THE EXHIBITION PHENOMENON WITH *PSEUDOMONAS AERUGINOSA* PHAGE 13

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(With 3 Figures in the Text)

INTRODUCTION

It was shown by Mead & van den Ende (1953) that an extract of a strain of *Pseudomonas aeruginosa* (*Ps.* LII) resistant to phages 5, 13 and 14 not only inhibited the formation of plaques by two of them (5 and 14) but also increased the plating efficiency of the third (13) when tested on a rough variant (*Ps.* LIII-3 bi) of the same strain. As the factor responsible for this latter effect appeared to differ in molecular size and complexity from known adsorption co-factors (e.g. Ca^{2+}, tryptophane) or specific phage growth co-factors (e.g. Ca^{2+}), the phenomenon seemed worth further study. This paper describes the conditions under which increased plating efficiency (exhibition) of phage 13 can be demonstrated and attempts an explanation of the effect. Some results with phage 14 are given for comparison and some properties of the active extract are described.

MATERIALS AND METHODS

Bacteria. The origins and characteristics of strains used have been described (van den Ende & Mead, 1952). The family relationship of the mutants of *Ps.* strain L and information about their sensitivity to phages 13 and 14 are given in Fig. 1. Bacteria were stored dry (Stamp, 1947) and fresh subcultures used for each experiment.

Bacteriophages. The origins and some properties of phages 13 and 14 have been described (van den Ende & Mead, 1952).

Purified extract of Ps. LII. Bacteria were grown during 13 hr. at 37° C. in 20 l. of H.I.D. medium (Mead & van den Ende, 1953) which was gassed with oxygen, and were collected in a refrigerated Sharples centrifuge. The supernatant fluid was neutralized with lactic acid, sterilized by filtration and used for a second run. The yields of moist bacteria were 140 g. from the first run and 120 g. from the second.

The bacteria (140 g.) were suspended in ice water with the aid of a blendor and stirred in an ice bath for 1 hr. after the addition of an equal volume of 0·5 N trichloracetic acid (TCA) (Boivin & Mesrobèanu, 1933). The mixture was centrifuged cold and the supernatant fluid at once partially neutralized with Na_2HPO_4 and finally adjusted with NaOH to pH 6·0. The centrifuged bacterial debris was re-extracted with TCA (700 ml. of 0·25 N) and the supernatant fluid collected. The combined neutralized extracts were concentrated by evaporation at about 30° C. and dialysed for 7 days against distilled water. A trace of precipitate was removed by centrifuging and the solution freeze-dried. The yield was 1·6–1·9 % of the moist

bacteria. For purification a 1 % solution in water was freed from a trace of protein with chloroform (Sevag, Lackman & Smolens, 1938), dialysed, concentrated to its original volume and brought to 80 % saturation with Na_2SO_4 at 20° C. The precipitate was collected by filtration (at 20° C.) and washed with 80 % saturated Na_2SO_4. The filtrate was brought to 90 % saturation and the precipitate collected and washed as before. The precipitates, dissolved separately in water, and also the mother liquor were dialysed, concentrated under reduced pressure and freeze-dried. The interfacial precipitate formed by the repeated shaking with chloroform was suspended in water and the organic solvent removed by evaporation under reduced

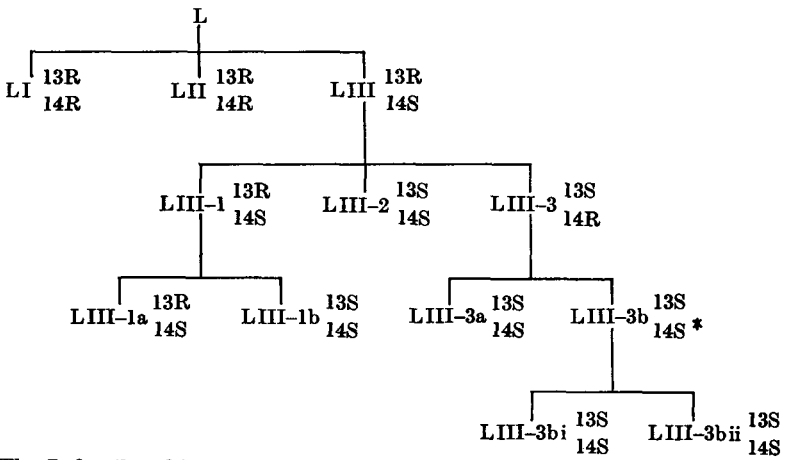

Fig. 1. The L family of *Ps. aeruginosa* mutants and their sensitivity to phages 13 and 14. S denotes sensitivity, R resistance to the indicated phages. * Sensitivity very slight.

Table 1. *The fractionation of the crude TCA extract of* Ps. *LII bacteria*

Fraction	Yield (%)	Inhibition (%)*
0 Crude extract	100	75
1 'Protein' fraction—water-soluble portion	6	44
2 Precipitate at 80 % saturation with Na_2SO_4	30	84
3 Precipitate at 90 % saturation with Na_2SO_4	32	88
4 Residue not precipitated	6	1

* Mean of two tests made with the fraction at a concentration of 5 μg./ml. against phage 14.

pressure. Undissolved material was removed by centrifuging and the supernatant fluid (which unavoidably contained some of the fraction not precipitable by chloroform) was similarly dialysed, concentrated and dried. The yields and inhibitory powers (for phage 14) of the fractions are given in Table 1. The ultra-violet absorption curves of the crude material and fractions (Fig. 2) show that impurities, probably derived from nucleic acid and absorbing at about 260 mμ, were eliminated from the active fractions which had a barely perceptible maximum at or near 275 mμ. Fraction 3 was used for nearly all the experiments to be described.

Extract of Ps. *LIII-1b bacteria.* This was prepared in the same way, but not purified. The yield was about 0·5 % of the moist bacteria.

Solutions and media. Buffered saline (BS) contained NaCl (8·5 g.), Na_2HPO_4 (31·5 ml. of 0·2 M) and KH_2PO_4 (19 ml. of 0·2 M) in water (1 l.) adjusted to pH 7·0.

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Difco medium (A) contained Difco nutrient broth (8 g.) and NaCl (5 g.) in water (1 l.) adjusted to pH 7·4. H.I.D. medium was described by Mead & van den Ende (1953). Standard diluent (SD) consisted of a mixture of equal volumes of A and BS. These were all sterilized by autoclaving.

Preparation of solutions of bacterial extracts. To ensure complete solution, weighed amounts were shaken vigorously with the solvent (normally BS) in a 'wrist-action' machine for 20 min. Solutions were usually prepared freshly for each experiment and contained 400–500 μg./ml. Dilutions were made in SD.

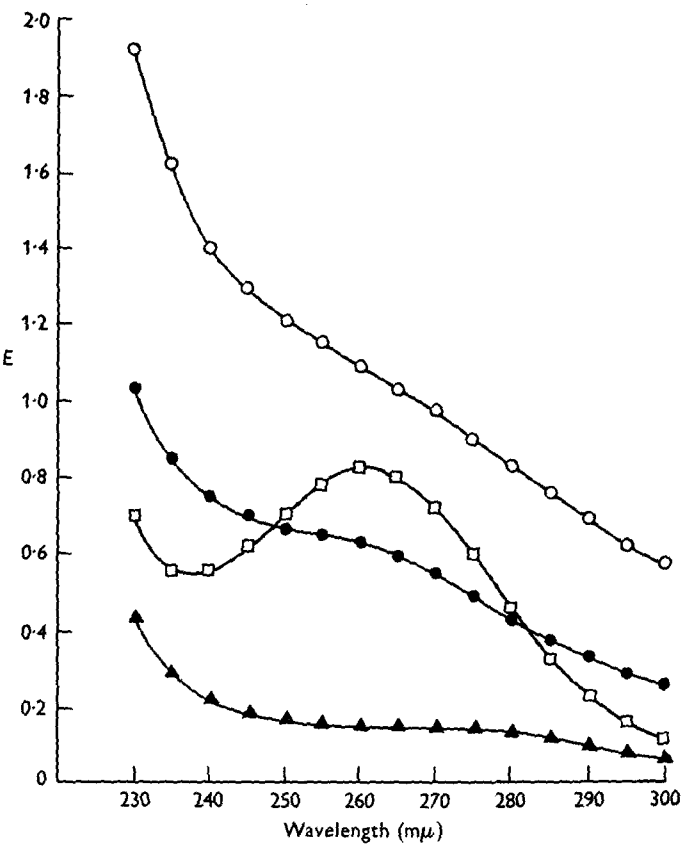

Fig. 2. Ultra-violet absorption curves illustrating the purification of the crude TCA extract of *Ps.* LII. ●—●, original extract; ○—○, 'protein' fraction; ▲—▲, fractions precipitated by 80 % and 90 % saturation with Na_2SO_4 (curves superimposed); □—□, fraction not precipitated by 90 % saturation with Na_2SO_4. Concentration 0·5 mg./ml. throughout in water.

Determination of phage inhibition or exhibition. The appropriate phage (14 for inhibition, 13 for exhibition) was diluted in A to about 2×10^3/ml. Control mixtures (C) were prepared by mixing equal volumes of diluted phage and SD. Test mixtures (E 1, E 2, etc.) consisted of equal volumes of diluted phage and SD containing bacterial extract. The mixtures were incubated (normally for 2 hr.) at 37° C. and plated, on *Ps.* LIII-3 bi unless otherwise stated, by one or more of the following methods: (*a*) the '50-dropper method' as described by Mead & van den Ende (1953); (*b*) the 'agar overlayer' method as described by Adams (1950), the

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bacterial suspension being obtained by emulsifying the overnight growth from 45 cm.^{2} of Hartley's agar in 5 ml.; (*c*) the 'flooded plate' method. Agar plates were flooded with a young culture and redried (as in the 50-dropper method) and the phage suspension was pipetted on to the surface, spread by tilting the plate, and allowed to soak in before incubation was begun. When the agar overlayer method was used, sufficient of a solution of the bacterial extract in SD was added to the 0·7 % agar forming the overlayer to give the same concentration as in the test mixture plated. The 0·7 % agar used for the corresponding control mixture received an equal volume of SD.

RESULTS AND DISCUSSION

For demonstrating exhibition Mead & van den Ende (1953) plated uniform drops of a suspension of phage 13 with or without an extract of *Ps.* LII on nutrient agar plates which had been flooded with a dilute culture of the sensitive strain *Ps.* LIII-3 bi (50-dropper method), and showed that more plaques appeared in the presence of the extract than in the controls.

Four experiments on the exhibitory activity of the new purified extract of *Ps.* LII (Table 2) confirmed the prozone effect previously reported and showed how

Table 2. *The relation between concentration of* Ps. *LII extract and percentage exhibition*

Concentration (μg./ml.)	Exhibition %				
	Test 1	Test 2	Test 3	Test 4	Average
800	71	71	78	64	71
200	99	108	155	99	115
50	80	92	99	108	95
12·5	67	80	95	78	80
3·1	82	51	68	47	62
0·8	70	36	24	57	47

little the percentage exhibition varied with concentration at values above about 1 μg./ml. This fact and the variability of the results have made quantitative work difficult.

Attempts to demonstrate exhibition by the agar overlayer method of plating were at first unsuccessful (Table 3), although the inhibition of phage 14 by the same extract was clearly shown (Table 4). Investigation of the differences between the 50-dropper and agar overlayer methods of plating revealed that the initial concentration of bacteria was the determining factor. Plates used for the 50-dropper method were flooded beforehand with a culture containing about 3×10^7 bacteria/ml. which was immediately poured off. The overlayers used for the alternative technique received about 4×10^9 bacteria/2·5 ml. of 0·7 % agar. Experiments (Table 5) showed that the efficiency of plating of phage 13 on *Ps.* LIII-3 bi by either method rises as the initial bacterial concentration is increased, whereas the plating efficiency of phage 14 (by the agar layer method) is not perceptibly affected. Exhibition is observed only when plating efficiency is lowered by a reduction in the number

Table 3. *Comparison of 50-dropper and agar overlayer techniques and two plating strains*

For each experiment control (C) and test (E) mixtures (the latter containing 10 μg./ml. of *Ps.* LII extract) were prepared, incubated in the usual manner and plated as indicated.

	Bacterial strain			
	LIII-3bi		LIII-1b	
	Experiment no.			
	1	2	1	2
Solution C by 50-dropper. Plaques per 0·1 ml.	46	85	157	165
Solution E by 50-dropper. Plaques per 0·1 ml.	90	166	166	201
Exhibition by 50-dropper method	90%	103%	5%	22%
Solution C by agar overlayer. Plaques per 0·1 ml.	130	171	165	209
Solution E by agar overlayer. Plaques per 0·1 ml.	122	173	161	195
Exhibition by agar overlayer method	−6%	1%	−2%	−7%

Table 4. *The influence of plating method on the inhibition of phage* 14

Concentration of *Ps.* LII extract (μg./ml.)	Inhibition % by	
	50-dropper method	Agar overlayer method
8	60	94
2	51	89
0·5	49	56
0·125	20	26

Table 5. *The influence of bacterial concentration on the plating efficiencies of phages* 13 *and* 14 *on* Ps. *LIII-3bi*

Bacteria from 180 cm.^{2} of nutrient agar which had been incubated overnight were suspended in 20 ml. of broth. This suspension and four dilutions prepared in broth were used to flood plates and prepare agar overlayers for the titration of phage 13. The agar overlayer experiment with phage 14 was carried out in the same way.

	Phage 13				
Bacteria/ml. × 10^{-10}	0·75	2·3	7·5	19	60
Plaques/0·2 ml. flooded plates	150	214	254	262	296
Plaques/0·2 ml. agar overlayer plates	132	166	202	234	249
	Phage 14				
Bacteria/ml. × 10^{-10}	0·23	0·7	2·3	6	18·4
Plaques/0·2 ml. agar overlayer plates	153	157	154	144	141

of bacteria and under these circumstances may be demonstrated by both methods of plating (Table 6).

Experiments with other strains of bacteria related to *Ps.* LII and *Ps.* LIII-3bi (Fig. 1) revealed two (*Ps.* LIII-2 and *Ps.* LIII-3*a*) on which exhibition of phage 13 could readily be demonstrated and a third (*Ps.* LIII-1*b*) on which little if any exhibition could be detected. When tested by the normal 50-dropper method (i.e. at bacterial concentrations that were probably sub-optimal), the plating efficiency of phage 13 was much higher on *Ps.* LIII-1*b* than on any of the other strains (Tables 3 and 7). These results confirmed the conclusion that exhibition occurs only under conditions of reduced plating efficiency.

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The poor adsorption of phage 13 on *Ps.* LIII-3bi in fluid culture noted by van den Ende & Mead (1952) has been confirmed by several experiments in which no adsorption could be detected by the usual method involving centrifugation and titration of unadsorbed phage. Cultures of this strain infected with phage 13 showed no abrupt increase in phage titre but a slow rise which continued for at least 24 hr. Turbidimetry indicated an inflexion in the growth curve 2·25 hr. after infection, with resumption an hour later of growth at a rate only slightly lower than that of the uninfected control. Phage stocks having titres greater than about 10^8/ml. were seldom obtained by fluid culture although suspensions containing 10^{11} phage/ml. were obtainable from confluently lysed plates. Such suspensions

Table 6. *Exhibition experiments with varying concentrations of bacteria*

Concentration of suspension bacteria/ml. $\times 10^{-9}$	Plaques/0·2 ml. Control	Plaques/0·2 ml. Test*	% exhibition
A. 50-dropper method			
12·5	170	178	5
0·2	91	170	87
B. Agar overlayer method			
27	147	153	9
0·54	96	96	0
0·3	79	97	23
0·125	45	63	40

* Concentration of *Ps.* LII extract 10 μg./ml.

Table 7. *The plating efficiency and exhibition of phage* 13 *on* Ps. *L variants*

Strain	Relative plating efficiency	Exhibition %
Ps. LIII-1b	100	4
Ps. LIII-2	43	31
Ps. LIII-3a	40	64
Ps. LIII-3bi	39	69

Each reading is the average of results of four experiments. The concentration of *Ps.* LII extract was 1 μg./ml.

could be filtered through sintered glass (5/3) without perceptible loss. The system *Ps.* LIII-3bi–phage 13 thus resembles in many respects the phage-semi-resistant strain systems described by Wahl (1953) in which only a proportion of the bacteria are phage-sensitive. Wahl has shown that in such systems both sensitive and resistant bacteria have a mixed progeny of sensitive and insensitive forms, and that the proportion of sensitive bacteria in any culture of a semi-resistant strain appears to be constant. Strains *Ps.* LIII-2 and *Ps.* LIII-3a have not been examined in detail, but as phage 13 plates on them with about the same efficiency as it does on *Ps.* LIII-3bi it is provisionally assumed that all three strains are semi-resistant. Strain *Ps.* LIII-1b, on which phage 13 has a higher plating efficiency, adsorbs this phage in fluid cultures at a measurable rate. In one experiment, for example, with a bacterial count of $1{\cdot}7 \times 10^8$/ml. and an initial phage

concentration of $1{\cdot}6 \times 10^7$/ml., 50 % adsorption occurred in 5 min. A fluid culture was lysed perceptibly 1·5 hr. and completely 2·5 hr. after infection, and simultaneously the phage titre increased a hundredfold. It appears that *Ps.* LIII-1 b is either fully sensitive to phage 13, or, if semi-resistant, yields cultures with a high proportion of sensitive bacteria. With phage 14, however, *Ps.* LIII-3 bi is the more sensitive, the average ratio of plaque counts on the two strains being about 0·7.

If the proportion of phage 13-sensitive bacteria in cultures of *Ps.* LIII-3 bi is as low as the adsorption measurements suggest, it is difficult to understand how clear visible plaques can be formed on this strain without assuming that the freshly liberated phage has some property like the nascent phage of Wollman & Stent (1952) enabling it to attack normally resistant bacteria. If this is the case, it must further be assumed that this mechanism fails in liquid cultures either because the time during which a phage remains nascent is short in comparison with the interval between phage-bacteria collisions or because dilution in fluid causes rapid dissociation from the phage of an adsorption promoter. It is of interest in this connexion that exhibition by the *Ps.* LII extract has not been observed in liquid cultures and

Table 8. *The dissociation of phage* 13*-exhibitor complex by dilution*

The following mixtures were incubated at 37° C. for 2 hr.

(i) 0·9 ml. phage 13 at $10/^7$ml. + 0·9 ml. SD.
(ii) 0·9 ml. phage 13 at $10/^7$ml. + 0·9 ml. SD containing 20 μg./ml. *Ps.* LII extract.
(iii) 0·9 ml. phage 13 at 4×10^3/ml. + 0·9 ml. SD containing 20 μg./ml. *Ps.* LII extract.
(iv) 0·9 ml. phage 13 at 4×10^3/ml. + 0·9 ml. SD containing 0·004 μg./ml. *Ps.* LII extract.
(v) 0·9 ml. phage 13 at 4×10^3/ml. + 0·9 ml. SD.

Samples from (i) and (ii) were diluted 1/5000 with a mixture of equal volumes of A and SD before being plated. (iii), (iv) and (v) were plated without further dilution. The results were as follows:

		Concentration of *Ps.* LII extract		
		10 μg./ml. throughout	10 μg./ml. diluted to 0·002 μg./ml.	0·002 μg./ml. throughout
% Exhibition	Experiment 1*	48	21	−8
	Experiment 2	116	18	−6
	Experiment 3	167	7	−2

* A less active batch of extract was used for this experiment.

that the phage-exhibitor complex appears to dissociate on dilution as indicated by the results in Table 8. Whatever the mechanism of plaque formation on semi-resistant strains, the observed rise in plating efficiency at increasing bacterial concentrations is probably due to the better chance that a phage particle will contact a sensitive bacterium. As the exhibitor increases plating efficiency on semi-resistant strains at low bacterial concentrations it may act by allowing infection of bacteria which are normally resistant.

Although neither phage 13 nor phage 14 forms plaques on *Ps.* LII at concentrations up to 10^7/ml., both are adsorbed by this strain in fluid cultures. Tests made by centrifugation and titration of unadsorbed phage indicated 47 % adsorption of

phage 13 and 40 % adsorption of phage 14 in 15 min. No explanation is offered for the resistance of *Ps.* LII to these phages, but exclusion by either phage carried lysogenically seems unlikely. *Ps.* LII is in fact lysogenic and cultures were induced by ultra-violet light, the optimum exposure, under the conditions described by Mead & van den Ende (1953) for the induction of *Ps.* LIII-3bi/14, being about 80 sec. There was, however, no detectable reduction in the titre of the liberated phage on treatment with active antiphage 14 serum. Moreover, the phage plated as efficiently on the resistant strains *Ps.* LIII-3bi/13 and *Ps.* LIII-3bi/14 as on *Ps.* LIII-3bi itself. As *Ps.* LII adsorbs phages 13 and 14, the trichloracetic acid extract may contain the corresponding receptors and probably inhibits phage 14 by the classical mechanism of phage-receptor combination for which Weidel & Kellenberger (1955) have recently provided new evidence by electron microscopy. For the operation of this mechanism neither partner of the phage-receptor combination must have a residual affinity for bacteria which can lead to adsorption and infection. It is, however, conceivable that particles carrying phage receptors liberated from bacterial cell walls by trichloracetic acid might retain an affinity for suitable bacteria as well as for phage, and that such particles when combined with phage might initiate infection of receptor-free bacteria. An extract containing particles having this dual affinity would thus cause exhibition of the kind encountered when phage 13 is plated on suboptimal concentrations of *Ps.* LIII-2, *Ps.* LIII-3a and *Ps.* LIII-3bi. Although these three strains appear to be equally semi-resistant (Table 7) exhibition is less marked with *Ps.* LIII-2, possibly indicating that the resistant forms of this strain are less well adapted to adsorb the exhibitor.

The failure of the *Ps.* LII extract to exhibit phage 14 implies either that the receptors for this phage are not attached to the same bacteriophile particles as the phage 13 receptors or that the phage 14-receptor combination cannot initiate infection if adsorbed. The two receptors have not been separated, but experiments (Table 9) have shown that the inhibitory properties of the *Ps.* LII extract are more sensitive to heat and acidity or alkalinity than the exhibitory properties, suggesting that the two functions are due to different particles or groups.

As *Ps.* LIII-1b is highly sensitive to phage 13 its extract might be expected to contain receptors which would cause exhibition or inhibition according to whether or not they were able in combination with phage 13 to attack resistant forms of the semi-resistant indicator *Ps.* LIII-3bi. Experiments (Table 10) showed that the crude extract exhibits phage 13 though less effectively than the extract of *Ps.* LII, and that it has no detectable effect on phage 14 to which *Ps.* LIII-1b is semi-resistant.

The presence of at least two precipitinogens in the purified extract of *Ps.* LII is demonstrable by the agar-gel diffusion technique of Oakley & Fulthorpe (1953). No serological cross-reaction could, however, be detected between the extracts of *Ps.* LII and *Ps.* LIII-1b and their heterologous antisera which, however, precipitated the homologous extracts at high dilution. This is surprising in view of the established connexion between other phage receptors and bacterial surface antigens and suggests that the phage 13 receptors on the two organisms, though alike in

Table 9. *The effects of heating* Ps. *LII extract at different* pH *values*

One ml. of a solution containing 400 μg./ml. of the extract was added to each of a series of tubes containing 9 ml. of an isotonic buffer or BS. One tube containing extract solution and BS was kept at 0° C. as standard. In one experiment subsidiary standards containing two-thirds and one-third of the concentration in the main standard were included. The remainder were heated as indicated and rapidly cooled. The mixtures were neutralized when necessary, diluted with BS to 20 ml., and kept at 0° C. till tested. For testing, a portion of each solution was diluted 1/10 with SD so that the final concentration of extract after mixture with phage (except in the subsidiary standards) was 1 μg./ml. Allowance was made for the temperature co-efficients of the buffers as indicated in the table.

Experiment no.	Concentration (μg./ml.)	Temperature (° C.)	Buffer	pH	Time (min.)	Exhibition (%) Test 1	Exhibition (%) Test 2	Inhibition %
1	1	4	BS	7	120	65	54	41
	0·67	4	BS	7	120	59	47	33
	0·33	4	BS	7	120	37	25	24
	1	58	BS	7	120	63	59	30
	1	58	*a*	8	120	77	53	10
2	1	4	BS	7	160	49		26
	1	60	*b*	3·9	160	41		8
	1	60	BS	7	160	44		11
	1	60	*c*	9·4	160	18		−13

Buffers: *a*, acetate-veronal-NaCl pH 8·5 at 17° C., pH 8·0 at 60° C.
b, phthalate-NaCl.
c, glycine-NaCl pH 10·5 at 20° C., pH 9·4 at 60° C.

Table 10. *Comparison between the inhibitory and exhibitory activities of extracts of* Ps. *LII and* Ps. *LIII*-1*b*

Extract	Concentration (μg./ml.)	% exhibition phage 13	% inhibition phage 14
Ps. LII	1	17	42
	2	48	45
Ps. LIII-1b	1	9	7
	2	20	−3
	8	35	0

Table 11. *The inactivation and re-activation of* Ps. *LII extract*

Ps. LII extract was dissolved in water (1 mg./ml.) and portions of the solution were diluted with equal volumes of A water, B 1·7 %NaCl. After 18 days' storage at 4° C. 2 ml. of A were freeze-dried and redissolved in 2 ml. BS to give solution C. Three days later a fresh solution N of the extract (0·5 mg./ml.) was prepared in BS. All solutions were diluted with an equal volume of BS for spectrophotometry (Fig. 3) and then further diluted to 1·5 μg./ml. with SD for testing as exhibitors of phage 13. (Final concentration after admixture with phage 0·75 μg./ml.)

Solution	% exhibition Test 1	% exhibition Test 2
N	41	26
A	0	−4
B	17	10
C	51	33

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promoting exhibition, are either dissimilar or not antigenic or present in amounts too small to be detected by the methods employed.

An interesting property of the purified *Ps.* LII extract is that, while giving active and apparently stable solutions in 0·85% NaCl or buffered saline, it loses both inhibitory and exhibitory properties instantly on solution in water. As the initial solutions were diluted extensively with saline before being tested, it is clear that the effect of water is on the substance itself and not on its interaction with the phage-bacteria systems. During its preparation the extract was thoroughly dialysed against distilled water on three occasions and was finally dried from the frozen state. It was deduced therefore that reactivation must take place during

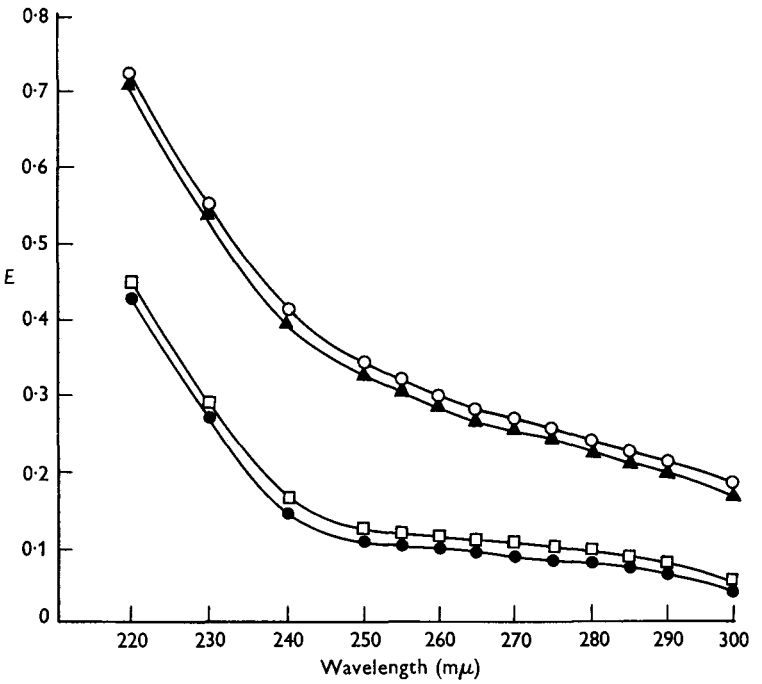

Fig. 3. Ultra-violet absorption curves illustrating the effect of solvent on the purified TCA extract of *Ps.* LII. ●—●, aqueous solution; □—□, aqueous solution to which NaCl had been added; ▲—▲, aqueous solution freeze-dried and residue dissolved in saline; ○—○, fresh solution in saline. Concentrations 0·25 mg./ml.

the freeze-drying. This was confirmed by the experiment recorded in Table 11, which also indicated that slow partial reactivation takes place if NaCl is added to the aqueous solution. Solutions in saline freshly prepared as described earlier are covered with foam, retain minute bubbles of air in suspension for several hours and are slightly turbid. Aqueous solutions have little foam, do not retain air bubbles in suspension and are almost clear. The apparent 'absorption' at short wavelengths (Fig. 3) of solutions in saline is probably due to light scattering and contrasts with the relative transparency of solutions in water. A possible explanation for this behaviour is that disaggregation occurs in the absence of salt, perhaps owing to electrostatic repulsion between particles of like charge. The effects of such charges

would be diminished by salt ions. Reversible disaggregation of large molecules, e.g. the haemocyanins, usually at acid or alkaline pH, is well known, but re-aggregation during freeze-drying is believed to be unusual.

SUMMARY

1. The efficiency of plating of phage 13 on the semi-resistant strain *Ps.* LIII-3bi decreases as the initial bacterial population on the plate is diminished. Addition of *Ps.* LII extract to the phage before plating with low bacterial concentrations increases the plating efficiency (exhibition effect).

2. An extract of a strain *Ps.* LIII-1b which is fully sensitive to phage 13 also causes exhibition of this phage on *Ps.* LIII-3bi.

3. It is suggested that dissolved phage 13 receptors present in extracts of *Ps.* LII and *Ps.* LIII-1b have an affinity not only for the phage but also for suitable bacteria and thus cause exhibition. Inhibition (e.g. of phage 14 by the *Ps.* LII extract) is due to phage receptors having no tendency to adsorb to bacteria.

4. The phage-exhibiting and inhibiting properties of a freeze-dried extract of *Ps.* LII active when dissolved in 0·85% NaCl are lost if the solid is dissolved in water and the solution so obtained further diluted with 0·85% NaCl. Activity is recovered slowly if NaCl is added to the aqueous solution, instantly if the solution is freeze-dried and the residue dissolved in 0·85% NaCl.

5. The inhibitory properties of the *Ps.* LII extract for phage 14 are more sensitive to heat and acidity or alkalinity than the exhibitory properties for phage 13.

6. The absence of cross-precipitin reactions between *Ps.* LII and *Ps.* LIII-1b suggests that the phage 13 receptors of the two organisms are non-antigenic or different.

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