RACKER

CELLULAR METABOLISM
AND INFE

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EDITED BY

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CELLULAR METABOLISM AND INFECTIONS

SECTION ON MICROBIOLOGY
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Preface

THE ORIGINAL PLAN of the symposium was to present a cross section of our knowledge of cellular metabolism in infected cells. In arranging this symposium it became apparent that the published data on this subject are few, scattered, and difficult to interpret. It might have been of some value to collect these data in a single volume and to assemble a pathology of infections at the metabolic level, but such an attempt seemed to be premature in view of the paucity of comparable data.

On the other hand important advances have been made in recent years in our knowledge of the metabolism and submicroscropic structure of animal tissues and microorganism. During the infectious process as well as in attempts at chemotherapeutic control, differences and similarities in the biochemistry of the host and the infectious agent assume a new significance. The concept of "unity in biochemistry" has for more than half a century oriented scientific thinking and experimentation in comparative biochemistry. The conspicuous lack of success of a rational approach to chemotherapy has served as a key witness for the "unitarians." However, the steadily growing recognition of the existence of alternate pathways, of qualitative and quantitative differences in enzymatic patterns, of differences in submicroscopic cell structure, permeability and rate of cell division have been quoted in favor of a "disunity in biochemistry." The assessment of those features that are not common to various cells might serve to provide us with a better understanding of the disease process as well as its control.

With these thoughts in mind the symposium was arranged and divided into two parts. One, on comparative biochemistry, dealing with differences in structural and metabolic patterns in hosts and parasites, the second dealing with metabolic aspects of the infectious process itself. The chapters in this volume represent the papers as they were presented at the symposium, though minor alterations in the sequence of presentation were made for the sake of continuity. In the first part some singular features of bacteria and helminths illustrating aspects of "disunity in biochemistry" were discussed by R. Y. Stanier and by E. Bueding, while the similarity of energy-yielding reactions was stressed by H. A. Krebs. The formation of adaptive enzymes has been studied for many years in microorganisms but was only recently firmly established in animal tissues; this subject was reviewed by

W. E. Knox. Some principles of a rational approach to chemotherapy were outlined by A. D. Welch.

The second part was devoted to a discussion of the infectious process. The peculiar environment of the host as a growth medium for bacteria was described by R. J. Dubos, while factors which contribute to bacterial diseases were analyzed by A. M. Pappenheimer, Jr.

Finally, aspects of virus infections were discussed. M. H. Adams reviewed the role of polysaccharides in the initiation of virus infections, and S. E. Luria summarized metabolic aspects of cytochemical and biosynthetic events in bacteria infected with phage. E. Racker dealt with alterations of cellular metabolism during some virus infections of animals and bacteria.

The discussions of the sessions were opened by the invited speakers B. Davis, S. S. Cohen and A. Lwoff. Their contributions as well as further discussions are included in this volume.

The invited speakers and discussants were selected not only on the basis of their outstanding contributions in the field of microbiology or biochemistry but also because it was expected that they would be free from fear of speculation and that they would follow the example of the turtle "who makes progress only when his neck is out."

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*Present address The Public Health Research Institute of the City of New York, New York, N. Y.

PART I

ASPECTS OF COMPARATIVE BIOCHEMISTRY

SOME SINGULAR FEATURES OF BACTERIA AS DYNAMIC SYSTEMS

By R. Y. Stanier, *Department of Bacteriology, University of California, Berkeley 4, California*

Il faut avouer, dit Micromégas, que la nature est bien variée. Oui, dit le Saturnien, la nature est comme un parterre, dont les fleurs. . . . Ah, dit l'autre, laissez-là votre parterre. . . . Elle est, reprit le secrétaire, comme une assemblée de blondes et de brunes, dont les parures. . . . Et qu'ai-je afaire de vos brunes? dit l'autre. . . . Elle est donc comme une galerie de peintures, dont les traits. . . . Et non, dit le voyageur, encor une fois, la nature est comme la nature. Pourquoi lui chercher des comparaisons? Pour vous plaire, répondit le secrétaire. Je ne veux point qu'on me plaise, répondit le voyageur, je veux qu'on m'instruise.

Voltaire, *Micromégas.*

I. INTRODUCTION

The comparative biochemist is a scientist who seeks, as a rule, for the common biochemical principles which, his articles of faith tell him, are expressed in all forms of life. Certainly the search has been a rewarding one; and during the past 25 years the articles of faith so clearly enunciated by Kluyver (15) have proved an excellent guide for exploring the metabolic machinery of the living cell. Time and again it has turned out that biochemical information obtained from the study of microorganisms is highly relevant to an understanding of biochemical processes in vertebrates or green plants, and studies on these higher forms of life have in turn clarified microbial biochemistry. The history of the discovery of accessory growth factors and the elucidation of their metabolic function provide perhaps the most striking instance of this biochemical interplay, but other examples could be selected from almost any branch of biochemistry. Out of such successes, there has grown in some quarters a view which may be crudely expressed by the statement that the liver cell and *E. coli,* the meristematic plant cell and the purple bacterium, are sisters under the skin, their biochemical differences being principally ones of minor detail. However, a little reflection, which can be greatly stimulated by a judicious use of the microscope, suggests that there are, after all, some very marked differences between a liver cell and *E. coli;* and it seems not unreasonable to assume that the evident morphological distinctness of these two kinds of cell is the

outward and visible expression of less tangible chemical differences, more subtle than the gross catabolic differences with which biochemists have mainly concerned themselves in the past. Eventually, we must try to understand the *uniqueness* of the species and the group in biochemical terms, and such an understanding is certainly a prerequisite to the analysis of host-parasite relationships.

II. BIOLOGICAL ATTRIBUTES OF BACTERIA AND BLUE-GREEN ALGAE

From the time of Ferdinand Cohn in the mid-nineteenth century, many microbiologists have felt that the bacteria and blue-green algae together occupy a rather isolated position in the living word. It is difficult to explain the basis for this taxonomic hunch in terms of a compact definition; van Niel and the author (36) attempted to do so about 15 years ago, when the formation of a separate kingdom, the Monera, was proposed for these two microbial assemblages, but the formal differential characters which we thought up have not stood the test of time. The matter must, therefore, be put in a vague and general fashion: there is something about the cell structure of bacteria and blue-green algae which is different from the cell structure of other microbial groups—the remaining algae, fungi, and protozoa—and of higher plants and animals. For one thing, the cytoplasm in living cells of bacteria and blue-green algae has a very characteristic and unusual appearance, which in itself is well-nigh diagnostic. There is a total absence of vacuoles, and streaming movements are never detectable.

The singularity of the cell structure of bacteria and blue-green algae can also be documented with reference to a few specific cytological features. In eubacterial swimming forms the contractile locomotor organelle, although designated as a *flagellum,* is not structurally homologous with the organelles variously termed flagella and cilia in protists, plants, and animals. In these higher groups, contractile locomotor organelles are always composed of a bundle of longitudinal fibrils, characteristically 11 in number, of which two are structurally distinguishable from the rest (10, 11). The bacterial flagellum consists of a single very fine fibril without any evidence of internal structural differentiation (14, 39); Astbury (3) has described it as a "monomolecular hair."

In blue-green algae and purple bacteria, the photosynthetic pigments are not localized in typical chloroplasts, but are found in structurally much simpler bodies of submicroscopic dimensions (5, 32).

The question of nuclear structure in both these groups is at present very far from settled. Mitosis has never been observed in a blue-green alga, and as for the bacteria, the bulk of the evidence favors the view that the nuclear equivalents may not be strictly comparable in organization and mode of division to a typical nucleus in higher organisms.

One other biological feature may well prove after more extensive analysis to distinguish these two groups from other organisms: this is the mechanism of gene transfer. Considering all work on the three bacteria—pneumococci, *Escherichia coli,* and the *Salmonella* group— where the problem has been most extensively explored, one cannot help being struck by the fact that gene transfer on the bacterial level seems to involve the transfer of a limited number of determinants; either unit characters, or blocks of characters which could be construed (38) as being located on single chromosomes.

The question I propose to examine is whether there are any distinguishing biochemical features, either structural or dynamic, that can be correlated with the biological features that appear to set bacteria and blue-green algae apart from other organisms. In this analysis, I shall not be concerned with biochemical specializations that occur in small groups within the bacteria and blue-green algae—chemoautotrophy or nitrogen fixation, for example—since such properties are rare even within the assemblage as a whole. What we shall try to find are singular biochemical *group characters.*

III. OCCURRENCE AND ROLE OF DIAMINOPIMELIC ACID IN BACTERIA AND BLUE-GREEN ALGAE

1. *Discovery and Distribution*

A new amino acid, α,ε-diaminopimelic acid (DAP), was isolated by Elizabeth Work (46) from hydrolysates of *Corynebacterium diphtheriae.* Subsequent observations by Work and others (2, 4, 47) showed that it occurred also in the cells of other bacteria; but it has never been found in hydrolysates of plant or animal materials. Recently Work and Dewey (49) undertook an exhaustive survey of the distribution of DAP in microorganisms; their results, somewhat condensed, have been summarized in Table I. DAP is found universally in Gram-negative true bacteria, in photosynthetic bacteria, and in the one myxobacterium examined. In the Gram-positive group, its distribution is more spotty. The micrococci, the streptococci, and the mycelial actinomycetes do not contain it, but it occurs in rod-shaped lactic acid bacteria, propionic acid bacteria, corynebacteria, mycobacteria, and rod-shaped sporeformers, with the single exception of *Clostridium tetani.* Of the Gram-positive bacteria which lack DAP, the mycelial actinomycetes contain a new amino acid which is structurally related to DAP, being a methyl-substituted homolog (48). This compound is not present in the cocci, however. The fact that DAP is absent from the spherical and present in the rod-shaped lactic-acid bacteria is particularly remarkable since these two groups show far-reaching physiological, nutri-

TABLE I

DISTRIBUTION OF DIAMINOPIMELIC ACID IN MICROORGANISMS, CONDENSED FROM DATA OF WORK AND DEWEY (49)[a]

	DAP Content
A. Unicellular Eubacteria	
α. Gram-negative groups	
1. Coliforms (8 spp., 20 cultures)	1 ++, 18 +, 1 tr.
2. *Pasteurella, Brucella, Hemophilus* (6 spp., 10 cults.)	tr. to ++
3. *Pseudomonas, Vibrio* (9 spp., 9 cults.)	1 ++, 7 +, 1 tr.
4. *Neisseria* (1 sp., 1 cult.)	+
5. Nonsulfur purple bacteria (4 spp., 6 cultures)	5 ++, 1 +
β. Gram-positive groups	
1. Streptococci, micrococci, sarcinae (7 spp., 8 cults.)	0
2. Rod-shaped lactic acid bacteria (1 sp., 1 culture)	++
3. Coryne- and propionibacteria (8 spp., 8 cultures)	++
4. Sporeforming rods (6 spp., 7 cultures)	2 ++, 3 +, 1 tr., 1 0
B. Actinomycetes	
1. Mycobacteria (4 spp., 4 cultures)	++
2. Mycelial actinomycetes (4 spp., 4 cultures)	0
C. Myxobacteria and Blue-green Algae	
1. Cytophagas (2 spp., 2 cultures)	+
2. Blue-green algae (3 genera, 3 spp., 3 cultures)	+
D. Other Algae	
6 species, representing 6 different phyla	0
E. Fungi	
19 species (Ascomycetes, Basidiomycetes, imperfects)	0
F. Protozoa	
1 flagellate, 1 ciliate	0

[a]On a dry-weight basis (whole cells) 0 = less than 0.02%, tr. = up to about 0.1%, + = 0.1–0.8%, and ++ = more than 0.8%.

tional, and biochemical similarities which have led bacterial taxonomists to place them in a single family despite their morphological differences.

DAP occurs in the three blue-green algae examined by Work and Dewey, but was not detected in representatives of six other algal phyla, in fungi belonging to the Ascomycetes, the Basidiomycetes, and the *Fungi Imperfecti,* in protozoa or in plant viruses. The presence of this amino acid is thus a sure indication of membership in the groups of bacteria and blue-green algae.

Studies on the intracellular distribution of DAP are still fragmentary,

but the data of Work and Dewey show that in certain bacteria it occurs in bound form as a protein constituent. This is further supported by Salton's (26) observation that DAP is one of the amino-acid constituents in some bacterial cell walls.

2. *Metabolic Role*

There is evidence for a metabolic role for DAP, as well as a structural one, in some bacteria. Davis (6) has found that certain lysine-requiring auxotrophs of *Escherichia coli* which respond only to lysine excrete large amounts of DAP into the culture medium. This nutritional finding is neatly correlated with biochemical observations. Dewey and Work (8) found that the wild type of *E. coli* synthesizes constitutively a DAP decarboxylase which converts DAP to lysine, and that this enzyme is lacking in the lysine-requiring auxotrophs of Davis which accumulate DAP. The evidence for DAP as a metabolic precursor of lysine is, therefore, good, although not conclusive.[1] In nonbacterial systems, the biosynthesis of lysine has received little study; but it is known that certain lysine-requiring mutants of *Neurospora* can grow when supplied either with α-aminoadipic acid or with α-amino, ε-hydroxycaproic acid (12), neither of which can replace lysine for any of the lysine-requiring auxotrophs of *E. coli* examined by Davis (6). Furthermore, lysine-requiring *Neurospora* mutants cannot use DAP as a replacement (50). In summary, then, it seems likely that the presence of DAP as a protein constituent in most bacteria is correlated with the possession of a biosynthetic mechanism for the manufacture of lysine which involves DAP as a precursor, and which differs from the pathway for lysine synthesis in *Neurospora.*

A very interesting question now presents itself. Considering that DAP appears to have both a metabolic and a structural function in *E. coli,* is it possible that bacteria which lack DAP as a protein constituent nevertheless use it as a precursor for lysine synthesis, thus ranging themselves *biosynthetically* with DAP-containing forms? An indication that this may be so is provided by the observations of Dewey, Hoare, and Work (7), who found active DAP decarboxylases in two Gram-positive cocci, *Sarcina lutea* and *Micrococcus lysodeikticus.* Lysine-requiring auxotrophs of these two bacteria have not yet been produced, so that nutritional confirmation of the suggested biosynthetic role for DAP is lacking. It must also be mentioned that *Streptococcus fecalis* and *Leuconostoc mesenteroides,* which require lysine for growth and lack DAP as a protein constituent, cannot use DAP as a replacement for lysine (50). Of course, in the absence of

[1] See Adelberg (1) for a critical evaluation of the criteria used to assign precursor roles in biosynthesis.