

history library of the British Museum and that of the Linnean Society of London, when their library catalogues were issued a few years back did not have this volume, would indicate that this is a rare work. The library of the Zoological Society of London lists the work in its catalogue, although the new species described in the "Transactions" are not noted in the *Zoological Record*. There are but two articles of

taxonomic interest in the volume, one by C. J. Wild, describing *Dendroceros subtropicus*, a new species of moss (pp. 49-50); and "Australian Lepidoptera: Thirty New Species," by Thomas P. Lucas, entirely moths (pp. 101-116).

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### DOUBLE STAINING BY THE CAJAL-BROŽEK METHOD

WHEN making durable microscopic slides, many different ways of dyeing are in use, of which some are of chief importance in cytological work. But the double staining is such that only quite definite parts of the cells, *e.g.*, chromosomes or nucleoles are stained by one color; whereas the other color stains another part, *e.g.*, cytoplasm. The method, which the author of this account wishes to describe briefly, has been worked out with a few alterations, by Ramon y Cajal. It has been used from time to time and still is used in zoological work, but only for the last three years in botanical studies, where it has been introduced by Professor Arthur Brožek with considerable success, in a modified form.

It is a cytological method, which stains the chromosomes splendidly, and when compared with Heidenhain's iron-hematoxylin method, so much used nowadays, has certain advantages, which will be pointed out below. By our method, the chromosomes are stained a bright red with magenta—which is basic fuchsin, while the nucleole and the other parts are stained sky-blue with picro-indigo-carmin. Picro-indigo-carmin is a mixture of 2 parts saturated aqueous solution of indigo-carmin and one part of picric acid. Both the magenta and picro-indigo-carmin are used in concentrated solutions.

The process of staining is quite simple and short. The preparation—paraffin sections mounted on supporting glass—is freed from paraffin by xylol and brought through a mixture of alcohol-xylol and then through a series of alcohol baths to water; after which the object is placed for 3 to 5 minutes in concentrated magenta. The exact time must be found out for various objects, although the preparation always appears quite right, if treated for the length of time mentioned above. At the expiration of this time, the object is rinsed in distilled water and immediately placed in picro-indigo-carmin, where it is left for 10 to 15 minutes. It is better to find out the precise time for various objects by experiment. Then the object is placed in very slightly acid water for about

one minute, into a beaker of distilled water with 1 to 2 minims acetic acid, after which an automatic differentiation is made with 80 per cent. alcohol. The slip is simply rinsed from the acid water, allowed to drain and plunged directly into 80 per cent. alcohol, where it remains until the red stain from the section remains, which can easily be seen with the naked eye. The time for differentiation is one half to one minute. Then the object is brought into 96 per cent. alcohol, 100 per cent. alcohol and a mixture of alcohol-xylol to xylol, and then mounted in Canada balsam. The whole process, from the placing in xylol to the mounting in Canada balsam, does not last more than 40 to 45 minutes. This is a great advantage when compared with Heidenhain's method, in which the procedure in the best case lasts for eight hours, and with careful working may even last for two and a half days. Another advantage is that the chromosomes stained with magenta can be distinguished from one another, even when they lie close to each other, or actually on each other. Such a method with hematoxylin is quite impossible on account of the covering color. A certain disadvantage of the method is that it can only be used successfully after sufficient acid fixing. It is best to use Nawashin's method, which is at the same time the most perfect, after fixing—15 parts 1 per cent. chromic acid, four parts formol and 1.5 parts glacial acetic acid. This method has been tried with success after fixing in highly diluted  $\text{HNO}_3$ . After using Němec fixing solution I., color tends to disappear—100 ccm 1 per cent. chromic acid and 8 ccm formol—but no change is apparent after using Němec II—50 ccm 1 per cent. chromic acid, 50 ccm 3 per cent. bichromate of potash, and 8 ccm formol; or after Regaud's method—80 parts 3 per cent. bichromate of potash and 20 parts formol. It proceeds badly after alcohol or formol fixing, but quite well after sublimate with acetic acid. There is no need to mention other fixing methods, which are for the greater part special. It is important that our method works excellently after Nawashin's fixing, which is most used to-day in plant-cytology. This method can be recommended, as it furnishes beautiful slides in a relatively short time,

which can be studied in all their details, drawn and excellently photographed with a green filter.

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### A NEW PARAFFIN EMBEDDING MIXTURE

DIFFICULTY in obtaining a paraffin of satisfactory consistency in which to embed and section biological material led to the development of the following formula. The use of neither of the substances added to the common paraffin is original with the writer (having been in fairly common use fifteen to twenty years ago) although the proportions and combinations may be.

A stock solution of crude rubber in paraffin is first made. Crude rubber is available in thin sheets, either smoked or unsmoked. Both kinds work equally well. The sheets of crude rubber are chopped up with a scissors and dropped in melted Parawax or any similar

common paraffin. The paraffin should be smoking hot and the mixture should be stirred occasionally. Three to four hours are required to completely melt the rubber. About 20 grams of rubber can be dissolved in 100 grams of paraffin.

#### EMBEDDING MIXTURE

Parawax .....	100 grams
Rubber-paraffin mixture .....	4-5 grams
Beeswax .....	1 gram

Filter through paper (paper towels serve this purpose excellently).

This mixture is pale yellow in color, does not crystallize readily, and is of a waxy consistency that sections unusually well. It has been in use in this laboratory for the past three years and has materially increased the success of large classes in micrology.

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## SPECIAL ARTICLES

### PHOTOPERIODISM AS A CAUSE OF THE REST PERIOD IN STRAWBERRIES

EXPERIMENTS made by the authors on the light requirements of strawberries have suggested an explanation of the cause of the rest period in this plant. The strawberry plant in northeastern United States becomes more or less dormant with the onset of low temperatures in the fall. Low temperatures alone are not responsible for dormancy, for many varieties remain dormant even when taken into a warm greenhouse in late fall. However, if plants are first given a low temperature rest period until about January 1, when brought into a warm greenhouse, all varieties grow vigorously, even though the days are still short. After the rest period has been broken, leaf growth and flower production is rapid. Plants covered with boxes in the field in January, so placed as to exclude all light, blossomed at about the same time as those in the open. Temperature and not light was the controlling factor after the rest period was broken.

If plants in the greenhouse are given additional light for several hours each night, beginning in November before they have had a low-temperature rest period, many but not all varieties grow vigorously. Each variety and species shows a characteristic response to the length of the daily light period at this time. If, however, the daily light period is lengthened beginning in early fall (September 1) before the days become short, the plants of all varieties we have tested make a vigorous vegetative growth throughout the entire winter and do not require a low-temperature rest period. Some varieties under such increased light periods produce some flower clusters, depending on

their particular daily light period response. In contrast, plants not given additional light start fruit bud formation with the onset of short days, and if the temperature is above 60° F., plants of many kinds go into the rest period.

In one test, plants of some 51 varieties were given electric light until 10:00 each night to supplement daylight, beginning September 1. Until February their growth was almost entirely vegetative. They produced an average of 0.7 flower clusters per plant by that time, while control plants under normal light but similar temperatures had averaged 3.8 clusters. By June 9, the control plants had averaged 20.2 clusters, while the plants under the lights had averaged 4.8 clusters each. Continued growth seems to be correlated with relatively constant exposure to the photoperiodic requirements of the particular variety. Growth in some form apparently can continue over a fairly wide range of light exposures. For ordinary varieties very short daily light periods initiate a rest period, short light periods result in continuous fruiting and longer periods in vegetative growth only. Rest periods then seem to result from nutritional conditions following exposures to short daily light periods. *Fragaria virginiana* and varieties adapted to the Northern and Eastern states require a rest period after exposure to days as short as 12 hours daily light. *F. chiloensis*, varieties of the Northwest derived in part from it recently, and Southern varieties do not need a rest period until the days are much less than 12 hours. In fact, after exposure to days as short as 9 hours at the relatively low temperatures used they still grew freely.