Improved Infrared Photography for Electrophoresis[1]

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The use of infrared light and infrared-sensitive plates for photographing pigmented protein solutions in electrophoretic work was first proposed by Treffers and Moore (*1*). A Nernst lamp or an ordinary tungsten lamp was suggested as a source of infrared light, but further specifications for the lamps or plates were not given. Examples of infrared photographs obtained by Moore for electrophoretic patterns of hemolyzed sera were presented by Abramson, Moyer, and Gorin (*2*). Longsworth (*3*) described interchangeable mountings for infrared lamps and the usual mercury vapor lamps. Recently, Kegeles and Gutter (*4*) showed that, with the aid of red-sensitive Eastman Kodak Spectroscopic Type 103-F plates, and a Wratten No. 105 filter for isolating the red lines of the mercury vapor lamp, satisfactory photographs of hemolyzed sera could be obtained without recourse to special sources of red light.

For electrophoretic studies on tissue extracts (*5*), we have found it necessary to use quite strong infrared illumination, since the protein solutions that we have been required to examine are often not only pigmented but also quite opalescent. It was not to be expected that the infrared radiation produced by the mercury vapor lamp would be generally adequate for this purpose. Tungsten projection lamps of the coiled filament type supplied strong enough infrared light but, because of the spaces between the coils, did not produce an even illumination of the entrance slit, a factor of importance when the Svensson-Philpot lens system is used.

The above problem was solved through the use of a tungsten projection lamp of the ribbon filament type. Such a lamp, having specifications suitable for electrophoretic work, is now available,[2] and is illustrated in Fig. 1. The filament is 2 mm wide, with an effective length of about 35 mm. Near one end of the filament is an expansion notch designed to keep the ribbon taut when it is heated. The rating is 7.5 v at 30 amp; a voltage transformer[3] is therefore required as an accessory. The lamp is fitted with a mogul prefocus base, is mounted in a horizontal position very close to the entrance slit, and is cooled with a blower. The bulb is turned so as to locate the filament support below

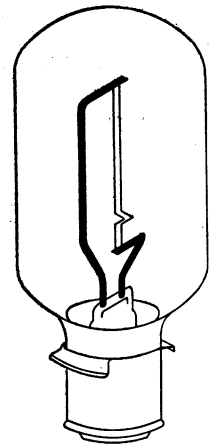

FIG. 1. General Electric ribbon filament lamp.

the filament. By the use of a mounting similar to the one used for the mercury vapor lamp, the two lamps are readily interchanged. Because of its limited life, the ribbon filament lamp is operated only when necessary.

For determining the efficacy of the ribbon filament lamp, a solution of hemoglobin and an extract of tumor (*5*) were used as test materials. These were placed in the electrophoresis cell in the usual way, and the boundaries that were formed were brought into view with the aid of a compensator. The boundaries were then allowed to diffuse to a desired degree before photographs were taken. In conjunction with the ribbon filament lamp, Eastman Kodak Spectroscopic Type I-N plates and a Wratten No. 25 filter were used. From the results, shown in Fig. 2 *a*, it may be seen that contrast across the hemoglobin boundary was completely eliminated, whereas that across the boundary of the tumor extract was only moderate.

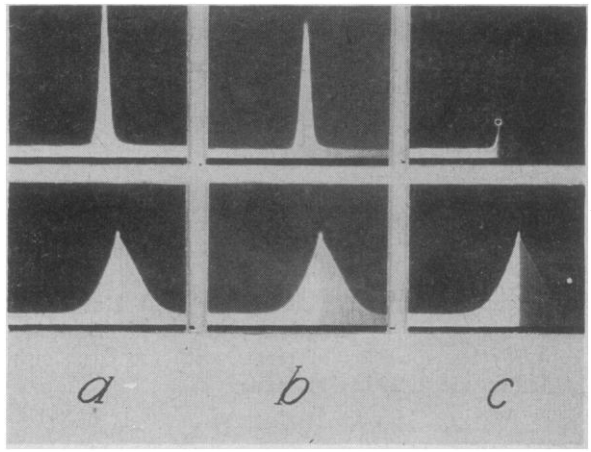

FIG. 2. Svensson-Philpot diagrams obtained with different light sources, photographic plates, and filters. Top row, pigmented hemoglobin solution. Bottom row, opalescent tumor extract. (*a*) Ribbon filament lamp, Type I-N plates, No. 25 filter, 2 sec exposure: (*b*) mercury vapor lamp, Type 103-F plates, No. 25 filter; 40 sec exposure for hemoglobin, 120 sec exposure for tumor extract; (*c*) mercury vapor lamp, Type IV-G plates, No. 77 filter, 10 sec exposure.

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[2] G-E projection lamp, Code No. 30A/T20/4. We wish to acknowledge the cooperation of R. E. Farnham, of the General Electric Company, Cleveland, Ohio, in directing our attention to this lamp. The diagram in Fig. 1 is reproduced with the permission of the General Electric Company.

[3] A suitable transformer may be obtained from Air Design, Inc., 241 Fairfield Ave., Upper Darby, Pa.

To compare the above results with those obtained under the conditions specified by Kegeles and Gutter, photographs were also taken with the mercury vapor lamp, Type 103-F plates, and the No. 25 filter. The No. 25 filter was, for the present purpose, equivalent to the No. 105 filter used by Kegeles and Gutter. The results obtained in these tests (Fig. 2 *b*) are quite comparable to those of Fig. 2 *a* except that the exposure required with the mercury vapor lamp was about 60 times as long. For photographing test samples which are more pigmented or more opalescent than those used here, the length of exposure needed with the mercury vapor lamp may become prohibitive.

In further tests, in which direct comparisons of the I-N and 103-F plates were made, it was found that the two types of plates were practically interchangeable, either with the ribbon filament lamp or with the mercury vapor lamp. The I-N plates possessed three significant advantages, however, in that they showed clearer backgrounds, sharper images, and less halation when overexposed.

Finally, to compare the results obtained with infrared light with those obtained with green light, photographs were taken with the mercury vapor lamp, Type IV-G plates, and a No. 77 filter. It is clearly apparent from Fig. 2 *c* that with the green light a high contrast across the boundaries and an incomplete recording of the images resulted.

References

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On the Evaluation of the Constants V_m and K_M in Enzyme Reactions

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Hofstee (*1*), discussing the evaluation of the constants, V_m and K_M, of the Michaelis-Menten equation, points out some disadvantages of the form of this equation proposed by Lineweaver and Burk (*2*). Hofstee's preferred equation (III) is identical, except for transposition of terms, with the form suggested by me in 1942 (*3*), namely:

$$v = V_m - K_M(v/s).$$

(I use Hofstee's symbols.) This form has the additional advantage in being that usually adopted for the regression equation. Statistical methods can then be readily applied to the evaluation of these constants, a matter of considerable difficulty with the equation of Lineweaver and Burk.

This formulation can also be used in the analysis of inhibition, the effects of which may be summarized as follows, using the classification of Ebersole, Guttentag, and Wilson (*4*), and putting $q = \left(1 + \frac{(I)}{Ki}\right)$

Type of inhibition	Slope	Intercept
II (competitive)	$q.K_M$	V_m
III (noncompetitive)	K_M	V_m/q
IV (uncompetitive)	K_M/q	V_m/q

Here the slope alone is sufficient to characterize the inhibition insofar as it is increased, unchanged, or decreased. This is not the case with plots of the type of Lineweaver and Burk, where the slope is unchanged in both Type III and Type IV. The error of the intercept is the error of the slope magnified by extrapolation and is therefore always greater. It is thus obviously advantageous to base conclusions on the slope, rather than on the intercept.

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Root Illumination and Flowering

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The reception of the photoperiodic stimulus which induces or promotes the flowering of plants appears to be localized chiefly in leaves, although there is some evidence that both aerial stems and rhizomes may participate in this reception (*1, 2*). Similarly, the reception of photoperiods which inhibit or retard flowering appears to occur principally in leaves. The absence of information concerning possible involvement of roots in such phenomena led the authors to perform an experiment on the effects of root illumination upon floral initiation and inflorescence growth.

Plants of *Amaranthus caudatus* L., a short-day species (*3*), were grown in greenhouse soil to the age of 6 weeks under long photoperiods to maintain them in vegetative condition and, after thorough root washing, were transplanted to especially constructed, trough-shaped boxes (Fig. 1) in such fashion that the root systems of the plants developed in a plane between the glass wall of the box and a sheet of finely woven glass fabric. Each trough was filled with a mixture of Vermiculite (two thirds) and peat moss (one third), and the mixture was watered daily throughout the experimental period with a complete three-salt nutrient solution with added micronutrients. The boxes were constructed in such fashion that opaque slides could be inserted against the glass sides; through manipulation of the slides it was possible to