

**New Fixatives for Plant Cytology.**

FOR the study of somatic chromosomes a fixative is required that penetrates quickly, but does not shrink the cytoplasm; that spreads the chromosomes for counting, and gives clear definition of constrictions and trabants. Carnoy spreads the chromosomes out well, but most of the definition is lost. Flemming and its modifications are most useful, giving very good results, but there are some plants for which they are not suitable. Some plants, however, cannot be fixed well by any of our present methods.

In the course of work with *Primula sinensis* a number of fixatives were tried, among others the following new formula, which was found to give excellent results:

1 per cent Chromic acid . . . . .	90 c.c.
Potassium bichromate . . . . .	1 gm.
Sodium sulphate . . . . .	0.5 gm.
Urea . . . . .	1 gm.
5 per cent Glacial acetic acid . . . . .	10 c.c.
2 per cent Osmic acid . . . . .	15 c.c.
Distilled water . . . . .	45 c.c.

The method employed is similar to that used with Flemming solution. Material is immersed in the solution for several minutes under an air pump to remove air and to aid penetration, and then it remains in the solution for a further twelve hours. After imbedding in paraffin, cutting and mounting in the usual way, the slides are bleached in 1 part hydrogen peroxide in 10 parts 70 per cent alcohol for two to three hours. They are then placed in an iodine solution for a minute or two to remove any remaining bichromate. Material can be stained with gentian violet (Newton's method), hæmatoxylin, or other cytological stains in common use.

Excellent results have been obtained with *Papaver*, *Melandrium*, *Datura*, and *Pentstemon levigatus* (with 96 chromosomes), and a comparative test of several fixatives, namely, Zenker, Flemming, Kihara, Navashin, and Allen's Bouin upon *Matthiola* ovaries, favoured my fixative both in regard to penetration and spreading of the chromosomes. For *Pisum* pollen mother-cells, whole buds are fixed in Carnoy for about 30 seconds; the Carnoy is then poured off and the fixation continued in the above fixative. This method is similar to Kihara's.

Although not used extensively on pollen mother-cells, the fixative has given good preparations by the smear method. The following, however, was found better for *Matthiola* pollen mother-cells:

1 per cent Chromic acid . . . . .	90 c.c.
Potassium bichromate . . . . .	3 gm.
Sodium sulphate . . . . .	1 gm.
Urea . . . . .	1 gm.
2 per cent Osmic acid . . . . .	20 c.c.
Distilled water . . . . .	50 c.c.

A third fixative used successfully with the smear method for pollen mother-cells is a mixture of Allen's Bouin and Champy in equal proportions. This was found useful for *Campanula* and *Solanum* species. The smeared slides, after washing and taking up through alcohols, must be left from two to three hours in a saturated solution of lithium carbonate in 70 per cent alcohol to remove any remaining picric acid.

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**An Isotope of Carbon, Mass 13.**

THE bands belonging to the Swan spectrum of carbon appear in the vacuum electric furnace at about 2400° C. At temperatures above 2600° they are strong and clear-cut, even the band at  $\lambda 6191$ , difficult to obtain in the carbon arc, being well defined. It has

been noted by one of us, during a variety of electric furnace investigations, that plates on which the band at  $\lambda 4737$  is very strong showed a faint band, not to be ascribed to a ghost, about 7.5 A. to the red of the strong band. The carbon arc shows a scattered structure in this region, which is suppressed in the furnace. It has, however, thus far failed to give the faint band, which in the furnace spectrum shows a distinct structure, essentially identical with the structure of the main band.

The recent discovery of isotopes of oxygen makes it very probable that other similar elements contain isotopes in small quantities. We have accordingly measured this faint band very carefully, using a first order exposure of a 15 ft. concave grating, the furnace being at about 2800° C. It is now generally agreed that the Swan bands are due to the neutral C<sub>2</sub> molecule, presumably C<sup>13</sup>-C<sup>12</sup>. We find that the new faint band corresponds quantitatively to that which should be given by an assumed C<sup>13</sup>-C<sup>12</sup> molecule. The constants of the Swan band system are known with great precision ("Int. Crit. Tables", 5, 411, and J. D. Shea, *Phys. Rev.*, 30, 825; 1927), and an accurate comparison with theory is therefore possible. With data of the precision now becoming available for oxygen, as well as in the present case, it is necessary to avoid various approximations which have commonly been used in previous work on isotopes. This will be fully discussed in later publications.

Because of irradiation due to the strong band, it is to be expected that the measured distance between the two heads will be slightly smaller than the calculated isotope shift for the head. This is in fact the case, this distance being measured as 2.020 mm. (= 7.520 A. = 33.44 cm.<sup>-1</sup>) as compared to a calculated isotope shift of 2.028 mm. (33.58 cm.<sup>-1</sup>). Fortunately, however, it is possible to distinguish six individual lines in the very faint isotope band. These have been identified as the unresolved triplets P<sub>26</sub> to P<sub>31</sub> (Shea's nomenclature). In the comparator P<sub>30</sub> and P<sub>29</sub> could be measured with reasonable accuracy, P<sub>28</sub> and P<sub>27</sub> less reliably, P<sub>26</sub> very poorly, and P<sub>31</sub> not at all. The corresponding triplets in the main band (also unresolved) could be measured with great precision. The observed isotope shifts in millimetres for P<sub>30</sub> to P<sub>26</sub>, with the calculated in parentheses in each case, are 2.059 (2.0515), 2.049 (2.0473), 2.037 (2.0429), 2.033 (2.0395), and 2.044 (2.0373) respectively. Multiplication by 16.58 gives the shift in cm.<sup>-1</sup>. The two good lines (P<sub>30</sub> and P<sub>29</sub>) give an average measured shift 0.082 cm.<sup>-1</sup> too large. Although with the present available data the fact may have no significance, it is interesting to note that this small discrepancy may be cancelled by assuming 12.0000 and 13.0026 for the two masses.

This 1.0 band ( $\lambda 4737$ ) is especially favourable for showing the faint isotope molecule, when one considers photographic intensity, position with respect to other bands (particularly CN), and size of the shift. We shall endeavour to get better plates, showing the isotope effect in other bands, and also showing more detailed fine structure. The present evidence seems, however, fully sufficient to establish the existence of an isotope of carbon, of mass 13. We cannot at this time make any statement as to its relative abundance, except to say that the isotope band is hundreds of times as faint as the strong band.

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