The concentration of drug in the blood was determined by Marshall's method[4] in a number of animals of Groups A and B. The values varied from 1.2 mg % to 3.6 mg % of the free drug calculated as sulfanilamide. Those animals which exhibited higher blood levels appeared to develop as extensive disease as those which exhibited low levels. Calculi were found in the kidney pelves of several of the animals in Group B and in the ureters of one of these animals. No renal calculi were found in any of the animals treated with N'-dodecanoylsulfanilamide, or in the controls.

Conclusions. N'-Dodecanoylsulfanilamide and sulfapyridine administered in large doses failed to exert any demonstrable inhibitory effect on experimental tuberculosis in rabbits infected with a bovine strain of tubercle bacilli.

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Electrophotometric Determination of Phosphorus by the Method of Fiske and Subbarow.

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The substitution of the filter photometer for the visual matching of solutions directs attention forcibly to the fact that many of the reactions employed in quantitative colorimetric microanalysis yield colors that are highly variable with respect to their rates of intensification and fading. An attempt to carry out Bodansky's directions[1] for the determination of serum phosphatase activity with a filter photometer* demonstrated the procedure of Kuttner and Lichtenstein[3] for the determination of phosphorus to be particularly troublesome in this respect. The essay was made during an excessively hot and humid period of the summer when the daily fluctuations of

[4] Marshall, E. K., Jr., *J. Biol. Chem.*, 1937, **122**, 263.

[1] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197; 1933, **101**, 93; 1937, **120**, 167.

* The instrument was constructed in this laboratory. It employs a Lange selenium photocell, type S 50.[2] The absorption tubes are matched test tubes, 20 x 175 mm. The source of light is an automobile headlight bulb; a current of approximately 1.6 amperes is drawn from a storage battery that is under a continuous trickle charge. A Lange "Multiflex" galvanometer is used.

[2] Lange, B., translated by St. John, A., *Photoelements and Their Application*, New York, Reinhold Publishing Corporation, 1938.

[3] Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1932, **95**, 661.

temperature sometimes amounted to 10°C. Under these circumstances it was found impossible to reproduce with satisfactory accuracy from day to day the standard curves expressing the relation of the phosphorus concentration of the solutions to their absorption of light. The difficulty manifested itself in the irregularity of the values that were obtained for K_1, the proportionality constant in the familiar equation, $C = 2 - \log G/K_1$, where C is the concentration of phosphorus; 2, the logarithm of 100, representing complete transmission of light; and G, the reading of the galvanometer. Efforts to modify the influence of temperature by icing the solutions before mixing were defeated by the condensation of a film of moisture on the absorption tubes.

The replacement of stannous chloride, the reducing agent in the Kuttner and Lichtenstein method, by 1-amino-2-naphthol-4-sulfonic acid, as recommended by Fiske and Subbarow,[4] led to more reproducible results: the effect of fluctuations of temperature was comparatively inconspicuous. However, the blue color did not attain maximum intensity within a short time, as we had hoped, but gained in intensity at a diminishing rate for as long as 72 hours, the limit to which we continued our observations. Hence, in order to attain the required degree of consistency it was necessary to make the measurements of absorption of light within a rather limited time interval selected arbitrarily with reference to the moment of mixing the solutions.

Considerable independence of timed readings and demonstrable increase of reproducibility was attained by recourse to a principle the application of which was suggested by Sunderman and Razek.[5] These investigators called attention to the fact that intensification or fading of a colored solution does not necessarily signify that the absorption of light in each and every region of the transmission spectrum of the solution is undergoing alteration. They demonstrated that with the passage of time the blue color that is developed in Benedict's sugar method[6] absorbs progressively more light of wave lengths between 400 and 580 millimicrons and progressively less in the region between 600 and 700 millimicrons; in a narrow zone bordering on 590 millimicrons there is no appreciable change with time, the degree of absorption in this region remaining constantly proportional to the concentration of sugar present in the solution.

Accordingly, we obtained serial curves representing the trans-

[4] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

[5] Sunderman, F. W., and Razek, J., *J. Biol. Chem.*, 1937, **118**, 397.

[6] Benedict, S. R., *J. Biol. Chem.*, 1931, **92**, 141.

mission of visible light by the Fiske and Subbarow blue color. The curves were traced at 15, 30, 75, 120, and 170 minutes after the solutions were prepared. The Hardy recording spectrophotometer was employed; each tracing required about 2 minutes to complete.† Comparison of these curves revealed (Fig. 1) that whereas a considerable increase of absorption of light occurred in the red region of the spectrum, the amount of absorption in a zone between 400 and 420 millimicrons in the violet portion of the spectrum remained appreciably more constant. On the basis of this information a combination of filters was selected that isolated a band extending in the visible spectrum from 400 to 430 millimicrons. The elements of the combination were obtained from the Corning Glass Company. The individual components were: Light Shade Aklo, No. 396,‡ thickness, 2.0 mm; Violet, No. 511, thickness, 3.0 mm; Red Purple Ultra, No. 597, thickness, 3.3 mm. The extended transmission

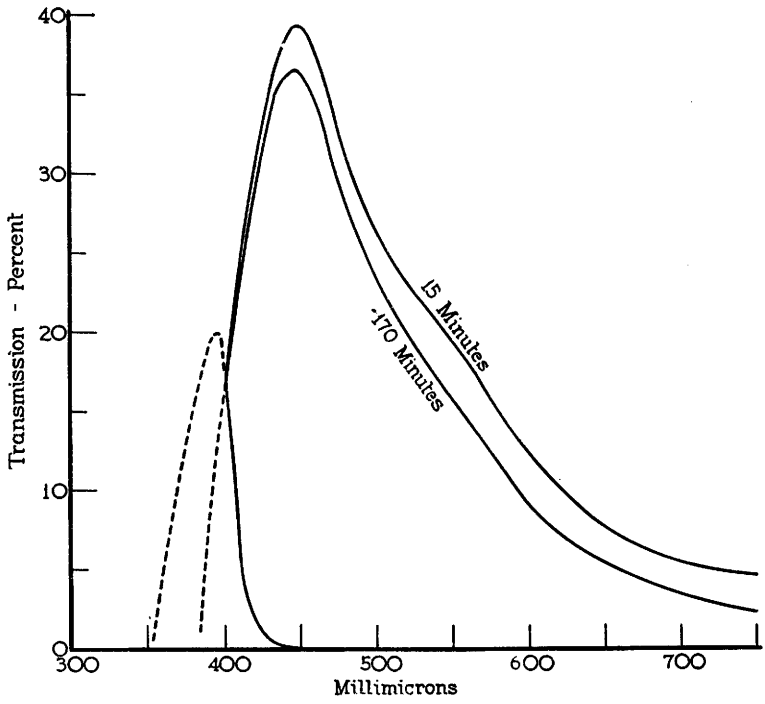

FIG. 1.

Per cent transmission in the visible spectrum (solid lines) and in the ultraviolet region (broken lines) by the Fiske and Subbarow blue color at 15 and 170 minutes after mixing the solutions, and by the filter pack.

† The Electrical Testing Laboratories, East End Avenue and 79th St., New York City.

‡ The numbers are the manufacturer's designation.

curves of the solution and the filter pack§ demonstrated that both media transmitted light in the ultraviolet region (Fig. 1). The extent to which transmission in this region changed with time was not investigated.

With these filters in place the solutions showed a slight but readily appreciable increase of intensity during 30 minutes after mixing but the change during the ensuing 60 minutes was negligible. Accordingly we have made it a practice to carry out the measurements of light absorption at any time within an hour, commencing 30 minutes after the solutions are prepared; this period may be extended to 3 hours without substantial loss of accuracy.

The absorption of light in the region isolated has been found to be such that even extremely pale solutions produce satisfactory deflection of the galvanometer. At the same time it is not so great that the concentration of phosphorus must be kept extremely low in order to avoid galvanometer readings that fall below the 10% mark on the scale. It requires about 700 μg per 100 cc of solution as introduced into the absorption tubes to yield this value. When plotted on semilogarithmic coördinates the regression line of galvanometer reading on concentration is so nearly linear throughout its entire extent that the regions between the 5 points that are determined in its preparation can be considered to be strictly linear without introducing significant error.

With these provisions, we have found it possible to make rapid and precise measurements of inorganic phosphorus by the method of Fiske and Subbarow with 0.2 cc of serum and of phosphatase activity by Bodansky's method with an additional 0.2 cc. Duplicate determinations require twice as much.

The precision of the technic is illustrated by the fact that the standard error of measurement (S.E.M) of the mean of 232 determinations of serum inorganic phosphorus in duplicate has been calculated to be 0.05 mg per 100 cc. This value was found to be consistent throughout a range that extended from 1 to 13 mg per 100 cc, indicating in approximate terms a 5% error at lowest concentrations, a 1% error at modal concentrations and an error of 0.5% at the higher concentrations. The S.E.M of the mean of 235 duplicate determinations of phosphatase varied from 0.2 Bodansky units in the range from 5 to 10 units to 0.6 units in the range from 40 to 90 units; thus the percent error varied between 3.2 and 0.9 as the amount of measured phosphatase activity increased.

§ Dr. Hugh Darby of the Department of Biochemistry kindly measured the transmission with a hydrogen discharge tube and a Spekker photometer in conjunction with a Hilger medium spectrograph.