type was not demonstrable in the nasal passages of adults and weanlings obtained directly from the commercial colony. Streptococci were certainly not present in the inocula which the mice received prior to the outbreak of lymphadenitis. Though direct evidence is lacking it may well be that the organisms were originally transmitted to the mice, by way of the nasal passages, from an infected attendant. What effect the preceding experimental treatment may have had on the mice is also uncertain. It is possible that they were more receptive to streptococcal infection than normal mice of the same age. The manifestations of the naturally acquired disease were fully reproduced, however, in the absence of the agents originally employed. Initial establishment of the streptococci in mice of different age groups was probably attended by a limited transmission by direct contact within each cage.

The experimental findings agreed essentially with the earlier results of Sonkin(2), and of Glaser, Berry, and Loeb(3) on the production of murine cervical lymphadenitis by hemolytic streptococci. There was a marked disparity, however, in the death rates resulting from the nasal deposition of the organisms. The susceptibility of the mice and the pathogenicity of the streptococci are variables that must be here considered. The present results were indicative of differences in the response of Princeton and unselected Swiss mice to streptococcal infection. Both of these strains differ genetically from Webster's selected Swiss strain that was used by the earlier workers. The several types of streptococci used in the current and the preceding series vary in their antigenic composition and probably differ in virulence.

Summary. Group A hemolytic streptococci were regularly isolated from the abscessed lymph nodes of Princeton mice during an outbreak of cervical lymphadenitis. The naturally acquired disease was reproduced in normal Princeton weanlings by nasal deposition of the organism in pure culture. The mortality rate was markedly increased, however, by nasal inhalation of the inoculum. In Swiss weanlings the morbidity rate of cervical lymphadenitis after deposition and the mortality rate after inhalation were much reduced. Nasal introduction of the streptococcus was attended by otitis media in 28 out of 45 Princeton wealings (62%) and 40 out of 55 Swiss (72%).

1. Boxmeyer, C. H., *J. Inf. Dis.*, 1907, v4, 657.
2. Sonkin, L. S., *ibid.*, 1949, v84, 290.
3. Glaser, R. J., Berry, J. W., and Loeb, L. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 87.
4. Nelson, J. B., *J. Exp. Med.*, 1953, v98, 433.

Received June 16, 1954. P.S.E.B.M., 1954, v86.

Protective Action of Tetanus Toxoid Unrelated to Active Immunization in Mice.* (21160)

LEON GOLDMAN, THOMAS B. TURNER, AND EDWARD S. STAFFORD.
From the Department of Microbiology, The Johns Hopkins University, Baltimore, Md.

Recent studies by us, as well as by others, indicate clearly that an individual previously immunized with tetanus toxoid enjoys a long-continued protection against the action of tetanus toxin, not only in respect of serum antitoxin levels, but in the ability to respond rapidly to a booster dose of toxoid. Even 10 to 11 years after the last immunizing injection, most persons respond to a booster dose of toxoid with a detectable rise in serum antitoxin within 3 to 5 days(1). While the brief lag period in antitoxin response after a booster dose of toxoid probably does not indicate lack of protection, nevertheless it might be useful to find means of supplementing the individual's immunity during this period other than

* Supported by a grant from the Whitehall Foundation.

by the injection of antitoxin, which when contained in horse serum leads to undesirable reactions in a high proportion of patients.

Krech(2), and Lemetayer, Raynaud, and their associates(3-5) found that tetanous toxoid administered to mice inhibited the action of tetanus toxin given 24 hours later. The present study is an extension of those observations.

Procedure. Highly purified tetanus toxin (Lot No. LCI 10-9-47) prepared by Dr. Louis Pillemer of Western Reserve University was used in a dose which varied between 1 mouse LD_{50} and 1 mouse mld. This dose regularly caused severe symptoms or death. The toxin used in each experiment was removed from −40°C, thawed, and diluted to proper strength in broth of pH 6.6 just prior to injection. Therefore, each experiment was performed with toxin of equivalent potency except for slight unavoidable dilution inaccuracies. That the variation in toxin strength was slight can be noted by comparison of groups of mice that received the same dose of toxin in different experiments. The toxin was contained in a volume of 0.2 ml and was injected into mice weighing 18 ± 2 g intramuscularly at the base of the tail on the right.

Symptoms were graded as follows:

Slight. Bulging of the left side of the abdomen because of spasm of the abdominal muscles on the right; sluggishness of right hind leg.

Severe. Spastic deflection of the tail to the right and paralysis of the right hind leg, often progressing to an inability of the mouse to right itself when placed on its back, and convulsions.

In severely paralyzed mice deaths occurring after the first week may have been due to lack of nourishment. Mice that showed severe symptoms but did not die recovered completely after many weeks.

Commercially available fluid tetanus toxoid was used in these experiments.† The toxoid, except when combined *in vitro* with toxin, was injected intramuscularly at the base of the tail on the left.

† The toxoid was supplied by the Lederle Laboratories Division, American Cyanamid Co., under the designation Tetanus Toxoid Fluid-Purogenated.

TABLE I. Effect of Varying Dose of Toxoid when Toxin and Toxoid Are Administered Simultaneously.

Dose of toxoid, ml	Deaths: Days after inj. of toxin 5	Deaths: Days after inj. of toxin 9	Severe symptoms: Days after inj. of toxin 5	Severe symptoms: Days after inj. of toxin 9
None	20/20*	—	20/20†	—
.2	1/20	15/20	20/20	20/20
.4	0/20	2/20	5/20	4/20
.8	0/20	0/20	1/20	1/20

* Numerator: No. of deaths. Denominator: Total No. mice inj.

† Numerator: No. with severe symptoms including death. Denominator: Total No. mice inj.

Results. Effect of varying dose of toxoid—toxin and toxoid administered simultaneously. When the toxoid was administered simultaneously with, or a few moments prior to the injection of toxin substantial protection was noted with each of the doses of toxoid employed, namely 0.2, 0.4, and 0.8 ml, as compared with mice which received no toxoid. This protective effect, which bore a direct relation to the size of the dose of toxoid, was somewhat greater in terms of survival than in terms of the absence of symptoms (Table I).

Effect of varying the interval between toxoid and toxin injection. Experiments were undertaken to investigate the effect of giving 0.4 ml of toxoid at varying intervals before the toxin. A high degree of protection is afforded mice given toxin and toxoid simultaneously, and somewhat less protection is afforded mice given toxoid 24 hours prior to the injection of toxin (Table II). Only slight protection was noted when the interval between toxoid

TABLE II. Effect of Varying Interval between Toxoid and Toxin Injection. Dose of toxoid 0.4 ml.

No. of days prior to inj. of toxin that toxoid was given	Deaths: Days after inj. of toxin 5	Deaths: Days after inj. of toxin 10	Severe symptoms: Days after inj. of toxin 5	Severe symptoms: Days after inj. of toxin 10
No toxoid	8/10*	10/10	10/10†	10/10
7	0/10	3/10	6/10	6/10
5	7/10	9/10	10/10	10/10
3	5/10	10/10	10/10	10/10
1	0/10	8/10	8/10	8/10
0	0/10	0/10	5/10	2/10

*† See footnotes, Table I.

TABLE III. Effect of Divided Doses of Toxoid at Daily Intervals.

	Deaths		Severe symptoms	
	Days after inj. of toxin			
	5	10	5	10
Group A	0/10*	5/10	3/10†	6/10
B	1/10	4/10	3/10	9/10
C	0/10	4/10	4/10	8/10
D	0/10	3/10	3/10	9/10
E	7/10	10/10	10/10	10/10

*† See footnotes, Table I.

and toxin injection was 3 days, and no protection was noted when the interval was 5 days. Protection exhibited by the mice receiving toxoid 7 days prior to the injection of toxin is taken as a manifestation of early active immunization.

Effect of divided doses of toxoid. The same dose of toxoid, namely 0.4 ml, was given in divided doses of 0.1 ml per day according to the following protocol. The day the toxin was given is referred to as zero day, and the minus and plus signs indicate days before or after zero day. All groups received the standard dose of toxin as described under Procedure.

Group A: 0.1 ml toxoid on −3, −2, −1, 0; Group B: 0.1 ml toxoid on −1, 0, +1, +2; Group C: 0.1 ml toxoid on 0, +1, +2, +3; Group D: 0.4 ml toxoid on day 0 only; Group E: No toxoid.

Each group that received divided doses of toxoid had significant protection similar in degree to the protection afforded mice receiving the same total dose (0.4 ml) on zero day (Table III). Moreover, there is no significant difference in the protection afforded groups A, B, and C. The results of this experiment should be viewed in relation to the previous one, since a single large dose given 3 days before the toxin seems to have less protective effect than divided doses given daily over the same period.

Effect of mixing toxoid and toxin in vitro prior to injection. When the standard dose of toxin was mixed with 0.4 ml of toxoid *in vitro* and the mixture immediately injected into mice intramuscularly at the base of the tail on the right the protection appeared to be greater than that afforded by the injection of toxoid at a site opposite that used for the injection of toxin. This effect was more apparent in terms of absence of symptoms than in terms of survival. Tetanus toxin mixed with diphtheria toxoid was used as a control and no protection was found (Table IV). Another experiment in which the toxin dose was very small yielded similar results.

Discussion. In addition to the well-known immunizing action of tetanus toxoid, these experiments and those of the authors previously cited, suggest that toxoid also inhibits the action of toxin by a mechanism not related to antibody formation. The experiments throw no direct light on the nature of this inhibitory action. Harvey(6) has shown that tetanus toxin is capable of peripheral action in the region of the neuromuscular junction, and postulates that the toxin affects the motor nerve ending with resulting derangement in the acetylcholine-cholinesterase relationship. It may be that these action sites of tetanus toxin have an equal affinity for toxoid, which when present occupies a proportion of the normal receptor sites thus serving to block the action of toxin. On the other hand, it is possible that the combination of toxoid and toxin results in inactivation of the latter.

Regardless of the underlying mechanism involved, it appears that in our experiments the toxoid was transported via the blood stream to the site into which toxin had been injected. It might be expected that the closer the toxoid can be approximated to the toxin depot, the more effective would be the protective value

TABLE IV. Effect of Mixing Toxoid and Toxin *In Vitro* Prior to Injection.

	Deaths		Severe symptoms	
	Days after inj. of toxin			
	5	10	5	10
No toxoid	5/6*	5/6	5/6†	5/6
Tetanus toxin + tetanus toxoid	0/6	1/6	0/6	1/6
Tetanus toxin + diphtheria toxoid	2/6	6/6	6/6	6/6
Tetanus toxin and tetanus toxoid at opposite sites	0/6	2/6	5/6	6/6

*† See footnotes, Table I.

of the toxoid, since toxoid could reach the sites of critical action by direct diffusions as well as through the blood stream. The results of the experiment in which toxoid and toxin were combined *in vitro* suggest that this consideration may be playing a role. Similarly, Raynaud, *et al.*,(4) reported that when toxoid and toxin were injected into the same leg local tetanus did not develop, although local tetanus did develop if the injections were made into opposite legs.

Another observation worthy of emphasis is the efficacy of small daily doses of toxoid in inhibiting the action of toxin. Injection of 0.1 ml of toxoid daily for 4 days commencing 3 days before, 1 day before, or on the day of toxin administration resulted in protection similar in degree to that afforded by the injection of the same total amount of toxoid, namely 0.4 ml, simultaneously with the injection of toxin, and the protection was substantially greater than when a single dose of 0.4 ml was given 3 days before the toxin injection. The fact that protection from small divided doses was just as great when the toxoid injections were started coincident with the injection of toxin, as when toxoid was begun 3 days before, would seem to eliminate the possibility that an accelerated active immune response was playing a role.

It should also be emphasized that the doses of tetanus toxoid used in these experiments were very large in relation to the body weight of the mouse, and it is not known to what extent these observations can be interpreted in terms of the problem in human beings. Certainly, this whole phenomenon should be explored first in larger animals.

Summary. 1. Mice injected with a single dose (0.4 ml) of fluid tetanus toxoid 24 hours prior to, or simultaneously with a small dose (1 LD_{50} to 1 MLD) of tetanus toxin exhibited a degree of protection against the action of the toxin. The protective effect varied directly with the dose of toxoid. Toxoid given 3 or 5 days before the toxin afforded little or no protection. 2. Small daily doses of toxoid (0.1 ml) given over a 4-day period commencing 3 days prior, one day prior, or on the day of toxin administration were as efficacious in inhibiting the action of toxin as an equivalent single large dose of toxoid (0.4 ml) administered coincident with the injection of toxin. 3. When toxoid and toxin were mixed *in vitro* prior to injection into mice, the protective effect of the toxoid appeared to be enhanced.

1. Turner, T. B., Stafford, E. S., and Goldman, L., *Bull. Johns Hopkins Hosp.,* 1954, v94, 204.

2. Krech, U., *Z. Immun.,* 1949, v106, 241.

3. Lemetayer, E., Nicol, L., Girard, O., Corvazier, R., and Cheyroux, M., *Ann. Inst. Pasteur,* 1950, v79, 672.

4. Raynaud, M., Lemeteyer, E., Turpin, A., Nicol, L., and Rouyer, M., *Acad. Sci., Paris,* 1951, v233, 586.

5. Raynaud, M., and Wright, E. A., *Nature,* 1953, v171, 797.

6. Harvey, A. M., *J. Physiol.,* 1939, v96, 348.

Received June 16, 1954. P.S.E.B.M., 1954, v86.