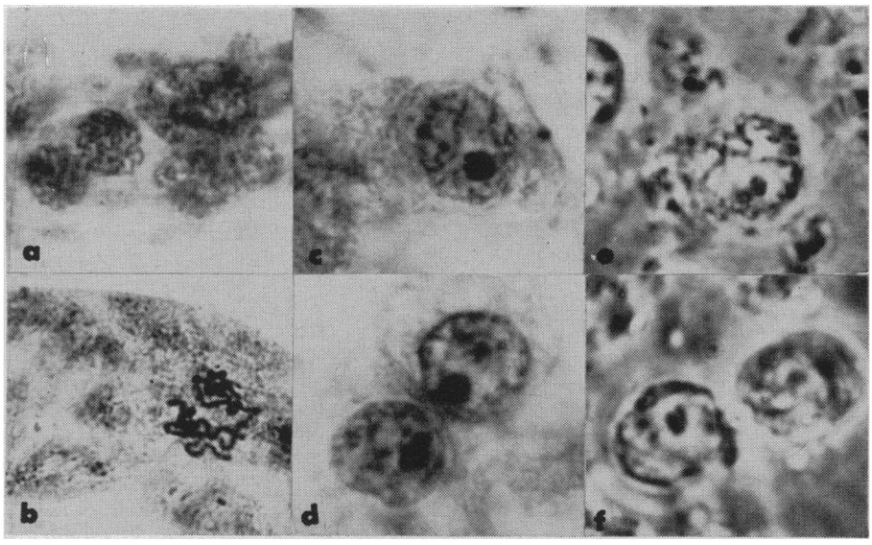

Fig. 1. Photomicrographs of cells of female *Parascaris* (*bivalens*). (*a–d*) Cells fixed in acetic alcohol and stained by Weigert's iron hematoxylin; (*a* and *b*) early and late prophase, respectively, of oögonial mitosis; (*c*) early pachytene stage; (*d*) early diplotene stage. Nucleoli appear as round black bodies. (*e* and *f*) Phase–contrast photomicrographs of living cells in approximately the same stages as *c* and *d*, respectively. (*a* and *b*, × 800; c–f, × 2000)

during meiosis in this species—namely, the aggregation of the two or four chromosomes it possesses (found, respectively, in subspecies *univalens* and *bivalens*) into a common chromatin mass; the occurrence of chromosomes in variable number; and more than one reduction division. Some of these noncanonical aspects have been clarified in more recent times and shown to be due in part to deficiencies in the interpretation of preparations obtained by faulty methods.

Recently it has been reported that *Parascaris* chromosomes are not seen as separate bodies in early meiosis and that they only differentiate themselves from a large chromatin mass, observed in early meiotic prophase, during stages corresponding to late pachytene or early diplotene in other species (*2, 3*). It was reported that before meiosis, in oögonial mitoses, the chromosomes presented large club-shaped ends which consisted of heterochromatin (*3*), and that this same heterochromatin formed the large chromatic mass of early meiosis and was afterwards "diminished" or eliminated in the somatic cells, during embryonic divisions (*3*).

We have found that these noncanonical findings are artifacts produced through deficient fixation. With appropriate fixation, the chromosomes are observed as separate bodies throughout the entire course of meiosis, no chromatin mass being visible in early meiotic prophase. On the other hand, in gonial mitosis the chromosomes do not show club-shaped ends. After several trials we found that in contrast to usual fixation procedure, which must be as rapid as possible, in this material the cells must be fixed gradually, without osmotic shock, to obtain good results. Acetic-alcohol fixative (glacial acetic acid, 1 volume; absolute alcohol, 3 volumes), strong AFA fixative [96-percent alcohol, 2 volumes; 40-percent formalin solution, 1 volume; glacial acetic acid, 0.1 volume (*4*)], buffered osmic acid, several mixtures of formalin, potassium bichromate, and cadmium or mercury chloride solutions all produce defective fixation when employed in the usual way.

We have obtained good results with the following procedure. The animals, secured from freshly killed horses and immediately brought to the laboratory in the intestinal fluid in which they had lived, were immersed in an isosmotic, 0.25*M* saccharose solution. The fixative was gradually dispensed from a dropping funnel into this solution, with stirring, so that 300 ml of fixative was mixed during 1 hour with the 150 ml of saccharose solution. The worms died within about 10 to 15 minutes after the fixative was first added. At the end of this hour of mixing the animals were immersed in pure fixative, and after another hour the cuticle was cut and the genital tract was exposed to the pure fixative overnight. Acetic-alcohol or strong AFA fixative generally gives good fixation. The second of these fluids is better for preserving nuclear morphology, but it may not preserve so perfectly the materials which give the Feulgen reaction (essentially deoxyribonucleic acid).

Figure 1 shows some aspects of oögonial mitosis and early meiosis, in which no chromatin masses or club-shaped chromosome ends are visible. Two photographs of living meiotic cells, obtained by means of phase contrast, are also shown. From rapidly dissected female ascaris, preparations of segments of the genital tract immersed in the body fluid of the worms were readily made and observed with phase-contrast equipment. The chromosomes were then seen to have the same appearance as after the fixation procedures described here. When fixative was added to these preparations, between slide and cover slip, aggregates and masses appeared and the entire nuclear content became distorted, while the materials of the chromosomes were in part dissolved and afterwards precipitated at random. These supravital observations leave no doubt that phenomena hitherto supposed to be the natural state of *Parascaris* chromosomes are indeed artifacts due to fixation. Our procedure opens the way to a detailed study of mitosis and especially of meiosis in these relatively large chromosomes, which are very interesting owing to their multiple, or so-called diffuse, centromere.

J. A. SERRA
P. G. C. PICCIOCHI
Institute of Zoology and Anthropology, Faculty of Science,
University of Lisbon, Lisbon, Portugal

References

1. E. Van Beneden, *Arch. biol.* (*Liége*) **4**, 265 (1883); R. Blanckertz, *Arch. Zellforsch.* **6**, 1 (1911).
2. R. B. Goldschmidt and T. P. Lin, *Science* **105**, 619 (1947).
3. J. Pasteels, *Arch. biol.* (*Liége*) **59**, 405 (1948); T. P. Lin, *Chromosoma* **6**, 175 (1954).
4. J. A. Serra, *Portugaliae Acta Biol. Sér. A*, 1 (1944).

1 August 1960

Callus Tissues from the Mosses Polytrichum and Atrichum

Abstract. Callus-like tissues, isolated from protonemal cultures of two species of mosses, grow vigorously and without marked differentiation on media containing sucrose, casamino acids, and coconut milk. On mineral agar and on media containing sorbitol the tissue from *Polytrichum* (found diploid) reverts to the growth pattern of apparently normal moss plants.

Relatively undifferentiated and continuously proliferating tissues of diverse vascular plants have been induced by one means or another in past years. These have been listed and described by Gautheret (*1*) under the general categories of *callus* and *tumor* tissues. No representative of the nonvascular plants is included. Allsopp (*2*) has described undifferentiated tissue aggregates from two species of liverwort and has noted that such growths, like

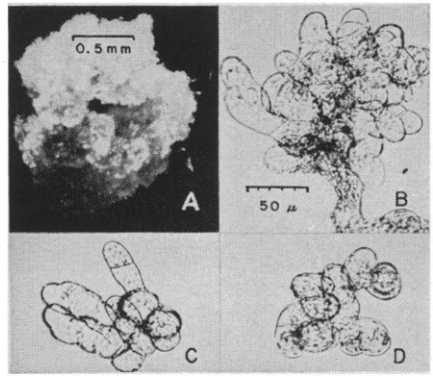

Fig. 1. Habit of tissue aggregate and highly magnified cell detail of callus from *Polytrichum commune* Hedw. *A*, Small mass of actively growing cells *in situ* in petri dish culture two months after planting spores on agar medium (Knudson's with 0.25 percent sucrose supplement). *B*, Single branch of callus aggregate with cells dividing in three dimensions. *C*, Rapidly growing and dividing peripheral cells from individual branch. *D*, Rounded cells produced in callus clumps during less active phases of growth. (Scale in *B* is applicable to all figures of cell detail, which are photomicrographs of unstained tissue made with light through amber filter.)

those in other plants, may be subcultured indefinitely in a continuous callus-like state.

In my studies (*3*), aggregates of callus-like tissue have been isolated from *in vitro* protonemal cultures grown from spores of the mosses *Polytrichum commune* Hedw. and *Atrichum undulatum* (Hedw.) Beauv. Tissue from the former has been subcultured and maintained in the callus condition for well over a year.

The tissue was originally isolated from agar cultures of *Polytrichum* grown on the modified Knudson's basal medium used in earlier experiments with ferns (*4*), supplemented by microelements (*5*) and containing 0.25 percent sucrose (herein called the isolation medium). (The sucrose is believed to have supported growth of the callus to detectable size, since it has rarely been found in mineral agar cultures.)

The callus aggregates are green and autotrophic. They first appeared 2 months after spores were planted, along with the first leaf buds. The tissue grew conspicuously more rapidly than normal protonemata or leaf buds. This rate of growth and undifferentiated habit continued after isolation and subculture. Aggregates of tissue left adjacent to normal protonemata and growing leafy plants displayed a sharp reduction in activity, followed by eventual loss of viability. Sporadic callus aggregates have occurred in subsequent cultures, some of which came from spores collected in widely separated localities.

The tissue aggregates have a rough exterior (Fig. 1*A*). The mass is soft and can be easily teased apart in water. The microscopic structure consists of multitudes of many-branched, semifilamentous processes, with cells dividing irregularly in three dimensions (Fig. 1*B*). Individual cells are elongate under conditions of rapid growth; subsequent cells become rounded as the pattern of division changes and growth becomes less vigorous (Fig. 1, *C*, *D*). All cells are parenchymatous (Fig. 1*D*), and no further differentiation regularly occurs on the isolation medium. It is believed that these aberrant tissues arise from protonemata directly or, possibly, from divisions related to bud formation.

Callus grown on the isolation medium displays a normal, green color (similar to that of plants on either mineral agar or soil); tissues growing on 1 percent sucrose are notably lighter green, but the growth rate is not significantly increased. Growth on sucrose-containing media continues in the absence of light, and the cells retain considerable green color for several months.

The isolation medium plus 0.20 percent casamino acids (Difco, vitamin-free) produces dark green aggregates at a growth rate markedly exceeding that on the former medium. The most rapid growth so far noted for the tissue occurs on agar containing only coconut milk (15 percent) as nutrient. The cells are light green, and the aggregates are very easily fragmented.

Naphthaleneacetic acid (NAA), when added to the isolation medium in concentrations of 1 to 2 mg/liter, induces formation of club-shaped processes on the periphery of the aggregates. The tips of these structures bear a rounded center of meristematic activity, surmounted by imperfect leaflike forms. This growth phase is maintained by continuous subculture on the medium which prompts their initiation; the structures revert to the basic callus pattern on the isolation medium. No progression to normal, leafy plants has resulted on media containing NAA. Cells quite yeastlike in pattern and loosely adherent result from growth on higher concentrations of sugar (1 percent, for instance) supplemented by 1 mg of NAA per liter.

Mineral agar media with sorbitol (500 to 2500 mg/liter) support growth only slightly better than mineral media alone. Adding sorbitol to the isolation medium produces better growth than that occurring on mineral agar, but inferior growth to that occurring on the isolation medium. However, the callus tissues may in time send out many normal protonemata which give rise to buds and slow-growing leafy plants. The growth pattern is here obviously shifted to a resumption of normal (with production of protonemata and buds), and the callus itself is ultimately inhibited by the leafy gametophytes.

Tissues maintained on mineral agar grow slowly, and after several months orderly protonemata from the peripheral cells may give rise to buds and normal plants, fully inhibiting growth of the callus. Subcultures of the latent callus may resume growth on sucrose-containing substrates—media very favorable for growth of normal plants. Leafy plants separated from the callus proper also assume normal growth.

Cytological workers (*6*) have shown the diploid chromosome number of *Polytrichum commune* to be 14. Dividing cells from the callus aggregates so far examined also have this diploid number.

Preliminary trials indicate that up to a tenfold increase in callus incidence may result from treatment of freshly planted spores with x-rays in dosages of about 5000 r. The naturally formed callus and that presumed to be induced through irradiation bear no evident differences in morphology, physiology, or cytology.

Similar undifferentiated tissues from *Atrichum undulatum* have been isolated from an experimental culture receiving 10,000 r x-ray dosage. Whether this tissue is "normal" or induced through irradiation is not yet established. The frequency of occurrence of callus in this species appears tentatively to be much lower than that in *Polytrichum commune*.

MAX WARD*

Biological Laboratories,
Harvard University,
Cambridge, Massachusetts

References and Notes

1. R. J. Gautheret, *La Culture des tissus végétaux* (Masson, Paris, 1959).
2. A. Allsopp, *Nature* **179**, 681 (1957).
3. This work was supported by grants from the National Science Foundation (G-6081 and G-8650) and was a part of a research project directed by Prof. R. H. Wetmore as principal investigator under the grants at the Biological Laboratories, Harvard University. The technical assistance of Miss Lydia Thomas is acknowledged, as is the aid of Dr. Otto T. Solbrig in the cytological determinations.
4. M. Ward, *Phytomorphology* **4**, 1 (1954).
5. J. P. Nitsch, *Am. J. Botany* **38**, 566 (1951).
6. A. P. Wylie, *Trans. Brit. Bryol. Soc.* **3**, 260 (1957).

* Present address: Glenville State College, Glenville, West Virginia.

12 July 1960