Infusion Agar which has been inoculated with 5 cc of an 18-hr culture of streptococci/100 cc of agar. Place 6 penicylinders at equidistance on each plate.

*Preparation of standard.* Six solutions of the standard are prepared in buffer pH 6.0 at concentrations of 1.0, 0.1, 0.08, 0.06, 0.04, and 0.02 units/cc.

*Preparation of sample.* Body fluids may be run undiluted, or any dilution necessary may be made in pH 6.0 buffer.

*Method of assay.* Eight plates are prepared for the determination of the standard curve. Each plate receives in the penicylinders 0.2 cc of each of the prepared standard solutions. Five plates are used for the test sample, each plate receiving 0.2 cc of two dilutions of the sample in duplicate. Likewise, each of the test plates receives 0.2 cc of each of the 0.1-unit and the 0.02-unit standard. All plates are incubated at 37° C for 18 hrs and the zone of inhibition measured.

*Calculation.* From the readings of the standard solution a curve is plotted. The readings of the test sample are plotted on this curve and the number of units calculated.

### References


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2. Johnson, Balbina A., Anker, Herbert, and Meleney, Frank L. *Science*, 1945, **102**, 376–377.


## An Apparatus to Facilitate Intravenous Injections in the Mouse[1]


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This paper describes a method of transillumination of the tail veins of mice and our results in using it for intravenous injections.

The equipment consists of the box shown in Fig. 1, a lamp, a hand lens, and a mouse holder fashioned from a Lucite base and an attached wire screen, shaped to hold the mouse. The mouse is permitted to run into the holder and is confined by a stopper with a hole at its base which allows the tail to protrude. The latter is conveniently fashioned from a one-hole rubber stopper. This holder is then placed on top of the box, which has clips to receive and keep it in place, as shown in Fig. 2. When the holder is in place, the tail is just over the slit that transmits the beam of light. This slit is about 1½″ long, and its width can be adjusted to the mean diameter of the tail by movable side slots. At its proximal end, the tail is held by a clip on the box, and the operator holds the tail at the distal end to keep it taut during the injection.


[1] This document is based on work performed under Contract No. W–31–109–eng–38 for the Manhattan Project at the Argonne National Laboratory, operated by the University of Chicago. The authors wish to acknowledge the helpful suggestions of William P. Norris, Thomas T. Tourlentes, and C. Phillip Miller.


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A curved bar of Lucite is used to transmit the light from its source to the tail. The curve is about 90°, and one end of the bar fits under the slit in the top of the box over which the tail is placed. The other end of the bar comes out of an opening on the side of the box. When a microscope lamp illuminates the Lucite end at the side of the box, the light is transmitted to the end under the slit in the box top, transilluminating the tail.

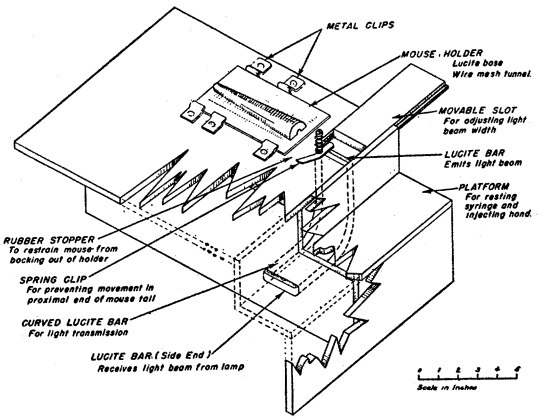


FIG. 1

The vein opposite the light is easily seen as a red cord. If the needle is introduced into the vein, the whole column of blood proximal to the needle will be replaced by the injectate. If, however, one penetrates either the fibrous band or through the vein into the core, injection is difficult and local blanching and subsequent swelling of the tail in this region is seen. There is a personal factor in

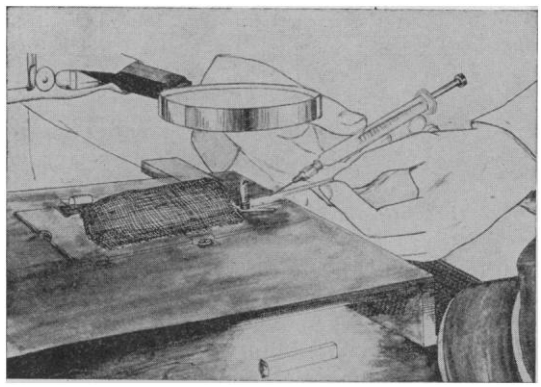

FIG. 2

acquiring the "feel" of keeping the needle superficial in order not to transfix the vein, but this can be overcome. We have found ½″ No. 27 needles most suitable.

In our experience it was possible with a few days practice to complete the injection of the tail veins of 90–95% of the animals. In a group of 170 we gave 22 injections/mouse over a period of 11 weeks. At the end of

this time the majority of the tails were free of venous sclerosis.

We believe the above technique renders practicable the multiple intravenous injection of mice.

## A Simple Self-leveling Drinking Well for Laboratory Animals

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The continuous supply of clean drinking water in adequate amounts to larger groups of rats and other small laboratory animals is both important and difficult of achievement. Automatic watering devices (*2*) are cumbersome, confine the cages to certain definite locations, and hence are rarely employed. Open water trays or cans are used by rats for "bathing" and are soon polluted with feces and urine. The water bottle with drinking stem (*3*), usually fastened outside the cage, eliminates this drawback but introduces many others. The size of the bottle is ordinarily limited to correspond with the dimensions of the cage and the amount of weight that can be safely suspended from its walls. As a rule, drinking bottles do not hold more than 50–300 ml of water. If 10 rats are placed in one cage, even the largest bottle of this type hardly accommodates the quantity of water required in one day, since, under the conditions of a moderate climate, the normal adult albino rat will drink about 30–35 ml of water in 24 hrs. Hence, the water bottle has to be dismounted, filled, and remounted at least once daily. Moreover, under certain experimental conditions the water demand often increases to a multiple of the normal intake. Rats with chronic tubular damage, for instance, frequently excrete up to 75 ml of urine per day (*4*) and consequently consume far more than 100 ml of water in 24 hrs.

In addition, many models of drinking bottles employed at present are not the commercial all-glass type (*1*), which, incidentally, are particularly difficult to fill although they are otherwise quite adequate, but consist of simple rubber-stoppered bottles which are attached to the cage in an upside-down position. A straight or bent glass tube which penetrates the stopper is introduced in the interior of the cage as the drinking stem. Not infrequently these tubes "run dry," and in the morning one may find the animals assembled around the empty stem of a full water bottle, frantically trying to obtain the much-needed supply of fluid. On the other hand, damage or loosening of the stopper will cause the water to drip out overnight; this water may be collected as "urine" by the zealous experimenter, causing him to ponder gravely on the sudden unexplained "diuresis" of his experimental animals.

It should also be kept in mind that drinking by licking the open end of a glass tube, which thereupon "hesitatingly" surrenders its contents drop by drop, is not a natural procedure and requires considerable effort. Apparently easily learned by the healthy rat and mouse, this form of drinking represents a heavy burden for the animal with a strongly increased water demand; it may become extremely difficult, if not impossible, to the sick or "drugged" animal which is frequently unable even to assume the typical position necessary for obtaining water from a tube. Animals so handicapped may still be able to drink from an open tray, placed close to the bottom of the cage. Obviously then, a full water bottle is no reliable indicator for the adequacy of the water supply in many toxicological and therapeutic studies. So-called unexplained discrepancies in toxicity figures and other observations reported by workers from different laboratories may be due in part to such hidden deficiencies in the supply of drinking water.

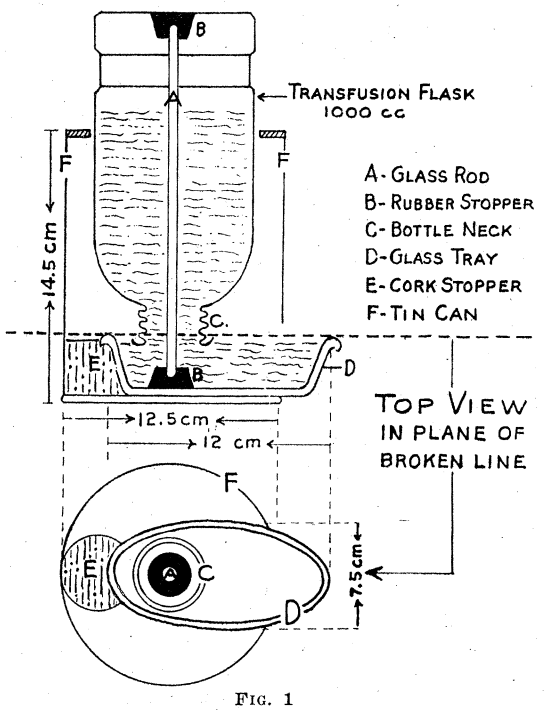


FIG. 1

This paper describes a simple drinking well which has proven its value after years of testing under varied experimental conditions. Basically, the well consists of a large, wide-mouthed glass bottle, such as a transfusion flask (capacity 1,000 cc or more), an old tin can (best with a removable cover), which just fits loosely over the flask, and an oval glass tray for bird feeding (obtainable in many 5- and 10-cent stores).

An aperture in form of a trapezoid, with the smaller of its parallel sides pointing downward, is cut in the side wall of the can near the bottom; the opening should permit the protrusion of a small part (about one-fifth) of the glass tray, which is firmly fixed inside the can and at its bottom with the aid of a large cork stopper in the manner illustrated in Fig. 1.

The flask is now filled with water and the can, with the glass tray securely fastened at its bottom, is slid