

THE EFFECT OF VARIOUS CULTURE MEDIA ON INFECTION,
GROWTH, LYSIS, AND PHAGE PRODUCTION OF
B. MEGATHERIUM

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The lysogenic strain of *B. megatherium* discovered by de Jong, produces two strains of phage (Gratia, 1936 *a*) which cause lysis of certain "sensitive" strains of the same organism. The C phage causes complete lysis in some media and gives clear plaques. The T phage usually causes partial lysis and gives cloudy plaques, due to overgrowth by a resistant lysogenic strain. These sensitive strains are non-spore-forming. They may recover their spore-forming habit and are then resistant to the phage. New lysogenic strains may be obtained from the sensitive strains by incubating the cultures after lysis has occurred. Under these conditions, a secondary growth takes place which consists of lysogenic cells (Gratia, 1936 *b*).

Such changes from sensitive to resistant strains are of general occurrence and usually are considered to be mutations, although it is known that the composition of the culture media is also important. (Cf. Price, 1949 *a*.) It was found, for instance (Northrop, 1951), that plaque formation by the sensitive strain of *B. megatherium* depends not only on the nature of the culture medium on which the organism is growing, but also on the culture medium from which the culture was obtained. Also lysis may occur on solid media, but not in liquid media. It has since been found that lysis in liquid media is even more dependent on the history of the cultures, than is plaque formation on solid media.

EXPERIMENTAL RESULTS

The results of a series of experiments in which the organism was grown on various culture media, and tested for infection, growth, phage production, and lysis, when mixed with T phage in different culture media, are shown in Table I.

The results show that cells, grown on any of the media tested and then added to 10 per cent peptone + T phage, are all infected, all undergo lysis, and all produce phage. The extent of growth before lysis begins (ΔB), however, depends on the media in which the culture was grown before transfer to 10 per cent peptone. Cells grown on 10 per cent peptone agar, V.I.B. agar, or liquid 10 per cent peptone grow very little before lysis starts, while those grown on

Y.E. agar or Y.E.¹ grow from 80 to 150×10^6 before lysis. All the suspensions produce about 5×10^9 phage/ml.

TABLE I
Effect of Media, on Which Culture Is Grown, On Growth, Phage T Production, and Lysis in Different Media

Average of 2 to 6 experiments each.

Media on which cells were grown	Media on which cells were mixed with phage T														
	5 or 10 per cent peptone agar	V.I.B. agar	Cells/chain	10 per cent peptone				Y.E.				V.I.B., T.P.B., hydrolyzed casein, or asparagine			
	Lysis	Lysis	After 1 hr.	Infection	ΔB 10 ⁶	$-\Delta B$ 10 ⁶	ΔP 10 ⁶	Infection	ΔB 10 ⁶	$-\Delta B$ 10 ⁶	ΔP 10 ⁶	Infection	ΔB 10 ⁶	$-\Delta B$ 10 ⁶	ΔP 10 ⁶
				per cent				per cent				per cent			
2 per cent peptone agar.....	+	-		100	40	20	7000	100	100	0	1000	0	100	0	0
10 per cent peptone agar.....	+	-		100	5	10	5000	100	100	0	5000	0	70	0	0
V.I.B. agar.....	-	-		100	10	15	10,000	10	100	0	0	0	100	0	0
Y.E. agar.....				100	80	50	7000								
10 per cent liquid peptone...	+	-	5	100	5	15	5000	100	140	0	5000	0	140	0	0
Y.E.	+	-	5	100	130	80	5000	20	120		500	0	100	0	0
V.I.B.															
Hydrolyzed casein }	+	-		100	50	30	1000	100	130	0	1000	0	160	0	0
Asparagine															
T.P.B.															

KM strain of *B. megatherium* grown 18 hours at 35°C. on media noted. Washed with H₂O and suspended in media noted. 30×10^6 B/ml. by turbidity. 5×10^8 T phage/ml. added and the culture shaken or incubated (agar) at 35°C. B/ml. by turbidity at 1 hour intervals. ΔB = maximum increase in B/ml. before lysis. $-\Delta B$ = decrease in B/ml. during lysis. $-\Delta B = 0$, indicates no lysis. Suspension plated for colonies on 5 per cent peptone 1 hour after addition of phage.

Per cent infection = 100

$$\left(\frac{\text{Colonies/ml. of control suspension (no phage)} - \text{normal colonies/ml. in phage} + \text{B suspension}}{\text{Colonies/ml. control suspension}} \right)$$

ΔP = plaques/ml. at time of lysis, or after 5 hours, if no lysis.

The hydrolyzed casein and asparagine media contained 5 mg./ml. and 0.01 M PO₄, 3×10^{-4} M Ca, 1.6×10^{-4} M Mg, and 0.25×10^{-4} M Fe.

Control tubes, containing no phage, were also prepared and the growth determined. The cell count increased to $200 \pm 40 \times 10^6$ in all the media.

Cells suspended in Y.E. plus phage all grow no matter what culture media they come from, but do not lyse, although the rate of growth, in some cases,

¹ In the first series of experiments, cells grown on Y.E. agar or in Y.E. did not lyse at any time when suspended in 10 per cent peptone + phage. After 5 months,

is less than that of the control tubes. Cells from V.I.B. agar, or from liquid Y.E. are only partly infected in Y.E. and produce little or no phage. Cells from the other media produce 1 to 5×10^9 phage/ml.

Cells suspended in V.I.B., T.P.B., hydrolyzed casein, or asparagine, are not infected, no matter which culture media they came from, and no phage is produced. The casein and asparagine media contained Ca, Mg, and Fe, but still no infection occurred. It appears that infection depends on the condition of the cell, as well as upon the presence of certain substances in the culture media.

Effect of the Culture Media on Infection, Growth, Lysis, and Phage Production in the Presence of Phage C

The results of these experiments are shown in Table II. The cells were grown in 5 per cent peptone agar, washed, and suspended in various media containing

TABLE II
Growth and Lysis of KM Culture in Various Media + C Phage

18 hour 5 per cent peptone agar culture, washed twice in water, suspended in media noted $\pm 1 \times 10^8$ C phage/ml.

Media	10 per cent peptone	Y.E.	Asparagine	T.P.B.
Per cent infected.....	100	100	100	0
$\Delta B 10^8$ { +.....	0	20	20	180
{ -.....	10	30	20	0
P/ml. 10^6	12,000	5000	7000	0

C phage. In this instance, the culture medium has less effect on the course of the reactions than in the case of phage T, described above.

Infection, lysis, and phage production occur in 10 per cent peptone, Y.E., or asparagine. No infection takes place in T.P.B., due, no doubt, to the high PO_4 concentration.

Table III shows the results of a series of similar experiments in which the cells from different culture media were suspended in 10 per cent peptone + phage T and plated for colonies on 5 per cent peptone agar, after 1 hour. The results show a marked difference between cultures from V.I.B. or 5 per cent peptone agar and those from Y.E. agar. The V.I.B. or peptone cultures gave 6×10^6 colonies/ml. from the control but only 3×10^6 colonies/ml. from the suspension containing phage; two-thirds of the colonies were infected (lyso-

during which time the stock culture was transferred every 24 hours on 2 per cent peptone agar, the cultures grew at the same rate as the control until a cell concentration of about 150×10^6 was reached, and then lysed.

genic) as shown by the transparent appearance after 48 hours' incubation. The presence of phage in these transparent colonies was checked by streaking on a plate prepared for phage determination.

The culture from Y.E., on the other hand, gave 5×10^6 colonies from the tube containing phage, and nine-tenths of these were infected.

In the culture from the V.I.B. or peptone agar, therefore, 50 per cent of the cells are unable to form colonies and only 30 per cent of the cells form lysogenic colonies, whereas in the culture from the Y.E. agar, only 16 per cent of the cells

TABLE III

Growth, Infection, Lysis, and Phage Production of B. megatherium in 10 Per Cent Peptone + T Phage after Preliminary Growth on Peptone, V.I.B., or Y.E. Agar

5 per cent peptone, V.I.B., or Y.E. agar inoculated from 18 hour 2 per cent peptone slant culture, 18 hours at 35°C., washed off in 10 per cent peptone, centrifuged, and washed in 10 per cent peptone, suspended in 10 per cent peptone. 25 to 30 $\times 10^6$ B/ml. 2 tubes of each culture. 5 $\times 10^6$ P T/ml. added to one tube. Both tubes shaken at 35°C. B/ml. by turbidity. Colonies/ml. by plating on 5 per cent agar. Infected colonies read after 48 hours. Average of six experiments.

Control ΔB = increase after 6 hours in control tube — no phage.

Phage $+\Delta B$ = maximum increase before lysis occurs.

$-\Delta B$ = maximum decrease during lysis.

	Control ΔB 10^6	+ phage		10 ⁶ col./ml. after 1 hr.			Normal 10^6	P/ml. 10^6
		$+\Delta B$ 10^6	$-\Delta B$ 10^6	Control Total 10^6	+ phage			
					Total 10^6	Infected 10^6		
5 per cent peptone agar or V.I.B. agar	180	5	10	6	3	2	1	2500
Y.E. agar	200	80	50	6	5	4.5	0.5	3000

are unable to form colonies and 80 per cent can form lysogenic colonies. Most cells from Y.E. agar, therefore, are able to grow on peptone agar, although they are infected, while fewer cells from peptone or V.I.B. agar are capable of growth after infection.

These results could be accounted for on the basis of selection, since the cultures were grown for 18 hours and there was a very large increase in the number of cells.

The same change in the behavior of the culture occurs, however, after an hour or so, if the cells are grown in Y.E. or 10 per cent peptone. Some growth must occur for the change to take place, but by the time the cell concentration has doubled, the behavior of the culture has changed.

The results of a series of such experiments are shown in Table IV. Cells from 5 per cent peptone agar were suspended in 10 per cent peptone + phage

T. These cells did not grow, and lysis occurred. The colony plate showed that 50 per cent of the cells formed colonies and two-thirds of these were lysogenic. The cells were grown in Y.E. for 1 hour, during which time there was an increase in cell count of about 30 per cent. They were then tested again for growth and lysis in 10 per cent peptone + phage T. They now increased from 30 to 120×10^6 cells per ml. before lysis started, 90 per cent of the cells formed colonies; 95 per cent of these were lysogenic. The cells were centrifuged out of the Y.E. and grown for an hour in 10 per cent peptone, and again tested for growth and lysis in 10 per cent peptone + phage T. They now grew less than those from Y.E. and only 30 per cent formed colonies, 95 per cent of which were

TABLE IV

Infection, Growth, and Lysis of Cells in 10 Per Cent Peptone + T Phage after Preliminary Growth on 5 Per Cent Peptone Agar, Y.E., or 10 Per Cent Peptone

Cells from 18 hour 5 per cent peptone agar suspended in Y.E. and shaken at 35°. Tested for infection and lysis in 10 per cent peptone ± T phage. Y.E. culture centrifuged after one hour and suspended in 10 per cent peptone. Shaken at 35°C. for 1 hour and tested for lysis, etc. Average of four experiments.

B grown on	ΔB Control 10 ⁶	+ phage		Colonies/ml. after 1 hr.		
		+ΔB 10 ⁶	-ΔB 10 ⁶	Control Total 10 ⁸	+ phage	
					Total 10 ⁸	Infected 10 ⁸
5 per cent peptone agar, 18 hrs.	200	0	10	6	3	2
Transfer to Y.E., 1 hr.	180	90	100	5.5	5	4.9
Transfer to 10 per cent peptone 1 hr.	185	60	60	4.0	1.2	1.1

lysogenic. The change from resistant to sensitive condition is therefore rapidly reversible and cannot be readily explained by selection. Control experiments showed that small amounts of Y.E. added to the peptone did not affect the result.

The idea of selection is also contradicted by the results of experiments with cultures isolated from single colonies. Cells from a peptone agar slant were plated on Y.E. agar and on peptone agar. Ten single colonies from each plate were then transferred to 10 per cent peptone and shaken at 35°C. until about 20×10^6 cells/ml. were present. 1×10^8 P/ml. were then added and the tubes shaken as usual. All 20 cultures grew slightly and then lysed. If the original culture contained both sensitive cells, which grow only on peptone, and resistant cells, which grow only on Y.E., it would be expected that the culture derived from colonies from Y.E. plates would be resistant and those from the peptone plates, sensitive. This is not the case. If selection does occur on the agar culture, the change must be readily reversible.

Apparently the cells grown on Y.E. contain some compound(s), possibly an adaptive enzyme, which allows them to continue to grow, after infection. The reverse explanation, that cells grown on peptone synthesize something which causes lysis, appears less probable, since many other media give the same result, whereas the effect of Y.E. is more specific.

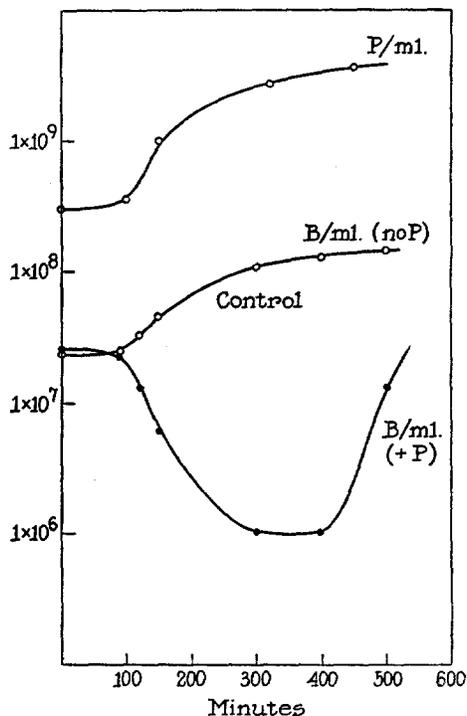


FIG. 1. Growth, lysis, and phage production of *B. megatherium* in 10 per cent peptone, after preliminary growth in 10 per cent peptone. Cells from 10 per cent peptone culture washed with 10 per cent peptone and suspended in 10 per cent peptone. Two tubes. 3×10^8 P/ml. added to one tube and same quantity of boiled P to the other. Both tubes shaken at 35°C . B/ml. by turbidity. P/ml. by plaque count.

In the case of *S. muscae*, Price (1949 a) has found that Y.E. contains some substance which increases lysis. The general result is similar, but with *B. megatherium*, the cells become more, instead of less resistant, after growth in Y.E.

Infection, Cell Growth, and Phage Production of Cells Grown First on Y.E. Agar or in 10 Per Cent Peptone and Then Suspended in 10 Per Cent Peptone + Phage T

The results of these experiments are shown in Figs. 1 and 2.

The culture previously grown in 10 per cent peptone, used in the experiment

reported in Fig. 1, undergoes almost complete lysis, and there is a concomitant increase in phage. 2.5×10^7 cells cleared, and 3×10^9 phage particles appeared, so that, under these conditions, about 100 phage particles are released per cell.

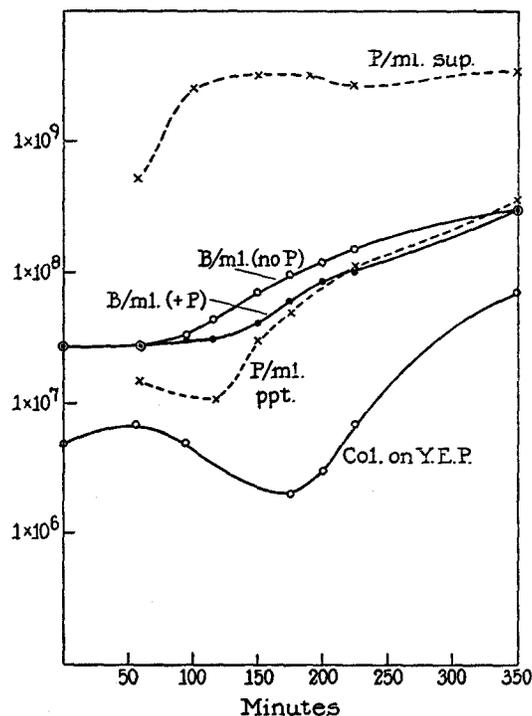


FIG. 2. Growth and phage production of *B. megatherium* in 10 per cent peptone, after preliminary growth on Y.E. agar. Y.E. agar plate inoculated from 2 per cent peptone slant, 18 hours at 35°C. Growth washed off with water, washed with 10 per cent peptone, and cells suspended in 10 per cent peptone. 3×10^8 P/ml. added and tubes shaken at 35°C. Control tubes same, but boiled phage added. B/ml. by turbidity. 1 ml. sample removed as noted. Diluted 1/10 in peptone, centrifuged, super, natant plated for phage. Precipitate washed with peptone, suspended in peptone and plated for phage on Y.E.P. and for colonies on Y.E.P. All colonies on Y.E.P. were translucent; *i.e.*, lysogenic.

This is about the same figure as that reported by Lwoff and Gutman (1950) for lysogenic *megatherium*.

The strain grown on Y.E. agar, under the same conditions, also forms about 3×10^9 phage particles (Fig. 2) without any lysis, so that if the calculation is carried out in the same way as in the preceding experiment, it is necessary to suppose that an infinite number of phage particles are formed per cell. If lysis is supposed to be represented by the difference between the control culture and

the infected culture, then the yield would be 100 particles per cell. If the calculation is made in the same way for the sensitive strain shown in Fig. 1, then the yield in that case would be about 25 particles per cell.

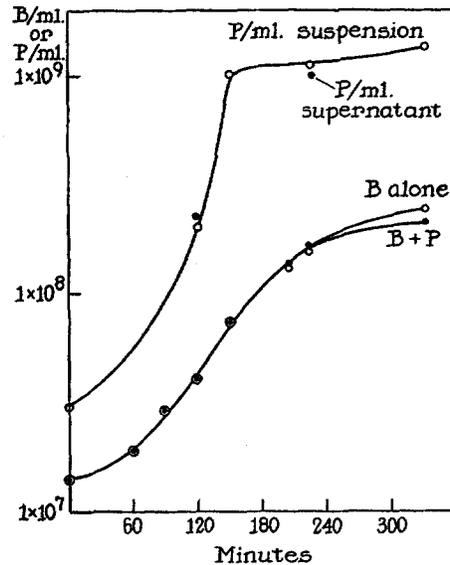


FIG. 3. Increase in cell count in Y.E. medium with and without phage. Cells from 18 hour 5 per cent peptone agar plate washed with Y.E. and suspended in Y.E. Two sets of six tubes each. 1 ml. phage added to one set and 1 ml. boiled phage to the other. Shaken at 35°C. B/ml. by turbidity. P/ml. by plaque count. Points are the average of the six individual determinations. The average deviation of the mean, for the turbidity measurements, was about ± 2 per cent.

Phage determination: In suspension.—Suspension diluted $\frac{0.1}{10} \times \frac{0.1}{100}$ in cold tap water. 0.5 ml. of the last dilution added to 3.5 ml. sensitive B suspension. 1 ml. hot agar added and 1 ml. poured on agar plate.

In supernatant.—1 ml. suspension added to 9 ml. cold water and centrifuged at once. Supernatant diluted $1/10^5$ and plated for phage as above.

Turbidity readings on $1/10$ dilution of suspension in cold water show no changes for at least 30 minutes.

The cells in this culture are all infected, as shown by the fact that the plaque count of the washed suspension is about the same as the cell count by turbidity. The plaque count is somewhat lower than the cell count by turbidity, since the organism grows in chains of about 5 cells each during the first part of the growth cycle. The colony count on Y.E.P. drops at the time the phage is increasing rapidly. The same effect was observed with the lysogenic culture (Northrop,

1951). It is due to the fact that Y.E.P. contains some inhibitory substance, which prevents the growth of single cells unless they are in good condition.

Growth and Phage Production in Y.E.

The results of an experiment in which cells from 5 per cent peptone agar were suspended in Y.E. with or without the addition of T phage are shown in Fig. 3. Six control tubes and six tubes containing phage were used. The points represent the mean of the measurements of the six tubes. The phage was determined

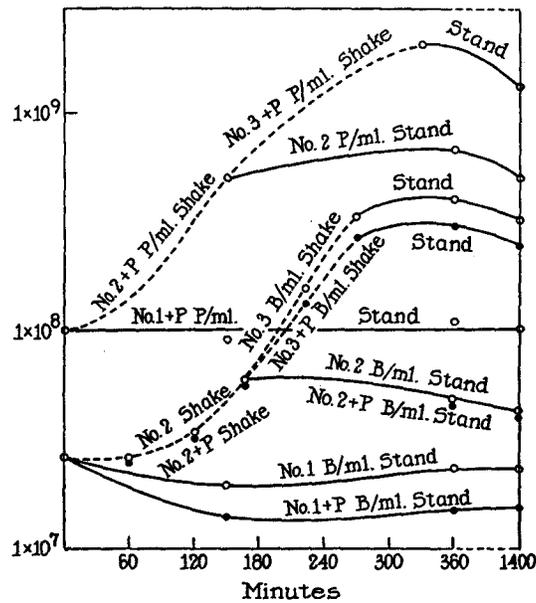


FIG. 4. Effect of shaking on cell growth, lysis, and phage production of resistant culture in Y.E. at 35°C.

both by dilution of the whole suspension and of the supernatant. The two figures agree, showing that no phage is produced during dilution (*cf.* Price, 1951). The objection of Lwoff and Gutman (1950) that lysis and phage production may occur during dilution, therefore, does not apply to this system.

The curves show that the infected culture grows within ± 2 per cent of the rate of the control culture. If it is assumed that the phage is produced by lysis of 2 per cent of the cells, which cannot be detected, then the yield per cell must be about 500. Similar results were reported by Price (1949 *b*) with *S. muscae*.

Effect of Shaking.—The growth and phage production of cultures shaken, or allowed to stand at 35°C. are shown in Fig. 4. Growth and phage production stop and lysis commences, as soon as the culture is allowed to stand. No phage

is produced during lysis, therefore, under these conditions. The lysogenic strain behaves in a similar way (Northrop, 1951, and Lwoff and Gutman, 1950).

Most of the experiments reported in this paper were carried out by Miss Marie King.

Experimental Procedure

Culture.—The *B. megatherium* culture (KM) used was obtained from Professor Krueger.

Difco agar, Difco bacto-peptone, Difco veal infusion medium (V.I.B.), and Difco tryptose phosphate broth (T.P.B.) were used. The peptone medium must be filtered after autoclaving in order to prevent agglutination of the cells.

The cultures were transferred as described previously (Northrop, 1951).

Yeast extract (Y.E.) and yeast extract peptone (Y.E.P.) were prepared as described (Northrop, 1939).

All synthetic media contained 0.01 M PO₄, 3 × 10⁻⁴ M CaCl₂, 1.6 × 10⁻⁴ M MgSO₄, and 0.25 × 10⁻⁴ M ferric ammonium sulfate.

All liquid cultures were shaken in a water bath at 35°C. at the rate of about 300 oscillations a minute.

Test for Lysis in 10 Per Cent Peptone

20 to 25 × 10⁶ cells/ml. suspended in 10 ml. 10 per cent peptone and 1 ml. phage solution containing 3 × 10⁹ P/ml. in 5 per cent peptone added. Tubes shaken at 35°C. and B/ml. determined by turbidity every half-hour for 5 hours. Results expressed as largest increase, or decrease in cell concentration during that time interval. For example,

$$\begin{cases} \Delta B = 20 \\ -\Delta B = 40 \end{cases}$$
 means that the cell content increased from 20 to 40 × 10⁶ B/ml. and then decreased to 0; *i.e.*, there was complete lysis.

SUMMARY

B. megatherium cells were grown in various culture media, centrifuged and washed, and suspended in other culture media containing "C" or "T" phage.

The per cent of infection, rate of growth, lysis, and phage production were determined.

The behavior of the system depends on the culture medium in which the cells were grown and also on the culture medium in which they were mixed with phage.

With the T phage it is possible to set up systems which yield the following results:

1. No infection, normal growth, no phage production.
2. Infection, normal growth, no lysis, phage production.
3. Infection, growth for several hours, lysis, and phage production.

4. Infection, no growth, lysis, and phage production.

The C phage system is less affected by changes in the culture medium.

The change in the behavior of the cells with T phage probably is not due to selection since it occurs without much growth of the culture, and is readily reversible.

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