two symposia sessions. A paper presented by Hans-Erich Reineck was particularly well received, as it was the first opportunity for many people in this country to hear this well-known German marine geologist. Reineck discussed the processes of formation, as well as the nature, of sedimentary structures in shallow-water sediments. The sedimentation in a particular environment is influenced by the type of deliverable sediment, physical processes and their intensity, rate of sedimentation, and biogenetic factors. The role of organisms in forming certain structures and destroying others was emphasized. K. O. Emery chaired a lively panel discussion which followed the formal papers.

The symposium on the role of detritus in estuaries, organized by Rezneat Darnell, was of particular interest to many biologists because of the recognition of dissolved and particulate organics as an important component in some aquatic food chains. Organic detritus was broadly defined as any material of biological origin which is in the process of microbial decomposition and which represents a potential energy source for consumers. Criteria were established for demonstrating nutritive significance of organic detritus, and the nutritive roles of various types of detritus were discussed. Investigations of particulate detritus in Georgia estuaries was reported by Eugene Odum and Armando de la Cruz. They indicated that the detritus derived from the *Spartina alterniflora* marshes is the chief link between primary and secondary productivity, because only a small portion of the net production of the marsh grass is grazed while in the living state. Results of the study demonstrated that the bacteria-rich detritus is nutritionally a better food source than the *Spartiana* tissue that forms the original base for the particulate detritus. Grover Stephens reviewed his work on dissolved organic material as a nutritional source for marine and estuarine invertebrates. The maldanid worm, *Clymenella torquata*, was provided with labeled glucose and amino acids. Measurements indicated that the free amino acids in the environment can make a very substantial contribution to the total requirements for reduced carbon in these worms. Studies of the relation between salinity and the accumulation of glycine in nereid polychaetes demonstrated that the process of uptake virtually stops at lower salinities. Such studies suggest that the accumulation of small organic molecules may represent an important source of reduced carbon for invertebrates in marine and estuarine environments.

The conference on estuaries reached a large, interdisciplinary audience and attracted outstanding talent to share knowledge, experience, and opinions with fellow workers and others in allied fields. Papers from the meeting will be published in the AAAS Symposium Series.

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Bacterial Structure and Replication

The fine structure and replication of bacteria and their parts was the main theme of a bipartite symposium during the 64th annual meeting of the American Society for Microbiology held in Washington, D.C., 6 May 1964. The purposes of the symposium were consideration of current knowledge, inclusion of new information of general interest, and speculation for exploration of new areas. Microbiologists from several European countries and Canada participated. Each of five invited speakers dealt with an aspect of bacterial anatomy or replication; and each was followed by an invited discussant who supplemented, complemented, or sometimes dissented from, the information given.

In discussing the cell surface, and particularly the arrangement of cell wall components, R. G. E. Murray (University of Western Ontario) contrasted the great variety of regular substructure seen in walls of gram-negative bacteria with the paucity of such organization in gram-positive organisms. He presented examples of elegant substructure in walls of *Spirillum, Rhodospirillum, Halobacterium, Lampropedia*, and *Nitrocystis*, and in the gram-positive exception, *Micrococcus radiodurans*. From two to five different layers of wall may often be distinguished; the correlation between the electron microscopic appearance and chemical information on different layers appear to be good. Photographs of true septal ingrowth in *Escherichia coli* during growth at elevated temperatures were shown, together with that of a mesosome. This was compared with the usual concept of "constrictive division" in gram-negative bacteria (as exemplified by *Nitrosomonas*) and of the idea of the lack of typical mesosomes in gram-negative organisms.

The lack of regular structure in the wall of gram-positive organisms was emphasized by P. Gerhardt (University of Michigan), who reviewed electron micrographic and permeability data. He indicated that a random meshwork of molecular strands and subsequent "heteroporosity" existed in the walls of bacilli. However, special techniques demonstrated that the exosporium consists of four close-packed layers, each with a hexagonally punctate lattice. Hair-like protrusions arise from the outer surface. Orderly structure would thus seem not to be limited to organisms of a particular staining reaction, even though such structure may not be usually nor readily apparent and may not necessarily occur in walls of vegetative cells.

G. W. Fuhs (University of Koln) reviewed the evidence suggesting that the bacterial nucleoid contains not more than one DNA unit of extended length, 1100 to 1400 μ, in the form of a closed circle and consisting of not more than two polynucleotide strands. He emphasized the continuous replication of DNA and its simultaneity with nucleoid separation. He demonstrated by serial sections the effects of different fixations on preservation of the architecture and detail of the nucleoid. Evidence from serial sections suggests that the DNA molecule (or "bacterial chromosome") may not be folded and refolded to form a true multistranded ring, but instead may be a series of fibrils linked together by proteins as in Kellenberger's model. Despite the intimate contact of nucleoid and cytoplasmic membrane—usually through the mesosome—the failure to see any special structure or arrangement at the point of contact led Fuhs to suggest that the mesosome does not play an active role in nucleoid division.

P. C. Fitz-James (University of

Western Ontario) disagreed. He referred to (i) the model of Ryter and Jacob by which the mesosome separates replicating DNA into two masses and to (ii) his own serial sections that show these organelles separating the chromatin by their attachments to the transverse septa at each end of the cell. He presented additional information on mesosome structure, position, and integrity derived from sections of *Bacillus* in penicillin, during spore formation, during the natural synchrony of spore germination, and in chilled and warmed cells. The suggestion was made that continuously replicating DNA may be continuously separated through its mesosomal attachment to the continuously expanding membrane-wall exoskeleton.

W. van Iterson (University of Amsterdam) reviewed the general structure of cytoplasm and compared the structure of bacteria with that of higher cells. Bacterial mesosomes (or membranous organelles) have been generally presumed absent in gram-negative bacteria and prominent in gram-positive organisms. Reduction of tellurite takes place in these "chondrioids" or "mitochondrial equivalents," and the bound reduced product can be detected in the electron microscope. It was also seen (in *Bacillus subtilis*) as thin rods at the cell periphery—although in *Proteus* it was found only as conglomerates of small elements contiguous with the plasma membrane. She suggested that the peripheral structures in both bacteria may function as basal granules or the cytoplasmic bases of flagella. She presented other micrographs suggesting that ribonucleoprotein does not usually occur in bacteria as separate rounded particles, but may be in linear arrays with numerous anastomoses. Ribonucleoprotein may be contiguous with the plasma membrane and possibly with the mesosome when present. In some organisms, helical fibrils from the nucleoplasm enter the cytoplasm and appear to participate in forming its net-like structure.

In his discussion, G. B. Chapman (Georgetown University) emphasized the bacterial cytoplasmic membrane and particularly its functions as the membrane septum and the mesosome. He noted the apparent absence of the latter in many bacteria (but not in *Escherichia coli*, as remarked by others) and their easy demonstrability in gram-positive bacteria. Discussions dealt with other derivatives of the plasma membrane (including chromatophores) and other constituents of the bacterial cytoplasm such as ribosomes and polysomes, fibrous structures, and various inclusions.

B. A. D. Stocker (Lister Institute, London) and H. Koffler (Purdue University) discussed bacterial flagella. These locomotor organelles can be seen by electron microscopy to be spiral filaments. Their subunits consist of proteins (flagellins). However, the organization of such proteins into "fibrils" or strands, the numbers of such fibrils about the hollow axis, and the coiling and pitch are variable or uncertain. Flagella arise from spheres or mushroom-shaped basal submembranous structures to which they are attached by hooks. Regions near the flagellar insertions reduce tellurite (discussed by van Iterson) and the hooks may contain RNA.

The flagellins, which have molecular weights from 20,000 to 40,000, lack cysteine, cystine and tryptophan; often contain an unspecified carbohydrate; and seem to lack most common mineral elements in any quantity. Those from thermophilic bacteria are more stable to heat and to a variety of denaturing agents than are those from

mesophiles. They contain less aspartic and glutamic acids. After acid disintegration of flagella, the flagellins can be reassembled under suitable conditions to reform spiral filaments which are indistinguishable from native flagella. Flagellar synthesis in vivo can be prevented in some strains by elevated incubation temperatures, and by inhibition of protein synthesis (for example, by chloramphenicol). The question of an intracellular pool of flagellin or of precursor is unsettled and may differ among genera of flagellated bacteria.

Additional surface components of some bacteria, the pili (or fimbriae), were discussed by C. C. Brinton, Jr. (University of Pittsburgh). He reviewed their chemical and morphologic nature, their inheritance, replication and development, and possible functions. Of particular interest was the discussion of the role of pili in phage resistance in some strains of *E. coli*, and the description of a new third type of pilus which is the apparent attachment site for Hf phage.

R. M. Cole (National Institutes of Health, Bethesda) reviewed the nature of bacterial cell wall replication as seen by the aid of specific immunofluorescence to wall antigens. The site and mode of initiation of wall replication differ in different bacteria (for example, a discrete single equatorial origin in *Streptococcus pyogenes* as opposed to multiple intercalated sites in *Salmonella typhosa*). Problems discussed in the light of this information concerned the nature of insertion of new wall and its relation to the mesosome; the simultaneity of time and site of synthesis of all the wall components as well as surface antigens; the differences between growth of peripheral wall and of septa, as seen in induced filament formation; and the need for other mechanisms to explain such phenomena as the overall thickening of walls of *Streptococcus fecalis*, which may occur under some conditions.

The latter was explained by G. D. Shockman (Temple University) as an example of an unbalanced growth situation. It did not essentially alter the concepts of normal replicatory mechanisms derived from immunofluorescent studies of cells growing in balanced steady states. Such wall thickening, first noted in the absence of essential precursors, (for example, valine or threonine) can also be produced by addition of selective inhibitors such as chloramphenicol. Also discussed was the possible manner of production of a site of weakness in the insoluble matrix of the replicating wall to allow insertion of new material; the role of autolytic enzymes in such a process and the sites and timing of their actions; and the need and existence of a primer or template for mucopeptide synthesis.

It can be said that the bacterial mesosome, in one relation or another, was the single topic of most concern to most participants and discussants. The importance of its occurrence (or ready demonstrability) in some organisms and not others is not known, nor is its actual relation to cell wall replication, to DNA replication, and to nucleoid separation. Its ability to reduce tellurite renews the question of "mitochondrial equivalence," but leaves unanswered the role of peripheral sites of tellurite reduction which appear related to flagellar origins. The reasons for complex and orderly substructure in walls of gram-negative bacteria, as opposed to those of most gram-positive organisms, are as obscure as the reasons for the seeming differences in their modes of wall replication and of cell separation after division. The na-

ture of the arrangement of bacterial DNA is not entirely clear, nor is it certain that it is actually linked to mesosome or to linear arrangements of ribonucleoprotein within the cytoplasm. Finally, further information is needed on correlation of anatomic and enzymatic data in cell wall replication, with particular emphasis on "normal" growth in balanced situations as compared with the mechanisms in unbalanced and "abnormal" states.

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Subunit Structure of Proteins

Biochemical and genetic aspects of the subunit structure of proteins was the topic of the annual symposium in biology held at Brookhaven National Laboratory, Upton, New York, 1–3 June 1964. Investigators from the fields of genetics and biochemistry convened to review progress in their particular areas and to integrate their findings into an understanding of proteins composed of two or more polypeptide chains and of the genes which govern such structures.

Of great importance, genetically, is the phenomenon of allelic complementation. Two differently mutated forms of a gene governing a single polypeptide chain cooperate to restore a function which is absent in cells containing only one of the defective genes. The biochemical explanation of such findings is that the function involved depends on an enzyme composed of identical polypeptides. A protein composed of two such polypeptides, altered in different places, may exhibit activity absent in a dimer composed only of identically altered chains. D. G. Catcheside's presentation of this phenomenon also pointed to the prevalence of proteins composed of identical subunits; he found that among 30 carefully analyzed genes in *Neurospora* more than one-half showed allelic complementation. It may well be that, aside from secretory proteins, the majority of proteins produced by the cell are composed of identical subunits.

One of the most intensively studied gene systems has been the histidine biosynthetic region of *Salmonella*. J. Loper, in discussing this system, pointed out that of eight enzymes governed by the region, four appear to be composed of subunits. Only two of the corresponding loci, however, exhibit allelic complementation. This indicates that dimeric composition, although essential for such complementation, does not by itself assure that the appropriate interactions will occur.

An interesting comparison of the histidine loci of *Neurospora* with those of *Salmonella* was made by Catcheside. Two corresponding loci in *Neurospora* and in *Salmonella* showed complementation in both cases. In addition, the loci corresponding to the two other *Salmonella* loci, which produce oligomeric products, did, in *Neurospora*, show the phenomenon. The other four histidine loci failed to show allelic complementation in either organism. However, in an analysis of a histidine locus in *Neurospora*, A. Ahmed pointed out possible pitfalls in this area; "polarity" mutants, which interfere with the for-