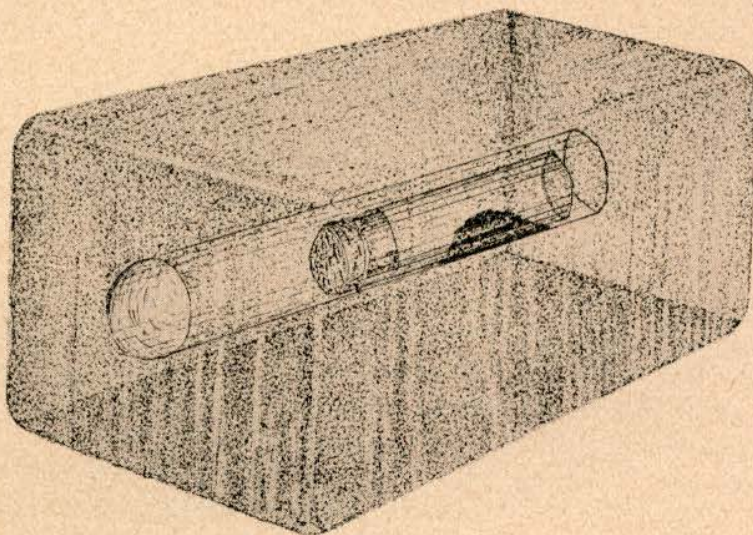


FIG. 2.—Specimen contained in a block of ice.

Another suitable method of packing in which a low temperature tends to keep the specimen fresh involves the use of a thermos bottle or jar. Specimen and container are previously chilled and the whole packed in some non-conducting material.

In many cases it is advisable to send blood smears along with the other material taken from an animal or carcass. For the preparation of smears, ordinary microscope slides are preferred, but when those are not available a piece of common window glass may be used. In either case the surface of the glass must be thoroly cleaned and freed from grease by the use of gasoline, alcohol, or ether. The smear is made by dipping the edge of a clean visiting card in the blood to be used and then passing this edge over the surface of the

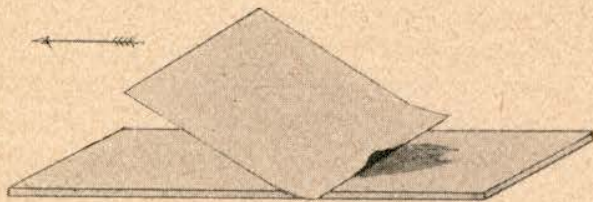


FIG. 3.—Method of preparing blood smears.

glass in such a manner that a very thin film of blood is distributed over it.

This is permitted to dry thoroly in the air and after dryness is secured the slide carrying the film is wrapped in a piece of plain paper or tinfoil, after which it is ready for shipment. It is always better to send more than one slide of the same animal, and care should be taken that they be not glued together by the omission of drying and wrapping.

Pus may be collected in a wide-mouthed, sterile bottle, closed with a sterile stopper; or it may be sufficient to collect a small quantity on a sterile swab, kept in a sterile test tube.

A swab is prepared by wrapping a bit of absorbent cotton on a piece of wire, placing it in a test tube, plugging the same with non-absorbent cotton, and sterilizing it by means of dry heat (kitchen oven). The method offers the advantage that, while small quantities of pus or discharge can be collected, there is no need of soiling the fingers of the operator. This is especially important when virulent discharge is suspected (glanders).

In the case of certain diseases, it is advisable to collect the blood on absorbent material and to permit it to dry rapidly by exposure to the air in order to encourage the rapid formation of spores. For this purpose rods of plaster of Paris, pieces of common chalk, or even clean paper can be used.

SEROLOGIC EXAMINATIONS

Serologic examinations for the detection of certain diseases are now widely employed. For this purpose small quantities of blood or serum from the suspected animals are desired by the laboratory worker. It is highly essential that during the collection and further manipulations the specimens be secured and kept in a perfectly sterile container. Sterile collecting needles or canulas and containers are thus prerequisite, while care must be taken to guard against intercurrent contamination. The samples from most of the farm animals are secured by the insertion of a hypodermic



FIG. 4. — Swab used for the collection of pus or discharge.

needle or canula into the jugular vein and under the usual precautions, which require no further description. Blood from swine may be obtained from the marginal ear vein by means of a hypodermic needle or by snipping a piece of the tail after a thoro cleansing of the parts. The blood is collected into the vial or bottle to be used for shipping.

To secure blood from chickens a few feathers are pulled from about the middle of the under side of the wing. A 2 cc to 5 cc hypodermic syringe armed with a 21 gauge needle is inserted into the large vein in the direction toward the tip of the wing. About 2 cc of blood is withdrawn and put into a small vial or bottle. The syringe and needle must be washed out with sterile water immediately after each sample is collected.

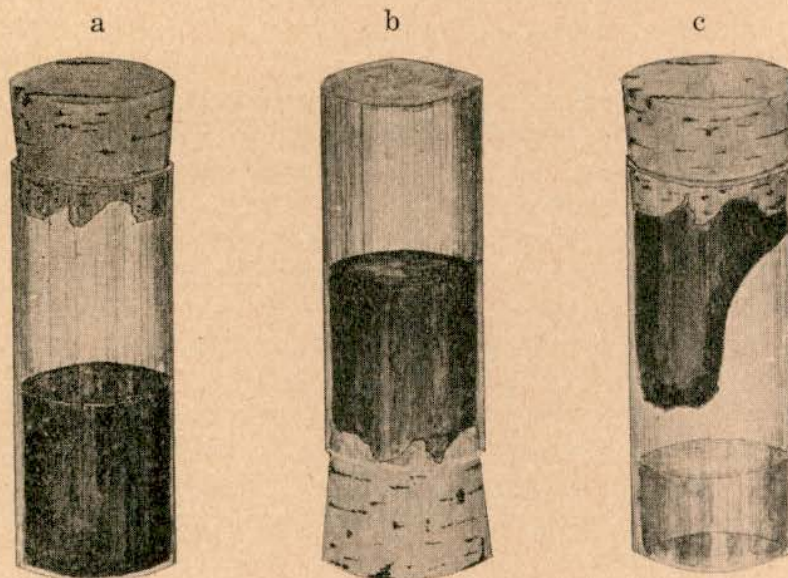


FIG. 5.—Method of procuring serum samples.
a. Vial partially filled with liquid blood.
b. Position for clotting of the blood.
c. Clot attached to stopper, and serum collected in lower part.

For the purpose of collecting, the following method is recommended. Prepare and sterilize the required number of heavy test tubes or shell vials, each one provided with a good cork stopper which is roughened on the lower surface. Permit to flow into each container from two to ten cubic centimeters of blood and close container by means of the stopper. While the blood is still liquid, the tube or vial is turned upside down and in this position the clotting of the blood is permitted to take place. When the coagulation is complete the tube with the blood clot in its upper part is again placed in its proper position and put in the cooler overnight. The serum will then

gradually flow to the bottom and the red clot remains attached to the cork. The latter is then carefully removed and the adhering clot is shaken off. The cork is replaced in the vessel, which is then ready for shipment. It is self-evident that bottles with a constricted neck cannot be used for this purpose.

Another method permits the use of ordinary small dispensing bottles. A two-ounce bottle, previously sterilized, is filled one-half full of blood and closed with equally sterile stopper. Before the blood clots, the bottle is placed in a slanting position and this is maintained until the clotting is complete. The container is again placed in the upright position so that the serum will collect in the free space within the vessel, from

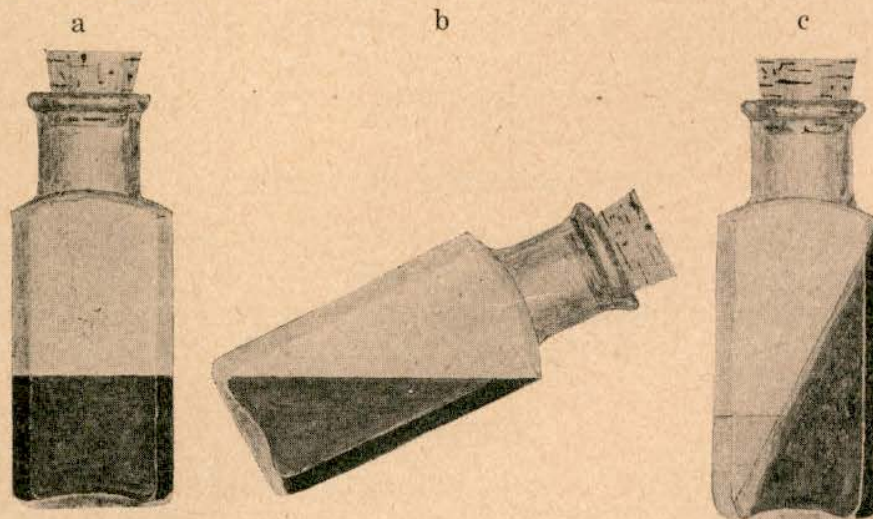


FIG. 6.—Method of procuring serum samples.
a. Vial partially filled with liquid blood.
b. Slanting position during clotting.
c. Sample after separation of serum.

which it can be readily removed by means of a pipette.

For the collection of blood from fowls a two-dram homeopathic vial is quite suitable for the purpose.

PARASITOLOGIC EXAMINATIONS

Laboratories are often appealed to for the detection and identification of animal parasites. The material required for this purpose varies largely with the problem confronting the person in the field.

In the case of the microscopic diagnosis of the parasitic skin diseases (scabies and mange), the specimens sent should consist of scrapings from the affected skin areas. As much as possible the rather recently affected parts, such as the margin of the diseased patches, should be selected for the collection of samples. When psoroptic mange is suspected the scrapings

may be made superficially; but in the case of presumed sarcoptic lesions, the scrapings must take in the entire epidermis. In suspected follicular mange it is usually sufficient to forward a small quantity of the pus squeezed out of the affected areas or pustules. Wide-mouthed vials are the best containers for shipment of this material, but when the latter is dry it can also be forwarded in tightly closed paper envelopes.

When discharge, pus, or similar substances are to be examined for evidence of parasites, they should always be sent in a considerable quantity if at all possible. Feces should be placed in a self-sealer and should have added about one-tenth volume of the formaldehyde solution of commerce.

Gross parasites, such as the intestinal worms and the like, should be placed in a 10 per cent solution of the formaldehyde solution as sold by the drug trade, in water to which a bit of common salt has been added.

MORE SPECIFIC DIRECTIONS

While the directions offered above are probably sufficient, it may not be amiss to consider briefly the special requirements for the collection and preparation of specimens pertaining to some of the more common problems which are to be referred to a laboratory.

TUBERCULOSIS

Tuberculosis is now more commonly diagnosed in the field by means of the tuberculin test than was formerly the case. As a result the laboratory is appealed to in special cases only. The material to be sent for the purpose of recognizing the presence or absence of this disease may vary. If taken from the dead animal, preference is, of course, given to any part which shows lesions and in particular to the enlarged lymph-nodes pertaining to the regions involved. If possible, the latter should be sent in their entirety, without having been cut into.

When the problem pertains to a living animal, the discharge or secretions of the organ or part suspected should be sent; while in exceptional cases it may be possible to secure, by excision or by the harpooning of the organ by means of a large straightened fishhook, sufficient material for a bacteriologic examination.

GLANDERS

The same practice should be followed in the case when a laboratory is to determine the absence or presence of glanders

in tissue or excretions. Since the more common use of mallein, laboratory examinations for this purpose have become less popular than they were at one time. When material is taken from the carcass, that which shows lesions is to be sent. This commonly involves the nasal septum, lungs, skin, and above all the enlarged lymphnodes. The last named as well as the still intact nodules of the skin are preferred. When the specimens are to be taken from the living animal, the suspected lymphatic enlargements or skin nodules must be opened in a sterile manner and the contents removed by means of a small spoon or curette or cotton swab and placed in a sterile container. The use of nasal discharge for the purpose of a bacteriologic diagnosis of glanders does not always lead to conclusive results, because even in actual glanders cases the specific organism is not constantly present in a viable form. Sometimes, however, this is all that can be obtained; and then it may be sent, even if there is prospect that the examination will have to be repeated.

Much of this kind of work is now unnecessary, because the mallein test as a rule renders a more prompt and equally definite decision. In addition to the bacteriologic test and the mallein test, the so-called complement fixation test can be made. This is a serologic test and for its purpose a blood or serum sample of the animal involved is to be collected and forwarded in the manner already described.

ACTINOMYCOSIS

When actinomycosis (lumpy jaw) is suspected, it is sufficient to send to the laboratory a bit of the pus which can be obtained from the lesions or to forward the affected tissue itself.

ANTHRAX

For the purpose of a laboratory diagnosis of anthrax, it is as a rule advisable to send a variety of specimens. In the first place, air-dried blood smears on microscopic slides (or common glass) should be submitted. Blood dried on a plaster of Paris rod, a piece of common chalk, or even a piece of paper, is a specimen extremely useful in the diagnosis of anthrax.

When material is taken during an autopsy, the shipment to the laboratory should, in addition, include the spleen or part thereof and the lymphnodes belonging to the region suspected of being involved. When an autopsy is not advisable (and this is commonly the case on account of infection danger), one of the ears of the carcass may be sent for examination.

When this is contemplated, a string or wire should be tightly drawn around the ear at its base and securely tied. The part is then cut off without disturbing the string, and the cut surface of the ear thoroly seared with a hot iron, in order to diminish the possibility of disease transmission by the specimen.

BLACKLEG

When material is to be sent to a laboratory for an examination for blackleg, the musculature of the carcass should be searched for discolored and frothy areas, and pieces taken from the same should be sent. It is not usually possible to find the blackleg bacillus in the blood or internal organs. Smears made from the affected muscle are desirable additions to the material sent.

HEMORRHAGIC SEPTICEMIA

The laboratory diagnosis of hemorrhagic septicemia requires fresh specimens of organs showing lesions as well as the heart and spleen.

HOG CHOLERA AND OTHER SWINE DISEASES

In hog cholera a bacteriologic diagnosis in the strict sense of the word cannot be made because the causative agent can be neither seen nor cultivated. Yet the problem of diagnosis in this disease sometimes seems to offer difficulties, and material is forwarded for an opinion. When this is required, organs or parts of organs showing lesions should always be included in the shipment, in addition to the kidney, bladder, stomach, intestines, and various lymphnodes. When possible it is always best to submit the carcass of an animal recently dead with the disease. This should be done in the case of any swine disease (aside from hog cholera) which may require laboratory examination as a means or aid in diagnosis.

POULTRY DISEASES

It is often desired that laboratory aid be given in the diagnosis of poultry diseases. In this instance, it is likewise preferable to have one or more recently dead fowls shipped for the purpose, packed according to the suggestions made.

RABIES

If the presence or absence of rabies is to be established by laboratory methods, the head of the suspected animal should be sent in its entirety and care should be taken that this be damaged as little as possible. It is needless to say that a dog's

head with the brain partially demolished by a close range shotgun charge will not be apt to reveal anything of a conclusive nature so far as rabies is concerned. Suspected rabies material should always be accompanied by a statement as to whether or not a person was bitten by the suspected animal.

ABORTION

In suspected abortion cases, the afterbirth or even the fetus may be submitted, provided the material be fresh and free from contamination. Blood or serum samples may be submitted for serologic examination, and they should be prepared according to the directions given above.