THE ISOLATION OF L TYPE CULTURES FROM BACTEROIDES WITH THE AID OF PENICILLIN AND THEIR REVERSION INTO THE USUAL BACILLI[1]

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It has been reported previously (Dienes and Smith, 1944) that in cultures of certain strains of *Bacteroides* colonies develop which in appearance, morphology, and properties of growth are similar to L_1 colonies in *Streptobacillus moniliformis*. These colonies develop from the bacilli in a similar manner in both species. The bacilli swell into large fusiform or round bodies, which grow either into L type colonies or return to the usual bacilli. Klieneberger (1948) has examined one of the *Bacteroides* strains studied by Smith and the author, and she observed both the similarity between the L type colonies of *Bacteroides* and those of *Streptobacillus moniliformis* and the derivation of these colonies from swollen bacterial forms.

The study of L type colonies is more difficult in *Bacteroides* than in *Streptobacillus moniliformis* because they develop only in the cultures of a few exceptional strains and only for a short period following isolation. Of 23 strains of *Bacteroides* studied in this laboratory, 9 were pleomorphic, and L type colonies were observed in only 4. They could be isolated in pure culture from 2 strains only, and only by keeping the cultures frozen in CO_2 ice was it possible to preserve their tendency to produce such colonies. The cultures of nonpleomorphic strains and of those which lost this property during cultivation consist of regularly shaped bacillary forms without any morphological peculiarities.

Shortly after penicillin became available Pierce (1942) observed that L_1 is highly resistant to it and can be readily isolated in pure culture by inoculating *Streptobacillus moniliformis* on media containing the antibiotic. Attempts made at that time by the author to isolate L type colonies from other bacteria with the help of penicillin were not successful. During the last two years, by studying the effects of high concentrations of penicillin and by using various media, the development of L type colonies has been observed in many species, and from some species it has been possible to isolate these forms in pure culture. This paper contains observations made with *Bacteroides*; those made with *Hemophilus influenzae* have already been briefly reported (Dienes, 1947*b*). According to the observations to be described, the addition of penicillin to the medium offers an easy method for the isolation of L type colonies from certain strains of *Bacteroides* in the same way as from *Streptobacillus moniliformis*.

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However, these observations imply more than the development of a new technical procedure; they indicate an unexpected effect of penicillin on bacteria. Penicillin apparently helps the isolation of L type colonies not only by suppression of bacterial growth but also by actual induction of the development of L forms in cultures in which they would not otherwise be seen.

Six strains of *Bacteroides* were used for the study of the effects of penicillin. One strain (132) had already been studied extensively. Two cultures of this strain were available: one which had been preserved in CO_2 ice and had remained pleomorphic, and another which had lost its pleomorphism as a result of continued transfers in broth. The second strain (Ph) was cultivated from a chronic infection of the knee joint. It was not pleomorphic and produced no L type colonies. The third strain (224) was isolated from a fistula of the abdominal wall and was also nonpleomorphic. Three strains (906, 133, and 701) were received from the Boston City Hospital through the courtesy of Miss Lamb. They were maintained by passages in meat tubes, and none of them produced L type colonies. All strains were gram-negative, nonmotile, nonsporeforming, anaerobic rods, and they were all isolated from suppurative processes in humans. To attempt further classification has not been regarded as necessary because at present it is impossible to decide whether the differences between the strains really indicate difference of species. Pleomorphism is a transitory characteristic in most strains and certainly does not indicate difference of species.

Thioglycolate broth cultures of the strains were transferred to agar plates containing concentrations of penicillin increasing from 1 to 5,000 units per ml. The medium was similar to that employed for isolation of pleuropneumonialike organisms. It is prepared by boiling nutrient agar with 2 per cent horse blood. After sedimentation of the precipitate, the supernatant is mixed with 20 to 30 per cent ascitic fluid. Anaerobiasis was produced as before by growing *Serratia marcescens* on a piece of agar on the lid of the sealed plate. Results obtained with the different strains varied to a great extent.

The first two strains (132 and Ph) produced L type colonies abundantly on penicillin plates. Bacterial growth was prevented by the smallest dose of penicillin tested (1 unit per ml). The bacteria transferred to the plates swelled up within a few hours into large round bodies, and L type colonies developed in the course of 2 to 3 days even with the highest concentrations of penicillin (5,000 units per ml). With strain 132, it made no difference whether the plates were inoculated with pleomorphic or nonpleomorphic cultures. Between September, 1947, and February, 1948, six experiments were conducted with these strains. The development of L type colonies varied considerably, but they grew in one or more plates in each experiment, more regularly in those with the highest concentrations of penicillin. In certain experiments some plates remained sterile while there was growth in others. For example, L type colonies developed abundantly in an experiment with 100 and 1,600 units and they were absent with 200 and 400 units. The variation is probably due in part to technical difficulties of anaerobic cultivation, and its cause was not further studied. The appearance of the plate with dense growth of tiny colonies and the structure of

the colonies as they appear unstained on the agar with moderate magnification is shown in photographs 5 and 8 (figure 1).

The colonies were transferred by cutting out a square of agar containing them and streaking it over the new medium. They grew equally well on media with or without penicillin. The agar squares are left on the fresh medium because growth often develops only beneath the square. On one occasion when transplants were made from a plate containing 1 unit of penicillin per ml, a few bacterial colonies developed in addition to L type ones. However, the latter type of colonies grew in transplants made from plates with higher concentrations of penicillin, and bacterial colonies did not develop either in the primary or in the subsequent transfers on agar. The number of colonies on ascitic agar is always low, although the colonies develop to a considerable size (1 to 1.5 mm) after 7 to 10 days' incubation. The most abundant growth can be obtained by restreaking the agar squares over the medium after 2 to 3 days' incubation and reincubating the plates. After 24 hours' incubation the colonies are very small and entirely embedded in the agar.

A few L type colonies developed from two of the strains received from the Boston City Hospital. Strain 906 produced a few bacterial colonies consisting of long filaments and a few L type colonies on plates containing 1 unit of penicillin. No growth was present with higher concentrations. Strain 133 produced a slight bacterial growth with 1 unit of penicillin per ml. Growth was absent with higher concentrations, but a few L type colonies developed with 50 units per ml. No L type colonies were obtained from these strains in two subsequent experiments. The third strain (701) received from the City Hospital and strain 224 isolated in our laboratory produced no L type colonies in two consecutive tests. In all experiments an area of the agar was inoculated with cultures of strain 132, which produced L type colonies abundantly in most of the plates.

The *Bacteroides* strains present a distinct individuality manifested in different degrees of pleomorphism, or in its absence, and in the tendency to produce L type colonies. The individuality of the strains is as marked with penicillin as without it. In some strains of *Bacteroides* penicillin induced an abundant growth of L type colonies; in others, only a slight growth or none at all. In the present experiments only one medium, ascitic agar, was used. Observations with other bacteria indicate that the composition of the medium and the origin of the serum added to it exert a marked influence on the results. The medium which was most successful with *Proteus* and *Eberthella typhosa* was a soft agar to which 10 per cent horse serum had been added. This medium also yielded a more abundant growth of L type colonies in *Bacteroides* than ascitic agar. It is probable that a large percentage of *Bacteroides* strains could be induced to produce L type colonies by experimenting with various media.

The L type colonies isolated with or without penicillin are similar in every respect. The appearance and growth of these colonies and the morphology and physical properties of the organisms are so characteristic and differ in so many respects from the parent culture that their identification causes no difficulty. The close similarity of these colonies to the L_1 was acknowledged by Kliene-

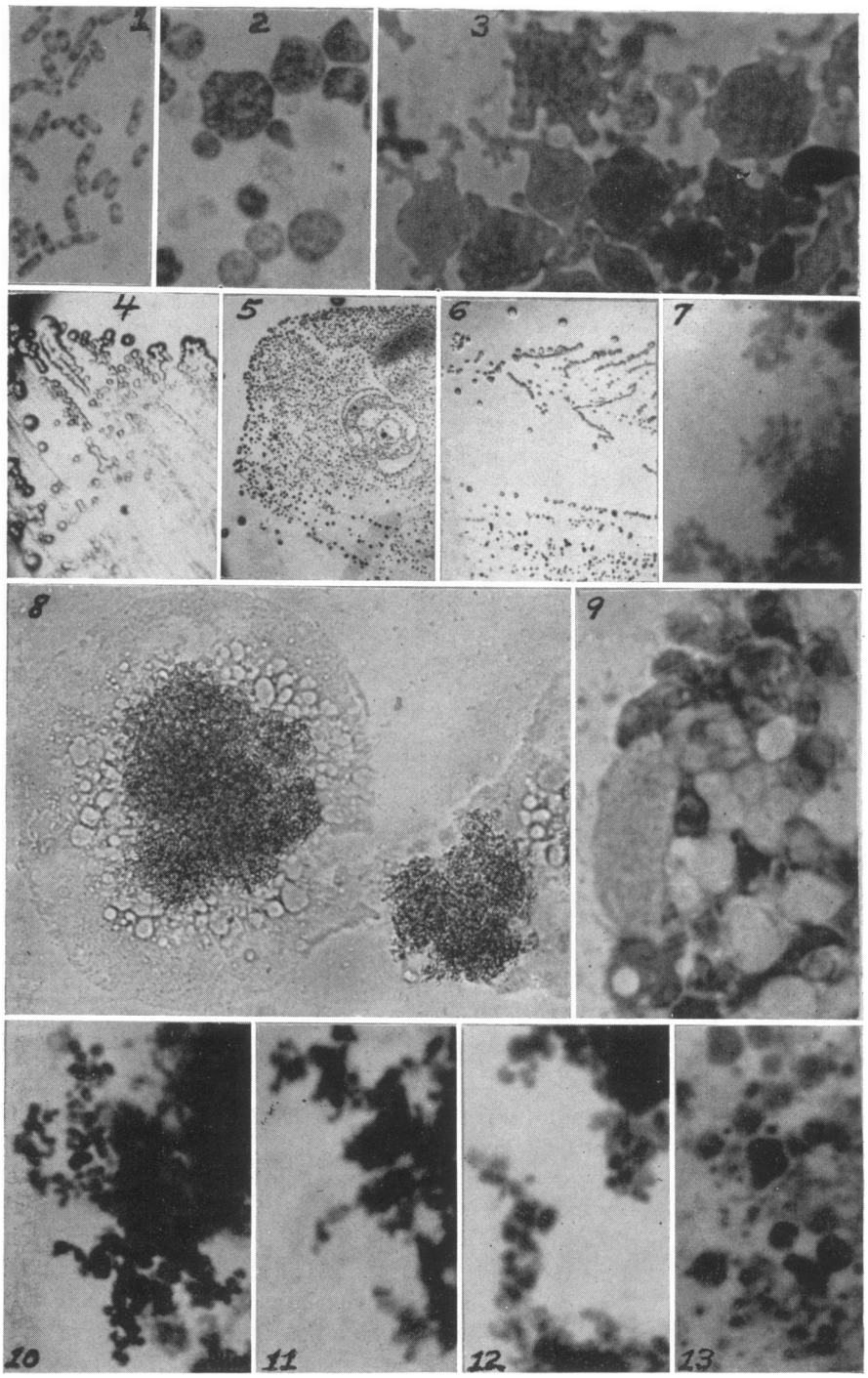

Figure 1

1. A nonpleomorphic broth culture of *Bacteroides* strain 132. × 2,000.
2. A culture similar to that in photograph 1 after 5 hours' exposure to 400 units penicillin per ml. × 2,000.

berger, and it is apparent in the photographs in figure 1. As in the case of L_1 (Dienes, 1947*c*), it is difficult to see and especially to photograph the shape of the small growing forms in the center of the colonies since they do not grow in one plane and cannot be transferred to a cover slip. In a successfully stained agar preparation very small bacilli, often with polar staining, and all transitional forms to the very large round bodies are clearly visible. Photographs 7, 10, 11, and 12 were made from the extending edge of the dense center of the colonies. Photograph 13 was made from an impression preparation of colonies from which the surface layer had been removed. Although the shape of the individual organisms cannot be seen so clearly in the photographs as would be desirable, both small bacillary and rounded forms are apparent. The shape and arrangement of the organisms are similar to those visible in photographs of L_1 and of pleuropneumonialike organisms made from similar preparations. The author cannot agree with Klieneberger's opinion that these organisms show no "cell boundaries" and grow as "thin shapeless slime." The form of the organisms is as distinct as the form of any other microorganisms, and their development follows a definite pattern.

The growth properties of the L type colonies isolated from *Bacteroides* and the L_1 are similar both on solid and liquid media. It is difficult to induce growth, and on a heavily inoculated plate only a few organisms will produce colonies. These develop slowly, but if they are not crowded they continue to grow for 5 to 7 days. Autolysis is often apparent, although the colonies continue to extend to the periphery. In broth, growth develops slowly and continues for several weeks as isolated colonies adhering to the sides of the tube, or in thioglycolate broth adhering to the agar particles.

Some insight into the mechanism by which penicillin exerts its influence on

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3. Transplant on ascitic agar from culture illustrated in photograph 2 after 17 hours' incubation. × 2,000.

4. Bacillary colonies of strain 132 on ascitic agar. × 2.

5. Tiny L type colonies on 10 per cent horse serum agar containing 1,600 units penicillin per ml inoculated with a nonpleomorphic culture of *Bacteroides* strain 132. Three-day-old culture. × 2.

6. L type colonies of strain 132 after the 15th passage on ascitic agar. Three-day-old culture. × 2.

8. L type colonies of strain 132. They have a dense center embedded in the medium with granular appearance and irregular contour. The periphery of the colony extends on the surface of the medium consisting of large round forms. These are apparent with this magnification only when they are transformed into large vacuoles. × 200.

9. The periphery of a colony similar to that in photograph 8. Many of the round bodies are not stained and the one in the lower left corner has a round vacuole. × 2,000.

7, 10, 11, and 12. These photographs illustrate the edge of the center of L type colonies spreading in the agar. This consists of small bacillary forms and small and somewhat larger round forms arranged in a way similar to bacilli growing into the agar. The short filaments with a knob or round swelling at the end can be seen often in cultures of pleuropneumonialike organisms. The organisms in the photographs are in their natural arrangement, although they are flattened by the drying of the agar. Photographs 7, 11, and 12, × 3,000; 10, × 2,0000.

13. Impression preparation after agar fixation stained with thionin. The surface of the colonies was eliminated and the impression made from the dense center. All transitions from very small to large round forms are present. × 3,000.

Photographs 1, 2, 3, 5, and 9 were made from wet-stained agar preparations; 7, 10, 11, and 12 from dry-stained agar preparations; 13 from an impression preparation after agar fixation. Photographs 4, 5, 6, and 8 were photographed from unstained agar plates.

the bacilli was obtained by study of the broth cultures of strain 132. In broth cultures of this strain under normal conditions of cultivation, pleomorphism is the result of the changes induced by the growth of the bacillus in the medium. The bacilli are not pleomorphic on solid media, and transferred into broth they start to multiply in the form of regularly shaped bacilli and short filaments. After 6 to 9 hours' incubation almost all bacilli develop central swellings at the same time, and during the following hours swell up to large bodies. Multiplication in bacillary form stops during this process. If the large bodies are transferred into fresh broth or agar at any time during the course of their development, they break up into small bacillary forms. They produce no pleuropneumonialike colonies. After the transformation of the bacilli into large bodies is complete in the broth cultures, both bacillary and L type colonies develop in transplants and the proportion of pleuropneumonialike colonies increases with the age of the cultures. The peculiar processes observed in the cultures are the response of the bacilli to the accumulation of metabolic products in the medium.

The culture of strain 132 that was used for the following experiments was propagated by passage in thioglycolate broth and lost its pleomorphism and ability to produce L type colonies. Throughout its whole development it consisted of small bacillary forms. To obtain a fresh growing culture, thioglycolate broth containing ascitic fluid was heavily inoculated with this strain and incubated for 3 hours. Increase of turbidity and gas production indicated vigorous growth. At this time enough penicillin was added to the cultures to bring the concentrations up to 100 and 400 units, respectively. Gas formation and increase of turbidity proceeded during the following hours, but were caused by an increase in the size of the bacilli and not by multiplication. Five hours after the addition of penicillin no usual bacilli were visible in broth; they had been replaced by large round bodies. At that time transfers were made on two ascitic agar plates. After incubation overnight one plate was opened, and the large bodies were found in all stages of transformation into bacilli. It can be seen in photograph 3 that the large bodies increased in size; they began to segment and bacillary filaments began to grow from them in multiple directions. Under the microscope this plate presented a picture similar to that observed in plates inoculated from young broth cultures of naturally pleomorphic strains. On the plate opened after 48 hours bacillary colonies were present in large numbers without admixture of L type colonies. Transferred to broth, these bacilli produced no pleomorphic forms.

The same broth cultures were transplanted after 2 days' exposure to penicillin. The large bodies were well preserved and stained deeply with methylene blue, but, instead of developing into bacilli, they produced L type colonies exclusively. These colonies grew well in transplants on ascitic agar plates both with and without penicillin. The appearance of the cultures was similar to that reproduced in photographs 5 and 8. The derivation of the L type colonies from the large bodies has previously been demonstrated by observation of this process in slide cultures. This method cannot be applied so well to large bodies produced by penicillin, because at the stage when they grow into L type colonies

only a few of them remain viable. However, in stained agar preparations it could be seen that in this case also the L type colonies came from the large bodies. The broth cultures have no other growing organisms besides the bacilli and the L type colonies evidently originate from them.

The influence of penicillin on nonpleomorphic broth cultures of strain 132 imitates closely the processes observed in pleomorphic broth cultures without penicillin. In both cases the swelling of bacteria into large forms and the growth of L type colonies are a response of the bacteria to the presence of chemical substances in the medium interfering with normal growth. This observation is of great interest because it indicates that the mechanism by which penicillin induces the production of L type colonies is probably closely similar to that by which these colonies are produced in pleomorphic cultures without penicillin.

The L type cultures isolated some years ago from strain 132 and from another pleomorphic strain O.H. could not be made to grow in liquid media. The cultures isolated with the help of penicillin both in strains 132 and Ph grew in thioglycolate broth containing ascitic fluid. This medium had not been used previously. To make a culture in broth, the colonies together with the superficial layer of medium were scratched off the surface of agar cultures and transferred into thioglycolate broth. The cultures were then sealed with liquid petrolatum. The colonies adhering to the agar continued to enlarge during the following days, and the metabolic activity of the culture was indicated by a slight development of gas. It was helpful at this point to break up the colonies by drawing them into a capillary pipette several times. In some cases, following this procedure, several hundred small colonies developed adhering to the agar suspended in the medium. The colonies were small but easily seen. Examined under the microscope they consisted of small to very large round bodies (up to 20 to 30 μ) most of which were empty and transparent; some were filled with granules. Similar empty blebs develop in cultures of the pleuropneumonia group of organisms and they are probably not living structures. The center of young colonies consisted of small forms. Growth was obtained in successive transfers in thioglycolate broth, but it was always slow and the gas production remained slight compared with that in bacillary cultures.

The most important observation made with the broth cultures was that sooner or later the usual bacillary forms reappeared in them. This observation is of considerable interest for the question of the nature of the L type growth, and one experiment will be described in detail.

L type colonies from penicillin plates inoculated with bacillary cultures were transferred on December 22 to ascitic agar plates without penicillin from both strains 132 and Ph. The cultures were transferred by cutting out agar squares containing the colonies and streaking them on fresh plates. Transfers were made at intervals of 3 to 5 days. The usual bacillary forms never appeared on the plates. Both strains were transferred from the agar plates into two thioglycolate broth tubes containing 10 to 20 per cent ascitic fluid from the 2nd, 3rd, 5th, 8th, 13th, 14th, and 15th passages on agar. The last transfer into broth was made on February 14, 54 days after the original isolation of the L type

growth. The broth tubes were stirred after 4 to 5 days by a capillary pipette, and at the same time transfers were made into fresh thioglycolate ascitic fluid tubes. Development of bacilli in the broth cultures was indicated by abundant gas formation and turbidity. The bacilli were examined for their morphology and growth under aerobic conditions. In all cases they presented the pleomorphism characteristic of certain freshly isolated strains of *Bacteroides*. This pleomorphism and the absence of growth under aerobic conditions is sufficient to identify them. A few cultures were more thoroughly examined and were found to be similar in every respect to the original strains. On agar they produced L type colonies in addition to bacillary ones. Contamination of the culture occurred only in 5 tubes out of the 43 used, both in original and secondary transplants. The contaminants did not produce gas in thioglycolate broth and grew in aerobic plates.

In table 1 are indicated the dates when bacilli were noticed in the broth cultures. They appeared in the tubes inoculated from the agar cultures after 4 to 33 days of incubation. Twenty-three transplants were made from the original tubes before bacillary growth was apparent; two were contaminated, but sooner or later bacilli developed in all the others. In some cases this happened only after long incubation of the subculture, in one instance after 64 days.

It is of interest that the bacilli which appeared in broth were pleomorphic in all cases and that after growth overnight the culture consisted of large round bodies and short filaments with large swellings. The cultures of strain 132, which had lost its pleomorphism, and strain Ph, which had never shown pleomorphism, both acquired a tendency to swell into large bodies similar to that observed in strain 132 immediately after isolation. One of the 132 cultures regained from L type colonies was propagated in broth cultures. The pleomorphism persisted in 5 consecutive transfers and disappeared in the sixth. This behavior is similar to that of the original strain. It has already been mentioned that the bacilli regained from the large bodies without passing through the L forms were not pleomorphic. Exposure to penicillin and to certain toxic salts induces the swelling of bacteria into large bodies, but these forms transferred on normal media reproduce bacteria of the usual morphology if they multiply at all. The passage of bacteria through the L forms is the only instance known to the author in which a tendency to pleomorphic growth, which persisted in successive generations, has been produced.

The reappearance of bacillary forms in the broth cultures is certainly not a result of contamination. The bacilli which reappeared were similar in every case, and the characteristic pleomorphism, anaerobic growth, and the production of L type colonies after transfer to agar proved their identity with the parent strain. The transfers and the examination of the cultures were always made by the author, and the media were tested for sterility. The thioglycolate broth was boiled immediately before inoculation. The ascitic fluid was also used for the cultivation of pleuropneumonialike organisms and of such routine specimens as joint and pleural fluids. *Bacteroides* never appeared in these cultures. At the same time that the thioglycolate tubes were inoculated from the third agar

transfer of L type colonies, two tubes were inoculated as a control with cultures of L_1 isolated several months before from a strain of *Streptobacillus moniliformis*. These tubes were stirred and transferred into two other tubes 3 days later. During the following days *Streptobacillus moniliformis* grew in all four tubes.

TABLE 1

Development of the usual bacilli in thioglycolate broth inoculated with L type cultures of Bacteroides strains 132 and Ph

NUMBER OF PASSAGES ON ASCITIC AGAR PLATES WITHOUT PENICILLIN BEFORE INOCULATION INTO BROTH (L TYPE CULTURES)	DATE OF TRANSFER INTO BROTH	DATE OF APPEARANCE OF BACILLI IN THE BROTH CULTURES
3	Jan. 5	Strain 132 Jan. 10
		132 Jan. 10
		Ph Jan. 10
		Ph Jan. 12
5	Jan. 13	132 Jan. 24
		132 Jan. 31
		Ph Jan. 19
		Ph Jan. 20
8	Jan. 24	132 contam.
		132 Feb. 5
		Ph Jan. 28
		Ph Feb. 1
13	Feb. 14	132 Mar. 11
		132 Mar. 18
		Ph contam.
		Ph Feb. 26
15th (strain 132)	Feb. 26	132 Mar. 4
		132 Mar. 15
14th (strain Ph)		Ph contam.
		Ph Mar. 20

The broth cultures were stirred and transferred into other broth tubes after 3 to 6 days' incubation. Bacilli appeared in only one tube before stirring (Jan. 28, 8th passage of strain Ph). Bacilli appeared in 21 of 23 thioglycolate tubes inoculated from the original broth cultures; 2 tubes were contaminated.

The four thioglycolate tubes inoculated simultaneously with L type colonies of *Bacteroides* later produced *Bacteroides*. The L type colonies in both cases reproduced the bacteria from which they originated.

DISCUSSION

L type colonies have been obtained without difficulty with the help of penicillin from certain strains of *Bacteroides*. The author hopes that the easy availability of these peculiar forms will attract others to their study. A high re-

sistance to penicillin is one more similarity between these colonies obtained from *Bacteroides* and the L_1 isolated from *Streptobacillus moniliformis*. The growth of L type colonies was not affected in either species by the highest concentrations of penicillin tested, 5,000 and 10,000 units, respectively. On the other hand, these colonies grew as well without penicillin. It is interesting that in both species this high resistance to penicillin is connected with colonies showing a close similarity in the type of growth on agar and in broth, in morphology and physical properties. However, metabolic activities of the L type cultures are different in the two species and similar to that of the parent organism, although in both cases they are greatly reduced in intensity.

The fact that penicillin favors the development of L type colonies in *Streptobacillus moniliformis* and *Bacteroides* is of considerable interest in connection with observations made with other bacteria. The isolation of colonies corresponding to the L_1 from *H. influenzae*, *E. typhosa*, and *Proteus* with the help of penicillin will be the subject of future papers. In these cases L type colonies could not be isolated without penicillin, although they are apparent in some cultures in a rudimentary form. If the L type colonies developing with or without penicillin are similar in two species, there is no reason to believe that in other species colonies with similar properties are of a different nature simply because at present they can be obtained only by the use of penicillin. The inducement of the growth of L type colonies is an unexpected effect of penicillin. This effect certainly represents more than mere inhibition of bacterial growth, but, since most of the information concerning it was obtained from the study of other species, its probable nature will be discussed in future publications.

The reappearance of bacilli in broth cultures of L forms of *Bacteroides* is important in ascertaining the nature of these forms, and it is an additional similarity between these forms and the L_1. The observations made with *Bacteroides* are similar to those reported by various authors on L_1 strains. Heilman (1941) studied several L_1 strains which returned to bacillary forms in broth during a certain period after isolation. Often several transfers in broth were needed for this purpose. The L_1 strain studied by Brown and Nunemaker (1942) returned in broth to the bacillary form after 120 transfers on solid media. Klieneberger (1940) observed the reappearance of bacilli in broth cultures, but she stressed the fact that after prolonged cultivation on solid media many strains became stabilized and lost the ability to return to the bacillary form. It is not apparent in the present experiments whether a similar stabilization in the L form occurs in *Bacteroides*.

Klieneberger tries to explain the reappearance of the bacilli in broth by the hypothesis that the bacilli are carried in the L_1 colonies as a contamination. A critical examination shows that this supposition does not agree with the observations. Both in *Streptobacillus moniliformis* and *Bacteroides* the L forms were transferred over long periods in pure culture on solid media, a fact which excludes the possibility that bacteria accidentally included in the cultures would persist without multiplication. The bacilli certainly do not multiply in the L type colonies in their usual form, because they do not form colonies on agar and

require long incubation in broth in order to be apparent. In both species the bacilli grow much faster than the L type cultures. The author has discussed the connection between the bacillus and the L_1 elsewhere (1947*a*). It is sufficient to say here that the L forms of *Bacteroides* are not only similar morphologically to the L_1 and that they are derived in a similar way from the bacilli, but that they return to the bacillary form in a similar manner. The L forms behave in both species as variant forms and not as organisms genetically different from the bacilli.

SUMMARY

The growth of six *Bacteroides* strains was studied on ascitic agar plates containing from 1 to 5,000 units of penicillin per ml of the medium. Growth in the usual bacillary forms was inhibited and L type colonies developed in abundance in two strains with all concentrations of penicillin. A few such colonies developed in plates inoculated with two other strains and none on those inoculated with the remaining two strains. The individual peculiarities of the strains in the development of L type colonies are as marked with penicillin as without it. The influence of the composition of the media in the development of L type colonies was not studied.

The processes induced by penicillin resulting in the growth of L type colonies are similar in many respects to those observed in pleomorphic strains without the use of penicillin. The bacilli, in a culture of the previously studied strain 132 after loss of pleomorphism, were induced by penicillin to swell to large forms. In the early stages of transformation these large forms transferred to penicillin-free media returned to the usual bacillary form; in a later stage they produced only L type colonies.

The L type colonies of two strains were induced to grow in thioglycolate broth. After prolonged incubation (4 to 64 days), the usual bacilli reappeared in all broth cultures. On agar plates development of bacilli was never observed in L type cultures. Before the L type cultures were inoculated into broth, they were transplanted on agar for a sufficient number of times to exclude the presence of bacilli accidentally included in them. The L type of growth in *Streptobacillus moniliformis* and that in *Bacteroides* are similar not only in their derivation from bacilli, in morphology, and in high resistance to penicillin, but also in the fact that, under appropriate conditions, they reproduce the parent bacilli in a similar way.

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