The National Research Council will be ready to consider further requests for research assistance this fall. Applications should be filed with the Committee on Grants-in-Aid before October 15, 1933. Action upon these applications will be taken during the latter part of December.

ISAIAH BOWMAN,
Chairman

SCIENTIFIC APPARATUS AND LABORATORY METHODS

LAMELLIBRANCH LEUCOCYTES AS LIVING MATERIAL FOR CLASSROOM DEMONSTRATION

INCIDENTAL to studies concerned with the vitality of Molluscan leucocytes *in vitro* it was noted that those of *Ostrea virginica* possessed several features especially recommending them for classroom use as demonstration material. These features were found to be also evident in Venus and Anodonta and are probably common to most other lamellibranchs.

After removing one valve, a capillary pipette may be thrust into the heart and a quantity of blood withdrawn. A single specimen will yield more than enough material for a large class. The leucocytes in a drop, when placed on a slide, contract to a spherical shape and clump together. In from five to ten minutes they begin to thrust out pseudopods and move away from each other, and will then remain active for at least several hours if the drop is sealed. Since these forms occur in a practically pure culture, if properly collected, they are particularly suited to student purposes.

If a few drops of the blood are placed in a dish of the same water from which the animals came, the leucocytes will go on living for several days without further attention.[1] As the leucocytes collect on the bottom they are readily recovered by means of a pipette when needed. The advantageous features of such material are evident and include the ease with which they are obtained, the simplicity of handling, the abundance of cells and their relative hardiness. As demonstration material they show amoeboid movement, ingestion of food, and in fact all the usual features for which free-living amoebae are customarily employed to illustrate. If left in a sealed drop of sera over night they will be found to have again contracted to a spherical form. The addition of some fresh water will revivify them a second time, at least. It seems that their length of life *in vitro* is almost directly proportional to the amount of fluid in their container.

The operative technique mentioned is not imperative, as these mollusks, if placed in a water that is allowed to warm slightly, will emit quantities of leucocytes. This is the well-known diapedesis or "bleeding" of oysters under suffocation.[2] Thus a single animal can be used repeatedly if a suitable aquarium is handy to which it may be returned. Samples taken this way frequently show contamination. For example, oysters obtained from a New York market yielded an *Amoeba* of the *limax* type from the exterior of the shell and *Valkampfia calkinski* or *V. patuxent* from the intestinal tract.[3] The latter two can be cultured easily on ordinary nutrient agar plates, yielding abundant parasitic material. Thus, such a demonstration may have a further advantage, showing on a single slide, from a single source, leucocytes, free-living and parasitic amoebae all migrated or ejected from one animal with no operative effort. Without considerable familiarity it is almost impossible to distinguish the living leucocytes from the parasitic amoebae.

Since the three genera, Ostrea, Venus and Anodonta, as well as other similar ones, are wide-spread, little difficulty should be encountered in obtaining material from various localities. The animals for most part are fairly hardy and may be kept in a suitable aquarium or obtained from a market or other sources as needed.

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EXAMINATION OF THE REVERSE SIDE OF MICRO-MOUNTS

ONE of the greatest needs of the acarologist, and others who mount minute arthropods on microslides, is to see the other side of their specimen. To turn the slide over and look through the one to two millimeters of glass is hardly satisfactory because the objectives are corrected for cover-glass thickness. Obviously, this may be overcome by securing an objective corrected for a cover-glass thickness of one or more millimeters. To have such a lens made up in America is prohibitive. Besides, the American four-millimeter objectives have such small working distances as to strike the slide before coming into focus. Finally I discovered that W. Watson and Sons, London, stock a four-millimeter (six-inch) parachromatic objective with a long working distance and that this objective

[1] Orton (1924, Fisheries Invest. Series II, 6) reported that the leucocytes of *Ostrea edulis* will live from 3 to 4 days in a dish, while we have had those of *Ostrea virginica* live for 6 days under similar conditions.

[2] This effect has been discussed by Orton (1924) ref. cited and Yonge (1928), *Jour. Mar. Biol. Ass.*, 15. The slight provocation that will cause this response can hardly be considered suffocation in the ordinary sense and certainly must take place frequently in a state of nature.

[3] Hogue (1915), *Arch. f. Protist.*, 35 (1922), *Jour. Exp. Zool.*, 35.

could be made up corrected for glass thickness of one millimeter and a tube length of one hundred and sixty millimeters for something like $20.

Thanks to an American Association for the Advancement of Science research grant, I was enabled to secure such an objective. After trying it out for the period of a year, I am able to recommend it to my colleagues as an important aid for the detailed study of micro-mounted arthropods. The definition of the lens is as good as the four-millimeter objective used in the ordinary way, so that one side of a specimen is as easily scrutinized as the other.

The next step in improving technique is to limit microslides to a standard maximum thickness, say 1.3 millimeters. If the thickness is less, it can be compensated by placing a cover glass on the slide (held by a film of water) to make up the necessary thickness.

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AN INEXPENSIVE THERMO-REGULATOR

A VERY satisfactory regulator for operating a small isothermal bath may be made from an ordinary Babcock skim-milk bottle filled with mercury. One lead of wire is placed in the side tube, which is plugged with cotton and a small cork. The other lead is connected with a short piece of platinum wire supported in the capillary of the bottle. The regulator can be easily adjusted by increasing or decreasing the volume of mercury and by varying the height of the platinum wire. The accuracy of control is estimated at 0.1 degree C.

If a more accurate instrument is desired a Wagner's improved skim-milk bottle, which has a smaller capillary, may be substituted for the Babcock bottle. Either bottle may be obtained at a cost of 80 cents and holds about 50 ml of mercury.

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ALABAMA POLYTECHNIC INSTITUTE

SPECIAL ARTICLES

EARLY CHANGES IN THE CEREBROSPINAL FLUID OF MONKEYS NASALLY INSTILLED WITH THE VIRUS OF POLIOMYELITIS

MICROSCOPIC examination of the cerebrospinal fluid from cases of epidemic poliomyelitis in man reveals an increase in the number of the white cells present. This change in the composition of the fluid is employed as a means of arriving at a diagnosis of suspected poliomyelitis in instances in which no muscular impairment is detectable.

A similar increase in the number of white cells present in the fluid arises in monkeys (*Macacus rhesus*) in which the virus of poliomyelitis has been instilled into the nasal cavities.[1] Advantage was taken of this interesting fact to determine the relation of the increase in cells to the appearance of symptoms of infection in the monkey. The cerebrospinal fluid was withdrawn by cistern puncture, just before instillation of the virus, and at intervals of 48 hours afterwards. This interval was chosen in order to avoid too frequent puncture and to insure an adequate flow of the fluid. Two instillations of virus, consisting of a suspension of glycerolated spinal cord and medulla of monkeys succumbing to acute, experimental poliomyelitis, were made on successive days.

The preliminary cell counts of the fluid of normal monkeys gave values as low as 12, but usually of from 20 to 30 cells per cubic millimeter. At the end of the first 48 hours, an increase of cells was already apparent in some, but not in all the monkeys receiving virus by the instillation method; the cell counts were around 70. At the expiration of the second 48-hour period, the rise in the count was more general, the cells fluctuating between 100 and 300 per cubic millimeter.

Up to this time, the instilled monkeys presented no detectable signs of infection. Beyond this period, depending on the potency of the virus and the susceptibility of individual animals, symptoms consisting of fever, ataxia and muscular weakness arose; and, as a rule, the cell count increased still further with the onset of symptoms. In passing, it should be stated that globulin also appeared in the altered cerebrospinal fluid.

Hence a similarity is found to exist, in advance of all other detectable signs of infection, between the changes arising in the cerebrospinal fluid of the nasally instilled monkeys and that yielded by cases of preparalytic poliomyelitis in man. On the other hand, it is obvious that the monkey is less responsive than are children to the presence of the virus in the nervous system, since, in contrast to children, during the early or preparalytic stage of infection, the animals remain to all appearances in a normal state.

The evidence is now strong that the virus ascends from the nasal membranes to the olfactory lobes of the brain, and then continuously by nerve conduction to the midbrain and spinal cord.[2] The early involvement of the cerebrospinal fluid in the pathological process, before any systemic effects of infection ap-

[1] S. Flexner, SCIENCE, 77: 413, 1933.

[2] S. Flexner and P. F. Clark, *Proc. Soc. Exper. Biol. and Med.*, 10: 1, 1912; H. F. Faber and L. P. Gebhardt, *idem*, 30: 879, 1933; *Jour. Exp. Med.*, 57: 933, 1933.