Some Methods for the Study of Plastids in Higher Plants.

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The following methods have been found to be satisfactory in the study of the primordia of chloroplasts, leucoplasts, and other apparently similar bodies in cells of liverworts and higher plants that are known under the name of chondriosmes.

Fixing.

Chrom-osmic acid is the fixing agent chiefly used, and in the following proportions:

Chromic acid, 1 %.....17 cc.
Osmic acid, 2 %..... 3 cc.
Glacial acetic acid.....3 drops

The specimens remain in this fluid from 36 to 48 hours, after which they are washed 12 to 24 hours in flowing water, or in several changes of water if flowing water is not available.

After careful dehydration the specimens are brought into paraffin, using chloroform as the solvent. Sections from 3 to 5 microns in thickness are cut, depending upon the nature of the tissue under consideration, and stained in the well-known iron-alum-haematoxylin stain. As a counter stain orange G dissolved in clove oil is sometimes very desirable.

Procedure with the Iron-Haematoxylin.

After the preparations have been freed from paraffin and from the solvent used in removing the paraffin (turpentine or xylol) by means of absolute alcohol, they are allowed to stand in the mordant from two hours to over night. As a mordant a 3 per cent. aqueous solution of the double iron salt is used (ferric ammonium sulphate $(NH_4)_2\ Fe_2(SO_4)_4 \cdot 24\ H_2O$. The preparations are now poured off with water and stained over night in a $\frac{1}{2}$ per cent. aqueous solution of haematoxylin. From the stain they are again poured off with water and destained with the above iron salt. The destaining is watched under the microscope. After the desired stain has been reached, and this

is determined by trial, the preparations are washed for about 15 minutes in gently flowing water. They are now dehydrated by treating with absolute alcohol, after which they may be counter stained with clove oil orange G or merely cleared in clove oil and cedar oil and mounted in balsam.

In case counter staining is desired, the process should be watched in order to avoid over-staining with the orange. In some cases the clove oil orange G need remain on the sections but half a minute. This stain may be removed by xylol, cedar oil, or pure clove oil, when the preparation is ready to be mounted in balsam.

By this method the primordia of chloroplasts and leucoplasts and other similar bodies are stained black or a blue-black.

The foregoing is much simpler than Benda's procedure, and it gives results that are as satisfactory. However, since it is desirable in cytological studies to check up one method with another, the procedure devised by Benda is recommended, although it is more tedious and time-consuming. The following modification of Benda's method has been used with excellent results:

1. Fix in chrom-osmic acid of the above-mentioned composition 24 to 48 hours.
2. Wash in water 1 to 2 hours.
3. Treat objects with equal parts pyroligneous acid (rectified) and 1 per cent. chromic acid 24 hours.
4. Treat with 2 per cent. solution bichromate of potassium 24 hours.
5. Wash in water 24 hours.
6. Bring into paraffin and section in case sections are to be made.
7. Treat with the iron mordant 12 to 24 hours.
8. Pour off with water and treat 10 to 20 minutes with alizarin.
9. Pour off with water and let dry in the air.
10. Stain now with Benda's crystal violet by warming gently to the point of forming vapor. Allow the preparation to cool for 5 to 10 minutes, after which pour off with water and let the preparation dry in air, standing the slide on end.
11. Destain with 5 per cent. acetic acid under microscopic control. This requires from a few seconds to a minute.
12. When the desired stain is reached, pour off with water, dehydrate with absolute alcohol, and counter stain, if desirable, with clove oil orange G. This stain is now removed with xylol or cedar oil, and the preparation is mounted in balsam.

As a result, the chloroplasts, chondrisomes, etc., are stained a deep blue, the cytoplasm a light orange or almost colorless, and the cell walls varying intensities of orange.

The writer has never been able to see the use of the treatment with alizarin, and this part of the process may be omitted. However, Benda's solution of alizarin is made as follows: Make a saturated solution of Kahlbaum's alizarin-sulfo-saurem Natron (mono.) in 70 per cent. alcohol. One cc. of this solution is added to 80 to 100 cc. of water.

Benda's crystal violet solution is made as follows:

Saturated solution crystal violet in 70% alcohol	1 part.
1% hydrochloric acid in 70% alcohol	1 part.
Anilin water	2 parts.