

Titration of Diphtheria Toxin and Antitoxin on Hamsters.

FAN CHUAN AND C. E. LIM. (Introduced by Hsien Wu.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

The use of guinea pigs in the titration of diphtheria toxin and antitoxin, while being a standard method, is not without its drawbacks. The animal is not always procurable and its cost of purchase and maintenance is comparatively high. The Ramon precipitation method is simple and economical, but the results are not always quite satisfactory or conclusive. Furthermore, we are not without misgivings in the titration of a biological phenomenon by purely chemico-physical means, at least not without further understanding of the nature of their correlation.

In an attempt to discover a method of titration which can be applied when guinea pigs are not available, we find that the results obtained by standardizing diphtheria toxin and antitoxin on hamsters are comparable to those obtained by the use of guinea pigs.

Throughout the experiment hamsters, *Cricetulus griseus*, weighing from 25 to 30 gm. are used. They are easily obtainable in this part of the world and are quite susceptible to diphtheria toxin. The diphtheria toxin we use is from the National Epidemic Prevention Bureau, Peiping. Its M.L.D. for guinea pig is 0.005 cc. and its L + dose 0.22 cc. The antitoxin is obtained from the same source and checked by us against the standard unit provided by the U. S. Hygienic Laboratory, Washington, as containing 350 units per cc. They are diluted to the required concentrations with 0.9% saline. In case both toxin and antitoxin are injected into the same animal, they are always mixed *in vitro* and allowed to stand one hour before injection. The total amount injected is 1 cc. and all injections are given subcutaneously.

Three sets of experiments are performed. One sample test from each set is reported in this communication.

The first set of experiments is to determine the minimal lethal dose for hamsters in terms of the M.L.D for guinea pigs. The smallest dose that will kill a hamster at the end of 3 days is found to be equivalent to 1/6 of a guinea pig M.L.D. as shown in the tabulation below:

Dosage of Toxin		Days of Survival
1.6	M.L.D.	1
0.5	"	2
0.25	"	2
0.20	"	2½
0.18	"	2½
0.166 (1/6)	"	3
0.15	"	Survived
0.14	"	"

The second set is to titrate a sample of antitoxin against toxin of known potency. Various amounts of antitoxin are mixed with 1/6 of a L + dose before injection. It is found that the amount of antitoxin that will delay the death of the hamster till the end of the third day after injection is 7/42 or 1/6 unit as given in Table I. It hap-

TABLE I.
Titration of Diphtheria Antitoxin on Hamsters.

Antitoxin		Toxin		Days of Survival
Dilution	Equivalent in 0.5 cc.	Dilution	Equivalent in 0.5 cc.	
1: 750	7/30 units	1.1:15	1/6 L + dose	Survived
1: 900	7/36 "	"	"	11
1:1050	7/42 "	"	"	3
1:1200	7/48 "	"	"	2½
1:1350	7/54 "	"	"	2

pens, in this case, as we have already shown that the minimal lethal dose for a hamster is 1/6 of a guinea pig M.L.D. Therefore, if we take it as a basis, we can calculate the potency of the antitoxin easily. Here 0.5 cc. of the antitoxin diluted 1:1050 protects the hamster from death till the end of the third day. If that amount contains 1/6 L + dose, the undiluted antitoxin must contain 350 units per cc.

Similarly, toxin can be titrated by mixing various amounts of its L + dose with an equivalent amount of antitoxin unit. Thus in Table II the amount of toxin that kills the hamster at the end of 3 days in the presence of, say, 1/6 unit of antitoxin is 1/6 L + dose. If we take this as the point of neutralization, we can calculate the L + dose for guinea pig. Since 0.5 cc. of the toxin diluted 1.1:15 contains 1/6 L + dose, the L + dose must be 0.22 cc. of the original toxin. This composite table, based upon the result of a large number of tests, also shows that the hamsters respond quite consistently to the toxin antitoxin mixture of different proportions. From this tabulation, it is also evident that with a known unit of antitoxin the L + dose of the toxin can be titrated, and similarly, with a known L + dose the antitoxin unit can be determined.

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.

ALBERT CARRUTHERS. (Introduced by Hsien Wu.)

From the Department of Biochemistry, Peiping Union Medical College.

The method in general use for the determination of glycogen in tissue¹ involves the use of strong KOH to effect a separation of glycogen from proteins. Other methods² 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 trichloroacetic 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 Pfüger, E., *Arch. ges. Physiol.*, 1909, **129**, 362.

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