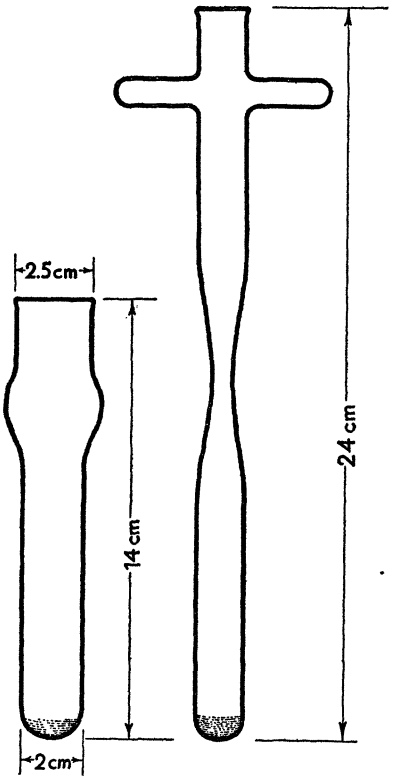

FIG. 1

We have modified it as shown in the sketch (Fig. 1) and have had it made of Pyrex glass to decrease the danger of breakage. A bulb holds the ground tissue and the salt solution used for suspending it. The plunger, which has been lengthened to prevent contamination of the top of the tube, has also been constricted to make the introduction of fluids easier. The projections on the top of the plunger make handles that can be easily grasped. The plunger must fit the inner side of the tube snugly but not so tightly as in a syringe. The bottom of the plunger is ground to fit the tube accurately, and the two should be numbered so that they can always be matched. The dimensions given are those we have found useful, but they may be modified according to the amount of tissue usually ground.

Since these grinders, when made in small quantities, cost about three dollars, a number can be kept on hand ready for use. The outer tube and plunger are sterilized separately; the former is plugged and capped, while the latter is inserted up to the cross pieces in a large plugged test-tube. The outer tube is then ready to receive tissue from an autopsy, and when convenient the sterile plunger can be inserted and the tissue ground. Salt solution or other diluent may be introduced with a pipette. The danger of contaminating the tissue or the hands of the worker is slight.

Since this grinder operates largely by pressure, organs containing much connective tissue can not be readily emulsified in it. Soft tissues, such as rabbit or guinea-pig spleen, liver, or brain, can, however, be rapidly ground to such a fine state that when diluted with an equal amount of salt solution they will pass through a No. 20 needle. It is sometimes necessary to remove coarse particles, which would plug the needle, by slow centrifugation. Cultures for bacterial counts or to secure isolated colonies from infected material can be made from the emulsions.

Recently we have found these grinders very useful for preparing chick embryo emulsions to be used in the Li-Rivers' method[2] of cultivating filtrable viruses, and for the preparation of uniform emulsions of dried tissues containing viruses.

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A SIMPLE APPARATUS FOR WASHING PROTOZOA

A SIMPLE method of washing Protozoa and other small objects in running water has proved so satisfactory in my own work that I believe other investigators may also find it useful. Its chief virtue lies in its simplicity.

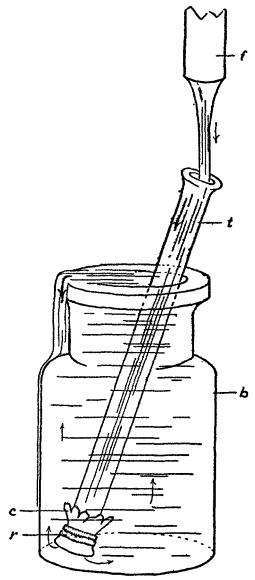
The apparatus described below is a modification of that used by Caullery and Chapellier as given by Langeron.[1] All that is needed is a section of glass tubing of convenient size, a scrap of bolting cloth, a rubber band and a bottle. The accompanying diagram will illustrate the set-up. A section of glass tubing (t) about 8 cm long and 5 or 6 mm inside diameter is heated at one or both ends and spread slightly after the manner of the bulb end of an ordinary pipette. A piece of bolting cloth (c) is tightly fastened over an enlarged end of the tube by means of a rubber band (r). No. 20 silk bolting cloth has proved quite satisfactory for average size Protozoa, while No. 25 serves for the smaller forms. This procedure is probably not suitable for the very smallest organisms.

The tube is placed, covered-end down, in a small bottle (b) of a size and shape that will allow the tube to extend well above the mouth, and provide a reasonably steady base. The organisms to be washed are placed in the tube by means of a pipette. As soon as the fluid has drained from the tube into the

[2] C. P. Li and T. M. Rivers, *J. Med. Exp.*, 52: 465, 1930.

[1] M. Langeron, "Précis de Microscopie," Maison et Cie., Paris, 1925.

bottle a small stream of water is allowed to flow into the tube, directly from the faucet (f) if the bottle can be placed within a few centimeters of it, or via an extension of rubber tubing, the end of which is supported a short distance above the glass tube. This procedure maintains a steady flow of fresh water over the material being washed as the column of water between the mouth of the bottle and the upper end of the tube exerts just enough pressure to cause a gentle flow through the tube. The waste escapes over the mouth of the bottle.



Modifications may easily be made to suit special needs. For example, if the tap water contains much foreign matter it may be strained by leading it through bolting cloth of finer mesh than that holding the organisms, before it is allowed to pass into the tube.

After washing, the material may be transferred to centrifuge or settling tubes for further manipulation. If preferred, it may be stained and dehydrated before removing from the apparatus by merely pipetting in the various fluids to be used, or by transferring the tube containing the organisms from one bottle to another containing the required reagents.

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SPECIAL ARTICLES

X-RADIATION AND REGENERATION IN AMBLYSTOMA

IT has been demonstrated within the past few years, especially by the work of Curtis[1] and his students, that x-radiation affects very markedly the regenerative activity in certain invertebrates. Coelenterates, planarians and annelids, for example, which under normal conditions readily regenerate lost parts, lose their capacity for regeneration when subjected to the influence of x-rays. Although considerable work has been done on the influence of x-radiation on regeneration in invertebrates, so far as the writer is aware no observations have been published on the effects of x-rays on the regenerative activity in vertebrates.

During the past two years the writer has conducted a series of experiments which deal with the effects of x-radiation on regeneration in embryos of Amblystoma, especially on regeneration of the fore limb. The fore limb of this animal is a convenient structure for use in experimental studies on regeneration, for the reason that under ordinary conditions it is very readily regenerated, and in embryonic stages of Amblystoma the regeneration takes place rather rapidly. The results of all experiments to date show conclusively that x-radiation in proper dosage will prevent normal regeneration of the limb in any stage of limb development.

When the limb bud is amputated at a very early stage of development, the amputation consists in the removal of merely the small mesodermal bud and its ectodermal covering. The wound heals quickly and, under normal conditions, regeneration of the limb bud and subsequent normal limb development takes place. However, the limb will fail to regenerate after amputation, if the embryo be given daily dosages of x-rays. Some growth takes place and a short spur-like appendage develops; but differentiation into upper arm, forearm and hand does not occur. Radiation appears to prevent differentiation during regenerative growth.

It is especially striking that a dosage of x-radiation which very definitely prevents limb regeneration has no apparent effect on normal limb growth and differentiation. If, for example, the limb bud on one side of the body is amputated at an early stage of limb development, and the embryo is radiated daily, the limb will fail to regenerate except for the development of a short spur-like appendage. In the same embryo, however, the unharmed limb bud on the opposite side of the body will grow and develop into a normal limb, apparently unaffected by the radiation. Other experiments, also, demonstrate this same phenomenon. For example, daily radiation of Amblystoma embryos has been begun in the blastula

[1] W. C. Curtis, "Old Problems and a New Technique," SCIENCE, 67: 141–149, 1928.