Investigation of the physical properties of tetanus toxoids

Toxoids are widely used in prophylaxis against disease and a knowledge of their nature is therefore of great practical significance. Tetanus toxoid is of particular interest in view of its spontaneous formation from toxin on standing, even at 0° C.

PILLEMER has purified tetanus toxin by low temperature methanol fractionation[1]. Ultracentrifugal examination of the purified toxin, which had aged, has shown two components with sedimentation constants of 4.5 and 7.0 Svedberg units due to the toxin and spontaneously formed toxoid respectively[2]. Further work on tetanus toxin, purified by multi-membrane electrodecantation (M.M.E.D.) and ammonium sulphate fractionation[3,4], has confirmed the presence of two components with sedimentation constants of 3.9 and 7.6 Svedberg units which differ slightly from those found by PILLEMER. In addition the diffusion constant of the toxin was measured using an analytical procedure[4] in which the concentrations of the original solution of tetanus toxin and its diffusates after increasing periods of time were measured. The partial specific volume of the toxin was calculated from the amino acid analysis and from this data the molecular weight and frictional ratio could be calculated (Table I).

TABLE I

PHYSICAL PROPERTIES OF TETANUS TOXIN AND ITS TOXOIDS

Substance	*Diffusion constant (cm²/sec)*	*Partial specific volume*	*Sedimentation constant (Svedberg units)*	*Molecular weight*	*Frictional ratio*
Tetanus toxin	$5.5 \cdot 10^{-7}$ **	0.749	3.9 (4.5)*	68,000** (78,000)*,**	1.41**(1.35)*,**
Spontaneous tetanus toxoid	$5.09 \cdot 10^{-7}$	0.749	7.6 (7.0)*	144,500 (132,900)*	1.20 (1.23)*
Formol tetanus toxoid	—	—	4.2	—	—

* Figures in brackets refer to the values of the sedimentation constants given by PILLEMER AND MOORE[2], and to the molecular weight and frictional ratios calculated with these values.

** These values are approximate in view of the fact that the diffusion constant of tetanus toxin was determined by a method involving toxicity measurements.

A preparation of tetanus toxin, purified by M.M.E.D. and ammonium sulphate fractionation, which was allowed to toxoid spontaneously, was found by ultracentrifugal examination to contain at least 95% of toxoid with a sedimentation constant of 7.6 Svedberg units (Fig. 1). The diffusion constant of this toxoid, as measured by the LAMM scale method[5], was found to be $5.09 \cdot 10^{-7}$ cm²/sec (Table II). Assuming the partial specific volume of the toxoid to be identical to that of the toxin (0.749)[4], a molecular weight of 144,500 and a frictional ratio of 1.20 could be calculated (Table I).

It was of interest to determine whether the toxoid formed by treating tetanus toxin with formaldehyde was the same as that formed spontaneously. Purified tetanus toxin was de-toxified with 0.25% formaldehyde at 37° C for 3 days. Ultracentrifugal examination of this toxoid showed the presence of two components with sedimentation constants of 4.2 and 7.6 Svedberg units (Fig. 2), indicating that the formol toxoid has a sedimentation constant of 4.2 Svedberg units.

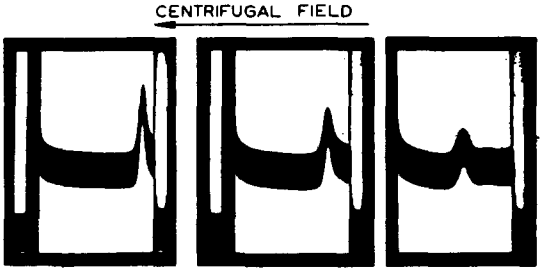

Fig. 1. Sedimentation diagrams of the spontanecusly formed tetanus toxoid after 8, 20 and 52 minutes at 60,000 r.p.m. The sedimentation constant of the toxoid is 7.6 Svedberg units.

The results of the investigation described above and of PILLEMER's previous work[2] indicate that the spontaneous formation of tetanus toxoid proceeds by the dimerisation of toxin molecules. The lower frictional ratio of the toxoid would suggest that this dimerisation proceeds in a side-to-side fashion and not end-to-end.

The tetanus toxoid by formaldehyde has approximately the same sedimentation constant as the toxin, itself, suggesting that, unlike the spontaneously formed toxoid, the formol toxoid

TABLE II

DIFFUSION CONSTANT OF TETANUS TOXOID

Time of diffusion (seconds)	*Diffusion constant (Inflection method) cm^2/sec*	*Diffusion constant (Method of moments) cm^2/sec*	*Average diffusion constant*
178,920	$5.07 \cdot 10^{-7}$	$5.17 \cdot 10^{-7}$	$5.09 \cdot 10^{-7}$
246,840	$4.85 \cdot 10^{-7}$	$5.00 \cdot 10^{-7}$	

has approximately the same molecular size as the parent toxin. This similarity in molecular size has also been shown for diphtheria toxin and formol toxoid, which have approximately the same sedimentation constants (4.6 Svedberg units)[6,7].

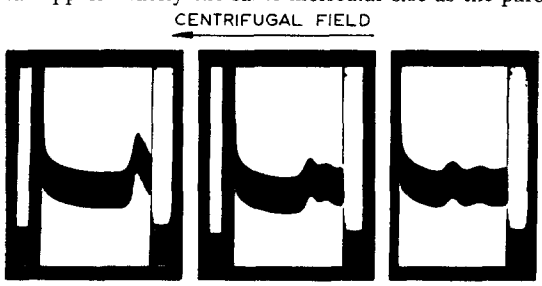

Fig. 2. Sedimentation diagrams of the formol tetanus toxoid after 12, 32 and 56 minutes at 60,000 r.p.m. The two components, which are presumably the formol toxoid and the spontaneously formed toxoid, have sedimentation constants of 4.2 and 7.6 Svedberg units respectively.

It would appear that de-toxification of tetanus toxin occurs as a result of two reactions: (i) Spontaneous conversion of the toxin (3.9 Svedberg units) to the toxoid (7.6 Svedberg units), and (ii) Interaction of formaldehyde to form a toxoid (4.2 Svedberg units). This latter toxoid is devoid of any tendency to dimerise and hence shows up in a reasonable concentration in the sedimentation diagram. The spontaneous formation of toxoid is both far slower and less complete than that induced by formaldehyde.

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The activation of chymotrypsinogen by subtilisin

The tryptic activation of chymotrypsinogen has long been a subject of great interest in the protein field. With the background provided by the observations of NORTHROP AND KUNITZ[1], and the investigations of JACOBSEN[2] concerning the mechanism of activation of this zymogen, the recent work of NEURATH *et al.*[3], and of the DESNUELLE group[4] has provided continuing progress in the understanding of the activation process.

Although trypsin is used as the initial catalyst in all of these studies, and further seems to be responsible for the activation of chymotrypsinogen in physiological systems, it is not the only enzyme capable of initiating the conversion of the zymogen to its active form. KUNITZ[5], in 1938, noted that culture media of the mold *Penicillium* contained a "kinase" capable of slowly activating acid solutions of chymotrypsinogen, and in 1951, ABRAMS AND JACOBSEN[6] observed that crude enzyme preparations from *Bacillus subtilis*, which had previously been shown to convert