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Hereditary Aberrancy in Growth of Some *Bacillus megaterium* Strains

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SUMMARY: Two hundred strains of *Bacillus megaterium* recently isolated from soil were tested for an antagonistic effect on a phage-resistant strain of the same species. Fifty-one strains gave a zone of inhibition on a lawn of the indicator strain. This effect was increased by a small dose of ultraviolet radiation which also caused 41 other strains to show an antagonistic effect. Sixteen strains which were completely lysed after u.v. irradiation also produced confluent lysis of the indicator strain. When grown in liquid media, these strains gave irregular growth curves owing to partial lysis during the exponential phase. Lysis was almost complete when such cultures were incubated after exposure to small doses of u.v. radiation. Lysis was always associated with accumulation of the antibacterial principal previously named 'megacin'. Irregular growth in liquid media and production of megacin were strictly correlated. However, only 2 megacinogenic strains were lysogenic and liberated phage. Populations of megacinogenic strains were examined by replica-plating for the presence of non-megacinogenic mutants. Spore suspensions of 4 megacinogenic strains contained non-megacinogenic mutants in various proportions depending on the strain and the particular sample. Non-megacinogenic mutants could not be made to lyse by exposure to u.v. radiation; did not produce any antibacterial effects; and all showed normal patterns of growth in various liquid media. It is supposed that megacinogeny and aberrant growth, as well as inducibility by u.v. radiation, of some *B. megaterium* strains are governed by a single hereditary unit (gene). It cannot be decided at present whether or not this unit is a highly defective prophage.

Some strains of *Bacillus megaterium* are capable of producing an antibacterial substance of protein character which has been named 'megacin' (Ivánovics & Alföldi, 1954, 1955). The production of this substance by growing bacteria is enhanced by irradiation with small doses of ultraviolet (u.v.) radiation which causes mass lysis and liberation of megacin. An interesting phenomenon appeared when organisms of a typical megacinogenic strain (no. 216) were grown with megacin-sensitive bacteria on the surface of solid media; plaques were formed which simulated the growth of lysogenic bacteria inoculated with an indicator strain. No phage particles could be detected in these plaques by either cultivation or electron microscopy (Ivánovics, Alföldi & Lovas, 1957).

We found recently that a considerable proportion of *Bacillus megaterium* strains showed an unusual pattern of growth in liquid medium since, after an initial period of normal growth, the turbidity of the culture decreased owing to mass lysis of the organisms. The lytic phase was in many cases only transient, as the turbidity later increased. The growth curves of these aberrant strains were variable and inconsistent, for even the same inoculum might give different curves when grown in different media. Furthermore, the results obtained in individual experiments carried out under apparently identical

conditions sometimes differed. The aim of the present investigation was to find out whether there is any relation between bacteriocinogeny and aberrant growth in *B. megaterium* strains. A study was also made to estimate the frequency of aberrant strains in soil. It will be shown below that a hereditary character may be responsible for aberrant growth.

METHODS

The methods were in general those used by Ivánovics & Alföldi (1955, 1957). Strains of *Bacillus megaterium* were isolated and identified as described by Ivánovics, Alföldi & Széll (1957) and were maintained by subculture on yeast-extract peptone (YP) agar. Strain 216 was described by Ivánovics & Alföldi (1955, 1957) and by Ivánovics, Alföldi & Lovas (1957), and it was maintained on yeast-extract casein-digest (YDC) agar. A streptomycin-resistant mutant (216S^r) was obtained on streptomycin agar.

Phages. These were the same as those described by Ivánovics, Alföldi & Széll (1957). 'Adapted (mutant) phages, highly specific for strains 216, 605 and 638, respectively, were isolated in the usual way from either phage M₁ or phage M_{ii}. The phages selected for adaptation had a relatively low efficiency of plating (e.o.p.) on the above strains. The e.o.p. of phage M₁ was 10^{-6} on strain 216 and 2×10^{-6} on strain 605, when the values for adapted phages was taken as 1. Phage M_{ii} had an e.o.p. of 10^{-5} on strain 638 before adaptation. The differences between the e.o.p. values of wild type and adapted phages provided a highly reliable means for identification of individual strains.

Nutrient media. By broth is meant horse meat broth containing 1 % (w/v) peptone (Richter, Budapest). Yeast extract peptone medium (YP) and yeast extract+enzymic digest of casein medium (YDC) have been described elsewhere (Ivánovics & Alföldi, 1955, 1957). Solid media contained 1.5 % (w/v) agar. Agar medium for the sporulation of *Bacillus megaterium* contained 0.1 % (w/v) enzymic digest of casein (Aminosol, Vitrum, Stockholm) as described by Tarr (1932).

Preparation of spore suspensions. Bacteria were grown either on Tarr's agar or on YP agar for several days at 37°. Spores formed in 95–98 % of the organisms under these conditions. Spores were washed twice with saline and stored in the refrigerator.

Titration of megacin. A strain of *Bacillus megaterium*, Mut-C, which is resistant to megaterium phages, was used (Ivánovics, Alföldi & Lovas, 1957). The indicator plates used for megacin titrations were also used in a screening test for iso-antagonistic (megacinogenic) strains. In this case, a drop of a young culture of the test strain was spotted on the surface of the indicator plate and irradiated with a high pressure u.v. lamp for 6 sec. before incubation.

The source of ultraviolet light. Two sources were used: a 'Hanau' high-pressure lamp used at a distance of 25 cm., as described by Ivánovics & Alföldi (1955, 1957); and a low-pressure lamp, emitting 7 erg./sec./mm.² at 2537 Å. at 1 m. on the optical axis.

Isolation of non-megacinogenic mutants from megacinogenic strains. Stock

spore suspensions or spores harvested from cultures grown from u.v.-irradiated spores were used. In the latter case, YP plates spread with *c.* 10^7 spores were exposed to the high-pressure u.v. lamp for 160–240 sec. After incubating the plates at 37° for 4–5 days, there were *c.* 30–200 colonies, consisting mainly of spores, which were washed off and stored in the refrigerator. This material is referred to below as ‘u.v. spores’.

One-tenth ml. samples of appropriate dilutions of spore suspensions, containing 40–70 colony-formers, were spread on several meat extract agar plates, the agar content being 2.5 %. After incubation overnight at 30°, the plates were placed in an incubator at 37° and kept there until the colonies were 1.5–2 mm. in diameter. These colonies were then tested by replica-plating (Lederberg & Lederberg, 1952) to nutrient agar plates covered with an agar layer containing megacin-sensitive bacteria. Immediately after transfer of the test colonies, the indicator plates were exposed to the ‘Hanau’ lamp for 6 sec. After further incubation, megacinogenic colonies produced either clear plaques or colonies surrounded by a growth-free zone. Colonies which did not show an antibacterial effect were re-streaked from the test plate and retested several times using young cultures in YP medium. Only isolates which did not show any antagonistic effect were accepted as non-megacinogenic.

Identification of non-megacinogenic isolates. *Bacillus megaterium* strain 216 was identified by the serological specificities of its cell wall, using rabbit antiserum in an agglutination test (Ivánovics, 1955). Resistance to streptomycin was checked by streaking on agar containing 20 µg. streptomycin/ml. agar.

The phage sensitivity of mutants was tested by spotting decimal dilutions of the phages on 10^7 organisms of the test strain incorporated in a soft agar layer. When highly specific ‘adapted’ phages were used, dilutions of the wild-type phage were included as a control.

RESULTS

The characteristics of aberrant Bacillus megaterium strains

Screening of strains by their iso-antagonistic effects. Iso-antagonistic effects between strains of *Bacillus megaterium* (Ivánovics & Alföldi, 1955) served as markers for defining groups among strains recently isolated from soil. The criteria of an iso-antagonistic effect was that the strain should produce a zone of inhibition on the surface of an indicator plate seeded with a phage-resistant strain of *B. megaterium*. Two identical indicator plates, each spotted with a suspension of the test strain, were prepared. One was u.v. irradiated before incubation, while the second, which was not exposed to u.v. radiation, served as a control. The test strains were then classified by their colonial appearance, as follows: (1) no inhibition around the colony; (2) an inhibition zone not exceeding 2 mm. in width; (3) a zone greater than 2 mm. in width; and (4) a growth-free area of diameter 10–14 mm. at the site of inoculation. Irradiation of the plates before incubation either induced or enhanced the iso-antagonistic effect with many of the strains. Table 1 gives 200 strains, recently isolated from

soil, classified in this way. Sixteen strains gave maximum inhibition on the indicator plates and these form the main subject of the following study.

Table 1. *Classification of 200 strains of Bacillus megaterium according to their iso-antagonistic effect*

No u.v. irradiation		After u.v. irradiation	
No effect	149	No effect	108
		Zone appeared	38
		Clear plaque	3
Zone less than 2 mm.	44	Same as without u.v.	5
		Zone increased	29
		Clear plaque	10
Zone broader than 2 mm.	7	Same as without u.v.	0
		Zone increased	4
		Clear plaque	3
Total	200		200

Cultivation of strains of Bacillus megaterium in liquid media. Media were inoculated from an overnight YP or YDC agar culture and growth of the organism followed by measuring the optical density of the cultures, aeration and turbidity measurements being carried out as described by Ivánovics & Alföldi (1955, 1957). For technical reasons, the experiments were ended after 10 hr. A typical megacinnogenic strain (no. 216; Ivánovics & Alföldi, 1955, 1957) was also studied. All the 17 strains which showed a marked iso-antagonistic effect in screening tests were grown several times in different liquid media during these investigations over a period of nearly two years. In one series of experiments, only 3 strains gave a normal curve, the remaining 14 being aberrant. By 'normal' growth, we mean logarithmic increase after a lag phase of 3 hr. The shapes of the aberrant curves were rather variable; curves from some typical experiments are shown in Figs. 1 and 2.

The growth curve of a given strain might vary markedly from one experiment to the next, although all experiments were carried out under the same conditions. Figure 3 shows variable results obtained with strain 605. The variability of the growth curves should not be related to any detail of the experimental technique. However, the medium generally had a marked effect on the outcome of the experiment. Thus, some strains gave very aberrant curves when grown in YP medium while the same inoculum grew normally in another medium, such as YDC medium. We are not able to give a reasonable explanation for the observed discrepancies between individual experiments so that the lysis of these strains of *Bacillus megaterium* has at present to be attributed to an 'uncontrollable' factor or factors.

Eight strains which did not show an iso-antagonistic effect even after exposure to u.v. radiation were also included for comparison. All these strains gave normal growth in all media tested. Furthermore, aberrant growth could not be produced even by a small dose of u.v. radiation. Similar results were obtained with 6 strains which showed a moderate iso-antagonistic effect

(i.e. a zone of less than 2 mm. diameter, not increased by u.v. irradiation). All the above observations support our assumption that a marked iso-antagonistic effect in the screening test was well correlated with aberrant growth in liquid medium. Also, the degree to which iso-antagonistic effects are enhanced by u.v. irradiation serves as an efficient marker for the isolation of strains exhibiting aberrant growth.

Fig. 1

Fig. 2

Fig. 1. Growth of *Bacillus megaterium* strain 594 in various media. Media were inoculated from YDC agar. Size of inoculum: 3×10^6 colony-formers. Amount of medium: 10 ml. ●—●, meat extract; ○—○, YP; ◐—◐, YDC.

Fig. 2. Growth of *Bacillus megaterium* strain 604 in various media. ●—●, meat extract; ○—○, YP; ◐—◐, YDC.

Investigation of the aberrant strains for lysogeny. Although the indicator strain used in the screening test for iso-antagonistic effects was assumed to be entirely resistant to megaterium phages, we had also to consider whether the antagonistic effect and its enhancement by u.v. irradiation was due to a carried temperate phage. The difficulty in detecting small amounts of temperate phage in cultures of strains which may simultaneously elaborate megacin is that the latter kills the indicator strain when lysates or supernatant fluids of the cultures are assayed for infective centres. This was overcome by separating hypothetical phage from the culture by high-speed centrifugation of supernatant fluids, after sterilization by streptomycin, and subsequent assay for free phage by plating the deposit on a streptomycin-resistant indicator strain.

Each of the 16 aberrant strains was examined. Cultures in YDC medium containing 2.5×10^8 – 3.5×10^8 bacteria/ml. were first centrifuged at 3000 rev./min. for 20 min. to remove most of the bacteria. The supernatant fluids were

collected and streptomycin (20 $\mu\text{g./ml.}$ suspension) added to prevent the growth of residual bacteria. Six ml. of supernatant fluid were then centrifuged at 100,000 g for 1 hr., the supernatant fluid discarded and the pellet resuspended in YP medium. The suspension was again centrifuged at 100,000 g for 1 hr., and the pellet suspended in 6 ml. YP medium containing streptomycin. One ml. of each sample was then plated with a streptomycin-resistant phage-sensitive indicator strain (*Bacillus megaterium*, strain 337a, 337b or KM) in an agar layer containing streptomycin. Fourteen of the 16 aberrant strains did not produce any plaques; that is, *c.* 10^9 organisms of these strains did not yield even one infective phage particle. Two strains (508 and 597) proved to be lysogenic and gave small numbers of infective centres, the observed yield being 1 plaque from 50 bacteria of strain 508 or from 400 bacteria of strain 597. The efficiency of the above method was checked by treating a suspension of the megaterium phage M_1 (10^{10} particles/ml.) in the same way; 98 % of the original phage was recovered. These results show clearly that most aberrant, that is megacinogenic, strains are non-lysogenic. Two strains yielded small numbers of plaque-forming particles, however, so that lysogeny and megacinogeny can occur simultaneously in some strains.

Table 2. *The rate of growth and lysis expressed in optical density values of irradiated and control cultures of 17 strains of Bacillus megaterium in YDC medium*

Young exponentially growing cultures at an optical density (OD) of 0.2 were u.v.-irradiated. After re-incubation the extent of residual growth is expressed by the peak value OD, and the extent of lysis is expressed by the OD values measured at the end of experiment. The *antibacterial titre* is expressed by the reciprocal of highest effective dilution of supernatant fluid. Zero means no antibacterial effect undiluted material.

Strain	Irradiated cultures			Non-irradiated culture		
	Peak value	At the end of experiment	Anti-bacterial titre	Peak value	At the end of experiment	Anti-bacterial titre
	Rel. optical density values			Rel. optical density values		
216	0.56	0.04	40,000	1.10	1.10	0
507	0.35	0.04	100	1.40	1.40	0
508	0.26	0.04	10	1.20	1.20	0
519	0.56	0.13	100			
527	0.25	0.04	1	1.30	1.30	0
575	0.50	0.20	10	1.30	1.30	0
594				0.20	0.07	100
597	0.50	0.15	10	1.30	1.30	0
598	0.50	0.11	100	1.30	1.30	0
604	0.27	0.09	10	0.30	0.10	1
605	0.28	0.03	10	1.40	1.40	0
637	0.50	0.13	10	1.35	1.35	0
638	0.60	0.40	1	1.40	1.40	0
659	0.25	0.04	10	1.05	1.05	0
669	0.23	0.04	10	1.30	1.30	0
675	0.22	0.05	10	1.20	1.20	0
681	0.30	0.08	10	1.30	1.30	0
689	0.53	0.17	100	1.40	1.40	0

Lysis induced by u.v. irradiation of cultures of Bacillus megaterium. Twenty ml. portions of YDC medium were inoculated from an overnight agar culture of the test strain and aerated by gentle shaking at 35°. When the viable count was $c. 3 \times 10^7/\text{ml.}$, 10 ml. were placed in a Petri dish and irradiated with a high-pressure lamp for 60 sec. The irradiated sample, and also a control un-irradiated culture, were then re-incubated. The megacin content of each culture was titrated at the end of each experiment. Table 2 shows that all the aberrant strains tested, lysed after irradiation and re-incubation. However, the amount

Fig. 3

Fig. 4

Fig. 3. Growth of *Bacillus megaterium* strain 605 in horse meat extract broth in several experiments. Date of experiments: ○, 30. v. 1956; ●, 5. vi. 1956; ●, 2. i. 1957; ○, 16. v. 1957.

Fig. 4. Growth of *Bacillus megaterium* strain 216 and its 216 meg⁻ mutant after u.v. irradiation. Megacin titres at the end of cultivation: 1/40,000 in lysate of 216. No antibacterial effect was shown by the supernatant fluid of strain 216 meg⁻. ○—○, strain 216; ●—●, strain 216 meg⁻.

of residual growth, that is, the maximum optical density after irradiation, differed considerably with different strains. In some cases, the un-irradiated sample also showed early lysis (see strains 519 and 594 in Table 2). It must be stressed that early and apparently spontaneous lysis occurred more often when broth or YP medium was used instead of YDC medium. The megacin titres of cultures lysed by u.v. irradiation also differed enormously according to the strain. The titres appeared to be influenced also by the degree of lysis and by the maximum growth that was reached before lysis. When no detectable lysis occurred, as in the case of the un-irradiated of each culture, no megacin formation could be detected.

Several attempts were made to detect the presence of infective phage particles in lysates of irradiated cultures but only two of the strains mentioned above yielded phage, namely, 508 and 597, whose cultures contained respectively 2×10^6 and 5×10^8 plaque-forming particles/ml. A number of strains which did not show an iso-antagonistic effect in the screening test were also

included in these experiments. None of them could be made to lyse by u.v. irradiation in various liquid media. Accordingly, no antibacterial effect was shown by supernatants of their cultures.

Analysis of populations of megacinogenic strains

General considerations. All the experiments described so far used inocula prepared from confluent growth on an agar slope and not from a single colony. The inocula were thus heterogeneous in origin. It should, however, be borne in mind that even when a suspension of vegetative *Bacillus megaterium* is used, colonies forming on solid media cannot be considered to be pure clones because of chain formation by this species. Homogeneous clones can only be obtained from a well-dispersed suspension of spores.

Spore suspensions were therefore used exclusively in the study of the genetic composition of the megacinogenic strains. Any vegetative organisms present (c. 5 %) appeared to be degenerate, for their staining characteristics suggested advanced lysis. These remnants of non-sporing organisms have been disregarded below, whether or not they were capable of forming colonies. It would have been easy to get rid of these organisms by heat shock but this treatment was avoided because we feared that some spores of low heat-resistance might be killed, so leading to a change in the composition of the population. Our aim has been to isolate non-megacinogenic mutants from strains which showed iso-antagonistic effects as well as aberrant growth when grown in liquid media. It was hoped that u.v. irradiation of stock spore suspensions would promote the appearance of such mutants, on the assumption that irradiation would lead to lysis of the megacinogenic members of the population (Ivánovics, Alföldi & Lovas, 1957).

Any clone isolated from a megacinogenic strain which did not show an iso-antagonistic effect when tested on indicator plates, even after u.v. irradiation, was designated 'meg⁻', provided this character remained stable on sub-culture. For example, 216 meg⁻ describes such a clone isolated from the megacinogenic strain 216. The identity of these clones was checked by their sensitivity to 'adapted' highly specific phages; or when these were not available, by the use of a set of megaterium phages differing in host range (Ivánovics, Alföldi & Széll, 1957). Other markers used were the serological character of the cell wall (Ivánovics, 1955; Ivánovics, Alföldi & Széll, 1957) and resistance to streptomycin. A non-megacinogenic mutant was accepted as a descendant of the test strain if it shared at least one marker character with the parent strain.

Experiments with strain 216. This strain is of particular interest because it is highly megacinogenic. Table 3 gives details of experiments carried out with 216, which was extremely sensitive to streptomycin, and with streptomycin-resistant mutant of it, designated 216 S^r. Both the mutant and parent stocks were attacked to the same extent by an 'adapted' phage. In 7 experiments, 8798 separate colonies were tested by replica-plating to indicator plates sown with a megacin-sensitive strain. Altogether, 52 colonies were found which did not produce an iso-antagonistic effect after small doses of u.v. radiation. These

strains were proved to be non-megacinogenic on further investigation. Thirty-two non-megacinogenic colonies were isolated from strain 216 S^r in the same way. All the non-megacinogenic strains possessed the same phage sensitivity, antigenic structure and resistance to streptomycin as the parent strain.

Table 3. *Frequency of non-megacinogenic mutants in spore suspension of strains 216 and 216 S^r of Bacillus megaterium*

No. of experiment	Strain	Spore material	No. of colonies examined	No. of non-megacinogenic colonies
1	216	Stock material	1167	2
2	216	'u.v. spore' 28/29	1016	0
3	216	'u.v. spore' 55/61	438	11
4	216	'u.v. spore' 69	985	7
5	216S ^r	'u.v. spore' 55/57	994	2
6	216S ^r	'u.v. spore' 56/72	1436	15
7	216S ^r	'u.v. spore' 56/74	2762	15

The non-megacinogenic state has proved to be stable, for no back mutants have yet been detected during a large number of subcultures. In one experiment, one 216 meg⁻ isolate was kept in the logarithmic phase of growth by serial transfer to fresh medium, samples of the culture being tested at intervals by plating in an agar layer with megacin-sensitive cells; but no megacinogenic cells were isolated from the several hundred million cells cultivated.

None of the 52 non-megacinogenic isolates could be induced to lyse by exposure of cultures in liquid medium (YDC) to u.v. radiation. Nor did the supernatant fluids of irradiated cultures show any antibacterial effect when tested on indicator plates after re-incubation, thus proving a complete lack of megacin production by these cultures. Figure 4 illustrates the outcome of such an experiment. Another observation made during cultivation of the non-megacinogenic mutants was that these invariably gave normal growth curves so that loss of megacinogeny appears to be paralleled by loss of an aberrant pattern of growth as well as by loss of inducibility by u.v. radiation.

Experiments with other megacinogenic strains. Experiments similar to those described above were carried out with 4 megacinogenic strains of different origin. Strain 597, besides being megacinogenic, liberated a temperate phage during exponential growth, and lysis after u.v. irradiation was accompanied by mass liberation of phage particles. This strain, and also strain 575, were identified by their patterns of sensitivity to a set of *Bacillus megaterium* phages. In the case of strains 605 and 638, highly specific 'adapted' phages were available for identification.

Table 4 shows that the proportion of non-megacinogenic isolates varied in different experiments. Two isolates were obtained from the lysogenic strain 597, one of which proved to have lost its lysogenicity, while the second carried the same phage as the parent strain. The lysogenic isolate was, however, no longer inducible by u.v. radiation.

A high proportion of non-megacinogenic mutants were isolated from strain

605. No differences were found when either stock or 'u.v. spore' material was used. We consider that it is unjustifiable to conclude from the difference observed between the frequency of non-megacinogenic mutants obtained in these two experiments that u.v. irradiation promotes the isolation of these mutants.

Table 4. *Frequency of non-megacinogenic mutants in spore suspension of individual megacinogenic strains of Bacillus megaterium*

Strain	Spore material	No. of colonies examined	No. of non-megacinogenic colonies
575	'u.v. spore' 56	1746	5
597	'u.v. spore' 54	1065	0
597	'u.v. spore' 56	1033	2
605	Stock material	359	35
605	'u.v. spore' 54	714	63
638	Stock material	652	0
638	'u.v. spore'	1086	22

Sensitivity of spores of the parent and the non-megacinogenic strains to u.v.-irradiation. Although wild type organisms were lysed when their exponentially growing cultures were irradiated and re-incubated, lysis did not occur when spores of these strains were irradiated and plated on agar. Spore suspensions of strains 216 and 216 meg⁻ were washed twice with saline, poured into Petri dishes to give a layer not more than 2 mm. thick, and then irradiated with a low-pressure mercury lamp while the dish was gently swirled by hand. At intervals during irradiation, appropriate dilutions were plated on YP or on meat-extract agar and incubated. There was no significant difference between the survival curves of megacinogenic and of non-megacinogenic spores, both appearing to be equally sensitive to u.v. radiation. This implies that spores are not induced to lyse by u.v. radiation even when they are plated immediately after irradiation. The shapes of the curves suggest a multi-hit mode of killing. These results are in sharp contrast with our previous observations on the inducibility of exponentially-growing cultures (Ivánovics & Alföldi, 1957).

DISCUSSION

Systematic investigations involving 200 strains of *Bacillus megaterium* recently isolated from soil provide further data concerning the occurrence of strains capable of producing an antibacterial principle identical or similar to that which has been described recently and named 'megacin' (Ivánovics & Alföldi, 1954, 1955). Nearly half these strains elaborated megacin after exposure to small doses of u.v. radiation. The release of the antibacterial principle follows partial or complete lysis of the cultures and is enhanced or induced by the small doses of u.v. radiation acting on growing organisms. Strains which were highly inducible also displayed irregularities in their growth curves when cultivated in different complete media. It is supposed

that the aberrant growth pattern of these strains is elicited by some unknown agents which exist or appear during logarithmic growth in liquid media. Megacinogeny and aberrant growth pattern are therefore different manifestations of some factor occurring in a large proportion of *Bacillus megaterium* strains.

The behaviour of these aberrant strains is in many ways reminiscent of the behaviour of lysogenic bacteria. Nevertheless only 2 of 16 aberrant strains yield infective phage particles from their cultures; the remaining 14 strains did not yield even 1 infective particle/10⁹ bacteria. Similarly, no infective particles could be demonstrated in cultures of megacinogenic strains after mass lysis had been induced by u.v. irradiation. Lysogeny and megacinogeny may coexist in the same bacterium as was shown in the case of 2 megacinogenic strains.

We think it can safely be said that megacinogeny is not necessarily associated with lysogeny, at least, not with a lysogenic relationship which results in the liberation of infective phage particles. However, the possibility of non-infective phage production is not excluded. A thorough study of strain 216, involving electron microscopy of lysing cells (Ivánovics, Alföldi & Lovas, 1957), did not reveal any structures reminiscent of defective phage production. Such a study, however, only excludes incomplete phage production at a morphological level and does not disprove a possible synthesis of some non-organized phage material governed by a highly degraded prophage.

As non-megacinogenic mutants were isolated from megacinogenic strains, it is safe to assume that there is a factor perpetuated in megacinogenic bacteria which governs the hereditary character of megacinogeny. This gene, whether located in the chromosomal or in the cytoplasmic substance of the organism, is responsible for phenomena such as inducibility to lysis, which in turn also shows itself by an aberrant pattern of growth in liquid media, as well as by megacin production. The genomes of *Bacillus megaterium* strains differ as to megacinogeny. Therefore, our observations may be of significance in the ecology of this species.

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