BACTERIAL CHEMISTRY and PHYSIOLOGY

BY

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PREFACE

This book is the outgrowth of lecture material which has been used for the past few years in a bacterial physiology course at the State University of Iowa. Since developments in this branch of science have recently been extremely rapid, it has become almost impossible to condense lectures and discussions to fit into one semester or even two semesters of classes. In the past some degree of success in meeting this problem has been achieved by supplying students with mimeographed tables, charts, and pertinent references for further reading. It has been thought, however, that if a book were available which outlined some of the past developments and other information concerning this science, more attention could be given in the classroom to general discussion of the more recent advances in the field. This material is thus compiled as a guide for a course which is being transformed from the lecture to the seminar type.

The students who are admitted to bacterial physiology at Iowa, as in most other universities, have diversified interests, but they all have had courses in biology and chemistry. For the most part they are advanced students majoring in chemistry, pharmacy, and the biological sciences. Although the subject matter in this book has been chosen and arranged primarily to fit the requirements and interests of these students, some thought has also been given to the needs of persons doing research in microbial chemistry.

An attempt has been made to restrict the discussion to the field of bacteriology, but mention is made from time to time of organisms which belong in other branches of science. Such references have been made in order to stress the fact that the physiological behavior of living matter is much the same in both the plant and the animal kingdoms. For example, we now know that the ability of plants and some bacteria to synthesize organic compounds from carbon dioxide is also a property of certain more fastidious forms of life. Likewise, some of the yeast enzymes responsible for alcoholic fermentation and those concerned with muscle glycolysis have much in common. Furthermore, the external supply of vitamins required by man is needed also by many insects, protozoa, and lower fungi. Since many other examples such as these can be cited, biologists are now beginning to appreciate the

fact that certain vital processes can be studied more easily by using microorganisms, and that the information obtained often has direct application to the more complex conditions in higher forms of life.

In citing references to the literature, I have endeavored to give the students a bibliography upon which they can build and also a means of following up for themselves any point in which they are interested. Much of the work from the early period of bacteriology has been omitted, because it has been adequately covered in Buchanan and Fulmer's three volumes, *Physiology and Biochemistry of Bacteria* (1928–1930), in Rahn's *Physiology of Bacteria* (1932), in Stephenson's *Bacterial Metabolism* (1939), and in other books and reviews. Among the great mass of recent publications many important reports have undoubtedly been omitted or overlooked, and others have been included which future events will show to be of no significance. In the absence of historical perspective, however, such errors in judgment are unavoidable.

Details of laboratory techniques are not included in the discussion of this book. For such information the student is referred to the original articles, and to such monographs as *Manometric Techniques and Related Methods for the Study of Tissue Metabolism* (1945) by W. W. Umbreit, R. H. Burris, and J. F. Stauffer, and *Laboratory Manual of Physiological Bacteriology* (1945) by F. W. Fabian.

In so far as possible, the nomenclature used for the bacteria mentioned in this book is that proposed in the Fifth Edition of *Bergey's Manual of Determinative Bacteriology* (1939). The names of genera and species of yeasts, molds, and other fungi are those that were used in the original articles.

The information on the pages that follow is the result of years of research by a large number of investigators, and much of it is widely scattered in the literature. My chief role has been that of a compiler and an editor.

An attempt has been made in the text to give credit for all illustrations and other materials used, and failure to acknowledge indebtedness to any writer or publisher is unintentional. I wish to thank specifically the following publishers, publications, and manufacturers for the use of copyrighted material: Academic Press, Inc., *American Journal of Hygiene*, *American Review of Tuberculosis*, American Sterilizer Co., *Biochemical Journal*, Biotech Publications, Cambridge University Press, Cornell University Press, Eastman Kodak Co., *Handbuch der Virusforschung*, His Majesty's Stationery Office, *Industrial and Engineering Chemistry*, Iowa Engineering Experiment Station, *Iowa State College Journal of Science*, *Journal of Bacteriology*, *Journal of Biological*

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SOME PHYSICO-CHEMICAL PROPERTIES OF BACTERIA AND THEIR ENVIRONMENT

THE COLLOIDAL STATE OF MATTER

In a study of bacterial chemistry and physiology it seems pertinent to consider first of all the colloidal state of matter, since basically many of the reactions of bacterial systems are dependent upon the colloidal phenomena that operate within these systems. We shall not attempt to present in this brief treatise an inclusive or mathematical description of colloids. For such information the books by Buchanan and Fulmer (1928), Kruyt (1930), Alexander (1926–1944), Gortner (1937), Weiser (1939), Bull (1943), and other books dealing with colloid chemistry should be consulted.

In 1861 Thomas Graham classified all substances into two groups, *crystalloids* and *colloids* (from the Greek *kolla*, gelatin or glue; *eidos*, like or semblance), depending upon their rate and ability to diffuse through water and parchment membranes. According to Graham, crystalloids passed readily through parchment membranes, whereas colloids did not. We now realize that this distinction between crystalloids and colloids is not tenable because many typical colloids, such as certain proteins, are crystallizable, and under proper conditions practically all crystalloids may be brought into the colloidal state. Thus, according to more modern conceptions, the term colloid is used to define substances with a characteristic kind of physical structure rather than to classify a particular type of matter.

Matter is said to be in the *colloidal state* when it is dispersed permanently and so finely that the individual particles, though larger than molecules, cannot be seen with the ordinary microscope. The medium in which the particles of a colloidal system are scattered is termed the *dispersion medium*, or *continuous phase;* and the scattered particles are called the *dispersed* or *discontinuous phase*. In studying colloidal systems, therefore, it should be emphasized that a heterogeneous system composed of at least two components (dispersed phase and dispersion medium) is under consideration.

Particle size is one characteristic of the colloidal state, and colloidal state may be defined (somewhat arbitrarily) in terms of particle size. The maximum size of colloidal particles is conveniently placed just below the lower limit of ordinary microscopic visibility, using the oil immersion objective. The minimum size of the particles is arbitrarily set above that of the average molecule. This means that the largest colloidal particles are below about 0.1 μ or 0.0001 mm. in diameter and thus invisible with the ordinary microscope, but are above 1.0 mμ or 0.000001 mm. The following diagram shows the relationship which exists between coarse suspensions, colloids, and molecular solutions:

	Suspensions		*Colloids*		*True Solutions*	
	Molecular aggregates (matter in mass)		Molecular aggregates		Molecules and ions	
Size or diameter:	>0.1 μ		0.1 μ		1.0 mμ	<1.0 mμ

It is generally believed that there is a continuous gradation of properties from coarse suspensions through the colloidal state to true solutions. There are, however, certain characteristic properties of matter in the colloidal state which are not exhibited by true solutions and which are shown in a negligible degree by gross suspensions. Certain of these properties will be briefly discussed later in this chapter.

Although all our known bacteria are larger than the upper limits of the colloidal system, many of the smaller organisms studied by the bacteriologist fall in this region and exhibit several properties of true colloids. It should be emphasized, however, that the properties of colloidal systems do not coincide strictly with the arbitrary boundaries described above. For this reason bacterial cells in various media behave in many respects like colloidal systems [Kendall (1925), Silbereisen (1939), McCalla (1940)].

Bacterial protoplasm, like that of all other living cells, is an intimate association of salts, carbohydrates, fats, and proteins. It is generally believed that the salts and carbohydrates of living protoplasm are, in part, in true solution and, in part, absorbed or otherwise bound to organic matter. The proteins exist as jellies, and the fats as emulsions. Water is the dispersion medium of all. Other substances, such as organic acids, not included in the above groups, can probably be regarded as minor constituents in most cases.

Although the living system includes all the component parts just enumerated, certain of these may be looked upon as representing the ultimate living substance itself with the other constituents functioning as nutrient material. But the importance of regarding protoplasm as a

highly organized and coordinated heterogeneous system, all parts of which are necessary to life, cannot be overemphasized. One of the most remarkable powers of protoplasm, which is due to its organization, is its ability to carry on simultaneously in the same cell a number of chemical reactions without one interfering with the other and without any evident boundaries of separation. Only a highly complex "organized" colloidal system with continuity of structure could be the site of such intricate and closely confined chemical reactions [Seifriz (1928)].

CLASSIFICATION OF COLLOIDAL SYSTEMS (LYOPHOBIC AND LYOPHILIC)

The broadest classification of colloidal systems is that devised in 1907 by Ostwald, who separated them on the basis of the physical state (gaseous, liquid, or solid) of the subdivided substance (dispersed phase) and the medium in which the particles of the subdivided substance were distributed. Thus the following nine systems are possible:

Dispersed Phase	*Dispersion Medium*	*Example*
1. Gas	Gas	Hypothetical case, since gases occur in a molecular state
2. Gas	Liquid	Foam, air in beaten egg white
3. Gas	Solid	Gaseous inclusions in minerals (meerschaum)
4. Liquid	Gas	Clouds, fog
5. Liquid	Liquid	Oil in water, cream
6. Liquid	Solid	Opal, pearl, water in paraffin wax
7. Solid	Gas	Smoke
8. Solid	Liquid	Colloidal gold
9. Solid	Solid	Ruby glass, black diamond

From a biological standpoint this classification is of little true value because it is too general and does not include the gels.

Another classification of certain colloidal systems is based on the attraction or affinity of the dispersed phase and the dispersion medium for each other. If the mutual affinity is small, the system is said to be *lyophobic;* whereas, if the mutual affinity is great, the system is called *lyophilic.* If water is the dispersion medium, the system is usually termed *hydrophobic* or *hydrophilic*, as the case may be; if benzene is the dispersion medium, *benzophobic* or *benzophilic;* etc. Gold neither dissolves nor swells in water, hence gold dispersed in water is a hydrophobic system. Gelatin and agar, on the other hand, take up water and swell to a marked degree; thus such colloidal systems are hydro-

philic. A given dispersed phase may be lyophobic in one medium and lyophilic in another. For example, starch forms a lyophobic system in alcohol and a lyophilic one in water.

In many of their physical properties, such as viscosity, lyophobic colloids differ only slightly from the pure dispersion medium. The particles carry a definite electric charge which can be changed only by special methods. Such colloids are flocculated by very small quantities of electrolytes and, when so precipitated, cannot ordinarily be brought back into the colloid state. The precipitation is therefore *irreversible*. Since the proteins and the higher carbohydrates form lyophilic systems, such systems are of much greater biological importance and interest than the lyophobic colloids. The viscosity of lyophilic systems is usually much higher than that of the pure dispersion medium. The particles carry an electric charge which may be changed by such simple measures as altering the *p*H of the solution. They require large amounts of electrolytes for their precipitation and, when precipitated, usually may be brought back into the colloidal state by the addition of fresh solvent. The precipitation is therefore *reversible*.

Colloidal systems possessing a high degree of fluidity are defined as *sols*. Lyophilic sols, under changing conditions of temperature and concentrations of disperse phase, of *p*H and of electrolytes, possess the property of imbibing large quantities of water and forming semi-rigid *gels*. These gels probably have a rather definite structure consisting in some cases of a network of disperse phase, or hydrated material, enclosing some of the dispersion medium. These properties of *imbibition* and *gel* formation exhibited by lyophilic colloids are probably responsible for the characteristic physical structure of protoplasm.

It can thus be seen that lyophilic and lyophobic colloids exhibit essential differences in their behavior toward the solvent and in their sensitivity to electrolytes. According to Kruyt (1930), the stability of lyophobic colloids depends entirely upon the charges on particles, which permit them to repel each other and thus prevent aggregation into coarser particles. If the charges on the particles are neutralized, or reduced below a certain critical value by an electrolyte, the particles coalesce and precipitate. Lyophilic colloids, on the other hand, possess two stability factors, charge and hydration, either of which is capable of preventing the aggregation and flocculation of colloidal particles. Neutralization of the charges on the particles of a lyophilic colloidal solution converts it into a neutral, or *isoelectric*, colloid which is stable as long as the particles remain hydrated. Dehydration of a charged, electrolyte-free lyophilic colloid by the addition of a dehydrating agent, such as alcohol, converts it into a lyophobic colloid exhibiting the

characteristic sensitivity to electrolytes. The precipitating action of large amounts of salts on lyophilic colloids is due to the fact that saturated solutions of the common salts act as dehydrating agents, thereby discharging and dehydrating the system at the same time. For further discussion of this subject see the section on the effect of electrolytes on colloids, pp. 19 to 22.

An additional system which should be considered in a study of colloidal systems is the *emulsions*. Emulsions are not necessarily true colloidal systems, if we limit our definition to the size of the particle concerned. Emulsions are drops of one liquid dispersed in the bulk of a second liquid. They usually consist of systems of oil dispersed in water or of systems of water dispersed in oil. Frequently the dispersion medium contains a lyophilic colloid which assists in stabilizing the emulsion. Very dilute emulsions could be classified as liquid-liquid colloidal systems, providing all the particles fell within the range of size characteristic of such systems. Most emulsions, however, contain oil or water droplets much larger than the upper limit of the truly colloidal field. Latex, milk, and mayonnaise are typical examples of emulsions. In each of these examples we are dealing not only with oil droplets suspended in an aqueous medium, but we have present at the same time lyophilic colloids in the form of proteins which stabilize the system. Therefore, a study of emulsions involves an investigation of not only the dispersion medium and the disperse phrase but also the nature and properties of the lyophilic colloid which acts as the stabilizer.

When viewed through the microscope, protoplasm (of cells larger than most bacteria) presents the appearance of an emulsion. Certainly there are droplets of fats and oils in all living protoplasm which are stabilized by the lyophilic colloids which are present. It is also true that there are solid particles in all protoplasm, so that protoplasm can be looked upon, in part, as a complex colloidal system, the dispersion medium being water and the disperse phase consisting of lyophilic (hydrophilic) colloids, lyophobic (hydrophobic) colloids, and fat droplets in the form of an emulsion.

CERTAIN CHARACTERISTICS OF COLLOIDAL SYSTEMS AND THEIR RELATIONSHIP TO BACTERIOLOGY

At this point it may be well to consider briefly several of the general properties of colloidal solutions and to point out their application to bacteriology.

1. Brownian Movement. In 1828 the English botanist, Robert Brown, announced that he had observed microscopically a continuous trembling motion when pollen grains were suspended in a liquid.

We now know that what Brown saw was the process which bears his name, the Brownian movement of particles, caused by the bombardment of the particles of the disperse phase by the molecules of the dispersion medium.

Particles exhibit Brownian movement only when below a definite size and in a medium of sufficiently low viscosity. Particles larger than about 4.0 μ exhibit no motion in water, and the viscosity of the surrounding medium determines the amplitude of motion for a particle of given size. For example, glycerin is about 800 times as viscous as water and, therefore, particles suspended in it do not move as freely as those suspended in water. In fact, particles larger than 1.0 μ to 2.0 μ do not exhibit Brownian movement in glycerin. In truly colloidal systems, where the dispersed phase cannot be seen with the ordinary microscope, dark-field illumination or the ultramicroscope must be used to demonstrate Brownian movement.

If the cells of many nonmotile bacteria are suspended in various media, they show Brownian movement when examined under the microscope, and particles in the protoplasm of the larger microorganisms are often in an active state of motion. However, Brownian movement may be inhibited or abolished in bacteria, as in certain typical colloids, by the addition of electrolytes.

For details of the historical phases and the theoretical significance of studies dealing with Brownian movement, the books of Kruyt (1930), Gortner (1937), and other authors on colloid chemistry should be consulted.

2. Sedimentation and Particle Weights. A particle suspended in a liquid medium tends to settle out under the influence of gravitational force. With particles in the colloidal realm the rate of sedimentation under gravity is negligibly slow because back diffusion acts in opposition to the gravitational force. However, if the force of gravity is increased by means of a centrifuge, the rate of settling is greatly increased. Ordinary laboratory centrifuges, which run up to 3,000 to 5,000 r.p.m., are usually sufficient to settle most bacteria. So-called supercentrifuges, which run at 40,000 r.p.m. and generate a force in excess of 42,000 times that of gravity, have been constructed. Such an instrument is useful for washing precipitates that settle very slowly, for sedimentation of certain particles, and for the preparation of certain sols; but it cannot be employed in the estimation of the size of colloidally dispersed particles because vibration in the apparatus sets up convection currents which modify the effect of the centrifugal force. To overcome this difficulty, Svedberg and coworkers [see Svedberg and Pedersen (1940)] have devised an apparatus called an

ultracentrifuge, which may be defined as "a centrifuge of low or high power in which convection does not occur and in which it is possible to measure any redistribution of the contents." In Svedberg's apparatus this redistribution is followed either by determination of the light absorption or the refractive index, and fields of 400,000 times that of gravity have been obtained.

Svedberg's ultracentrifuge has been used extensively to determine the "particle" or molecular weights of proteins, celluloses, other biochemical compounds, dyes, soaps, etc. For the technical details of the method the book by Svedberg and Pedersen (1940) should be consulted. In Table 1 are given the particle or molecular weights of a number of substances as obtained or quoted by Svedberg and associates from data on sedimentary velocity and diffusion.

TABLE 1

MOLECULAR OR PARTICLE WEIGHTS (M) OF VARIOUS SUBSTANCES

[From Svedberg and Pedersen (1940) and Others]

Substance	M *
Tuberculin polysaccharide	9,000
Scarlet fever toxin [1]	4,000–13,000
Cytochrome c	15,600
Mycobacterium phlei protein	17,000
Lactalbumin	17,400
Gliadin	27,500
Mycobacterium tuberculosis (human) protein	32,000
Pepsin	35,500
Pectins: Apple, pear, plum	25,000–35,000
Pectins: Orange	40,000–50,000
Zein	40,000
Insulin	41,000
Cellulose acetate in acetone	50,000–250,000
Hemoglobin (man)	63,000
Diphtheria toxin [2]	74,000
Yellow enzyme	82,000
Diphtheria antitoxin [2]	113,000
Serum globulin (man)	176,000
Antipneumococcus serum globulin: rabbit	158,000
Antipneumococcus serum globulin: man	195,000
Antipneumococcus serum globulin: horse	910,000
Antipneumococcus serum globulin: cow	910,000
Antipneumococcus serum globulin: pig	930,000
Thymonucleic acid	200,000
Catalase	250,000
Edestin	310,000
Rubber sols	400,000–435,000
Urease	480,000
Hemocyanin from different sources	400,000–6,700,000
Bushy stunt virus	7,600,000
Bacterial glycogen from the avian tubercle bacillus [3]	12,100,000–13,200,000
Tobacco mosaic virus protein	17,000,000–42,500,000

* Some of these values are only apparent molecular or particle weights.

[1] Barron, Dick, and Lyman (1941).

[2] Petermann and Pappenheimer (1941).

[3] Chargaff and Moore (1944).

The initial cost of the Svedberg ultracentrifuge is so great that its use is greatly restricted. In recent years, however, relatively inexpensive qualitative ultracentrifuges have been constructed by Beams and others [see review by Beams (1938)] based on the principle of a rotor driven by compressed air and spinning on a thin cushion of air—the spinning top of Henriot and Huguenard (1925). Beams has also developed "suspended rotor" types of ultracentrifuges driven by air, steam, or electricity, which have been adapted and improved by various workers to suit their special problems. With such instruments centrifugal forces up to 4,000,000 times that of gravity have been obtained, the maximum value being limited only by the tensile strength of the material from which the rotor is made.

By means of the ultracentrifuge the size and distribution of particles in sols may be determined. Considerable use is also being made of the ultracentrifuge to purify and study viruses, enzymes, and certain components of the bacterial cell.

3. Diffusion, Osmotic Pressure, and Imbibition. The kinetic energy of molecules causes them to diffuse in all directions. Thus, when a gas is set free in a chamber, it will in time be distributed equally throughout; and, when a crystal of ordinary salt is placed in a glass of water, it soon becomes uniformly dissolved in the water. Such movement of gases and dissolved substances from a region of high concentration to one of low concentration is called *diffusion*. When diffusion takes place through a membrane or through a gel, the process is called *dialysis*. Dialysis is frequently used to purify colloidal sols, the membrane restraining the movement of the colloidal particles while permitting crystalloids and ions to pass through into the external solvent phase. By the introduction of electrodes into the dialysate and the external water, and the passage of a suitable electric current, the removal of diffusible matter may be hastened. This process is known as *electrodialysis* or *electro-osmosis*.

It has already been mentioned that Graham in 1861 used the phenomenon of diffusion as a basis for differentiating between crystalloids and colloids. Crystalloids readily diffused through parchment membranes, whereas colloids did not. It should be emphasized again, however, that this distinction is not sharp. It is only a question of degree, since ions migrate at different speeds, and even some typical colloids, such as egg albumin, diffuse very slowly through certain membranes. However, membranes can be prepared which are sufficiently dense to retain the egg albumin molecules.

The diffusion constant (D, the specific diffusion rate) of an ion or molecule may be defined as the amount of solute which will diffuse

unit distance across unit area under a concentration gradient of unity in unit time. The following diffusion constants of several crystalloidal and colloidal substances are old values but are widely quoted in the literature:

DIFFUSION CONSTANTS

(D = cm.^{2}/sec. $\times 10^5$)

Hydrogen ion	32.5	Rennin	0.066
Sodium ion	4.51	Egg albumin	0.059
Urea	1.01	Emulsin	0.036
Glucose	0.57	Diphtheria toxin	0.014–0.06
Cane sugar (sucrose)	0.31	Diphtheria antitoxin	0.0015–0.05
Svedberg's nuclear gold sol	0.27	Tetanolysin	0.037
Pepsin	0.07	Antitetanolysin	0.0021

From the foregoing data it will be seen that the diffusion constant of Svedberg's nuclear gold sol, which has a particle size just within the lower limits of the colloidal realm, is only slightly less than that of sucrose, which is ordinarily regarded as a true crystalloid. Accordingly, we should expect some of the gold sol to pass through a membrane, and this is what actually happens. On the other hand, the extremely low value for the antitetanolysin would indicate that practically none of this substance will diffuse, and this is actually the case.

Osmotic pressure is defined several different ways in the literature [Eyster (1943)]. In general, it may be defined as the diffusion pressure of a solvent diffusing through a semipermeable membrane. Solutions of equal osmotic pressure are said to be *isotonic;* those having a greater osmotic pressure than the standard are said to be *hypertonic;* and those with less osmotic pressure are called *hypotonic* solutions.

When a sugar solution is enclosed in a parchment bag and immersed in water, a hydrostatic pressure is set up in the bag because of the entrance of water. The incoming water is in excess of the outgoing water because of the inability of the sugar to diffuse out and set up an equilibrium. The greater the number of sugar molecules, the greater will be the difference between the incoming and the outgoing water, and therefore the greater will be the hydrostatic pressure developed within the bag. The amount of this hydrostatic unbalance is termed the osmotic pressure. It may be measured simply by observing the rise of sugar solution (because of increase in its volume by the incoming water) in a tube or manometer into which the solution is allowed to expand directly. Protein molecules and colloidal particles in general are large and occupy considerable space compared to crystalloids. Consequently, in equimolecular concentrations of crystalloids and colloids, there will be fewer colloids per unit volume in the parchment bag.