A Method of Injecting Small Laboratory Animals by the Ophthalmic Plexus Route.* (29420)

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Intravenous injection of small laboratory animals such as mice, rats, hamsters, and guinea pigs is a somewhat difficult technique, especially when repeated injections in a short space of time are required. Moreover, the tails of rats and mice occasionally become scarred, making further injections impossible. In this laboratory, hamsters and mice (strains C57BL/6 and B10D2) are used for experimental purposes. Since the mice are black, the difficulty of using the tail veins as a route for injecting material is increased. In the case of the hamsters and guinea pigs, it is not easy to make injections by a convenient intravenous route. The ophthalmic plexus has been used successfully in obtaining mammalian blood(1,2,3,4) while Berger (5) has let blood from the eyes of frogs. These observations suggested, therefore, that the same route could be utilized for injecting material intravenously. In the demonstration of passive cutaneous anaphylaxis(6), as an example of its practical application, the orbital sinus route is advantageous, since leakage of the antigen dye mixture is wasteful and disorderly when attempted tail vein injection is unsuccessful.

Material and methods. Adult C57BL/6 mice were used for this demonstration. Each animal was lightly etherized and placed dorsal side up on a plain surface with the head turned laterally. Anesthesia is unnecessary during routine injections; however, immobilization was required for X-ray purposes. The index finger and thumb of the left hand were placed on the anterior region of the head, thus holding the animal quite firmly. At the same time the eyelid was pulled ventrally in a motion which results in a protrusion of the eyeball. This position exposes the anterior internal angle of the eye and allows entrance of the needle with the bevel edge toward the head. The needle is then pushed gently until the bone in the sphenoid region is reached. By sliding downward on this bone, a ledge or depression is felt which corresponds to the point where the ophthalmic veins converge. These veins, when punctured, allow injection of the material. Successful entrance into this plexus is assured provided only the venous system is punctured. Forcible entry will damage the corresponding arteries and may even produce a fracture of the bones in that region, resulting in a nasal hemorrhage and loss of injected material.

The dispersion of the inoculum was observed by using radiological angiographic contrast media. Mice were etherized and placed in position for injecting 0.2 cc of Hypaque® 50% diatrizoate sodium. The injections were made into the ophthalmic plexus by means of a 1 ml tuberculin syringe attached to a 15-inch length of PE10 polyethylene tubing at one end, which had the shaft of a 25″ × 5/8″ disposable needle inserted in the other end. This was done to minimize the direct exposure of the operator to the X-rays while showing the needle *in situ* during the injection. In one mouse the needle was inserted into the right eye and 4 seconds after injecting the hypaque, a film was made. The needle was then removed and a second shot taken at approximately 15 seconds, followed by a final film at 30 seconds. A Picker portable X-ray machine was used with Kodak Blue Brand films in screen casettes at 40 KVP, 40 inches target distance and 0.1 second with about 15 M.A.

Results. A normal C57BL/6 mouse is shown in Fig. 1 while the route of the dye can be seen in Fig. 2. The ophthalmic plexus has been entered, the material has been passed *via* the anterior and posterior facial veins into the external jugulars, then through

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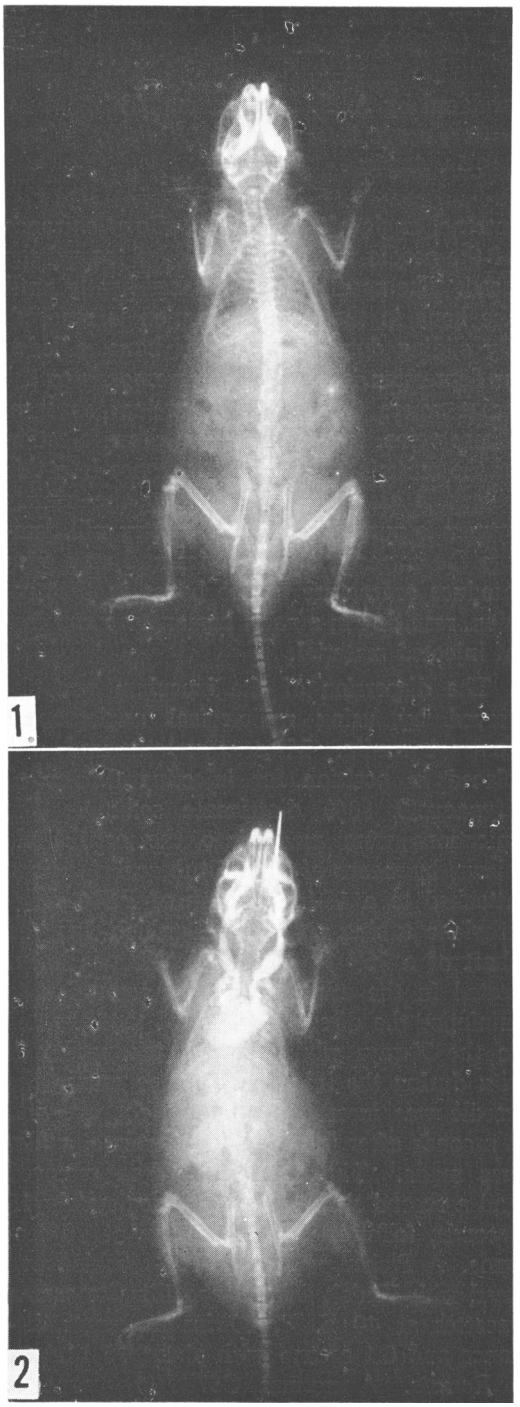

FIG. 1. X-ray of a normal C57BL/6 mouse.

FIG. 2. X-ray of C57BL/6 mouse, showing dispersion of photo opaque dye injected by orbital sinus route. Position of needle can be seen in the orbit, also material as it entered the heart via external jugulars and left and right vena cavae.

the left and right superior vena cavae into the heart. Initial renal entry can also be observed. The dye appears to have crossed over from the right to the left side of the head, perhaps due to the anastomosis of the vessels in the area and to the abnormal pressure caused by the syringe. Probable arterial flow can be observed in the area of the Circle of Willis although it is difficult to ascertain with certainty which vessels were involved. A time lapse angiogram could be used to demonstrate this fully, but from these pictures it appears that the dye does enter the circulatory system immediately without undue leakage. After 30 seconds there is no radiological evidence of the dye.

Discussion and summary. A method is described of injecting small laboratory animals by a venous plexus in the orbital cavity. This plexus, when entered with reasonable care, facilitates the introduction of materials into the circulation rapidly and without leakage. The tissues surrounding the area of the eye heal rapidly and the eyesight of the animal is not impaired.

Over 100 mice and 50 hamsters were injected in this laboratory and the success of the plexus route suggested it could be utilized in other mammalian species such as rats and guinea pigs. Rats required more manipulation and orientation of the needle than mice or hamsters, but once the plexus was located, no difficulty was encountered in introducing physiological saline (1.5 ml). In the case of the guinea pigs, injections were made directly through the nictitating membrane because of the difficulty in extruding the eyeball. However, the ledge of the sphenoid bone was easily located, thus allowing injection of saline.

From observations made while injecting different species *via* the ophthalmic plexus, it became clear that the anatomical variance in the arrangement of the blood vessels necessitated finding the area of a large vein. In bleeding the animals, probably one small vessel, when severed, is sufficient to obtain blood from the resulting pool, whereas a larger vein is required for injection. Once located in each animal this site should prove invaluable for

easier introduction of drugs, hormones, and cell preparations.

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The First Isolation of St. Louis Encephalitis Virus from Mosquitoes in Florida. (29421)

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The virus of St. Louis encephalitis (SLE) has been isolated from man in Florida during 1952(1,2). Since 1959, three recognized outbreaks of SLE which have occurred in the Tampa Bay area of the state have been studied with progressively more detail(3). During 1961, the first intensive collection of mosquitoes was made for virological examination. In this report are presented the details of the initial isolation and identification of SLE virus from Florida mosquitoes. This strain, recovered one year prior to the large number of SLE virus isolations from mosquitoes during the widespread 1962 epidemic(4, 5), was used to prepare hemagglutinating (HA) and complement-fixing (CF) antigens for the study of that outbreak.

Materials and methods. Mosquitoes which were collected in standard battery-operated New Jersey (N.J.) light traps were placed in vials, sealed and transported to the laboratory on dry ice. Pools of 50 mosquitoes or less were made by species, trap locations and collection dates, triturated in 4 ml of 10% rabbit serum-saline diluent, pH 7.4-7.6, and 0.02 ml inoculated intracerebrally (i.c.) into 2-4 day old mice.

Sensitivity of the virus to the action of sodium desoxycholate was determined by the method described by Theiler(6).

The techniques utilized for preparing HA and CF antigens from the isolate and for carrying out the hemagglutination-inhibition (HI) test were essentially those described by Clarke and Casals(7).

Complement-fixation tests were performed with 2 units of antigen and 2 exact units of complement; total volume 0.6 ml. Titers were based on the highest dilution of serum showing 3 or 4+ fixation of complement.

Serum-neutralization (SN) tests were performed by the constant serum, varying-virus technique. Mixtures of serum and virus were incubated at 37°C for one hour and 0.03 ml inoculated i.c. into groups of 6 weanlings Swiss white mice, Webster strain.

Isolation. Of 961 mosquitoes collected during a 2-week period, 2 pools designated P15 and P16 yielded a viral agent. These were from a single collection trapped on November 8, 1961 near Lake Maggiore in the southern part of St. Petersburg, Pinellas Co., Fla. This area is predominantly a hardwood swamp, a portion of which is utilized as a zoo and botanical garden.

Mosquitoes in the 2 virus-containing pools were identified as *Culex species*. Further classification was impossible because the scale pattern of the mosquitoes had been disrupted by the turbulence of the trap-collecting mechanism.

On the 9th day following inoculation, suckling mice injected with suspensions of 2 pools of *Culex sp* were either ill, moribund or dead. A 10% brain suspension prepared from sick and moribund animals was passaged to additional suckling mice. Illness