organism (Tunnicliff coccus). In view of Hadley's[3] claim that certain cultures of microorganisms possess simultaneously, filtrable and non-filtrable forms, it was considered a possibility in the case of the Tunnicliff coccus but was unsupported by experimentation.

Special media have nothing to do with causing the Tunnicliff coccus to become filtrable or reinducing it subsequently to become non-filtrable. The interchangeable metamorphosis is determined by natural environmental factors (living tissue) on the one, and by artificial growth conditions on the other hand.

Finally Tunnicliff's coccus of measles is capable of existing in two states, filtrable *(in vivo)* and non-filtrable *(in vitro)*.

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Eosin-Light Injury of Erythrocytes. 1. The Influence of Certain Ions.*

ROY H. TURNER.

From the Department of Medicine, Tulane University of Louisiana, New Orleans.

To investigate the problem of photo sensitization in pellagrins, need has been felt for quantitative *in vitro* methods. The observations here described were made with the hope of aiding in the development of such methods.

As a sensitizing agent yellow eosin (National Analine and Chemical Company) has been used. The light source was a nitrogen-filled tungsten filament lamp of 1500 watts, operated at 115 volts by alternating current. The suspension of erythrocytes was contained throughout exposure to light and measurement of degree of hemolysis in standard Pyrex test tubes of 16 mm. outside diameter with walls 1.5 mm. thick. Equal exposure of each tube was insured by placing the tubes in a device to be described in detail elsewhere. This apparatus moved tubes in a circle 30 cm. in diameter about the light source at the approximate center, while the tubes were immersed in a constant temperature waterbath at 37.5° C. and received light through plane windows of plate glass 3 mm. thick. The layer of tap water between the tube and the plate glass was 2 mm. where the tube's circumference came closest to the window. Adequate provision for aeration and maintenance of suspension was

[3] Hadley, P., *J. Infect. Dis.*, 1927, **40**, 301.

* Aided by grants from the David Trautmann Schwartz Research Fund.

provided. Human blood from finger prick was allowed to fall into 0.85% NaCl solution made sufficiently alkaline to give a pink color with phenol red by the addition of 0.1 N NaOH and washed by centrifugation 3 times in specimens of the same solution. This cell suspension was added in 0.2 cc. quantities, measured from pipettes of the between-marks type into 5 cc. quantities of the various solutions which were under study. The final concentration of erythrocytes was about 15,000 per mm^3; it was kept close to this figure by adjusting the concentration of the heavy suspension as was indicated by photometer readings of the trial dilutions. All solutions were isotonic as determined by the hematocrit method.

In the present communication hemolysis is used as the criterion of erythrocyte injury. The degree of hemolysis was estimated by measuring the light transmission of the cell suspension. This principle was introduced by Ponder.[1] A photoelectric tube photometer, which measures light transmission of solutions and suspensions in 16x155 mm. test tubes, was used. This instrument will be described in detail elsewhere. Properly calibrated it has proved highly satisfactory for following the progress of hemolysis.

In order to determine the effect of hydrogen ion concentration upon eosin-light hemolysis, buffer solutions of mixtures of primary and secondary potassium phosphate were prepared containing 1-200,000 eosin. Fig. 1 shows the results of one experiment in which the exposure to light was for 40 minutes. It will be noted that within the ranges of hydrogen ion concentration studied there was a tendency for hemolysis to become less rapid as hydrogen ion concentration diminishes. Repetition of this experiment with different concentration of eosin and different time of exposure has given similar results.

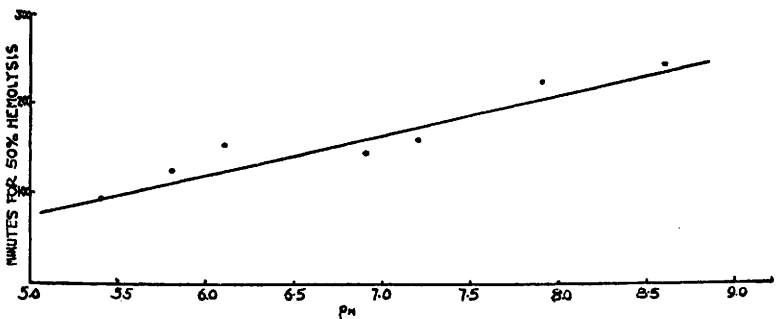

FIG. 1.

[1] Ponder, E., *Proc. Roy. Soc.*, 1923, **95**, 382.

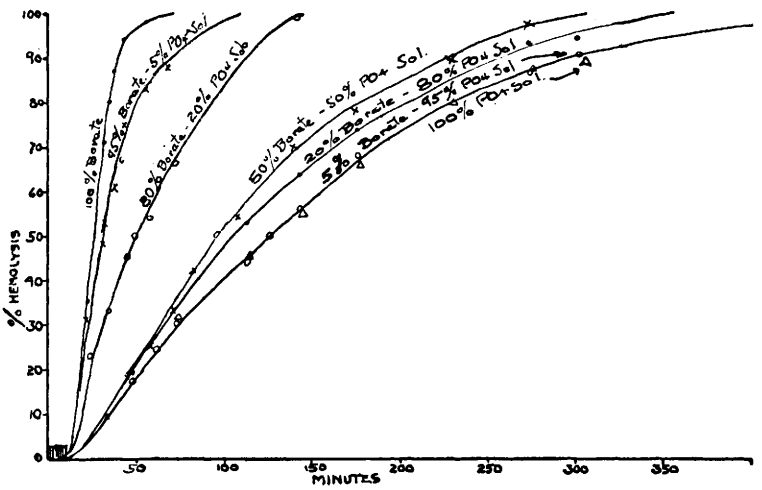

FIG. 2.

A buffer solution containing sodium borate and hydrochloric acid of pH 7.5 was prepared. Its effect on eosin-light hemolysis was compared with that of a potassium phosphate buffer of the same pH, each with an eosin concentration of 1-10,000. The influence on hemolysis of the 2 solutions and various mixtures of the 2 is shown for one experiment in Fig. 2. Borate served to accelerate and phosphate to retard hemolysis. The velocity of hemolysis was not demonstrably different in solutions in which the sole buffer salt was potassium phosphate than those containing 5 volumes per hundred of borate buffer solution. Had light dosage been sufficient to have caused more rapid hemolysis in these particular solutions a difference might have been demonstrated.

In another series of experiments each tube contained 2 cc. of sodium borate-HCl buffer, 3 cc. of solution of neutral salts and the usual 0.2 cc. of erythrocyte suspension. The borate solution had an eosin content sufficient to give a final concentration of 1-50,000. The results of one such experiment which may be accepted as typical are shown in part in Fig. 3. The neutral salts thus tested were: KCl, NaCl, and Na_2SO_4. Potassium phosphate was included in the forms of a buffer solution of pH 7.5. Solutions of $CaCl_2$, and $MgCl_2$ were mixed with solutions of NaCl so that the final concentration of calcium was 24 mg. per 100 cc. and magnesium 31 mg. per 100 cc. Hemolysis in the calcium and magnesium solutions was at a velocity not measurably different from that taking place when NaCl solution without either calcium or magnesium chloride was employed.

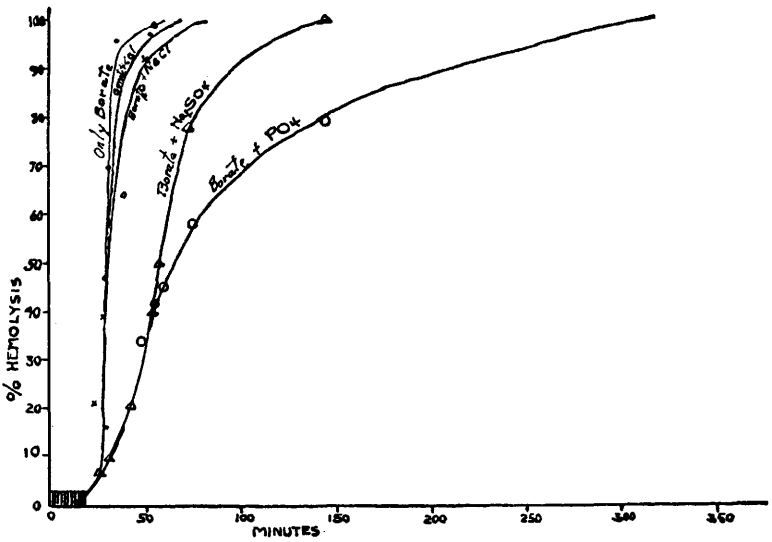

FIG. 3.

As control all suspensions in the various solutions studied containing eosin but unexposed to light were observed for hemolysis. In only one solution was hemolysis noted. That was in the phosphate buffer of pH 5.4. Proper correction in the velocity of hemolysis for the exposed specimen was made. Cell suspensions in the solutions under study containing no eosin were also exposed to light for the same duration as those containing eosin. In no instance was hemolysis observed.

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Infection of Kittens with Endamoeba histolytica by Direct Injection of Cultures into the Ileum.

HENRY E. MELENEY AND WILLIAM W. FRYE. (Introduced by E. C. Faust.)

From the Department of Preventive Medicine and Public Health, Vanderbilt University School of Medicine, and the Tennessee State Department of Public Health.

In the course of experiments to test the relative pathogenicity for kittens of a number of strains of *Endamoeba histolytica* in culture, rectal injection proved unsatisfactory because of the small percentage of kittens which became infected. The technique of rectal injection was simply to withhold food on the day of injection, and inject the sediment from a rich culture by means of a rubber-tipped