fine adjustment one of the pipettes is then brought momentarily into the drop of spore suspension and a considerable amount of the liquid with its spore load is taken into the pipette by capillary action. With the mechanical stage the chamber is moved to a clear spot on the cover slip, and a certain amount of liquid expelled, by means of a long blowing tube, to form a small drop on the cover slip. This procedure is continued until a drop is obtained which contains a single spore. The second pipette is then brought into this drop and the spore removed. The drop of sterile agar medium is brought into position and the isolated spore deposited on it. As the spores are isolated, the cover slips with the agar drop and its single spore are sealed with sterile vaseline onto deep hanging drop slides, the well of the slide containing sufficient moisture to prevent drying of the agar, and the whole is incubated at the desired temperature.

Early results were most discouraging, as none of the single spores germinated. It was deemed advisable to germinate the spores in malt extract broth, and to isolate germinated spores shortly after the emission of the germ tube, in the hope that growth, once started, would be continued. That the procedure may be successfully applied, at least to some of the common, vigorous forms, is indicated in the accompanying table.

An experienced operator can, with considerable ease and with absolute certainty, isolate 20 or more germinated spores in the course of from three to five hours with the use of this micromanipulator method, so that it is apparent that single spore cultures of many organisms can be accumulated with considerable rapidity.

GROWTH OF SINGLE GERMINATED SPORES

Organism	Germinated spores isolated	Spores continuing growth	Per cent. continuing growth
(Ascospores)			
Aspergillus fischeri	8	5	62
(Conidia)			
Aspergillus fischeri	17	10	59
(Conidia)			
Aspergillus nidulans	8	3	37
Aspergillus sydowi	12	6	50
Botrytis sp.	8	7	87
Hormodendron sp.	12	12	100
Monilia sitophila	8	8	100
Penicillium digitatum	12	3	25
Penicillium islandicum	8	7	87
Syncephalastrum sp.	11	8	73
Trichoderma sp.	9	5	55
Totals	113	74	Av. per cent. = 65

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A METHOD FOR DETERMINING THE VOLUME OF SMALL PIECES OF TISSUE

A SERIES of test-tubes of varying sizes have each a capillary tube drawn off from one side, as shown in Fig. A. With the tube retained in a perpendicular position fluid is run into the tube to a point above the lower outlet and then brought down to this exact level by air pressure exerted through the mouth of the tube. For greatest simplicity direct mouth pressure has satisfactorily served this purpose.

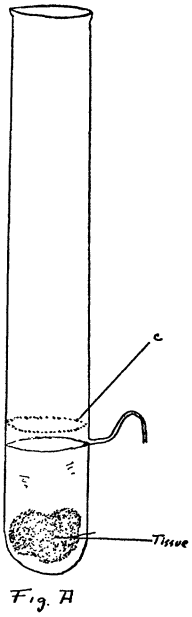

Fig. A

The tissue is then immersed in the fluid, and in accordance with the principle of fluid displacement, a new level is established at "C." By a procedure similar to that described above, the displaced fluid is collected through the capillary tube and its volume estimated.

Temperature and barometric corrections are hardly necessary, since in volumes as small as can be measured by this method the errors are negligible.

By this method volumes as small as one tenth of a cubic centimeter have with ease been estimated. It is, however, essential to use a test-tube whose diameter is only slightly larger than that of the tissue in order to secure a maximal rise of fluid and minimize error.

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ALOXITE AS AN ABRASIVE FOR GRINDING BONE SECTIONS FOR HISTOLOGICAL PURPOSES

SECTIONS of dry bone for histological study are prepared usually by one of the following methods: Grinding on a lathe, on compact pumice stone, on sand—or carborundum—paper of different grades of fineness, and lastly, on or between stones of suitable fineness. These methods are suitable but slow and tedious.

In the course of preparation of bone sections, the writer has found that aloxite powder (crystalline alumina) possesses exceptional abrasive properties for rapidly reducing bone to any desired thinness.

The technique here given has been found most satisfactory and particularly suitable for classroom use be-

cause the student can grind sections for himself quickly and inexpensively. Thin sections of about 1 mm in thickness are sawed with a hack saw and given to the students who grind them on glass plates (discarded lantern slides make suitable plates) with aloxite powder and water. The quantity of water used is just enough to maintain the powder in a pasty consistency during the grinding. Ordinary corks are used to hold the sections during the process. The grinding should be conducted by frequently alternating the surfaces, thus insuring more uniform thinness and better finish. When the sections become transparent or of tissue-paper thinness, they are polished by dipping in water, blotting dry and rubbing carefully with finger on a carborundum or greenstone hone. This last step is necessary so as to fill the lacunae and canaliculi with débris. The sections are mounted, as usual, in gelatin.

Comparative trials show that aloxite used as described cuts twice as fast as the fastest of the other above-mentioned abrasives. No more than ten minutes should be required to completely grind and mount a section.

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SPECIAL ARTICLES

THE CENTRIOLE OF THE AMPHIBIAN LEUCOCYTE

IN 1926 Belar published his observations on the centrioles of the perihepatic leucopoietic tissue of Salamandra. These studies were made by the Flemming-hematoxylin method and demonstrated the fact that a cell in the interphase contains two centrioles which have been derived directly from telophasic division of a single centriole at the pole of the mitotic spindle and that during a subsequent prophase these two centers of the non-dividing cell separate and become located at the poles of the spindle. Belar's series of figures illustrating this genetic continuity of the centriole is so completely demonstrative that it is difficult to understand why these very significant results have not received more attention in recent discussions of the central body problem.

I have repeated Belar's observations on Salamandra and have extended the study to other Urodeles, namely: Amphiuma, two species of Amblystoma, Triturus and Siren. Material was fixed in the fluids of Champy, Flemming, Benda, Helly, Bouin, and a saturated solution of corrosive sublimate in normal saline. Staining was by means of iron hematoxylin, Kull's acid fuchsin-thionin-aurantia and Benda's alizarin-crystal violet. The facts are demonstrated by any of these combinations, but the Benda method is most useful, since it stains the centrioles a distinctive color, unlike that of any other structures in the cell.

This extensive reexamination completely confirms Belar's results and in my opinion incontestably establishes the fact that the centrioles maintain direct visible genetic continuity through all the numerous cell generations in the differentiation of the mature leucocytes. This general conclusion holds for the six types examined, but in minor details there are differences among the genera. The following brief description refers, in all its details, only to Amphiuma.

The myelocyte in the interphase contains a single large aster. The Golgi apparatus is closely applied to this structure, and chondrioconts in its vicinity are oriented so that their axes are radial to its center. At the focal point of the astral rays is a spherical zone that stains considerably more heavily than the general cytoplasm, and which I shall more or less arbitrarily call the centrosome. Outside this zone and considerably excentric with reference to the focal point of the astral radiations are two granules which stain very intensely and which I consider are most accurately termed the centrioles. These have so great an affinity for hematoxylin, acid fuchsin or crystal violet that slides can be extracted until the centrioles are the only stained objects outside the nucleus. Both centrosome and centrioles are well preserved and may be stained after such fixatives as Champy's fluid, which preserves not the least trace of an aster. In early prophase the centrioles seem to enlarge slightly and then they move apart. A spindle forms between them, and upon breakdown of the nuclear membrane the chromosomes become arranged in the metaphase plate. The centriole at this stage is exactly at the focal point of the spindle fibers, but I wish to forestall any possibility of a suggestion that it is their coagulated focal point by stating that in Benda preparations the spindle fibers are light orange, while the centriole is a brilliant purple. The centriole in this material is far more clear-cut and definite than any I have ever seen. Its size and spherical shape are constant. In the early telophase, after disappearance of the polar part of the spindle, the centrosome and astral zones are reformed around the centriole, and in middle telophase it can first be clearly seen that the centriole has divided. At first these division products are very small and close together, but in late telophase they move apart somewhat and enlarge to the size which they maintain throughout the interphase.