## Squash Preparations of Living Root-Tip Cells

THE cells of plant root tips cannot be observed satisfactorily in squash preparations without removal of the intercellular cement. This is normally accomplished by the Feulgen hydrolysis, namely, by hydrolysis in *N* hydrochloric acid for a suitable period at 60° C. More recently, some authors have treated fixed root tips with pectic enzymes[1,2,3]. It was thought that it might be possible to separate the living cells with a pectinase complex and so be able to study mitosis and the sites of nucleic acid in living cells, and also the effects of fixation and of other treatments. Experiments were made to investigate this possibility.

Root tips of *Vicia faba* were used. The enzyme preparation was provided by Dr. R. G. Tomkins, of the Ditton Laboratory, East Malling, and is prepared as follows. *Penicillium digitatum* is grown on bran; the culture is then dried and an aqueous extract is made by mixing one part of the dried material with three or four parts of distilled water. The mixture is well shaken, allowed to stand for one hour, and filtered. The apical four to five millimetres of a root tip is excised, well washed by agitation in distilled water, and placed in the strong solution of the enzyme for twenty-four hours. It is removed, again washed in distilled water and put into a freshly prepared enzyme solution diluted to one-fifth of its original strength. After twenty-four hours the second cycle of washing and enzyme treatment is repeated. The root tips may then be teased and the cells separated.

Since the living cells do not resemble normal Feulgen preparations, it was necessary, in order to verify the normality of such cells, to fix and stain them in acetic carmine and, if required, follow this with a Feulgen stain. Normal mitotic figures were then seen.

After separation of the living cells, following enzyme treatment, it is possible to float the coverslip off in Ringer's solution and to keep the cells as a suspension in this solution in a small specimen tube. The use of Ringer's solution is indicated by its osmotic properties and lack of nutrient for growth of micro-organisms, for no attempt has been made to attain an aseptic technique.

Samples of such a suspension of cells, stained with acetic carmine, suggested that after maceration the cells complete the mitotic cycle in which they were at the time of maceration. At first the number of mitotically active cells declines to zero. This might be a shock effect, since afterwards mitosis reappears and the frequency of divisions increases. Mitotic figures have been observed four days after maceration.

These living, isolated cells do not resemble the cells separated by Feulgen hydrolysis; but they appear to be exactly similar to the intact cells of an untreated root teased mechanically. They also resemble the cells of root tips fixed by Newcomer's method[4]. They differ from fixed and Feulgen hydrolysed preparations mainly in the presence of many mitochondria which obscure other structures.

It is believed that the enzyme solution attacks the intercellular cement and, by thus weakening the tissues along intercellular cleavage lines, causes the cells to separate when teased with needles followed by gentle tapping on a coverslip placed on the tissue. If the enzyme treatment has been insufficient, the mechanical treatment bursts the cells owing to the lack of cleavage lines.

The main fault of this method, as developed at present, is the danger of growth of micro-organisms. This is minimized by washing the tips thoroughly in water and by frequent changes to fresh enzyme solutions. If the tips can be grown aseptically, this trouble may be overcome.

I wish to express my gratitude to Dr. D. G. Catcheside for his advice and guidance in this work. I also wish to acknowledge the encouragement and advice given me by Prof. J. T. Randall and Dr. Honor B. Fell, of the Medical Research Council Biophysics Unit, King's College, London.

J. CHAYEN

Botany School,
Cambridge,
and
Medical Research Council Biophysics Unit,
King's College,
London, W.C.2.
July 14.

[1] Emsweller, *Stain Tech.*, **9**, 109 (1944).
[2] McKay and Clark, *Stain Tech.*, **21**, 111 (1946).
[3] Hohl, *Stain Tech.*, **23**, 129 (1948).
[4] Newcomer, *Amer. J. Bot.*, **33**, 684 (1946).

---

## New British Records of Two Rare Deep-Sea Fishes: *Oxynotus paradoxus* Frade and *Aphanopus carbo* Lowe

THROUGH the courtesy of Mr. W. Wilson of Milford Haven and of the staff of the Lowestoft Fisheries Laboratory we have received three deep-sea fishes taken by the *Richard Crofts* in 330 fathoms at a point 250 miles W. by S. of St. Ann's Head on March 10.

The first species is the small shark *Oxynotus paradoxus* Frade[1], represented by a female of total length 895 mm. (Specimen IX). The type was described from the coast of Morocco, and another was obtained in the Gulf of Gascony; the remaining known records are all British and the specimens are in the British Museum except where otherwise stated:

| | | | | |
|---|---|---|---|---|
| I | April | 1931 | ♀ | 70 miles W.N.W. of Fastnet, S.W. Ireland[2]. |
| II | April | 1931 | ♀ | Black Rock, N.W. Ireland, at 160 fathoms[2]. (Three specimens, of which only one reached the B.M.) |
| III | March | 1933 | ♀ | Scourie Bank, 5 miles south of Handa, North Minch, N.W. Scotland. (Royal Scottish Museum, Edinburgh, No. 1933.23.) |
| IV | Feb. | 1934 | ♂ | Messrs. Barrow, Billingsgate Market. Possibly from Irish waters, *via* Milford Haven. |
| V | July | 1934 | ♂ | Irish Atlantic Slope, 53° 40′ N., 11° 15′ W., at 145 fathoms[3]. |
| VI | May | 1935 | ♀ | Labidee Bank, 50° 30′ N., 8° 20′ W., in 60–65 fathoms. (National Museum of Wales, Cardiff, No. 35.527.1). |
| VII | April | 1938 | ♀ | 52° 30′ N., 12° 30′ W., in 290 fathoms. (National Museum of Wales, No. 38.293.1.) |
| VIII | March | 1945 | ♀ | "Trawled in deep water, landed at Milford Haven." (Probably from Irish Atlantic Slope.) |

The general concentration of these records along the edge of the Atlantic Slope during the late spring, apparently unrelated to any seasonal localization of the fishing fleets, led us to consider the possibility of an inshore migration at this time of year. The six specimens available to us have been examined for food-contents; but all were practically empty and the few particles present so well digested and finely divided as to afford no evidence. The flattened belly and tiny ventral mouth with prominent lips and specialized dentition suggest a bottom-feeding habit, and that breeding rather than feeding would, therefore, be a more likely migration