may be inhibited in tissue extracts by some macromolecular particles. The L virus is larger, has a more limited host range, and may be related to the genome of the leukemic cell.

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Technic for Intravascular Injection and Bleeding of Newborn Rats and Mice.* (24365)

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Recent quantitative studies of immunological tolerance have focused attention on the need for special technics of bleeding and injection of embryonic and neonatal animals. Billingham and Brent(1) found that a higher proportion of tolerant mice was produced when homologous cells were injected by the intravenous route into the newborn than by other routes of administration. Their method of intravenous injection requires considerable skill and is not suitable for bleeding neonatal mice. Terasaki and Cannon(2) have devised an ingenious technic for cross-transfusion of blood in embryonic chicks. An intravascular method of injection for rat embryos of 13 to 17 days was described by Grazer and Clemente(3) but it has a relatively high mortality rate. The intracardiac technic described here for bleeding and injection of newborn rats and mice was accompanied by mortality of less than 5% in over 600 newborn rats, and 40 newborn mice. This technic has been advantageously employed in studies of transplantation immunity(4), and heterologous tumor cell tolerance.

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Methods and materials. The animal is immobilized on an injecting board ventral side up (Fig. 1) with masking tape. It is important to stretch the subject as it is masked down, which helps to bring the heart closer to skin surface. The injecting equipment consists of a mounted Luer Lock Tuberculin syringe connected to a 15-inch length of P 50 polyethylene tubing by means of a plastic tubing adapter (Clay Adams size A). The 15-inch length can be conveniently calibrated for vol-

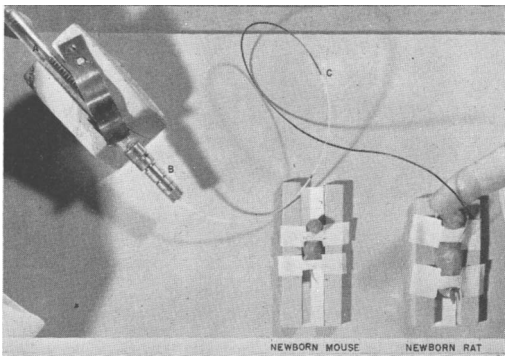

FIG. 1. Injection apparatus and procedure. (A) Luer Lock Tuberculin Syringe. (B) Plastic tubing adapter (Clay Adams Size A). (C) P 50 polyethylene tubing.

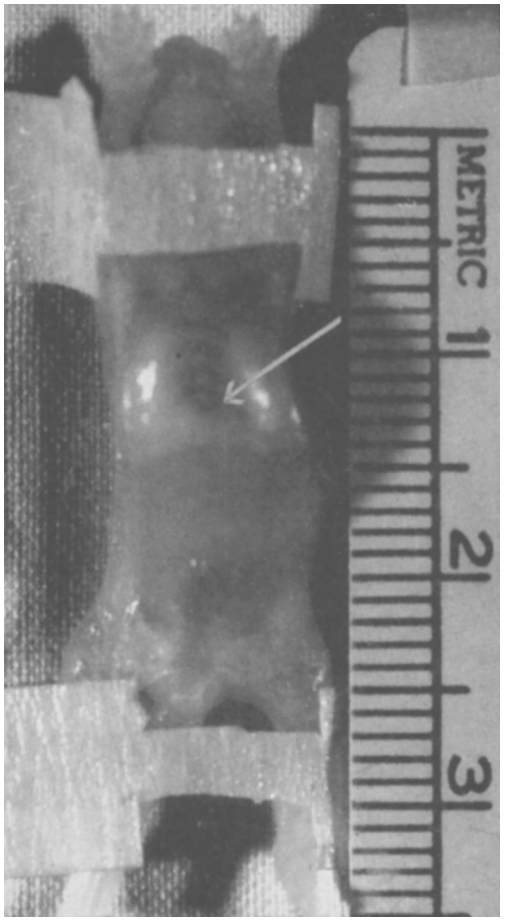

FIG. 2. Infra red photograph of newborn mouse demonstrating cardiac silhouette. Tip of white arrow is the anatomical site for injection.

umes up to 0.1 ml. A small length (½inch) of P 10 tubing is slipped inside the distal end of the P 50 tubing to serve as an adapter for the 30 gauge needle stock which is obtained by cutting off the hub of a 30 gauge needle. Before injecting or withdrawing blood, we rinse the tubing with anticoagulant (normal saline and heparin) which is then drawn back into the syringe. The air space thus created separates the material in the syringe from the blood being drawn. The needle is inserted at right angles to the skin surface of the immobilized animal to a depth of about 3 mm just to the left of the midsternal line and approximately 1 mm above the costal margin (Figs. 2, 3). As soon as the needle is in the heart, the blood pressure readily pumps the blood into the tubing and it is no longer necessary to hold the needle. The free hand is immediately used to support the weight of the tubing. This procedure allows the animal to make small movements without dislodging the needle or traumatizing the heart. Rapid injection of cell suspensions can be made without danger to the animal. However, this technic also allows the injections to be performed over a relatively long period of time. Momentary aspiration can be performed occasionally to confirm that the needle is in proper intracardiac position. The newborn rat will tolerate removal of .075 ml of blood or an injection of the same volume. Above this volume the mortality is considerably increased. Newborn rats will readily tolerate intracardiac injections on several successive days. In the same manner, they may be bled

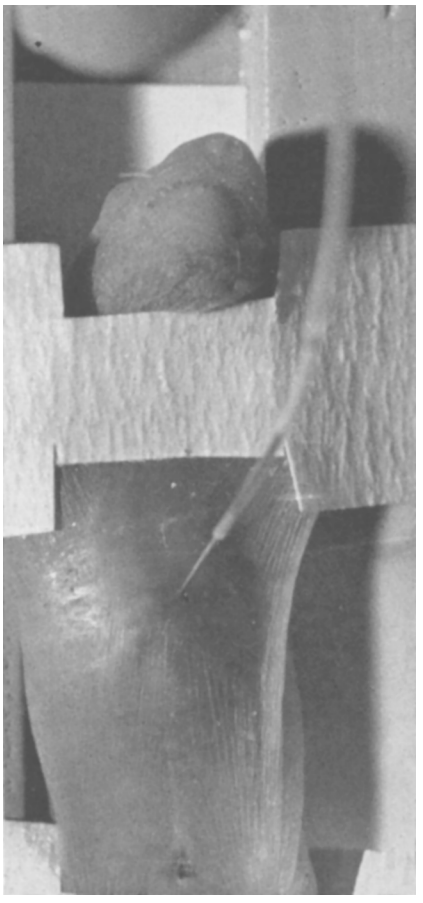

FIG. 3. Close up of injection procedure with newborn rat.

and reinjected a few minutes later without difficulty. The newborn mouse easily tolerates the removal of .025 ml of blood or the injection of .05 ml of cell suspension.

There are several advantages that this technic provides over the intravenous route. One person can perform the operations without the aid of an assistant, and the inexperienced injector can become quite proficient after the first few attempts. Because of the size of the heart and the transparency of the skin, no magnification is required as with intravenous injections. There are no post-injection hematomas, and there is no need to apply pressure after withdrawal of the needle.

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Enumeration of Vaccinia Virus Particles in Crude Extracts of Infected Tissues by Electron Microscopy.* (24366)

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A previous report indicated that accurate counts of vaccinia virus particles could be made in unpurified suspensions by diluting the crude 10% extract 1:100 and sedimenting the virus out of the diluted sample onto a collodion coated grid. Counts were then made in the electron microscope(1). It was felt that, even in the presence of much non-viral debris, vaccinia particles could be discerned clearly enough for quantitative determinations. Other studies, however, have questioned the validity of this general procedure(2). The question is whether there is some dilution range of the crude extract in which replicate counts of virus are feasible and in which counts are linear with dilution. The present paper analyzes a large number of vaccinia virus counts performed on crude 10% extracts of virus-infected chorioallantoic membrane and rabbit skin tissues utilizing count-dilution curves and demonstrates that accurate and reproducible virus particle counts are obtainable in these non-purified suspensions.

Materials and methods. Virus and virus extracts. Vaccinia virus was obtained from North Carolina Dept. of Health as a calf lymph suspension and maintained in this laboratory by passage on chorioallantoic membrane of 12-day-old embryonated eggs and on skin of rabbits. Egg membranes were removed 66 hr after inoculation and areas showing confluent vaccinia lesions were cut out and extracted in glass tissue grinder with 5% horse serum in normal saline. The extract was then centrifuged at 1600 rpm for 5 minutes. The supernate was removed, divided in aliquot parts and stored at –60°C. The egg membrane virus used was the 5th egg passage of the original calf lymph suspension. Rabbit skin passages were begun with this 5th passage egg membrane virus and harvests made as described by Parker and Rivers(3). After the pulp was extracted to 10% by weight with 5% horse serum in saline in glass tissue grinder, this extract was then centrifuged at 2600 rpm for 10 minutes. The supernate was then removed, divided into aliquot samples and stored at –60°C. *Virus particle counts.* Tubes containing the 10% vaccinia virus extracts were processed for counting immediately after thawing and thawed samples were never refrozen. If a small precipitate was present in the thawed sample, the tube was

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