EXPERIMENT No. 3

Animal Inoculation

Animal inoculation was the first method of virus cultivation and for many years was the only means of virus propagation. This method is not as convenient and is also more expensive than embryo method. Besides serving to propagate the virus, the inoculation of various animal host is important in the identification of an unknown virus.

To confirm the infectious nature of a disease it may be necessary to reproduce the clinical illness in another member of the same species; either by placing healthy susceptible animals in direct contact with those showing symptoms of infection or by inoculating groups of healthy and immune (vaccinated) animals by a suitable route with a material obtained by a sick animal. If the later procedure is adopted it is necessary to insure that the material capable of transmitting the disease does not contain other microorganisms (example bacteria) which produce a concurrent infection and complicate the clinical picture. To insure that the inoculum is bacteriological sterile the material must be filtered or treated with antibiotics.

In medical virology, monkeys are frequently used for inoculation since it is possible to reproduce in these species many human diseases which show similar clinical symptoms and comparable development.

In veterinary medicine the same species or atleast those closely related to the host animal should be used and in diseases such as swine fever, which show a high degree of host specificity the use of same species may be obligatory.

On the other hand many viruses including some members of the togavirus group can be grown successfully and will produce clinical disease in a wide variety of laboratory animals such as dogs, cats, mice, rats, guinea pig, rabbits and hamsters as well as producing abnormalities of developing chicken embryo and a variety of cell culture.

Animal inoculation is used in distinguishing between viruses, which produce similar lesion such as foot and mouth disease and vesicular stomatitis of cattle. In such cases calves and horses are inoculated. Calves are susceptible to both viruses while horses are insusceptible to foot and mouth disease virus but readily contact vesicular stomatitis.

The natural host is still being used for studies of pathogenesis, immunology, vaccine trials, diagnosis and chemotherapy.

Rabies diagnosis in some case must be made by the inoculation of the laboratory animals because Negri bodies can not always be demonstrated in the brain of the infected animaK

Viruses which produce encephalitis are usually inoculated intracerebrally, the pox viruses intradermally, and those of the respiratory group intranassaly . example of other types of inoculation include inoculation into the scarified cornea of a rabbit, scarified epithelium of the mouth and tongue of calves or the foot pad of the guinea pig.

In poultry, inoculation of live virus vaccine is made into the defeathered follicles on the leg, the web of the wing, or in the case of laryngo tracheitis vaccine, into the cloacal bursa.

Before using experimental animals the operator should make himself familiar with the general directions for the care and management of laboratory animals.

The experimental animals must be healthy, before they are used and that they be properly cared for during the course of the experiment. Not only are laboratory animals prone to a wide variety of bacterial, viral and parasitic diseases, which may complicate the result of a particular experiment; but they are also liable to harbor various latent or subclinical infections, which may be activated under the stress of a particular experiment. These difficulties are largely overcome if specific pathogen free stock, or gnotobiotic facilities are available before and during an experiment the operator should personally examine the animals once or preferably, twice daily for the detection of the

signs of the disease or other abnormalities. Where ever possible rectal temperatures should be taken and recorded daily by means of a blunt clinical thermometer.

The experiment animals are used for the following purposes

1. Virus isolation
2. To study pathogenecity and host immune reactions.
3. To test and develop viral vaccines.
4. To raise monoclonal or polyclonal antibodies.

1. Virus isolation - For diagnostic purposes the experimental animals are still used for example mice in rabies and louping ill disease diagnosis.

2. To study pathogenicity and host immune reactions- This is studied in homologous host example pig in swine fever. The cost of using the homologous host is very high and therefore inbred experimental animals are used instead of homologous host. Example inbred mice used in African swine fever. The laboratory animals used as models are -

(a) Rabbit - the rabbits were used by the Pasteur to adopt street virus of rabies. In malignant catarrhal fever virus these animals react in the similar manner as the cattle.

(b) Guinea pig - guinea pigs react to foot and mouth disease virus when inoculated intradermaly in the footpad. Primary vesical is formed on the footpad and secondary vesicals appear in the mouth following viraemia.

(c) Ferrets - ferrets are used in the study of pathogenesis of distemer virus.

Other laboratory animals are also used in virus study or in the preparation of antisera against different viruses.

3. To test and develop viral vaccines - Mice, guinea pig, rabbits are used for attenuation of virus strains as well as for testing vaccines. Foot and mouth disease virus vaccine is initially tested in guinea pigs and finally in cattle and pigs.

4. **To raise monoclonal or polyclonal antibodies-** Various routes are employed -to inoculate experimental animals with virus infected material. The usual routes are Intracerebral, Intranasal, Intradermal, Intramuscular, intravenous and subcutaneous, the route of inoculation largely depends upon the nature of virus; its possible affinity for the tissue, age and species of experimental animal.

Preparation of inoculum

The material used for anima! inoculation may consist of filtered or unfiltered suspensions of organs or exudates. If the materials are unfiltered, it is important to add antibacterial substance such as penicillin and streptomycin, to prevent contaminating or associated bacterial agent from becoming established. This is specially important in intracerebral inoculation, some bacteria which are ordinarily considered non pathogenic may cause infection when directly introduced into the brain tissue of the living animal.

A general procedure for the Preparation of inoculum is as follows -

1. The infected tissue or exudate is removed from the animal with sterile instrument and placed in a sterile container.
2. The tissue is cut into small pieces and placed in a sterile mortar.
3. An abrasive such as sterile alundum (90 mesh) is sprinkled over the tissue which is ground to a paste and then suspended in tryptose phosphate broth or other buffered liquid to make a 10-12% suspension.
4. Centrifugation at 3000 rpm for 3-5 minutes will clarify the material so that the supernatant can be used in a syringe for injection.
5. After centrifugation the supernatant fluid is transferred to another tube and the antibiotics are added.
6. A period of 15-30 minutes incubation at room temperature is allowed before the mixture is injected. However if the material is collected relatively free of contamination it can be inoculated immediately into animals and chicken embryos.

Inoculation technique

The usual routes of commonly employed for the inoculation of the viruses into experimental animals are - intravenous, intramuscular, subcutaneous, intradermal, intraperitoneal, intracerebral and Intranasal. In

addition, other routes of inoculation viz. intraplantar, intradermolingual, corneal, scarification, cheek pouch and peroral may also be employed in certain specialized cases.

The sizes of the hypodermic needles are expressed as s.w.g. x inches. The abbreviation s.w.g. denotes standard wire gauge, which is the diameter of the needle. The larger is the s.w.g. number the smaller will be the needle diameter, thus 26 s.w.g. needle is a very fine one as compared with a 18 s.w.g needle. The length of the needle from the mount to the point is given in inches.

Route of Inoculation Intravenous -

Monkey, rabbits, mice and rats may be inoculated by this route. Inoculation into an ear vein may be done with a fine needle, otherwise a vein in the leg should be used for inoculation. In monkey femoral vein is used for inoculation.

1. For inoculation into rabbits, the ear should be warmed by applying warm water to dilute the vein.
2. The injection site shouid be cleaned with alcohol.
3. When the alcohol dries up, a one inch 26 s.w.g needle is inserted into the marginal vein and when it is felt that the needle is inside the vein the plunger is slightly withdrawn until a little blood is withdrawn. This will ensure that needle is in the correct position.
4. Then the inoculum is injected slowly. The average dose is 0.5 ml but upto 2.5 ml may be injected.

Mice and rats may be injected into the caudal vein.

1. The tail should be immersed in warm water (55℃) to dilate the caudal vein.
2. Inoculation is done with a 1 inch 26 s.w.g needle.
3. Volume of 0.5 ml in rats and 0.2 ml in mice is injected.

In chick, inoculation is done into the humoral vein

1. One assistant should hold the bird in supine- position with the wing well spread.

2. Enough feather are plucked to expose the vein where it passes along the inner side of the wing in the humeral region.

3. Inoculation is done with a 1 inch 26 s.w.g needle

4. The needle is inserted into the vein and the proper placing is ascertained by moving the needle to and fro and slightly withdrawing the plunger to see if some blood is withdrawn.

5. After which the inoculum is injected upto 5 ml volume may be injected.

Intramuscular -

It is very simple inoculation for which a suitable muscle may be used and usually thigh muscle is selected. In rodents, inoculation is done into the gastrocnemius muscle with a % inch 26 s.w.g needle. The needle is inserted about 2-3 mm into the flesh part and 0.05 ml is gently inoculated. In rabbits 1ml in one leg may be inoculated.

Subcutaneous

Any area of the animal where the skin is loose may be used. In rabbits and mice injection may be between the shoulders. A fold of skin should be punched between the under finger and the thumb and injection made through the fold. In large animals the loose skin along the flank may be used.

Intradermal

Any suitable site may be chosen. The hair is removed to wash the site with water. The injection is made by inserting the needle horizontally at an angle so that the needle does not penetrate deep makes the injection.

Intraperitoneal

All the laboratory animals are suitable for this inoculation, the inoculation is made to one side of the midline of the lower abdomen. As the needle is withdrawn the skin around it should be gently pinched together and held for a few seconds to prevent the inoculum from leaking back. For mice, the animal is held horizontally, the ventral surface uppermost and the needle is inserted (1 inch 23 s.w.g.) through the abdominal wall about 5 mm lateral to the mid line, at an angle of about 30° from the horizontal. The needle is

inserted 1 cm. deep to avoid puncturing of the viscera. A volume of 0.5 to 2 ml is usually injected although upto 5 ml may be inoculated.

Intracerebral

In a suckling mouse no assistance is required. The mouse is held firmly on the bench with the left hand in the sitting position of the mouse (dorsal surface uppermost) a % inch 26 s.w.g. needle is inserted vertically through the left cranial wall at a point equidistant from the anterior margin of the ear, the posterior angle of the eye and the cranial mid line. The needle is inserted to about 2 mm, 0.01 ml is inoculated and the needle withdrawn.

In an adult mouse and other animals, anesthesia is required. In an adult mouse, the animal is anaesthetized with ether and placed on the bench, The remaining procedure is the same as described, above for a suckling mouse. The needle is inserted about 2 to 3 mm, 0.03 ml is inoculated and the needle is withdrawn.

For rabbits and monkeys, drilling of a hole is required for inoculation to be done.

Intranasal

Mice and ferrets are usually employed for this route and inoculation is done with a V2 inch 23 s.w.g. needle which should have a blunt tip. The animal is anaesthetized, the animal is held keeping the head up, the needle is brought to the nostril and the required inoculum (0.05 ml in mice & 0.1 ml in ferrets) is dropped slowly.

Intraplanter

The route is employed for such disease as mousepox and foot and mouth disease in which case the planter pad (foot pad) is inoculated. For foot and mouth disease guinea pig are employed. A 1/ $^{1/2}$ inch 22 s.w.g. needle, whose tip has been made slightly blunt by a little grinding is used. The assistant should hold the guinea pig. The toes are held with the left hand by keeping them pressed with the thumb and the needle is inserted with the right hand. Several tunnels are made intradermally and inoculum injected. The volume of inoculation is 0.1 ml in each pad. It is also called interadermal pad inoculation.

Intradermolingual

This route is specially used for inoculating apthovirus (foot and mouth disease virus) in cattle. No sterilization of the inoculation area is done. The tongue of the animal is withdrawn and held tightly by taking the tip of the tounue in the grip by the assistant. A 1/$^{1/2}$ inches 26 s.w.g. needle is inserted intradermally into the tongue by keeping the needle horizontal to the tongue surface and making tunnels. It starts from the inner side of the tongue making several tunnels and then coming towards the tip about five lines of the tunnels with five tunnels in each line are made.

Corneal scarification

This route is employed in rabbit. No sterilization of the inoculation area is required. The animal is anaesthetized with ether and placed on its side. One drop of inoculum is placed on the corneal surface and gently scarified with the scalpel blade.

Cheek pouch

Hamsters are inoculated by this route. No sterilization of the inoculation is done. The animal is anaesthetized with dither and its mouth open using the left thumb and the index finger the cheek pouch is held, the animal leg suspended and the mucous membrane of the cheek pouch is exposed for inoculation. A V2 inch 26 s.w.g. needle is introduced into the pouch tissue as superficially as possible and slowly 0.1ml inoculated. This route is employed for studying the oncogenic viruses for their ability to produce tumors in hamsters.

Peroral

This route is employed in a few circumstances. The usual procedure is to hold the head high, to open the mouth, and to drop the inoculum into the posterior part of the buckle cavity and to keep the head high for some time and allow the animal to swallow the content.

Bleeding techniques

The experimental animal may be bled either by vein puncture or by cardiac puncture. The vein puncture is preferred when small volume of blood are to be recovered.

Monkey

Bleeding is done from the femoral vein using a syringe with a 1½ inch 21 s.w.g needle attached. The animal should be anaesthetized before bleeding.

Rabbit

For routine purpose where a small volume of blood is required, bleeding may be done from the marginal ear vein without any anesthetic. To distant the vein and make it clearly visible the area is swabbed with xylene or alcohol.

If a larger volume of blood is required, bleeding is done from the heart. The animal is placed in supine position and the position of the heart is located by palpating with finger. This may be done by keeping the fingers slightly over the left thoracic wall. The needle is inserted through the chest wall toward the heart and as soon as the needle touches the heart the heartbeat is felt. The needle is pushed inside the heart in one stroke and the plunger is slightly withdrawn so that blood entire the syringe freely . The required volume of blood is withdrawn and the needle is removed.

Rat, mouse, hamster

For a small volume the bleeding may be done from the retro ocular plexus of veins of the eye. A pasture pipette is specially prepared for this purpose. A pasture pipette is taken and the tip is cut with a glass cutting foil at a place so that the diameter of the tip is about 1ml. The tip is ground to make it blunt so that it may not cause any injury. The assistant will hold the animal keeping the dorsal surface uppermost. The eyelids are open with the left thumb and index finger and the tip of the pasture pipette is placed with the right hand in the orbital cavity without damaging the eye. This will result in bulging of eye to one side. The pipette is pushed further so that its tip touches the reto-ocular plexus and the pasture pipette is rotated and then slightly the pressure of pipette is loosened to allow the blood to be sucked in by capillary action. After the blood is sucked in, the pasture pipette is withdrawn and the blood is transferred into a small tube.

When a large volume of blood is required, it is collected either from the heart or by sacrificing the animal.

Chicken

Blood is withdrawn from the humeral vein with a 1 inch 21 s.w.g. needle. The bird is placed in a supine position with the wing well spread. The feathers are plucked to expose the vein. The needle is inserted in the vein and its correct position is ascertained by withdrawing the plunger when the blood will flow in the syringe. The required volume of blood is collected and the needle is withdrawn.

Cattle sheep goat

Blood is collected from the jugular vein. The hair is removed and the jugular vein is pressed with the left thumb so that the flow of blood is stopped and the vein becomes prominent. The needle is inserted and the blood will flow immediately with force if needle is placed rightly. The desired volume of blood is collected and the needle is withdrawn.For bleeding large volumes of blood,bleeding canula may be used.

It may be an advantage to withdraw blood samples at regular intervals from experimental animals to detect the possible development of specific antibodies (eg.Q fever) and to cull and carry out post mortem examination of some animals during the course of the experiment. Clinical symptoms, the development of visible lesions, abnormal behavior and all deaths, whatever the cause, should be carefully observed and recorded. At the termination of an experiment involving infectious material, all bedding utensils cages,carcasses and tissues should be removed, burned, sterilized or thoroughly cleaned by the most appropriate method. Animals infected experimentally must be held in separate isolation rooms and all persons handling infected animals, cages or other contaminated materials must pay strict attention to personal cleanliness.

Special care must be taken when performing inclusions if the material is believed to contain virulent viruses or when specimens obtained from experimentally infected animals are being processed for further passage in animals or inoculated into eggs or cell cultures.