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MICROBIAL AGGREGATION

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PREFACE

Somewhere I have read that an author's prefatory notes are usually written last, if at all written, and that they are usually read last, if at all read. (It must have been in somebody's preface.) And yet they come, by definition, before the main text. First shall be last and last first. This one is no exception.

A preface has a number of uses. Here I will use the device for the most part as a means of acknowledging my debt to those who helped me assemble this book. For this is nothing more than an aggregation, conscious and unconscious, of the words and ideas of others and a bit of mine, chiefly the string to bind them together.

I originally attempted to cover all microbial aggregation systems known and studied to date, but halfway in the writing process, it appeared that to do just that, with justice, required at least two volumes. Thus, the present volume covers in detail bacteria and yeasts. A second volume will comprise the cellular slime molds, the filamentous fungi, algae, and protozoa. Nonetheless, an overall perspective of microbial aggregation as fundamental form and function is presented here to include systems still to be treated in detail. Such an overview, I feel, is demanded by the subject matter.

There is a long list of people I have to thank. First among them is Byron F. Johnson of the National Research Council of Canada in Ottawa. A good many of his suggestions, I have unabashedly appropriated. Without him around to read patiently the drafts as the typewriter spewed them, this little book would have been doubly difficult to write and shape. Among those I have worked with in the laboratory, Bong Yul Yoo of the University of New Brunswick in Fredericton has helped me much by way of many insightful discussions. Both Dr. Johnson and Professor Yoo have been most generous collaborators in other projects.

With deep appreciation, I like to bare my indebtedness to Teena Walker and Susan Levy-Rick. Without their help, intellectual and manual, I would have given up gathering the materials for this volume; instead, I would have reached for the nearest waste basket. Writing may be physically accomplished with one hand, left or right, but the making of a book of this kind takes more than a pair of hands. It requires friends, who do not just watch as the author bleeds. The more difficult references were collected with the kind assistance of Margaret Schade and Noreen Brady.

In addition, the book and I profited much from corrections, comments, and criticisms (some caustic, others kind, never with a hint of malice) from other colleagues, who took some of their precious time to read segments of the manuscript: Isabelle Boisclair, Jack Christ, J. R. Colvin, Patricia Douglas, Allen P. James, C. V. Lusena, Brian L. A. Miki, F. Moranelli, Alain Vaisius, as well as others who would prefer to remain anonymous for fear that their contribution might be only minor. I disagree that their contribution is small. Anne Daley, Chris Gobey, and Lynda Boucher took turns to type the final script for the printer. The original illustrations were drawn by Celia Clyde and Denise Ladoucer. The photographic plates were prepared by Harry Turner. I would like to thank them all.

As well, I would like to thank all the authors who have consented to the re-use of their original materials. They are individually acknowledged at the appropriate places in the text. Here I will just list their names in alphabetical order: M. Achtman, C. E. Ballou, J. T. Bonner, T. D. Brock, M. Crandall, J. P. Duguid, G. M. Dunny, G. Gerisch, R. J. Gibbons, R. B. Gilliland, R. N. Greenshields, B. D. Hartong, E. Helm, W. Heumann, J. Hodgkin, J. Kohli, R. P. Levine, V. L. MacKay, S. A. MacKay, D. Malchow, T. R. Manney, B. L. A. Miki, W. L. Orton, P. C. Newell, N. H. Poon, A. Pühler, H. Reichenbach, G. G. Stewart, N. W. Taylor, R. S. W. Thorne, A. Tomasz, Y. Tsubo, H. van den Ende, M. J. Vold, L. J. Wickerham, N. Yanagishima, B. Y. Yoo, and M. Yusa.

In spite of the many forms of assistance generously given by associates, the book is far from perfect. Of course, my friends are not responsible for the imperfections. That responsibility is greedily reserved by the author for himself. The fool and his book are never parted.

This book is not for my wife, Mia, and the children, Maria and Raoul — even as they suffered as I wrote. There is world enough, I hope, to write another book just for you. You are most welcome to read this one — or even to show it to friends, yours, mine, and ours — provided they are not minors or prudes.

Gode B. Calleja

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Chapter 1

GATHERING THINGS TOGETHER

I. INTRODUCTION

A first book on an ill-defined subject must begin with definitions. For, in spite of the literature it has generated, microbial aggregation remains an ill-defined subject. This book and a subsequent volume are an attempt to gather in two bundles the scattered literature on the subject.

II. AGGREGATION DEFINED

Aggregation is the gathering together of units to make a larger unit (Figure 1). The resultant larger unit is also called an aggregation. In addition, the state or condition of being gathered together is also known as aggregation. The clumping of cells in liquid culture is aggregation. The resultant clump is an aggregation, or an aggregate, of cells. In the first sense, aggregation is a process, a function, the gathering together. In the second, it is a product, a structure, the larger unit. Such a melding of function and structure, despite the mutual disrespect of the physiologist and the morphologist for each other, appears to be a common feature of many languages.

The gathering together and the units may be concrete or purely conceptual. A compilation such as this book is therefore an aggregation. A cell is an aggregation of organelles, an organelle of molecules, a molecule of atoms. Society is an aggregation of individuals. A forest is an aggregation of trees. A sentence is an aggregation of words, a word of letters. The mathematical operation of addition is aggregation. The resultant sum is an aggregation, too, and so is any collective noun or pronoun. However, we shall concern ourselves here not merely with either grammar or numerical manipulation, but primarily with the concrete and the palpable, in particular, the aggregation of microbial cells.

The Milky Way is an aggregation of stars, but only structurally and conceptually. Its origins are not likely due to an aggregation process. Concatenated paper dolls make an aggregation (Figure 2). They are the product of a process not of aggregation but of paper cutting. A chain of bacilli, another concrete example, may be thought of as an aggregation, conceptually, but even in the conceptual sense, the process of chain formation among bacilli is not aggregation. Instead, it is properly called cell division or even cell multiplication, which is outside our present concern. Aggregation is addition, mathematically and rhetorically.

III. CELL AGGREGATION DEFINED

With that in mind and with the unabashed assumption that aggregation is a fundamental condition, function, and structure in biology, we shall define "cell aggregation" as the gathering together of cells to form fairly stable, contiguous, multicellular associations under physiological conditions (Figure 3). To various investigators, it is also known as adhesion, adherence, agglomeration, agglutination, association, autoagglutination, clumping, coagulation, coherence, cohesion, flocculation, flocculence, flotation, isoagglutination, sedimentation, stickiness. The resultant structure is also called agglomerate, agglomeration, aggregate, aggregation, agglutinate, agglutination, clump, cluster, coagulum, coremium, film, floc, flock, grex, head, pellet, pellicle, plasmodium, rhizomorph, ring, sclerotium, slime, slug, strand, stroma, synnema.

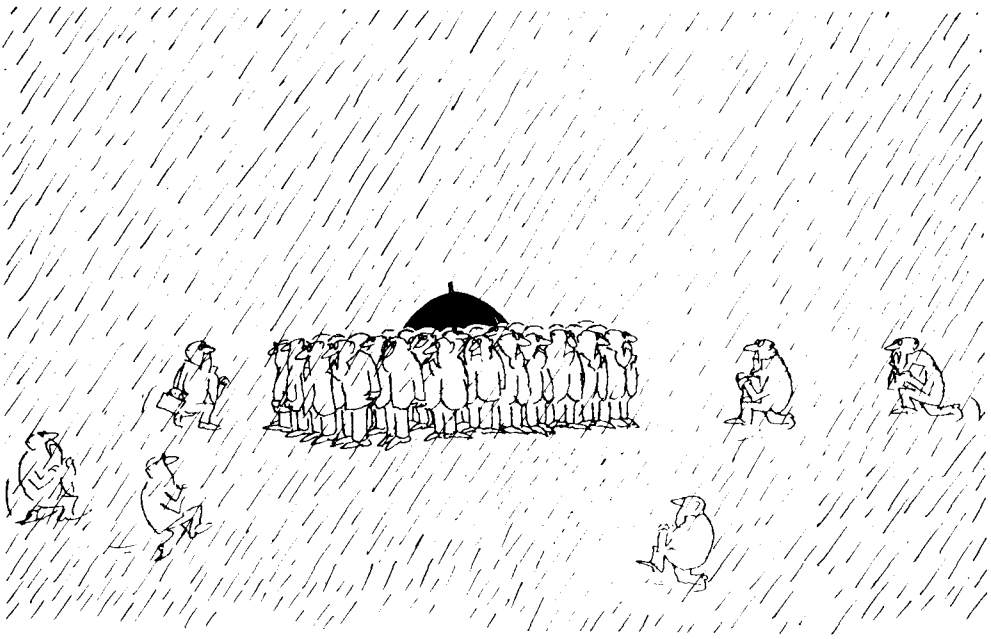

FIGURE 1. Aggregation, nonmicrobial. (Reprinted by permission of the *Bulletin of the Atomic Scientists*, a magazine of science and public affairs. Copyright (c) by the Educational Foundation for Nuclear Science, Chicago.)

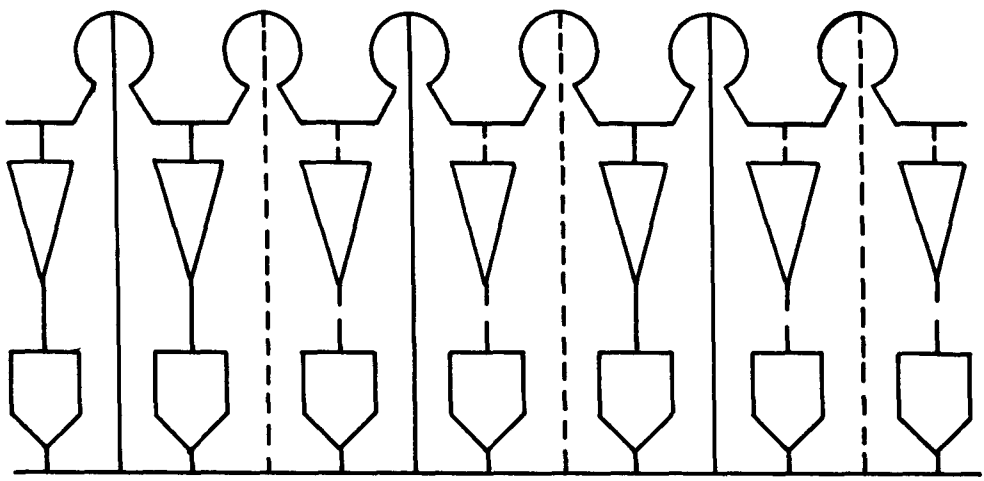

FIGURE 2. A catena of paper dolls. An aggregation structure that is not a product of an aggregation process.

The abundance of names, although a source of minor confusion, must not be viewed as a hindrance to the unification of the field of study. Rather, it is in keeping with the richness of the English language, especially with regard to words describing collectives or aggregations of animals. A few of these more colorful names are a bale of turtles, a charm of finches, a clutter of cats, a congregation of plovers, a cowardice of curs, a cry of hounds,

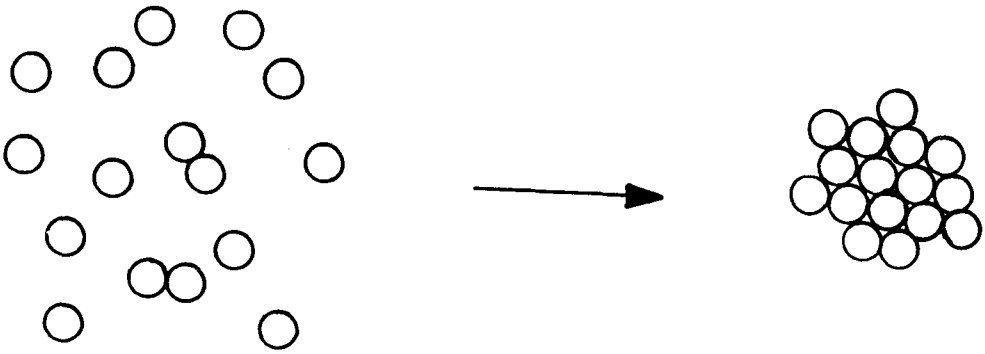

FIGURE 3. A generalized schema for cell aggregation. Cells are represented as spheres.

a drift of hogs, an exaltation of larks, a gaggle of geese, a leap of leopards, a murder of crows, an ostentation of peacocks, a parliament of owls, a pod of whales, a pride of lions, a shrewdness of apes, a skulk of foxes, a sleuth of bears, a spring of teal, an unkindness of ravens, a watch of nightingales.[1,2] In like manner, we may speak of an aggregation of myxobacteria, a clump of cells, a floc of yeast, a slug of amoebae, a strand of hyphae.

The definition contains two principal elements: physical movement and stable multicellular contacts. Both need to be present in any cell aggregation system.

Movement may be either directed (active), like chemotaxis in the cellular slime molds, or stochastic (passive), like random collision in a shaken liquid culture of bacteria (Figure 4). However it is achieved, there must be movement to allow cells to come together. This condition presupposes a state, prior to aggregation, in which the cells are disperse.

There must be actual physical contacts among the aggregated units, rather than merely conceptual grouping or lumping. Mere proximity of units, or even juxtaposition, is not good enough. Contacts must be more intimately close than close encounters of any kind. Moreover, they must be intercellular. Adsorption of cells onto inert surfaces fails to satisfy the definition. Furthermore, contacts must be multicellular. The minimally plural condition is not sufficient. Nor is the number of cells prior to aggregation strictly singular. The reason is more of convenience than of grammar: if we were to include pair formation (mechanistically, but not formally by our definition, the minimum aggregation), then all conjugation systems would have to be included, surely, not an easily managed lot. A lower limit of ten cells is a convenient, albeit largely arbitrary, size for a minimum group. It appears reasonable for certain systems, sex-directed flocculation in fission yeast, for example.

The multicellular condition connotes multivalency of the involved cells. It is clear that the final aggregated structure is determined by the combining power, or valence, of the individual components (Figure 5). Monovalent cells can only form pairs, divalent cells only chains (open or closed).

As in gametic agglutination of unicellular algae, contacts may be transient, but must last long enough and be strong enough to be experimentally describable. An aggregation that does not result in conjugation or cell fusion must be able to withstand Brownian buffeting under physiological conditions. A sediment of nonflocculent yeast cells, for instance, when gently agitated, disperses as a fairly homogeneous suspension in wort, but that of flocculent cells disperses as discrete flocs.

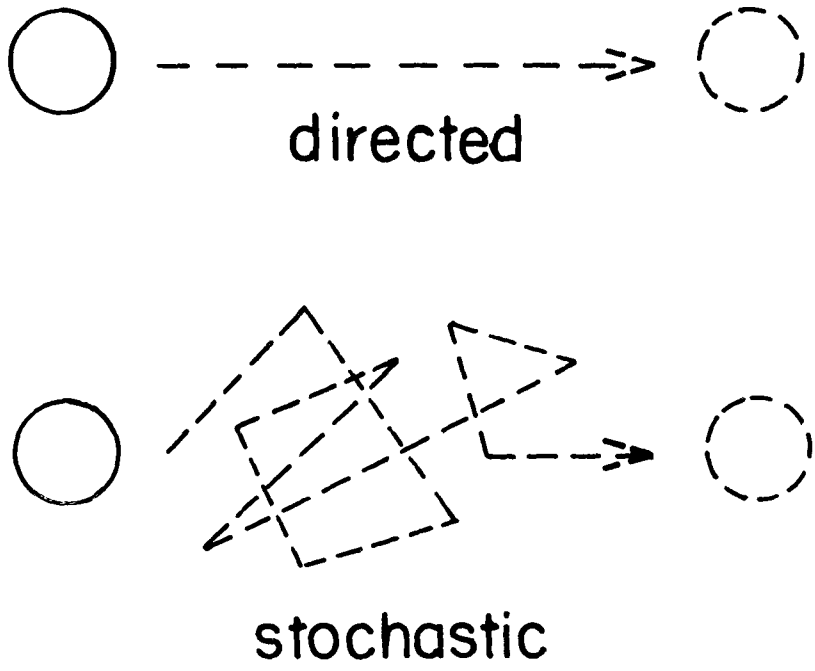

FIGURE 4. Movement of cells from one site to another.

IV. EXCLUDED PHENOMENA

Listed below are phenomena that may be, and have been in the literature, confused with cell aggregation. Some of them are graphically illustrated in Figure 6. They are things with which we shall not be concerned. For convenience, they are grouped as follows:

1. Noncellular aggregation: colloidal suspension, crystallization of viruses, gel formation, macromolecular self-assembly, polymerization, precipitation of molecules
2. Multicellular or multinuclear condition due to failure of progeny to effect complete separation after cell division or due to failure of cells to divide after nuclear division: chain, coenocyte, colonial form, filament, fruiting body, mycelium, packet, plasmodium, pseudomycelium, syncytium
3. Grouping (due to growth, buoyancy, or gravitation) under nondispersive conditions: bloom, colony on agar, focus of infection, pellet, pellicle, sediment
4. Association with noncellular objects: adherence, adhesion to glassware, adsorption onto solids, phage adsorption, stickiness
5. Interaction short of the multicellular condition: anastomosis, cell fusion, conjugation, copulation, heterokaryosis, mating, pair formation, palmella formation, zygote formation
6. Tropic response without subsequent stable contact: aerotaxis, chemotaxis, phototaxis, pellicle, swarm
7. Aggregation provoked by substances not usually found as components in the culture medium nor produced by the organism: agglutination, flocculation, and sedimentation provoked by antibodies, exotic lectins (e.g., concanavalin A, ricin, wheat germ agglutinin), native or denatured enzymes (e.g., lysozyme, ribonuclease), serum proteins (e.g., bovine serum albumin), artificial flocculants (e.g., bentonite, borate, undefined clays), and synthetic polymers (e.g., polyethyleneimine, polylysine)

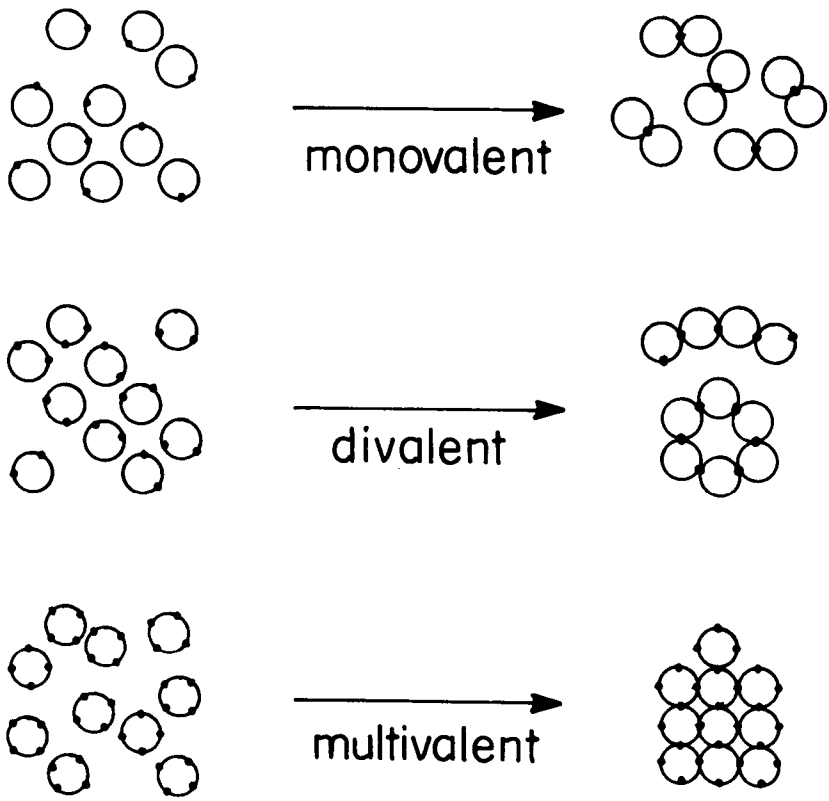

FIGURE 5. Valency of cells determines final aggregated structure. Dots represent potential and actual sites of union between cells.

8. Aggregation provoked under obviously nonphysiological conditions: agglutination, coagulation, denaturation, precipitation, and sedimentation due to strong acids and alkalis, organic solvents, centrifugation, dehydration, desiccation, heat, and lyophilization
9. Phenomena directly or indirectly associated with cell aggregation: anastomosis, cell fusion, competence, conjugation, copulation, fruiting-body formation, gametogenesis, growth, hyphal elongation, mating, meiosis, sexuality, sporulation, taxis, zygote formation

V. CRITERIA FOR INCLUSION

The following criteria are derived from the definition and the enumerated exclusions. To be included in a list of cell aggregation phenomena, a system must be (1) active (there is a change from a disperse condition to an aggregated condition), (2) inducible (there exists a physiological situation in which aggregation is absent), (3) stable (as an aqueous suspension), (4) intercellular (adhesion to noncellular surfaces is excluded), (5) multicomponent (a minimum group of ten cells), and (6) spontaneous (compatible with the life cycle of the organism and not due to obviously nonphysiological perturbations).

Notwithstanding the expedient limitations of the definition and the exclusion of many situations, cell aggregation remains a widespread condition in the microbial world. It is

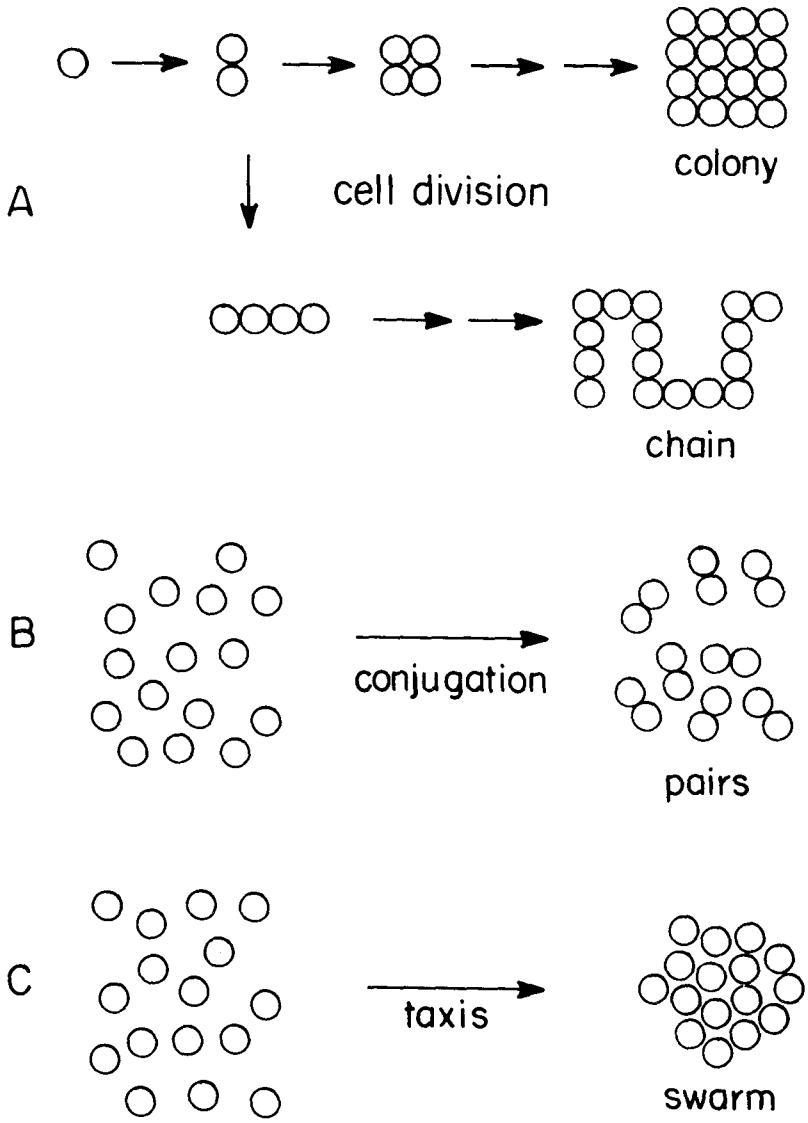

FIGURE 6. Phenomena that are related to, but excluded from, cell aggregation as defined in the text. (A) is excluded because the multicellular condition is achieved by a process that is more properly termed cell division; (B) because the final structures fall short of multicellularity; (C) because the final structures are units not in intimate material contact; (D) because the contacts are not intercellular; (E) because the process of achieving the multicellular condition is not physiological; (F) because the multicellular condition is elicited by exotic polymers; (G) because the final structure is achieved only under nondispersive conditions.

found among bacteria, yeasts, cellular slime molds, filamentous fungi, algae, and protozoa. In addition to well-known phenomena such as flocculation of brewers' yeasts, aggregation of cellular slime molds, and agglutination of gametes in chlamydomonads, the definition includes mating-aggregate formation during bacterial conjugation, bacterial star formation, agglutination associated with competence for bacterial transformation, dental plaque formation, aggregation in myxobacteria, sex-directed flocculation in fission yeast, and sexual agglutination in *Hansenula, Saccharomyces,* and other yeasts. It also includes mating re-

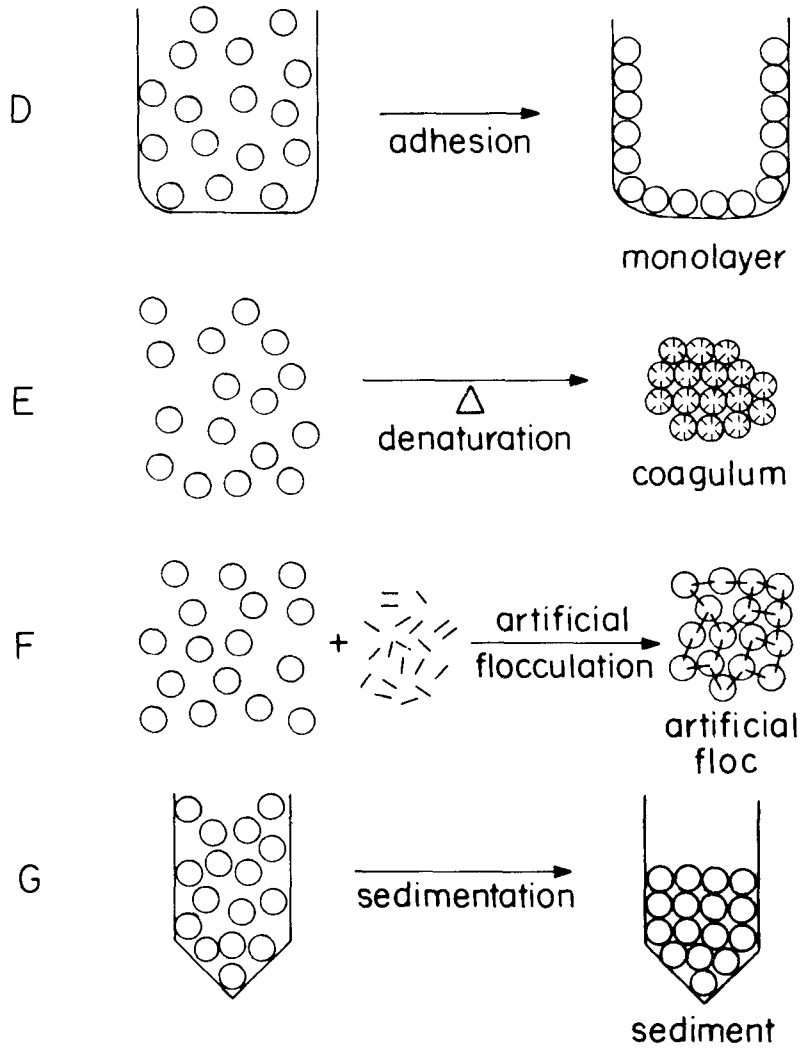

FIGURE 6.

actions in protozoa and the formation of strands, synnemata, rhizomorphs, sclerotia, and coremia in the filamentous fungi. As you well see, we have not run out of subject matter.

VI. REVIEWS REVIEWED

Of cell aggregation that is nonmicrobial, many reviews of general coverage have been written. A few of these must be mentioned, if for no other reason than to direct the reader to them.[3-7] Microbial systems, in contrast, have been less dealt with in broad terms, perhaps because of their apparent diversity.

Microbial aggregation has been reviewed from various vantage points. Hoffman[8] in 1964 treated bacterial aggregations as models of morphogenesis. His concept of aggregation, however, remains exactly that, that which we have dismissed as purely conceptual. The bacterial aggregations he reviewed are cell division, failure of cells to completely separate

after cell division, chain formation, and colonies on agar, items excluded from the definition. Indeed, the review virtually confines itself to these phenomena.

Morris's treatment,[9] in 1966, of aggregation in yeasts likewise includes colony formation in addition to flocculation and sexual aggregation. Strangely enough, the formation of pseudomycelia is also entertained as aggregation and labeled asexual conjugation.

The literature on the role of polymers in microbial aggregation was ably summarized in 1973 by Harris and Mitchell.[10] Systems discussed include bacterial aggregation by synthetic and natural polymers, formation of dental plaque, yeast aggregation during conjugation and fermentation, and aggregation in heterogeneous microbial communities, with emphasis on soil ecology and waste treatment. The ecological and practical import of microbial aggregation is underscored.

A different approach was made in 1974 by Reissig[11] in viewing the cell surface as a seat of cellular regulation and recognition. Touching on many different aspects of ektobiology,[12] his admirable synthesis covers the whole range of the microbial spectrum and, among other things, includes bacterial conjugation, sexual agglutination in yeasts, mating reactions in protozoa and algae, chemotaxis, and bacterial transformation. The emphasis is information content of the cell surface, but the significance of microbial aggregation comes out very clearly.

The survey by Atkinson and Daoud[13] in 1976 is from the standpoint of fermentation process engineering. It is quite useful because of its detailed handling of methodology, mechanisms, and process applications. However, a great majority of the flocculation systems described are outside the scope of our present concern.

Ottow's review[14] in 1975 on fimbrae and pili peripherally touches on bacterial aggregation, and so does Smith's review[15] in 1977 on microbial surfaces in relation to pathogenicity.

The subject of mating-type interactions in microorganisms was extensively reviewed in 1977 by Crandall[16] for the new series *Receptors and Recognition.* Most of the systems covered fall within bounds of our definition. The coverage, however, is limited to sexual systems.

In the same new series is an anthology, edited by Reissig[17] in 1977, of contributions from various laboratories on microbial cell-cell interactions. It includes aggregation in the cellular slime molds, bacterial chemotaxis, bacterial transformation, mating-aggregate formation in bacteria, and mating reactions in *Saccharomyces, Chlamydomonas,* and the ciliates. A summary by the editor gives the reader a broad overview of the subject.

Fungal aggregation in its various guises has been dealt with adequately by Burnett[18,19] in his book on mycology. Other reviews on fungal aggregation from various biased viewpoints are those by Hawker,[20] Garrett,[21] Butler,[22] Willetts,[23] and Chet and Innis.[24] A review by Carlile and Gooday[25] in 1978 on cell fusion in fungi and myxomycetes embraces a good number of fungal aggregation systems.

By far, the most adequately covered systems are those of brewers' yeast and the cellular slime molds. It is no accident that it is in these systems where there is most intensive research activity. Reviews exclusively concerning these systems will be dealt with in chapters devoted to them. For the moment, it is sufficient to note that, in Bonner's book on the cellular slime molds,[26] cell aggregation is treated as if it were a virtual monopoly of Acrasiales, among the eukaryotes, and of myxobacteria, among the prokaryotes.

VII. THE STRATEGY OF THE MONOGRAPH

The recent flurry of reviews, general or specialized, on microbial aggregation is a welcome sign of a growing interest in the subject among researchers of diverse prejudices. Still, there is the need to gather together the many scattered bits of information concerning the subject. The need to gather the bits into a monographic whole becomes more pressing as the clutter

and the confusion intensify. Because many associated and more complicated phenomena, including unidentified objects, have been mistaken for microbial aggregation, it has become imperative that a unifying treatment of the subject in rather broad and comprehensive terms be written, if only to put into proper perspective the extent and the meaning of a general biological phenomenon that has been too long neglected.

This monograph, an expansion of an earlier compilation on the nutritional aspects of microbial aggregation,[27] limits itself to microbes and to cell aggregation as defined above and as defined in the earlier review. The word "microbes" will be taken to mean organisms that are commonly accepted as cellular microorganisms by students of microbiology. Much space has already been spent on quibbling over definitions. There will be no further attempt to quibble in a taxonomic manner. Instead, the book will attempt to approach microbial aggregation as a unified subject and as a fundamental structure and a fundamental function in biology. As a way of emphasizing diversity, a number of chapters will be devoted in some detail to the better studied systems. Cellular slime molds, filamentous fungi, algae, and protozoa will be dealt with in a subsequent volume. In this volume, bacterial and yeast aggregation systems will be examined in detail. In a final chapter of this volume, these diverse systems will be gathered once again into an aggregated whole. The objective is to permit us, so to speak, to see the forest for the trees, the exaltation for the larks.