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Large-Scale Production of Tetanus Toxoid

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The production of tetanus toxoid in a large culture vessel was described by R. O. Thomson (Nature **180**:1126, 1957). By employing a semicontinuous procedure, 70 liters of culture every 48 hr could be produced. When fully continuous methods were attempted, the organism tended to lose toxigenicity.

J. R. Hepple (J. Appl. Bacteriol. **28**:52, 1965) was able to obtain large volumes of high potency material by batch cultivation in tanks, but did not report any yields.

Results of preliminary experiments with a protein-free medium for tetanus toxin production (W. C. Latham, D. F. Bent, and L. Levine, Appl. Microbiol. **10**:146, 1962) suggested that this procedure could be scaled up to larger volumes. Therefore, experimental work was undertaken in our laboratories to adapt the method to our particular needs and equipment.

The production medium was prepared, following the procedure of the Massachusetts Public Health Biologic Laboratories (Latham, *personal communication*), in a 100-gal, steam-jacketed, glass-lined fermentor, equipped with temperature control and a two-bladed gear-driven agitator, and with proper connections for introducing inocula and withdrawing samples. The medium was sterilized at 118 to 122 C for 1 hr, cooled rapidly to 36 to 39 C, and inoculated immediately.

Seed cultures of *Clostridium tetani* (Massachusetts strain) were maintained at −70 C in 1.0-ml amounts in glass ampoules. One or four of these ampoules were thawed and inoculated into 200 to 300 ml of Brewer's thioglycolate broth contained in 500-ml bottles. After incubation at 37 C for 24 to 30 hr, these cultures were used to inoculate the tank.

Tank cultures were incubated for 7 to 8 days at 36 to 39 C. Either nitrogen or air was introduced to the culture surface within the tank through a sterile filter stack packed with glass wool.

TABLE 1. *Tetanus toxin production in a fermentation tank using the Massachusetts strain of Clostridium tetani*

Expt no.	Inoculum age and vol	Culture vol	Growth period	Aeration	Results	
					Toxin Lf/ml[a]	L+ doses/ml in mice[b]
		liters	*days*			
1	29 hr, 200 ml	200	7	Nitrogen to culture surface	100	50+, 75−
2	25 hr, 250 ml	200	7		40	300+, 400−
3	24 hr, 500 ml	300	8	Air to culture surface	40	200+, 300−
6	24.5 hr, 300 ml	300	7		10	100+, 150−
8	31 hr, 250 ml	200	8	Air to culture surface, intermittent agitation	40	200+, 300−

[a] Toxin titer (Lf/ml) established by flocculation with tetanus antitoxin.
[b] An L+ dose is that quantity of toxin which, when mixed with 0.1 unit of antitoxin, will kill a mouse in 72 to 90 hr.

Results are summarized in Table 1. Satisfactory toxin yields were obtained in both 200- and 300-liter volumes with gas atmospheres of either nitrogen or air. The titer of 100 Lf/ml reported for experiment 1 is questionable, and may represent a nonspecific flocculation, sometimes seen in flocculation tests.

The surface-to-volume ratio in culture vessels is an important factor in tetanus toxin production (W. C. Latham, D. F. Bent, and L. Levine, Appl. Microbiol. **10**:146, 1962). This is to permit the

TABLE 2. *Purification and potency of tank-produced tetanus toxoids*

Expt no.	Results			
	Crude toxoid titer (Lf/ml)	Purity (Lf/mg of N) after alcohol fractionation	Overall yield	Antitoxin units produced in guinea pigs[a]
			%	
2	40	1,190	60.0	4+
3	25	1,137	69.4	2+, 4−

[a] Refined toxoids contained 12 Lf/ml + 1.0 mg/ml of $AlPO_4$; a 4-week immunization period was used.

escape of gaseous products of metabolism which might exert an inhibitory effect on the elaboration of toxin. In our experiments, the gas flow was maintained at sufficient volume to exert a slight positive pressure on the culture, to prevent a vacuum from developing within the tank, and to expel gaseous metabolic products with the effluent air stream.

It was reported by W. C. Latham et al. (Appl. Microbiol. **10**:146, 1962) that heating time is also important in the preparation of this culture medium, and is incidental to its sterilizing function. Extending the medium sterilization time to 90 min (experiment 8) had no effect on toxin yield. The optimal heating time remains to be determined.

Samples from tank cultures 2 and 3 were detoxified by adding 0.3% (final concentration) formaldehyde solution and incubating at 37 C for 21 days. Considerable purification was effected when the crude toxoids were refined by the method of L. Pillemer et al. (J. Exptl. Med. **88**:205, 1948). Experimental vaccines containing 12 Lf units of toxoid and 1.0 mg of $AlPO_4$ adjuvant per ml were prepared and tested for potency by standard NIH Division of Biologics Standards methods. To be acceptable, this type of product must produce 2 antitoxin units per ml of serum in guinea pigs in not more than a 6-week period. Our results (Table 2) show that both toxoids exceeded the minimal requirement of serum antitoxin levels in a 4-week period.

It thus appears that satisfactory toxoid can be produced in the manner described. In addition, the Massachusetts medium would seem to be the medium of choice for this purpose, since all ingredients are commercially available and the absence of protein in the medium may result in a more refined product.