The Two-Wavelength Method of Microspectrophotometry

II. A Set of Tables to Facilitate the Calculations*[‡]

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(Received for publication, October 29, 1957)

ABSTRACT

The calculations required for two-wavelength measurements are time consuming and laborious. In order to circumvent this limitation of the method, a set of tables which combined four operations into one has been designed and is reproduced within. The tables are based on Patau's formulae. The two transmission readings obtained according to the photometric method provide the coordinates which lead directly to a value for the relative absorbance. The product of this absorbance and the area of the photometric field gives the relative amount of chromophore in the field. The range of transmission values covered in the table corresponds to the effective range of the two-wavelength method.

In spite of the fact that Patau (1) greatly simplified the calculations for the two-wavelength method, they are still very laborious, and take at least as long as the measurements. Because the operations involved are a mixture of division, subtraction, multiplication, and reference to a table, they are not prone to further simplification. After casting about for a suitable method, it was decided to construct a set of tables which would combine as many of the operations as possible. It was realized that the tables would be cumbersome and would take considerable time to prepare; however, they have long since repaid the effort put in to them, and they can now be offered to others using the two-wavelength method.

Patau's formulation of the two-wavelength method can be stated in the following equations:

$$m_t = \frac{BL_a C}{k_a \ln 10}$$

$$C = \frac{1}{2 - Q} \ln \frac{1}{Q - 1}$$
$$Q = \frac{L_b}{L_a} = \frac{1 - T_b}{1 - T_a}.$$

B is the area of the photometric field, m_t is the total amount of chromophore, *T* is the transmission, and *k* is the absorptivity. Patau has supplied a table giving *C* in terms of *Q*. The subscripts *a* and *b* refer to two wavelengths such that $k_b = 2 k_a$.

In practice, one determines two transmission values for a field of a specified area. Each of these transmissions is subtracted from one; then the ratio Q is determined. C is obtained from Q using Patau's table, and the relative amount of chromophore is calculated from BL_aC . The new tables have been based on Patau's calculations and have been designed so that L_aC is obtained directly from the two transmissions.

The tables were constructed by calculating the product L_aC for every pair of two-digit transmissions likely to be met in practice. The intermediate values, corresponding to even-numbered three-digit transmissions, were estimated by interpolation; they are correct to within ± 0.2 per cent. The arrangement of the tables, and the intervals between the numbers are such that the additional interpolation necessary for the various combinations involving odd-numbered transmissions can

^{*} This work was carried out during the tenure of a British-American Fellowship of the American Cancer Society, Inc., and a Fellowship from the Jane Coffin Childs Memorial Fund for Medical Research. Support was also obtained from the Sloan Fund.

[‡] Grateful acknowledgement is made to the S. S. Fels Fund for paying part of the cost of publishing the tables.

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J. BIOPHYSIC. AND BIOCHEM. CYTOL., 1958, Vol. 4, No. 4

be made with ease. In Table VIII, only two, instead of four, interpolated values are given for the vertical scale.

To use the tables, first find the transmission value T_b on the vertical scale, and then the value T_a on the horizontal. The number in the body of the table corresponding to these coordinates is $L_aC \times 10^3$. Thus, $T_b = 0.752$, $T_a = 0.826$, $L_aC =$ 0.259. The values for T_b cover a range from 0.320 to 0.880, and those for T_a extend from 0.480 to 0.930. Occasionally, values will be obtained which are outside the scope of the tables. This may be because of their magnitude, or because they have an unusual ratio to each other. Since the tables extend well beyond the effective range of the twowavelength method, such values should be considered with caution.

If it is at all possible to do a series of measurements with a constant field-size, the relative amount of chromophore can then be L_aC and no further calculations are necessary. Otherwise, L_aC must be multiplied by the area of the field used for each measurement.

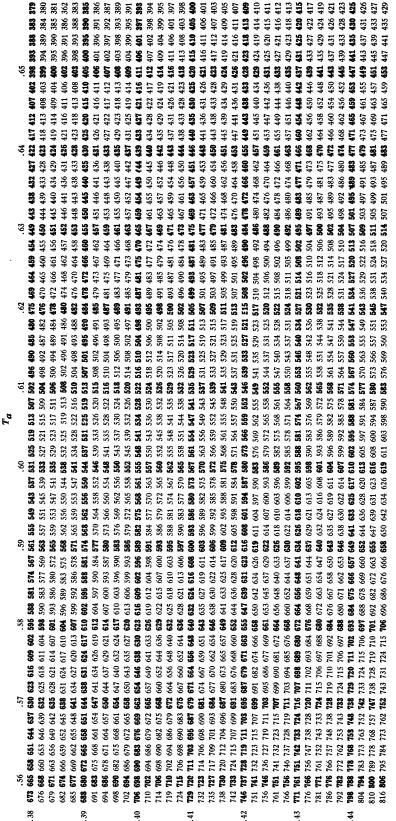
SUMMARY

A set of tables is provided which greatly facilitate the calculations associated with the twowavelength method of photometry.

Bibliography

1. Patau, K., Chromosoma, 1952, 5, 341.

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TABLE III

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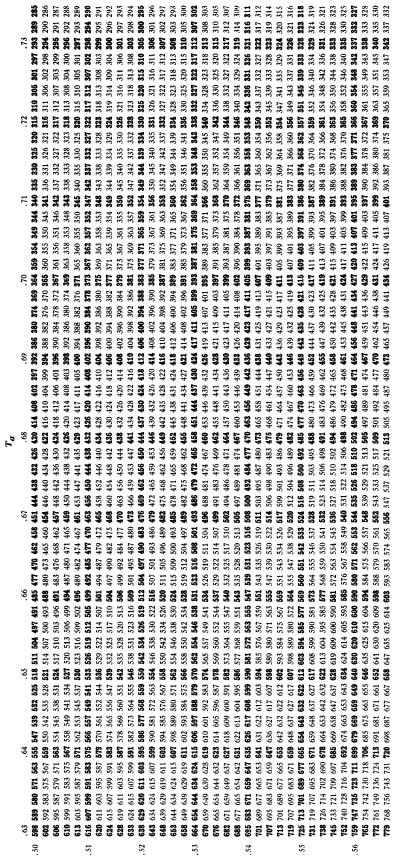


TABLE IV

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