When α is small, $\sin 2\alpha = \tan 2\alpha = \frac{n_2 B}{A}$,

$$R^2 = \surd(A^2 + n_2^2 B^2) = A, \text{ approximately.}$$

Hence $$\mu = \surd A + \iota \frac{n_2 B}{\surd A}.$$

Here $n_2 B / \surd A$ is the coefficient of absorption of the solution, and is proportional to n_2, the number of molecules of the absorbent substance present per unit volume. Since it is essential that (19) should possess a well-marked maximum value for a certain wave-length (that of the light absorbed), additional evidence is obtained in support of the introduction of a viscous term into the equations to the atomic vibrations.

The equation for the square of the refractive index of a substance like crystallized copper sulphate, which possesses marked selective absorption without exhibiting selective reflection to any extent, might be represented by an equation of the form of (18); τ_2, &c., now referring to the conditions of motion of a particular atom, the motions of the remaining atoms giving rise to the terms involving τ_1, &c.

"A Photographic Investigation of the Absorption Spectra of Chlorophyll and its Derivatives in the Violet and Ultra-violet Region of the Spectrum." By C. A. SCHUNCK. Communicated by Dr. E. SCHUNCK, F.R.S. Received March 17,—Read March 24, 1898.

[PLATES 3, 4, 5.]

As is well known from the investigations of Soret,* Gamgee,† and others, hæmoglobin and its coloured derivatives show a characteristic absorption band lying between the lines G and M of the solar spectrum. The band has been shown to vary in position between narrow limits; in some derivatives it is nearer the red, and in others nearer the violet, end of the spectrum, and is of all the blood absorption bands the most stable.

The very near relationship that has been shown to exist by Schunck and Marchlewski‡ between phylloporphyrin (a chlorophyll derivative) and hæmatoporphyrin (a hæmoglobin derivative), and the remarkable resemblance of their absorption spectra—one may almost

* 'Arch. des Sciences Phys. et Nat.,' vol. 61, p. 322; vol. 66, p. 429.

† 'Arch. des Sciences Phys. et Nat.,' Dec., 1895.

‡ 'Roy. Soc. Proc.,' vol. 59, p. 233.

say they are identical—led Tschirch to the belief that there must also exist a like resemblance in the violet and ultra-violet region of the spectrum. Tschirch in his investigations* shows this to be the case, and finds a band in phylloporphyrin (termed by him phyllopurpuric acid) in the same position as the band observed in hæmatoporphyrin, and this corresponding to the characteristic band which has been shown to exist in the blood colouring matter derivatives. But further on I will show that this particular resemblance in these two derivatives is only partly correct, the band in the phylloporphyrin I examined being double, though occupying the same position as the single one of hæmatoporphyrin.

Tschirch (in the same memoir) makes the further important discovery that this band is not confined to phylloporphyrin alone, but that it is distinctive of the chlorophyll derivatives generally, and that it occupies (in the derivatives he observed) the same position (varying between narrow limits) as that shown to exist in the blood derivatives, and, like the blood band, the new chlorophyll band shows much greater stability than any of the other bands, no matter what chemical changes the derivatives have undergone.

The chlorophyll derivatives investigated by Tschirch are phyllopurpuric acid (impure phylloporphyrin), phyllocyanic acid (phyllocyanin), and its zinc, copper, and iron compounds, in all of which he finds a single band corresponding in position with the band in the hæmoglobin derivatives, in the zinc and copper compounds shifted slightly towards the red end of the spectrum, and he also finds it indicated in the living leaf, whilst in chrysophyll and carotin he observes three bands situated between the solar lines F and H and in identical positions. By the kind permission of Dr. E. Schunck I have had access to his beautiful collection of chlorophyll derivatives, and have been able to examine spectroscopically in the *pure* state solutions of chrysophyll, carotin, chlorophyll, phyll-oxanthin, phyllocyanin and its zinc and copper compounds, alka-chlorophyll, phyllotaonin, ethyl-phyllotaonin, and phylloporphyrin, and a specimen of hæmatoporphyrin kindly sent me by Dr. L. Marchlewski. In all there appears a characteristic absorption between the solar lines F and M, which confirms the statement of Tschirch that the chlorophyll derivatives, like the hæmoglobin derivatives, give a very characteristic absorption in this region of the spectrum, and considering the very near relationship which exists between phylloporphyrin and hæmatoporphyrin, is further evidence of the supposition that there exists something in common to both chlorophyll and hæmoglobin, the two great colouring matters of biological importance in the vegetable and animal kingdoms.

* 'Berichte der Deutschen Botanischen Gesellschaft,' vol. 14, Part 2, p. 76, 1896.

As I have been able to examine many more derivatives than the former investigator, and as the results of my observations differ from his, inasmuch that I find only some of the derivatives are characterised by the *single* band, whilst in others two are apparent, and in chlorophyll itself and chrysophyll three, and also that my method of procedure differs from his, it will perhaps be of interest to give the results of my experiments as compared with his.

Method of Procedure.

The spectroscope was a single prism one of Iceland spar, which just divided the sodium lines. The lenses were of quartz, the focal length of the collimator lens being 12·5 inches, and that of the camera lens 42 inches. The dark slide which held the plate was movable at fixed intervals, so that seven exposures could be taken on the one plate. The solutions were examined in a glass cell with parallel quartz faces, placed in front of the slit, the whole apparatus being set up for me by Mr. A. Hilger, of London. The source of light used was a Welsbach incandescent mantle of sixty-candle power, no chimney being used, placed 8 inches distant from the slit. For reference lines a hydrogen vacuum tube was used, from which the lines F, S′, L, and H′ were obtained, and the violet potassium line $K\beta$ was at the same time thrown in by volatilising a little of the salt in a Bunsen burner in front of the slit. The plate used was Messrs. Cadett and Neal's "lightning," pyrosoda being adopted as the developer. The exposure given in each case was half an hour, which was found to be the most advantageous after various trials of different times of exposure had been made. Under these conditions the photographs extended distinctly as far as the solar line Q. On each plate a spectrum of the source of light used was thrown in for a comparison with the light absorbed by the solutions of the different derivatives. In every case the solutions had to be excessively diluted before the characteristic absorption became apparent on the photographic plate, so dilute that, with the exception of chrysophyll, carotin, and chlorophyll, only a faint indication of colour was visible in the solutions to the eye by transmitted light, and of the bands in the visible region of the spectrum, only the first, the characteristic one in the red, was visible, and that in the majority of cases only faint. In the case of the hydrochloric acid compounds of phylloporphyrin and hæmatoporphyrin one might say the solutions were colourless, and yet these two derivatives give the *single* band more pronounced and better defined than any of the others. As a very slight difference in the strength of the solutions gave an appreciable difference in the resulting absorption spectra, three solutions of *slightly* varying strengths were photographed of each derivative, and the most charac-

teristic of each were then selected and photographed together as depicted in the plates. In no case on dilution were any further bands observable, and in all I find this particular distinctive absorption situated between the lines F and L in chrysophyll, carotin, and chlorophyll, and in the other derivatives between the lines G and L, the mean position of the bands being situated at the $K\beta$ line, varying sometimes slightly towards red and in others towards the violet. The main difference in my procedure to that of Tschirch is that I have made use of artificial light in the place of direct sunlight. By this means I have been able to reproduce the bands as regards their definition and their relative intensity in a far more distinct manner, and though my photos. do not extend so far as his, mine going to Q and his to S, yet as the characteristic absorption does not extend further than M this is not of great consequence.

Chlorophyll, Chrysophyll and Carotin.

The chlorophyll solution was prepared in the usual way. Fresh leaves were extracted with boiling alcohol, and the solution filtered off from the fatty deposit which usually forms on standing. When the solution is diluted so that in the normal chlorophyll spectrum of four bands only the characteristic one in the red is visible when received by the spectroscope, and a photograph is now taken, three bands are found lying between the lines F and $K\beta$ (Plate 3, figs. 1 and 2). The first two bands are the bands usually numbered 5 and 6 of the normal chlorophyll spectrum, and can be seen on dilution by the eye when sunlight is used, and the first one, 5, when artificial light is used. But the third band in the violet, having its centre situated about the line *h*, has not, I believe, been observed before. In weaker solutions still, one only gets a general absorption in the ultra-violet, no further bands being discernible. As is well known, chlorophyll solutions prepared from the leaves of different plants vary slightly in their absorption spectra, which depends upon the amount of acid present in the leaf. In the spectrum of the purest chlorophyll solutions, the fourth band situated about the line E is extremely faint; the purer the solution the fainter it is, but where the least trace of an acid is present this band appears darker than the third, situated between the lines D and E, and on the solutions standing becomes darker still, while the third becomes fainter, and has moved further away from the red end. If, however, a chlorophyll solution be exposed to the action of direct sunlight for a few hours until the colour has become brown, or if a few drops of a *strong* acid, as hydrochloric, be added, and the solution be allowed to stand for a few days, a further change in the spectrum takes place, by the formation of a fifth band situated between the lines

E and F. According to the investigations of Schunck and Marchlewski,* these changes may be explained by the supposition that phylloxanthin is formed in the first case, and phyllocyanin in the second. These changes take place in a greater or less degree in every chlorophyll solution on standing, according to the amount and strength of acid present originally.

I have found in all the various chlorophyll solutions I have examined, even in those which from the commencement gave the fourth band darker than the third, and those in which the same change had taken place on standing, that no change had taken place in the violet, the only change being that more and more of the ultra-violet rays are absorbed. In the purest solution the total obscuration started at the line N, while in others the total obscuration commenced at the line H_2. But in the case of a solution which had been exposed to the action of direct sunlight and which showed the fifth band, then the bands in the violet had disappeared altogether, leaving only a general absorption in the ultra-violet. As will be seen from Plate 5 (figs. 2 and 3), the second and third of these violet bands are identical in position with the two bands found in phylloxanthin, while the first corresponds in position to a new sixth band I have found to exist in the spectrum of phyllocyanin when the solution is examined in a more concentrated form than usual, and which appears distinct but faint when photographed.

The chrysophyll was obtained by Schunck's method† and examined in an alcoholic solution; the carotin crystals were obtained from the carrot root by the process of Arnaud, and was likewise examined in an alcoholic solution. In each three very distinct characteristic bands were found, which agrees with the statement of Tschirch, but the bands from my photographs are not in identical positions, those in the carotin being slightly shifted towards the violet end.

Like Tschirch, I find that both these derivatives are very transparent to the ultra-violet rays. From Plate 3 (figs. 2 and 3) it will be seen that the three chrysophyll bands occupy intermediate positions compared to the three chlorophyll bands, which seems to point to the supposition that chrysophyll does not exist in chlorophyll solutions as such, but under certain conditions only is formed by decomposition of a derivative,‡ and then crystallises out, or that if it does exist in the solutions it must be in a very small quantity, otherwise the bands would overlap each other, and the result would be a total obscuration, which I have only found to be the case in a chlorophyll solution exposed to the action of direct sunlight.

* 'Roy. Soc. Proc.,' vol. 57, p. 321.

† 'Roy. Soc. Proc.,' vol. 44, p. 449.

‡ Hansen, 'Die Farbstoffe des Chlorophylls,' 1889, p. 58.

Phylloxanthin, Phyllocyanin and its Compounds.

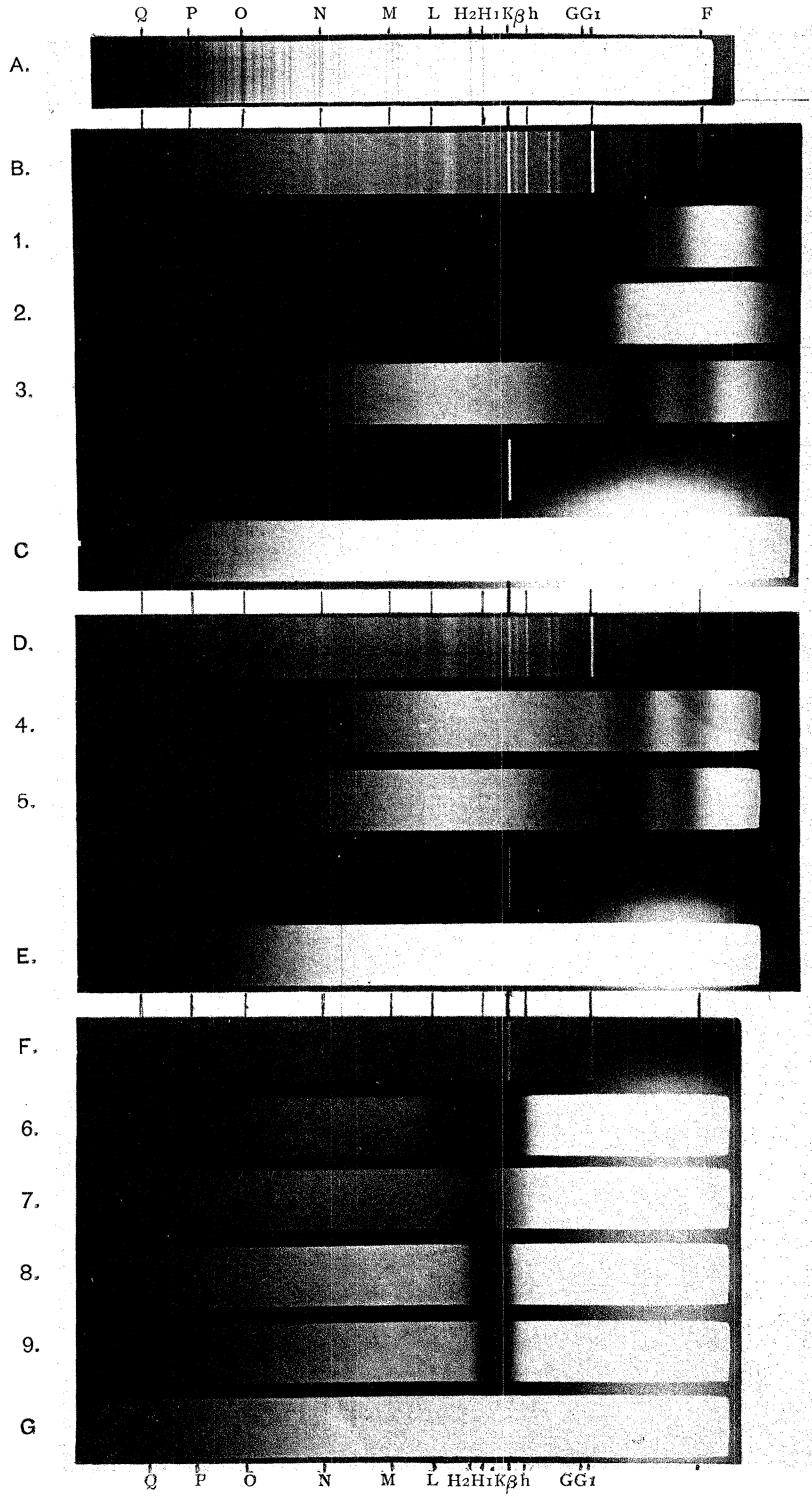
Phylloxanthin and phyllocyanin, the two leading chlorophyll derivatives obtained by the action of hydrochloric acid (Schunck, "Contributions to the Chemistry of Chlorophyll")* give in alcoholic solutions in the violet region of the spectrum, in the former case two bands, and in the latter one (Plate 5, figs. 3 and 4). As has already been pointed out, the two phylloxanthin bands are identical in position with the second and third of the chlorophyll ones in the violet, the band in phyllocyanin being moved slightly towards the violet end, this band being situated between the lines *h* and H^2. In both cases the solutions have to be exceedingly dilute before the bands become visible on the photographic plate, the only band remaining visible to the eye in the visible part of the spectrum being the first, the characteristic one in the red, and that now only appears faint. From the investigations of Schunck,† phyllocyanin plays the part of a weak base, and combines with strong acids, the compounds, however, being unstable and easily decomposed even by water, and, like other bases, giving definite double compounds of great comparative stability, into which metals and acids, especially organic acids, enter as constituents.

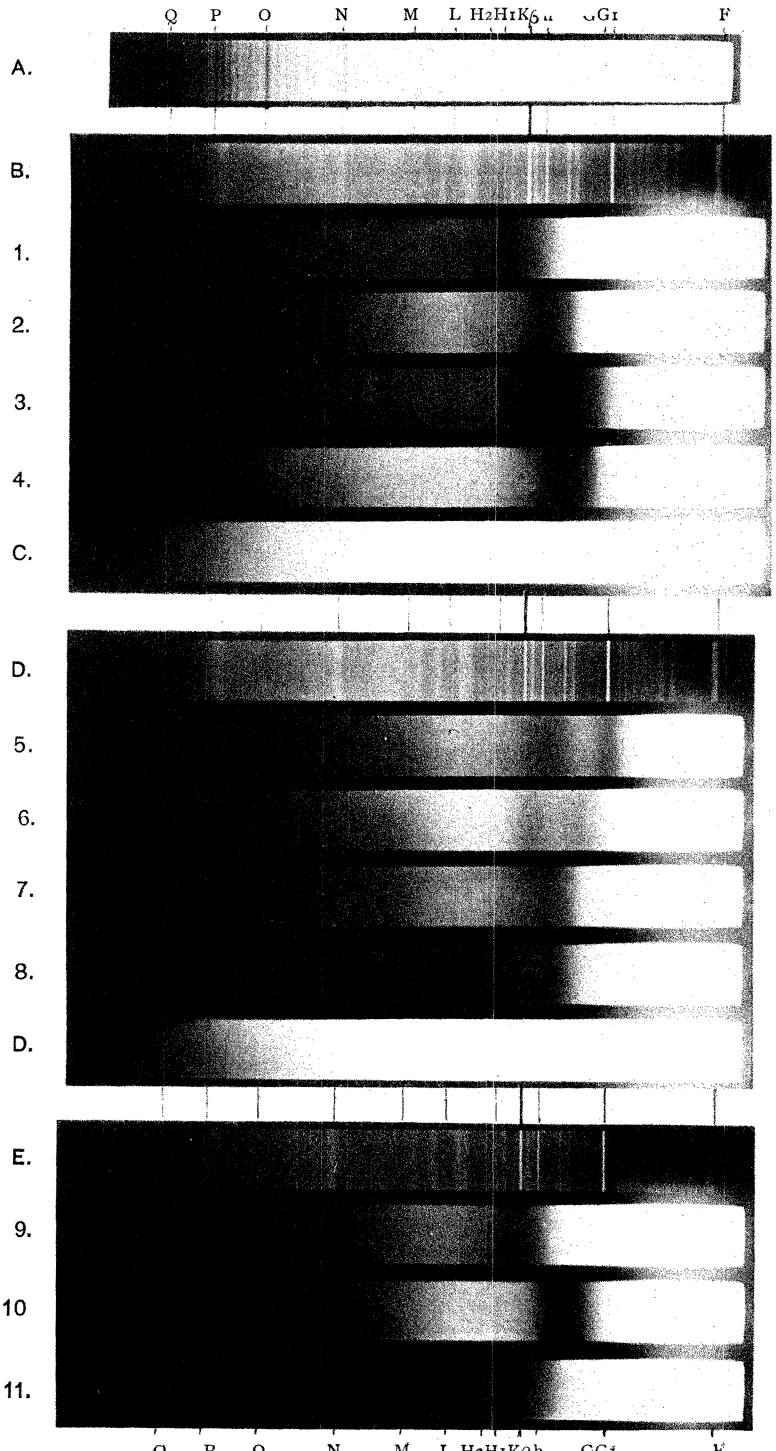
I have examined the compounds formed by dissolving phyllocyanin in *anhydrous* acetic acid, hydrochloric acid, and sulphuric acid, and find a more pronounced band in each than even in the case of an alcoholic solution of phyllocyanin, but in each compound the band is shifted slightly towards the red end of the spectrum compared to the band in phyllocyanin itself (Plate 4, figs. 1, 2, 3, and 4).

Of the compounds formed by phyllocyanin with metallic salts, the one with zinc carbonate in alcohol, with zinc acetate in acetic acid, and the one with cupric acetate in the same solvent were examined. In each the characteristic absorption was noticeable in the same region of the spectrum. In the zinc carbonate compound two very distinct bands were found corresponding very nearly in position with the two in phylloxanthin, the shifting being towards the violet end (Plate 4, figs. 5 and 6), the zinc acetate compound, on the other hand, gave only one band, corresponding in position with the band of the anhydrous acetic acid compound of phyllocyanin (Plate 4, fig. 7), whilst in the case of the cupric acetate compound two badly defined dark bands were apparent, the division between the bands corresponding about to the position of the phyllocyanin band (Plate 4, fig. 8). In both these series of compounds, as in the case of phylloxanthin and phyllocyanin, the solutions had to be very dilute before the characteristic absorption became apparent on the photographic

* 'Roy. Soc. Proc.,' vol. 39, p. 348; vol. 50, p. 306.

† 'Roy. Soc. Proc.,' vol. 39, pp. 354 and 356.


Q P O N M L H2H1Kβh GG1 F
A.
B.
1.
2.
3.
C
D.
4.
5.
E.
F.
6.
7.
8.
9.
G
Q P O N M L H2H1Kβh GG1


Q P O N M L H2H1K
F
A.
B.
1.
2.
3.
4.
C.
D.
5.
6.
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8.
D.
E.
9.
10
11.
Q P O N M L H2H1K h GGi F

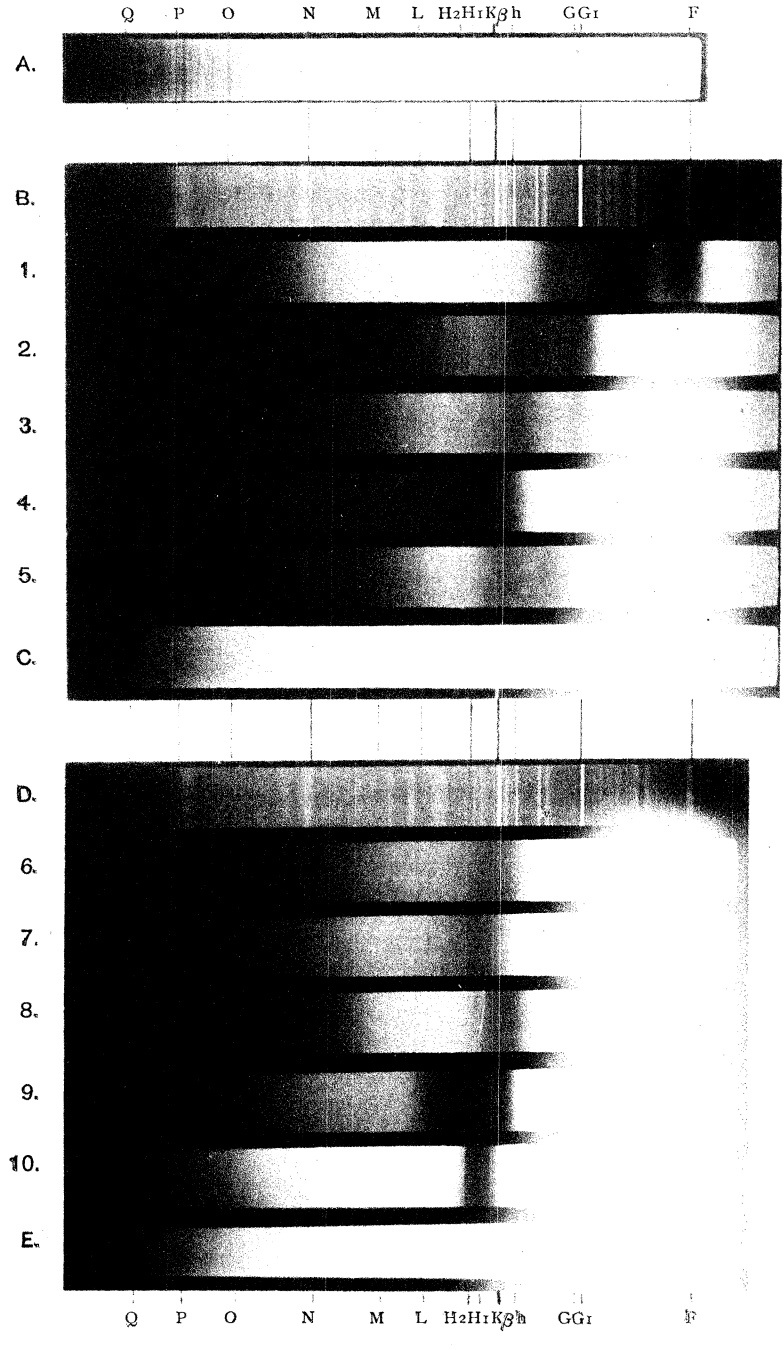

Q P O N M L H2H1Kβh GG1 F
A.
B.
1.
2.
3.
4.
5.
C.
D.
6.
7.
8.
9.
10.
E.
Q P O N M L H2H1Kβh GG1 F

plate. Here the results of my experiments differ from those of Tschirch, who found but one band in the zinc and copper compounds, but the discrepancy may have arisen from the fact that he does not state in his memoir what acid was in combination along with the metal with phyllocyanin. That the zinc carbonate compound of phyllocyanin should give two bands in the violet region corresponding very nearly in position and relative intensity with the two of phylloxanthin, and therefore, with the second and third violet chlorophyll bands, is interesting from the fact that of all the chlorophyll derivatives the spectrum of the phyllocyanin zinc carbonate in the visible region corresponds more closely than any of the others with that of chlorophyll, and from the deductions arrived at from this similarity by Schunck* as regards the functions of chlorophyll as being a carrier of carbonic acid in the plant, just as hæmoglobin serves to convey oxygen in the animal economy.

Alkachlorophyll, Phyllotaonin, Ethyl-phyllotaonin, Phylloporphyrin.

In his "Contributions to the Chemistry of Chlorophyll" Schunck has discovered that by the action of alkalis upon chlorophyll, the above definite derivatives are produced in a crystalline form.† Alkachlorophyll, phyllotaonin, and ethyl-phyllotaonin all give the *single* band in a pronounced and well-defined manner, corresponding very nearly in position with the band in phyllocyanin, viz., at about Kβ, in phyllotaonin it being shifted slightly towards the violet, and in the other two a trifle towards the red end of the spectrum (Plate 5, figs. 6, 7, and 8). Alkachlorophyll also forms a definite crystallised sodium salt. In an alcoholic solution the band appears considerably shifted towards the red, but a watery solution gives the band much obscured, in the same position as the one in the alcoholic solution of alkachlorophyll itself (Plate 4, figs. 9, 10, and 11).

Phylloporphyrin on the other hand gives a double band, intense and fairly well defined, the less refrangible one having its centre situated at the Kβ line (Plate 5, fig. 9). Phylloporphyrin forms a compound with acids, which has a spectrum in the visible region quite distinct from the very characteristic one of phylloporphyrin itself, which consists of seven bands, while the acid compounds only show three bands.‡ On examining the hydrochloric acid compound in the violet region, a corresponding change is also found in the spectrum, and instead of the double band, a single very intense

* 'Annals of Botany,' vol. 3, pp. 65—120.

† 'Roy. Soc. Proc.,' vol. 39, p. 355; vol. 44, pp. 449—454; vol. 50, pp. 312—316; vol. 57, pp. 316—319.

‡ Schunck and Marchlewski, 'Roy. Soc. Proc.,' vol. 57, p. 319.

one takes its place, situated between the other two (Plate 5, fig. 10). This band is the most pronounced and the best defined one in the whole series, and only becomes visible on the photographic plate in excessively dilute solutions, so dilute that one might say the solution was colourless to the eye when viewed by transmitted light.

Phylloporphyrin and Hæmatoporphyrin.

On comparing the spectra of phylloporphyrin and hæmatoporphyrin in this region, and also those of their hydrochloric acid compounds (Plate 3, figs. 6, 7, 8, and 9), it was found that hæmatoporphyrin gave only a single band, but situated in the same position as the double one of phylloporphyrin. On this point the results of my experiments differ from those of Tschirch, who, as stated above, found in both a *single* band occupying the same position. In the hydrochloric compounds of hæmatoporphyrin, however, a single band of the same pronounced character as that in phylloporphyrin was found, the one in hæmatoporphyrin, as will be seen from the figures, being slightly shifted towards the red end of the spectrum, which is interesting from the fact that just in the same way are the bands in the visible region of the spectrum of these two compounds shifted, this constituting their only spectroscopic difference.

In conclusion, my thanks are due to Dr. E. Schunck and Dr. L. Marchlewski for the valuable assistance they have given me in many details in connection with this investigation.

I hope in a further paper to investigate more particularly the spectroscopic behaviour in the same region of the spectrum of the yellow colouring matter accompanying chlorophyll in leaves and allied colouring matters obtainable from other sources than the leaf, for instance, carotin.

"On Photographic Evidence of the Objective Reality of Combination Tones." By R. W. Forsyth, A.R.C.S., and R. J. Sowter, A.R.C.S. Communicated by Professor Rücker, Sec.R.S. Received March 29,—Read May 5, 1898.

[Plates 6, 7.]

In the following paper we propose to describe a series of photographs which prove the objective reality of difference and summation tones. The work was suggested to us by Professor Rücker, and we have used the method of detecting these tones which has been described by Rücker and Edser in the 'Philosophical Magazine' for April, 1895.

Explanation of Plate 3.

A. Solar Spectrum.
B. Reference Lines.

1. Chlorophyll in Alcohol.
2. Chlorophyll Diluted in Alcohol.
3. Chrysophyll in Alcohol.

C. Spectrum of the Incandescent Mantle.
D. Reference Lines.

4. Chrysophyll in Alcohol.
5. Carotin in Alcohol.

E. Spectrum of the Incandescent Mantle.
F. Reference Lines.

6. Phylloporphyrin in Alcohol.
7. Hæmatoporphyrin in Alcohol.
8. HCl Salt of Phylloporphyrin in Water.
9. HCl Salt of Hæmatoporphyrin in Water.

G. Spectrum of the Incandescent Mantle.

Explanation of Plate 4.

A. Solar Spectrum.
B. Reference Lines.

1. Phyllocyanin in Alcohol.
2. Phyllocyanin in Anhydrous Acetic Acid.
3. Phyllocyanin in HCl.
4. Phyllocyanin in H_2SO_4.

C. Spectrum of the Incandescent Mantle.
D. Reference Lines.

5. Phylloxanthin in Alcohol.
6. Phyllocyanin Zinc Carbonate in Alcohol.
7. Phyllocyanin Zinc Acetate in Acetic Acid.
8. Phyllocyanin Cupric Acetate in Acetic Acid.

D. Spectrum of the Incandescent Mantle.
E. Reference Lines.

9. Alkachlorophyll in Alcohol.
10. Sodium Salt of Alkachlorophyll in Alcohol.
11. Sodium Salt of Alkachlorophyll in Water.

Explanation of Plate 5

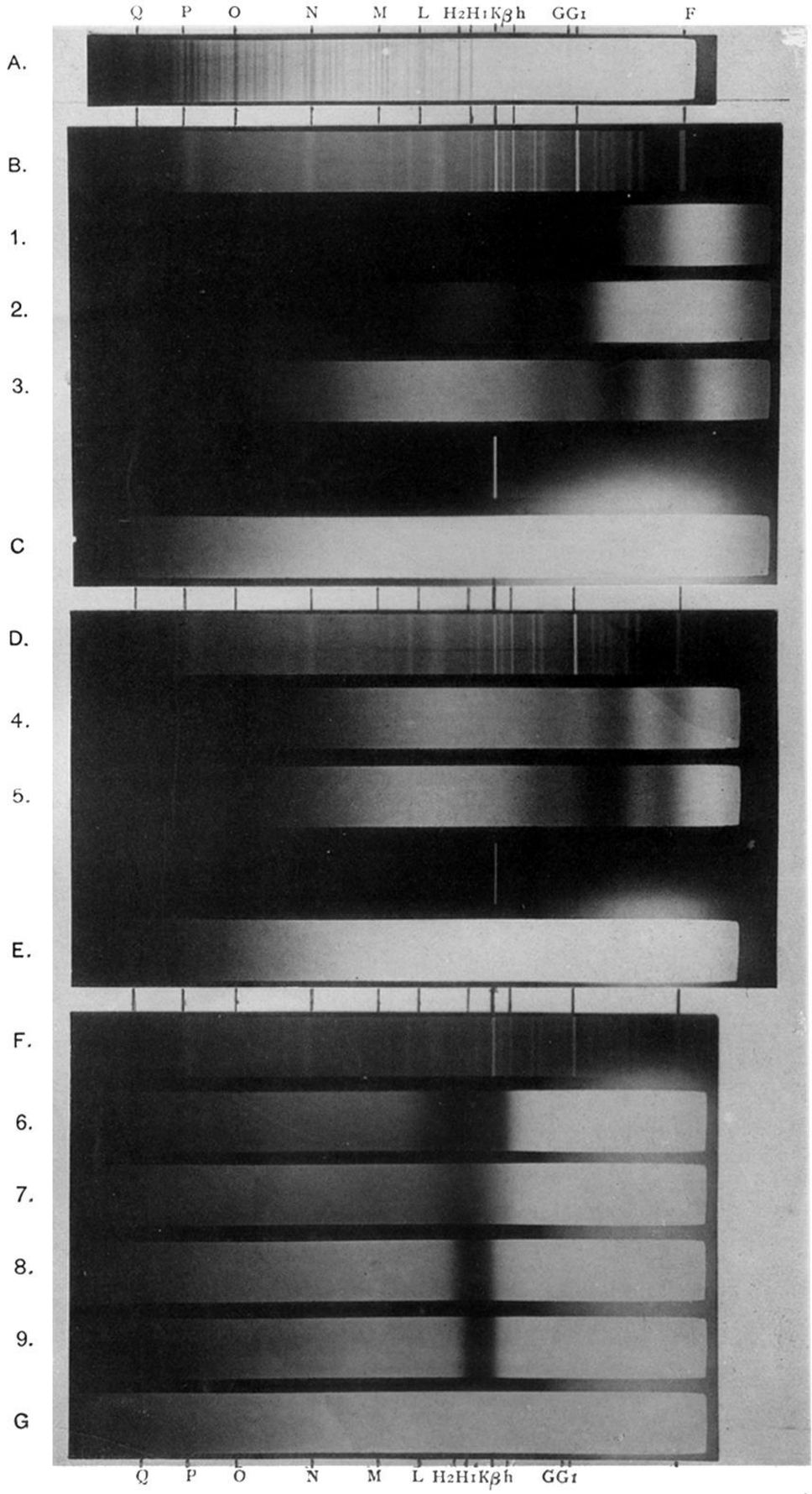
A. Solar Spectrum.
B. Reference Lines.

1. Chrysophyll in Alcohol.
2. Chlorophyll in Alcohol.
3. Phylloxanthin in Alcohol.
4. Phyllocyanin in Alcohol.
5. Phyllocyanin Zinc Carbonate in Alcohol.

C. Spectrum of the Incandescent Mantle.
D. Reference Lines.

6. Alkachlorophyll in Alcohol.
7. Phyllotaonin in Alcohol.
8. Ethyl-Phyllotaonin in Alcohol.
9. Phylloporphyrin in Alcohol.
10. HCl Salt of Phylloporphyrin in Water.

E. Spectrum of the Incandescent Mantle.



Explanation of Plate 3.

A. Solar Spectrum.
B. Reference Lines.

1. Chlorophyll in Alcohol.
2. Chlorophyll Diluted in Alcohol.
3. Chrysophyll in Alcohol.

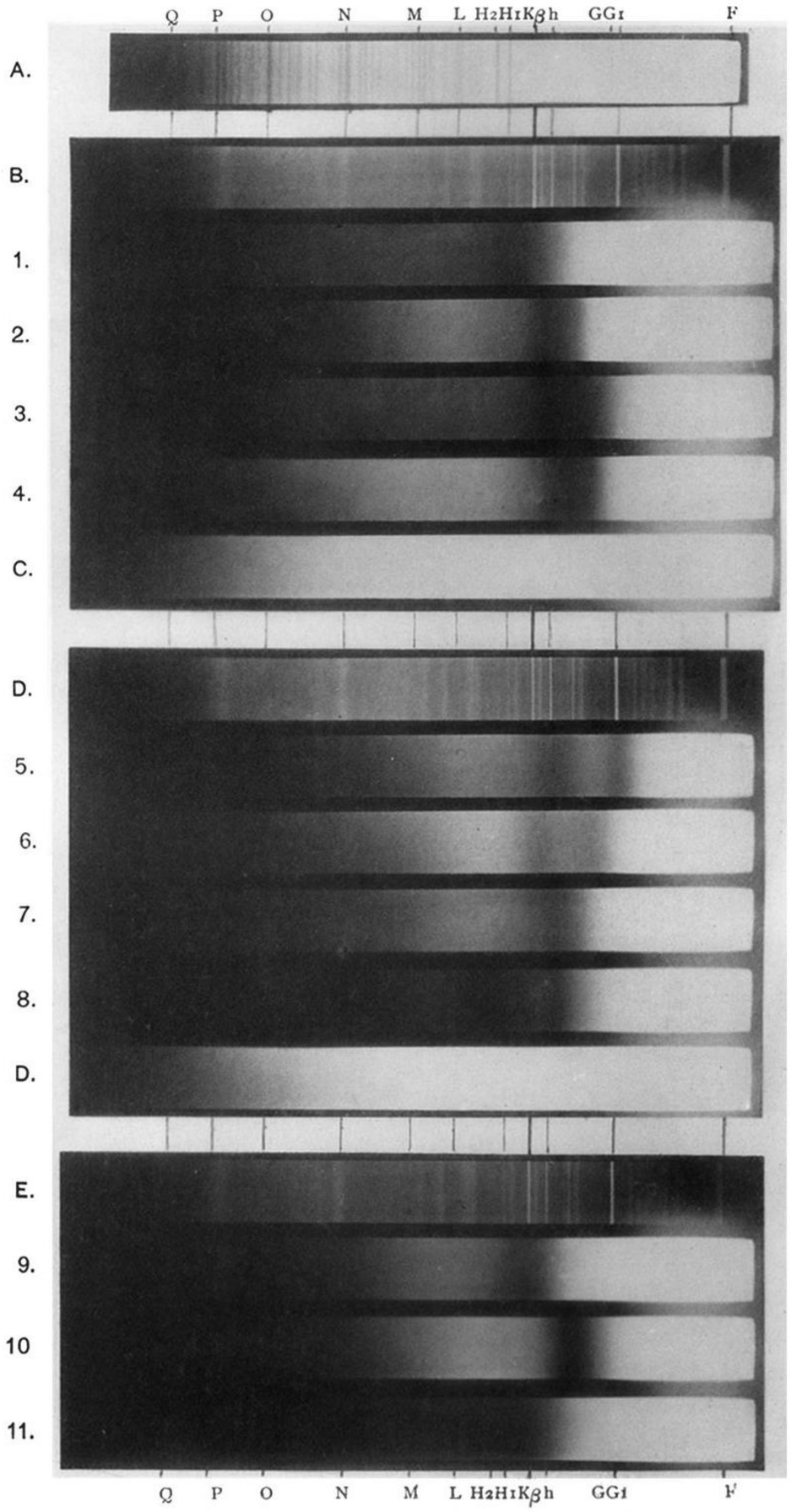
C. Spectrum of the Incandescent Mantle.
D. Reference Lines.

4. Chrysophyll in Alcohol.
5. Carotin in Alcohol.

E. Spectrum of the Incandescent Mantle.
F. Reference Lines.

6. Phylloporphyrin in Alcohol.
7. Hæmatoporphyrin in Alcohol.
8. HCl Salt of Phylloporphyrin in Water.
9. HCl Salt of Hæmatoporphyrin in Water.

G. Spectrum of the Incandescent Mantle.



Explanation of Plate 4.

A. Solar Spectrum.
B. Reference Lines.

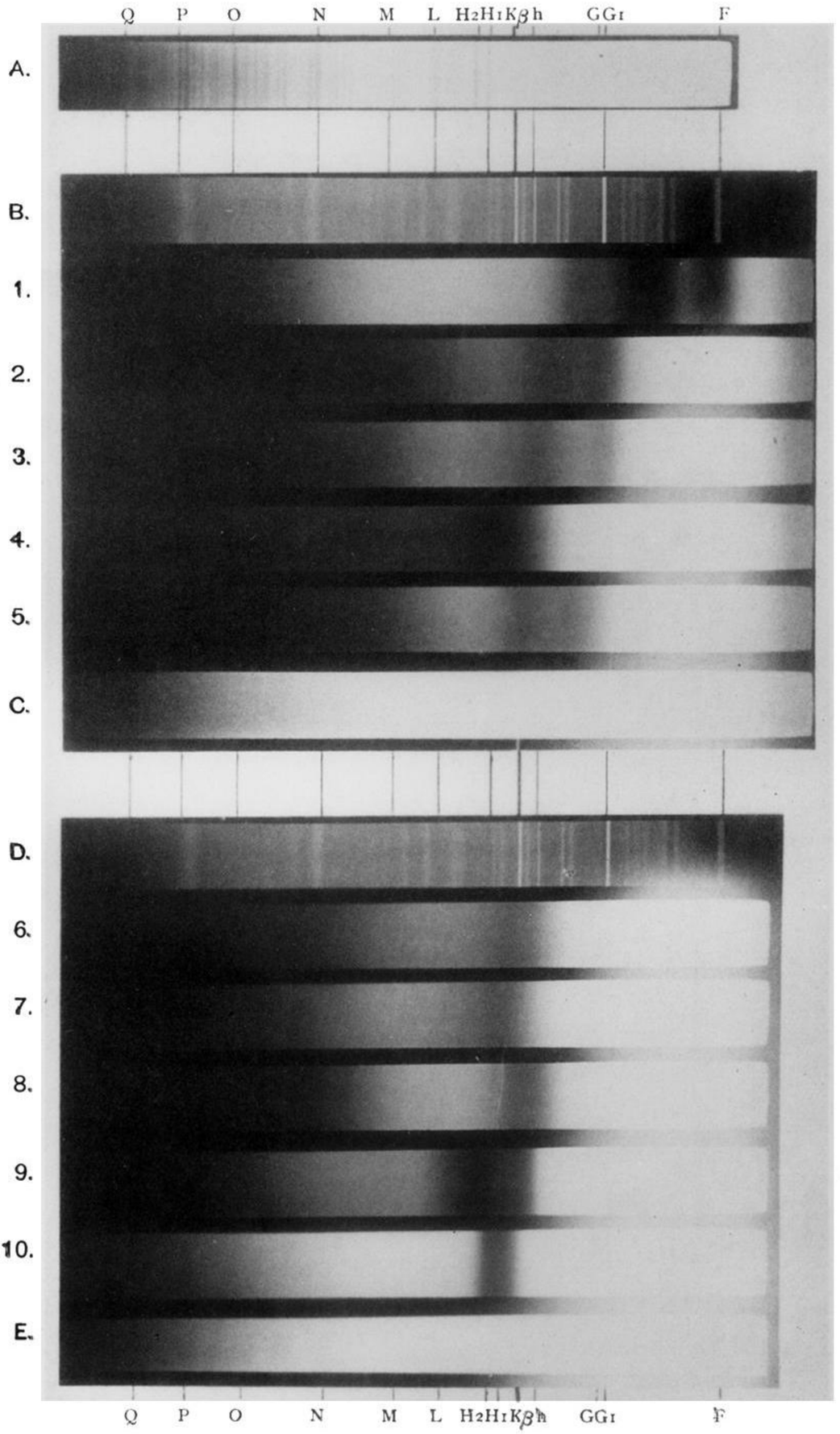
1. Phyllocyanin in Alcohol.
2. Phyllocyanin in Anhydrous Acetic Acid.
3. Phyllocyanin in HCl.
4. Phyllocyanin in H_2SO_4.

C. Spectrum of the Incandescent Mantle.
D. Reference Lines.

5. Phylloxanthin in Alcohol.
6. Phyllocyanin Zinc Carbonate in Alcohol.
7. Phyllocyanin Zinc Acetate in Acetic Acid.
8. Phyllocyanin Cupric Acetate in Acetic Acid.

D. Spectrum of the Incandescent Mantle.
E. Reference Lines.

9. Alkachlorophyll in Alcohol.
10. Sodium Salt of Alkachlorophyll in Alcohol.
11. Sodium Salt of Alkachlorophyll in Water.



Explanation of Plate 5

A. Solar Spectrum.
B. Reference Lines.

1. Chrysophyll in Alcohol.
2. Chlorophyll in Alcohol.
3. Phylloxanthin in Alcohol.
4. Phyllocyanin in Alcohol.
5. Phyllocyanin Zinc Carbonate in Alcohol.

C. Spectrum of the Incandescent Mantle.
D. Reference Lines.

6. Alkachlorophyll in Alcohol.
7. Phyllotaonin in Alcohol.
8. Ethyl-Phyllotaonin in Alcohol.
9. Phylloporphyrin in Alcohol.
10. HCl Salt of Phylloporphyrin in Water.

E. Spectrum of the Incandescent Mantle.