the purpose by the Rockefeller Foundation. The index of Volume 2 will be mailed in the fall, and the others will follow as rapidly as the necessary editorial work can be accomplished. With the indexes of the earlier volumes completed, each subsequent index should be available reasonably promptly after completion of the corresponding volume. Specific announcements will be made from time to time in current numbers of *Biological Abstracts*.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF COLOR FILTERS IN COLORIMETRIC ANALYSIS

IT is a common difficulty in colorimetric work to encounter conditions in which a color match between a solution of an unknown and one of a pure substance is very imperfect. Although in recent years more and more use has been made of limiting the portion of the visible spectrum used for this work, it is probable that not enough workers realize the advantages to be gained by such limitations or the ease of their application. Among recent methods utilizing selected portions of the spectrum can be mentioned those described in the articles of Kennedy, Flesche and McClendon, Gregory and Pascoe, Folin and Malmros, Cavett and Armstrong.

Kennedy[1] made a careful spectrophotometric study of blood solutions, and on the basis of the study advocated the use of a light filter for the determination of hemoglobin as carbonyl hemoglobin, using a "neutral grey" filter as a standard. Flesche and McClendon[2] used the same light filter, but employed the Newcomer glass as a standard.

Gregory and Pascoe[3] made a careful spectrophotometric study of the color produced by the reaction of bile salts with furfural in sulfuric acid of a definite concentration, and determined the most marked absorption band of the resulting product. They then obtained light of the same wave-length as that absorbed in this band by means of a neon lamp and appropriate color filters.

The above methods employing light approximating monochromatic represent undoubtedly the best method for obtaining a color match between two colored solutions, since no other substance with absorption in other regions can have any effect on the color match obtainable. However, a spectrophotometric analysis of the colors produced by a given reaction requires special equipment not available to all research workers, and certainly not available to many clinical laboratories. As will be shown, excellent results can be frequently obtained without the use of a laborious technique.

Bausch and Lomb was unable to produce a glass that would give the same spectrum as does acid hematin. A divergence of the curves given by the glass and by the solution was encountered in the longer wave-lengths. A good match was obtained by blocking out the longer lengths with a blue glass filter. Armstrong[4] found that use of this same blue glass allowed a good match of colors in his method for the determination of fluorine. In these cases, while careful studies were made of the absorption spectra involved, it was not found necessary to use monochromatic light, the mere shutting out of one end of the spectrum being sufficient restriction.

Folin and Malmros[5] were troubled by contamination of the blue color of colloidal ferric ferrocyanide by varying amounts of potassium ferricyanide. The latter substance absorbs rays at the short end of the spectrum, while the Prussian blue absorbs the longer rays. The authors mentioned found by trial that they obtained a close match of the two fields when the amount of light of short wave-length was cut out or decreased by transmission through yellow paper. In this laboratory, when their method is used it is found more convenient to block out violet and blue rays by passage of the light through a jar of potassium ferricyanide solution or of picric acid solution.

Recently workers in this laboratory have adopted the following simple procedure when difficulty is encountered in securing a color match between an unknown mixture, as blood or urine, and a standard pure solution. Results have been excellent. Cavett[6] employed this method, finding that a color filter gave a much better match between standard and unknown in Hanke and Koessler's analysis for histidine. Other workers are now using filters in procedures to be described in future papers.

Carry out the color reaction with a fairly strong pure solution on the substance for which analysis is to be made. Pour the resulting solution in a flat-

[1] R. P. Kennedy, *Am. J. Physiol.*, 78: 56, 1926; *ibid.*, 79: 346, 1927.

[2] B. A. Flesche and J. F. McClendon, *Soc. Exp. Biol. Med.* 25: 791, 1929.

[3] R. L. Gregory and T. A. Pascoe, *J. Biol. Chem.*, 83: 35, 1929.

[4] W. D. Armstrong, *Soc. Exp. Biol. Med.*, 29: 414, 1932.

[5] O. Folin and H. Malmros, *J. Biol. Chem.*, 83: 115, 1929.

[6] J. W. Cavett, *J. Biol. Chem.*, 95: 335, 1932.

sided vessel, and examine its absorption spectrum by means of a simple hand spectroscope, using the same light source later to be used for the colorimeter. Dilute the solution, and inspect again. Note the region of the spectrum in which the maximum change of absorption occurs. Next examine, with the same light source, several light filters or solutions of various chemicals, until one is found which transmits light in the region of absorption by the above solution, but which absorbs as completely as possible all other light. Superimposition of the filter and the strong standard solution should shut out practically all visible rays. If the color filter finally adopted is of glass or other solid material, it can be placed over the eyepiece of the colorimeter, cut to fit inside the ocular tube of the colorimeter, or placed over the source of light. If the color filter is a solution, it can be placed in a flat-sided glass jar, as a museum jar, which is then placed in front of the colorimeter, or can be placed in accessory cups, of equal depth, one of which is placed under each colorimeter cup. A colored light source, as a neon lamp, may be used.

Where it is desired to block out the long end of the spectrum, a Bausch and Lomb blue hemoglobin filter can be used. An aqueous solution of picric acid blocks out the short end. These two superimposed allow practically no light to pass, except two faint bands in the green and in the yellow, respectively. A potassium chromate solution acts similarly to a picric acid solution. A solution of nickel sulphate blocks out both ends of the spectrum, and transmits the middle portion. If desired, a set of glass or gelatin filters may be purchased from an optical supply house, and will be found quite convenient. In many instances, however, they will be found not at all superior to the above-mentioned improvised filters, and ones similar to them, available in any laboratory.

R. H. HAMILTON, JR.

MEDICAL SCHOOL,
UNIVERSITY OF MINNESOTA

SPECIAL ARTICLES

PROBABILITY OF THE PRESENCE OF A SEX ANTAGONISTIC SUBSTANCE IN URINE

IT has been frequently observed in this laboratory that certain extracts of normal, as well as of pathological urines, when injected into immature animals, inhibit the development of sexual organs. The results first obtained were not very uniform, as the treatment was never extended beyond a week. By extending the injections longer we obtained in a series of experiments typical sex organ inhibition. One of such experiments of 32 days' duration will be described here in detail.

Six male and six female rats of about the same initial weight were placed on an artificial food mixture consisting of casein, starch and salts with the daily addition of 0.5 gm dried yeast, 2 drops of cod-liver oil, 1 gm of dried milk and 400 mg of lard. The animals were divided into three groups, controls and two groups receiving daily subcutaneous injections of two different urine extracts, representing 250 cc of urine daily. The extracts were prepared in the following way.

Mixed male and female urines[1] (non-acidified) were extracted for 48 hours in a continuous extractor with ether. The ethereal extracts were evaporated and the residue extracted with as many cc of water, that one cc of extract represented one liter of the original urine used. The second extract was prepared from the water-insoluble material, by dissolving the residue in a corresponding amount of peanut oil. The two extracts, prepared fresh every few days, showed the following general characteristics. The aqueous extracts were of a brown-yellow color and deposited on standing a brown oxidation pigment. They contained probably small amounts of the cortical suprarenal hormone and traces of female hormones. The oil-soluble extracts, on the other hand, contained no detectable amounts of andronin (male sex hormone), but relatively large amounts of theelin-like substances, which were, however, mostly insoluble in alkali. The animals were weighed every 4 days and the food intake determined every second day. The food intake was fair in all experimental groups and the growth of females was comparable in all the groups. These facts would practically eliminate the probability of toxic effects of the injections. After 32 injections the animals were sacrificed and the weight of testicles and seminal vesicles in the males, and of ovaries and uteri in females was determined in absolute figures, as well as per gm of body weight. The table below summarizes the experiment, the figures representing the average of two animals.

As contrasted with short experiments which show sometimes stimulation and sometimes inhibition of development of sex organs in males and a constant stimulation in females, the described typical experiment of longer duration leaves the males in the infantile stage, while a marked retardation is noticed in females. The difference in the degree of influence in the 2 sexes is tentatively explained by the compen-

[1] Our thanks are due to the authorities and the head nurse of Hôpital Stell, Fondation Edward Tuck, in Rueil-Malmaison for supplying us with urines of patients for our investigations.