

quent tests were made on mice to determine whether loss in toxicity occurred during the Warburg runs. This was not the case.

Work on the effect of this toxin on glycolysis is now in progress.

Conclusions. Low concentrations of *Triturus* toxin had no effect on the respiratory rate of rat brain, kidney and liver slices *in vitro*. In the presence of 85 M.U. per ml in the case of liver, and of 850 M.U. per ml in the case of kidney and brain slices, slight transient stimulation of respiration occurred, but it is not clear that this could be attributed to the action of the toxin.

13342

Application of "Falling Drop" Measurement of Density to Quantitative Determination of Antibody in Immunological Reactions.*

W. J. NUNGESTER AND S. ROBERT LEWIS.

From the Hygienic Laboratory, University of Michigan, Ann Arbor, Michigan.

The serological methods for measuring antibody concentration are based on secondary reactions which are indirect in nature. The determination of the amount of protein precipitated by a specific antigen, a method first described by Wu and associates,¹ and since developed by Heidelberger and Kendall,² estimates directly the amount of antibody protein contained in a solution. The only serious disadvantage of this method is the time required.

It occurred to us that the rapid "falling drop" measurement of density for ascertaining protein concentration, as developed by Barbour and Hamilton,³ might be applied to the quantitative determination of antibody. The technic of this test has been adequately described, and no essential modification has been made by the authors. The most troublesome source of error was maintaining a sufficiently constant temperature in the water bath.

The antisera used in these experiments were freed of any suspended particles by centrifugation and the supernatant fluids used. The antigen, Type I pneumococcus polysaccharide prepared accord-

* These studies were aided by a grant from the Horace H. Rackham School of Graduate Studies.

¹ Wu, *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 853; 1929, **26**, 737.

² Heidelberger and Kendall, *J. Exp. Med.*, 1929, **50**, 809.

³ Barbour and Hamilton, *J. Biol. Chem.*, 1926, **69**, 625.

TABLE I.
Comparison of Falling Drop Rate of Antiserum and Precipitable Nitrogen, Following Reaction of Antiserum with Varying Amounts of Antigen.

Antiserum ml	Antigen, mg	Precipitable nitrogen, in mg*	Falling drop rate,† sec. per 20 cm	Ratio of falling drop rate to precipitable N
1.0	.00	.00	34.2	
1.0	.05	.28	36.6	127
1.0	.10	.32	38.6	121
1.0	.15	.33	39.4	120
1.0	.20	.33	39.3	120
1.0	.25	.33	39.4	120

*Estimated by difference.

†Average of 5 determinations on supernatant fluids after precipitates were removed by centrifugation.

ing to the method of Heidelberger, Kendall and Scherp,⁴ was dissolved in 0.85% saline. Dilutions in which 1.0 ml contained from 0 to 0.25 mg of polysaccharide were all adjusted to the same density by the addition of solid sodium chloride. Equal amounts of the antigen dilutions and antibody were mixed, and allowed to stand overnight in the icebox; the precipitates were removed by centrifugation and the supernatant fluids tested for nitrogen by the Kjeldahl method as modified by Pregle.⁵ The precipitable nitrogen was deter-

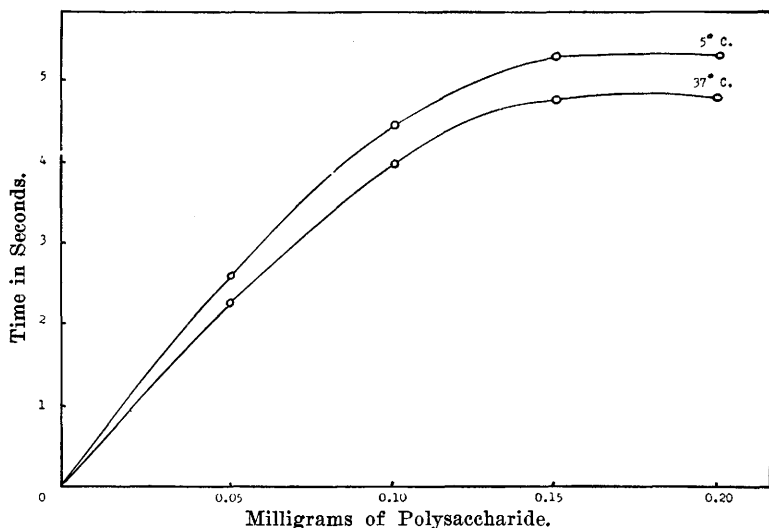


CHART 1.

Effect of Temperature of Incubation on Extent of Antigen-antibody Reaction, as Measured by Falling Drop Method.

⁴ Heidelberger, Kendall and Scherp, *J. Exp. Med.*, 1936, **64**, 559.

⁵ Pregle, *Quantitative Organic Micro Analysis*, Philadelphia, P. Blakiston's Son and Co., 1934.

mined by difference. Simultaneously, falling drop measurements were made to determine the densities of the supernatant fluids. The results of a typical experiment are given in Table I. The last column of this table shows the ratio of falling drop time to precipitable nitrogen. The constancy of this value indicates a close parallel between the data obtained by the two methods.

In the course of these studies, it was noted that the temperature at which the antigen-antibody system was incubated influenced the reaction as measured by the falling drop method. This is in accordance with Heidelberger's findings,⁶ and the results are plotted in Chart 1.

Summary. The falling drop method devised by Barbour and Hamilton for the determination of the concentration of protein solutions has been found applicable to the estimation of the antibody content of antipneumococcus serum. After the precipitate has been separated from the reacting mixture by centrifugation, the density of the supernatant fluid can be determined in less than one minute by this method.

13343

A Silver-glass Micro-electrode for Use in a Micromanipulator.

GEORGE P. FULTON. (Introduced by F. H. Pratt.)

From the Department of Biology, Boston University, Boston, Mass.

In an investigation of the neuro-motor mechanism of small blood vessels,¹ the author needed to make micro-electrodes for the stimulation of small nerve fibers. The non-polarizable type (micro-salt-bridge), described by Ettisch and Péterfi,² Taylor,³ Gelfan,⁴ and Field,⁵ was not necessary. Various polarizable types described in the literature were found to be inadequate. The platinum-quartz micro-electrode, devised by Taylor³ and used by Pratt and Reid⁶

⁶ Heidelberger, *Bact. Rev.*, 1939, **3**, 41.

¹ Fulton, G. P., and Lutz, B. R., *Science*, 1940, **92**, 223.

² Ettisch, G., and Péterfi, T., *Pflüger's Archives*, 1925, **208**, 454.

³ Taylor, C. V., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, **23**, 147.

⁴ Gelfan, S., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **23**, 308.

⁵ Field, M. E., *Skand. Arch. f. Physiol.*, 1935, **72**, 175.

⁶ Pratt, F. H., and Reid, M. A., *Science*, 1930, **72**, 431.