NOTES ON MICROSPECTRA.

I. The Absorption Spectrum of Euglena.

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The opacity or transparency of a certain substance for monochromatic radiation plotted against wave-length yields a so called absorption or transmission curve. The finite integral of this function between two definite wave-lengths represents a certain fraction of the corresponding integral for the source of light used. This fraction is the percentage of energy absorbed (absorption curve) or transmitted (transmission curve) by the absorbing substance. The absorption curves are therefore sometimes incorrectly called energy curves.

The absorption curve of the living leaf has been determined, more or less accurately, by various authors. The best and most recent values available are those of Ursprung,[1] in whose paper the older literature will be found. Ursprung used a linear thermocouple to measure the absorption of green leaves (*Acer*, *Tradescantia*) at various places of the spectrum (10 $\mu\mu$ apart).

The ideal object for the study of these absorption curves would be the isolated plastid, excluding the optical interference of air, water, cellulose walls, and other substances. Certain euglenids approximate this condition.

With the aid of an Abbe prismatic microspectral ocular (E. Leitz), which had been provided with adjustable slits for both absorption and comparison spectra, the authors were able to obtain spectrum photographs of minute objects (limit 10 $\times$ 15 μ). The source of light used was a 6 volt "Mignon" lamp which ran on a battery. An Eastman panchromatic film was used throughout. The procedure was simple. Exposures were taken with and without the organisms in the micro-

[1] Ursprung, A., *Ber. bot. Ges.*, 1918, xxxvi, 73.

scopic field, the photographs developed with pyro developer in absolute darkness.

The opacity of the negatives for various regions of the spectrum was determined with the aid of a modified Boys' radiomicrometer.[2] This combination thermocouple and galvanometer was constructed in the following way: Soldered on to a strip of silver foil (12 mm^2.) are a thin silver wire, and at the other corner, a thin bismuth wire. The free ends of the wires are joined to a thin copper coil, which serves as galvanometer coil and to which a galvanometer mirror is attached. The resistance of the copper coil about equals the resistance of the rest of the system. The apparatus is steadied by a glass capillary and suspended on a quartz fiber. The coil is brought between the poles of a powerful permanent magnet. The silver foil is blackened by three coats of India ink, the entire apparatus properly insolated.

A constant light source was obtained by running a 6 volt lamp on a 104 volt storage battery current with tungsten lamps (which illuminated the scale) in series. The film moved slowly over an adjustable slit.

The density of the plate is proportional to the logarithm of the reciprocal galvanometer deflection. Adjustment of the nul point by correspondence of the fog reading on the film to 100 scale divisions simplifies the calculations materially. The spectra were measured at intervals of .7 mm.; at critical points intermediate readings (down to .07 mm.) were taken. The Amici prism was calibrated with the aid of three Fraunhofer lines of the solar spectrum with Hartmann's simplified interpolation formula.[3] This formula expresses the wavelength λ as a function of a linear distance d as follows:

$$\lambda = \lambda_0 + \frac{c}{d_0 - d}$$

in which c and d_0 are constants; d represents the distance of the lines measured. The position of the Fraunhofer lines 486 $\mu\mu$ (H), 527 $\mu\mu$ (Fe) and 589 $\mu\mu$ (Na) was established on the radiomicrometer curve

[2] Boys, C. V., *Phil. Tr., Series A*, 1889, clxxx, 159, 183. See also Paschen, F., *Ann. Phys.*, 1893, xlviii, 272.

[3] Hartmann, J., *Astrophys. J.*, 1898, viii, 218.

of a solar spectrum. Taking 486 $\mu\mu$ as an arbitrary zero we found

λ	d
486$\mu\mu$	= 0
527	= 18.8
589	= 39.4 divisions.

The constants were determined with the aid of these three sets of data

$$486 = \lambda_0 + \frac{c}{d_0} \quad (1)$$

$$527 = \lambda_0 + \frac{c}{d_0 - 18.8} \quad (2)$$

$$589 = \lambda_0 + \frac{c}{d_0 - 39.4} \quad (3)$$

The constants proved to be:

$$\lambda_0 = 214.9$$
$$d_0 = 143.1$$
$$c = 38794.4$$

The distance between 486 $\mu\mu$ and the maximum film density in the red for a *Euglena* spectrum proved to be 57.8 divisions. The wave-length of the absorption maximum was therefore determined at

$$\lambda = 214.9 + \frac{38794.4}{143.1 - 57.8} = 214.9 + 454.8 = 669.7 \ \mu\mu$$

The low dispersion in the red increases the probable error. The value is very close to that obtained by other authors.

Ursprung's curve was transformed to the same abscissæ, the ordinates expressing transparency instead of extinction. Ursprung's curve comprises 24 determinations, in most cases 10 $\mu\mu$ apart, in the range investigated by us. (See Fig. 1.)

The nature of our negative allowed for 56 determinations in the same region (700–450 $\mu\mu$) for *Euglena*. With good negatives the number of determinations could easily be increased tenfold. The following table gives the location of the absorption maxima for *Euglena* as compared with Willstätter's (visual) determination for a living leaf.[4]

[4] Willstätter, R., and Stoll, A., Untersuchungen ueber Chlorophyll, Berlin, 1913, 62.

Data for *Euglena*.	Willstätter.
670$\mu\mu$	693–663$\mu\mu$
—	643
626 } 613 }	625–611
586	592–569
543	551–535
528	520– end absorption.
476	
468	

No trace of band 643 $\mu\mu$ could be found. This is probably due to the steepness of the curve in this region. More determinations with better negatives should reveal this band. The obvious advantage of our method is shown in the above table. The accompanying figure represents Ursprung's data (*A*), our data for *Euglena* (*B*), Willstätter's determination for the living leaf (*C*) and for an aqueous suspension of Chlorophyll *a* (*D*). The absorption bands are all drawn in black. The great similarity of Ursprung's and our curve is apparent.

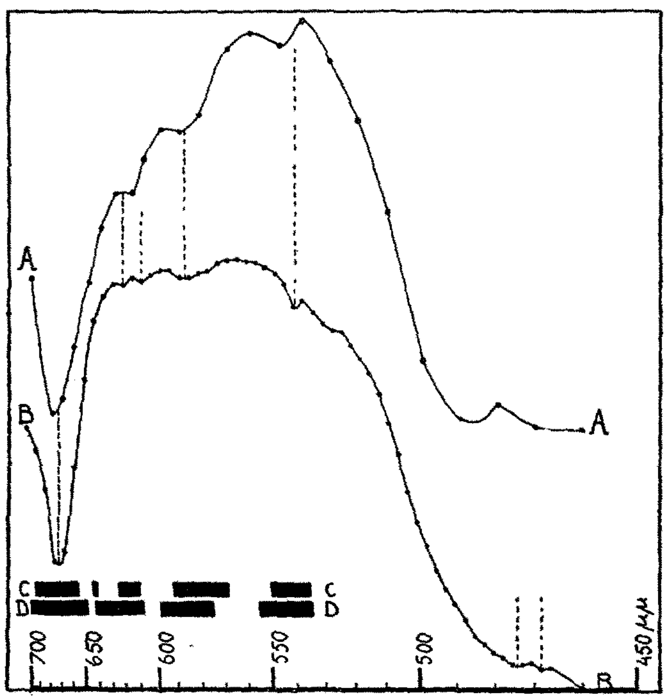

FIG. 1.