

SIGNIFICANCE OF COLLOIDAL CHEMISTRY IN PHYSIOLOGY

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I. INTRODUCTION

Were we to put today to a large number of physiologists the question, "How much, in your opinion, will the laws of colloidal chemistry illuminate and explain physiological phenomena?" we would get a great diversity of opinion. We would find on one extreme those who believe that the newer branch of chemistry has not led and will not lead to any considerable advance in physiology and, on the other, those who see almost all the problems of biology rooted in colloids and almost all the phenomena peculiar to the living organism finding explanation in the simple laws of heterogeneous systems. With colloidal chemistry in many of its phases a new science and with many of its fundamental laws even now just being established, it is evident that the application of its principles to the complex phenomena of living organisms is a thing to be done with some caution.

The application of the laws of homogeneous solutions to biology has already answered many questions and is still to answer many more. It is likewise evident that it will leave perhaps a greater number, unanswered or explained only on the assumption of vital principles. The laws of heterogeneous solutions are now having their turn at vital problems with excellent prospects of solving many of these along physical chemical lines. To date, however, the significance of colloidal chemistry in biology is more a matter of hope and excellent outlook than of actual accomplishment. This prospect can

best be put by a quotation from Höber (1)*: "Biology experiences extraordinarily valuable stimuli as soon as many of its old problems are considered from the standpoint of colloidal chemistry. Consequently every attempt, in any degree possible, to interpret those problems, hitherto not completely understood, into colloidal processes is not only desirable but to be demanded. The materials of the biologist offer sufficient reason for this. For the microscopist must daily consider colloids, when he deals with the effects of fixing, macerating, and staining reagents upon the structures of protoplasm, for visible evidence of these effects rests mainly upon the visibility of solid or precipitated colloids; the medical chemist deals particularly with colloidal albumin bodies: whoever undertakes problems of metabolism finds that most of his riddles are bound up, in a large measure, with colloidal ferments and any one who undertakes in these days the analysis of any fundamental physiological phenomenon will observe that it is not in vain that protoplasm consists largely of colloids, that all of the more delicate and grosser membranes, which serve for the isolation and regulation of biochemical processes, are colloidal membranes. It may be emphasized that a study of immunity reactions presupposes a knowledge of colloidal properties, since toxins, antitoxins, alexins, agglutinins and lysins are colloids."

II COLLOIDAL NATURE OF LIVING CELLS

The structure of the protoplasm, its organs and the parts produced by it, has been the phase of biology of perhaps most general interest to which the knowledge of colloids have contributed. The students of colloids are coming more and more to view the protoplasm and its individual organs as typical hydrosols or as hydrogels, and it must be acknowledged that they are accumulating much evidence for this conception. From this viewpoint the following are the more prominent of the questions worked upon. In how far are the structures of the cell and its accessory parts hydrosols and how far hydrogels? What are the structure of gels and sols in general, including the protoplasmic gels and sols? Are colloidal structures complex enough to satisfy the demands of protoplasmic processes.

In answering the first question Czapeck (2) states that cell membranes, gums and starch grains are gels, as well as a number of protoplasmic organs, such as nuclei and many chromatophores.

*Figures in parenthesis refer to the bibliography at the end of this paper.

Kite (3), in work marked by excellency and delicacy of technique, has done much toward answering this question. By using an adaption of the Barber pipette as a dissecting knife he has cut away various portions of a considerable number of different sorts of plant and animal cells, determining the consistency, elasticity, and other physical characters of various cell organs. His work shows that protoplasmic parts are much more frequently gels than is generally supposed, and that the same organs in cells of different organisms may have very different characters. The nucleus of *Asterias* egg is a free flowing sol covered with a very tough consistent membrane and bearing a nucleolus of rigid cohesive granular gel. The nucleus of *Spirogyra* is a gel of rather slight viscosity bearing a network of granules and strands of greater consistency. The nucleus of epithelial cells of *Necturus* is a gel of greater viscosity bearing areas of rigid granules, giving an appearance of threads. Chloroplasts and many other organs show great range of viscosity, varying from sols to consistent gels. Kite finds that in general the animal cells are more viscous and consistent than the corresponding organs of plant cells.

There is no reason for believing that there is a transformation from gels to sols and vice versa, with a change of conditions in protoplasm as in vitro. The conditions important in producing such transformations are temperature changes, changes in hydrogen, hydroxyl, and salts ion content.

As to the second question—structure of gels—there are two rather distinct pictures. One was developed by Bütschli and confirmed and extended by Van Bemmelen (2, p. 10). They consider gels to be solid colloids with solid dispersal medium and fluid disperse phase. This is Bütschli's well-known foam structure. The frame work of the gel consists of the gel forming materials with little water imbibed, surrounding numerous spherical cavities filled with a water rich hydrosol of the gel forming material. This foam structure can be seen with the microscope in many hydrogels, especially after displacing the water with alcohol or after slight drying. The mesh size of the foam structure varies considerably with the sort of gel studied and in some is apparently ultramicroscopical. The people holding this view of gel structure extend it to hydrosols as well, so even the more fluid portions of the protoplasm are conceived as foam-like.

This picture of hydrosols is very different from the more strictly colloidal conception (1: pp. 312-316). According to the latter view hydrosols consist of discrete particles of the colloid dispersed in water. In the hydrophyllous group the

particles are more or less swollen by the absorption of water. The advocates of this view extend this conception of hydrosols to hydrogels. In the gel the imbibed or hydrated particles crowd upon each other, giving high viscosity.

It is estimated that in a 6 per cent casein solution the swollen submicrons or amicrons occupy about 60 per cent and in a 9.4 per cent solution about 80 per cent of the space of the total solution. There are several reasons offered for postulating this structure. Both a gelatin solution too dilute to gel and one sufficiently concentrated to gel show numerous submicrons as they cool, but no difference between the two can be detected by the ultramicroscope. Each can be flocculated by salt additions with the same relative effectiveness of the ions. There are points of similarity between the phenomena resulting from mixing with water certain hydrating crystalloids like sulfuric acid and glycerin and those of swelling gels. High dispersion of these gels along with their hydration gives them many of the characters of true solutions. The degree of dispersion of the gelatin is not known, but it is certain that haemoglobin forms a molecular solution.

It is not believed that the foam structure of Bütschli and VanBemmelen is an ever present structure of gels. It is considered that the foam structure is one of several deformations produced in gels by a variety of reagents or by pressure and temperature changes. A number of workers have studied separations of this type produced in gels and sols. We may draw a good illustration from Hardy's work (4). He found that when gelatin was treated with concentrated sublimate or other killing or fixing agents a variety of structures appeared depending upon the concentration of gelatin and the reagent used. From dilute solutions treated with sublimate the gelatin falls out as individual granules, from medium concentrations as a network of granules and in concentrations of 5-7 per cent or above a foam structure appears. The more concentrated the gel the thicker the walls of the foam and the smaller the enclosed cavities. In 10 per cent gelatin the cavities were 7μ in diameter and in 50 per cent gelatin 2.5μ in diameter. These reactions were reversible or irreversible, depending on the reagent used. Protoplasm shows very similar behavior in all respects with perhaps a greater sensitive to reagents, both as to deformation and irreversibility, and these reactions may in large part explain the various theories of protoplasmic structures. It seems established now that these reversible flocculations occur in normal living protoplasm. The irreversible ones, if extensive, lead to death.

In protoplasm the multiple vacuolate condition may have another origin as is shown by Bensley (5), in his late work on the canalicular systems of plant and animal cells. He concludes with the sentence, "I regard, therefore, the canalicular system as the true condition *intra vitum* of the vacuolar apparatus in these cells of the root tip, and believe that the multiple vacuole condition is of secondary origin due in most cases to injury of the cell." Here while the origin of the multiple vacuolate condition is different from that found by Hardy in gelatin gells, it is nevertheless a deformation of the prevailing structures.

In answer to the third question above, Are colloidal structures complex enough to satisfy the demands of protoplasmic processes? it should be stated that many reactions are occurring in a cell coincidentally—reactions of the most diverse character and of antagonistic types. On this basis Hofmeister (6) believed there must be a special structure offering compartments separated by membranes of special permeability characters to isolate and regulate these reactions. This he thought doubly necessary for cells with few special organs. The foam structure of protoplasm cares amply for this chemical necessity, especially if the walls of the foam are endowed with permeable qualities varying with conditions and with location in the cell.

Some of the students holding to the more strictly colloidal conception offer a number of arguments against this view. Protoplasmic movements argue against such a structure. The ultramicroscope generally fails to reveal foam structure in gels and sols *in vitro* as well as in the protoplasm. Such structures are apparently not ever present, but are formed as a result of certain accompanying conditions.

As our *intra vitum* staining methods improve and as new methods of the direct study of the living protoplasm are developed, such as the dissecting method of Kite, we are finding more and more bodies of micronic size in the cell. These are included under a variety of names such as mitochondria, chondriosomes, etc. If we remember that colloidal particles of this nature generally surround themselves with special membranes, which give them peculiar permeability characters, it is easy to see that here again is the possibility of the isolation and regulation of chemical reactions. Aside from these the ordinary plant cell has plastids, vacuoles, or canalicular systems and nuclei.

If in the frame-work and cell wall systems of plants in general such a simple structure is primary, there must also exist a secondary and grosser structure which determines the anisotropy as manifested in unequal swelling along the several axes due to water absorption. Wood, for instance, swells most tangentially, **less radially and least longitudinally**, while a great variety of unequal swelling appears in wall structures showing hygroscopic movements. In some of these cases only a single wall is involved, showing anisotropy to exist in rather minute structures whether primary or secondary. The multivacuolate conception of gel structure lends itself more readily to the explanation of this anisotropy, for with water gain and loss the vacuoles may show greater dilation and contraction in certain axes than in others (7: pp. 745-552).

The thing of greatest biological interest in the study of hydrosols and hydrogels, including the protoplasm, is not any specific structure found, but the capacity for the assumption of one or another structure with variation in condition. The reaction is now reversible and now irreversible. While such modifications are brought about with relative ease in gels and sols in vitro, it seems that living protoplasm and its constituents are even more labile. This is illustrated by Lepeschkin's (8a) work showing that slight pressure will cause a reversible flocculation of cell proteins of *Spirogyra* and greater pressure, a permanent coagulation and death. Pressure alone does not coagulate protein in vitro. According to Lepeschkin some of the proteins of plant cells essentials to life would coagulate in a few hours, or at most a few days, at 20° C, if there were not dispersion processes in the living cell counteracting the coagulation processes (8b). The proteins in vitro at the same temperature require thousands of years for coagulations.

The way investigators of colloids are attempting to change our conception of the structure of living matter is well illustrated by the work on the ordinary green chloroplast. Earlier work indicates a definite structure of this organ, but different investigators give very different pictures. One speaks of the cytoplasmic stroma as a sponge-like framework with definitely organized granules of pigment filling the cavities, another of the pigment itself in the framework with the protein filling the cavities, and a third of the surface distribution of the pigment. Liebaltd (9) has lately studied the structure of this organ from the standpoint of colloids and finds that the normal living chloroplast is generally homogeneous when viewed either with the microscope or the ultramicroscope. Allowing

for oil droplets and starch grains she speaks of the lipid pigment phase as distributed through the hydroid protein phase to amicronic dimensions. This distribution is easily deformed by various reagents giving all types of structure formerly claimed for the organ. Excessive water absorption caused by opening the cells and bringing the chloroplasts directly into contact with water or even by long soaking of the entire leaf, produces a variety of deformations. As shown by staining reactions, water never produces a complete separation of the two phases. Such altered structures appear frequently in nature and they probably result from peculiar water relations of the plastid. Surface tension active substances as well as other fixing and killing agents in sufficient concentration cause a complete separation of the two phases. This emphasizes the great danger of concluding as to the structure of a living cell from fixed and stained material. (See also citation 4).

Other evidence is offered for the colloidal state of chlorophyll in the living plastid. It has been known for a long time that chlorophyll in an alcohol solution is readily decomposed by sunlight. This has called for an explanation of the apparent or real light stability of chlorophyll in the illuminated leaf. Wiesner believed that protective action of fatty bodies and continual synthesis account for the maintained amount, while Reinke suggested that chlorophyll forms a light stable compound with proteins of the plastid. Iwanowski (10) accepts neither of these views, but thinks the light stability of chlorophyll is explained on the basis of the colloidal condition in the plastid. He found chlorophyll extract in 49 per cent alcohol far more light resistant than that in 95 per cent alcohol. In the former case it is in colloidal solution, in the latter molecular. He also shows that as the colloidal concentration of the solution increases and the degree of dispersion correspondingly decreases light stability rises. Finally in the concentration and degree of dispersion probably existing in the chloroplast, it is light stable. While the Iwanowski conception has evidence in its favor there is also no important evidence against the Reinke view. Iwanowski also finds evidence for carotin and xanthophyll of the plastid protecting the chlorophyll against light destruction. Herlitzka (11), using carefully purified chlorophyll, finds that it shows little if any fluorescence in colloidal solution, while this property is very evident in a molecular solution. The lack of marked fluorescence in the plastid is probably due to the colloidal state of the pigment as well as to the turbidity of the plastid, formerly offered as the explanation.

III SOME GENERAL CHARACTERISTICS OF CELL COLLOIDS

The protoplasm is optically isotropic, while some of its products (starch grains, cell walls and crystalloids) are optically anisotropic. It is believed that the double refraction of starch grains and cell walls can be explained on the basis either of impregnating materials, as in the cuticle, or tension within the gels of these structures; and that the property furnishes no argument in favor of their crystalline structure as against their hydrogel nature (1). With time, however, it seems that the line between crystalline and amorphous bodies becomes distance. (12: pp. 66-75).

The colloidal constituents of the living cell bear negative charges. As a result living cells themselves are electronegative. This has been shown for lecithin, chlesterin, proteins, chlorophyll, blood corpuscles, bacteria and spermatozoa.

When Graham divided matter into two classes—crystalloids and colloids—he thought these two divisions were quite distinct. One of the principal characteristics for distinguishing the two classes was their diffusibility through gelatin or other colloidal membranes. We know that the two classes of substances are not so distinct but that colloidal solutions range in their degree of dispersion from suspensions with microscopically visible particles, on one extremity to molecular dispersions on the other. The hydrophyllous colloids of the living body, which are of greater interest to the biologist, are very highly dispersed, approaching in general the molecular state. It is now well known, for example, that hæmoglobin forms a molecular solution. Along with this very high dispersion they have the character of diffusing with ease through a variety of colloidal membranes. Egg albumin and hæmoglobin diffuse through gelatin plates. Pepsin diffuses into cubes of coagulated albumin. The immunity bodies diffuse through a great variety of colloidal membranes (gelatin and agar plates, dead intestine walls and others). This raises the question of how they are retained within the living cell. This question becomes especially urgent if Ruhland (13) is correct in his conclusion that for colloidal solutions the protoplasm acts as an ultra filter. We shall later see his explanation for the retention of certain enzymes by the cell and their localization within it.

IV WATER RELATIONS OF CELL COLLOIDS

There are certain characters of hydrophyllous gels and sols that are assuming great importance physiologically. One of the more significant is the water absorbing power of these as affected by various reagents. Water absorption by gels and

sols is often spoken of as hydration, although this is strongly condemned by Van Bemmelen as incorrectly emphasizing chemical union of the water with the colloid. A great part of the knowledge in this field we owe to the efforts of Martin Fischer (14). The more significant facts known for gelatin and fibrin are: acids, alkalies and urea greatly increase water absorption; some anions of alkali metals increase water absorption (Rhodonate Cl, Br, NO_3 .) and others decrease it (SO_4 , PO_4 , Tartr., Citr.) (2: p. 43); salts in general inhibit the hydrating effects of acids and bases and the total effect of the salt is due to the joint action of the two ions; non-electrolytes show influence on the hydration effects of acids and alkalies; sugar and other non-electrolytes reduce greatly the hydration effects of urea, while electrolytes show little influence. Fischer finds these reagents act on the hydrophyllous colloids of the body, especially the proteins of the cells and fluids, exactly as they do upon gelatin and fibrin. He concludes from his work that dropsy in the animal body is produced by the presence of agents that favor water absorption by colloids of the body. Amongst these, acids and urea are the more important. Intravenous injections of salts, alkalies and dextrose are effective in reducing edema by dehydrating tissues and fluids of the body on the same basis as they act on gelatin and fibrin. In fact he finds the cause and therapeutics of dropsy tied up with water absorption by body colloids. From this extensive work Fischer concludes that absorption by colloids and modification of it by various factors play the main part in water relations and secretions in the animal body; while osmotic pressure is of very minor importance. This is a reversal of the general view and brings colloids into prominence in contrast to crystalloids, which, although they act by the help of colloidal semipermeable membranes, are the main source of osmotic pressure.

Whatever may be true in the animal, one who knows the very extensive literature on the water relations of plants cannot give osmotic pressure such a minor role there. It is probably just as true that the significance of water absorption by colloids has been greatly underrated in plant physiology. This is due to the over shadowing prominence given to osmotic pressure and the resulting turgor pressure. We generally think of the plant cell with its large vacuole and cell wall as pre-eminently an osmotic machine. One is surprised when Borowikow (15) announces that a root placed in .01 M. HCl elongates twice as fast as in distilled water. It behaves much like a piece of gelatin, although it ought to exhibit osmotic

activity at its optimum. The elongation is of course temporary for the high concentration of acid soon proves fatal. Lower concentrations of acid increase the rate of elongation, but less considerably and for a longer period. There seems to be evidence for the view that rate of growth in plants is regulated to a considerable degree by organic acids formed (15, 16, 18). Whether this is due in the main to the hydration effect of the H^+ , or whether the acids also favor the formation and activation of enzymes, or perhaps other processes are effective, is not established.

Acids and bases increase the rate of water absorption by seeds and they are good forcing agents for many seeds (16, 17, 18). This forcing effect in some cases is due to increased water absorption by the seed coat. In other seeds the reagents have this and other effects upon the embryos. I shall be surprised if the often mentioned forcing powers of neutral salts for seeds is not due in many cases to its effect upon water absorption by gels of the seed coats and the endosperm. Assuming that these gels are electro-negative one would expect such salts as lithium, sodium and potassium chlorate, nitrate or chloride to increase water absorption while salts involving calcium or sulphate might have the opposite effect.

As yet, however, we know very little about the role of these reagents in water absorption by carbohydrate gels, which are so prominent in seed coats. There are peculiar cases (19) recorded, such as the power of potassium salts to increase the water absorption and cause the final dissolution of the cell walls in the growing zone of roots.

Calcium salts alone or in combination with other salts on the other hand, maintain the proper consistency of the walls and integrity of the roots. In fact Cranmer finds that in general potassium salts greatly favor the absorption of water by cell walls and inhibit the evaporation of water from them; while calcium salts have the opposite effect. The effect of these salts upon water absorption and retention by the cell wall is similar to their effect upon transpiration which indicates that they modify the latter process, at least in part and perhaps in the main, quite independent of the protoplasm.

Calcium ions tend to maintain the proper consistency of the intracellular cements of animal cells, while potassium ions lead to their dissolution (1).

In this connection we should mention certain effects of calcium and other bivalent and even trivalent ions upon the protoplasm. They reduce the permeability of the protoplasm

(plasmahaut?) to salts of the alkali metals and many other substances (20, 21), thus probably reducing the toxicity of these substances to the organism. This effect has been described under the term antagonism. Recognizing Cranner's contribution, wall as well as the plasmahaut effects may be involved in antagonism¹. It is also not proved that antagonism results alone from permeability change. It may include adsorption phenomena (2: p. 60; 21) or something quite different, such as induced or stimulus effects rather than direct physical changes.

We might summarize what we have said on the relation of various substances (ions and molecules, especially of salts, acids, bases and anesthetics) upon colloids of the living cell. In certain concentrations and mixtures they maintain the consistency and other physical characters of cell colloids necessary for the proper functioning of the organism. Höber and others emphasize in this connection the colloids of the plasmahaut and believe that the effect of these substances upon the irritability, permeability (35), etc., of the organism can largely be explained through their effects upon this organ (1: pp.—). Cranner (19) would emphasize in addition their importance in maintaining the proper consistency of the cell walls in plants; while Martin Fischer sees no less significance in their effects upon all colloids of the organism, including sols as well as gels.

Besides the general nutrient function of salts in plants, such as the use of nitrates, phosphates, sulphates and magnesium for the synthesis of proteins, nucleoproteins, lecithin, chlorophyll, etc., all salts or rather all ions of salts have effect on the physical characters of the colloids of the organism. The necessity of calcium for most plants can best be explained today by its function in maintaining the proper consistency of the colloids of the organism wall and protoplasm. We have much yet to learn concerning calcium in this regard and even more concerning potassium, magnesium and other ions².

There is evidence that some very important morphogenic or development changes are brought about by changes in the amount of water held by cell colloids at the time particular processes are occurring in the organism. Certain work indicates

1. Indeed it is not established that the plasmahaut alone determines the entrance of materials into the cell. Other layers of the protoplasm may be involved and in plants the permeability characters of walls and change of these with conditions should not be neglected. This is especially emphasized by recent study of many walls in seed coats showing peculiar osmotic characters in part at least resembling those of the plasmahaut.

2. This is not denying still other functions of salts in plants.

that sex may in some cases be determined by the water content of the egg colloids at the time of fertilization. Eggs of frogs fertilized when laid give about half and half male and female. If they soak in the watery fluids of the oviduct of the female for some hours or days before fertilization they give predominantly (sometimes 100 per cent) males. If toad eggs are fertilized as soon as laid they produce about half males and half females, but if dessicated before fertilization females dominated, in some experiments 9 to 1. In hybrids between certain pigeons Riddle finds high water content of the yolk generally correlated with maleness and low water content with femaleness (22).

On the plant side also there are results that may be interpreted as meaning that water content of cell colloids of various primordia have a prominent part in determining the course of development of those primordia. According to MacCallum (23) the markedly different type of leaves in *Proserpinaca palustris* developing in water and air is due to differences in water contents and Klebs (7: p. 488) finds that in flowering plants high water and salt supply (especially nitrates) leads to vegetative growth, while low water and salt (especially nitrates) along with high carbohydrates induced reproduction. The nitrates here belonging as they do to the least hydrated end of the lyotropic anion series may act similarly to greatly increased water supply by inducing greater hydration of the cell colloids¹.

From these illustrations the question naturally arises: Why does the degree of hydration, or water imbibition, by the protoplasm play such an important role in the course of development of the primordia? The question is not capable of answer at present. It should be pointed out, however, that many other physical characters (viscosity, degree of dispersion, etc.) of the colloidal mass vary correlatively with water supply².

In the mammal nervous control and internal secretions, acting perhaps through correlations, are two very important morphogenic determiners. If the degree of hydration of colloids turns out to be as significant as the illustrations above indicate we have here a point of great interest.

Schroeder (2: p. 42) has pointed out a character of colloids that has much of interest to the plant physiologists. He finds that a gelatin gel is in equilibrium with a saturated atmosphere when it contains about 40 per cent of its dry weight in water

1. This interpretation is quite different from that given by Klebs.

2. Here some will contend that turgor pressure rather than hydration of colloids is the determining condition.

and with liquid water when it contains about 1100 per cent. If gelatin fully imbibed in a saturated atmosphere, is placed in water a rapid absorption occurs and the liquid water equilibrium is finally approximately reached. The reverse process takes place when a gel fully imbibed in liquid water is placed in a saturated atmosphere. Filter paper absorbs about equal amounts from saturated atmosphere and water. This character of gelatin and other gels is not certainly explained physically, but that does not concern the biologist so much as the question of whether the living body behaves similarly. Martin (24 and unpublished work) has found an exact counterpart of this behavior of gels in the pollen of alfalfa. The pollen has a very narrow range of water supply necessary for its germination. This is the main factor in the uncertainty of seed production—a matter of considerable economic significance in the United States. In distilled water the grains swell rapidly and burst. In a saturated atmosphere they germinate rather abundantly; but will not germinate in an atmosphere appreciably below the point of saturation. A number of experiments indicate that the final water equilibrium is an important factor here, although the rate of water absorption is likely of some significance. Shull (unpublished work) has found for several sorts of seeds that they contain much more water when in equilibrium with distilled water than when in equilibrium with saturated atmosphere.

If the entire plant acts like a piece of gelatin it should transpire to a saturated atmosphere when its lower end is in water. Dixon (25) claims that this occurs. This character of gels may be acting along with raised temperature due to absorption of radiant energy in maintaining transportation in plants of tropical rainy regions. It is a common thing in plant physiology to grow what are naturally soil or water roots in a saturated atmosphere and assume that they are in a normal condition. Aside from the rate of water absorption we have this character of gels raising a question as to the amount capable of being absorbed. Every worker in plant physiology has observed peculiar characters in roots grown in saturated atmosphere, such as reduced rate of elongation and profusion of root hairs. This gel character of plants may also play a part in guttation.

V DIFFUSION IN A COLLOIDAL MEDIUM

Peculiar diffusion and precipitation phenomena occur in colloidal substrata. Küster (26) attempts to relate these causally with the most diverse features of plant anatomy. His point of

departure is the Liesegang ring system. This concentric ring system is produced in a few hours after placing a droplet of 80 per cent AgNO_3 on a 5-10 per cent gelatin plate bearing one per cent $\text{K}_2\text{Cr}_2\text{O}_7$. The rings consist of Ag_2CrO_4 and become more definite and more distinct from each other as the distance from the drop increases. The explanation of this phenomenon is still in question, although it may be due to the colloidal **substratum** making possible great supersaturation before the labile equilibrium is reached. The bands formed in gelatin in capillary tubes show definite rhythm and polarity. Aside from the Ag_2CrO_4 bands there are others caused by impurities in the gelatin which Küster terms small rhythms in contrast to the great rhythms of Ag_2CrO_4 . Contact with the dish in contrast to free gelatin, tensions, pressures and many other conditions, produce a great variety of precipitation figures. In fact patterns can be produced that resemble closely all the various structures appearing in mottled leaves, in the arrangement of vascular elements of plants and the markings of tracheae. Küster emphasizes the fact that the pattern differentiation of Ag_2CrO_4 precipitate shown in the gelatin plate is a self differentiation occurring under constant environmental conditions. This is taken to show that similar periodic or rhythmic structural changes in the cells may be independent of rhythm in the environment, a matter of self-differentiation. The work also indicates the possibility of separating such differentiation from vitalistic peculiarities. The fact that a simple diffusion process in gelatin gives complex polarized precipitation patterns leads the author to conclude that perhaps a complex pattern in the organism may likewise be referred to a simple diffusion process in a colloidal matrix rather than explained by appeal to complex regulative action of living protoplasm.

Rhythm in plant activity is held by many to be generally related to environmental rhythm. Others feel that rhythm is a necessity of the very nature of protoplasmic activity; activity must be followed by rest. An examination of the facts that Küster offers shows the possibility of rhythms in the organism independent of the inscrutable features of protoplasm. In short, internally determined rhythms may be matters of relatively simple physical and chemical laws. Bringing this third possibility of structural rhythm and polarity into prominence is the great contribution here. The work really has not explained in terms of process of formation a single plant or animal structure.

If the explanation of Ostwald mentioned above is correct, it means that a colloidal medium of the type of a gel favors

the maintenance of supersaturation, or of a metastable equilibrium. Likewise it seems to favor supercooling as does any capillary system. This last feature of colloids is claimed to be of importance in protecting the organism against death from low temperatures (1: pp. 50, 58, 27). The full discussion of this point, pro and con, would lead us, however, too far away from the main topic under consideration. A colloidal medium then seems to favor these two "Überschreitungserscheinungen," supersaturation and supercooling, and perhaps through these explain certain behavior of organisms.

VI ENZYMES AS COLLOIDS

Enzymes which play the main role in the metabolism of living organism, have many characters indicating their colloidal nature. They are readily adsorbed by fine suspensions or other colloids. This adsorption is probably due in part to their surface tension activity, and in part to their electrical characters. In the first instance they show themselves closely related to the hydrophyllous colloids. In the second they manifest weak suspensoid characters (2: pp. 95-127).

Even the strongest argument against their colloidal nature—the fact that they never have been prepared in a pure state and that we therefore do not know their true nature—is an argument favoring it also, for it is an outcome of high adsorption capacity.

Grüss was able to separate the various enzymes of a plant organ by placing a drop of its juice on a stretched filter paper. As the drop spreads the enzymes arrange themselves in rings about its center in order of ease of adsorption by the paper. That the electrical charge of the enzyme particle is sometimes a factor in its adsorption is shown by the following reactions: kaolin, a negative suspension, will not adsorb invertase, a negative colloid, but *tonert*, a positive suspension, adsorbs invertase readily. Kaolin will adsorb malt diastase only if the diastase is acid in reaction, or is a positive colloid. In a neutral medium malt diastase, like trypsin, pepsin, and ptyalin, is nearly amphoteric, so the adsorption of all is more a matter of surface tension. Filtering of enzymes through charcoal or repeatedly through filter paper lowers their activity due to adsorption by the filter. The colloidal nature of enzymes has been called into play as a means of explaining their retention within the living cell. Hoffmeister believed their colloidal character prevented them from diffusing through membranes generally and especially through

protoplasmic membranes. On this basis he conceived that they were not only held within the cell, but also localized within the separate compartments of the multivacuolate protoplasm, as he pictured it. It has been shown, however, that enzymes are very highly dispersed colloids and that they diffuse readily through 10 per cent gelatin and Ruhland (13) believes through cell walls and protoplasm generally. This author thinks retention within the cell is due either to adsorption or more likely to actual chemical union with the protoplasm. He believes that this also accounts for the localization of enzymes within the cell.

In many of their activities enzymes behave like colloids. The question has often been raised whether their main function is a typical colloidal reaction or more of the nature of reactions in molecular solutions. The evidence generally is in favor of the second. In the first case diffusion should play the main role and the speed coefficient for 10°C rise in temperature should not exceed 1.25. For lipase, invertase, catalase, and tryosonase the coefficient is about 1.5, but for most enzymes it is from 2 to 3. Where the coefficient is as low as 1.5 diffusion between the two phases of the system (aided by increased Brownian movement of the enzymes and reduced viscosity of the medium) may be the main rate determining process; but where the coefficient lies between 2 and 3 the rate is probably determined by a reaction typical of homogeneous systems. The action of enzymes is, however, very complex, involving adsorption by the substratum and often by products of their action, and many other disturbing factors. As a consequence we are far from a quantitative statement of the kinetics of enzymes.

VII IMMUNITY BODIES AS COLLOIDS

In general when the physiological significance of colloids is discussed the characters and behavior of immunity bodies are used as illustrations. These show much in common with enzymes and many of the questions concerning the colloidal nature of the one has been asked and answered similarly for the other. Space does not permit the discussion of the colloidal characters of this group of substances and besides an excellent statement can be found in many texts (1, 2, 28).

VIII SOME COLLOIDAL PHENOMENA OF SOILS

There is a large number of reactions in the soil that are colloidal in nature and of great interest to the plant physiologist. The soil is the environment of approximately one-half

of each land plant and, so far as many processes are concerned, the more interesting, if the less well understood, half. Flocculation, a process by which clay soils are rendered more penetrable and given a better oxygen supply, is a typical reaction between suspensoids and suspensions and electrolytes. It is well illustrated in delta formations at the mouths of rivers where the silt and clay constituents of the fresh water are brought into contact with the salts of the sea water and flocculated. The effect here is mainly due to the cations of the salt acting on the negatively charged soil particles. Deflocculation, brought about by the addition of excess of flocculating salts, is a well known reaction of suspensions and suspensoids included under the term peptisation (1 : p. 293-295).

The dissolution and removal from the soil of lime carbonate, its natural sweetener or neutralizer, and the final accumulation of acid in it, is in part at least due to flocculation. The positive ions of the inorganic salts are absorbed by the organic and inorganic negative soil particles and the latter are flocculated, leaving free in the soil the strong inorganic acids corresponding to the negative ions of the salt. The soil particles thus flocculated may be of microscopical size, or suspensions, or of ultramicroscopical size—true colloids; but the principle of the reaction is the same in either case. Flocculation, in part at least, explains the mysterious humic acids of the soils that have been so much discussed without certain identification and so far as it does account for the acidity shows that the acids are largely not organic but inorganic.

Daikuhara (29) has lately brought evidence to show that the injurious effects of adding fertilizer salts to many soils of Japan and Korea, poor in lime, are due to the fertilizer salts freeing complex acid salts of aluminium and iron from adsorbed condition on the colloids of the soil. If lime accompany the fertilizers very beneficial effects results from them. He implies that the same may be true in America where acid soils are so prevalent.

Litmus paper as a qualitative test for soil acidity has its short-comings in the differential adsorption of the two ions of the organic salts of the litmus by the suspensions and suspensoids of the soil on one hand, and by the colloids of the paper on the other—typical adsorption processes of suspensoids. The common quantitative tests for soil acidity are rendered unsatisfactory on similar grounds (30).

Of course, no one will claim that the total loss of lime carbonate from the soil (about 800 lbs. per acre per year) along

with the final assumption of the acid character by the soil is due solely to the acids set free by the flocculation of organic and inorganic suspensoids of the soil. There are a great number of acid forming processes going on in the soil; carbon dioxide production of the respiration and fermentation of soil organisms and nitric acid production by the nitrifying processes (oxidation of ammonia salts to nitric acid by nitrosomonas and nitrobacter) may be mentioned as two important ones.

As one passes from the coarse sands to the finer clay he finds a continual rise in the specific surface presented by the soil—that is, a continual rise in the total surface of the soil particles per unit volume. The high specific surface of the finer soils is due in part to particles of micronic or microscopic size, but in large part to the ultramicroscopic or submicronic particles—soil colloids, inorganic and organic. The great specific surface developed in fine soils, especially those with considerable colloidal constituents brings about much adsorption or concentration of dissolved and suspended substances on the surface of the soil particles. This surface accumulation is due in part to electrical effects typical of suspensoids and in part to the surface tension active substances in accordance with the Gibbs-Thompson law. Whatever the cause of these surface concentrations the result is that many new reactions are set up between the concentrated materials themselves and between them and the soil particles. We often speak of sandy soils being poor in plant nutrients, while clays are rich. This is largely due to the surface phenomena and the reactions resulting from them.

In fact any good soil shows many of the characters of a colloidal medium, or is essentially a colloid, as is well expressed by a quotation from Russell's Monograph (31) on Soil Conditions and Plant Growth, "It is a mistake to suppose—and this point cannot be too strongly emphasized—that the medium on which the soil organisms live, and which is in contact with the plant roots, is the inert mineral matter that forms the bulk of the soil. Instead, the medium is the colloidal complex of organic and inorganic compounds usually more or less saturated with water, that envelops the mineral particles. It is therefore analogous to the plates of nutrient jelly used by bacteriologists, while the mineral particles serve mainly to support the medium and control the supply of air and water and, to some extent, the temperature."

IX TOPICS IN COLLOIDAL CHEMISTRY AND THEIR BEARING UPON
PHYSIOLOGY

Almost any topic commonly discussed under hydrophyllous colloids can also be discussed in important biological relations. Let us select three of a considerable number of such topics for brief consideration: hysteresis, protective action of hydrophyllous colloids, and the anion series of alkali salts.

Hysteresis, or spontaneous ageing changes, so common in colloids has many points of physiological interest. These are well illustrated *in vitro* by the slow or rapid flocculation of any colloidal solution; by the reaction between immune bodies becoming irreversible with time and by changes in the vapor tension of gels, continuing for weeks or months after their formation. No doubt many changes in the living or non-living parts of the organism fall under this head.

Seeds in dry storage lose their power to germinate gradually—some even after a year and others only after a century and a half. This is likely due to slow coagulation of embryo proteins (32). Seeds of *Amaranthus retroflexus* will not germinate when harvested, but will do so after a month or two of storage under uniform conditions. This is a result of spontaneous ageing changes in the gels of the seed coats. Such ageing changes have also been observed in spores. Heating the oat coleoptile to 39°C for one hour lowers the rate of photo-perception at 20°C fourfold. After four hours at 20°C the old speed is regained. This recovery has been interpreted as a matter of hysteresis in cell colloids rather than elimination of poisons formed at the higher temperatures, although the latter interpretation is possible. The main virtue of classifying some of the spontaneous changes in the organism under the term hysteresis is not that it furnishes in itself an explanation of them. It takes these problems out of their connection with vital qualities and places them on the basis of a colloidal substratum capable of physical and chemical study.

Protective colloids are colloids of the hydrophyllous type which have the character of forming a film about the particles of suspensions or suspensoids and preventing their flocculation. Blood serum often bears uric acid far above the saturation point. Albumins of the serum of the blood acting as protective colloids keep the droplets of uric acid in suspension. The droplets vary in size from amicros to microns, depending upon the degree of supersaturation. The high content of calcium phosphate in milk is probably explained on a similar basis. Gall stones and bladder stones are in part due to short-

age of protective colloids for holding certain insoluble substances in suspension.

Hofmeister showed that certain anions of alkali salts decrease the amounts of water absorbed by gelatin gel, while others increase it. The sulfate, tartrate, citrate, and acetate anions cause shrinkage, while bromides, nitrates and rhodanates cause swelling. Starting with this determination a great amount of later work has led to a rather definite arrangement of the anions of alkali salts in relation to this as well as other characters of colloids and to a number of physical and chemical processes in homogeneous solutions (1: pp. 309-326). The following are interesting colloidal applications of this series. In salting out hydrophyllous colloids, the sulfate-tartrate end is most effective, while certain anions of the opposite end of the series cause dissolution. The same order holds in the productions of gels from sols. The cations show less range in effectiveness, but they too can be arranged especially in regard to precipitation of colloids, in definite order reversed, however, with a reversal in the charge. We generally consider tropic movements in plants, so far as their underlying causes and processes are concerned, as amongst the most complex of plant phenomena. Porodko (33) shows in a recent publication that the effectiveness of the anions of salts of alkali metals in producing positive chemotropism follows almost exactly this series: Tartrate > citrate > sulfate > acetate > chlorate > chloride > nitrate > iodide > cyanide. The effectiveness of their cations for positive chemotropism also approximated their effectiveness for the precipitation of colloids in an acid medium, thus: rubidium > caesium > potassium > lithium > sodium. Bromide does not cause positive chemotropism. These and a number of other facts discovered by Porodko indicate that dehydration or flocculation of colloids bears a fundamental relation to chemotropism. Work by Fischer and by Schley indicates similar significant relations between geotropic response and water absorption by colloids (12, 34). To date, however, the results on both chemotropism and geotropism in this line are only suggestive and open the possibility of attacking these complex phenomena of plants on a definite basis of the physics of colloids.

X CONCLUSIONS

In closing let us attempt to make a few summary statements: In discussing the topic colloidal chemistry in relation to biology, one faces a relatively new science with a still newer application to another science. The application to date

has been made only at occasional points and not consistently over the whole field. Hence the discussion takes more the nature of isolated illustrations than of a summary, but these illustrations bring into prominence a few very important facts and open up situations of great promise.

The living portion of the organism and many parts produced by it are in the main colloidal and in their behavior obey the laws of colloids. Rational explanations in this field cannot be expected when these basal facts are neglected.

Therefore many of the phenomena of the living organism and some of the phenomena of its environment, which entirely lack explanation on the basis of homogeneous systems, are common processes of colloidal systems.

Although at present the application of colloidal chemistry to biology offers much more of bright prospect and excellent promise than of accomplishment, it is certain that as the application progresses many processes that had to be included under the unexplained group of vital processes will find partial or complete elucidation on the basis of laws of colloids.

But even after colloidal chemistry consummates its possible accomplishments in biology, the vitalist will still have legs, although quite different legs, yet legs to stand upon.

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