experiments the 2 gm. navy bean meal was suspended in 20 cc. of disodium phosphate solution of pH 8.9 containing 0.2 gm. of trypsin (Fairchild's). Willstätter and Waldschmidt-Leitz's[6] titration method was used and the figures represent increases over those obtained at the beginning, expressed in cubic centimeters of 0.1, normalcy representing 1.4 mg. amino N per cc. In pepsin digestion changes in the polypeptide N and in trypsin digestion, changes in the amino-acid N served as a measure of proteolytic acceleration.

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Investigations on the Pathogenesis of Tetanus.

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We have observed that 10 to 20 times more tetanus antitoxin given intravenously is required to protect guinea pigs and rabbits against a multiple lethal dose of the toxin injected intramuscularly than intravenously. Table I gives the results of a representative experiment.

TABLE I.

Comparison of the Amounts of Antitoxin Required to Protect Guinea Pigs against 10 Fatal Doses of Tetanus Toxin Injected Intramuscularly and Intravenously.
Tetanus Toxin—N. Y. City Board of Health Laboratories Lot 47 Vial C.
Antitoxin " " " " " " Lot 284.

Antitoxin 1 cc. intravenously, dilutions	Toxin 0.1 cc. 1:200			
	G.p. No.	Intramuscularly	G.p. No.	Intravenously
1:50	507	LT, partial		
1:100	506	LT, "		
1:200	505	LT, complete		
1:500	504	LT, "		
1:1000	503	LT, "		
1:2000	502	LT, GT *3		
1:4000	501	LT, GT *6		
1:5000			511	0
1:10,000			510	0
1:20,000			509	0
1:40,000			508	GT *5

LT—local tetanus.
GT—generalized tetanus.
0—no symptoms.
*—death, number following indicating day after injection.

[6] Willstätter, R., and Waldschmidt-Leitz, E., *Berichte*, 1921, **54**, 2988.

In further experiments we found that free antitoxin was present in the blood of the intramuscularly-injected animal at the time of death. These observations, we believe, have an important bearing on the pathogenesis of tetanus. They show that the death of the animal cannot be due to toxin reaching other muscles or the central nervous system by way of the circulating blood.

After excluding the Danysz phenomenon as a factor in our results we were faced with the alternative that either, contrary to Abel's[1, 2, 3] conception, tetanus toxin reaches the central nervous system by way of the peripheral nerve, or that a secondary toxic substance is formed in the muscle which is not neutralized by antitoxin. The first substance we suspected was acetylcholine. This phase of the work is still going on.

In searching for this hypothetical substance, toxin and muscle were mixed *in vitro*. It was found that when injected intramuscularly, the "muscle toxin" was 4 to 8 times more lethal than the pure toxin and 8 times more effective in causing local tetanus. In Table II we present a protocol of one of the many experiments we have carried out with this phenomenon.

The mixture is not more toxic than the pure toxin when injected intravenously. We also found that irrespective of the toxicity,

TABLE II.

Comparison of Lethal Dose of Muscle-toxin and Plain Toxin.

Preparation of Muscle-toxin—5 gm. of muscle from rear extremity of exsanguinated guinea pig triturated with 2 cc. of tetanus toxin (Eli Lilly Company) in mortar with aid of 2 cc. of sterile sand. Mixture centrifuged at 2000 r.p.m. for 15 minutes. Dilutions made from supernate, which was usually slightly in excess of 2 cc.

1 cc. injected intramuscularly in calf region of rear leg. Dilutions:	G.p. No.	Muscle-toxin	G.p. No.	Plain Toxin
1:2,000	692	LT *2	30	LT *2
1:4,000	710	LT *2	545	LT, GT *3
1:8,000	715	LT, GT *3	616	LT, complete
1:16,000	731	LT, GT *4	631	0
1:32,000	803	LT, GT *7	648	0
1:64,000	711	LT, complete		
1:128,000	756	0		

LT—local tetanus.
GT—generalized tetanus.
0—no symptoms.
*—death, number following indicating day after injection.

1 Abel, John J., Evans, E. A., Jr., Hampil, B., and Lee, F. C., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 84.

2 Abel, J. J., Hampil, B., and Jonas, A. F., Jr., *Ibid.*, 1935, **56**, 317.

3 Abel, J. J., and Hampil, B., *Ibid.*, 1935, **57**, 343.

equal amounts of muscle toxin and pure toxin were neutralized by equal amounts of antitoxin *in vitro* and *in vivo*. This shows that the hypothetical secondary substance is not present in the mixture of muscle and toxin, although this does not preclude its production in the living muscle. It is also clear that the amount of toxin in the mixture is not increased. The mechanism of the potentiation of the toxin by the muscle is still under investigation.

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Further Studies of Agar-slant Tissue Cultures of Typhus Rickettsiæ.*

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In a preliminary note published in these PROCEEDINGS,[1] the writers described an agar-slant-tissue method for the cultivation of Rickettsiæ, both of the murine and of the classical types of typhus fever.

Further study of this method has led to modifications and standardizations which are reported herewith in order to facilitate its use in the hands of others. Preliminary experiments by Pinkerton with Rocky Mountain Spotted Fever virus indicate a possibility that the method may be successful with Rickettsiæ other than those of typhus.

The medium as now used differs from the original only in proportions of ingredients. We have found that results are most regular when the agar is prepared as follows:

A "Tyrode" solution of the ordinary formula is made up double strength throughout, except that only 1 gm. of bicarbonate of sodium is used per liter. The agar used is a Difco granular Bacto-agar.

A mixture of 150 cc. double strength Tyrode and 150 cc. of horse serum with 8 cc. of phenol red solution is filtered through a Seitz

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[1] Zinsser, Hans, Wei, H., and FitzPatrick, Florence, PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 604.