Kuttner-Cohen method for this analysis in the Bodansky procedure. The incubation procedures were according to directions. All analyses were made in triplicate.

Table I shows the results of these comparisons, the inorganic phosphate determinations being omitted for lack of space. It was evident, however, that the inorganic phosphate was estimated with essentially equal accuracy by the 2 methods, as judged by a comparison of the calculated values with those determined in the various mixtures of plasma; the mean percentage error was 0.75% for the Fiske-Subbarow method and 1.23% for the Kuttner-Cohen method. The results of this study also fail to show any superiority of the Bodansky method over the Jenner-Kay method for plasma phosphatase so far as accuracy of calculated vs. determined values is concerned. The range of errors for the Bodansky method was from 0.10 to 16.53% with a mean error of 4.36%, while the range of errors for the Jenner-Kay method was 0.80 to 13.76%, with a mean of 5.04%. The choice of method seems to be one of personal preference rather than significantly greater accuracy when applied in routine analysis. When only a few samples are to be analyzed the shorter incubation period of the Bodansky method is an advantage. For a larger series the longer incubation period of the Jenner-Kay method is definitely advantageous in routine work. Inasmuch as both methods are empirical and, in our judgment, equally accurate, it is unfortunate that there does not seem to be a more uniform ratio between the units arrived at by the methods. While the ratio of Jenner-Kay to Bodansky units is roughly 2:1 in our series, the range for individual samples is from 1.49:1 to 4.10:1.

7443 C

Fading of Methylene Blue-Acetone Solutions by Ultra-Violet Light.

WILHELM STENSTROM AND CARL E. NURNBERGER.

From the Laboratory of Biophysics, University Hospital, Minneapolis, Minn.

Recently Nurnberger and Arnow[1] found that solutions of methylene blue in water irradiated under a mercury-arc therapy lamp had their absorption spectra changed quantitatively when the rays from the lamp were filtered through quartz. If, however, the rays shorter than 270 millimicrons were absorbed by a suitable filter, then prac-

[1] Nurnberger, Carl E., and Arnow, L. Earle, *J. Phys. Chem.*, 1934, **38**, 71.

tically no change in the absorption spectrum, either in the visible or ultra-violet, occurred for exposures 2 hours in length. It was, therefore, concluded that the fading of methylene blue in water is not a satisfactory indication of ultra-violet ray intensity in general.

Leonard Hill[2] and Webster, Hill and Eidinow[3] used the fading of methylene blue in acetone solutions when exposed to ultra-violet light to measure the intensity of the "physiologically active" part of the spectrum of sun light. Hill found his solutions to be most sensitive to wave lengths shorter than 320 mμ. In this respect, methylene blue in acetone and methylene blue in water behave alike, though it seemed indicated that the sensitivity of the former solution extended to longer rays.

Accurate intensity measurements require knowledge about the response of the indicator to the different wave lengths of the light used. The usual and essential procedure in the use of bolometers, thermopiles, photoelectric cells and other instruments used to measure intensity of radiation is to determine beforehand their sensitivity to different spectral regions. The same care, however, is not commonly observed when colored solutions are used, and therefore, the results are only approximate and are unsatisfactory for ultra-violet therapy. Colored solution indicators with known spectral sensitivity have, however, some advantages over the instruments mentioned. Particularly, the simplicity of the experimental apparatus gives to the method a more extensive usage than can be had with more costly equipment.

A convenient method for the determination of the color fading results from the characteristic absorption of visible rays. Methylene blue in aqueous solutions has absorption bands with a maximum at 670 mμ.[4] A measure of the amount of light absorbed at this peak can be used to calculate the quantity of methylene blue changed by the ultra-violet irradiation. These measurements are commonly made by means of a spectrometer in combination with some type of photometer. We used a Bausch and Lomb constant-diviation spectrometer and a polarization photometer of the Konig-Martin type.

The irradiated solutions were made from a 0.1 mg. per cc. standard solution of Merck's medicinal methylene blue in distilled water. Solutions of 3 different concentrations were used. Two of them consisted of 5.8 cc. and 58.0 cc. respectively, of the standard plus

[2] Hill, Leonard, *Strahlentherapie*, 1929, **34**, 117.

[3] Webster, A., Hill, Leonard, and Eidinow, A., *Lancet*, 1924, **206**, 745.

[4] Stenstrom, W., and Lohmann, A., *Radiology*, 1931, **16**, 322.

30 cc. of acetone added to distilled water to make 100 cc. of solution. The third consisted of 5.8 cc. of the standard added to distilled water to make 100 cc. of solution. The solution which contained 58.0 cc. of the standard had nearly the same concentration of methylene blue and of acetone as Hill's solutions (loc. cit.). The solutions were kept in the dark all of the time except when samples for irradiation were taken.

The chamber used to hold the solutions during irradiation was a cylindrical quartz vessel, 1.3 cm. high and 4 cm. outside diameter. The plane parallel ends were 2 mm. thick. The solutions were exposed from one of these ends. The liquid was poured in through a small quartz tube sealed to one side. The quartz chamber was immersed in a water bath to keep the solution temperature approximately constant. The water in the bath just reached the top face of the cell. A short rubber capsule was fitted tightly over the open end of the side tube to prevent evaporization and to keep the bath water from entering the chamber.

The solutions were irradiated with different parts of the spectrum of a mercury arc therapy lamp, which operated on 65 volts and 4 amperes d. c. Various glass filters were placed directly over the quartz chamber. The transmission of the filters: quartz, blue-purple Corex "A", clear Corex,* clear Corex "D" and window glass was measured by means of a quartz spectrograph and a recording

TABLE I.

% of Methylene Blue Faded by Rays from Different Parts of the Ultra-violet Spectrum.

.0058 mg. per cc. in water and 30% acetone.

Solution Temp.	Distance Inch.	Min. Exposure	Filters plus 2 mm. of quartz	10% or less Transmission	% Faded
			mm.	mμ	
26.6 C°	12	19	0	230	69.9
26.9	12	19	5 Blue-purple Corex "A"	258	28.2
27.2	12	21	6 Clear Corex	265	14.0
26.8	12	19	4 Clear " "D"	290	1.2
27.0	12	19	2 window glass	321	0.0
			.0058 mg. per cc. in water		
27.5	12	165	0	230	51.4
27.8	12	165	5 Blue-purple Corex "A"	258	5.8
28.0	12	165	6 Clear Corex	265	0.0
29.2	12	165	4 Clear " "D"	290	0.0
29.0	12	165	2 window glass	321	0.0
			.058 mg. per cc. in water and 30% acetone.		
28.5	12	160	0	230	92.9
29.0	12	387	5 Blue-purple Corex "A"	258	90.0
29.0	12	722	6 Clear Corex	265	93.6

* This piece of clear Corex was obtained from the Department of Physiological Chemistry, University of Minnesota. Its exact classification is not known, but it is probably an old piece of clear Corex "A."

micro-photometer. Their transmission thus obtained is in good agreement with the values commonly recited in the literature.

The results of a group of experiments are recorded in Table I. In column 5 are listed the maximum wavelengths of the ultra-violet spectrum which are transmitted by the filters by no more than 10%. For example, the combination, quartz and window glass, will transmit no more than 10% of the radiant energy of wavelengths shorter than 321 mμ. Two points of interest are demonstrated by the tabular values. First, the per cent of methylene blue in acetone changed,

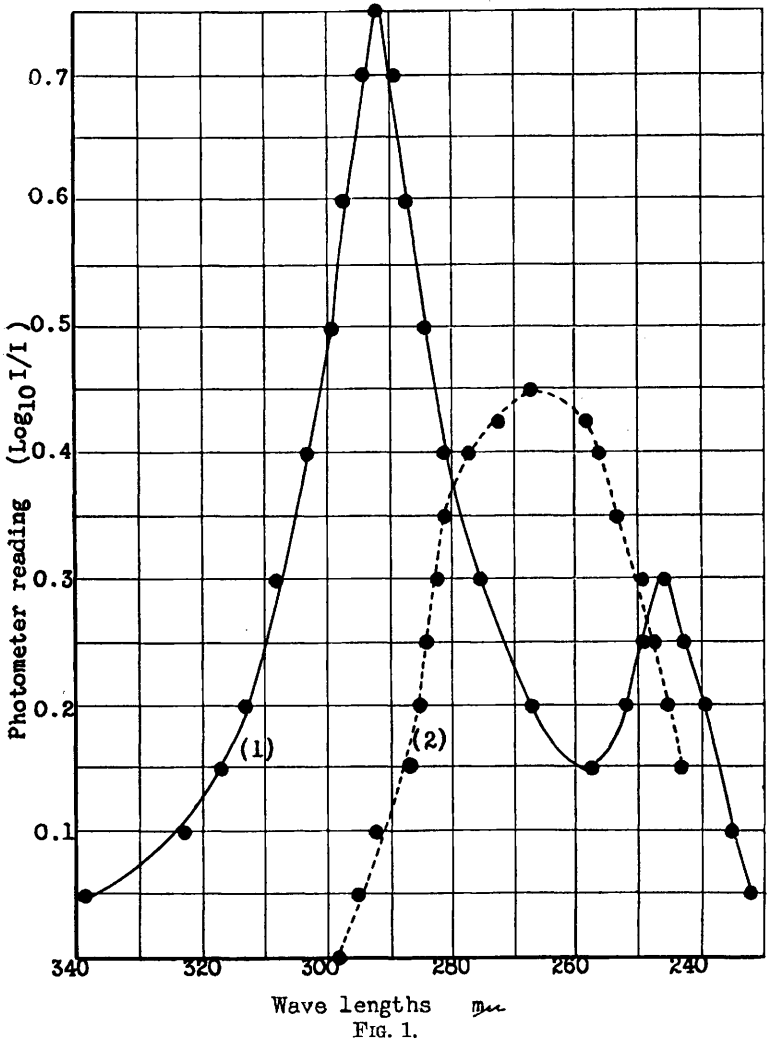

FIG. 1.

Absorption spectra of (1) methylene blue in water and (2) 0.2% acetone in water.

as determined by its absorption of visible light decreases rapidly when waves shorter than 290 mμ are absorbed by the filters. Second, the presence of acetone increases the effectiveness of wavelengths between 250 and 300 mμ to change the methylene blue. This apparent increase of sensitiveness of methylene blue to these rays when in the presence of acetone may be expected from an analysis of the absorption spectrum of the substances. Their absorption of ultra-violet light is shown in Fig. 1.† The pronounced absorption of acetone with a maximum at 267 mμ may result in the transfer of the absorbed energy from the acetone molecules to those of methylene blue, and in this manner increase the fading of the solution. The absorption of methylene blue around 290 mμ does not seem to contribute to the fading. It has been suggested by other investigators that acetone is decomposed into acetic and formic acids and that these acids produce the color change. This assumption, however, is not supported by the results of the following experiments: First acetic and formic acids were added to aqueous methylene blue and fading occurred promptly. Then acetone was irradiated under the conditions described previously and added to aqueous methylene blue. No more fading occurred than was expected by dilution alone. Therefore, it would seem that both acetone and methylene blue have to be present in the same solution at the time of irradiation in order to produce significant increases in the color change. It is of interest to note here that when a sample of .0058 mg. per cc. of methylene blue in 30% acetone was irradiated with X-rays practically no color change occurred. Irradiation of the same concentration in water without acetone under the same exposure conditions resulted in an almost colorless solution.

Ultra-violet rays longer than 290 mμ have an almost negligible effect as compared to rays shorter than 260 mμ. The "physiologically active" rays, usually designated as the region between 310 and 290 mμ, therefore, have an insignificant part in the fading of methylene blue by the light from a mercury arc lamp. Thus, a scale of skin erythemas in terms of units of color change of the solution would be subject to wide fluctuations. Since there is a skin erythemal effectiveness of wavelengths shorter than 270 mμ,[5, 6] any regular relationship between erythemal production and methylene blue fading is more likely to occur in the shorter wavelength

† The acetone curve agrees qualitatively with that of Victor Henri.

[5] Hausser, K. W., *Strahlentherapie*, 1928, **28**, 25.

[6] Luekiesh, M., Holladay, L. L., and Taylor, A. H., *J. Opt. Soc. Am.*, 1930, **20**, 423.

region. It might be possible to establish some kind of scale between visible skin reactions and solution color changes by use of a filter which would absorb rays below 280 mμ.

In the course of the work it was noted that solutions of methylene blue in acetone were not only faded by ultra-violet light but also were subjected to a color change from blue to green to a pale yellow. This series of colors became more apparent as the initial concentration of methylene blue was increased. The color change reversed when the solutions were removed from the ultra-violet light. If the green color predominated at the end of an exposure then the reverse change was rapid. Consequently, the solutions had to be transferred quickly from the irradiation chamber to the photometer cell and the match points read immediately. The yellow colored solutions, however, required several days to change from yellow to green to blue. The peculiar behavior of the color adds to the uncertainty of intensity determinations by this method. It is particularly true when fading is estimated in the simplest way; that is, when a comparison of the color intensity of the irradiated solution with a set of standard solutions of different concentrations is made without the help of any measuring instrument.

Summary. 1. The quantity of methylene blue in acetone solutions faded by ultra-violet from a mercury arc lamp decreases when the shorter wavelengths are absorbed by filters. 2. Addition of acetone to aqueous methylene blue solutions increases the effectiveness of the rays up to 300 mμ. 3. An aqueous solution of methylene blue and 30% acetone is not suitable for measurements of physiologically active ultra-violet radiation from an arc unless a suitable filter is used which reduces the intensity below 280 mμ. 4. Methylene blue acetone solutions undergo a color change from blue to green to yellow which reverses direction when the solutions are removed from the irradiation beam.