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Organization of Developing *Escherichia coli* Colonies Viewed by Scanning Electron Microscopy

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Colony growth was initiated by inoculating minimal glucose agar with 1-μl. spots of a plasmid-free *Escherichia coli* culture and incubating at 32°C. Inoculations took place over a 3-day period, at the end of which the plates were fixed and dried for scanning electron microscopy. In this way, it was possible to examine the surfaces of colonies ranging in age from 0 to 68 h. Macroscopically, the colonies were organized into different concentric zones, and several morphological features could be seen to develop over this period. These included a shallow depression ring marking the site of inoculation, a deeper indentation ring whose position moved outward as the colony grew, an expanding plateau region between the two rings, a mound outside the indentation ring, and a flat brim extending onto the substrate which was either present or absent at different times. Microscopically, a variety of cell morphologies and cell arrangements were detected. Upon inoculation, the bacteria accumulated at the periphery of the inoculation spot but showed no other kind of order. For the first 7.5 h, all bacteria were rod shaped; at the end of this initial phase, a high degree of alignment was seen in the cells at the colony edge. By 24.5 h, both shorter more ovoid cells and longer filaments had begun to appear, and large multicellular arrays had formed. At later stages of colony development, morphologically distinguishable zones involving cells of different shapes and sizes had formed, and these zones often marked the boundaries of macroscopic features. The edges were particularly interesting and at 68 h displayed very sharp saw-toothed boundaries between concentrically organized groups of bacteria. There were some transient irregularities in the concentric organizations of growing colonies, and one colony had entered upon a distinct developmental pathway.

Examination of fully developed bacterial colonies at both the macroscopic and microscopic levels has revealed a high degree of morphological and biochemical organization (1, 2, 12, 15–19). Large-scale patterns included both sectorial and concentric elements. Each sectorial feature was assumed to represent a phenotypically distinguishable group of bacteria descended from a common ancestor. Each concentric feature represented a group of phenotypically distinguishable bacteria which had many different ancestors but shared a common position in the history of colony development. Some aspects of morphogenetic regulation are known to be heritable since colonial structure is taxonomically specific, and pedigree analysis of *Pseudomonas putida* cultures has shown that variations which changed certain features of colony organization were maintained in clonal lineages (19). It was frequently observed in those pedigree studies that hereditary changes could simultaneously affect more than one aspect of colony organization, and variant subclones were obtained which showed novel patterns of biochemical activity, multicellular aggregation, and regulation of colony spread. Similar results on the heredity of pattern specificity have subsequently been obtained with *Escherichia coli* cultures (unpublished data) and can be found in the literature on *Candida* and *Saccharomyces* spp. (20, 24). Observations such as these indicated that regulatory systems could control the behavior of groups of procaryotic cells and that it should be possible to monitor specific multicellular morphogenetic events during bacterial colony development. This idea is not unprecedented. Other authors have proposed similar ideas based on studies of colony structure and variation (1, 5, 12).

There are a number of studies of the early stages of bacterial colony development in the literature (7, 10, 13). These studies generally involved cultures growing on special slides or chambers and were limited to the first few hours of growth when most of the individual bacteria could be distinguished. To provide information about cellular changes at later stages of colony development, the scanning electron microscope (SEM) was used to examine the surface structures of *E. coli* colonies growing on agar medium in normal petri dishes. Previous work had shown that the SEM could be used to provide detailed information at the cellular level about the structure of mature *Pseudomonas putida* colonies without gross distortion of colony organization (18). The present study extended the earlier results by examining the colonies produced by a single culture at different stages of development, thereby documenting how patterns of multicellular organization could change over time.

The background for these SEM studies was an investigation of bacterial morphogenesis on agar substrates, using time-lapse photography and video recording. The time-lapse studies concerned the growth of *E. coli* colonies and the formation of swarm colonies by *Proteus mirabilis*. The results significantly influenced my descriptions and interpretations of the SEM pictures in this report and thus require a brief summary. Kinetic analysis of living *E. coli* colonies revealed that expansion was not a uniform process but rather that different phases of activity could be discerned. To obtain the colonies for SEM examination, plates were inoculated with spots containing 10^4 to 10^5 bacteria because this procedure was found to yield highly reproducible colonial structures at known locations on an agar surface (19). Each combination of a particular culture and a particular substrate generally led to a well-defined pathway of colony development after this kind of inoculation. When the medium and

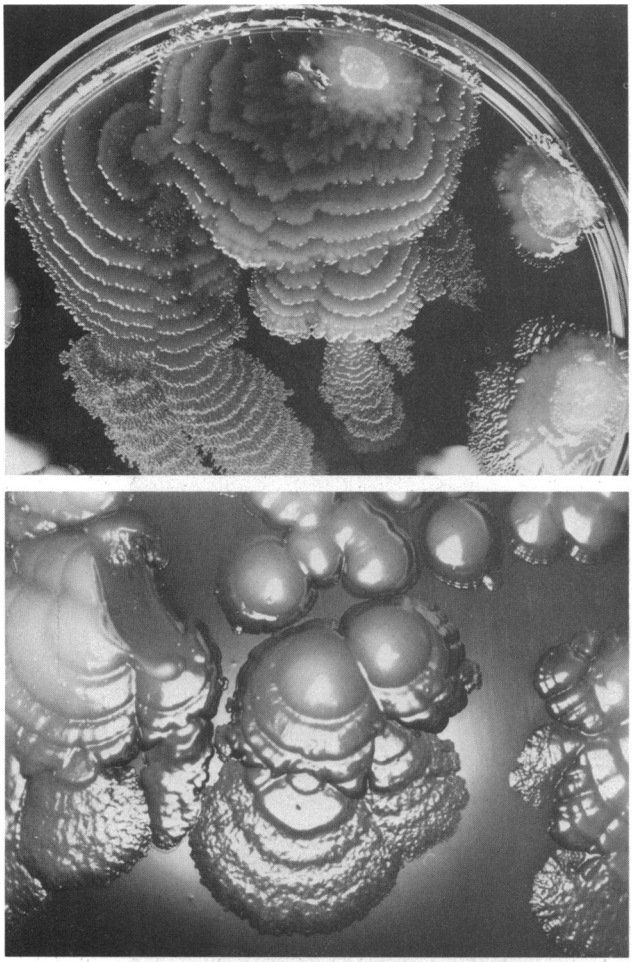


FIG. 1. Comparison of *P. mirabilis* swarms and *E. coli* colonies. (Top) Kanamycin-resistant *P. mirabilis* exconjugants swarming over tryptone-yeast extract agar containing kanamycin. (Bottom) Colonies of a urinary tract isolate of *E. coli* after streaking on MacConkey agar. Both panels show terracing and continued outward expansion by sectorial subpopulations. The top panel was previously published in *ASM News* (March, 1984, cover photo; 14).

culture were the same as those used in the current SEM studies, the developmental sequence included the following phases: active swimming of bacteria in the spot before it dried, preferential accumulation of cells at the edge of the inoculated zone, multiplication of bacteria in the inoculated area (about 0 to 3 h after inoculation), especially pronounced development of the outermost ring to create a shallow craterlike structure (about 3 to 8 h), and then a period of outward colony expansion and development. During the period of outward expansion, a depression developed just inside the thick ring at the edge of the inoculated area (about 10 to 12 h); there was subsequently little visible growth in the central region, and the structure of the outer zone of active colony expansion showed variation over time in the patterns of bacterial aggregation. Viewing the time-lapse recordings (in particular the periods of initial multiplication and the start of outward expansion) gave the impression that each phase of colony development served as a preparation for succeeding phases. The temporal and spatial specificities of structural changes observed macroscopically in growing *E. coli* colonies were reflected in low-magnification SEM pictures and made it seem likely that there would be a corresponding series of changes seen at the microscopic level.

Proteus swarm colony morphogenesis is particularly favorable for developmental analysis because it is a much more rapid and extensive process than growth of *E. coli* colonies. A swarm colony originating from a spot of less than 10^4 bacteria can cover a 9-cm-diameter petri dish in about 12 to 16 h at 32°C (and in even less time at 37°C). Many aspects of swarm colony development have been described in the literature (3, 6, 8, 9, 11, 14, 21–23). The aspects that are

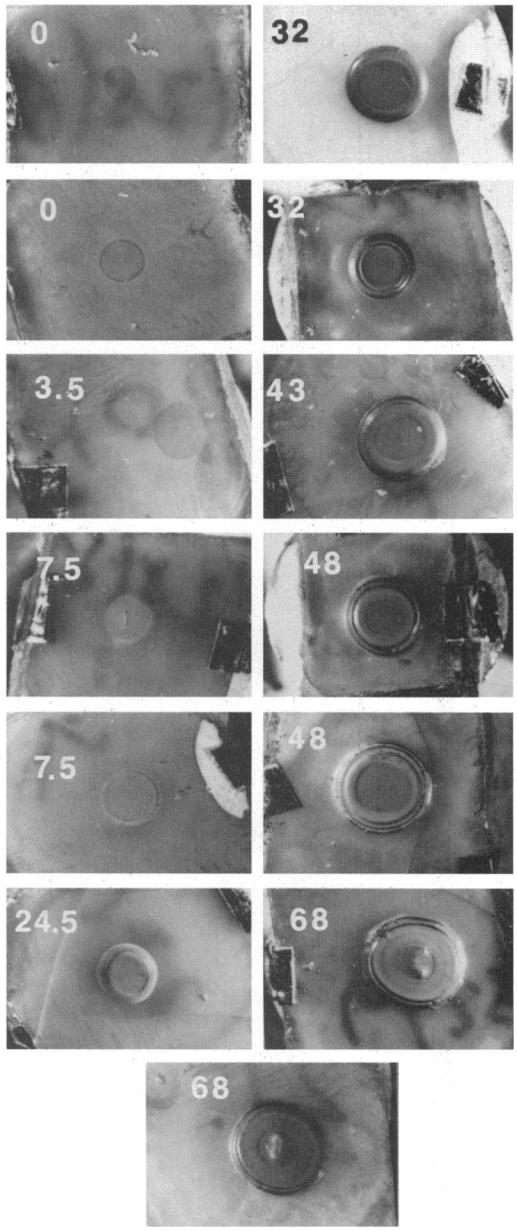
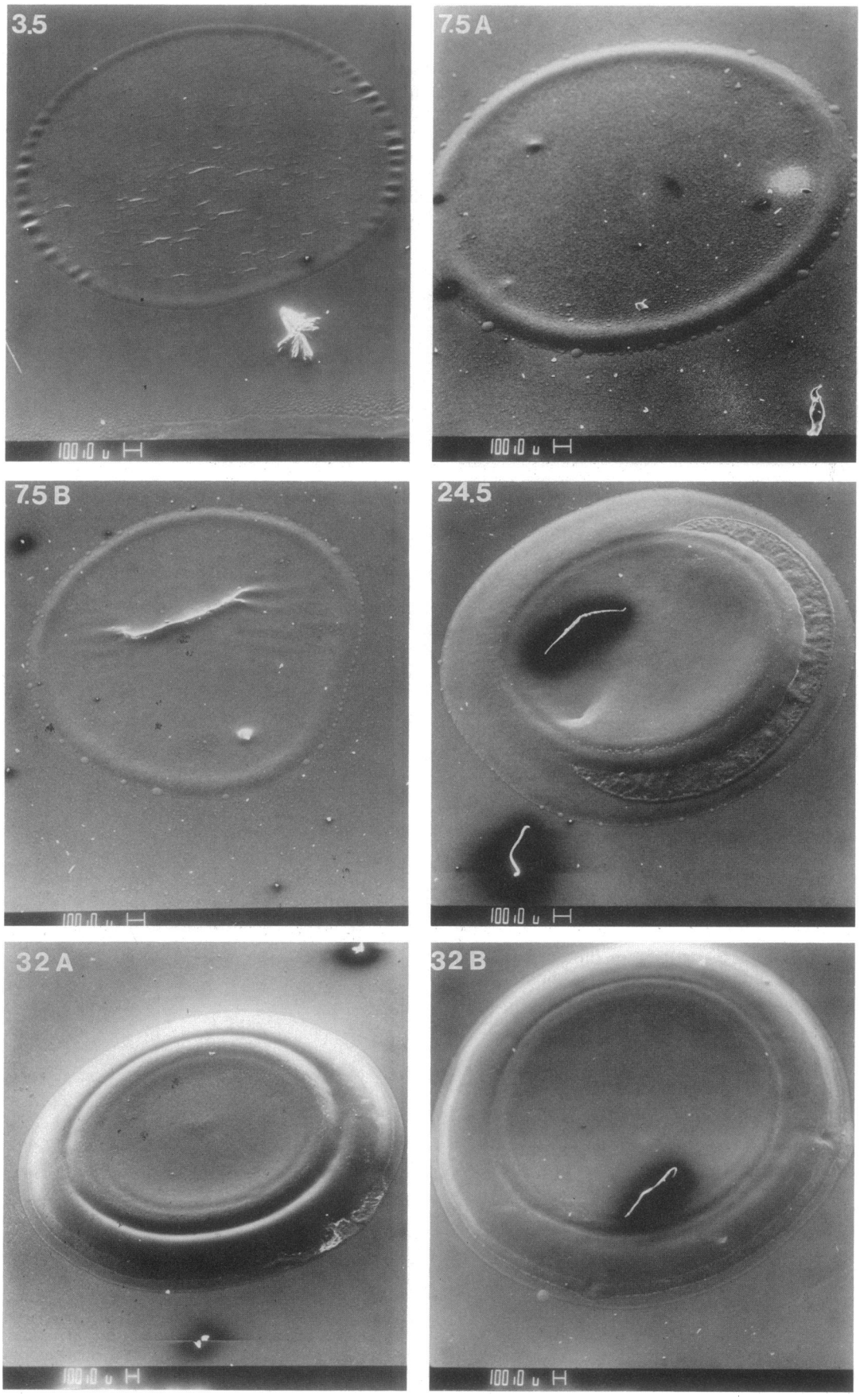


FIG. 2. *E. coli* colonies mounted for SEM examination. The age of each colony in hours is indicated at the upper left. The 0- and 3.5-h samples are rather indistinct. Discontinuities can be seen on the surfaces of some of the samples, such as a crack on one 7.5-h colony, a depression zone on the 24.5-h colony, a small crack on one 32-h colony, a small crystal on the 43-h colony, and cracks in the central regions of both 68-h colonies. Aside from these blemishes, overall structure was well preserved. Magnification, × 2.5.


3.5
7.5 A
7.5 B
24.5
32 A
32 B

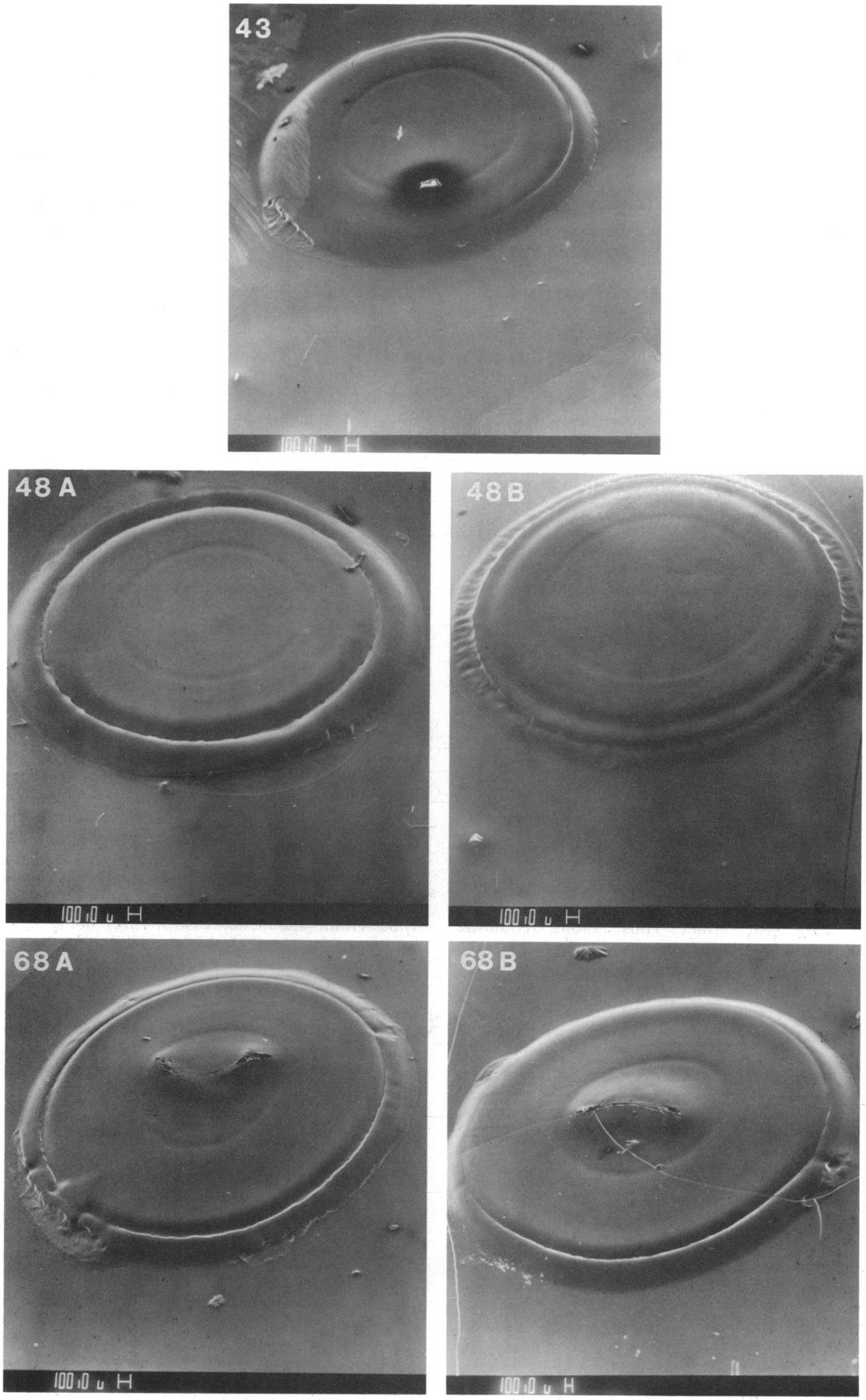


FIG. 3. Low-magnification SEM pictures of *E. coli* colonies. The age of each colony in hours is indicated at the upper left. Duplicate colonies are labeled A and B for cross-referencing. The 0-h samples could not be visualized at this magnification in the SEM. The significance of various structural features is discussed in the text. The blemishes noted in Fig. 2 are visible here, and prominences within the 24.5-, 32B-, and 43-h colonies and outside the 32A colony produced burn spots (bright features surrounded by a very dark region) in the pictures due to distortion of the electric field at these points. As mentioned in the text, the ripples on the sides of the 3.5-h colony were caused by handling of the sample before complete drying. Since the magnification scale of the SEM is not accurate at low magnifications, see Fig. 2 for the sizes of each colony.

particularly relevant here are (i) the formation of specialized swarm cells which accumulate in parallel arrays at the edges of growing *Proteus* colonies before the cells spread out over the substrate in groups (3, 8, 9, 21) and (ii) the rhythmic nature of cell amplification, swarm cell spreading, and development of structure in the main colonial mass. These rhythms are most obvious in (but are not limited to) the alternations between periods of active swarm cell migration and periods of apparent quiescence at the colony periphery (6, 11). If it is assumed the *P. mirabilis* swarm colony development may usefully serve as a model for spatially more restricted morphogenesis by nonswarming bacteria, it would be logical to anticipate a parallel series of cellular specializations and temporal changes in structure during the growth of *E. coli* colonies. The points that can be cited to justify this assumption are (i) the taxonomic closeness of *P. mirabilis* to *E. coli* and other members of the family *Enterobacteriacaea* (4), (ii) the similarity of early colony development as seen in *Proteus* sp. and in nonswarming enterics (7, 10, 13, 14), and (iii) the striking similarities often observed between the topographies of *P. mirabilis* swarms and *E. coli* colonies (Fig. 1).

The results presented below confirm the expectations of the time-lapse studies and provide further evidence that many (perhaps all) bacterial species can participate in highly orchestrated developmental processes involving millions of differentiated cells.

MATERIALS AND METHODS

Bacteria. M7124 is an F^- *thi* Δ(*argF-lac*) U169 strain of *E. coli* K-12 that has been used as a standard bacteriophage and plasmid host for 18 years.

Microbiological and sample preparation. These methods were as described in previous publications (16–19). As mentioned in the introduction, spot inoculations were used to initiate colony growth rather than stabbing, streaking, or spreading dilute suspensions. Spot inoculations are more uniform than stabs or streaks, and they can be directed at a specific place on the agar surface. Thus, colonies could be well spaced to avoid mutual inhibitory effects and also to facilitate sample preparation. Moreover, much of the detailed study of hereditary transmission of pattern specificity has used colonies growing from spots (19; unpublished data), so that there was a particular reason to look more closely at colonies whose development had begun this way. The ages of fixed colonies are given as the interval between inoculation with a 1-μl spot of culture containing about 10^4 CFU and the start of the fixation process by exposure to OsO_4 vapors (18). All colonies were inoculated from a single tryptone-yeast extract broth M7124 culture kept at room temperature in a closed vial. To obtain colonies of different ages (but otherwise grown under similar conditions), all cultures were inoculated onto three PA-glucose agar plates. Inoculations were made at irregular intervals over a 3-day period, and up to six colonies were inoculated at different times on each PA-glucose agar plate. The plates were incubated at 32°C. As soon as the last spots had dried, all the plates were fixed in parallel. At each time point, duplicate colonies were inoculated (sometimes on the same plate, sometimes on different plates). After preparation, 13 of the original 18 inoculations yielded fixed specimens suitable for examination in the SEM. Duplicate samples were obtained from the 0-, 7.5-, 32-, 48-, and 68-h (prefixation) inoculations; single samples were obtained from the 3.5-, 24.5-, and 43-h inoculations.

RESULTS

Macroscopic patterns. Because it was difficult to deliver 1 μl precisely with an Eppendorf pipette, the individual inoculation spots differed somewhat in size. There was no apparent change in colony size upon fixation and drying, and a good idea of the pattern of colony expansion can be obtained from photographs of the mounted fixed samples (Fig. 2). Several cracks and prominences that arose during preparation are visible in these photographs, and these preparation defects can also be seen in low-magnification SEM pictures of the same samples, where they sometimes produced "burn" spots on the picture (Fig. 3). The 0-h samples fixed immediately after inoculation could not be visualized at low magnification in the SEM.

As observed by light microscopy, the bacteria in each inoculation spot formed a disk, with cells concentrated at the edge. At 3.5-h, the disk had filled in somewhat, and by 7.5-h, the outermost edge of the colony had developed into a raised ring around the growth area. (The ripples at either side of the 3.5-h colony seen in Fig. 3 were preparation artifacts caused by handling the agar before it had fully dried. Their artifactual nature is evident from the alignment of these ripples with cracks inside the colony and by comparison with the unrippled portions of the colony perimeter.) It is possible to see that the outermost edges of the 7.5-h colonies have begun to produce a narrow flat brim extending onto the substrate (most clearly visible at about seven o'clock on the 7.5A colony), and a number of blebs of extracellular material are also visible around the perimeters of the 7.5-h colonies.

At 24.5-h, much more structure was visible in the growing colony. The depression which had been seen to develop in the time-lapse recordings at the perimeter of the inoculation site had developed into a well-defined ring surrounded by a wide flange marking the extent of colony expansion. This depression ring was a constant feature of colonies produced by 1-μl spot inoculations with M7124 cultures. As colony growth proceeded, the position of the ring did not change, and it conveniently marked the outlines of the inoculating spot. Homologous depression rings could be seen in the low-magnification SEM images of all the older colonies. One striking feature visible on this 24.5-h colony was the crescent of more complex surface texture emerging from the central growth area and extending over about half of the expansion flange. The significance of this kind of crescent in young *E. coli* colonies is not known, but geometrically similar spreading arcs of activity have been observed during the development of *P. mirabilis* swarm terraces.

By 32 h, colonial surface morphology had become even more highly differentiated, with multiple concentric features clearly visible in each of the colonies examined. An indentation ring had developed between the shallow depression ring and the outermost part of colony 32A, and it appears that a similar zone had just started to form in colony 32B. Unlike the structure I have termed the depression ring, the position of this indentation ring was not fixed with respect to the site of inoculation but moved outward as the colonies became larger. In addition, not every colony displayed this structure (it was not observed in one of the 48-h colonies). The outermost region beyond the indentation ring in the two 32-h colonies was subdivided into an internal rounded zone (or mound) and an external flat brim where the bacteria encountered the substrate. At a slightly higher magnification, the concentric arrangement of at least seven different zones is quite distinct (Fig. 4).

In the 43-h colony examined (Fig. 3 right), the same basic

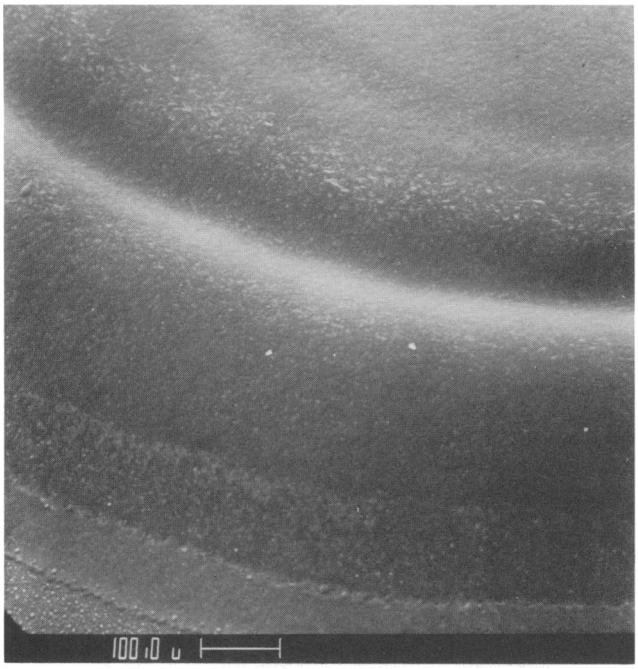


FIG. 4. The outer region of the 32A colony in Fig. 3. The agar substrate is visible in the lower left corner, and the central depression ring can be seen at the upper right. At least seven concentric zones are distinguishable in this picture (from the center outward): the central plateau, the depression ring, a kind of speckled mound, the indentation ring, another mound, a thin speckled band, and the brim extending onto the substrate.

colony structure has been slightly modified in two ways. (i) The zone between the shallow depression and the indentation ring has grown wider, and (ii) around most of the colony perimeter there was very little flat brim extending onto the substrate. This brim appeared to expand and contract at different times during colony development, suggesting the operation of a repeating process at the colony edge possibly analogous to the alternation between swarming and consolidation seen at the edges of *P. mirabilis* swarm colonies.

The two 48-h colonies displayed certain similarities, but they also were the only pair of duplicates examined which were clearly different in a major morphological feature, namely, the structure of the outermost region. In the 48A colony, this outermost region resembled that of the 32-h colonies in having an indentation ring surrounded by a mound and a flat exterior brim. In the 48B colony, however, this outermost region was quite different, with no indentation ring and mound but having instead a flat disk surrounded by a fluted rim structure. The two 68-h colonies, on the other hand, were remarkably similar in structure, each displaying a very wide zone between the shallow depression and indentation rings and having very short brims at the edges. In both cases small areas were visible at the edges which had more extensive flanges emerging onto the substrate. In the 68A colony, the position of such a flange coincided with the edge of a raised sector expanding outward over the indentation ring. The similarity of the edge structures in these two colonies at the microscopic level was quite remarkable and will be documented below.

Microscopic patterns. Each colony had a very intricate structure. To keep the investigation within manageable limits and to routinize colony-to-colony comparisons, a few features on each were chosen for particular study. These included the colony edges, where the actual expansion over the substrate was occurring; successive zones moving inward from the edges toward the central regions; and the boundaries or highlights of macroscopic structural discontinuities, such as sectors or concentric rings.

As mentioned above, a limited degree of organization appeared in the initial inoculation spots because of bacterial accumulation at the edges (Fig. 5a). When viewed at high magnification, the bacteria around and inside the edges of the 0-h colonies were not easy to discern and appeared to be scattered about without apparent order (Fig. 5b and c). The relative lack of distinctness in cell outlines when liquid suspensions of *E. coli* cells were deposited on agar had also been observed by light microscopy. It was only after a couple of hours of incubation (at about 32°C) that individual

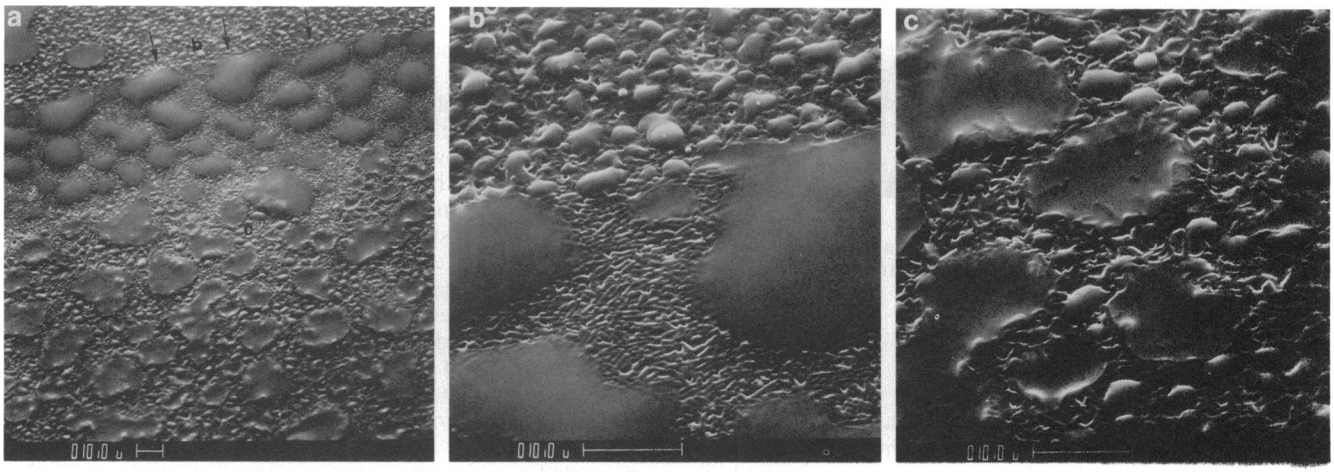


FIG. 5. Three views of an inoculation spot fixed immediately after drying onto the agar substrate. (a) The edge of the dried spot which can be distinguished at lower magnification by the ring of large blebs; arrows mark the position of the boundary between the inoculated area (below) and the agar substrate (above), and lowercase letters mark the positions of the higher magnification views shown in the other two panels. (b) The edge seen at higher magnification, with the agar substrate at the top and densely packed but irregularly arranged cells at the bottom. (c) Just inside the inoculation spot below the ring of densely accumulated cells; many fewer bacteria are barely visible among the blebs.

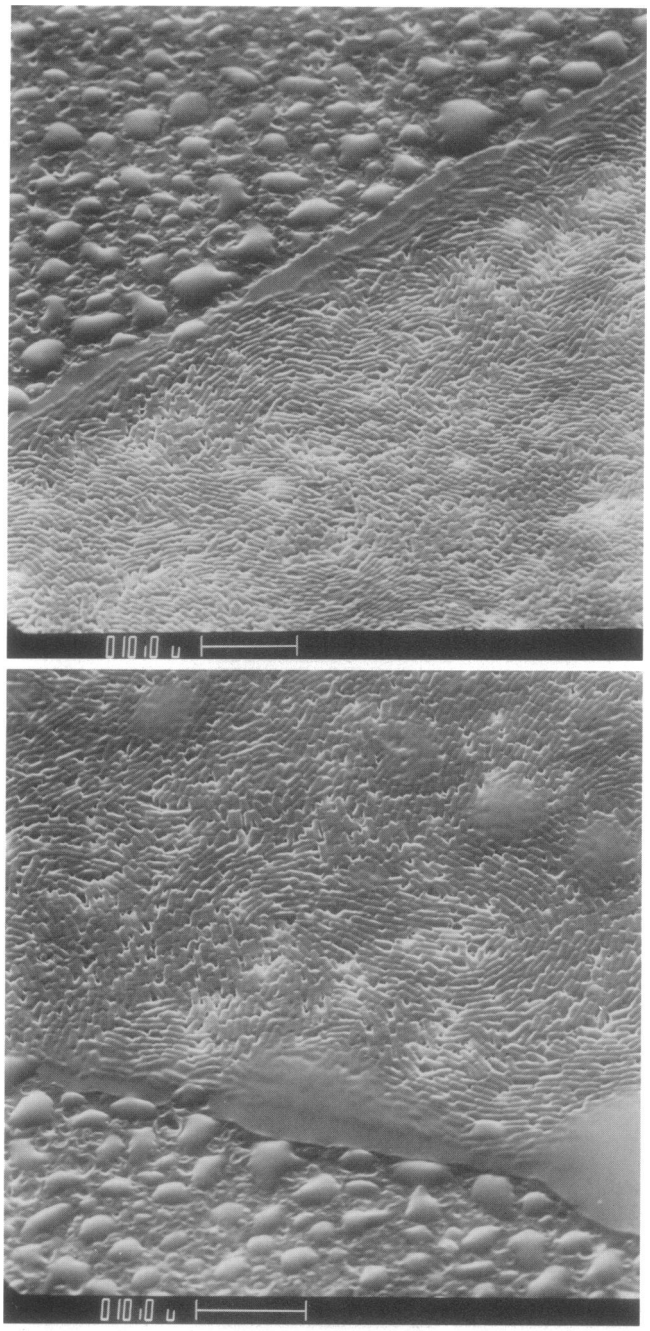


FIG. 6. Two regions at the edge of the 7.5A colony. These pictures were taken at about eleven o'clock and seven o'clock on the colony shown in Fig. 3. Note the parallel arrays of bacilli and the definition of the colonial boundaries by a rim of extracellular material.

bacteria showed up distinctly on the surfaces of normal petri dishes with bright-field transmitted light optics. This enhanced definition occurred before cell division. Such observations indicate that processes take place in the first hours after inoculation which lead to changes in cell structure (thereby enhancing the contrast between the bacteria and the agar) in preparation for subsequent cell divisions on the solid substrate. By 3.5 h, cell multiplication had produced a confluent population which displayed some degree of palisading and ordered alignment of cells around the edge (data not shown). Similar kinds of cell alignments in very young colonies have been described in the literature (3, 7, 10, 13). By 7.5 h, the order at the colony edges was even more marked, and some zones involved hundreds or more cells arranged in curving parallel arrays (Fig. 6). Often the cells at the very edge were aligned tangentially to the colony perimeter. In these first few hours of colony growth, all the cells had the bacillary form considered characteristic of *E. coli* and very long cells were not observed.

In 24.5-h and older colonies, the relatively simple patterns described above developed into more complex microscopic as well as macroscopic patterns. By scanning across the crescent region of the 24.5-h colony, for example, a variety of cell morphologies and arrangements was observed to be localized at specific places (Fig. 7). Palisaded bacilli were again seen at the edge, sometimes associated with large blebs of extracellular material (Fig. 7b), but zones containing cells with a more ovoid or coccoid form were observed at the outside edge of the crescent (Fig. 7c), and then bacilli were again seen at the inside edge of the crescent (Fig. 7d) and in the central plateau region of this colony (Fig. 7e). In some cases, the apparently round cells may have represented bacilli seen end-on, and there were several clusters of such cells in all the older colonies probably composed of groups of bacilli oriented perpendicularly to the colony surface. However, in many zones both ends of the shorter ovoid cells could be distinguished (as in Fig. 7c). The sizes of the parallel arrays of bacillary cells were much larger and more highly ordered at 24.5 h than were those at 7.5 h (data not shown) and occasional longer filamentous cells appeared (e.g., bottom right of Fig. 7c and top middle of Fig. 7e). These same trends toward multiple cell morphologies and large ordered multicellular arrays were even more pronounced in the 32-h colonies (Fig. 8).

At 43 h, the structure of the colony edge was especially intriguing (Fig. 9). This colony appears to have been captured somewhere between two phases displaying brims extending onto the substrate, since both 32-h and 48-h colonies had significant structures of this kind. In a region at about three o'clock on the low-magnification picture (Fig. 3), the edge displayed a kind of sawtooth pattern with thin layers of ovoid cells in contact with the agar (Fig. 9). A similar sawtooth pattern was later observed at the edges of the 68-h colonies.

The two 48-h colonies contained a wide range of multicellular patterns (see Fig. 10 and 11). Pictures taken along a diameter extending upward from the bottom flange toward the top edge of the 48A colony are shown in Fig. 10 (a through f). The individual panels of this figure depict the following quite distinct zones: (a) regularly palisaded short bacilli at the very edge and just above them very irregularly arranged longer bacilli apparently encased in extracellular material, (b) clustered ovoid cells in the mound between the flange and the indentation ring, (c) mixtures of bacilli of different sizes and orientations in the central plateau above the inoculation site inside the shallow indentation ring, (d and e) very short cells with occasional filaments within the deep indentation ring at about twelve o'clock, and (f) palisaded bacilli at the very top edge. The most noteworthy distinguishing microscopic feature of the 48B colony was the presence of very long bacilli among fields of ovoid cells at the tops of the radially oriented ridges in the outer fluted rim (Fig. 11). These filaments were generally very rare in this outer region of the colony, and there appeared to be some

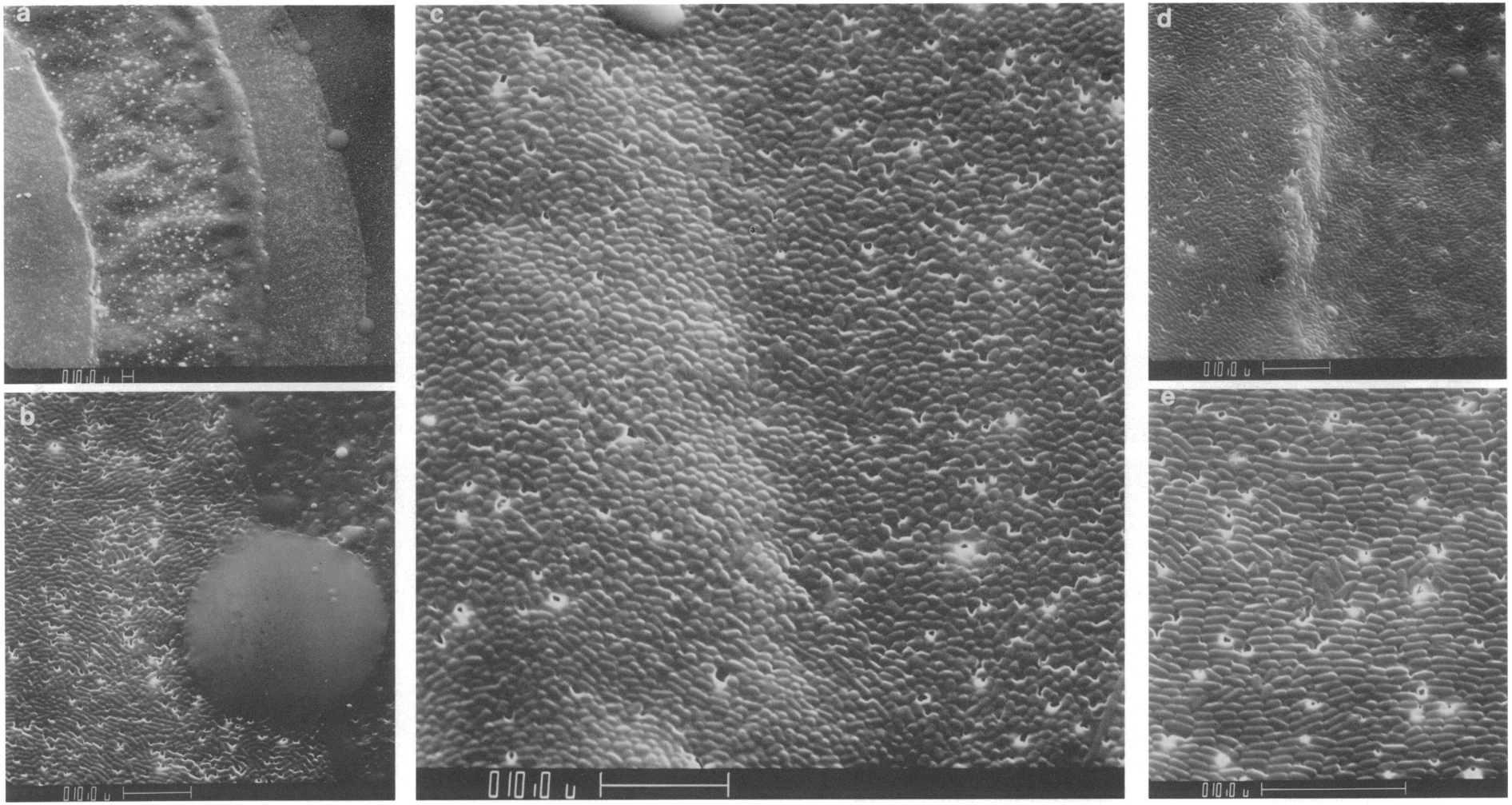


FIG. 7. Different regions of the 24.5-h colony. (a) Relatively low-magnification picture of the right side of the colony, showing a segment of the crescent structure visible in Fig. 3. (b) Close-up of the edge and extracellular bleb at two o'clock in panel a. (c) Just inside panel b at the outer edge of the crescent. (d) Inside panel c at the inner boundary of the crescent. (e) In the central plateau of the colony (not visible in panel a). Note the differences in cell shapes and alignments in the various panels.

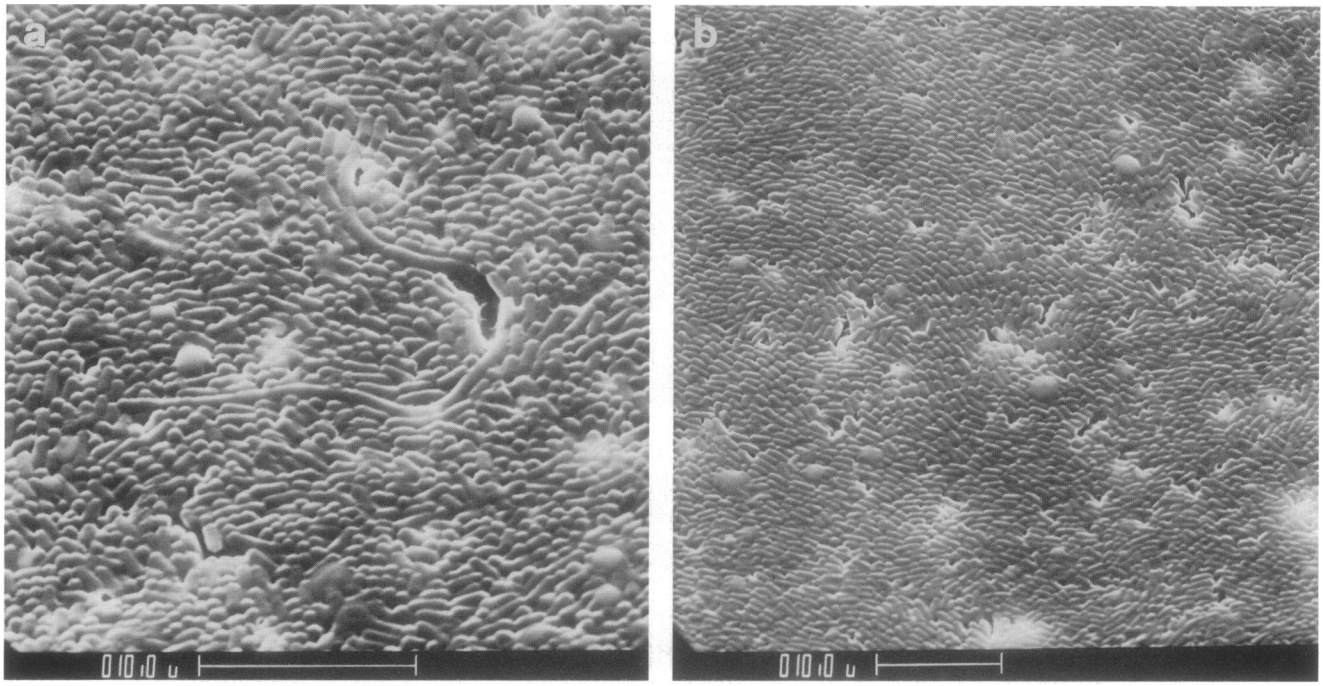


FIG. 8. Two views of the 32A colony. These pictures were both taken in the central plateau. Panel b was located closer to the depression ring. Note the long, partially septated bacillus in panel a and the high degree of order in panel b.

pattern to their distribution because they could readily be located by searching the ridge tops.

The oldest colonies examined, 68 h old, were similar to each other. Both showed various regions of the kinds observed in the 48-h colonies and illustrated in Fig. 10. The one labeled 68A was unique among the colonies examined here in that it had a prominent sector marked by a very sharply defined boundary between distinct multicellular arrays (Fig. 12). Both 68-h colonies had sawtooth concentric patterns at the edges, and successively higher-magnification

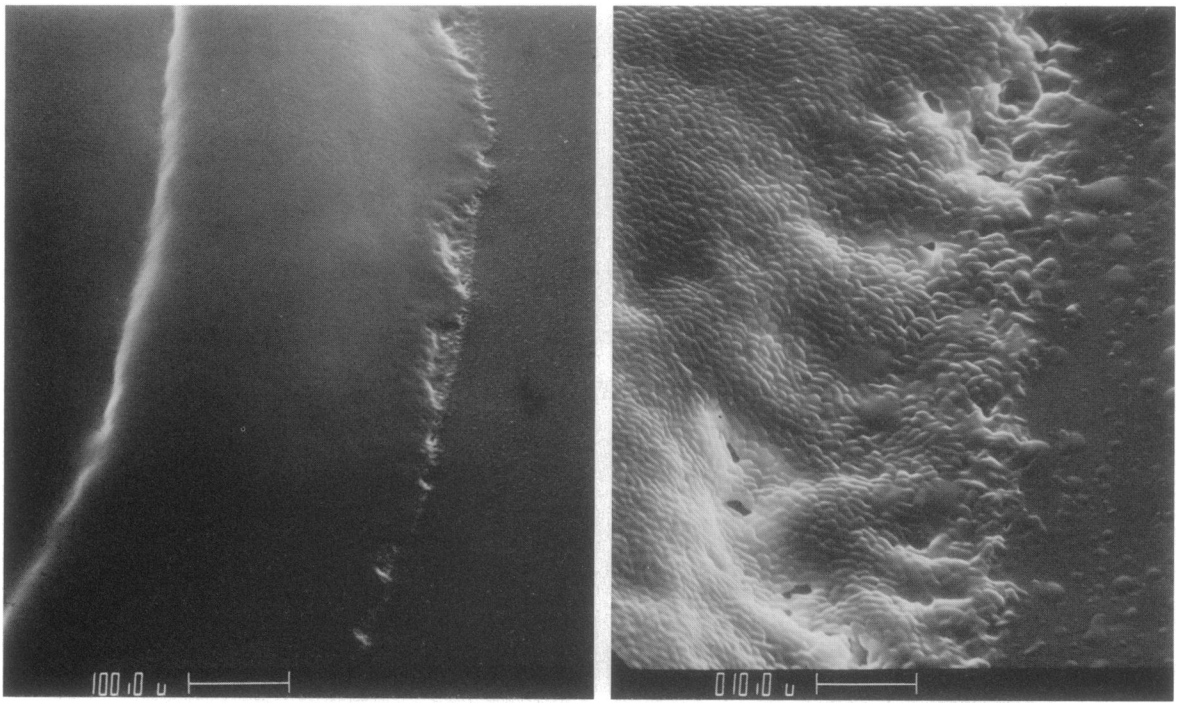


FIG. 9. One region at the edge of the 43-h colony. The panels show two magnifications of the same region at three o'clock on the colony in Fig. 3. Note the sawtooth pattern of the overall edge structure and the ovoid shapes of the bacteria.

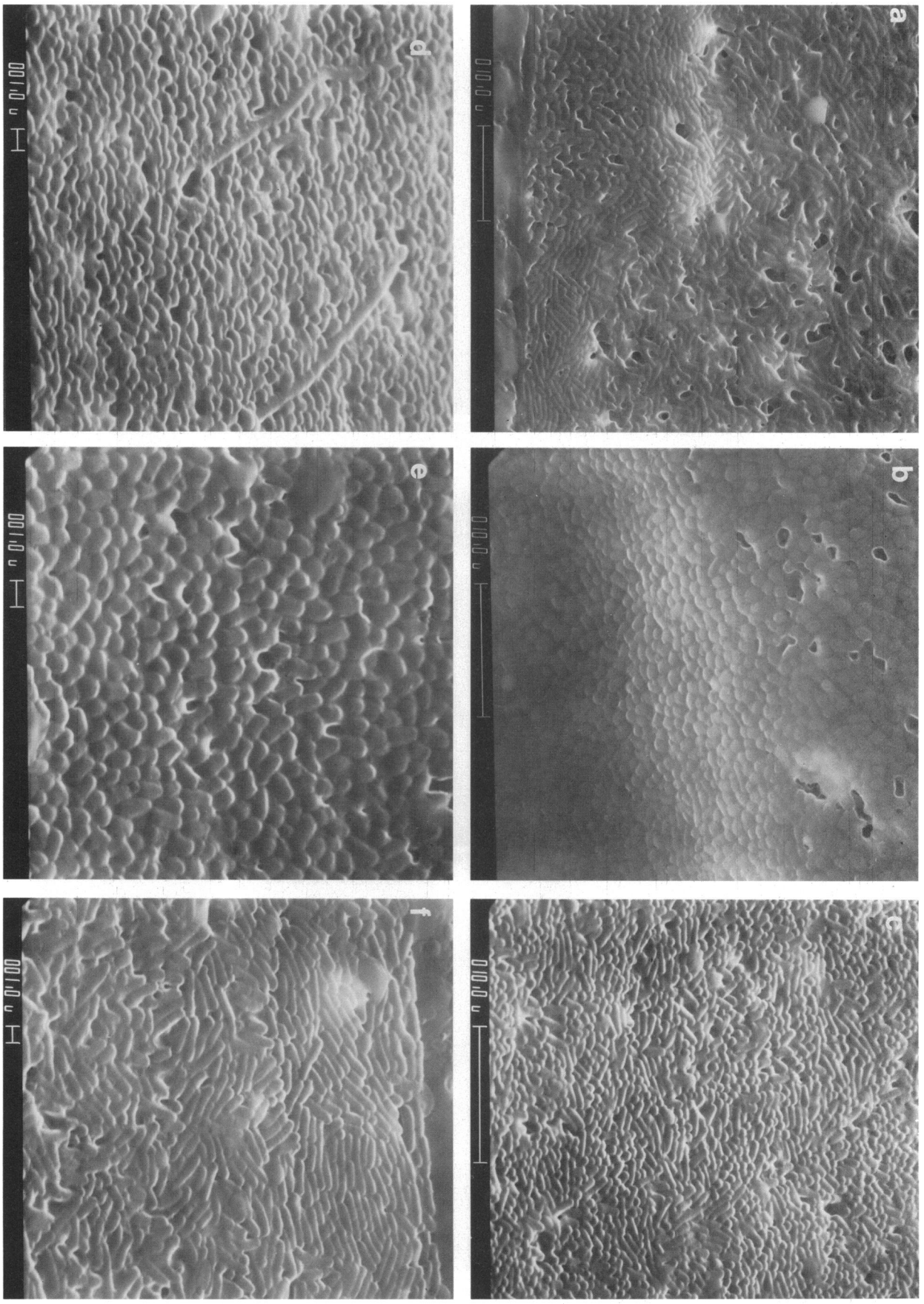


FIG. 10. A series of views along a line from the bottom edge (a) to the top edge (f) of the 48A colony shown in Fig. 3. See the text for the location of each panel. Note the wide range of cellular morphologies and multicellular arrangements in different zones of this colony.

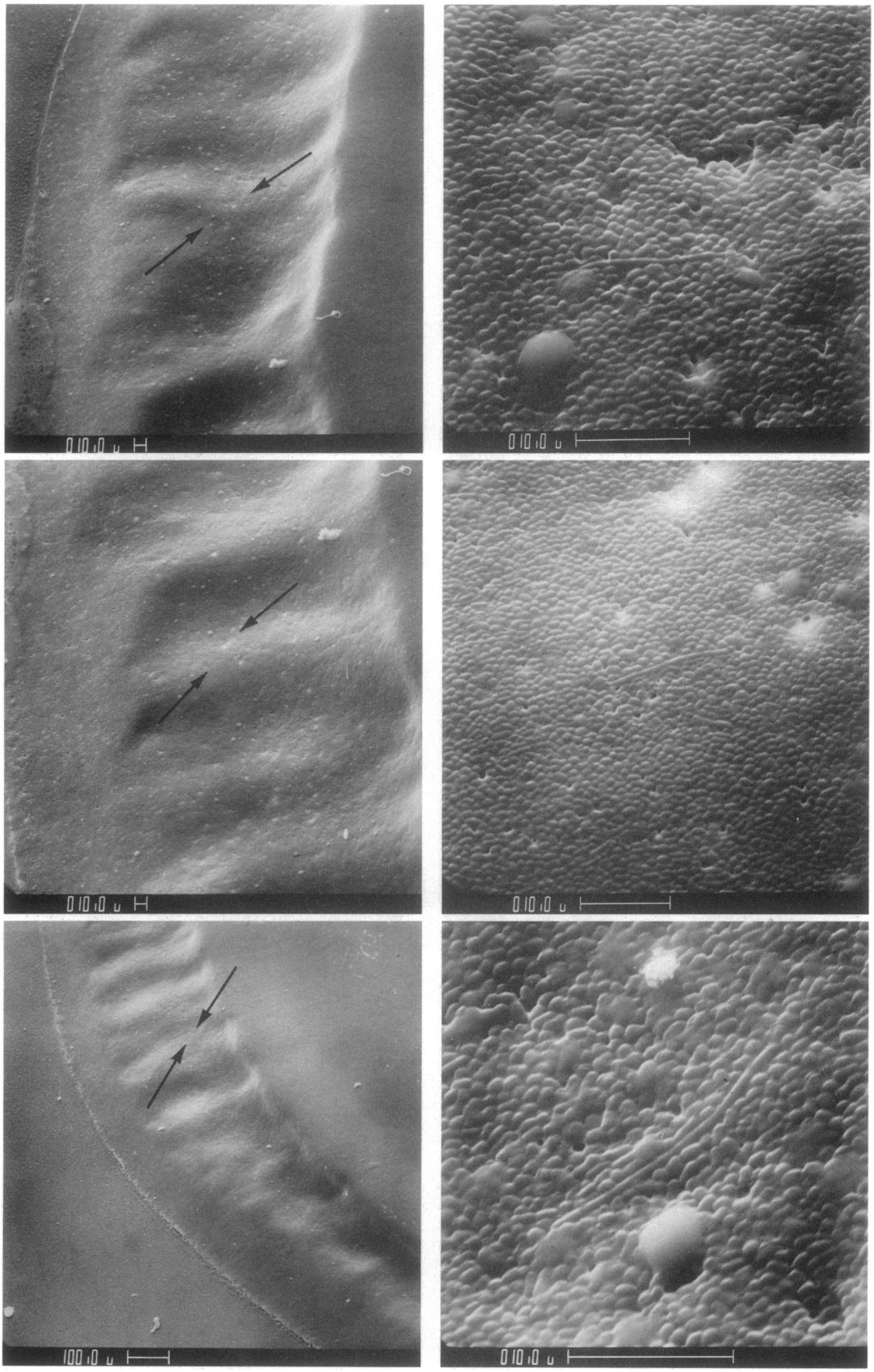


FIG. 11. Three sites on the left side of the 48B colony shown in Fig. 3. The lower-magnification pictures at the left show the fluted ridges characteristic of the outer region of this colony, and the arrows on each panel indicate the position of the corresponding higher-magnification picture at the right. Note the radially oriented long cell nestled in a field of small cells on top of each ridge.

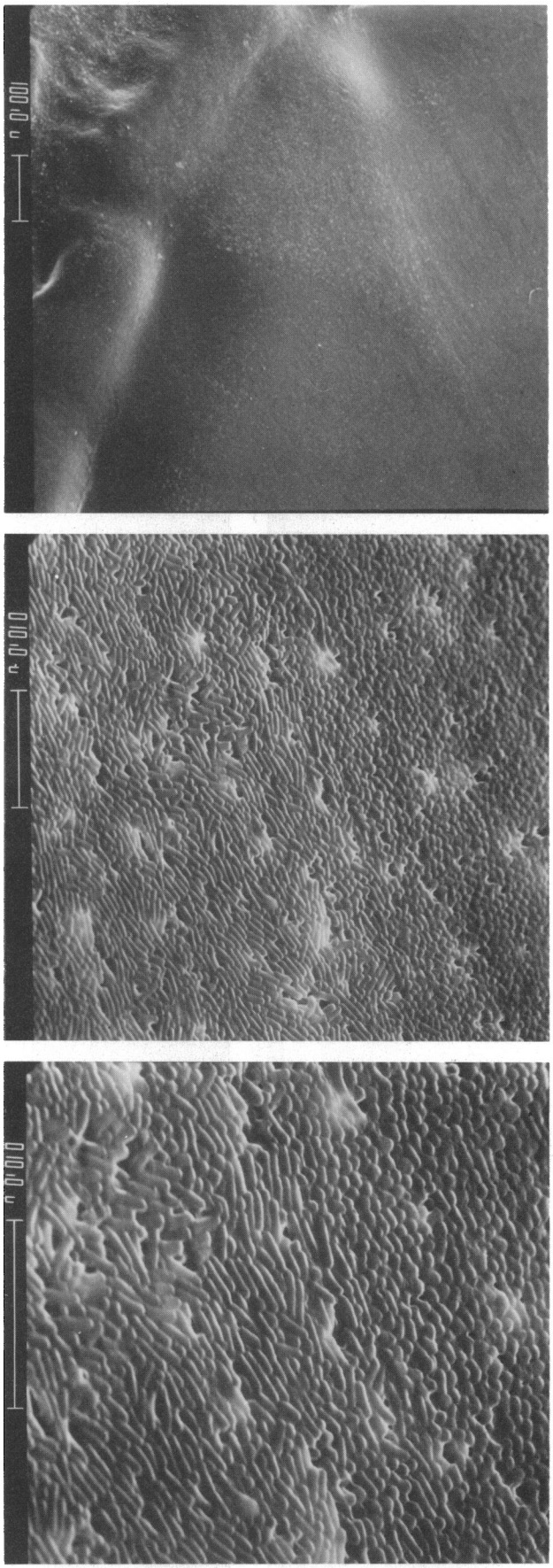


FIG. 12. The sector visible on the 68A colony. The three panels show successively higher-magnification pictures to zoom in on the top edge of this sector. Note the sharp definition of the upper boundary of the sector.

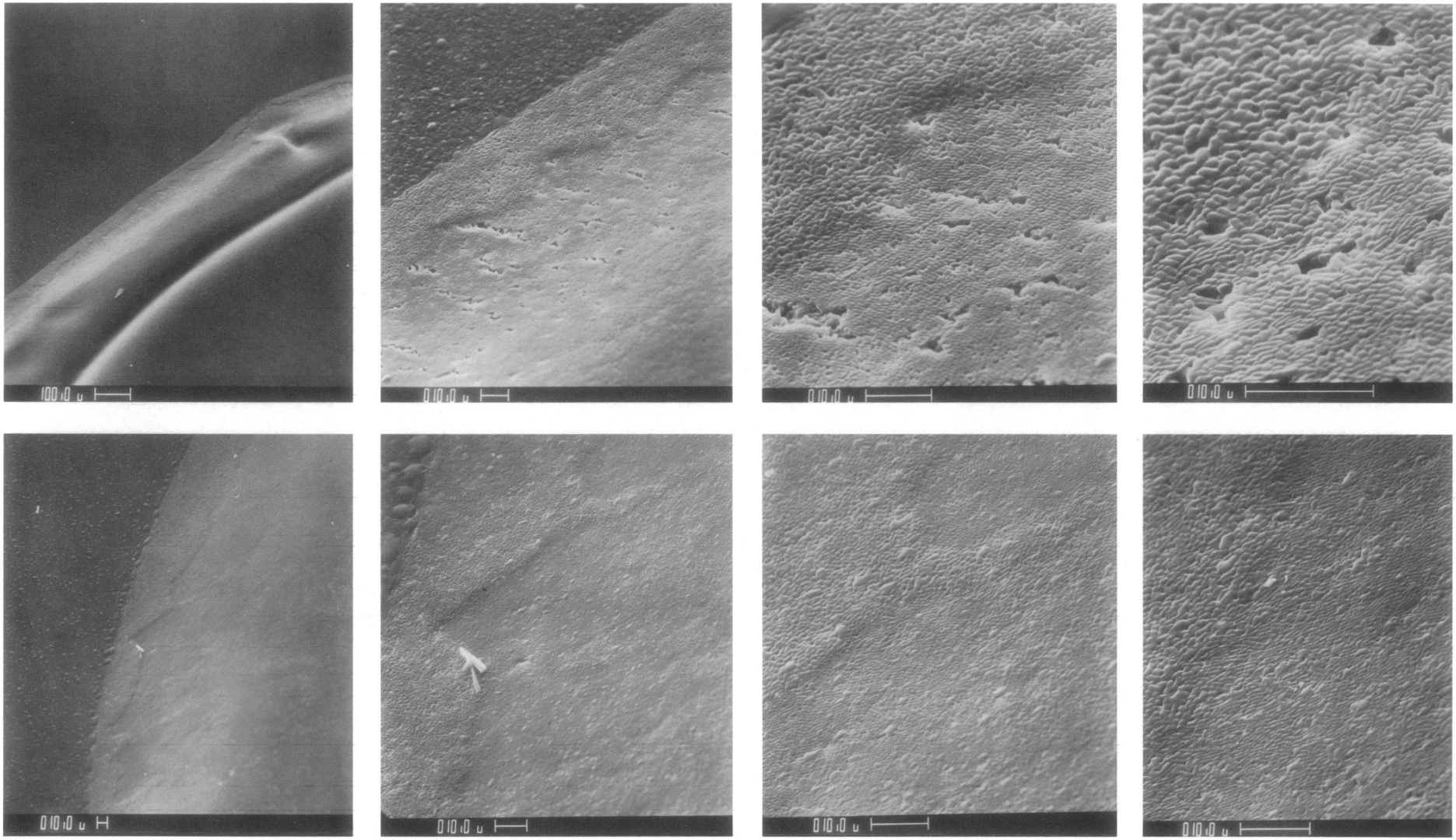


FIG. 13. The edges of the two 68-h colonies. The top row shows successively higher-magnification pictures that zoom in on a particular region of the edge of the 68A colony, and the bottom row shows similar views of the edge of the 68B colony. Note (i) the sharp change from parallel arrays of bacilli inside the brim to irregular groupings of larger ovoid cells at the very edge and (ii) the jagged nature of the demarcation between these two multicellular arrangements.

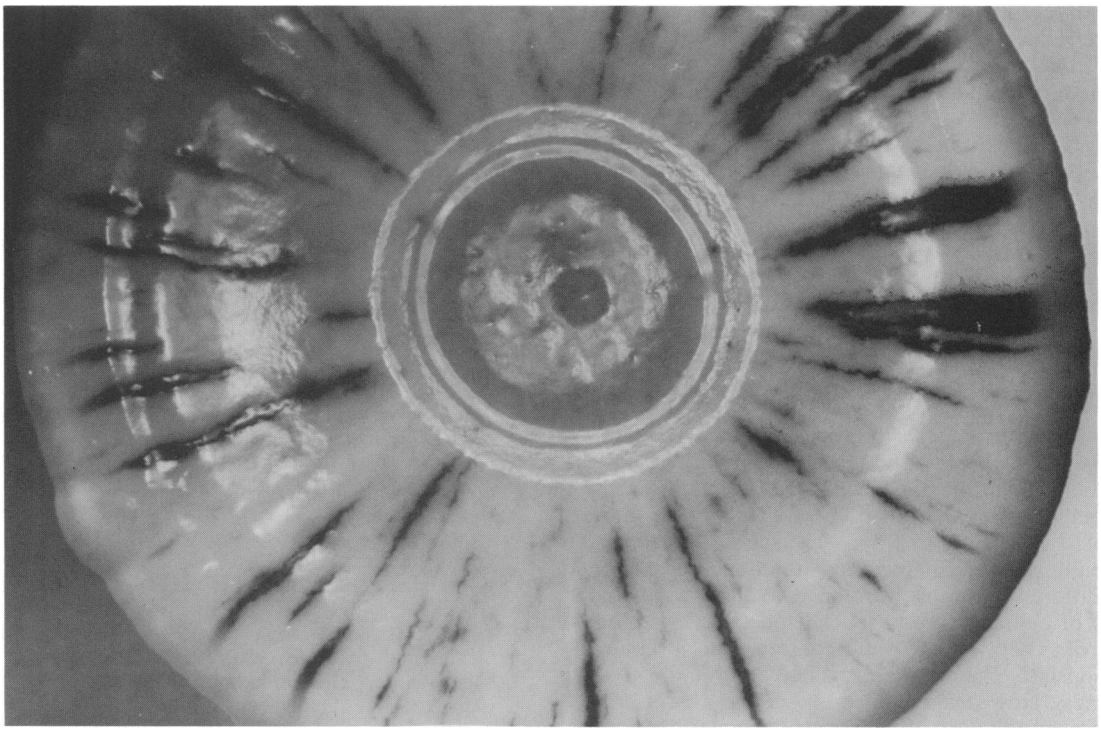


FIG. 14. A colony produced by spotting 1 μl of a culture of an M7124 derivative carrying a Mu*dlac* element on β-galactosidase indicator agar. This colony was 10 days old and measured 14 mm across. The picture was taken at low magnification with a WILD M420 Makroskop fitted with coaxial circularly polarized light optics and with additional lateral illumination from a fiber optics unit. At the magnification used, the coaxial illumination did not cover the specimen completely, so that surface structure is clearly visible only in the central region and outward toward nine o'clock on the edge. Note the depression ring in the central region and the section of the indentation ring visualized by the coaxial illumination at the left.

pictures of these edges showed a sharp transition from an inner zone of palisaded bacilli to an outer zone of larger, less regularly oriented ovoid cells (Fig. 13). These sawtooth patterns were reminiscent of that seen at the edge of the 43-h colony (Fig. 9).

DISCUSSION

Because SEM pictures show the structures of fixed, dried material, it is important to ask how reliable a guide such pictures are to the structures of the original living material. Some aspects of this question were already addressed with respect to SEM images of *Pseudomonas putida* colonies (18), and the conclusion can be repeated that fixed material is not the same as living material but that patterns in fixed material prepared without loss of overall organization (as done here) do reflect patterns in the living material. In the case of *E. coli* colonies, two further similarities (one microscopic and one macroscopic) between observations on living and fixed material provide added confidence in the reliability of the preparation methods used: (i) the palisades of bacteria at the edges of young colonies (Fig. 6; references 6, 9) and (ii) the depression and indentation rings in older colonies (Fig. 3 and 14).

Several general features of the low-magnification SEM pictures deserve special emphasis. They are the increase in concentric structural complexity with age, the good overall circular symmetry of the colonies at all ages, and the appearance of specific irregularities at various points around the colony circumference (e.g., the crescent in the 24.5-h colony and the flanges and extensions in the 32-, 43-, 48-, and 68-h colonies). With a single exception in the 68A colony, these irregularities did not have the shapes of sectors, and no relics of such inhomogeneities were visible in successively older colonies. Thus, these irregularities appear to represent transient features of the growth process which were compensated for as morphogenesis proceeded. Some of the macroscopic features which developed with age, such as the shallow depression ring around the inoculation site and the deeper indentation ring inside the rim, were almost always permanent aspects of colony structure once they had appeared. Other features, such as the breadth of the plateau zone between these two rings, changed in a regular way that was related to overall colony growth. Still other features, such as the brim extending outward onto the substrate at the colony perimeter, appeared to alternate in a repetitive manner that was reminiscent of swarm cell spreading in *Proteus* sp. The steady growth of some concentric structural features and the periodic changes in other features emphasize the progressive nature of colony development. The directed nature of this progressive morphogenesis was highlighted by observing the exceptional rim structure on the 48B colony. The fact that this structure was uniform around the entire circumference meant that this colony had entered a developmental pathway distinct from that of the other colonies examined, even though the 48B colony was inoculated with the same culture as they were. Thus, some different choice must have been made earlier in development that influenced the way morphogenesis proceeded over the whole colony.

The high-magnification pictures presented here showed that growth of *E. coli* colonies was accompanied by the formation of sharply delineated zones containing bacterial

populations differentiated from each other with respect to cell sizes, cell shapes, and patterns of multicellular alignment. Occasionally these zones were organized as sectors. However, the predominant mode of differentiation observed in these young colonies was concentric and thus independent of cell lineages. These observations on colony microstructure relate directly to earlier work on the control of large-scale aspects of colony morphology (15–17, 19), because it was the general rule that macroscopic boundaries, such as sector edges and concentric changes in colony structure, were marked by microscopic boundaries between bacterial groups that differed in cell size, cell shape, and cell alignments. The results thus provided an affirmative answer to the question of whether populations of nonswarming bacteria display identifiable changing patterns of multicellular organization as colonies develop.

While answering a general question about procaryotic morphogenesis, the observed patterns raised many detailed questions about function and regulation. What roles did differential cell division and postdivision growth play in the formation of the various cell types observed? Were the morphologically distinguishable cells functionally and biochemically different from each other? Did the various distinct groups of cells carry out specialized tasks in colony building, or did they assume such characteristic organizations in response to chemical heterogeneity within the growing cell masses? How did regular multicellular arrays originate, and why were they so obvious in some zones but not in others? What coordinated the activities over time of the many different cell groups throughout each colony to produce a regular colony morphology? The utility of the present observations is not in providing any answers about underlying mechanisms controlling procaryotic morphogenesis but lies rather in defining some questions to be addressed. The observation that different fields composed of many thousands of cells sharing size, shape, and orientation appeared at specific times and places during colony development was important because it demonstrated the need to investigate systems that regulate the group behavior of large numbers of bacterial cells. The observation that some features of colony structure departed from regularity was equally important because it demonstrated the need to investigate how decisions and adjustments are made during development to produce regular colony morphologies.

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LITERATURE CITED

1. **Axelrad, C.** 1903. Ueber morphologie der colonien pathogener bakterien. Z. Hyg. Infektionskr. **44**:477–497.
2. **Bisset, K. A.** 1939. The mode of growth of bacterial colonies. J. Pathol. Bacteriol. **48**:427–435.
3. **Bisset, K. A., and C. W. I. Douglas.** 1976. A continuous study of morphological phase in the swarm of *Proteus*. J. Med. Microbiol. **9**:229–231.
4. **Buchanan, R. E., and N. E. Gibbons.** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
5. **Busila, V.** 1940. Beiträge zu dem studium der bakterienlagerungen in mikroskopischen kolonien. Zentralbl. Bakteriol. Hyg. Abt. Mikrobiol. 1 Orig. A **146**:124–128.
6. **Douglas, C. W. I., and K. A. Bissett.** 1976. Development of concentric zones in the *Proteus* swarm colony. J. Med. Microbiol. **9**:497–500.
7. **Graham-Smith, G. S.** 1910. The division and post-fission movements of bacilli when grown on solid media. Parasitology **3**:17–53.
8. **Hoeniger, J. F.** 1964. Cellular changes accompanying the swarming of *Proteus mirabilis*. I. Observations on living cultures. Can. J. Microbiol. **10**:1–9.
9. **Hoeniger, J. F.** 1965. Development of flagella by *Proteus mirabilis*. J. Gen. Microbiol. **40**:29–42.
10. **Hoffman, H., and Franck, M. E.** 1961. Form and internal structure of cellular aggregates in early *Escherichia coli* microcultures. J. Gen. Microbiol. **25**:353–364.
11. **Kvittingen, J.** 1949. Studies of the life-cycle of *Proteus* Hauser. Acta Pathol. Microbiol. Scand. **26**:24–50.
12. **Legroux, R., and J. Magrou.** 1920. Etat organisè des colonies bactèriennes. Ann. Inst. Pasteur (Paris) **34**:417–431.
13. **Nutt, M.** 1927. The method of division of the rough and smooth type of colonies among bacilli of the *Salmonella* group. J. Hyg. **26**:44–48.
14. **Roelcke, K., and W. Bartram.** 1939. Ueber die wuchsformen des bacterium proteus. Zentralbl. Bakteriol. Hyg. Abt. Mikrobiol. 1 Orig. A **143**:247–252.
15. **Shapiro, J. A.** 1984. Transposable elements, genome reorganization and cellular differentiation in gram-negative bacteria. Symp. Soc. Gen. Microbiol. **36**(Part 2):169–193.
16. **Shapiro, J. A.** 1984. The use of Mu*dlac* transposons as tools for vital staining to visualize clonal and non-clonal patterns of organization in bacterial growth on agar surfaces. J. Gen. Microbiol. **130**:1169–1181.
17. **Shapiro, J. A.** 1985. Photographing bacterial colonies. ASM News **51**:62–69.
18. **Shapiro, J. A.** 1985. Scanning electron microscope study of *Pseudomonas putida* colonies. J. Bacteriol. **164**:1171–1181.
19. **Shapiro, J. A.** 1986. Control of *Pseudomonas putida* growth on agar surfaces, p. 27–69. *In* J. R. Sokatch (ed.), The bacteria, vol, 10. Academic Press, New York.
20. **Slutsky, B., J. Buffo, and D. R. Soll.** 1985. High-frequency switching of colony morphology in *Candida albicans*. Science **230**:666–669.
21. **Sturdza, S. A.** 1975. Expansionserscheinungen der proteuskulturen. I. Die schwärmexpansion. Zentralbl. Bakteriol. Hyg. Abt. Mikrobiol. 1 Orig. A **233**:505–530.
22. **Sturdza, S. A.** 1977. Expansionserscheinungen der proteuskulturen. II. Die verschiebungexpansion. Zentralbl. Bakteriol. Hyg. Abt. Mikrobiol. 1 Orig. A **238**:444–474.
23. **Williams, F. D., and R. H. Schwarzhoff.** 1978. Nature of the swarming phenomenon in *Proteus*. Annu. Rev. Microbiol. **32**:101–122.
24. **Winge, Ö.** 1944. On segregation and mutation in yeast. C. R. Trav. Lab. Carlsberg, Ser. Physiol. **24**:79–95.