rate *in vitro* in the same way as it affects the metabolic rate of the intact animal and the factors which determine the metabolic level *in vivo* seem still to be present in the surviving tissues cut out of the organism.

13341 P

Effect of *Triturus* Toxin on Respiration of Rat Tissues *in vitro*.

FREDERICK A. FUHRMAN AND JOHN FIELD, 2D.
Department of Physiology, Stanford University.

Twitty and coworkers[1] have shown that a very active toxin is present in the yolk of *Triturus* ovarian eggs and embryos. Some of the chemical properties and physiological actions of this substance have been described by Horsburgh, Tatum and Hall.[2] Among other actions the toxin paralyzes motor and sensory nerve endings and evokes central respiratory paralysis in the anesthetized cat.

Since it appeared possible that the effects on nervous tissue might be attributable, in part at least, to depression of oxidative metabolism, we have investigated the effects of graded concentrations of the toxin on the respiration of rat brain (cerebral cortex) slices. For comparison, similar experiments were carried out on kidney cortex and liver slices from the same animals. Methods of preparation of tissue slices differed somewhat from those used previously[3] and will be described elsewhere. Oxygen consumption was measured in cmm, N.P.T., per mg wet weight per hour, by the Warburg manometric method. A relatively pure preparation of the toxin was kindly furnished us by Drs. van Wagtendonk and Tatum.

Dried powder containing the toxin was taken up in Ringer's-phosphate-glucose[3] and brought to pH 7.2. The final concentrations in the respirometer vessels ranged from 0.43 mouse units (M.U.) per ml[2] to 850 M.U. per ml. Eight adult male rats were used, and at least 2 determinations were made at each dosage level. The lowest

1 Twitty, V. C., and Elliott, H. A., *J. Exp. Zool.*, 1934, **68**, 247; Twitty, V. C., and Johnson, H. H., *Science*, 1934, **80**, 78; Twitty, V. C., *J. Exp. Zool.*, 1937, **76**, 67.

2 Horsburgh, D. B., Tatum, E. L., and Hall, V. E., *J. Pharm. and Exp. Therap.*, 1940, **68**, 284.

3 Crismon, J. M., and Field, John, 2d, *Am. J. Physiol.*, 1940, **130**, 231.

concentration was about 8 times the dosage required to kill a 20 g mouse in 10 minutes,[2] assuming uniform distribution in the body. The corresponding concentrations of pure toxin cannot be stated exactly, but there is evidence[4] suggesting that it ranged from about 0.006 to 1.3 mg per ml. The dried powder containing the toxin was free from proteins, peptids, fats, basic amino acids and alkaloids. Reducing sugar, minerals and some dialysable nitrogenous compounds, including neutral and acidic amino acids were present.[4]

It was found that the respiration of brain and kidney slices was affected only by the highest concentration used (850 M.U. per ml), while the respiration of liver slices was stimulated slightly with 85 M.U. per ml. These results are summarized in Table I, where it is shown that when any difference did appear between the control and experimental rates of oxygen consumption it was rather small and transient.

TABLE I.

Comparison of Respiratory Rates of Rat Brain, Kidney and Liver Slices in the Presence of High Concentrations of *Triturus* Toxin with That of Controls (Arbitrarily Taken as 100). Time Indicated is After Start of Run.

	850 M.U.		85 M.U.		"Inactivated"*	
Tissue	30 min.	120 min.	30 min.	120 min.	30 min.	120 min.
Brain	111	104	99	96	118	115
Kidney	134	107	100	97	108	84
Liver	174	113	119	128	108	60

*850 M.U. reduced to 100 M.U. by treatment with alkali. Brought to pH 7.2 before use.

It is not clear that this effect could be attributed to the toxin. Concentrations of 85 and 850 M.U. involved the presence of 1.33 to 13.3 mg of organic matter respectively, which might have served as additional substrate. When a solution of 850 M.U. per ml concentration was partially inactivated,[4] so that the strength was reduced to 100 M.U. per ml, there was no initial stimulation, but the respiratory rate decreased below the control levels after 120 minutes. This appears to indicate that more complex changes than simple inactivation of toxin occurred, and that the inactivated preparation was not an adequate control for testing the hypothesis that substrate rather than toxin was responsible for the initial rise in respiration with high doses.

In all cases pH measurements were made with a glass electrode before and after the runs, and little change in pH occurred. Fre-

[4] Tatum, E. L., and van Wagtendonk, W. G., unpublished.

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 antiserums 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.