Results of the kind here obtained might possibly be explained as due to a difference in hydrogen ion concentration of the dyes used. It seemed quite unlikely that this explanation was the correct one. The problem had already been studied and it had been found that by changing the hydrogen ion concentration of the agar used for divided gentian violet plates, the normal selective activity of this dye was in no way affected. If the pH of the agar were such that growth of the organisms was at all possible, the dye produced the expected selective result, no matter what the pH was.

In order, however, to check up this fact again, the experiments with *Bacillus X* and *B. anthracis* just described, were repeated, using—instead of the dyes—distilled water adjusted to hydrogen ion concentrations, corresponding to those of gentian violet, acid fuchsin (old Grübler) and acid fuchsin (new Grübler). These experiments were entirely negative, the fluids tested being without any effect on the growth of the organisms. The reverse selective bacteriostatic properties of these dyes are not, therefore, to be explained by variation in hydrogen ion concentration.

3043

Standardization of typhoid vaccine by photometric methods.

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Owing to an inquiry from Dr. Joseph W. Smith, Jr., of the Army Medical School, as to the suitability of the photomètre to estimate the strength of bacterial suspensions, a series of investigations were undertaken. Since the completion of this work, Dr. Smith[1] has published two articles on this general subject. From the result of our own experiments we are in accord with his critical statements in regard to the accuracy of the counts made by

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[1] Smith, Joseph W., Jr., *Am. J. Pub. Health*, May, 1925, 433; *J. Infect. Dis.*, 1925, xxxvii, 385.

the Wright's method in standardizing bacterial vaccines. Further, we would emphasize the error introduced in laboratories employing women, by their use of the normal erythrocyte count for men, rather than the actual erythrocyte count for the worker. Where the vaccine prepared is an autogenous one the errors are of lesser importance. However, with stock vaccines for administration to several individuals either for treatment or for immunization, the need of at least reasonable precision in standardization is generally recognized.

The present communication is based on the examination of ten bacterial suspensions made from different cultures of the typhoid bacillus, the strength of the suspension being estimated first, by the microscopic counting method of Wright on dry film, second, by the reading for the absorption of light in the photomètre, and third, by the reading for the diffusion of light in the same instrument. For the count, blood slides were made and counted, a dilution of 5,000 million organisms per 1 cc. of vaccine, prepared in accordance with the average of the counts, and later an additional check introduced, by counting the final 5,000 million dilution in similar manner. The stock solution was stored in a special bottle devised to prevent contamination, and permit easy removal of any desired amount, without opening the bottle. The photometer was the photomètre of Vernes, Bricq and Yvon, described in detail elsewhere[2] and devised by them for use in the Vernes flocculation test for syphilis. Curves were plotted from the resulting readings for the absorption and the diffusion of light. We observed a greater variation in the absorption than in the diffusion, pointing to the ability of the former more readily to detect any slight change, and therefore to be considered the more delicate test of the two. As the number of organisms decreased the readings became closer. These lower readings represent the amounts ordinarily used in vaccines, so it would appear that the chance of a significant error in photometric standardization is negligible. The complete paper will appear in the *Journal of Infectious Diseases.*

[2] Baylis, Adelaide B., Sheplar, Adele E., and MacNeal, Ward J., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 1-5.