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Scanning Electron Microscopy of Fruiting Body Formation by Myxobacteria

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Scanning electron microscopy was used to follow fruiting body formation by pure cultures of *Chondromyces crocatus* M38 and *Stigmatella aurantica*. Vegetative cells were grown on SP agar and then transferred to Bonner salts agar for fructification. Fruiting in both species commences with the formation of aggregation centers which resemble a fried egg in appearance. In *Chondromyces* the elevated center or "yolk" region of the aggregation enlarges into a bulbous structure under which the stalk forms and lengthens. At maximum stalk height the bulb extends laterally as bud-like swellings appear. These are immature sporangia and are arranged in a distintive radial pattern around the top of the stalk. This symmetry is lost as more sporangia are formed. *Stigmatella* does not form a bulb; rather the yolk region of the aggregation center projects upward to form a column-like stalk which is nearly uniform in diameter throughout its length. At maximum stalk height, the terminus of the stalk develops an irregular pattern of bud-like swellings. These differentiate into sporangia. Stalks of 2-week-old mature fruiting bodies of both species appear to be cellular in composition. Stereomicrographs suggest orientation of these cells parallel to the long axis of the stalk. Stalks of 8-week-old fruiting bodies of *Chondromyces* were acellular and consisted of empty tubules, suggesting that the cells undergo degeneration with aging of the fruiting body.

Under appropriate but only partially defined conditions, fruiting myxobacteria exhibit an elaborate life cycle. It is characterized by the transformation of swarms of rod-shaped vegetative cells into aggregation centers. The centers ultimately project above the surface as fruiting bodies. Fruiting bodies may be simple or complex in structure. Among the simplest are the spherical-shaped, sometimes constricted at the base, and stalkless forms produced by *Myxococcus* species. These are composed wholly of resting cells held together by slime. By contrast, species of *Chondromyces* and *Stigmatella* produce complex fruiting bodies consisting of numerous sporangia (cysts) borne at the terminus of distinct stalks. Only the sporangia contain resting cells and are usually separated from the stalk by an extension of variable length termed a pedicel. A slime layer encompasses all parts of the fruiting structure.

Fruiting bodies of myxobacteria were first represented in the literature as drawings (5, 17-19). Later workers used light microscopy to record their observations (9, 11-14, 16). However, light microscopy failed to provide sufficient depth of field for detailing the relatively larger and more complex fruiting structures. With the advent of scanning electron microscopy (SEM), a tool became available which provided significant increases in both depth of field and resolution. Recognizing this potential, several workers studied myxobacter fruiting bodies using SEM (2, 3, 10, 15). With the exception of recent work by Campos and Zusman (3) and Shimkets and Seale (15), previous studies concentrated on observations of mature fruiting bodies of several genera, with only a limited number of developmental stages included.

The present study employs SEM to follow the development of pure cultures of *Chondromyces crocatus* M38 and *Stigmatella aurantica* from vegetative swarms to mature fruiting bodies.

MATERIALS AND METHODS

Organisms and cultural conditions. *C. crocatus*, M38, was obtained from H. McCurdy and *S. aurantica* from W. Fluegel. Both organisms were grown on SP agar plates (7) for 5 to 7 days at room temperature (21 to 25 C). For fructification, swarms were removed from SP with a transfer loop and gently spread on the surface of Bonner salts (1) plus 15 g of agar (Difco) per liter.

Electron microscopy. Growth was stopped at various developmental stages by 8% glutaraldehyde vapors. Exposure to the vapor was for a minimum of 4 h at room temperature. Additional fixation and initial dehydration were accomplished by placing small blocks of agar containing glutaraldehyde-fixed growth in a graded ethanol series. The blocks were immersed upright in ethanol only to the depth required to cover growth. The ethanol was then exchanged for amyl acetate in a graded series. Subsequently, the blocks were placed in small cups made from filter paper and saturated with amyl acetate.

Drying was accomplished by the critical point method in liquid CO_2. The dried blocks were mounted on specimen stubs with double adhesive "transparent" tape and were metal-coated in a vacuum evaporator, first with silver and then gold, to a thickness of 10 mm each.

The specimens were examined with a Cambridge Stereoscan Mark IIA SEM.

RESULTS

The scanning electron micrographs shown in Fig. 1 and 2 represent the sequential development of fruiting bodies by *C. crocatus* M38. Figure 1a shows four early stages of vegetative cell aggregation. The earliest stage is slightly elevated and barely discernible from the background swarm. Individual cells within the youngest aggregation centers show no apparent orientation (Fig. 1b). As the aggregation centers continue to develop, the central portion differentiates, resulting in a fried-egg appearance (Fig. 1c). Some degree of orientation is exhibited by the peripheral cells of this stage. The central portion or "yolk" region of the fried egg enlarges into a bulbous structure (Fig. 1d and e). Cells composing the bulb were often parallel to adjacent cells but showed no directed orientation with respect to the bulb (Fig. 1f). The bulb is well developed and at or near its maximum dimensions as stalk formation begins. Figures 1 d–f show the initial stages of stalk formation, whereas Fig. 1g shows a stalk near maximum height and with little increase in its bulb dimensions.

At maximum stalk height the bulb enlarges laterally, the top surface becomes flattened, and bud-like swellings appear (Fig. 2a and g). These swellings develop into immature sporangia arranged in a distinctive pattern (Fig. 2b). This symmetry is lost as additional sporangia form (Fig. 2c and d). Mature sporangia are separated from the stalk by pedicels (Fig. 2c). Figure 2f is a higher magnification of a pedicel and of the cells composing it. Occasionally more mature stalks produce sporangia below the main sporangial cluster (Fig. 2c and d).

During preparation breaks and cracks sometimes occurred due to the fragile nature of the fruiting bodies. These defects permitted limited observation of cells beneath the thick slime layer. Figure 2d shows a mature fruiting body with a break in the slime cover near the stalk base. Closer examination of this region revealed cells oriented parallel to the long axis of the stalk (Fig. 2e).

Figures 2g and h summarize sporangia formation after elongation of the stalk to maximum height. A significant increase in stalk diameter became apparent at this stage. This is especially well evidenced by Fig. 2g.

Figures 3, 4, and 5 depict the developmental stages of *S. aurantica* fruiting bodies. Initial stages resemble those of *Chondromyces* except that the background swarm cells are less obscured by slime and the aggregation centers are roughly three times larger in diameter (Fig. 3a). The background swarm cells appear directionally oriented. Figure 3b shows a fried-egg stage with peripheral cell orientation. Occasionally, a pronounced ring of cells was observed to encircle the yolk region of this stage (Fig. 3c and e). Cells in the yolk region of all early aggregation stages viewed were tightly packed but showed no apparent directed orientation (Fig. 3f).

Continued upward development of the yolk region forms the stalk. Its diameter remains nearly constant throughout its length (Fig. 3c and d). Once the stalk attains maximum height, randomly scattered bud-like swellings appear at its tip. Figure 4a shows the bud-like swellings as they begin to form and Fig. 4b depicts stalks viewed from the top with swellings at various stages of sporangial development. The irregular distribution of the bud-like swellings is shown in more detail in Fig. 4c. Mature fruiting bodies averaged four sporangia but ranged from one to seven or more in number (Fig. 4d). The sporangia are attached to the tip of the stalk by pedicels of variable length. A heavy slime layer enveloped the cells composing the pedicels, but they were typical of those contained within the sporangia (Fig. 5b).

As in *Chondromyces*, the mature fruiting bodies of *Stigmatella* were heavily encased in slime. Stalk slime is thickest and relatively smooth at the base (Fig. 5a). It becomes thinner and assumes a more rugose appearance on the upper stalk and sporangial surfaces. Figure 5c shows a broken mature fruiting body stalk and the cells composing it. High-magnification stereomicrographs of the interior of this stalk show the cells to be aligned parallel to the long axis of the stalk (Fig. 5d). Heavy slime surrounds the

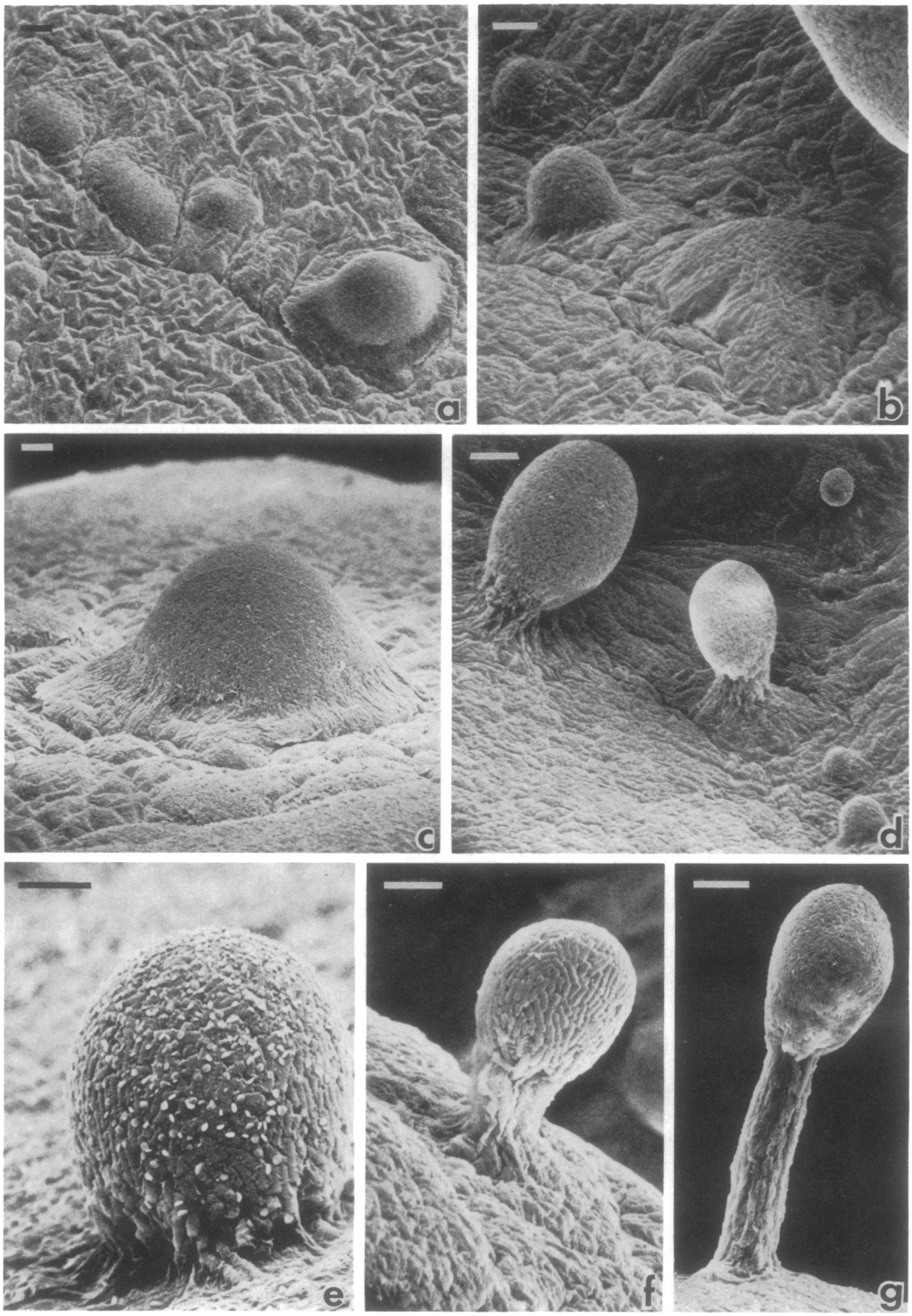

FIG. 1. *Early developmental stages in fruiting body formation by C. crocatus. (a) Initial stages of vegetative cell aggregation; bar = 20 μm. (b) Higher magnification of the youngest stage; bar = 5 μm. (c) Fried-egg stage showing orientation of peripheral cells; bar = 10 μm. (d) Four stages of bulb formation; bar = 10 μm. (e and f) Initial stages in stalk formation; bar = 5 μm. (g) Stalk at maximum height; bar = 10 μm.*

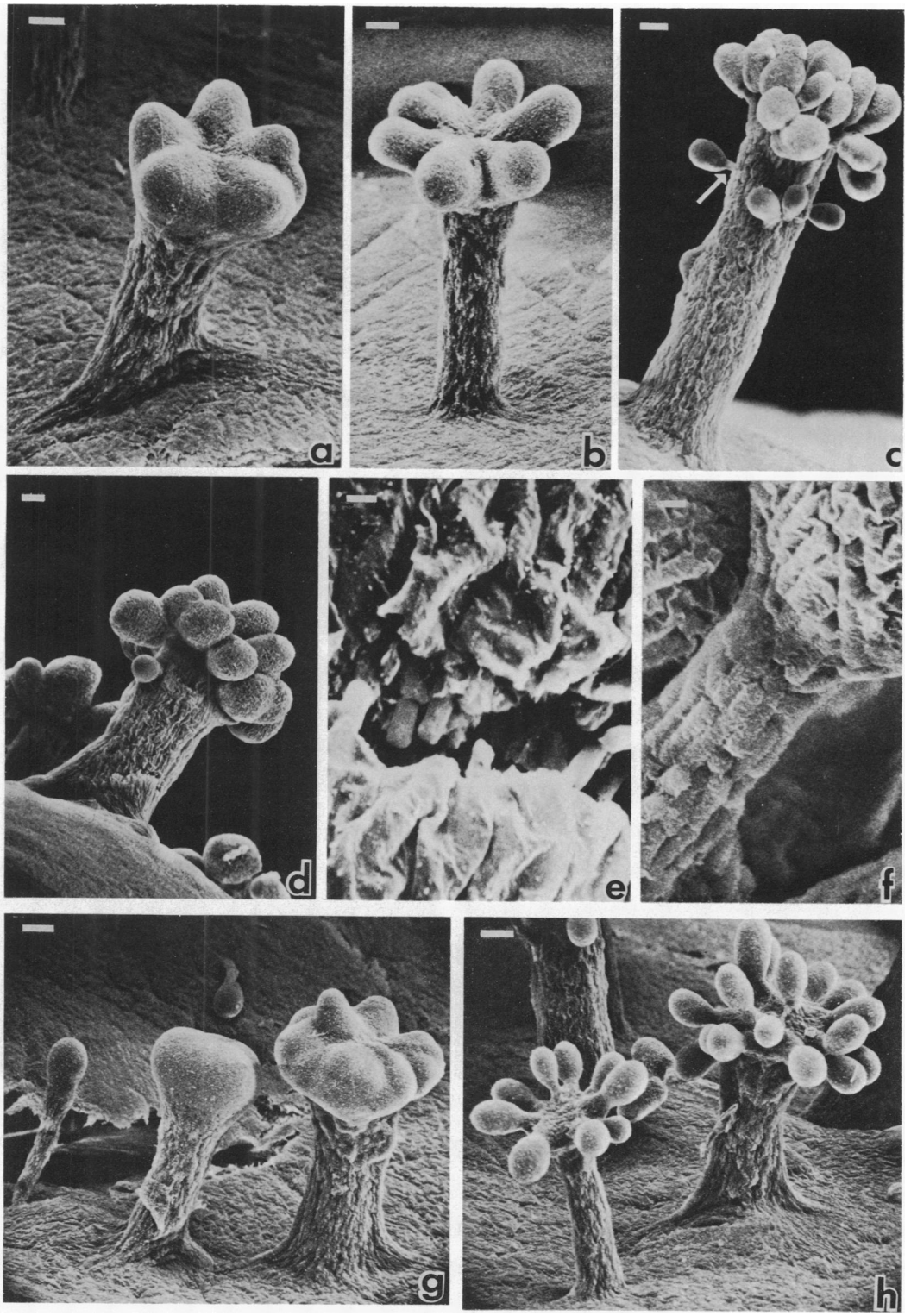

FIG. 2. *Late developmental stages in fruiting body formation by C. crocatus. Bars = 10 μm; except in (e and f) bars = 1 μm. (a and b) Initial stages of sporangia formation. (c) Mature fruiting body with sporangia midstalk. Arrow indicates pedicel. (f) Higher magnification of pedicel and cells composing it. (d) Mature fruiting body with a break in the slime layer near the base. (e) Higher magnification of break showing cells beneath the slime layer. (g and h) Summary of sporangia formation after elongation of stalk to maximum height.*

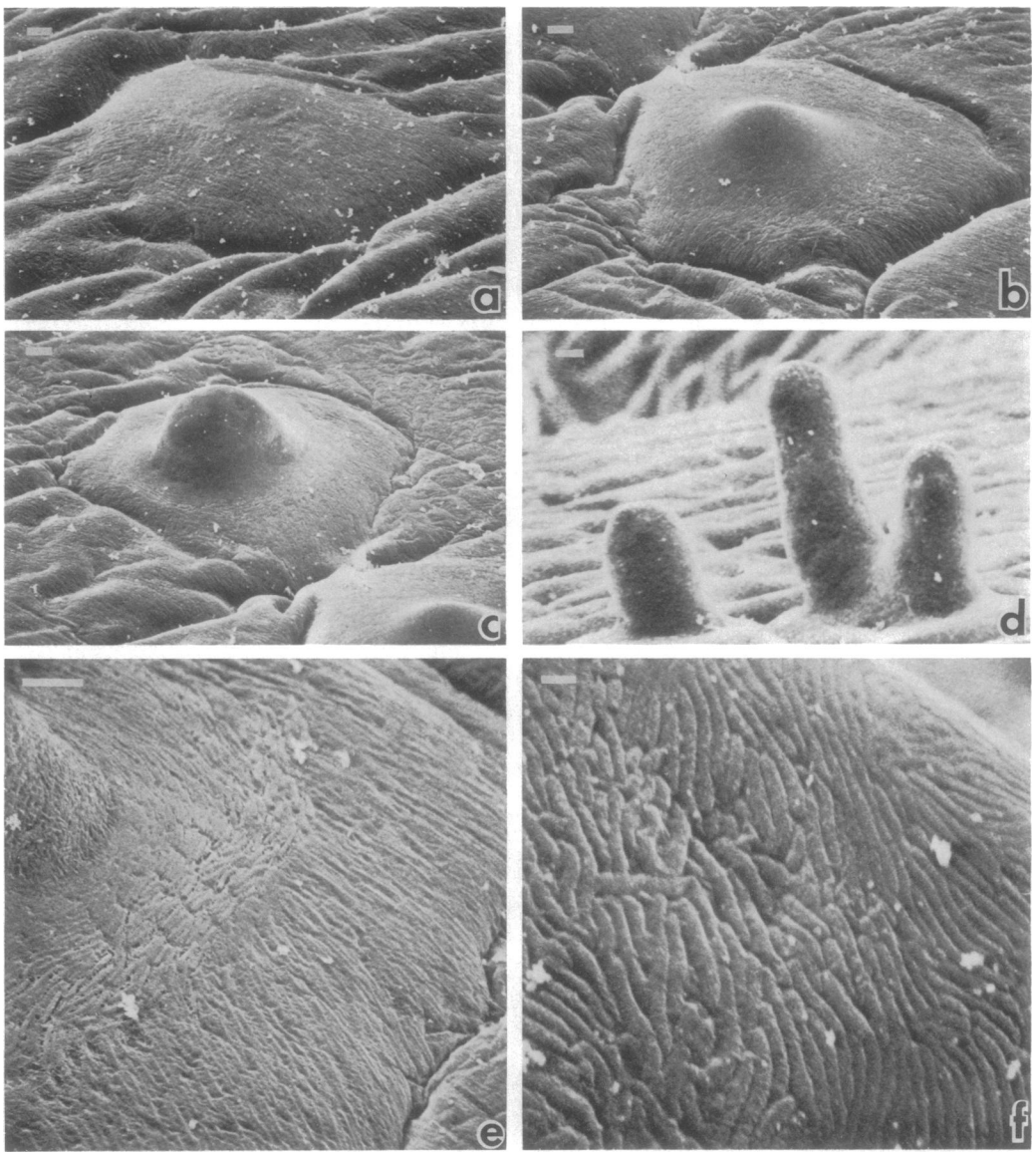

FIG. 3. *Initial stages of fruiting body formation by S. aurantica. Bars = 10 μm; except (e and f) bars = 5 and 1 μm, respectively. (a) Young aggregation center preceding yolk region formation. (b and c) Development of yolk region. (d) Three stages of stalk formation. (e) Higher magnification of ring of cells encircling yolk region of fried-egg stage shown in (c). (f) Higher magnification of tightly packed cells in yolk region.*

individual cells and forms "holes" from which cells were pulled when the stalks were broken.

Mature *Stigmatella* fruiting body stalks exhibited considerable variation in their gross appearance. Branching of the stalks was observed most frequently in clusters of fruiting bodies (Fig. 4b). Isolated fruiting bodies were seen to branch less frequently (Fig. 4d).

DISCUSSION

Considerable effort was spent developing techniques for specimen preparation which would assure specimens with minimum drying artifacts. Our experience showed that fixation by submergence in liquid glutaraldehyde gave good results with several species of living *Myxococcus* and of *Cystobacter fuscus*, but

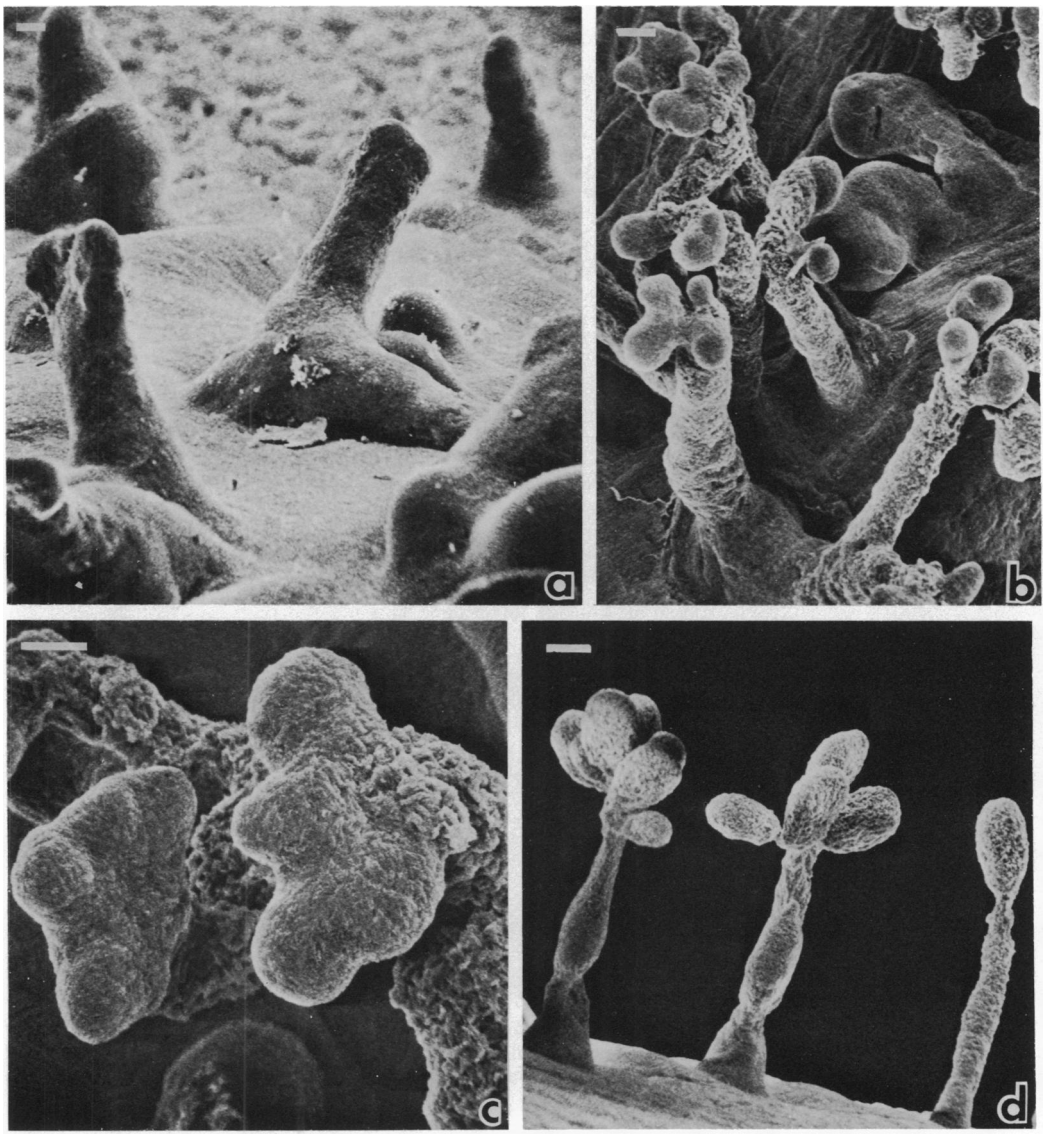

FIG. 4. *Final stages of S. aurantica fruiting body formation. Bars = 20 μm; except in (c) bar = 10 μm. (a) Terminus of stalks with bud-like swellings. (b) Immature sporangia differentiating on branching stalks. (c) Higher magnification of two clusters of four immature sporangia each. (d) Three mature fruiting bodies.*

resulted in grossly distorted fruiting bodies of living *Stigmatella* and *Chondromyces*. Osmium fixation also proved unsatisfactory for the same reason. Despite careful preparation, contaminating debris of an unexplained origin occasionally occurred (Fig. 1e).

The early stages of fruiting body development of *S. aurantica* and *C. crocatus* M38 closely parallel each other. Both begin by forming young aggregation centers which are barely distinguishable from their background swarms. Those in *Stigmatella* are noticeably larger in diameter, usually on the order of three times. In both species, the central region of the aggregation center enlarges faster than the outer, causing the aggregations to assume a fried-egg appearance. It is immediately after the fried-egg stage that significant and consistent differences appear between the two species. In *Chondromyces*, a bulbous structure forms from the yolk region, enlarges to its maximum dimensions, and then appears to be pushed from

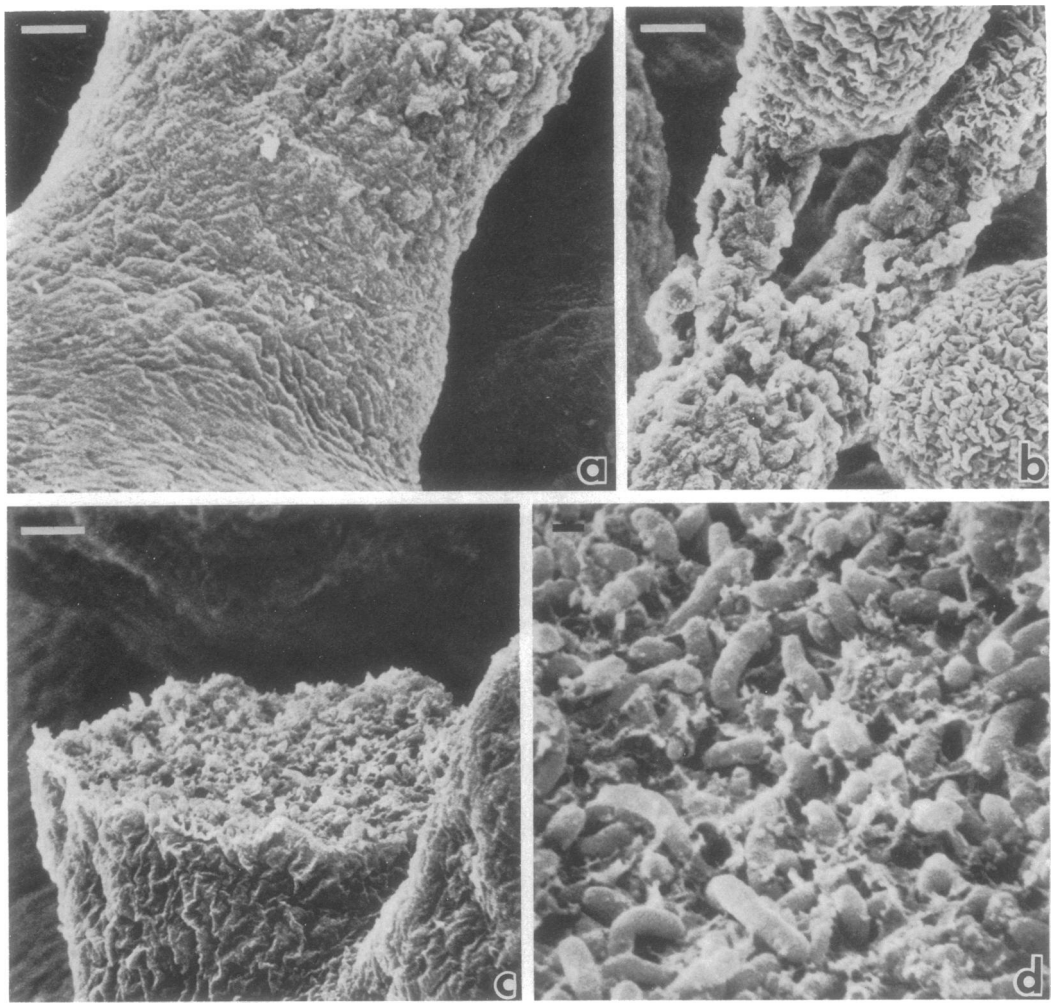

FIG. 5. *Details of stalks and sporangia of S. aurantica. Bars = 5 μm; except in (d) bar = 1 μm. (a) Base of mature fruiting body showing heavy slime covering. (b) Terminus of stalk showing details of pedicels and sporangia. (c) Interior of mature stalk broken near base. (d) Higher magnification of (c) revealing cells embedded in slime. Holes remain from which cells were pulled at the time of the break.*

beneath by the slender developing stalk. *Stigmatella* stalk development differs in that no distinct bulb is formed. Instead, the yolk region extends upward, forming a column the diameter of which remains nearly constant along its entire length.

Dworkin (4) stated that fruiting body formation may occur when "aggregated cells produce slime in a random, disoriented fashion. That slime which is exposed to air dries and hardens, providing a barrier to further expansion in an upward or sideways direction. The slime is thus excreted downwards, lifting the cell mass above the surface." This proposed mechanism of formation is based, in part, on transmission electron microscope observations of *C. crocatus* (8) and *S. aurantica* (20). Those studies indicated the fruiting body stalks to be acellular (or acellular with some degenerative cells) and tubular in nature. In both organisms, the tubules appeared to be arranged parallel to the long axis of the stalk. Our study demonstrated the presence of cells, arranged parallel to the long axis of the stalk, in developing and in 2-week-old mature fruiting body stalks. However, in an attempt to prepare a specimen by freeze drying, we observed empty tubules in several broken stalks of 8-week-old *Chondromyces* fruiting bodies. Thus, the presence of cells in young mature stalks lends limited

support to McCurdy's speculation (8) that cells in an aggregation orient themselves in a vertical position and then raise themselves above the surface by the excretion of slime. The slime thus forms a system of parallel tunnels through which later-entering cells may pass. The stalk height increases as more cells enter the tunnels and contribute their slime. The absence of cells in our older *Chondromyces* stalk preparations is consistent with the transmission electron microscope studies and suggests that aging of the mature fruiting body results in death and degeneration of the stalk cells, leaving only their hardened slime cases to form the stalk. Unfortunately, our work neither fully supports nor refutes either hypothesis and the question of stalk formation remains unsolved.

Once sporangia formation begins, the diameter of the stalk of *Chondromyces* increases significantly. By comparison, *Stigmatella* stalks undergo no apparent increase in diameter and, in fact, often appear to decrease slightly during maturation. It is not within the scope of this paper to speculate on the mechanism(s) involved in these seemingly opposed developmental patterns.

Since branching of the stalks of *Stigmatella* was observed to occur most often under conditions of crowding, we suggest that some branching, at least, is the result of anastomosis of adjacent stalks. Branching of *Chondromyces* stalks was not observed at anytime during our investigations.

In both species, sporangia formation commences when the stalk reaches maximum height. This is consistent with the observation of Kühlwein and Reichenbach (6) in their time-lapse photomicrography study of *Chondromyces*. In *Chondromyces*, the bulb undergoes dramatic enlargement in a lateral direction resulting in a flattened top. This directional enlargement is primarily due to the initial developing sporangia. The distinctive radial pattern which results becomes obscured as additional sporangia develop and mature. The lack of bulb formation by *Stigmatella* may contribute to the random formation of immature sporangia as opposed to the radial pattern displayed by *Chondromyces*.

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