Spectrophotometry in the Far Ultraviolet

Although the use of the photomultiplier as a detecting device has greatly increased the resolving power of the spectrophotometer, it has also magnified certain potential errors because of its sensitivity (*1*). Photomultiplier types vary in their response to light of different wavelengths. The R.C.A. 1P28 photomultiplier, commonly used in commercial instruments, has a spectral response of between 2000 and 7000 A, with a maximum at 3500 A. Consequently, it "sees" all of the light which is not absorbed by the chromophore—namely, (i) the stray light inherent in the monochromator (*2*) (the amount varies greatly with the design and condition of the instrument used and the nature of the light source); (ii) those extraneous or unwanted wavelengths that appear in the spectral band isolated, on either side of the central "monochromatic" wavelength measured (the amount varies with the width of the slit); (iii) any fluorescent light emitted by the chromophore; and (iv) the fluorescent light emitted by fused silica cells.

Since Beer's law is valid only when monochromatic light is used, spectrophotometric measurements become increasingly subject to error with decreasing wavelengths for the following reasons. In the far ultraviolet, not only does the angle of dispersion of the prism of the monochromator increase rapidly but also the brilliance of the usual light source and the sensitivity of the photomultiplier decline simultaneously (manifestations caused largely by their fused silica envelopes) with the result that the slit must be opened more rapidly than theory indicates to balance the photoelectric bridge circuit. Consequently, with decreasing wavelengths, the ratio of stray light to monochromatic light increases rapidly, and the spectral band isolated is no longer effectively monochromatic.

Attempts (*3*) have been made to correct for stray light in this region by measuring the unabsorbed light emerging from concentrated solutions from which all the monochromatic light presumably had been selectively absorbed by the chromophore. Such corrections are themselves subject not only to the errors which are inherent in measurements of optical density in concentrated solutions but also to those arising from the fact that the incident monochromatic light is never completely absorbed.

The ideal solution to the problem would be the complete elimination of the stray light inherent in the monochromator as well as that caused by excessively large slit widths. Since the far ultraviolet is a spectral region in which many biochemically important compounds show characteristic absorption, it seemed worth while to develop a technique for the accurate study of this property. Such a technique might serve as a means of identifying these compounds, give information concerning their structure, and provide a tool for following the enzymic reactions in which they participate.

The technical details of a spectrophotometer for use between 195 and 230 mμ have been described (*4*). The instrument was designed to minimize the abovementioned sources of error. Stray light of wavelengths longer than 280 mμ has been eliminated by the use of a "solar blind" photomultiplier (R.C.A. developmental type C7180) whose photocathode has a high photoelectric work function (*5*). Also, the slit width has been kept sufficiently small to give a satisfactory approximation of monochromatic light. This has been achieved by a proper balance between a brilliant light source (a Hanovia mercury-xenon arc, 250 w) and the photoelectric systems. The monochromator used was a Leiss single monochromator (*6*). Double monochromation in this system would serve only to reduce the optical efficiency of the instrument.

For work in the far ultraviolet, especially designed crystal quartz cells (*7*) were used for two reasons. First, the transmission of light in the far ultraviolet was demonstrated to be significantly greater with crystal quartz cells than with fused silica cells of the best available quality. With the solar blind photomultiplier and mercury-xenon arc, it was found that crystal quartz cells transmitted, at 195 mμ, 22 percent more energy than did fused silica cells; at 210 mμ, 4 percent more; at 220 mμ, 2 percent more; at 240 mμ, 6 percent more; and at 260 mμ, 1 percent more. Second, all of the many fused silica cells tested, and other samples of highly purified silica as well, showed a characteristic fluorescence which was not found in crystal quartz cells. The increased absorption observed at 240 mμ is caused by the excitation of fluorescence in silica, the maximum point of excitation being found at 244 mμ. The emission of this energy is said to be in the violet (*8*). This fluorescence will introduce an error into absorption spectra taken in fused silica cells when a photomultiplier employing either an S-5 or an S-13 surface is used.

Conformity with Beer's law was demonstrated when the optical densities of solutions of adenylic acid, observed at 210 mμ, were plotted as a function of concentration. Many absorption spectra of biochemically important compounds, including the nucleotides and the aromatic amino acids, have been taken; some reveal fine structure previously unobserved in this region (*9*). It is anticipated that the photoelectric technique described will eliminate the difficulties currently being found in following the course of enzymic reactions by observing difference spectra in the far ultraviolet in solutions of high optical density (*10*).

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References and Notes

1. A. H. Mehler, *Science* 120, 1043 (1954); I. Fridovich *et al.*, *ibid.* 125, 1141 (1957).
2. In instruments in which the light is doubly monochromated the amount of stray light is greatly reduced, at the sacrifice of optical efficiency, but is never completely eliminated.
3. L. J. Saidel, A. R. Goldfarb, W. B. Kalt, *Science* 113, 683 (1951).
4. R. E. Hansen and M. V. Buell, in preparation.
5. For other regions in the ultraviolet, suitable photosurfaces are described by V. K. Zworykin and E. G. Ramberg [*Photoelectricity and its Applications* (Wiley, New York, 1950), p. 99].
6. Purchased from Carl Leiss, Berlin, Germany, through the agency of the Photovolt Corp. This instrument employs a Littrow prism, together with two parabolic mirrors for monochromating and collimating the light.
7. Made by the Crystal Optics Co., 4319 N. Lincoln Ave., Chicago, Ill.
8. P. Pringsheim, *Fluorescence and Phosphorescence* (Interscience, New York, 1949), p. 507.
9. A paper on these spectra is in preparation.
10. We are indebted to Howard H. Hess, optical engineer for the Crystal Optics Company, for advice on optical problems and to K. G. Benford, electronics engineer of the Research Institute, University of Chicago, for advice on electronic problems. This work was supported by a grant from the National Institutes of Health [A-646(C)].

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Cultivation of Adult Mouse Mammary Gland in Hormone-Enriched Synthetic Medium

As a part of our laboratory research program that is concerned with the factors responsible for the induction, maintenance, and neoplastic transformation of hyperplastic nodules in the mammary gland of mice of the C3H/He CRGL strain, we have been attempting the cultivation *in vitro* of tissues from various growth stages of the adult gland, normal and abnormal. Adult mouse mammary epithelium generally has been difficult to grow *in vitro* (*1*); however, Lasfargues (*2*) has recently obtained successful growth in tissue culture. Moreover, Hardy (*3*) observed some mammary duct growth but no alveolar development in organ cultures of ventral body wall from embryonic mice. This report describes the successful maintenance *in vitro* of adult mammary gland in synthetic medium with added purified hormones.

The Chen adaptation (*4*) to liquid medium of the organ culture method of Fell (*5*) was employed, and the synthetic culture medium "199" (*6*) was used. The cultures were incubated at 37°C, generally for 5 days, with the *p*H of the medium remaining at about 8.4. The hormones used were estrone, progesterone, cortisol, growth hormone, and