

# ELECTRON MICROSCOPIC STUDY OF LEAF TISSUE

BY: LESLIE WARD POWRIE

BOTANY HONOURS 1979

## SUMMARY

By the use of relatively simple preparative techniques it is possible to carry out detailed study on the micro-structure of plant tissues and thus gain deeper understanding of the physiology of the plant. Some micrographs of leaf tissue of Rhoeas sp. and Chlerodendron sp. leaf sections are included in the paper with discussion.

## INTRODUCTION

A great deal of progress has been possible in gaining understanding of the physiology and other aspects of biological specimens through a study of micro-structures and organelles by means of microscopes. Thus the microscope has been invaluable in the <sup>re</sup>searches of scientists into the details of living systems.

When working at the microscopic levels it is necessary to use great care in preparing specimens for study. This is particularly true for studies carried out with the Electron Microscope, since a small speck of dust can, by virtue of it's being magnified hundreds of thousands of times, prove to be a major problem to the resolution of the specimen concerned. Thus care is needed to avoid contamination of sections or specimens to be studied under the Electron Microscope.

When using the Transmission Electron Microscope where the electron beam passes through the section of the specimen it is necessary to have very thin sections in order to have the least possible absorption of the electrons. Yet it is necessary to also have a section which is sufficiently dense to disperse the electrons which are to be

*split infinitives!!*

registered on the scintillation system on the microscope such that one can see what is on the section. A further need for thin sections is that if the section is too thick, organelles overlying other organelles will cause decreased resolution due to there being other organelles superimposed. In order to have such thin sections a sophisticated instrument is needed to cut the sections. The ultramicrotome which is used is capable of cutting sections of the order of  $5 \cdot 10^{-8}$  m <sup>50 nm</sup> thick. It is then necessary to increase the scattering potential of the section by 'staining'. This is done by causing heavy metals to adhere to the section. These heavy metals are easily excited to release secondary electrons which are recorded on the screen of the microscope. No!  
not in  
TEM.

This paper briefly describes some techniques in use for the preparation of sections for viewing under the Transmission Electron Microscope.

#### MATERIALS AND METHODS

For details of the preparation techniques used the reader is referred to the schedule attached as Appendix 1.

Leaf tissue of the plant Rhoeas sp. was prepared by the author and leaf tissue of Chlerodendron sp. was used as prepared by M Collins as a comparison.

The tissue was fixed in 3% glutaraldehyde solution, post fixed with 1% Osmium Tetroxide and set in Epon. The material was then sectioned on an ultramicrotome to obtain sections of about 70 nm.

While the Osmium applied in the Osmium Tetroxide post fix acts to scatter electrons, further staining was carried out using Uranyl Acetate and Reynold's Lead Citrate stain. Prior to this staining and following the sectioning the sections were placed on copper grids.



The compositions of the solutions used are given in Appendix 2. The ultramicrotome used was a Reichert OMU-3 Ultramicrotome, and the sections were viewed <sup>in</sup> under a JEM-100S Transmission Electron Microscope.

### RESULTS

The included electron micrographs are a selection of those obtained as representative of some of the leaf tissue of the two species concerned. Some xylem tissue was found for the Chlerodendron (Fig. 3), but further very similar sections are shown for both species with essentially similar organelles.

Figures 1 to 3 show general cell structures whilst the remainder of the figures show details of organelles and membranes. In each case the scale is shown with a line scale superimposed on the micrograph.

Abbreviations of cell organelles and structures are as follows:

|     |                        |    |                      |
|-----|------------------------|----|----------------------|
| C   | Chloroplast            | N  | Nucleus              |
| Cr  | Chloroplastic ribosome | NE | Nuclear envelope     |
| CE  | Chloroplast Envelope   | no | Nucleolus            |
| CG  | Chloroplastic globule  | P  | Pit                  |
| Ch  | Chromatin              | r  | Ribosome             |
| CW  | Cell Wall              | S  | Stroma               |
| Cyt | Cytoplasm              | St | Starch               |
| G   | Granum                 | ST | Secondary thickening |
| IS  | Intercellular space    | T  | Tonoplast            |
| M   | Mitochondrion          | Th | Thylakoid            |
| ML  | Middle Lamella         | V  | Vacuole              |

As can be seen from Fig. 1 and 2, the cells for both species have much the same composition with regard to organelles, although the distribution appears on the whole to be different. The vacuoles of Rhoeas sp. appear to be less densely packed than those of



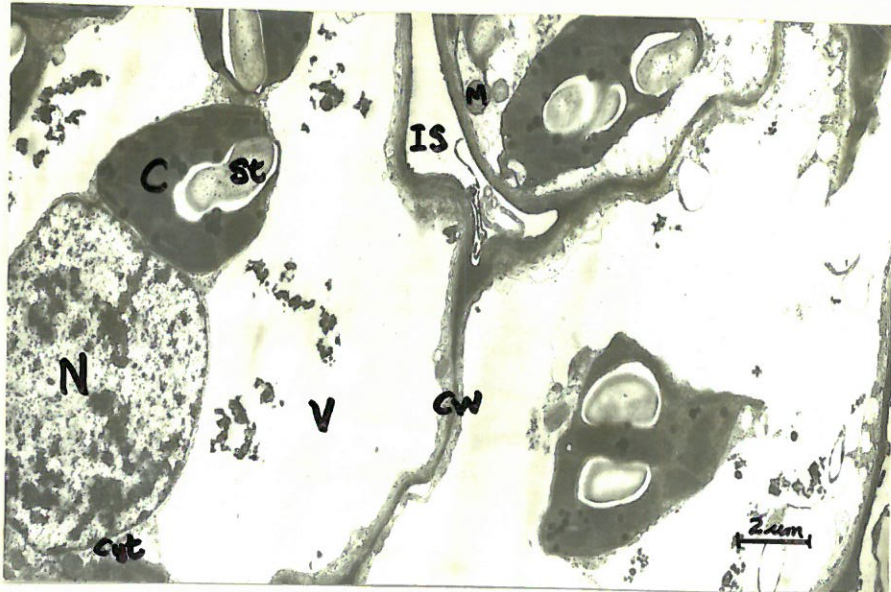


FIG. 1: Section of Rhoeas sp. leaf. Overall cell structure can be seen.

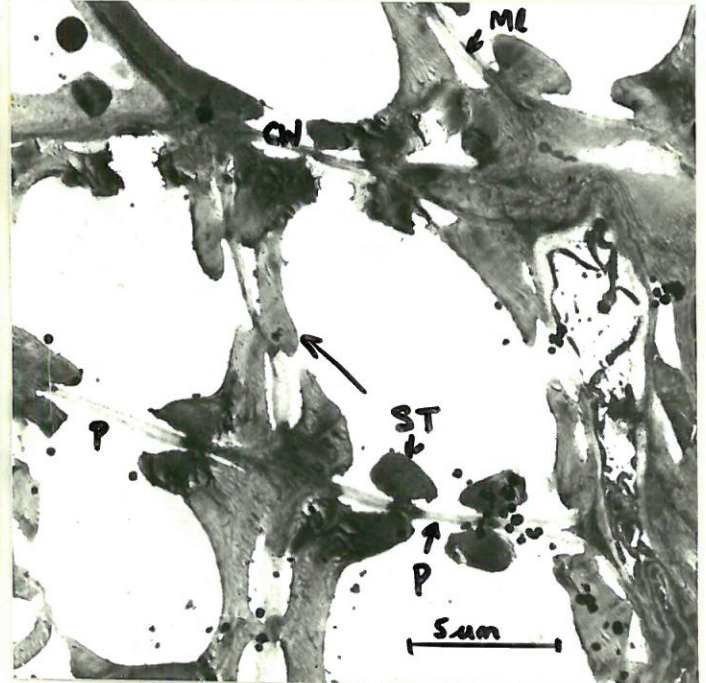
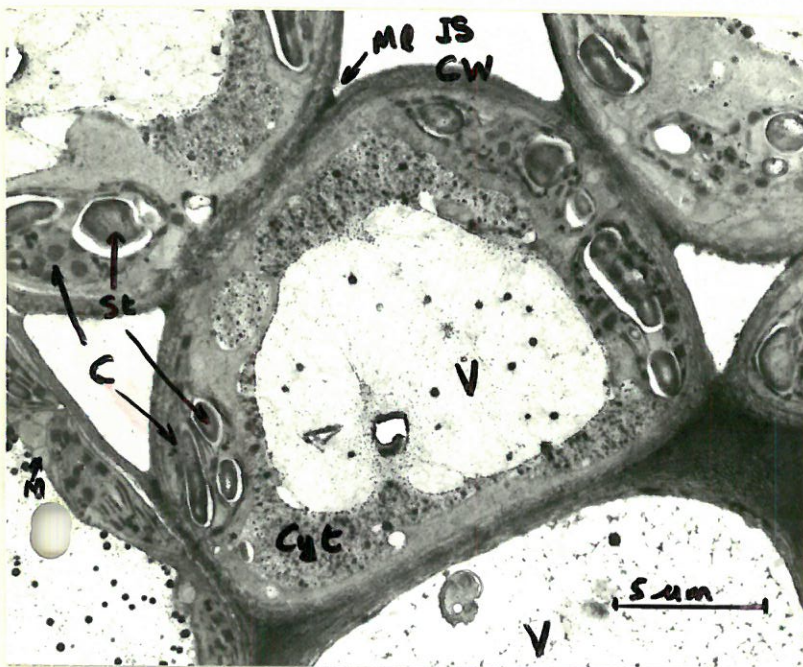


FIG. 2, 3: Leaf section of Chlerodendron sp. with overall cell structure visible. Fig. 3 shows specifically xylem tissue of the conducting stele of a leaf vein. (Courtesy M Collins)

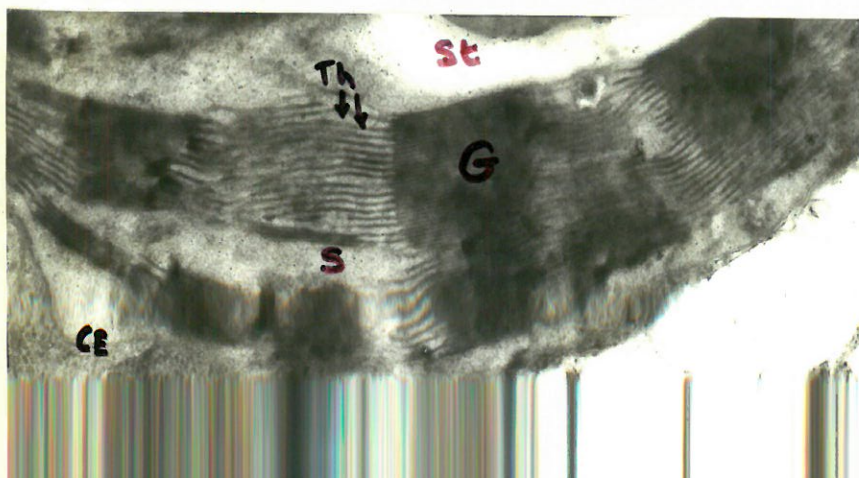


FIG. 4: Detail of the Chloroplast structures of Chlerodendron sp. leaf tissue. Clearly visible are the thylakoid membranes.



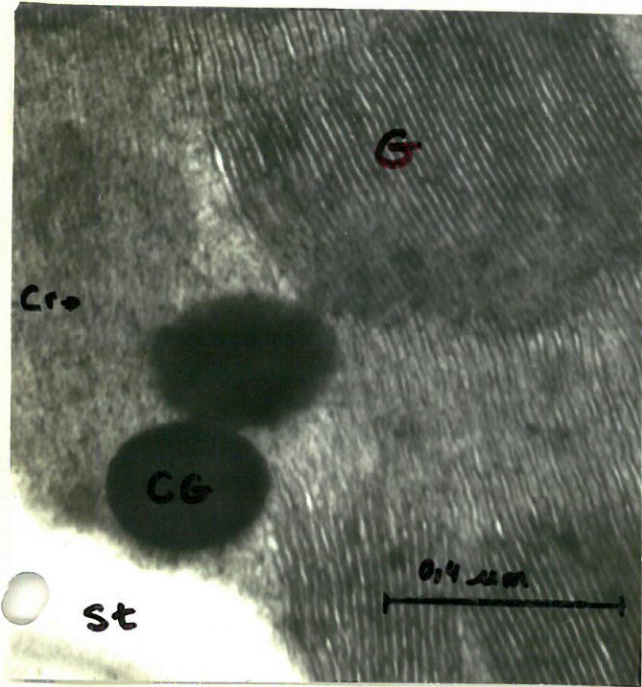


FIG 5: Detail of some of the chloroplast structures of the leaf tissue chloroplast of Rhoeas sp.. The grana with thylakoid stacks and some thylakoid membranes between grana (small thylakoids) can clearly be seen. Also visible are Chloroplastic globules the structure, composition or function of which are not known.

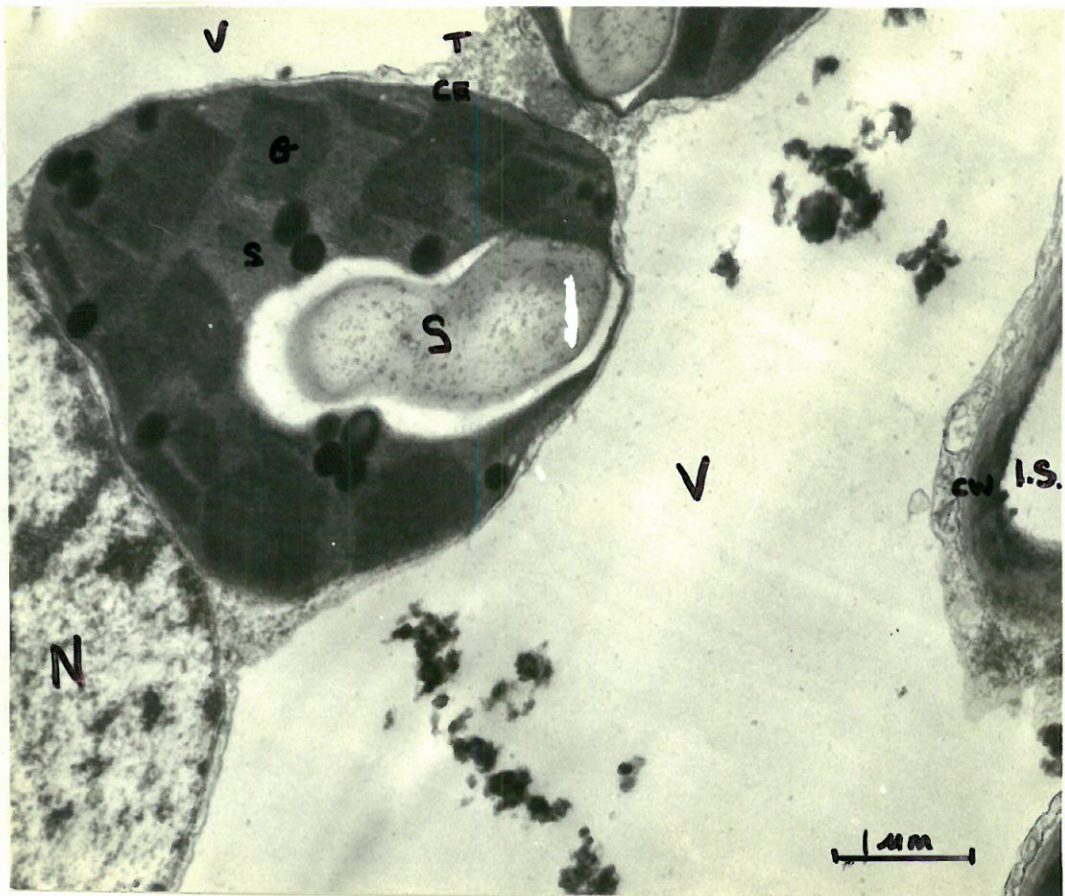


FIG 6: Part of a leaf cell of Rhoeas sp. where a whole chloroplast and part of the nucleus can be seen. As can be seen, the vacuole appears to have a fairly non-dense composition. The starch within the chloroplast would normally not take up stain but appears to be 'nucleate'.





FIG 7: Section of leaf tissue of Rhoeas sp. showing mitochondria and a portion of a chloroplast.

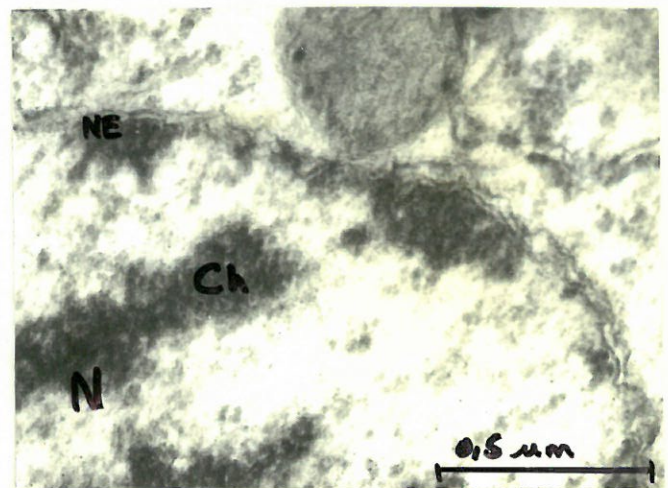
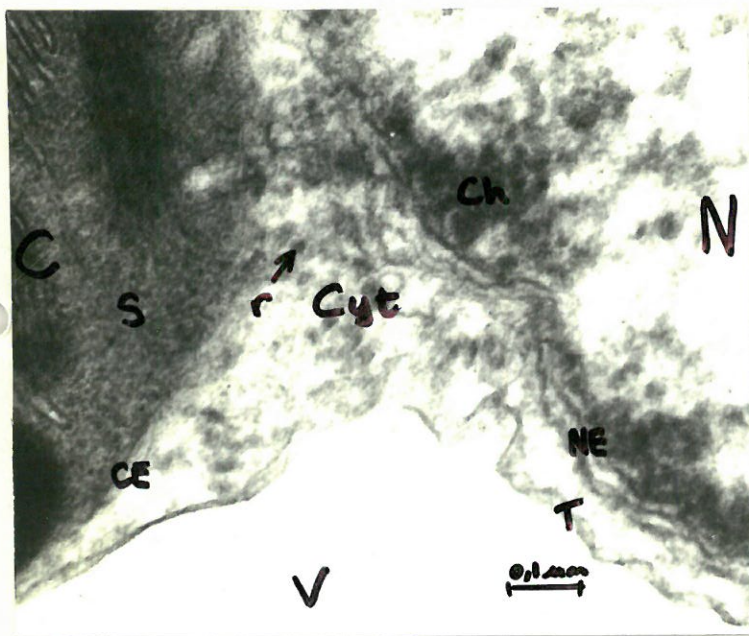


FIG 8, 9: Section of leaf tissue of Rhoeas sp. where some membrane features are visible. The nucleus and chloroplast have double unit membranes whilst the tonoplast is a unit membrane.



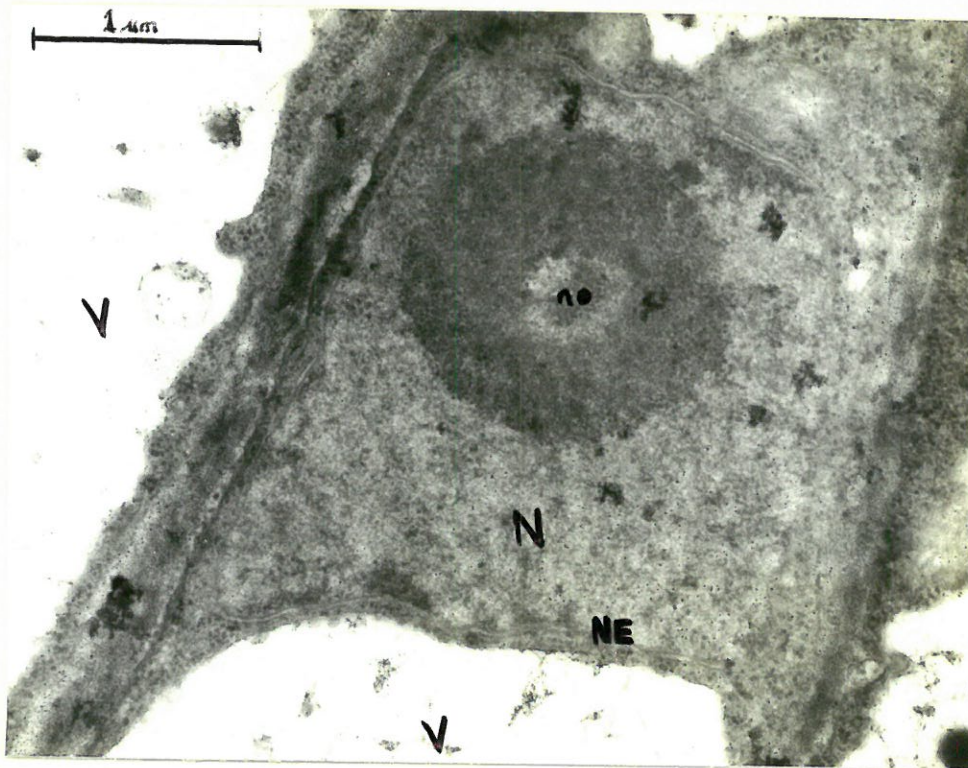


FIG 10: Section of Chlerodendron sp. leaf tissue showing some detail of the nucleus and nucleolus. The nuclear membrane is also visible. (Courtesy M Collins.)

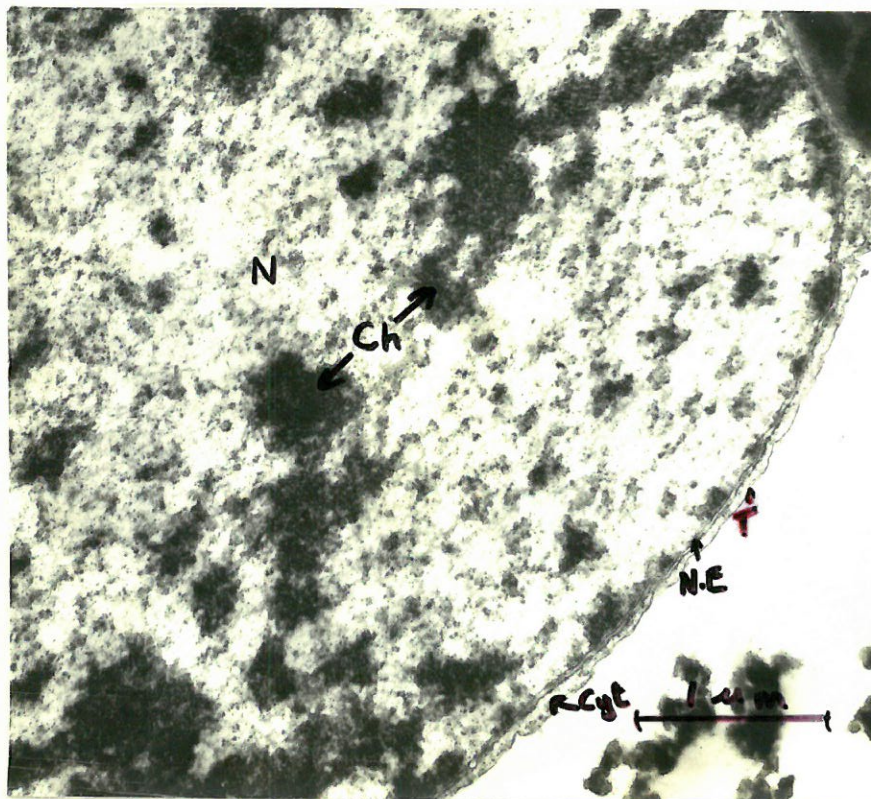


FIG 11: Section of Rhoegas sp. leaf showing the nucleus of one cell in part. The double unit membrane of the nucleus can clearly be seen next to the single unit tonoplast.



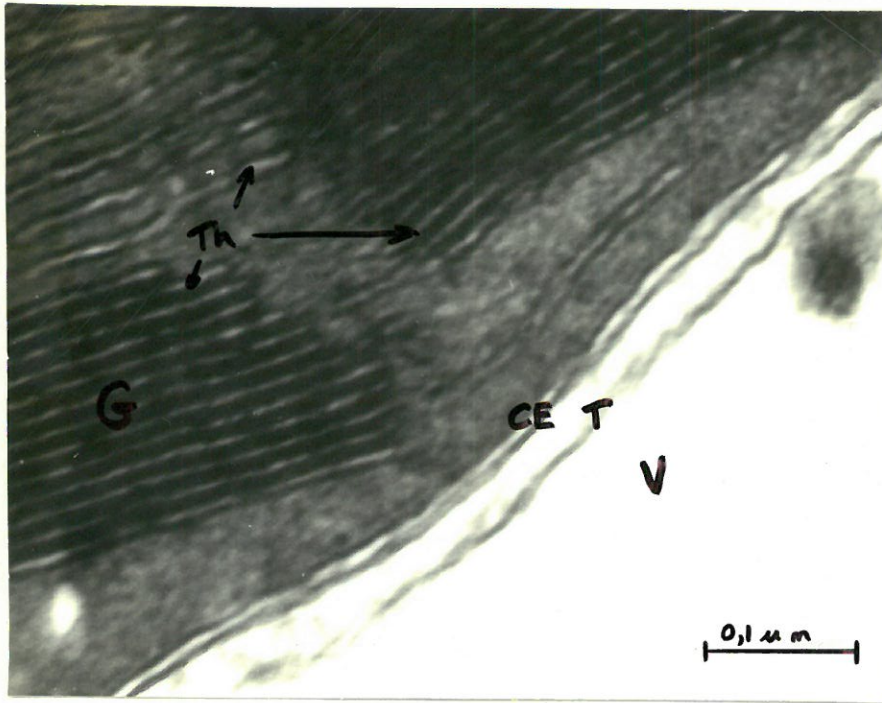


FIG 12: Electron micrograph of a part of a chloroplast of Rhoeas sp. leaf tissue. The double unit membrane of the chloroplast is shown with the single unit membrane of the vacuole (Tonoplast). The thylakoids are quite clearly demonstrated.

#### DISCUSSION

As was mentioned above, the cellular structures of Rhoeas sp. and <sup>12r</sup>Chelodendron sp. are very similar. Yet, with more careful analysis many small and consistant differences would doubtless be found. Mention was made that it appears that the vacuoles of Rhoeas sp. are quite clear of particles when compared with the vacuoles of <sup>12r</sup>Chelodendron sp. and yet this could simply be a result of sectioning or at least preparation. Some of the heavy deposits showing up as black in the sections could be deposits of lead crystals arising from the staining with lead citrate. These crystals are generally distinct as appearing to stand out from the section, so that the distinct round particles in the nucleus are probably not lead crystals. ?

The membrane structure is quite clearly shown as double layer: for the nucleus and chloroplast whilst the tonoplasts are clearly unit membranes. It is unfortunate that no clear views were obtained of the plasmalemma. In the micrograph of the vascular tissue (Fig 3) and some other micrographs (Fig 1, 2) the middle lamella can clearly be seen and in Fig 2 it can be seen that the middle lamella extends



into the intercellular airspace in the one place.

In the caption to Fig 6 the comment is made that the starch grain in the chloroplast appears to be 'nucleate' due to the fact that it is stained in the centre. This phenomenon is evident in the sections of both species, yet in some text books the starch grains are shown as being fully unstained.

In the fine structures of the chloroplasts the thylakoid membranes can be seen as being stacked in the grana and the thylakoid membranes interconnecting grana stacks are clear. The latter are called small thylakoids.

Thus it can be seen that the transmission electron microscope affords a great deal of access to data on the fine structures of biological specimens. The one micrograph represents a 200 000X magnification (Fig 12) of the chloroplast envelope and some of the thylakoid membranes. This shows but a small degree of detail available by using the electron microscope. As was stated above definition can be of the order of  $2 \mu m$  whilst in this micrograph the definition is not that fine. It would appear too that there was some movement of the specimen whilst the picture was being taken and as a result the detail which is possible was not realized.

*Handwritten notes:*  
 "fine?"  
 $\approx 1.5 - 2 \text{ \AA}$   
 "max."  
 $\approx 0.2 \text{ nm}$

The great advantages afforded by the availability of such equipment as the electron microscope is a great boon to the modern scientist.

#### REFERENCES

Ledbetter M C, K R Porter (1970) Introduction to the Fine Structure of Plant Cells. Springer - Verlag Berlin.

Robards A W (1970) Electron Microscopy and Plant Ultrastructure. McGraw Hill London.

*Own micrographs good. 'Introduction' & 'materials & methods' could have been more extensive & detailed. A number of factual errors & peculiarities of wording.*

60%