SIMPLIFIED METHODS FOR MICRO-INCINERATION OF TISSUES

MICRO-INCINERATION of sections of isolated cells or tissues reveals with remarkable clarity the distribution of mineral ash in various parts of the cell. Various parts of the tissues are recognizable;[1] stages of mitosis can be traced;[2] the structural details of ciliates are found to be clearly represented.[3,4] It is a valuable cytological method and should be more widely used.

Many workers are undoubtedly deterred by the apparent need for special furnaces. A quartz tube furnace heated by gas or by electric coil, and with porcelain or platinum supports to prevent warping of the slide, has been recommended.[1,2] Emphasis has been placed on a relatively exact furnace temperature, for instance, 625°–630° C.,[5] and on careful shielding of the slides from dust by means of the quartz tube. The use of special quartz slides has also been recommended.[5]

We have experimented in this laboratory with simplified methods of ashing for cytological purposes. It was found[4] that an ordinary muffle furnace gave very good results. The temperature could be accurately regulated and the sections were effectively protected from dust by inverting Gooch crucibles over the slides. Using this method on *Paramecium,* the finest details could be made out: cilia, basal granules, differentiation between ectoplasm and endoplasm, etc.

Recently an even simpler method has been found to be entirely satisfactory. An ordinary hot-plate with exposed coils, which retails at about two dollars, was used instead of a furnace. The slides were set on the fire-clay rack holding the coils and covered with Gooch crucibles. The slides were incinerated at a dull red heat for about three to six hours. Sections of *Paramecium, Termopsis* and various rat tissues were incinerated with excellent results by this method. No trouble was experienced from warping of the slides when an ordinary hard-glass type was used. Shrinking and cracking of the sections is largely prevented by doing the initial heating slowly, taking two to three hours to bring the slide up to red heat. This may be done with a rheostat or by placing a heavy unglazed porcelain plate between the slide and the hot plate.

The whole procedure is as follows: Fix in formol-alcohol,[3] dehydrate, embed in paraffin, section and spread on a slide by the usual methods. Incinerate three to six hours (two to three hours initial heating, one half to three hours at red heat), cool and seal a cover slip in place with paraffin. Examine with a dark-field microscope, or, if that is not available, by oblique illumination against a black background.

This method is easier than many of the cytological methods commonly taught and does not need to involve any apparatus not found in the ordinary histological laboratory. The simplified micro-incineration method is splendidly adapted for routine laboratory use for class demonstration of the distribution of mineral ash in cells.

R. F. MACLENNAN

DEPARTMENT OF ZOOLOGY
STATE COLLEGE OF WASHINGTON

SPECIAL ARTICLES

DEMONSTRATION OF THE CENTRAL BODY IN THE LIVING CELL

IN recent publications, Wilson and Huettner[1] offered conclusive evidence, based on observations of fixed preparations, of the existence of central bodies in Drosophila eggs It is the purpose of the authors to add new evidence of the existence of central bodies based on living material. These findings were obtained in the course of studies, now in progress, on the pole cells of developing eggs of *Drosophila melanogaster.*

In making these studies, the method introduced by Child and Howland[2] for observing the living Drosophila egg was employed. The opaque, chitinous chorion was first removed with a sharp needle, leaving the naked egg with its shiny, transparent and tough vitelline membrane intact. The egg was then placed on a coverslip on which a thin layer of Ambroid cement had been previously spread and baked to complete dryness. Ambroid solvent (amyl acetate) was next applied on the ambroid near the egg in such quantity as to allow only a thin film of dissolved ambroid to spread under the egg. Since amyl acetate is a very volatile liquid, the ambroid under the egg dried in a short time, leaving the egg securely fastened to the coverslip. The egg was now surrounded by a drop of any desired medium and the coverslip was inverted, placed on a depression slide and sealed with vaseline.

Using this method, the living egg could be observed, with the aid of the microscope, under any desired magnification. Great care was taken that the egg should suffer no injury while being prepared for observation. Such injury could occur:

[1] A. Policard, *Protoplasma,* 7: 464, 1929.
[2] G. H. Scott, *Bull. d'Histol. Appl. Phys. et Path.,* 7: 251, 1930.
[3] E. S. Horning and G. H. Scott, *Jour. Morph.,* 54: 389, 1933.
[4] R. F. MacLennan and H. K. Murer, *Jour. Morph.,* (in press).
[5] E. S. Horning, *Jour. Cancer Research Comm.,* Univ. Sydney, 4: 118, 1932.
[1] SCIENCE, 73: 447–448, April 24, 1931, and *Zeitsch. f. Zellforsch. u. Mikr. Anat.,* Vol. 19, No. 1, 1933.
[2] SCIENCE, in press.

(1) In peeling the eggs. Repeated experience showed that an excellent criterion for judging the injured eggs was the presence of granules, in Brownian movement, within the micropyle. It was often found that, although there was no other apparent injury, when the micropyle contained granules the egg did not develop in a normal manner. On the other hand, when there was no visible injury and these granules were absent, the egg invariably developed normally.

(2) In the application of amyl acetate. Care was taken that the amyl acetate should not wash ambroid over the egg, for, on drying, a film of ambroid covering the egg would exert great pressure and thus injure it.

(3) By excessive desiccation. This was avoided by preparing the egg for observation very rapidly and by sealing the coverslip to the depression slide with vaseline. Another precaution that was taken was to place a drop of the medium on the bottom of the depression.

Only after proper orientation can the pole cells be observed to best advantage. Since they are more concentrated dorsally, the best view of them was obtained when the egg was placed with its dorsal (more or less concave) surface against the coverslip. The pole cells begin to bud off at the posterior end of the egg at the time of the 8th or 9th cleavage. As soon as they push off, they divide, so that by the time the egg is in the 11th cleavage, 10 to 12 pole cells are present. The pole cells are particularly advantageous for the observations of central bodies since they are large, since they are free from yolk spheres and since the surface of the nucleus of the pole cell is free of any extraneous granules which are found scattered through the cell.

With a magnification of 1,500 times, in many of the pole cells on the upper periphery of the pale, hyaline nucleus, two spherical bodies opposed to each other were observed. The distance between these bodies varied. When they were close together, they seemed to lie within a single vacuole. When they were some distance apart, each one appeared to be enclosed in its own smaller vacuole. (Wilson and Huettner[1] described such a small clear area about the central body, in fixed preparations, at the beginning of prophase). These bodies were identified as the central bodies by their behavior during division of the cell, by their position in the cell and by their distinctive appearance. They possessed a slight vibratory motion so that one could get both of them in focus at the same time for only a very short interval. The central bodies in each pair were of the same size. On the whole they seemed to be less transparent than the ordinary granules which are present in the cytoplasm of the pole cell.

The central bodies may also be identified near the nuclei of blastodermal cells. These cells are not as favorable for observation as the pole cells, since they are much smaller. Moreover, it is easy for one to confuse the central bodies with the mitochondria and other cell inclusions which swarm about the nuclei. For the same reason, the behavior of the central bodies, during cell division, can not be followed in the blastodermal cells.

During divisions of the pole cells the central bodies were seen to move apart along the periphery of the nucleus. This movement was gradual at first, but as the nucleus lengthened, they moved apart more rapidly. Although they were not actually seen to divide, shortly after this, the nucleus still being elongate, 2 pairs of central bodies were observed, one pair near each end of the nucleus. The central bodies of each pair kept shifting their positions with respect to each other. This accounts for certain conditions found in fixed preparations. For example, in telophases of the nuclear cleavages, at each end of the cleavage figure, the two pairs of central bodies are not placed similarly but may occupy any conceivable position with respect to each other.

The central bodies were observed best at the stage in the development of the egg where there are about 10 to 12 pole cells. At this time they are undergoing division and are not crowded to such an extent that they overlap each other. They were seen in the pole cells of eggs developing in various external media, namely, 33 per cent. sea water, 0.5 M. glycerine and pure egg albumin. In each medium, the eggs developed quite normally, *i.e.,* larvae hatched out in the normal length of time.

These observations, though they are of a preliminary nature, demonstrate that the central bodies have an actual existence in the living cell. They have a distinctive appearance and behavior in the pole cells of the normally developing egg of *Drosophila melanogaster*. One can also observe them in the cells of the blastoderm. They are not as obvious in these cells, however, for cell inclusions such as mitochondria, which are very numerous, fill the cells.

ALFRED F. HUETTNER
MORRIS RABINOWITZ

NEW YORK UNIVERSITY

A STUDY OF CANNED SHRIMP WITH REFERENCE TO THE PRESENCE OF VITAMINS A, B AND D

QUALITATIVE tests on wet-pack and dry-pack canned shrimp[1] show that vitamins A[2] and D are present in

[1] After shrimp are cooked and weighed into cans, wet-