TABLE II.

Titration of Diphtheria Toxin and Antitoxin on Hamsters.

Antitoxin in Standard Unit	Toxin in Guinea Pig L + Doses				
	1/8	1/7	1/6	1/5	1/4
1/8	D S	D D	D D	D D	D D
1/7	S S	D D	D D	D D	D D
1/6	S S	S S	D D	D D	D D
1/5	S S	S S	S S	D D	D D
1/4	S S	S S	S S	D D	D D

S—Living at the end of 3 days. D—Dead at the end of third day.

These experiments indicate that hamsters may be used for the titration of diphtheria toxin and antitoxin for preliminary tests when guinea pigs are not available.

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The Determination of Glycogen in Tissue.

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The method in general use for the determination of glycogen in tissue[1] involves the use of strong KOH to effect a separation of glycogen from proteins. Other methods[2] for treatment of tissue have not proved satisfactory.

A suitable method, however, has been found by treating boiling water extracts of tissue (liver only has been employed thus far) with trichloracetic acid. A filtrate from this mixture gives quantitative values for tissue glycogen. The use of KOH is rendered unnecessary and the procedure saves considerable time, particularly when amounts of tissue of more than a gram are involved.

Procedure: Tissue is weighed and plunged into boiling water for about 2 minutes when it is ground thoroughly and re-boiled. The

[1] Pflüger, E., *Arch. ges. Physiol.*, 1909, **129**, 362.

[2] Fränkel, S., *Arch. ges. Physiol.*, 1892, **52**, 125.

mixture is cooled and diluted to a known volume and after mixing it is centrifuged. The creamy supernatant fluid is removed (the bulk of the proteins are insoluble) and an aliquot part added to a definite volume of trichloracetic acid (we have employed an equal volume), allowed to stand 15 minutes and filtered. Trichloracetic acid of strengths varying from 2 to 5% has been found satisfactory but with 2% acid it has been necessary to refilter. A second filtration has in this case always given satisfactory solutions. To an aliquot part of the filtrate 3 volumes of alcohol and a pinch of N.Cl are added, and the mixture is allowed to stand, preferably overnight. The deposit obtained after centrifuging is a clean white precipitate and the alcoholic fluid is perfectly clear. The deposit is washed twice with 80% alcohol (containing NaCl), once with 95% alcohol, once with absolute alcohol and once with ether. The precipitate is dissolved in water and hydrolyzed. Three methods, Folin-Wu,[3] Folin-Wu,[4] and Hagedorn and Jensen[5] have given identical values for the glucose in the hydrolysates. The values obtained by this method have been compared with those given (a) after treating the 75% alcohol precipitate with 60% KOH and reprecipitating with alcohol, (b) after adding alcohol to the aqueous extract to make 75%, and proceeding as in (a), and (c) by treating a sample of the same tissue with alcohol, and then proceeding as in (a). Identical values for glycogen have been obtained.

The limits of the method have not yet been worked out, but satisfactory results are obtained when about 5 gm. of liver tissue are extracted with water and the volume made to 50 cc. The glycogen in this volume has varied from 150-200 mg.; that is, the original tissue has contained 3-4% of glycogen. It is desirable to keep the volume of the aqueous extract reasonably low to facilitate complete precipitation of glycogen subsequently.

[3] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[4] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.