CONCENTRATION AND PURIFICATION OF B. MEGATHERIUM PHAGE

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B. megatherium phage, like staphylococcus phage, is inactivated by repeated high speed centrifugation (Northrop, 1938; Hotchin, 1954). This method of purification has the additional disadvantage that large volumes of material cannot be handled.

B. megatherium phage is precipitated from peptone solution by 0.4 saturated ammonium sulfate. The precipitate is filtered off, washed, and dissolved in a small volume of 1 M sodium acetate. A few γ of trypsin are added and this solution kept at 0° for several days. The protein content of this solution is now nearly equivalent to that calculated from its active phage content. The phage may be precipitated from this solution by the slow addition (with stirring) of an equal volume of saturated magnesium sulfate. A fine silky precipitate forms which consists of highly refractile irregular particles and fibers. This precipitate contains all the phage activity. The number of plaques formed by 1 mg. nitrogen of this precipitate is very nearly equal to the number calculated from the size (Murphy, 1954) of the phage particle. If a solution of this precipitate is heated or made acid so as to inactivate the phage, the protein then appears as a flocculent amorphous precipitate.

Experimental.—An example of the method is shown in Table I.

Experimental Procedures

All filtrations, washings, etc., were carried out as described in Crystalline Enzymes (Northrop, Kunitz, and Herriott, 1948).

N determinations.—1 ml. of solution added to 10 ml. 2 per cent (cold) trichloroacetic acid. The suspension is allowed to stand 20 minutes at 20°C., centrifuged, and the precipitate washed twice with cold 2 per cent trichloroacetic acid. The precipitate is then analyzed for nitrogen according to Lanni, Dillon, and Beard (1950).

The precipitate prepared in this way contains the nucleic acid as well as the protein. The actual weight of the phage particle is therefore approximately six times the nitrogen figure.

TABLE I

Concentration and Purification of Megatherium T Phage

	No.	Volume	Phage		Protein N ml.	Phage γProtein N
			Ml.$^{-1}$ 10^{10}	Total 10^{13}		
					gamma	*10^{10}*
500 ml. 5 per cent peptone + 15 × 10^6 *megatherium*-sensitive,/*ml.* Bubble O_2 at 35°. 1 × 10^7 T phage/ml. added when B/ml. = 1 × 10^8. Lysis after 2 hrs. (Stock phage).....	1	500	4	2		
Add 500 gm. standard supercel, filter (suction, Whatman No. 3 paper).............	2	500	4	2		
500 ml. No. 2 + 230 ml. saturated ammonium sulfate (≈0.4 saturated)—4 days 0°, slight brown flocculent precipitate. Add 1 gm. standard supercel, filter (suction, Number 3 paper). Precipitate—wash three times on paper + 50 ml. ½ saturated, $MgSO_4$. Wash through paper + 50 ml. 1 M sodium acetate—clear, bluish, slight Tyndall cone.............................	3	50	35	1.7	30	1.2
Add 1.0 γ crystalline trypsin/ml. Stand 0°, 2 days..................................	4	50	35	1.7	8	4
Stir in 50 ml. saturated $MgSO_4$—silky precipitate with highly refractile irregular particles. Stand 20°, 1 day. Add 1 gm. standard supercel, filter (suction, Number 3 paper). Precipitate—wash twice + ½ saturated $MgSO_4$, dissolve through paper with 20 ml. 1 M sodium acetate—clear, slightly bluish, faint Tyndall cone.........	5	20	60	1.2	13	4.5
Calculated from size of phage particle (Murphy, 1953) (assuming 15 per cent N)..						5.80

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