# OBSERVATIONS ON LYSOGENESIS IN B. MEGATERIUM AND ON MEGACINE, THE ANTIBACTERIAL PRINCIPLE OF THIS BACILLUS SPECIES

By

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In studying lysogenesis in B. megaterium, our attention has been focussed upon strains isolated from soil, animal excrements and air. At the same time experiments were carried out with standard lysogenic strains well-known in the literature. We observed that some of the strains isolated produced an antibacterial principle. In this respect, two of our strains displayed a particularly remarkable behaviour in that their fresh cultures lysed on the action of a small dose of ultraviolet light, and the resulting lysate exerted a more or less intense antibacterial effect on B. megaterium strains. It was found that the antibacterial principle was not a phage, but should be regarded as a substance of protein character as yet largely undefined. We suggested to term it megacine [1]. In the present paper we shall discuss our observations in detail.

## Material and methods

*B. megaterium strains.* The strains isolated were identified on the basis of their morphological and cultural characteristics [2, 3]. In addition, serological studies [4, 5] were made for the presence of D-glutamylpolypeptide [6]. The strains were isolated in the course of recent years, and have been maintained by monthly transfer on horse-meat peptone agar. Besides, suspensions of their washed spores have been stored in the frozen state in order to exclude any potential changes of the strains.

Sixteen of the strains have been studied for years for their cultural qualities and antigenic structure. The pertaining observations will be published elsewhere.

The strain labelled NRRL B-938 was kindly supplied by *C. W. Hesseltine* (Peoria). The following standard laboratory strains of B. megaterium were utilized: the «mutilate» and lysogenic strains 899 [1], both sent us most kindly by professor *A. Lwoff* (Paris); strain 899, a sporogenous and lysogenic one, and strains 899 IId and 7—0—11, both lysogenic, were received from the collection of *H. J. Welshimer* (Richmond), to whom we are greatly indebted. In this paper the latter strains are marked by the letter «W» in distinction from the others.

*Media.* Besides horse-meat peptone agar, a considerable number of different media were used. Of these only those will be mentioned which yielded invariably reproducible results. The yeast extracts employed in nutrient media were prepared by acid or neutral extraction.

The «acid» extract of baker's yeast was prepared as described by *Lwoff* and *Gutman* [8]. The «neutral» yeast extract was made as follows: 2 kg of baker's yeast were suspended in 8 litres of tap water, then autoclaved at 110° C for 30 minutes. The supernatant obtained after sedimentation was made to pass through an asbestos filter.

Acid hydrolysate of casein: 100 g of technical casein of good quality were suspended in 500 ml of 20 per cent hydrochloric acid and heated on a sand bath for 24 hours. The hydrochloric acid was distilled in vacuum, the remains dissolved in 200 ml of water, adjusted with N NaOH

to pH 3,4, and the volume made up to 1 litre with distilled water. The hot solution was repeatedly treated with Norit until it turned perfectly colourless after filtration. The hydrolysate was kept under toluol at room temperature.

YC medium: To every 100 ml of «acid» yeast extract 50 ml of acid hydrolysed casein solution and 20 ml of M/15 Soerensen phosphate buffer solution of pH 7,2 were added and made up to 1 litre with water. The pH was adjusted to 7,2.

YP medium. In 200 ml of «neutral» yeast extract 10 g of Witte's peptone were dissolved and the volume made up to 1 litre. The pH was adjusted to 7,2.

Medium 9. In 200 ml of «neutral» yeast extract 5 g of enzyme hydrolysed casein, the commercial *Protolysate* (Mead Johnson & Co.), were dissolved. The volume was made up to 1 litre and the pH adjusted to 7,2. The agar media were prepared by adding 15 g of agar to each litre of the liquid media described above.

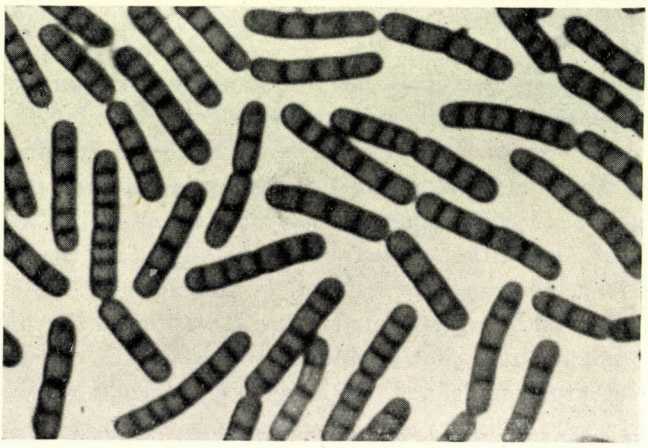


*Fig. 1.* Stained preparation of 10-hour culture of B. megaterium strain 213 obtained in medium 9. (Stained according to Gutstein with methylene violet after mordanting with tannic acid. Completely intact multicellular rods)

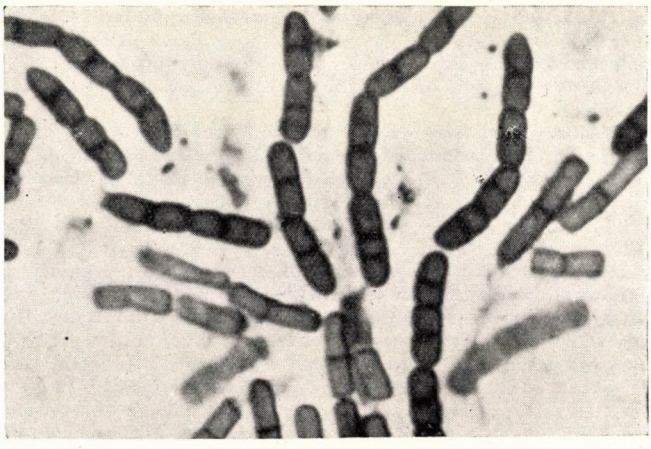


*Fig. 2.* Stained preparation of culture of B. megaterium strain 216. (Gutstein's staining. Besides intact rods with distinct cellular outlines, there are a great number of more or less desintegrated organisms with unstained cellular walls)

*Preparation of bacterial cultures and estimation of their growth.* Cultures prepared in liquid media were invariably aerated by shaking. Erlenmeyer flasks were used in preparing the cultures. A 10 cm long sealed glass tube 14,5 to 14,7 mm in diameter was fused slightly inclined onto the side of each flask. On tilting the flask part of the culture was taken up by the tube. The tube fitted into the corresponding recipient of our optical densimeter so that the growth of the culture could be easily checked in every period. Not more than 20 ml of medium were filled in each 100 ml flasks.

An electromagnetic shaker performing 110 oscillations per minute at an amplitude of 3 cm was used in aerating the cultures, at a temperature of 34° to 35° C.

In measuring culture growth, an «Orifot» microphotometer with a photoelectric vacuum cell was employed. A frontal extension was constructed to this apparatus to hold the tubes fused onto the flasks, or test tubes of an equal diameter. Measurements were carried out in monochromatic light by applying an orange colour filter. Optical density, expressing the density of the

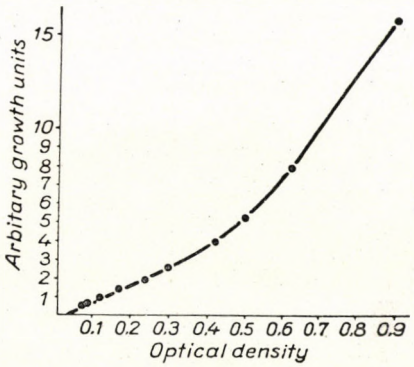



*Fig. 3.* Calibration curve plotted on the basis of an approximately 10-hour culture of B. megaterium strain 213

bacterial suspension, is determined by the logarithmic value of the ratio between incident light and light that passed through. Up to values of 0,350 to 0,400 the optical density of the culture follows the Beer—Lambert's law. Beyond these values, the curve indicating growth deviates from the straight line. Since in a few exceptional cases some means had become necessary whereby to compare quantitative data concerning also some other properties of suspensions with optical density values exceeding above, a calibration curve of the B. megaterium suspension was prepared. An approximately 10-hour culture of B. megaterium strain 213 prepared in medium 9 was used in plotting this curve. Although in the course of the experiments reported below we worked chiefly with strains 119 and 216, for the purposes of the calibration curve we thought them less suitable than strain 213, because in their 8 to 10-hour cultures a considerable number of bacilli were observed to disintegrate spontaneously, whereas in equally old cultures of strain 213 apparently intact multicellular organisms characteristic of B. megaterium were found, consisting of 4 to 8 units (Figs. 1 and 2).

Fig. 3 shows the calibration curve plotted on the basis of varying dilutions of a culture of optical density 0,9 from strain 213.

The following procedure had been adopted in selecting the so-called «growth units» of arbitrary character shown on the ordinate. An 1 : 16 dilution of the culture of strain 213 with an optical density of 0,120 was plated on agar surface. It was found that $10^7$/ml colonies developed of the diluted culture. This was regarded as the «growth unit». The «growth unit» values, which with the aid of the curve may be computed from the determined values of optical density, do not by any means represent in each case the actually prevailing conditions ; they, at the utmost, approximate them. Yet, although influenced by the extent of chain formation and the degree of cellular desintegration, the «growth unit» is still more characteristic of the growing bacterial culture than is optical density.

*Source of ultraviolet light.* A «Hanau» mercury lamp, factory number SR 300 M 197557 was used. Its performance data, as measured by I. Kecskeméty and L. Szalay, to whom our thanks are due, were found to be as follows. The energy passing in one sec through 1 $cm^2$ surface at 25 cm distance in the optical axis amounted to $7{,}3 \cdot 10^5$ erg, of which $3{,}3 \cdot 10^5$ erg fell to the spectral range below 3500 Å. The lamp was used without a filter, i. e., heterochromatic rays were applied in the course of experiments.

## Experimental results

### *Lysogenicity of B. megaterium strains*

Cultures obtained in YC medium by aeration for 10 to 16 hours were diluted to 0,300 optical density. Serial dilutions of $10^{-2}$, $10^{-3}$, etc. were prepared from the suspensions. The same medium was invariably used as a diluent. From similarly obtained cultures of the phage-sensitive «mutilate» strain a dilution of an optical density of 0,400 was prepared and 0,8 ml of this were added to 0,2 ml of each culture dilution tested. One ml of YC medium containing 1 per cent agar which had been melted and cooled to 60° C was added to these mixtures and layered on YC agar plates.

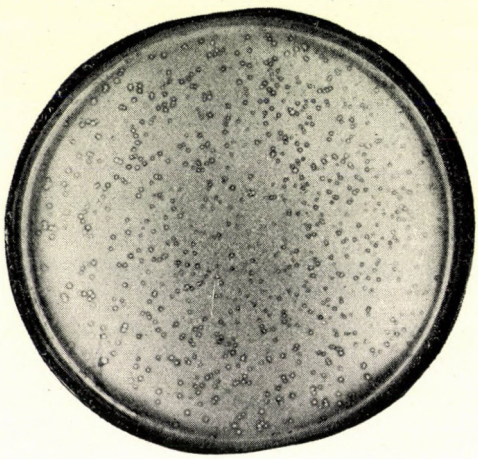


*Fig. 4.* Effect of strain 216 on growth of the «mutilate» strain (x0,5, approx.)

The standard lysogenic strains 899 [1], W899, W899 IId and W7-0-11 yielded without exception numerous isolated plaques. Apart from these phage colonies, plaques containing a central colony were frequently found. Of the 16 B. megaterium strains isolated by us, 15 have proved not lysogenic. A similar behaviour was displayed by the strain labelled NRRL B-938. One of our strains (strain 56) isolated from air yielded a considerable number of phage plaques.

Two of our strains, 119 and 216, behaved in a particularly remarkable manner. They had been isolated 2 or 3 years ago from soil samples and mouse faeces, respectively. On pouring a dilute suspension of strain 216 together with the «mutilate» suspension into a thin layer of agar the patterns shown in Figs. 4 and 5 were obtained.

It is striking that there are no plaques to be seen, whereas around the isolated colonies zones free of growth are visible everywhere. On identification

these colonies proved to be of strain 216. When the colonies surrounded by a zone, and their immediate environments, were removed, added to dilute «mutilate» suspension and then cultivated, there was no dissolution to be observed. On repeating the above experiment with the culture so obtained, a result identical with those just described was obtained.

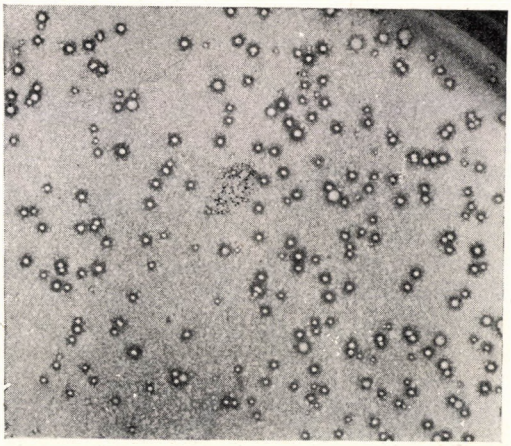


*Fig. 5.* A portion of Fig. 4 enlarged about 2,5 times

With a view to subjecting the phenomenon described to a closer study YC agar plates seeded with the «mutilate» were spotted with 0,03 ml of 8 to 16-hour YC cultures of various B. megaterium strains. Each culture was made in duplicate. Half of the plates was incubated at once, while the others were exposed to UV light from a distance of 45 cm, for 45 seconds before incubation. (Table I summarizes the behaviour displayed by our strains on plates not exposed to UV light.)

**Table I**

*Behaviour of B. megaterium strains spotted onto surface of YC agar coated with «mutilate» strain*

| Characteristics of strain | Number of strains | Behaviour of colony at site of spots |
|---|---|---|
| Non-lysogenic ..... | 4 | Giant colony with distinct margins without inhibition zone |
| Lysogenic ..... | 2 | Giant colony with distinct margins without inhibition zone |
| Non-lysogenic ..... | 10 | 0,5 to 1,5 mm wide inhibition zone around giant colony with distinct margins |
| Lysogenic ..... | 2 | 0,5 to 1,5 mm wide inhibition zone around giant colony with distinct margins |
| Non-lysogenic ..... | 3 | At least 2,5 to 3 mm wide inhibition zone surrounding giant colony with distinct margins |

Quite conspicuous differences were observed on our plates ir radiated with UV light for short periods. They are shown in Figs. 6 and 7.

It attracts attention that no growth whatsoever is noticeable on the «mutilate» layer spotted with strains 119 and 216 and irradiated. Strain 299, on the other hand, although usually developing without a zone, can be seen to have become surrounded by a broad inhibition zone upon the effect of irradiation. Growth of the lysogenic strains — also of strain 899 — exposed to the effect of UV light differed in no way from that of non-irradiated cultures.

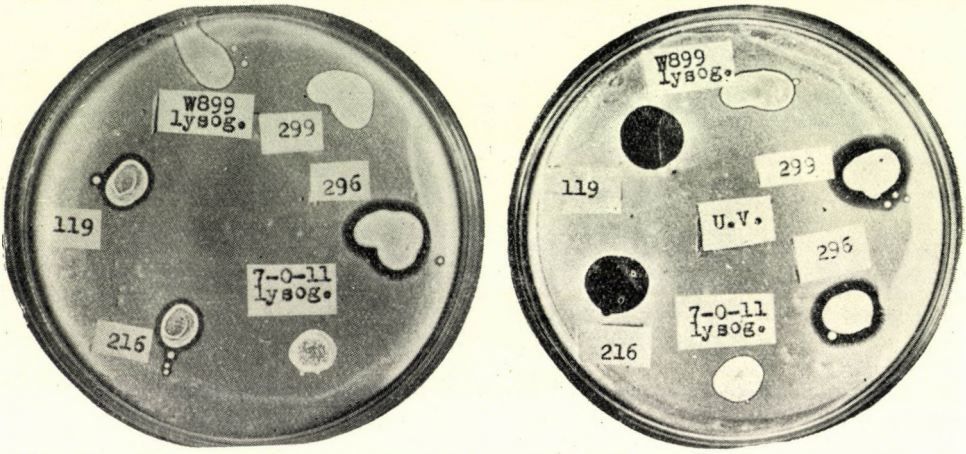


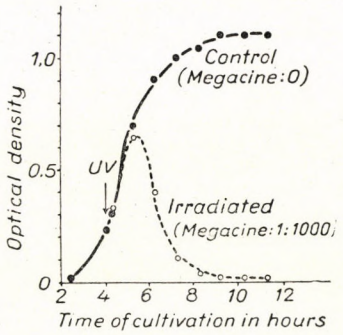

*Fig. 6.* Culture of different strains dripped on plate seeded with «mutilate». Not irradiated
*Fig. 7.* Culture of different strains dripped plate seeded with «mutilate». After irradiation

The growth-free agar areas of strain 119 and 216 developed after irradiation with UV light, were scraped off and added to young «mutilate» cultures in YC medium, and then incubated for 16 hours. The culture was diluted as already described, and assayed for the presence of phage particles. On plating strain 216 in dilution of $10^{-1}$ against the «mutilate» indicator surrounded by a zone appeared, but no free plaques. In an experiment carried out with strain 119, the same dilution yielded neither zone-surrounded colonies, nor phage plaques. All this appears to indicate that the amount of the antibacterial principle was not increasing in the presence of cells of a sensitive strain.

### *Conditions of the formation of the antibacterial principle*

Our experiments reported in the foregoing show that certain strains of B. megaterium produce, in various degree an antibacterial principle capable of inhibiting the growth of the «mutilate». It is striking that upon the effect of UV light some of the strains not only became incapable of growth but at

the same time inhibit in their proximity the development of indicator bacteria. In order to gain more information about this phenomenon, experiments have been carried out with strains 119 and 216 in liquid media. It needs to be emphasized that the effect of irradiation with UV light was found to depend to a very large extent on the composition of the media. Since so far we have succeeded to a limited extent only in elucidating the effect of the composition of media upon the phenomenon described in this paper, we must confine ourselves to experiments giving unequivocal results.



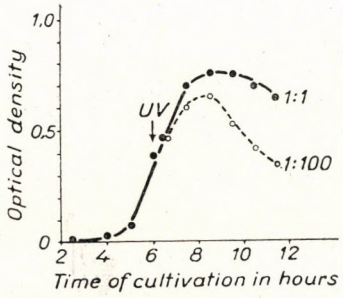
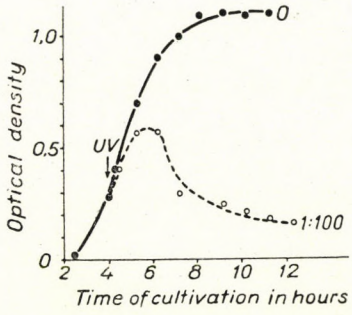




*Fig. 8.* Experiment in medium 9 (Centrifugate of control culture displayed no antibacterial action)
*Fig. 9.* Experiment in YP medium. (Numerals next to curves indicate titre of megacine)



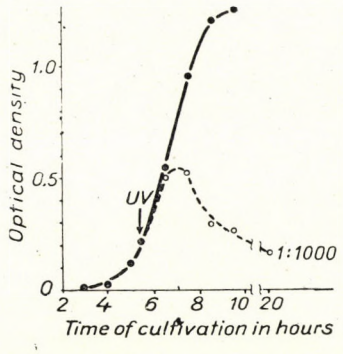




*Fig. 10.* Experiment in YC medium. (Centrifugate of control contained no megacine)
*Fig. 11.* Experiment in undiluted «neutral» yeast extract. (Centrifugate of control contained no megacine)

In essence, our procedure was as follows. A 16-hour culture obtained on YP agar was washed with saline and the suspension adjusted to an optical density of 0,400. This served as inoculum. Twenty ml of the medium sterilized in an Erlenmeyer flask with a tube fused onto its side were inoculated with 0,1 ml of suspension and incubated under shaking. On having attained the desired stage, 10 ml of culture were removed, poured into a sterile Petri dish,

and irradiated with UV light from a distance of 25 cm. During irradiation the suspension was constantly stirred. The irradiated suspension was then placed into a sterile flask and reincubated simultaneously with the non-irradiated suspension. From time to time, the degree of optical density was determined.

In determining the antibacterial principle which had formed and which hereafter will be termed megacine, the following procedure was employed. One half ml of young culture of the indicator strain (optical density 0,400) was poured on a YP agar plate dried. Serial dilutions prepared from the lysate, 0,02 ml of each dilution were dripped onto the plate and incubated for 8 to 10 hours. The highest dilution giving a distinct spot of few millimetres in diameter was considered to be the titre.

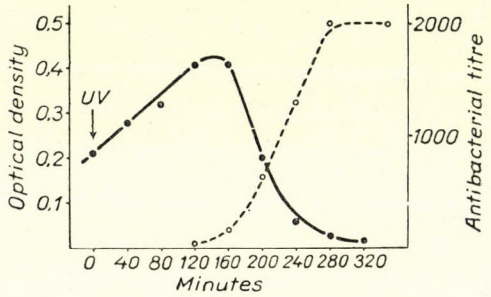



*Fig. 12.* Behaviour of irradiated culture and release of megacine. (Growing culture of an optical density of 0,200 irradiated with UV light for 35 secs.; after reincubation, titre of megacine determined in supernatant of samples withdrawn from time to time. Indicator strain 213. Unbroken line indicates optical density; broken line, titre of megacine)

The importance attaching to the composition of the media was revealed by the experiments presented in Figs. 8 to 11. In these experiments, young cultures of strain 216, grown in different media of an approximately identical optical density, were exposed to UV light for 30 seconds, and reincubated. At the end of the experiments, the antibacterial titre of the centrifugate of the culture was determined by means of the «mutilate» strain. Depending on the nutrient medium employed, marked differences could be established in the growth of both the control and the irradiated cultures. In addition, substantial differences were noticeable in the amount of the antibacterial principle that had formed. In YP medium, the control culture was observed to stop growing very soon; upon the action of UV light lysis was found to be slow and only partial, and the amount of megacine formed to be very small. Most characteristic of all were the conditions in medium 9. Lysis started as early as in the second hour following irradiation, and in about four hours it was almost complete. The lysate of the same culture was found to be effective up to high titre. In the not irradiated culture not even traces of the antibacterial principle were found. Intermediate results between these extreme types were seen in experiments carried out either in «neutral» yeast extract or in YC medium.

Since the most distinctive features had been observed in medium 9, it was decided to continue studying the mechanism of the phenomenon in this medium exclusively. Fig. 12 demonstrates the lysis and the appearance of megacine as a function of time. With advancing lysis, the amount of megacine gradually increases, reaching its peak when dissolution of the culture is almost complete.

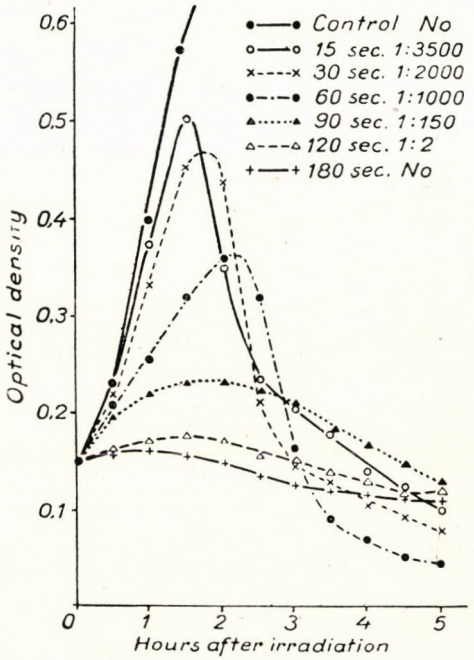



*Fig. 13.* Growth of cultures irradiated with UV light for different periods of time, and amount of megacine formed. (Antibacterial effect titrated with supernatant of culture centrifuged at end of experiment using indicator strain 213)

In the following experiment, to which Fig. 13 refers, young cultures of strain 216 grown in medium 9 were exposed to UV light for different periods of time, and reincubated individually. It was found that in very young cultures (optical density 0,150) irradiation lasting for 15 seconds sufficed to elicit the characteristic phenomenon, and to induce the production of a great amount of megacine. By raising the UV dose, residual growth became more and more decreased. On application of great UV doses (120 to 180 seconds), there was hardly any growth observable following irradiation, and lysis, too, was noticed to be insignificant. Under these circumstances no production of antibacterial principle was observed. As conclusion of these experiments it may be stated that megacine was found to have formed in the greatest amount when residual growth had come closest to the exponential phase of the control culture, and was followed by sudden lysis.

Apart from the composition of the medium and the dose of UV light, formation of the antibacterial principle was also affected by the age of the culture.Suspensions of strain 216 cultured under identical conditions for different periods of time were diluted with medium 9 to the same optical density (0,180), and irradiated with UV light for 30 seconds. The titre of the lysate obtained after reincubation was determined in strain 213. The titre of the lysate of the youngest ($4^1/_2$-hour) culture, with an original optical density of 0,180, was found to be 1 : 1500. The older, $5^1/_2$-hour culture of an original optical density of 0,480 displayed a behaviour exactly the same as that of the preceding one. Diverging from these results were those of our experiments that had been started with cultures older than 6 hours and of an optical density of 0,770 and 0,950, respectively. Although they hardly differed in residual growth and lysis, yet the amount of megacine formed was considerably less in them than in the previous ones.

Conditions similar to those described above were noted to prevail in the experiments carried out with strain 119. Upon UV irradiation, the young culture prepared in medium 9 became gradually dissolved and the resulting lysate was found to be markedly antibacterial against B. megaterium strains.

It was also attempted to induce the formation of the antibacterial principle with chemical compounds instead of UV irradiation. Our pertaining experiments with strain 216 in medium 9 yielded the following results. On adding to each 1 ml of culture of an optical density of 0,270 1 mg of sodium thioglycolate, lysis started at the end of the second hour and proceeded somewhat slower than usual. The titre of the lysate was 1 : 20 000. The lysate obtained simultaneously with UV irradiation was of the same effect. Induction only set in if the thioglycolate was added to the culture already in the state of growing. If added to the medium before inoculation, the rate of bacterial growth was found to be perfectly normal. Induction has failed with 2,5 mg/ml of Na ascorbate or with trypaflavine diluted to $2{,}10^{-6}$ or $4{,}10^{-6}$, respectively.

The inducibility of strain 216 by UV light to produce the antibacterial principle under optimum physiological conditions seems to be a hereditary quality of the strain. All our attempts at making it to lose this character have failed. The streptomycin resistant variant of the strain tolerating as much as 200 $\mu$g/ml of the antibiotic, was also inducible with UV light. We also failed to «cure» the strain by serial passages for long periods of time in a citrate medium. In *Wahl*'s citrate medium [9], by transfers made in 2 to 3 day intervals, the strain was maintained through 58 passages. The culture isolated from the last passage proved to be inducible with UV light, and its lysate was found to contain the antibacterial principle. The 58th subculture of strain 899(1) serially passaged in *Wahl*'s medium gave, on the other hand, no indication of any lysogenic character.

*The properties of the antibacterial principle megacine*

Depending on the experimental conditions and the indicator strain employed, the antibacterial effect of the lysates differed very considerably. Under optimum conditions titres of 1 : 10 000 to 1 : 20 000 could be established. As a rule, they were, however, lower. To some extent, the effect was also influenced by the composition of the medium used at titration. YP and YC media proved to be the most appropriate ones. The lysates were found to exert an effect on all the B. megaterium strains that had so far been studied. Over and above the 21 B. megaterium strains dealt with in the foregoing, our studies were extended to 20 additional strains, some of them freshly isolated, some others coming from established collections. All 41 strains, without exception, were sensitive, although in this respect very essential individual differences were noticed between them. Table II presents our findings concerning some of these strains.

**Table II**

*Titres against B. megaterium strains of megacine preparations obtained from strains 119 and 216*

| Indicator strain | Antibacterial titre | |
|---|---|---|
| | 119 | 216 |
| «Mutilate» ..... | 1 : 2500 | 1 : 2500 |
| 899(1) ..... | 1 : 10000 | 1 : 10000 |
| 213..... | 1 : 10000 | 1 : 10000 |
| 119..... | 1 : 160 | 1 : 160 |
| 216..... | 1 : 320 | 1 : 320 |

Note : Titration was performed on YP agar.

As seen in Table II, the two megacine preparations obtained from strains 119 and 216 exerted the greatest effect on the lysogenic strain 899(1) and on strain 213. It is very interesting that the productive strains themselves proved also sensitive to the antibacterial principle, although to a considerably lesser extent than the others.

This fact alone would suffice to contradict the phage character of the principle, but apart from the experiments already described the following observations also speak against it.

Two ml of the lysate obtained from strain 216 and active upon the «mutilate» strain up to a dilution of 1 : 3200, were added to 8 ml of a «mutilate» culture, of an optical density of 0,210, prepared in YC medium, and then reincubated. After addition of megacine, the optical density of the young culture ceased to increase, moreover, it began to decrease slowly. Thus, by the end of the third hour, it decreased to 0,140, by the end of the fifth to 0,130 ; and in the 18th hour it was found to be almost completely lysed, yielding a value

of 0,060. With a sample taken at this stage, an attempt was made to demonstrate the presence of phage in the usual way, but with no success. The antibacterial titre of the supernatant, as determined after centrifugation of the culture, was 1 : 256. This value was in fair agreement with that expected on the basis of the dilution (1 : 320). Thus, under the experimental conditions described, the principle did not increase in amount.

Passing through Seitz EK filter did not reduce the antibacterial effect of the lysates. Megacine does not pass parchment and is very slightly diffusible in agar. The principle was precipitated by ammonium sulphate at 75 per cent

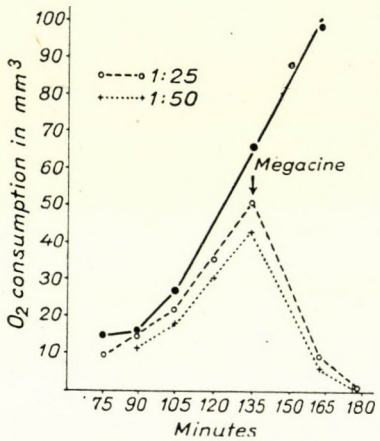



*Fig. 14.* $O_2$ consumption of growing strain 213 after addition of megacine. (The individual values are the results of 15-minute determination)

saturation. Commercial trypsin concentrate at pH 8 inactivated it within a short time.

The antibacterial effect of the lysates was decreased only at extreme pH values. To a borate buffer series of different pH values lysate was added at the ratio of 1 : 10, and the mixture kept in a water bath at 30° C for 30 minutes. Under these conditions its effect did not decrease between the pH values 3,34 and 11,01. With growing alkalinity, at pH 12, only 50 per cent of the effect remained, and only 3 per cent in a buffer of pH 12,9. In buffers of 2,61 and 1,42, 25 per cent of the original effect was left untouched.

The antibacterial action of megacine proved to be of bactericidal character. This already indicated by our experiment carried out in the Warburg manometer, the results of which are shown in Fig. 14.

As seen in this diagram, megacine added to a growing bacterial culture immediately reduced its respiration; in 50 minutes the suspension completely ceased consuming $O_2$.

In order to study its bactericidal effect, various dilutions of the lysate were added to cultures of strain 213 developed in YC medium. Samples were

then withdrawn at different intervals and diluted 1 : 100 with saline containing 20 per cent medium. This stock served for further dilutions to count the viable bacteria by plating 0,1 ml of each of the appropriate dilutions on YC agar surface. Fig. 15 shows the bactericidal effect of the lysate of strain 216.

It can be seen in Fig. 15 that the bactericidal effect manifested itself within a very short time ; however, even at the end of the experiment 0,23 and 0,63 per cent of the bacteria, respectively, were still viable.

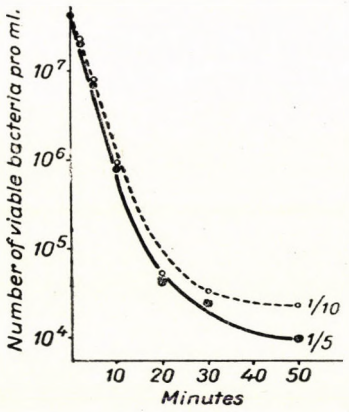



*Fig. 15.* Bactericidal effect of megacine prepared from strain 216 on suspension of strain 213

The megacine preparation obtained from strain 119 behaved somewhat differently. Here the 1 : 5 and 1 : 10 dilutions were much more active than in the previous experiment. The 1 : 25 and 1 : 50 dilutions, on the other hand, displayed a very limited bactericidal effect (Table III).

**Table III**

*Bactericidal effect of megacine obtained from strain 119 on suspension of strain 213*

| Dilution of lysate | Viable bacteria after | | |
|---|---|---|---|
| | 2 minutes | 10 minutes | 30 minutes |
| 1 : 5 | $15 \cdot 10^6$ | $40 \cdot 10^3$ | $<10^{3}$* |
| 1 : 10 | $90 \cdot 10^6$ | $21 \cdot 10^5$ | $11 \cdot 10^4$ |
| 1 : 25 | $135 \cdot 10^6$ | $90 \cdot 10^6$ | $85 \cdot 10^6$ |
| 1 : 50 | $170 \cdot 10^6$ | $130 \cdot 10^6$ | $115 \cdot 10^6$ |
| 0 (control) | $176 \cdot 10^6$/ml | | |

* No colonies developed from the 0,1 ml explanted from the lowest (1 : 100) dilution used in determining the bacterium count.

## Discussion

In the last few years, our knowledge of lysogeny has been increased rapidly and the phenomenon has been given a general biological interpretation, mainly by workers of the Pasteur Institute, whose theoretical conception and hypothesis have been summarized in *Lwoff*'s recently published excellent review [10].

*Lwoff* and his associates have based their conception for the most part on their experiments carried out with the B. megaterium strain 899(1) prompted by their studies; which by today can be regarded as classic, we thought it justified to investigate some other strains of this bacillus species; this all the more as knowledge concerning the lysogenic strains of B. megaterium is still deficient in several respects. Thus, for instance, the mechanism of the process is far from clear by which *den Dooren de Jong* obtained the phage-sensitive, so-called «mutilate» strains [11]. Equally little is known about the ecology of the lysogenic B. megaterium strains. This was what has made us to study strains occurring in nature.

Concerning the distribution in nature of lysogenic B. megaterium strains *den Dooren de Jong* wrote [12]: «Ich wage sogar die These auszusprechen, dass die Megaterium-Stämme ohne Ausnahme Bakteriophagen produzieren . . .». Yet, in the course of his experiments only three out of 18 strains proved to be lysogenic. The reason of this was ascribed to the many technical difficulties attaching to the verification of the lysogenic character of a given strain, for, in demonstrating lysogenesis, the sensitiveness of the individual «mutilate» strains as well as the selectivity of the phages produced are to be taken into account, factors which do not always permit the creation of favourable conditions. In support of his above statement, *den Dooren de Jong* aligned his observations made during the appearance of secondary colonies, the «mutilates»; and the morphological changes of a degenerative character he had seen in the cells studied.

According to *Cowles* [13], lysogenic B. megaterium strains rarely occur in nature. Not one did he succeed in isolating.

Of the 16 strains isolated by us from various soil samples, animal excrements, and the air, one only proved to be lysogenic. On the other hand, while studying our strains another, hitherto unknown, type of lethal biosynthesis has been observed. It seems that among B. megaterium strains to be found in nature this phenomenon occurs more frequently than lysogenesis. As its cause we succeeded in pointing out a soluble principle of protein character. Our observations were in many respects similar to the so-called bacteriocinogenesis described in certain strains of E. coli and Ps. pyocyanea. *Gratia* [14, 15] was the first to draw attention to the possibility of an antagonism existing among the individual strains of E. coli. This observation was subsequently carefully studied by *Fredericq* [16], and other investigators [17, 18]. For the antagonism, a material of protein character was held responsible. *Jacob* et al. [19] demon-

strated that there also exists an E. coli strain the culture of which gradually lysed upon irradiation with a small dose of UV light, and that the arising lysate exerted a marked bactericidal effect on certain sensitive strains. Quite recently, *Jacob* [20] observed a similar phenomenon in one of his Ps. pyocyanea strains, and termed this antibacterial agent pyocine.

The results of our studies on B. megaterium resemble the above in many ways. We, too, observed that there exist strains which, although in a slight degree, are antagonistic to the «mutilate» used as an indicator, without any kind of induction.

Particularly those two strains have caught our attention, of which it could be established already from tentative tests that they become dissolved on the effect of UV irradiation and, in addition, inhibit the growth of the «mutilate» cells in their vicinity. Sytematic studies enabled us to observe the effect on induction of physiological conditions, primarily of the composition of the medium. The antibacterial principle in the lysate of bacteria dissolved by the optimum dose of UV light was found to possess a protein character. For this antibacterial principle, which on account of both its mode of formation and its biological properties should be classed as bacteriocine, we venture to suggest the designation, megacine. The antibacterial spectrum of megacine is very narrow. Besides B. megaterium, no other bacterium species had been found sensitive to it in our preliminary investigations [1]. Later, however, when these experiments were extended to include several strains of other Bacillus species, we did come upon a few sensitive ones. Certain coccus strains, rather frequent in the air, were also found to be sensitive to megacine [21].

There is, however, an essential point in which the effect of megacine differs from that of the bacteriocins already known. While only certain strains of the corresponding bacterium species are sensitive to pyocine or the colicines, megacine was found to exert an effect on all the 41 B. megaterium strains studied. Most striking was the finding that megacine also acts on the homologous strain, i. e., on the one that produces it. Yet it is certain that the megacine-producing strains are considerably less sensitive to it than are others.

Megacine formation is not correlated with secretion, but with lethal biosynthesis. Only in inducible strains is this markedly perceptible. Following optimum UV irradiation, the optical density of the cultures increases substantially, and it is only at the end of the second hour that lysis of the bacteria suddenly sets in. By virtue of an increase in the so-called «growth units», computed by utilizing the values of optical density, megacine formation and, therewith, disruption of the bacteria may be preceded by two divisions. This induction was successful with thioglycolate, but failed with sodium ascorbate. Our experiments with chemical inducing agents cannot yet be regarded as completed.

Megacine synthesis recalls in several of its bearings the knowledge we possess of the lysogenesis of B. megaterium. In both cases strains inducible

and non-inducible with UV light have to be taken into account. The experimental data at our disposal do not as yet furnish us with sufficient information as to whether or not the two phenomena are correlated genetically.

On the basis of the mode of action displayed by colicine and pyocine, respectively, studies undertaken by *Jacob et al.* [19, 20] revealed that one particle of these materials was sufficient to destroy a sensitive cell. In their view, the bacteriocins mentioned above are adsorbed irreversibly onto the receptors of the sensitive cells, reminding one of the mode of action displayed by phages. We do not consider the results of our experiments with megacine suitable for similar computations. In this connection, the greatest difficulty is caused by the fact that B. megaterium is not an unicellular organism, cell wall formation being an earlier process than division. The foregoing would make it appear that the bactericidal behaviour of the individual megacine preparations is not quite identical either. Thus, when megacine obtained from strain 216 had been added to dense bacterial suspension, there arose a state of equilibrium, which was conspicuously reminiscent of the experiments made by the cited authors with colicine and pyocine, respectively. On the other hand, in adequate concentrations, the preparation derived from strain 119 exerted its bactericidal influence as long as the experimental conditions permitted the demonstration of live germs in the mixture. Whether the cause of the differences in affinity is or is not correlated with the antigenic structure of the megacine-producing and the indicator strains, represents a question which cannot be answered before more will be known of the antigenic structure of B. megaterium.

## Summary

1. Out of 16 B. megaterium strains isolated by us from soil samples, animal excrements, and the air, one only proved to be lysogenic.

2. On the other hand, a great number of the strains displayed an antagonistic effect of a non-phage character on the «mutilate» employed as indicator. This phenomenon is in no connection with the strain being lysogenic or non-lysogenic.

3. In three strains the antagonistic action was found substantially to increase on irradiation with a small dose of UV light. The cells of two strains (119 and 216) dissolved completely, with simultaneous enhanced production of the antibacterial substance.

4. The same effect of UV light was exerted also on cultures prepared in liquid media. However, success in this respect greatly depended on the composition of the medium and the age of the culture. Formation of the antibacterial substance was most intensive when an adequately small dose of UV light had been used for induction. The phenomenon could be elicited also by the addition of sodium-thioglycolate.

5. Under optimum conditions, lysis of the UV irradiated culture began in the second hour. It is estimated that in the period of residual growth the cells pass through two divisions, when the antibacterial principle, which is designated as megacine, is being formed. Megacine formation is thus the effect of lethal biosynthesis and its release coincides with cellular dissolution.

6. Megacine possesses properties indicative of protein character.

7. Megacine proved to be bactericidal against all the 41 B. megaterium strains studied. The strains producing the antibacterial substance are also sensitive to it. In this respect megacine substantially differs from the so-called bacteriocins already known, viz. the colicines and pyocine, which act on certain sensitive strains only.

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## О ЛИЗОГЕНЕЗЕ ШТАММОВ B. MEGATERIUM И О МЕГАЦИНЕ — АНТИБАКТЕРИАЛЬНОМ НАЧАЛЕ ЭТОГО ВИДА БАЦИЛЛ

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### Резюме


1. Из 16 штаммов B. megaterium, выделенных из проб земли и испражнений животных, а также из воздуха, лизогенным оказался только один.

2. В противоположность этому, большинство штаммов оказывало антагонистическое действие не фагового характера на «мутилат», используемый в качестве индикаторного штамма. Это явление не связано с лизогенностью или нелизогенностью штаммов.

3. У трех штаммов значительно повысилось антагонистическое действие при облучении ультрафиолетовыми лучами в малой дозе. Клетки двух штаммов (№№ 119 и 216) совершенно растворились и вместе с тем значительно повышалось производство антибактериального вещества.

4. Ультрафиолетовые лучи действовали также и на культуры, полученные в жидких средах. Успешность этого, однако, в большой степени зависит от состава среды и от возраста культуры. Наибольшее количество антибактериального вещества образовывалось в случае ультрафиолетового облучения в надлежаще малой дозе. Впрочем это явление вызывалось также и путем добавления тиогликолата натрия.

5. В оптимальных условиях под ультрафиолетовым облучением культуры начинают растворяться во втором часу. В фазе резидуального прироста происходит еще, приблизительно, двухкратное деление клеток, в процессе чего образуется антибактериальное вещество, названное нами мегацином. Итак, образование мегацина является следствием летального биосинтеза и освобождение мегацина совпадает со временем растворения клеток.

6. Мегацин обладает свойствами протеиноподобных веществ.

7. Мегацин оказался бактерицидным в отношении исследованного до сих пор 41 различного штамма B. megaterium. Штаммы, производящие антибактериальные вещества, являются также чувствительными к мегацину. С этой точки зрения мегацин значительно отличается от известных до сих пор, так называемых бактериоцинов, колицинов и пиоцинов, которые оказывают действие только на некоторые чувствительные штаммы.