tervals for 5 weeks. A rabbit was given intradermally 0.2 cc. of identical dilutions of 2 vaccines stored under similar conditions. During a storage of 5 weeks in ice chest, the titer of the vaccines gradually dropped from 1:10,240 to 1:1,280. No difference in the potency of glycerol and saturated salt solution vaccines was noted. Titration of vaccines kept at room temperature showed that during a period of 5 weeks the titre of glycerol vaccine decreased from 1:10,240 to 1:160 and that of saturated salt solution to 1:320. This slight difference was considered insignificant, although somewhat more intense reactions resulted from the vaccine preserved in saturated sodium chloride solution.

Dysentery Shiga bacteriophage, titre $1x10^{-10}$, was treated with an excess of sodium chloride and with an untreated control, distributed into tubes, sealed with paraffine and placed at 37°C. Titration of both lots of phage was performed from time to time for 3 months, by photometric measurements of turbidity[3] of young cultures of *B. dysenteriae* Shiga in broth to which phage was added. At the end of 3 months the potency of control phage had fallen from $1x10^{-10}$ to $1x10^{-5}$, while that of the salt-preserved phage dropped only to $1x10^{-8}$. This difference indicates that sodium chloride has a definite value in preserving the potency of phage. Its favorable effect has been further demonstrated by determination of the incubation time (time interval between the addition of phage and onset of bacterial lysis), using dilutions varying from $1x10^{-4}$ to $1x10^{-10}$. At the end of 3 months the incubation time of any dilution used in case of phage preserved with sodium chloride was 16 minutes shorter than that of the control lot. This indicates that the potency of salt-preserved phage was 400 times higher than that of the control phage.

8364 C

Use of Chinese Hamster for Testing the Virulence of C. Diphtheriae.

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The use of animals for the determination of virulence of *C. diphtheriae* is often necessary in the study of cultures obtained from patients or suspects. Frequently it is also desirable to use animals in

[3] Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 488.

certain epidemiological studies, *e. g.,* in determining the incidence of carriers. To employ guinea pigs in large numbers is expensive. In order to find a cheaper laboratory animal, the Chinese hamster *Cricetulus griseus,* has been selected. Hamsters (field mice) are easily procurable in this part of the world and their susceptibility to diphtheria toxin has been reported from this laboratory.[1] The purpose of the studies here reported was to conduct comparative tests to determine the reactions of hamsters and of guinea pigs to cultures of *C. diphtheriae* recently isolated from patients. It is necessary to mention in this connection that as the hamsters are readily susceptible to pyogenic infections, pure cultures of diphtheria organisms are prerequisite in using these animals. Fortunately the use of simple tellurite blood agar plates[2] readily yields a pure culture of this group of organisms.

Preparation of bacterial emulsions. Isolated cultures were separated into 3 groups: (1) Pure cultures of typical *C. diphtheriae,* with bulging polar bodies; (2) cultures with only a few of the organisms carrying typical polar bodies, and (3) cultures of diphtheria-like organisms without polar bodies. The morphology of the organisms was determined in each instance before the cultures were injected into animals. Pure cultures were obtained by plating on 0.04% potassium tellurite blood agar plates and single typical colonies were picked. The culture was then grown on Loeffler's slant for 24 hours and the growth was emulsified in 2 cc. of normal saline. Of this emulsion 0.2 cc. was injected into guinea pigs and 0.1 cc. into hamsters.

Inoculation of guinea pigs. The usual intradermal technique was followed. A control animal received 1000 units of antitoxin 24 hours before, while the test animal received 250 units 4 hours after the injection of organisms. Five to 7 tests may be made on one large-sized guinea pig. Readings were made daily for 3 days. With the exceptions to be noted, frank necrosis, at the site of inoculation of the virulent cultures, was produced invariably whereas the control animals remained entirely normal.

Inoculation of hamsters. Hamsters weighing from 20 to 30 gm. were used. Control animals received intraperitoneal injections of 50 units of antitoxin 24 hours prior to the subcutaneous injection of cultures. The animals were kept in separate cages and were observed for 5 days. It was found that test animals injected with

[1] Fan, C., and Lim, C. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 226.

[2] Horgan, E. S., and Marshal, A., *J. Hyg.*, 1932, **51**, 441.

virulent cultures usually died within 50 hours, most of them before 40 hours. Autopsy of these hamsters revealed only adrenal congestion. Those that survived 5 days were discarded.

Results. One hundred and two different cultures recently isolated from patients were thus tested. The results are presented in Table I.

TABLE I.
Comparison of Results in Diphtheria Virulence Tests on Guinea Pigs and Hamsters.

Morphology	Virulence	Total No.	No. in agreement	No. not in agreement: In favor of guinea pig	No. not in agreement: In favor of hamster
Typical	Virulent	51	48	2	1
	Avirulent	9	9		
Atypical with few polar bodies	Virulent	6	6		
	Avirulent	13	12		1
Diphtheria-like organisms	Virulent	3	1	1	1
	Avirulent	20	19		1
Total		102	95	3	4

It can be seen from the table that the results obtained were fairly comparable. Two out of 3 discrepancies in which guinea pigs excelled were due to the death of the control and test hamsters. This might have been obviated if more than one set of animals had been used for each specimen. It may be noted that the use of guinea pigs was not entirely without error; in 2 instances, control pigs showed definite necrosis, in one, the test animal failed to react, and in a fourth, the test animal showed a reaction to a non-virulent culture. All these have been checked with the result that the guinea pigs agreed well in all 7 instances with the hamsters.

Discussion and Summary. The experiments demonstrate that hamsters react much in the same way as guinea pigs to cultures of *C. diphtheriae* or of diphtheriae-like organisms. There was complete agreement in 95 out of 102 instances. To use hamsters instead of guinea pigs possessed the advantage of giving clear-cut results because these animals invariably died from the virulent organisms and survived the non-virulent ones. Equivocal results from intradermal tests on guinea pigs due to secondary infection from field cultures, or to injections being made too deep into the skin, could thus be avoided.

Other advantages are that 10 hamsters may be purchased for the cost of one guinea pig, and that the control hamsters require much

less antitoxin. Objections may be raised in that pure cultures are necessary, thereby causing possible delay. This is only of theoretical interest, because usually it is only in doubtful and convalescent cases of diphtheria that this test is required, and a delay of one day does not limit its usefulness. Furthermore, one must remember that in making the conventional test on guinea pigs a delay of 24 hours is also necessary after the control guinea pig has received antitoxin.

As a result of these studies it has been shown that Chinese hamsters are suitable animals for the routine determination of the virulence of *C. diphtheriae*. The use of these animals instead of guinea pigs is very much less expensive and in certain respects gives more reliable information.

8365 P

Photodynamic Action of Methylene Blue on Diphtheria Toxin.

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The effect of photodynamic action of certain dyes on various substances, among which may be mentioned different types of animal cells, viruses[1, 2, 3] and soluble toxins of both bacterial[4] and animal origin,[5] is a well-known phenomenon. Workers generally agree as to the extreme susceptibility of these substances to the photodynamic action of dyes, the optimal concentration of the dye employed, and the relatively short time required for exposure, but disagree with regard to the antigenicity of substances so treated. The present study aims to investigate the reaction of diphtheria toxin to the photodynamic action of methylene blue upon which subject little study has been made. Diphtheria toxin is looked upon as a more suitable material than either tetanus toxin or viruses on account of its greater stability and ease with which quantities given can be accurately measured and properly controlled.

The technique employed is essentially similar to those of other workers.[1, 5] Methylene blue is selected since it has been more thor-

[1] Perdrau and Todd, *Proc. Roy. Soc. Bull.*, 1933, **112**, 277, 288.
[2] Perdrau and Todd, *J. Comp. Path. and Therap.*, 1933, **46**, 78.
[3] Shortt and Brooks, *Indian J. Med. Res.*, 1934, **21**, 581.
[4] Lippert, *J. Immunol.*, 1935, **28**, 193.
[5] Shortt and Mallick, *Indian Med. J. Res.*, 1935, **22**, 529.