Selected topics in colloid chemistry with especial reference to biochemical problems / by Ross Aiken Bortner.

THE GEORGE FISHER BAKER
NON-RESIDENT LECTURESHIP IN CHEMISTRY
AT CORNELL UNIVERSITY

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Professor of Agricultural Biochemistry
in the University of Minnesota

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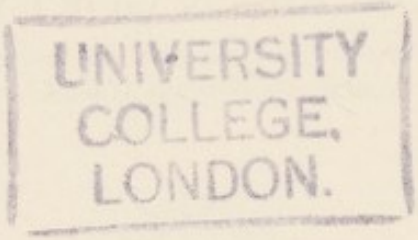

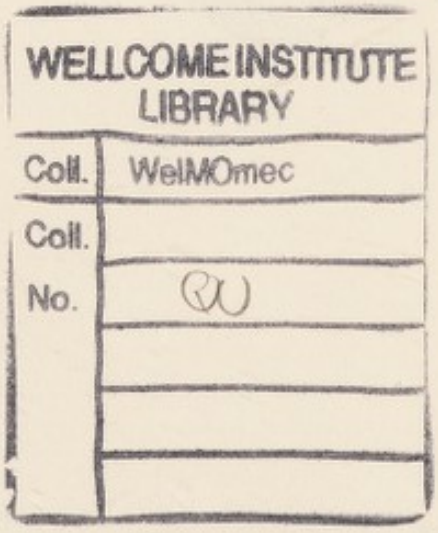

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To

WILDER DWIGHT BANCROFT

TO WHOM AMERICAN SCIENCE IS DEEPLY INDEBTED FOR HIS CONSTANT INSISTENCE, IN SEASON AND OUT OF SEASON, AND OFTEN IN THE FACE OF OPPOSITION, ON THE IMPORTANCE OF COLLOID PHENOMENA AND SURFACE REACTIONS IN ALMOST EVERY FIELD OF PURE AND APPLIED CHEMISTRY

PREFACE

THIS BOOK covers the content of a series of lectures delivered in the Department of Chemistry of Cornell University during the first semester of the academic year 1935-1936 at which time the author held the George Fisher Baker Non-Resident Lectureship in Chemistry.

During the past quarter of a century there has been developing a vast literature in the field of colloid chemistry. An exhaustive survey of all of the important aspects of colloid behavior would require a much more complete treatment than could be given in a short series of lectures. Consequently no claim is made that the present volume is an adequate treatment of the nature and behavior of colloid systems. On the other hand it is specifically designed to present an introduction to certain fundamental phenomena characteristic of such systems and, above everything else, to present the author's viewpoint and interpretation as to how and why these phenomena should be of interest to biochemists, physiologists, and biologists. Some of the interpretations which the author has made are probably debatable. So much the better! Science will stagnate only when all scientists come into complete and harmonious accord, and when all will agree that only one interpretation can be drawn from a given series of data. If any of the theories or interpretations of data noted in this volume shall stimulate a researcher to undertake further investigations, then the author will feel that the task of writing has been worthwhile.

The author is convinced that in the study of the phenomena characteristic of colloidal systems we will find clues which will enable us to understand many of the reactions characteristic of living organisms and life processes. Sir W. B. Hardy "once saw a cell divide," and he spent the rest of his life in a study of colloid and surface phenomena in order to answer his own question: "Why does a cell divide?" In his last paper he pleads for others to carry on and "to take the findings of physics and chemistry and faithfully to apply them to the riddle of this impossible elusive living slime in its coat of many colors."

In the author's text book, "Outlines of Biochemistry," John

Wiley and Sons, Inc., New York, 1929, some of the fundamental properties of colloid systems have been discussed more completely than seemed necessary for the present volume. In addition other colloid phenomena are considered there which it has not been deemed necessary to treat in the present series of lectures. The author is indebted to his publishers for their permission to utilize in the present manuscript certain of the figures and tabular material from his "Outlines" and also for their permission to present in this volume discussions which, in some instances, parallel somewhat similar discussions in the larger text book.

In concluding this foreword may I express my sincere appreciation to my many friends in the faculty and student body of Cornell University, and especially to Professor Papish and the staff of the Department of Chemistry, for a delightful four months period of sojourn with them in their great university. It was a period of intellectual stimulation that I shall long remember. Fortunate indeed is Cornell University to have the endowed George Fisher Baker Non-Resident Lectureship and more than fortunate is the one who is invited to fill this Lectureship and to enjoy the hospitality of perfect hosts.

Ross Aiken Gortner

St. Paul, Minnesota
August, 1936

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SCIENTIFIC GENEALOGY

Provost Mann, Professor Papish, members of the staff, and students of Cornell University: I feel honored and at the same time very humble as I stand here this evening. Honored because it is generally recognized throughout the chemical world that selection as the George Fisher Baker Lecturer at Cornell University is a reward that comes only to a select few. I thank you for deeming me worthy of this honor. At the same time I feel extremely humble as I think of those illustrious chemists who have preceded me in this position and I only hope that I may in some small degree measure up to your expectations.

I am sure that every member of every chemistry faculty, and certainly every director of every chemical laboratory in America, violates the Tenth Commandment of the Mosaic Law every time that the George Fisher Baker Lectureship at Cornell University comes to his attention. The Tenth Commandment reads, "Thou shalt not covet," and I am sure that every institution of learning in America, if not in the entire civilized world, covets for itself a lectureship such as has been established here. May I congratulate Cornell University on the possession of this lectureship and pay tribute to the memory of George Fisher Baker who so generously provided for the endowment of this lectureship. I can think of no finer memorial for a man than that he make provision for the extension of knowledge within the halls of our universities, now, and in the days that are to come. The generations of students who have passed and who will pass their time in the laboratories and classrooms of this magnificent building should never forget the generosity of the man who made these material facilities possible. They should regard his gift to them during their student days as only a loan which, if it is at all possible, they should some day repay with interest, by following his example and applying a portion of whatever earthly wealth they may accumulate, to the advancement of knowledge for the benefit of generations still unborn.

Think for yourself—Who is remembered throughout the length and breadth of America, the millionaire whose body lies beneath a marble temple upon which his name is carved or, for example, James

Smithson who left his fortune to advance science in a young and struggling nation? The Smithsonian Institution is his monument, one of the greatest monuments in all the world, one beside which marble sarcophagi pale into insignificance.

One of the requisites of the Baker Lectureship is that the incumbent deliver a public address. I have ascertained that the subject need not lie in the chosen field of the speaker's specialty. I have accordingly selected for this evening the topic, "Scientific Genealogy," partly because the title is perhaps somewhat cryptic and may have excited your curiosity enough to attract you away from a game of bridge to attend what may prove to be a rather dull lecture, and partly because the title accurately describes what has been a hobby of mine for the past many years, and which will continue to occupy my leisure moments for many years to come. In order that I may be sure that you will see things as I see them, it will be necessary for me to digress somewhat from the usual plan of a public address. It is usual for the lecturer to select some topic of broad scientific interest, and to summarize the field and perhaps philosophize on future trends. Such addresses are usually very impersonal. I must needs, if I am to succeed in my plan, be at times intensely personal, and I must relate certain intimate personal experiences, not because they happened to me, but because I personally know how important they were; and since I can testify to their happening, perhaps I can convince you of the validity of my thesis. In other words, I am going to adopt the good old methodist practice of testifying and preaching at the same time, and since each preacher must have a test, I have selected the one that reads:

"By their fruits ye shall know them."

Genealogies are found among the world's earliest recorded writings. In the fifth chapter of Genesis we read: "And Adam begat Seth, and Seth begat Enos, and Enos begat Cainan, and Cainan begat Mahalaleel, and Mahalaleel begat Jared," etc. Sir Flinders Petrie comments on the records of descent which are graven on the monuments of ancient Egypt, and Breasted, when he uncovered Ur of the Chaldees, found a record of the genealogy of those ancient kings. Ancestor worship extends back beyond the dawn of history, so that it is not surprising that when the antiquarian uncovers old hieroglyphics or finds bricks with cuneiform or punctiform writing upon them he is very likely on deciphering them to find only the record that x was the son of y and that in turn x begat z.

But genealogies change their fashion with the passing of ages. Today it is the fashion to trace descent largely through the paternal line; long ago the maternal line was regarded as all important, probably because of a greater certainty of the records.

Unquestionably in the early civilizations there was a belief that physical prowess, or leadership, or beauty, or wisdom, or power for evil were specific attributes which descended from father to son or from mother to daughter as the case might be. The right of a prince to reign in his father's stead, or the inability of a serf to rise above serfdom because his father had been a serf, both unquestionably had their origin in this belief of the mechanism of inheritance.

The science of genetics is one of the youngest of our sciences. Less than a century ago Gregor Mendel made his remarkable experiments, but, like Willard Gibbs, or Thomas Graham, he thought and wrote far in advance of his time, so that it was only within the lifetime of many of us that Correns, Bateson, Tschermak, and DeVries, essentially independently, rediscovered "Mendel's Law" which lies at the foundation of our modern concepts of inheritance.

And what has Mendel's Law told us about our recorded genealogies? As usual the law is relatively simple, but its results are extremely complex. The law tells us that the characters of an individual are due to a combination of the characters received from each parent, and that in the second generation there is again a segregation of characters, the segregation taking place more or less at random in accordance with the laws of probability. Within a completely inbred line it is theoretically possible for the geneticist to eventually develop progeny which are extremely uniform, the so-called "pure lines," but insofar as man is concerned, we are hopelessly heterozygous, to use the geneticists' term, and the chances of a particular trait being descended invariably from father to son, or from mother to daughter, are in general rather remote unless the trait should fall in those relatively rare classes of "simple dominants," or sex-linked inheritance.

If then *all* of our ancestors have contributed to the heterozygous individual, ourself, what sort of a mixture do we each represent? We each had two parents, four grandparents, eight great grandparents, and when we go back for ten generations we find that there are one thousand and twenty-four individuals, and in fifteen generations thirty-two thousand seven hundred and sixty-eight individuals in our *direct* line of descent.

These fifteen generations would cover a span of years only back to the landing of the Pilgrims on Plymouth Rock, and each descendant of that group, if he had his direct genealogy complete would have over thirty thousand names upon the record. He who proudly claims to trace his ancestry back to the battle of Hastings would need entries of more than thirty-five billion names to record those in his *direct* line of descent. Note that this is only the direct line, and does not include the probably more than one hundred billion additional persons in the brothers and the sisters and the cousins and the uncles and the aunts. Since the present population of the entire world is only approximately two billion, and since a hundred years ago it was less than one billion, and probably at the time of the battle of Hastings did not exceed five-hundred million, it is obvious that within any given race there has been, perhaps unwittingly, but of necessity, an enormous amount of inbreeding. Within a given race of people living in a localized geographical environment over a period of several centuries the probability of mating with some one who is not a distant blood relative is extremely remote. The same blood lines have separated only to come together again in a later generation. This has tended to perpetuate and accentuate hereditary characteristics and at the same time tended to make the human race hopelessly heterozygous.

But to return to the more than one thousand direct-line ancestors that each one of us has had in the last ten generations: Any one of these more than one thousand persons may have supplied those particular genes which are responsible for some particular characteristic or trait that we as individuals exhibit. How differently such a genealogy would read from the ten names in a record that, "Adam begat Seth, who begat Enos, etc."

Is it any wonder, therefore, that each one of us is an individual different from all others in our personal characteristics? Even if germinal constitution alone were accountable for behavior, we would anticipate that mankind would exhibit an almost infinite variety of physical and mental types.

But I did not intend to digress so far into the realm which my friends, the geneticists, have preempted. They, perhaps, will take me to task for suggesting that in educated man at least there is another factor equally important with inheritance and that that factor is *environment*. Well do I realize that environment has been largely ruled out as a factor in inheritance. In spite of the fact that Darwin

based his thesis of evolution on the survival of the fittest and the adaptation of a species to environment, geneticists in general seem to feel that environment does not modify the germ plasm. Perhaps they are right. You will note that I say "perhaps," I still have mental reservations, and I rejoice that Henry Fairfield Osborn has recently raised some momentous questions in regard to the processes of evolution in the Titanotheres, questions which apparently involve "use and disuse" and a changing environment through long geologic ages. If I read Osborn correctly, he regards environment as a major factor in directing evolutionary changes. The shovel-tuskers developed shovels instead of tusks because more and more they came to depend upon aquatic plants growing in shallow estuaries for their food supply. Osborn's thesis differs in detail from that of Weismann, but it seems to me, a layman in this field, that there is much in common in the two viewpoints.

But here again we are wandering afield. I do not intend to discuss environment as an evolutionary factor nor as a factor which may modify the germ plasm. What I do wish to insist upon is that both heredity *and* environment are equally essential to the full development of the potentialities of an individual organism. With favorable heredity in a favorable environment we have maximum development, with either favorable heredity in an unfavorable environment or with unfavorable heredity in a favorable environment the full development of the organism is thwarted.

Some of you are perhaps familiar with the scrubby mesquite which grows in the deserts of our Southwest. In Arizona, its native home, it is a thorny desert shrub, or at the best, a low stunted tree. In Hawaii, on the other hand, where it was introduced a number of years ago, it grows into a stately tree comparable to many of our elms in this region. The mesquite had the same hereditary potentialities in each location, but in Hawaii the environment permitted it to realize its hereditary possibilities. Many similar examples could be cited from both plants and animals in which environmental influences outweigh and in many instances apparently nullify the latent potentialities of the germ plasm, and man is no exception to this environmental influence.

At the 1932 meeting of the American Society of Naturalists, Alexander Weinstein of Johns Hopkins University, read a paper entitled, "Palamedes." This address was later published in Volume 67 of the American Naturalist for 1933. I recommend its reading to you.

It was a brilliant, somewhat satirical address, the theme of which was that genius has but little chance if the individual is born in the lower economic classes, not because the hereditary potentialities for genius are not present but because the environment does not permit of their expression.

Thus Weinstein says, "It is of course true that the richer classes have contributed proportionally a greater number of eminent men than have the poor. But this is due in part at least to their better opportunities and there is no evidence that it is due to anything else." (p. 234)

He continues, "There is a wide-spread notion that great ideas come as inspirations and that their coming can not be controlled or even predicted. It is true that great ideas often come at unexpected moments; but I doubt whether they ever come without preparation. They are the result of long intensive work, of complete absorption in a subject. Often the idea does not come until after the worker has rested from his labor; and this inactive period is probably the source of the opinion that labor is unnecessary. Now, obviously, complete absorption in a subject is possible only where there is leisure; and this means that it is all but impossible for a really poor man who has no leisure, to do great creative work."

Weinstein then quotes Sir Arthur Quiller-Couch, Professor of English Literature at Cambridge University, in support of this thesis. Sir Arthur writes, "What are the great poetical names of the last hundred years or so? Coleridge, Wordsworth, Byron, Landor, Shelley, Keats, Tennyson, Browning, Arnold, Morris, Rossetti, Swinburne—we may stop there. Of these, all but Keats, Browning, Rossetti were university men; and of these three Keats, who died young, cut off in his prime, was the only one not fairly well-to-do. It may seem a brutal thing to say, and it is a sad thing to say; but as a matter of hard fact, the theory that poetical genius bloweth where it listeth, and equally in poor and rich, holds little truth. As a matter of hard fact nine out of those twelve were university men; which means that somehow or other they procured the means to get the best education that England can give. As a matter of hard fact, of the remaining three you know that Browning was well-to-do, ... Rossetti had a small private income. . . . . There remains but Keats, ... These are dreadful facts, but let us face them. It is—however dishonoring to us as a nation—certain that, by some fault of our commonwealth, the poor poet has not in these days, nor has had for

two hundred years, a dog's chance. Believe me . . . . . we may prate of democracy, but actually a poor child in England has little more hope than had the son of an Athenian slave to be emancipated into that intellectual freedom of which great writings are born."

Writing great poetry or painting beautiful pictures is not the usual outlet for a man's energies after a weary day of toil. According to the laws of probability, insofar as the heredity factors of the germ plasm are concerned, there must have been many great poets, painters, and scientists born during the last two hundred years in England's and in our own lower economic classes. *They never had a chance! Their environment annulled their hereditary potentialities.*

One of my friends is one of the world's great scientists. His father was illiterate, denied schooling as one of an oppressed minority in one of the provinces under the Russian czars. He came to America when the boy was only a few years old, and here, in the new world, the boy had a chance to secure an education. He led his classes throughout grade and high school and secured a scholarship which enabled him to attend one of our great universities. Here, for the first time in the history of that institution, he captured every prize for scholarship for which he was eligible in every year. He was graduated with the highest honors and today is one of a chosen few leaders in his field of science.

What chance would that boy have had in the environment in which his father was reared? He would now be an illiterate Russian peasant. What chance would he have had if the American system of education had not afforded him the opportunities which he received? The germ plasm which he received from his ancestors possessed the hereditary possibilities for scientific genius—perhaps his father had them—who knows? In one case the *environment* permitted the *development* of the *potentialities*, in the other case the environment *utterly inhibited* any possiblity of development.

Here in America, as elsewhere in civilized nations, we have schools, colleges, and universities. But of what are these schools, colleges, and universities constituted? Not of buildings, not of landscaped grounds, not of endowments, not of material things, no—not even of most of the students, but rather of *teachers*; these are the essential nucleus of any center of learning. And unfortunately not *every* teacher—only a chosen few. Those who contribute *more* than just guiding a daily task! Those who lead a student into a *new world!* Who challenge the imagination, those who sometimes seem to demand almost the im-

possible but who show the student the path whereby the heights may be attained.

Possibly my thinking along these lines was inspired by a conversation which I had some years ago with Guy Stanton Ford, Dean of the Graduate School, at the University of Minnesota. It just happened that the morning paper had carried notices of the death of the chief physicist of a great industrial organization and of the death of Professor Bumstead, of the Department of Physics, of Yale University, who had died while en route to New Haven from the winter meetings of the American Association for the Advancement of Science. Our conversation drifted to these two losses to American science, and I shall never forget Dean Ford's comment. He said, "Tomorrow there will be another chief physicist of that great industrial corporation, but there will never be another Professor Bumstead at Yale!" And then he added, "However much we may ponder as to the future, those of us who spend our lives in the universities can be sure of *one* kind of immortality—the immortality of the teacher who lives on in his students and in his students' students in succeeding generations of scholars." And that statement started me on the "hobby" which I mentioned.

Now if you will pardon me, I would like to become personal. I have said that not all teachers, but only a chosen few, make a deep impression upon the developing student. As my thoughts have reverted to my own grade and high school teachers, I find that I remember only two names. Odd as it may seem the one is that of my first grade teacher, the other that of my teacher in the seventh grade, Miss Jones and Mr. Bovee. Why I remember Miss Jones I do not know, but I can visualize her today almost as well as I could more than forty years ago. I have a feeling that she was a great teacher; she certainly knew how to make children love her. Mr. Bovee was a junior at the University of Nebraska, teaching for a year to secure funds so he could return and complete his college course. He was majoring in biology at Nebraska, and I have often wondered whether the major interest of that particular teacher has in any way influenced me in being attracted to the biological aspects of chemical science. I do know that one of my classmates in the seventh grade later became a student at the University of Nebraska and made a brilliant record under Professor Barbour in the field of paleontology Somehow or other I feel that I owe much to Miss Jones and Mr. Bovee.

When I come to my college teachers—only three names stand out in my mind. Two of these I revere for both *personality* and *subject matter*, one for *personality* alone. This latter man was a poor teacher, he had an inadequate grasp of his subject matter, he allowed the classes to loaf along at their own pace, and we took advantage of that trait, but from the *human* standpoint he was one of the grandest men I have ever known. We boys all went to him with our problems, and his office door and the door of his home were always open to "his boys." From him I learned how to live in a man-made world, how to deal kindly with my fellow men, how to submerge self in order to accomplish the common good. He was our father confessor, and in spite of the fact that I still feel the lack of his classroom knowledge, nevertheless, I would not exchange his philosophy of life for all the science technics that he might have taught me.

The college where I matriculated was a small denominational school, with less than two hundred students of collegiate grade. When I matriculated I had no idea of what constituted a college education. My mother was merely following the death-bed wish of my father who, as a missionary dying in Africa, had whispered, "If possible give the boys an education." That whisper was, I believe, a major factor in my environment. Without it I might well today be a farmer on the old homestead in Nebraska.

In this small college, on registration, the entire faculty of perhaps fifteen persons were seated around a table in the library. Each student contacted each faculty member and secured permission to register for specific courses. I glanced over the catalogue and saw "Chemistry." The title meant little to me, so I approached the chemistry instructor and asked, "What is chemistry like?" Never shall I forget Dr. Alway's reply, the first words he ever spoke to me, "Young man, if you stick at it long enough, some day you'll be able to do something that nobody ever did before!" Before I knew his name and before he knew mine, the gauntlet had been thrown down. The challenge of research was thrown at me before I knew what research was! The wings of imagination were loosened! And then and there I resolved to accept the challenge, if the power lay within me! I knew there was one subject for which I wished to register.

And here may I pay humble tribute to a great teacher of chemistry fresh from the inspiration of Victor Meyer and Ludwig Gattermann at Heidelberg. In a little denominational college, with utterly inadequate facilities, with tinners' blow torches for blast burners and alcohol

lamps for other experiments, he kept research ever before his students. He always set the goal at the utmost limit of possible attainment, and from the small enrollment in that little college, in the few years that he taught there, inspired by his leadership and enthusiasm for research, came a number of men who hold responsible positions in the field of chemistry today. I said earlier that material facilities do not constitute a university, that universities are made of teachers! Professor Alway was one of the chosen few. I know that he was a major factor in the environment of the little group of students that worked with him and that almost worshipped him! I am confident that they were as much the product of their environment as of their heredity.

Again in my post-graduate work I came in contact with many teachers, but relatively few of these stand out in my memories today. In the office of one of these, late one night, the conversation turned to the great developments in the history of chemistry. He remarked how it was that synthetic organic chemistry had been the dominating field of the science from 1828 to approximately 1900, how then physical chemistry seemed to be sweeping to the front, and he ventured the prediction that the application of organic chemistry and physical chemistry technics to the problems of life processes would be one of the outstanding opportunities for the young chemists of the rising generation. Just how much that evening's talk influenced me I do not know, but I do know that I distinctly remember the details of that conversation, whereas the details of many others in that same room have faded from my memory.

So much then for my immediate teachers, but who were their teachers, what teachers were major factors in their environment? In other words, I know who my scientific fathers were, who were my scientific grandfathers and great-grandfathers, and so on back as far as chemical history can be traced? It is an interesting hobby to attempt to trace ones scientific genealogy. Fortunately biographers have made it easy to trace certain lines of descent. In other instances gaps have been found which I have not been able to bridge, so that my own scientific genealogy is still far from complete. In my leisure hours in future years I may be able to fill in some of the gaps, but I have had great satisfaction in finding among the names of my scientific ancestors such great teachers as

Charles F. Chandler
Heinrich Rose
Edward C. Franklin
Friedrich August Kekulé
Johannes Wislicenus
Justus von Liebig

Ludwig Gattermann	Robert Bunsen
Wilhelm Ostwald	Joseph Louis Gay-Lussac
Victor Meyer	Friedrich Wöhler
August Wilhelm von Hofmann	Jöns Jacob Berzelius
Adolph von Baeyer	Claude Louis Berthollet

and among biologists,

Karl Pearson	Alexander von Humbolt
Francis Galton	Georges Cuvier

Surely a group of scientific ancestors of which one can be pardonably proud! Incidentally pursuing a hobby of this sort is an excellent means of developing an historical view of one's field of science.

These men unquestionably were likewise in a large measure the products of their environments, in all probability inspired by some great teacher, although that teacher's name may be unknown to us today. It is of interest to note that Mitscherlich went to the University of Heidelberg in 1811 not to study chemistry but rather to study oriental languages, expecting to conduct research in the antiquities of Persia. From 1811 to 1817 he studied oriental languages and had essentially completed his thesis, a study in old Persian philology. Then, in order to prepare himself for research in the orient, he attended certain classes in medicine at Göttingen. Here he took a course in chemistry which was taught by Friedrich Stromeyer, the discoverer of cadmium. The young orientalist promptly abandoned his first love and threw his whole interest into chemistry. His name now stands high among the great chemists of the world—again the environment prevailed.

And so I might continue with illustration after illustration, in each of which we would find the influence of some great teacher who had made possible the full development of the latent possibilities of some one of his students.

I have little patience with the educator who believes that every freshman who enters our colleges or universities should at the time of his initial registration know exactly what he intends to be and exactly what course of study he will follow. In most cases the young students are entering an entirely new environment, and ample latitude should be permitted so that the new environment may supplement rather than repress their hereditary potentialities.

Likewise I have little patience with the idea that each student should be moulded around a fixed curriculum. We are all individuals,

no two of us think or react exactly alike, and in assisting one of these individuals to prepare himself for the future, we should take care to preserve his individuality and to supplement in every way all of his latent possibilities.

Some years ago a young man came to the University of Minnesota as a freshman. He had no idea as to what career he wished to follow. His father had simply sent him to the university. It was the thing to do! In the line before the registrar's window he fell into conversation with the boy ahead of him, discovered that he too was entering the University for the first time and that he planned on registering in the Engineering College. So our young man decided he too would register there. He failed nearly every course in the first quarter. As a special dispensation he was permitted to again register to repeat those courses in the winter quarter, and again he made a hopeless record. He returned for the spring quarter work only to find that he had been barred by the students' work committee from registering in Engineering. He appealed the decision to one of our adminstrative officials, explaining that he could not go home with a record of failure; by some means he must be permitted to register in the University.

It happened that it was about closing time, so that particular administrative official invited the boy to take a walk with him while they talked over his problem. The walk led along the riverbank, and the faculty member noticed that the boy often stopped to examine some particular tree, that he commented on the symmetrical shape of an unusually perfect tree, so the University official suggested that since the boy was barred from registering in Engineering, he transfer for at least a quarter to Forestry. The boy protested strenuously, but since that seemed the only solution of his problem, he finally acquiesced. Once in Forestry he proved to be a brilliant student. He swept everything before him, and upon graduation was selected as Forest Manager for a great tropical forest owned by one of America's great industrial organizations. One cannot always be sure that a student does not possess the aptitude for scholarship. It may be that he is groping in a, to him, uninspiring environment.

And sometimes chance seems to decide a career. Francis Galton started out to be a pharmacist. Since he had inherited scientific potentialities he conceived the idea that in order to be a good pharmacist he should be personally acquainted with the physiological effects of the drugs which he would dispense. So he began testing their efficacy upon himself and being a methodical individual, he

began with the beginning of the alphabet. All went well until he reached "cr." The next drug was croton oil, and ill advisedly, but perhaps fortunately for posterity, he took a full drop. His interest in personal experimental medicine stopped then and there, and his interest in pharmacy disappeared, instead he turned to biological phenomena and became one of the outstanding biologists of all time.

Again may I become personal—All through my college and post-graduate years I prepared myself to teach organic chemistry. I never had a lecture from an instructor in biological chemistry in my life, and when about to receive the doctorate, I began to look around for a position. I was offered the opportunity to teach organic chemistry in a college close to a great industrial center. At almost the same time I received the offer to do full-time research in a biological research institution. I knew practically nothing of biology, so I deliberately accepted the research position, partly because it involved full-time research, but largely because it would force me to learn something of a science of which I was almost wholly ignorant. I fully expected to stay in such work for only a short time and then return to the field for which I had trained myself. Here, in a new and strange environment, I came in contact with some outstanding biologists, especially the late J. Arthur Harris. He probably has influenced my career as much as any one. I had been in this new field for only a short time when I realized that I was in it for the rest of my life.

But what about the man who took the organic chemistry post that I might have taken? There, close to an industrial center, before chemical engineering had fully developed, he made many industrial contacts as a consultant. Today he heads the Department of Chemical Engineering in one of our great universities. Had he been located in the environment of the biological research institution or had I gone into the environment which he entered, the present interests of either one of us might well have been reversed.

And now in closing—Why do teachers teach? I fondly believe that in many instances the same motives move the teacher that move the missionary. As a rule the teacher does not teach for material compensation but rather for that spiritual satisfaction which comes from watching the latent possibilities of his students develop, and now and then finding that a rose is blooming on what appeared to be a weed in the corner of his garden.

The highest, the ultimate reward of the true teacher is not reached when all of the students can write perfect examination papers, when

they can all recite parrot-fashion all of the facts and theories enumerated in the textbook. The real joy comes when one, or two, or perhaps half a dozen students who have come under his guidance catch the fire of inspiration, become impatient with the meager fragments of knowledge which are handed out in the classroom, and start out for themselves to push back the barriers of the unknown. Then the teacher can pass on to younger hands the torch which he has been carrying and can feel assured that his life and his teaching have not been in vain, that some one more gifted than he will carry on into succeeding generations.

I do not know the human genealogy of Faraday, I care not to know it, but I do know that Sir Humphry Davy once said that his greatest discovery was Michael Faraday, and if we were to trace the scientific genealogy of our scientists, we would find *that* much more important than their blood genealogy. The teacher, the researcher, lives in his students and in their students in succeeding generations. That is the teacher's reward. That is true immortality.

Chapter I

THE BEGINNINGS OF THE SCIENCE†

Although alchemy is centuries old, the science of chemistry as we know it today is of relatively recent origin. It was only a little over a hundred years ago (1828) that Wöhler synthesized urea from ammonium cyanate and demonstrated for the first time that a vital force was not necessary for the formation of "organic" compounds.

Organic chemistry had its beginnings in biochemistry, and the early organic chemists were intensely interested in the chemistry of the fats, carbohydrates, pigments, proteins, etc. In the majority of cases, however, the naturally-occurring organic compounds proved to be too complex for the earlier organic technics, so that "pure" organic chemistry turned aside to study synthetic reactions and group behavior. Only recently have the leaders in organic chemistry returned to the original plan, and the acclaim which greets such names as Willstätter, Hans Fischer, Windaus, and Karrer attests the success which is being made in the structural chemistry of compounds formed by living organisms.

It is much less than a century ago that the great triumvirate of Van't Hoff (1852-1911), Wilhelm Ostwald (1853-1931), and Walther Nernst (1864-) is credited with laying the foundations of modern physical chemistry. What a structure has been erected thereon! The progress of our present materialistic civilization is in no small measure due to the developments in physical chemistry and its ally—applied physics. On every hand we meet the comforts, conveniences and, we now believe, necessities that have grown from the applications of the knowledge gained in research in physical chemistry.

Coincident with the fathers of organic chemistry (Wöhler 1800-1882; Liebig 1803-1872) another chemist was exploring a new field

† This chapter, essentially as printed here, was originally prepared as a part of a symposium on colloid chemistry and delivered at a meeting of the Division of Colloid Chemistry of the American Chemical Society, Chicago, September 12, 1933. It was later published in the *Journal of Chemical Education*, Vol. 11, No. 5, pp. 279–283, May, 1934, and with slight modifications is reproduced here by permission of the editors of that journal.

of the science. Thomas Graham was born December 21, 1805 and died September 16, 1869. His researches lay almost exclusively in the field of physical chemistry, although he had died before any of the physical chemistry triumvirate, Nernst, Van't Hoff, Ostwald, had begun to publish. If anyone can be truly said to be the father of physical chemistry, that credit should go to Thomas Graham. A perusal of his collected papers[1], comprising 46 titles in all, reveals a continuity in objectives, a persistence in following up details, a scientific imagination far in advance of his time, an ability in apparatus technic, and an uncanny clearness of interpretation of experimental data, all of which leaves the reader more and more amazed at the things which he accomplished and the views which he expressed. It is no wonder that the name and works of Thomas Graham have not received the credit which they deserve. Like Willard Gibbs he lived and thought so far in advance of his time that full appreciation of his viewpoint and accomplishments could only come after the passage of many years and when his readers were sufficiently grounded in the fundamentals to understand the meaning of his words. Even today this remarkable man does not receive the credit which I believe is his due, probably because relatively few persons have actually read all of his original papers.

Graham has been rightly called the Father of Colloid Chemistry. His Bakerian lecture, "On the Diffusion of Liquids"[2], delivered December 20, 1849, and which required 78 printed pages (a lecture which would probably be unduly long for our modern audiences) may be regarded as the beginning paper in colloid chemistry. However, it is only the summary, and the philosophical discussion, of his researches on diffusion which began with his first paper at the age of 21, entitled "On the Absorption of Gases by Liquids"[3], which paper led rapidly into his studies of diffusion, first with gases in 1829[4] and later with liquids and finally culminated with his paper[5] in 1861 entitled "Liquid Diffusion Applied to Analysis," which is essentially modern in its colloid terminology and colloid-chemical viewpoint. In

[1] Thomas Graham. Chemical and Physical Researches. Collected by R. Angus Smith and printed for presentation only by Edinburgh University Press. 1876. lvi+660 pp. 1 portrait.

[2] Graham, Thomas. *Phil. Trans.* (1850) 1–46, 805–836, (1851) 483–494. [*Analen* 77: 56–89, 129–160 (1851); *Phil. Mag.* 37: 181–198, 254–281, 341–349 (1850).]

[3] Graham, Thomas. *Annals of Philosophy*, 12, 69–74 (1826).

[4] Graham, Thomas. *Quart. J. Sci.*, 2; 74–83 (1829).

[5] Graham, Thomas. *Phil. Trans.* 1861, pp. 183–224.

almost every paper of Graham's we find that he is investigating the phenomena of diffusion, and in following whole heartedly this one objective in all of its ramifications he uncovered fact after fact so that when the proper time came his conception of the colloidal state of matter rested upon a firm experimental foundation.

This series of lectures is on the general topic of "Colloids in Biochemistry." I believe the purpose of this initial lecture will be best served by calling attention to some remarkable comments which Graham made regarding colloid chemistry in general and especially the rôle of colloids in living processes.

In 1830, when Graham was only 25 years old, he published a paper in volume one of the Quarterly Journal of Science[6], entitled "The Effects of Animal Charcoal on Solutions." This paper is an outstanding contribution in the field of adsorption. He notes that bone black had already been used to decolorize syrups in the process of sugar manufacture, but that hitherto the only study which had been made of bone black was to remove colored matters from solution. He therefore studied the action of animal charcoal, de-ashed by boiling with dilute hydrochloric acid until silica only remained in the ash, and followed by complete washing to the absence of acid reaction, on a number of solutions, mostly of the inorganic type. In this paper he shows that such charcoal removes the blue color from ammoniacal copper solutions and that the copper could not be removed from the charcoal by subsequent elutriation with strong ammonia. He points out that silver is adsorbed from a solution of silver nitrate and that crystals of metallic silver appeared on the surface of the charcoal; that iodine was removed from a solution of iodine and potassium iodide and that the carbon could be dried at a relatively high temperature without iodine vapors appearing. If, however, the iodine-charcoal mixture were heated strongly in a closed flask the iodine could be resublimed, but later on cooling, the charcoal readsorbed the iodine vapors. He found that the charcoal adsorbed chlorine from a solution of the gas in water but that on the surface of the charcoal the chlorine was converted into hydrochloric acid. However, perhaps the most striking comment in this entire paper is his vision in that early period of chemical science, as to the effect of adsorption on procedures in analytical chemistry. He states:

> The same property is possessed by other solid bodies, in a state of minute division, as when newly precipitated, although not in so great a degree. And, in analytic researches, its interference must be guarded

[6] Graham, Thomas. *Quart. J. Sci.*, 1: 120–125 (1830).

against, as it may contribute, in some cases, to increase the weight of precipitates.

Certainly no modern text in the field of analytical chemistry can state the facts more succinctly than this, and in many texts describing analytical procedure the possibility of adsorption is still wholly ignored.

Graham's 1861 paper[5] shows that he had a clear-cut appreciation of the differences which exist between the colloidal and the crystalloidal states of matter, and interwoven with his comments on his experimental data we find him philosophizing on the probable effects of such phenomena in living processes. Thus he states:

> I may be allowed to advert again to the radical distinction assumed in this paper to exist between colloids and crystalloids in their intimate molecular constitution. Every physical and chemical property is characteristically modified in each class. They appear like different worlds of matter, and give occasion to a corresponding division of chemical science. The distinction between these kinds of matter is that subsisting between the material of a mineral and the material of an organized mass . . . The phenomena of the solution of a salt or crystalloid probably all appear in the solution of a colloid, but greatly reduced in degree. The process becomes slow; time, indeed, appearing essential to all colloidal changes . . . . . It may be questioned whether a colloid, when tasted, ever reaches the sentient extremities of the nerves of the palate, as the latter are probably protected by a colloidal membrane, impermeable to soluble substances of the same physical constitution.

and in commenting on the fact that the colloids appear to possess high molecular weights:

> The inquiry suggests itself whether the colloid molecule may not be constituted by the grouping together of a number of smaller crystalloid molecules, and whether the bases of colloidality may not really be this composite character of the molecule.

In these quotations we see his primary interest in the phenomena of diffusion and his keenness in suggesting what appears to be certainly the reason that colloid sols and gels are essentially tasteless. The fact that strychnine and quinine adsorbed on hydrous aluminum silicates are tasteless is a relatively recent[7] (1913) observation. These quotations have been almost universally interpreted to mean that Graham believed that there was a sharp discontinuity between colloids and crystalloids—that these represented different *kinds* of

[7] Lloyd, J. U. "Concerning the Alkaloidal Reagent Hydrous Aluminum Silicate." Circular. Cleveland, Ohio. October 1, 1913.

matter. A careful perusal of his writings, however, shows that such was *not* Graham's view. He recognized that one and the same substance may, under different sets of conditions, be either colloidal or crystalloidal. Thus he says in 1861:

> A departure from its normal condition appears to be presented by a colloid holding so high a place in its class as albumen. In the so-called blood-crystals of Funke, a soft and gelatinous albuminoid body is seen to assume a crystalline contour. Can any facts more strikingly illustrate the maxim that in nature there are no abrupt transitions, and that distinctions of class are never absolute?

As early as 1854 Graham[8] observed that animal membranes, gelatin, etc., remove water from strong alcohol and concentrate the alcohol. He states:

> There can be no doubt, therefore, that gelatin *per se* separates water from alcohol.

His interest in diffusion phenomena then comes to the fore, and he points out that by exposing dilute alcohol in a bladder to the atmosphere causes the water to pass through the membrane and evaporate from the surface of the bladder, its place being constantly supplied with fresh water from the dilute alcohol within, thus concentrating the alcohol within the bladder. He explains this as being due to the fact that water will wet the animal membrane, whereas alcohol is much less efficient in wetting this substance.

This interest in diffusion and the application of diffusion phenomena to physiological processes runs through many of his papers. Thus in 1850 he[2] says:

> Chloride of sodium appears 20 times more diffusible than albumin. . . . The experiment appears to promise a delicate method of proximate analysis peculiarly adapted for animal fluids. The value of this low diffusibility in retaining the serous fluids within the blood-vessels at once suggests itself.

In the same paper he suggests that diffusion may well be a factor in plant nutrition.

> The mode in which the soil of the earth is moistened by rain is peculiarly favourable to separations by diffusion. The soluble salts of the soil may be supposed to be carried down together, to a certain depth, by the first portion of rain which falls, while they find afterwards an atmosphere of nearly pure water, in the moisture which falls last and occupies the

[8] Graham, Thomas. *Brit. Assoc. Rept.* Part 2, p. 69 (1854).

surface stratum of the soil. Diffusion of the salts upwards into this water, with its separations and decompositions, must necessarily ensue. The salts of potash and ammonia, which are most required for vegetation, possess the highest diffusibility, and will rise first. The preeminent diffusibility of the alkaline hydrates may also be called into action in the soil by hydrate of lime, particularly as quicklime is applied for a top-dressing to grass lands.

And in 1861 in speaking of the coefficient of diffusion he states:

It is easy to see that such a constant must enter into all the chronic phenomena of physiology, and that it holds a place in vital science not unlike the time of the falling of heavy bodies in the physics of gravitation.

Graham's studies in diffusion led to his speculating on the nature of osmosis and the mechanism whereby water moves across an osmotic membrane. Several years ago when I wrote a text on biochemistry I thought I was formulating a more or less novel illustration of the mechanism of this water flow by suggesting hydration and dehydration of the opposite sides of the membrane with a consequent unbalanced hydration equilibrium within the membrane[9]. However, Graham in 1861 had stated the hypothesis even more clearly than I. He says:

It now appears to me that the water movement in osmose is an affair of hydration and dehydration in the substance of the membrane or other colloid septum, and that the diffusion of the saline solution placed within the osmometer has little or nothing to do with the osmotic result, otherwise than as it affects the state of hydration of the septum. . . . The degree of hydration of any gelatinous body is much affected by the liquid medium in which it is placed. . . . Hence the equilibrium of hydration is different on the two sides of the membrane of an osmometer. The outer surface of the membrane being in contact with pure water tends to hydrate itself in a higher degree than the inner surface does, the latter surface being supposed to be in contact with a saline solution. When the full hydration of the outer surface extends through the thickness of the membrane and reaches the inner surface, it there receives a check. The degree of hydration is lowered, and water must be given up by the inner layer of the membrane, and it forms the osmose. . . . . The inner surface of the membrane of the osmometer contracts by contact with the saline solution, while the outer surface dilates by contact with pure water. Far from promoting the separation of water, the diffusion of salt through the substance of the membrane appears to impede osmose, by equalizing the condition as to saline matter of the membrane through its whole thickness.

[9] Gortner, R. A. Outlines of Biochemistry. Wiley and Sons, New York. 1929. cf. p. 246.

And in the same paper:

The substances fibrin, albumen, and animal membrane swell greatly when immersed in water containing minute proportions of acid or of alkali, as is well known. On the other hand, when the proportion of acid or alkali is carried beyond a point peculiar to each substance, contraction of the colloid takes place. Such colloids as have been named acquire the power of combining with an increased proportion of water, and of forming superior gelatinous hydrates, in consequence of contact with dilute acid and alkaline reagents. . . . When so hydrated and dilated, the colloids present an extreme osmotic sensibility. Used as septa, they appear to assume or resign their water of gelatination under influences apparently the most feeble. It is not attempted to explain this varying hydration of colloids with the osmotic effects thence arising. Such phenomena belong to colloidal chemistry, where the prevailing changes in composition appear to be of the kind vaguely described as catalytic. To the future investigation of catalytic affinity, therefore, must we look for the further elucidation of osmose.

Graham, of course, knew nothing of the Donnan equilibrium. Thermodynamics was far in the future, nevertheless from his studies of diffusion and colloid behavior he predicted fairly exactly the mechanism for the secretion of hydrochloric acid by the gastric mucosa. In his 1861 paper he wrote:

The secretion of free hydrochloric acid during digestion—at times most abundant—appears to depend upon processes of which no distinct conception has been formed. But certain colloidal decompositions are equally inexplicable upon ordinary chemical views. To facilitate the separation of hydrochloric acid from the perchloride of iron, for instance, that salt is first rendered basic by the addition of peroxide of iron. The comparatively stable perchloride of iron is transformed, by such treatment, into a feebly-constituted colloidal hydrochlorate. The latter compound breaks up under the purely physical agency of diffusion, and divides on the dialyser into colloidal peroxide of iron and free hydrochloric acid. The super-induction of the colloidal condition may possibly form a stage in many analogous organic decompositions.

In 1864 Graham introduced the now universally used terms of "sol," "gel," and "peptization," and in each instance commented on analogies to physiological processes. Thus, in a paper dealing with the properties of silicic acid he says[10]:

If I may be allowed to distinguish the liquid and gelatinous hydrates of silicic acid by the irregularly formed terms of *hydrosol* and *hydrogel* of silicic acid, the two corresponding alcoholic bodies now introduced may be named the alcosol and alcogel of silicic acid.

[10] Graham, Thomas. *J. Chem. Soc.*, 17: 318 (1864).

Graham then continues to investigate the behavior of silica gel in replacing the liquid with other liquids such as alcohol, ether, benzene, carbon bisulfide, glycerol, concentrated sulfuric acid (in which case the gel is successively placed in stronger and stronger acid until concentrated acid is reached), and he points out that in each case no permanent compound is formed but that we are still dealing with jellies of silicic acid. He further points out that sulfuric acid can be exchanged for nitric, acetic, formic acids, etc. He then makes the following general comment:

> The production of the compounds of silicic acid now described indicates the possession of a wider range of affinity by a colloid than could well be anticipated. The organic colloids are no doubt invested with similar wide powers of combination, which may become of interest to the physiologist. The capacity of a mass of gelatinous silicic acid to assume alcohol, or even olein, in the place of water of combination, without disintegration or alteration of form, may perhaps afford a clew to the penetration of the albuminous matter of membrane by fatty and other insoluble bodies, which seems to occur in the digestion of food. Still more remarkable and suggestive are the *fluid* compounds of silicic acid. The fluid alcohol-compound favours the possibility of the existence of a compound of the colloid albumin with olein, soluble also and capable of circulating with the blood. The feebleness of the force which holds together two substances belonging to different physical classes, one being a colloid and the other a crystalloid, is a subject deserving notice. When such a compound is placed in a fluid, the superior diffusive energy of the crystalloid may cause its separation from the colloid. Thus, of hydrated silicic acid, the combined water (a crystalloid) leaves the acid (a colloid) to diffuse into alcohol; and if the alcohol be repeatedly changed, the entire water is thus removed, alcohol (another crystalloid) at the same time taking the place of water in combination with the silicic acid. . . . The process is reversed if an alcogel be placed in a considerable volume of water.

And in the same paper in speaking of the formation of sols of silicic acid Graham states:

> The solution of these colloids, in such circumstances, may be looked upon as analogous to the solution of insoluble organic colloids witnessed in animal digestion. . . . Liquid silicic acid may be represented as the "peptone" of gelatinous silicic acid; and the liquefaction of the latter by a trace of alkali, may be spoken of as the peptization of the jelly. The pure jellies of alumina, peroxide of iron, and titanic acid, prepared by dialysis, are assimilated more closely to albumin, being peptized by minute quantities of hydrochloric acid.

As I have already indicated, Graham thought and wrote far in

advance of the chemical thought of his time. For fifty years after Graham's death the phenomena of colloid chemistry were largely neglected as a separate field of study. In 1893, only two papers dealing with the properties of matter in the colloidal state appeared in all the world's literature. One of these[11] dealt with colloidal gels of lead tartarate, barium sulfate, and lead sulfate, the other[12] with the behavior of colloidal sols at the critical temperature of the dispersions medium.

Even ten years later in 1903 only 23 papers or patents dealing with colloids appear in the entire world chemical literature. Of these 23 titles, eight are either directly or indirectly associated with biochemical study. The names of W. B. Hardy, Jean Perrin, Wo. Pauli, G. Bredig, and Herbert Freundlich which appear this year bespeak the advent of a new era in the study of colloids.

Pauli[13] was initiating his studies of the interaction of proteins and electrolytes. Hardy[14] showed that the electric charge on blood globulin was reversed in passing from an acid to an alkaline solution and that "radium rays" [α particles (?)] caused a coagulation of the electronegative sol and an increased fluidity with less opalescence in electropositive sols. Zacharias[15] contended that textile fibers were colloids and regarded the dyeing process as an adsorption reaction. In a patent application he uses colloidal tin oxide in the tanning of hides. Bock[16] suggests that since HCN destroys the ability of colloidal platinum to decompose hydrogen peroxide, perhaps enzyme reactions may be analogous to the catalytic activity of colloidal metals. Garrett[17] investigated the viscosity of some colloidal solutions including gelatin, albumin, and SiO_2 using both outflow and pendulum types of viscometers with temperature and concentration as variables. He suggests that sols such as these consist of two phases, one colloid-rich, the other colloid-poor, and finds that at a given concentration temperature control alone is not sufficient to give reproducible viscometric values.

Perrin's[18] paper is one dealing with the theory of colloidal sols in which he discusses electric charge, particle size, surface tension,

[11] Schiff, H., *Chem. Ztg.*, 17: 1000 (1893).
[12] Schneider, E. A., *Z. anorg. Chem.*, 3: 78 (1893).
[13] Pauli, Wo., *Beitr. chem. Physiol u. Path.*, 3: 225 (1903).
[14] Hardy, W. B., *Proc. Cambridge Phil. Soc.*, III, 12: 201 (1903).
[15] Zacharias, P. D., *Z. Farb. u. Text. Chem.*, 2: 233 (1903).
[16] Bock, F. *Öster. Chem. Ztg.*, 6: 49 (1903).
[17] Garrett, H., *Phil. Mag.*, [6] 6: 374 (1903).
[18] Perrin, J., *Compt. rend.*, 137: 564 (1903).

cohesion and coagulation, Bredig[19] discusses electrophoresis, Muller[20] the classification of colloids, and Freundlich[21] the flocculation of sols by electrolytes.

Only one paper[22], and that dealing with a gold sol, is of American origin.

In my undergraduate and graduate lectures in chemistry (1902–1909) I do not remember ever hearing the word "colloid" mentioned. Certainly it was never stressed as an important field of study. I believe that the first time that colloid chemistry was ever seriously called to my attention was in the summer of 1910 when Dr. Bancroft was arranging a symposium for the fall meeting of the American Chemical Society. At that time I was connected with a biological research institution, and Professor Bancroft invited me to present a paper on "Colloids in Biology." Obviously I did not wish to reply that I did not know what he was talking about, so I took the usual procedure which is followed in such cases and pleaded the pressure of other work which prevented my complying with his request. However, I distinctly remember the embarrassment I felt on receiving his letter, and I resolved then and there to see if colloids *did* have any relationship to biological phenomena.

Today the behavior of colloid systems is taught in most undergraduate curricula, and a knowledge of colloid chemistry is essential to an adequate understanding of the principles of physiology and biochemistry.

The chemical literature of the world is replete with papers dealing with the behavior of colloid systems. Medicine, industry, and the arts have profited greatly by advances in this field. Many of the technics of the other branches of chemistry and physics have been adopted by the colloid chemist to advance knowledge in his chosen field. The Nobel prize has been awarded to four men, Jean Perrin (1925–26), The Svedberg (1925–26), Richard Zsigmondy (1925–26), and our own Irving Langmuir (1932), who have made notable contributions to the theory and practice of colloid chemistry. No longer does a scientist primarily interested in the study of the phenomena of colloidal systems have to defend himself from the ridicule of his fellow chemists. The colloid chemist has acquired a modicum of respectability. More and more the biochemist and the physiologist

[19] Bredig, G., *Z. Electrochem.*, 9: 738 (1903).
[20] Muller, A., *Z. anorg. Chem.*, 36: 340 (1903).
[21] Freundlich, H., *Z. physik. Chem.*, 44: 129 (1903).
[22] Blake, J. C., *Am. J. Sci.*, [4] 16: 381 (1903).

are coming to realize that nearly all reactions of living organisms take place in matter in the colloidal state and that if the intimate problems of life are ever to be solved the solution can only come about through the aid of those who are versed in the intricacies of colloid behavior.

Graham recognized this as early as 1861 and perhaps it is fitting to close this introductory lecture with another quotation from that master. In speaking of the properties of colloids as opposed to crystalloids Graham says:

> Their peculiar physical aggregation with the chemical indifference referred to appears to be required in substances that can intervene in the organic processes of life. The plastic elements of the animal body are found in this class. As gelatin appears to be its type, it is proposed to designate substances of the class as *colloids*, and to speak of their peculiar form of aggregation as the *colloidal condition* of matter. Opposed to the colloidal is the crystalline condition. Substances affecting the latter form will be classed as *crystalloids*. The discussion is no doubt one of intimate molecular constitution. . . . The colloidal is, in fact, a dynamical state of matter; the crystalloidal being the statical condition. The colloid possesses ENERGIA. It may be looked upon as the primary source of the force appearing in the phenomena of vitality.

Chapter II

WHAT IS COLLOID CHEMISTRY

Colloid chemistry deals with a system of matter, not with a kind of matter. It is often erroneously stated that Graham believed that colloids constituted a different kind of matter, but it is evident from a reading of his original papers that he recognized that there was a continuous gradation from matter in true solution to matter in the colloid state. Graham coined the word, "colloid," from the Greek (κολλα) Kolla, meaning *gelatin* or *glue*, and (ειδος) Eidos, meaning *like*, so that the class is named from one of its components. It should be emphasized that in colloid chemistry we are dealing with a heterogeneous system composed of at least two components, one of which is known as the disperse phase and the other as the dispersions medium.

Earlier definitions of colloid systems stated in substance that colloid systems arise whenever one component is dispersed in another, the dispersion being coarser than molecular. We now know that this is not necessarily true, for there are certain substances, for example, egg albumin, the molecules of which are so large as to yield in solution colloidal systems which at the same time are molecularly dispersed.

We owe to Wo. Ostwald the following diagram showing the relationships which exist between coarse suspensions and molecular solutions and the colloid state:

Matter in mass	Colloids	Molecules and ions
	0.1μ	$1.0\ m\mu$

It will be noted that the boundaries of the colloid state are defined by size limits. The upper limit was originally selected as representing approximately the lower limit of the resolving power of the better grades of the ordinary microscope. The lower limit of 1.0 $m\mu$ was likewise arbitrarily selected. We now know, however, that this size represents a diameter somewhat greater than the diameter of the

ordinary molecules and ions, so that in general, with few exceptions, particles in the colloid state represent aggregations of molecules which are not visible in the field of the ordinary microscope.

It should be evident from the above diagram that there is probably a continuous gradation of properties from coarse suspensions through the colloid state to true solutions. There are, however, certain characteristic properties of matter in the colloid state which are not exhibited by true solutions and which are shown in a negligible degree by gross suspensions, and these are the properties toward which the colloid chemist directs his attention. It should likewise be emphasized that there is some particular degree of dispersion within which a given substance will show maximum colloidal properties. This has often been referred to as the "optimum zone of colloidality."

Graham originally defined the colloid state on the basis of diffusibility, stating that crystalloids diffused at an appreciable and readily measurable rate, whereas colloids showed little or no diffusibility. Later the optical behavior in part superseded diffusibility, and the term *micron* came into general use for fine suspensions containing particles visible in the ordinary microscope, *ultramicrons* for particles not resolvable by the ordinary microscope but visible in the ultramicroscope, and *amicrons* for particles not distinguished by the ultramicroscope.

Since we are dealing with two-phase systems, it is possible to classify such systems in eight general groups depending upon the physical state of the disperse phase and the dispersions medium. Thus, we may have solid-in-solid, examples of which are ruby glass (gold in glass) and the black diamond (amorphous carbon in crystalline carbon); solid-in-liquid, an example of which is a suspension of clay in water; solid-in-gas, an example of which is smoke, particularly the blue haze following a forest fire; liquid-in-solid, examples of which are the opal and pearl, in the one instance water dispersed in SiO_2, in the other in $CaCO_3$; liquid-in-liquid, an example of which is the milky latex of the milkweed; liquid-in-gas, an example of which is the ordinary fog; gas-in-solid, examples of which are the structural colors, the iridescent blues and greens of certain feathers and insects, the pigment in these instances being a dull brown but the iridescent colors being produced by the structure of the keratin or chitin trapping thin films of air; and gas-in-liquid, examples of which are foams. The ninth theoretical system, gas-in-gas is not realizable because this system yields only molecular dispersions.

The above classification on the basis of the physical properties of the two phases is not entirely satisfactory. For example, in the case of liquid-in-liquid it is altogether possible that in many systems we are dealing with what is essentially a solid-in-liquid system. When liquids are subdivided into the extremely fine divisions characteristic of the colloid state, surface compression upon spherical droplets may reach relatively enormous values, and such compression forces probably in some instances cause the liquid particle to become to all intents and purposes transformed into a solid. Likewise the behavior of the various systems does not in general show sharp breaks between the various classes noted above. These facts have necessitated a search for a better method of classification, and it appears preferable to divide all colloid systems into two general classes based upon the affinities which exist between the disperse phase and the dispersions medium. The general terms, *lyophilic* and *lyophobic*, have come into general use. A lyophilic system may be defined as one in which there is mutual solubility between the two phases. An example of such a system is rubber-in-benzene. Benzene dissolves to a certain extent in rubber so that the particle becomes solvated and swells. However, the "solution" does not in general proceed to the point of true molecular dispersion, although there may be small amounts of the rubber molecularly dispersed in the dispersions medium. On the other hand, gold-in-water forms a lyophobic system, water neither dissolving in the gold particles nor gold dissolving to any appreciable extent as a molecular dispersion in the dispersions medium.

Lyophilic and lyophobic are general terms. In many instances it is preferable to use specific terms such as hydrophilic and hydrophobic, indicating that water is the dispersions medium, alcopihilic and alcophobic, indicating that alcohol is the dispersions medum, etc. The biological chemist, however, is in general dealing with systems in which water is the dispersions medium, and accordingly generally employs the terms, hydrophilic and hydrophobic.

Sub-classes under each of the major groups are *sols*, *gels*, and *coagula*. Sols may be defined as colloid systems possessing a high degree of fluidity. From the physical standpoint they appear to be essentially "solutions," but when studied by colloid chemical technic, it is apparent that the particles of the disperse phase have diameters lying within the colloid realm. Gels are colloid systems showing a greater or less degree of rigidity. Presumably all gels possess a struc-

ture and require a finite amount of energy to distort this structure. The more rigid gels show many of the characteristics of solids, but it should be emphasized that there is a border-line region where the sols grade almost imperceptibly into gels and that there is no sharp line of demarcation between the two classes. Coagula are sometimes classified with the gels. Coagula result whenever the disperse phase of a lyophobic system has been flocculated or precipitated by appropriate technic. Such a precipitate is usually rather bulky, an appreciable amount of the dispersions medium adhering to the precipitated material. The behavior of such coagula or precipitates, however, is quite different from the true lyophilic gels, and it would seem desirable to group the precipitates from lyophobic sols in a different class.

Under both sols and gels we can have sub-classes depending again upon the nature of the dispersions medium. Thus, we may have hydrosols or hydrogels, alcosols, or alcogels, benzosols or benzogels.

The advantages of the classification into lyophilic and lyophobic systems over that of "solid-in-liquid" or "liquid-in-liquid" can be illustrated by the fact that a rubber hydrosol behaves, from the colloid chemical standpoint, as though it were a solid-in-liquid system, *i.e.*, it is lyophobic, whereas a rubber benzosol behaves as though it were a liquid-in-liquid system, *i.e.*, it is lyophilic. Similarly starch-in-alcohol is lyophobic, whereas starch-in-water is lyophilic. We are thus freed from a preconceived notion as to the physical state of the disperse phase and are permitted to classify any particular system from observations which we can make upon that particular system as it exists under our methods of preparation.

Chapter III

SOME BASIC CONCEPTS

Methods of Preparation

If the colloid realm is defined as systems containing particles which lie in size between those systems characterized by gross suspensions and true solutions, it is obvious that one can approach the colloid realm from either direction. However, in order to secure a stable system, conditions must be so chosen that when the particles have reached colloidal dimensions they will retain their size characteristics, *i.e.*, the particles will not further dissolve nor will the particles grow larger at a rapid rate. In order to realize these conditions, it is necessary that a colloidal particle shall have a solubility below a certain minimum. It is therefore relatively easy to form stable colloid systems from substances which have an extremely low solubility (*ca.* 1 mg. per liter), whereas more soluble materials form relatively unstable colloidal systems, the particles of which redissolve and recrystallize until eventually relatively coarse crystals result which separate out.

Furthermore in order to produce a colloidal system which is relatively concentrated, it is necessary that a large number of nuclei be formed essentially simultaneously. Von Weimarn has pointed out conditions which are essential for nuclei formation. He has given us the formula:

$$W = K\left(\frac{C - L}{L}\right) \qquad (1)$$

as defining conditions for nuclei formation from true solutions, where L is a solubility, C is the concentration, (C−L) is accordingly the supersaturation, W is the velocity of nuclei formation, and K is a constant characteristic of the particular material. Accordingly the velocity of nuclei formation is proportional to the ratio which exists between supersaturation and solubility and as L approaches a negligible value, the ratio $\frac{C-L}{L}$ becomes large with a proportional in-

crease in W. It is thus obvious that an extremely insoluble material, such as AgBr can readily be obtained in the colloidal state, whereas more soluble materials, such as NaCl are not stable with water as the dispersions medium. If, however, alcohol or benzene is the external phase, it is possible to secure stable colloidal sols of NaCl because of the low solubility of the salt in such liquids.

Following the formation of nuclei the stability of the system is determined by the rate of crystal growth, probably in accordance with the Noyes-Nernst formula:

$$V = \frac{\Delta}{l} S\,(C-L) \qquad (2)$$

where V is the velocity of crystal growth, Δ is the diffusion coefficient, l is the length of the diffusion path, S is the surface area, and (C−L) is the supersaturation as in (1). Formula (2) holds for only a given instant of time, inasmuch as the surface area is increasing with increased crystal growth and the length of the diffusion path is likewise increasing with decreasing supersaturation and (C−L) is decreasing continually to a limit of zero.

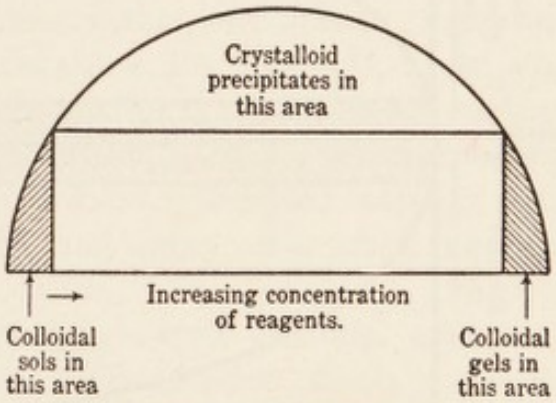


FIG. 1. A diagrammatic representation of the effect of reagent concentration on the physical state of the resulting precipitate

Under ideal conditions if $(C-L) \leqq 0$ at the end of nuclei formation there is essentially no supersaturation in the mother liquor, accordingly crystal growth is at a minimum and the system is stable and the particle size is small. If (C−L) is relatively large following the initial nuclei formation, a colloidal system containing large particles may result, and if (C−L) is still larger, a colloidal system may persist for a limited period of time with particles rapidly growing larger until eventually their size becomes microscopic and they precipitate.

Fig. 1 shows diagrammatically the relationship between the concentration of two reagents which react to produce an insoluble precipitate, such for example as equivalent concentrations of ferric chloride and tri-sodium phosphate to form ferric phosphate, or ferric chloride and potassium ferrocyanide to form Prussian blue.

It will be noted that a colloidal sol results from the interaction of dilute solutions and that a colloidal gel is formed from the interaction of the very concentrated solutions but that in the intermediate ranges of concentration a crystalline precipitate is formed which can be readily filtered off and washed upon the filter. This behavior of these reacting systems is of great importance in analytical chemistry, and it is obvious that in case one wishes to determine the weight of a precipitate, one should so choose the concentration of the interacting reagents as to avoid the production of particle size lying within the colloid realm. If, on the other hand, one wishes to determine by colorimetric or nephelometric technics the amount of precipitate which has formed, it is almost essential that one use extremely dilute solutions so as to obtain a stable colloidal sol. Thus, in the colorimetric determination of HCN, the stable blue sol which is read in the colorimeter should practically never be more concentrated than that represented by 5 mgs. HCN per 100 cc. Otherwise, in more concentrated solutions the sol, Prussian blue, will flocculate.

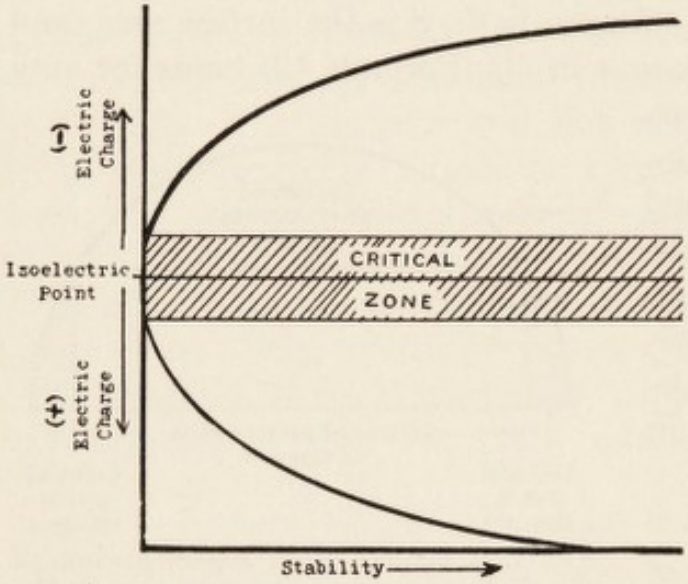


FIG. 2. A diagrammatic representation of the relationships between colloidal stability and electric charge and the area of the "critical zone"

The volume of liquid in which a given precipitate is formed may have a great influence on the physical characters of the precipitate, and because of the changing particle size in the precipitate with increasing concentration (or dilution), the surface contamination of the precipitate by the materials present in the mother liquor will vary appreciably.

Electric Charge:—All stable lyophobic systems and most stable lyophilic systems contain particles which possess an electric charge. This charge may be either positive or negative, depending upon the nature of the particle and the nature of the dispersions medium. Under electrokinetics (*vide infra*) we will later discuss certain fundamental properties of the electrical force on surfaces. It is sufficient

to indicate at this point that the charge may arise either (a) by the "capture" of an ion (adsorption, *vide infra*) or (b) by direct ionization or (c) by contact electrification. We are certain that both (a) and (b) are involved in the origin of the charge. We are not so certain in regard to (c). Fig. 2 shows diagrammatically the influence of the electric forces upon colloid stability. It will be noted that there is a region close to the point of electrical neutrality (the isoelectric point) within which the system is relatively unstable. This region has come to be known as the critical zone. Above this region in the diagram the system is stable, the particles possessing a negative charge. Below this region the system is likewise stable with the particles possessing a positive charge.

It should be emphasized here that in the above discussion the phrase, "critical zone," and the words, "stable" and "unstable," are used in a relative sense, for in all colloid studies *time* must be considered as a fourth dimension. Time is just as important in defining conditions of colloid study as is concentration, and in many instances it is more important than is temperature. For example, under one condition the system may be stable for ten minutes, under another for ten days, and under still another condition for ten years. Obviously the critical zone will be broader for a ten-year stability than it will be for a ten-minute stability, and many other properties will similarly vary with time.

Most substances form negatively charged colloidal sols with water as the dispersions medium. If a substance of low dielectric constant, *e.g.*, turpentine, is used as the dispersions medium, most substances form positively charged sols. The complex bases, metallic hydroxides, metallic oxides, basic dyes, and basic proteins, such as histones and protamines, normally form positively charged sols in water. Starch, cellulose, gums, the usual proteins, oil droplets, and inorganic colloid systems, such as sulfur, gold, platinum, silver, etc., form negative hydrosols. In the case of sulfur we probably have a sulfur particle stabilized by the adsorption of an SH^- ion having the composition $[S]_x^{SH^-}$. The hydrogen and hydroxyl ions are common stabilizing ions, water being a constant reservoir for such ions.

The nature of the positively charged micelles may be illustrated by ferric hydroxide. The composition of the particle may be either $[Fe(OH)_3]_y[Fe(OH)_2]_x^+ \ OH^-$ or $[Fe(OH)_3]_y[Fe(Cl)_2]_x^+ \ Cl^-$, where x and y may represent continuous numerical series varying over relatively wide ranges, *i.e.*, they are not stoichiometrical units, in

the first case the charge arising by direct ionization of the ferric hydroxide, in the second case arising by ionization of ferric chloride, which has been adsorbed by the ferric hydroxide. It is generally recognized that "ferric hydroxide" sols usually contain traces of ferric chloride. This has given rise to what has been called the "complex theory of colloids" which assumes that an extremely insoluble substance, *e.g.*, gold, silver, platinum, ferric hydroxide, etc., always has associated with it a residual portion of a readily ionizable salt which by dissociation gives rise to a positive (negative) charge upon the micelle with a corresponding charge of the opposite sign in its immediate vicinity in the dispersions medium. Whether or not the charge arises by direct ionization or by direct adsorption is immaterial for the purposes of the present discussion.

A rather striking example of the capture of an ion has been utilized in certain recent analytical technics, *e.g.*, by titration of chlorides with silver nitrate, using dichlorofluorescein as an indicator.[1] The anion of the dichlorofluorescein is colored, consequently as soon as a trace of excess of Ag^+ is present, the silver chloride becomes positively charged, and this positively charged particle adsorbs the anion of the dye so that the precipitate suddenly changes from white to an intense red. The reaction is much more sensitive than the old chromate indicator and has the additional advantage over the chromate of reacting in dilute acid solutions.

A given ion in low concentration may stabilize a colloid system, whereas in a larger concentration it may cause the system to flocculate. This behavior is due to the differential effects of the anions and the cations and to their effect on the water relationships. It is, of course, obvious that opposite electrical charges neutralize each other. Accordingly colloid systems which possess a positive charge are flocculated by the addition of negatively charged colloid systems, and similarly it is the anions of an electrolyte which most markedly affect positively charged colloid systems, whereas the cations most profoundly affect negatively charged colloid systems. Since it is impossible to add ions possessing only a single charge, the effect which is observed upon the addition of electrolytes is the joint effect of stabilization brought about by the like-charged ion and the flocculation produced by the ion of unlike charge. These considerations bring us to two phenomena which are of major interest to the in-

[1] Kolthoff, I. M., Lauer, W. M., and Sunde, C. J., *J. Am. Chem. Soc.*, 51: 3273 (1929).

vestigator working with biological material, (1) the purification of biochemical compounds by the "replacement of solvent" and (2) peptization.

Purification of Biochemical Compounds by Replacement of Solvent. A great many biochemical compounds, such as proteins, starch, pectins, etc., are more or less readily dispersible in aqueous media to form hydrosols. As a rule such hydrosols are flocculated by the addition of alcohol and other organic solvents which are miscible with water. The flocculation is produced by a dehydration of the colloidal micelle without greatly affecting the magnitude of the electrical charge. Accordingly the coagulum which is produced disperses rather readily when water is added. It is a common practice to purify such biochemical preparations by repeated precipitation with alcohol followed by redispersion with water, the procedure usually ending by washing the precipitate thrown down by alcohol first with absolute alcohol, followed by drying with absolute alcohol and ether. In the process of the repeated precipitation, soluble impurities, both of organic and inorganic types, remain in the mother liquors, and it often happens that after two or three precipitations the electrolyte content of the final hydrosol becomes so greatly reduced that either no flocculation occurs when the alcohol is added or only a small amount of coagulum is thrown down. This results in the formation of an alcosol which may be stable for a long period of time. Frequently valuable preparations have been discarded with the mother liquors, and it is accordingly always essential to test mother liquors for the complete precipitation of proteins, starches, pectins, etc., if they are being purified by the replacement of solvent method. If stable organosols are formed when alcohol is added to precipitate a hydrosol, one can usually bring about precipitation of the desired chemical compound by adding a drop or two of saturated lithium chloride to the alcosol. Lithium chloride is preferable to most other electrolytes, since it is soluble in alcohol.

Peptization. The classic example of peptization is the precipitation of silver bromide through the interaction of silver nitrate and potassium bromide, although the formation of almost any very insoluble precipitate could be utilized to illustrate this phenomenon. Fig. 3 is a diagrammatic illustration of peptization showing the influence of the ions on the sign of the charge of the micelle, conditions which are necessary for isoelectric precipitation, and the "salting out" of a coagulum at high salt concentrations. It will be observed

that there is only one point at which the precipitate consists of isoelectric AgBr and that this point occurs when there is an exact equivalence of silver and bromide ions. If bromide ions are in excess there is present in the mother liquor a colloid sol having the composition $(AgBr)_xBr^-$, negatively charged and stabilized by the bromide ion. If to such a system a larger amount of potassium bromide is added, this sol will be precipitated but will not consist of pure silver bromide but rather of silver bromide containing an excess of adsorbed bromide ions. If such a precipitate is washed so as to reduce the content of precipitating electrolyte, a part of it will redisperse into

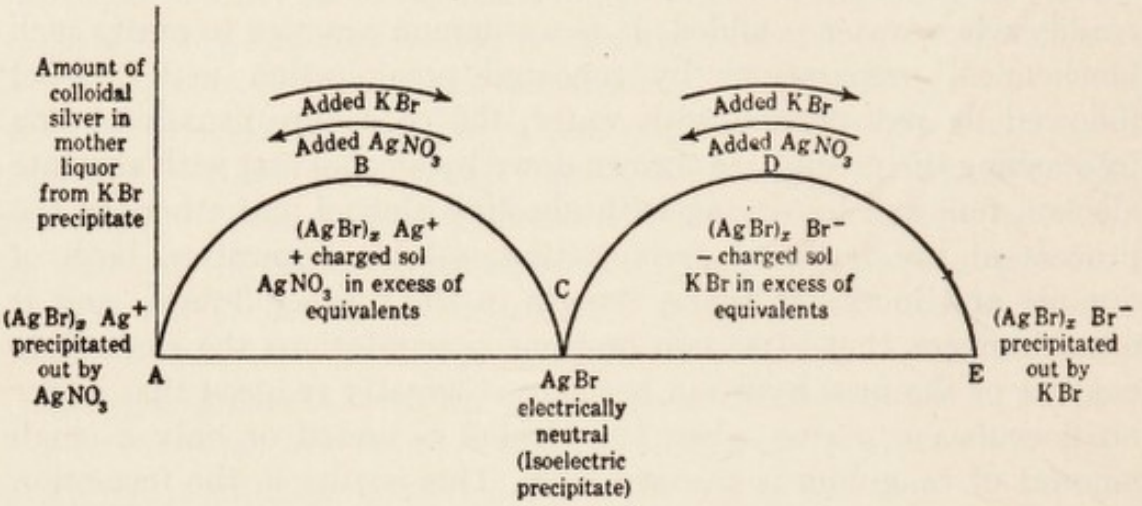


FIG. 3. A diagrammatic representation of the peptization behavior of excess $AgNO_3$ or KBr on freshly precipitated AgBr

a colloidal sol and pass through the filter paper. Similarly if the silver ion is in excess, we will obtain first a positively charged silver bromide sol where the micelles have the composition $(AgBr)_xAg^+$, and with still larger amounts of silver nitrate present this sol will be coagulated, but here again the precipitate will be redispersed in part when the coagulating electrolytes have been removed by washing. For the accurate determination of either silver or bromine by weighing silver bromide, it is very essential that isoelectric conditions be reached. The nicety of manipulation necessary to bring this about can be ascertained by reading details of almost any paper dealing with the determination of atomic weights where silver is the reference standard.

Many biochemical sytems, such as proteins, starchs, gums, mucilages, tannins, pectins, etc., often exhibit unusual or at least extreme peptization behavior. Many of these systems have been

defined or classified in terms of "solubility" or "insolubility" and since these all form lyophilic sols, the "solubility" and "insolubility" are in reality peptization or non-peptization.

Perhaps the three protein classes of albumins, globulins, and glutelins may serve as illustrations. Albumins are defined as proteins which are soluble in water and coagulable by heat. Globulins are defined as proteins which are insoluble in water, soluble in dilute salt solutions, and coagulable by heat. Glutelins are defined as insoluble in water, insoluble in dilute salt solutions, soluble in dilute solutions of the fixed alkalies, and coagulable by heat. From the above definitions one would anticipate that it would be relatively easy to make a sharp separation of proteins belonging to these three classes, providing that they occurred (as they often do) in the same biological material, and the literature is replete with papers describing the properties of proteins belonging to these various classes which have been isolated from a given material.

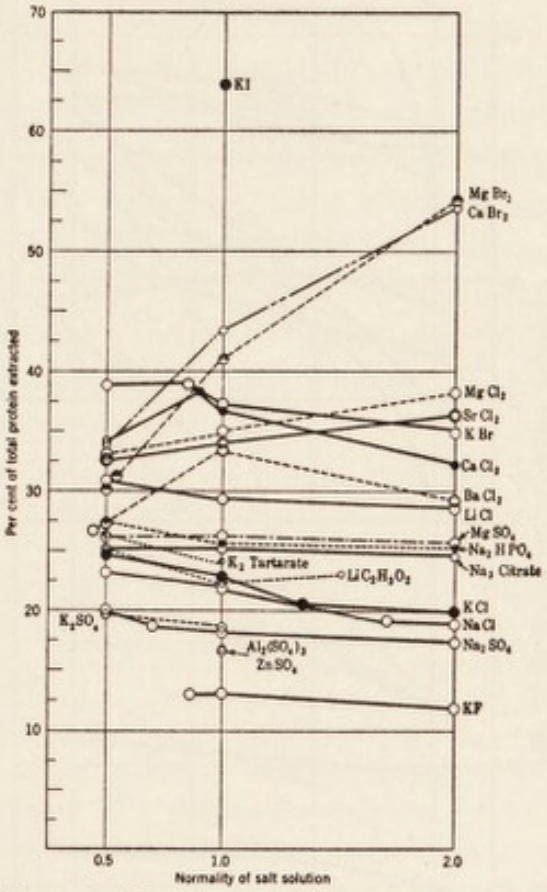


FIG. 4. Showing the peptization behavior of a series of salts in various concentrations on the proteins of wheat flour (Data of Gortner, Hoffman, and Sinclair)

In 1927, Hoffman and Gortner[2] showed that when wheat flour was extracted with 5 per cent potassium sulfate solution and with 10 per cent sodium chloride solution and the extracts were later dialyzed to precipitate the "globulins," the ratios of albumin to globulin obtained by the two different extraction methods differed widely, and they accordingly raised the question as to the meaning of a "dilute salt solution" in the definition of the globulins. Later a comprehensive study was undertaken as to the influence of specific electrolyte solutions on the extraction of proteins from wheat flour.

[2] Hoffman, W. F., and Gortner, R. A., *Cereal Chem.*, 4: 221–229 (1927).

Gortner, Hoffman, and Sinclair[3,4] studied the influence of twenty-one different inorganic salts, most of them in four different concentrations, on twelve different wheat flours, and they found that each salt in each concentration showed, in general, distinct peptization behavior, and what was perhaps still more surprising the flours differed widely among themselves with respect to the peptization behavior of any given salt solution of any particular concentration. Fig. 4 shows the average percentage of protein which was extracted from the twelve wheat flours by the various concentrations of salt solutions. It will be noted in the case of the series of the normal solutions of the potassium halides that KF extracted approximately 13 per cent of the proteins, KCl approximately 23 per cent, KBr approximately 37 per cent, and KI approximately 64 per cent. All existing data indicate that the maximum amount of true "albumin" and "globulin" which can be present in wheat flour probably does not exceed 10 per cent of the total protein. Obviously, therefore, in the experiments which have been cited the salt solutions were peptizing proteins which belong to other classes. These studies also made it evident that the term "solubility" as used in the definition of the protein classes was not synonymous with the term "solubility" as used in

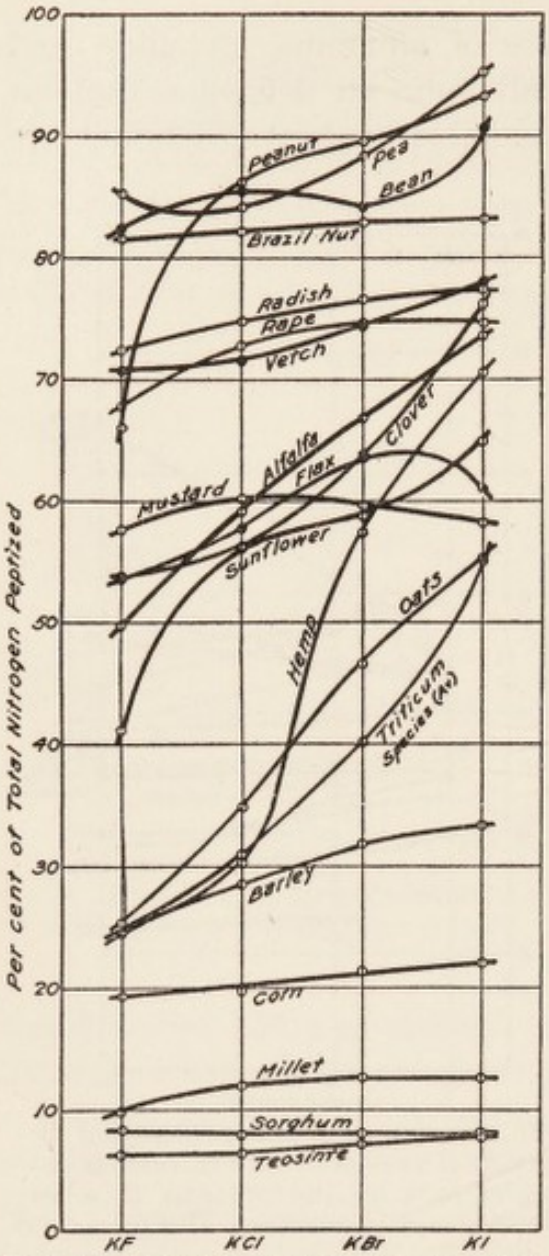


FIG. 5. Showing the peptization behavior of 0.5 M solutions of the potassium halides on the protein complex of various seeds and grains (Data of Staker and Gortner)

[3] Gortner, R. A., Hoffman, W. F., and Sinclair, W. B., *Colloid Symposium Monograph*, 5: 179–98 (1928).

[4] Gortner, R. A., Hoffman, W. F., and Sinclair, W. B., *Cereal Chem.*, 6: 1–17 (1929).

crystalloidal chemistry but rather represented peptization, and accordingly the fractionation of proteins by means of partial dispersibility must be treated from the colloid chemical standpoint.

Following the studies by Gortner, Hoffman, and Sinclair, Staker and Gortner[5] studied the peptization of the protein complex in various seeds and grains, utilizing as peptizing media distilled water and 0.5 molar solutions of KF, KCl, KBr, KI, and K_2SO_4. Here again they found extreme differences in the albumin and globulin ratios dependent upon the particular salt solution which was used for extraction. Fig. 5 shows the peptization behavior of the various seeds and grains which were examined with respect to the percent of total nitrogen which was peptized by the various salt solutions. This study again emphasized the fact that protein "solubility" is in reality peptization.

Shortly after the studies of Gortner, Hoffman, and Sinclair, Sørensen[6] published an extensive paper on the constitution of soluble proteins as reversible dissociable component systems. The gist of this paper is summed up in the following quotation:

> Soluble proteins consist of a series of complexes or components, reversibly combined, which makes their constitution expressible by the ordinary formula $A_xB_yC_z$ . . . A, B, C and so on each marking complete complexes, mainly polypeptides, yet in some cases also containing other groups, for example phosphorous ones, whereas the affixed indices x, y, z and so on mark the amount to which the indicated complex is present in the entire component system. Within each complex all the atoms and atom groups are linked together by main-valencies, whereas the various complexes in the whole component system are comparatively loosely and reversibly knit together by means of the residual valencies which each component must be assumed to possess, and the strength and nature of which must depend on the chemical composition of the component in question as well as on its physical properties, above all on its dimensions and the resulting shape and surface. But all things considered, the linkage between the components must be supposed to be comparatively slight and of such a nature that alterations in the composition of the solution (salt content, hydrogen-ion activity, alcohol content, temperature) may give rise to reversible dissociations of the involved component systems and interchange of components between the same. When these alterations in the composition of the solution are so suited as to render possible in sufficient quantities the formation of a component system insoluble or sparingly soluble under the new conditions, such a system will be formed and precipitated. In good accord with this is the fact that through suitable proceedings it has been possible to effect a reversible fractionation in the case of all hitherto investigated proteins. In the main, the fractions

[5] Staker, E. V., and Gortner, R. A., *J. Phys. Chem.*, 35: 1565–1605 (1931).
[6] Sørensen, S. P. L., *Compt. rend. trav. lab. Carlsberg*, 18 (No. 5): 1–124 (1930).

gained possess indeed the properties of the initial material, yet both the physical properties and the chemical composition are more or less modified from fraction to fraction because of their varying contents of the different components.

The above quotation means that Sørensen and other workers in his laboratory have apparently been successful in separating certain proteins which have hitherto been regarded as distinct entities into fractions which differ from each other in physical properties and perhaps to some extent in chemical composition, although the degree of difference is much more apparent in the physical properties. Sørensen accordingly believes that when these various fractions are recombined and held together more or less loosely by secondary valence forces the properties of the original protein reappear.

There is, however, another way of attacking the problem, *i.e.*, to fractionate a protein by a specific technic and then *without recombining* the fractions to rework them by a standard technic and to see if now the various preparations are markedly different or whether they approximate uniformity with the original material. Such a study was undertaken by Sinclair and Gortner.[7] These authors studied the fractionation of various preparations of gliadin by molar solutions of KCl, KBr, and KI. They found that gliadin was markedly peptized by the KI solutions, somewhat peptized by the KBr solutions, and only slightly peptized by the KCl solutions. They then exhaustively extracted a large sample of gliadin with KI solution and secured a fraction which was "soluble" in the KI solution and another fraction which was essentially "insoluble." These two fractions were then separately electrodialyzed so as to remove all of the electrolyte. They were then reworked by the original method for the preparation of gliadin (dispersing in 70 per cent alcohol and precipitating, pouring into absolute alcohol and ether, and repeating this process several times, finally drying at a low temperature). Two samples of gliadin were thus obtained, one of which had been so completely peptizable by the KI solution as to yield a perfectly transparent non-opalescent hydrosol and the other fraction which had been essentially "insoluble" in the presence of N/1 KI solution. These should represent extreme fractions. However, the reworking of these fractions by the original "standard" technic for the preparation of gliadin yielded preparations both of which now reflected on subsequent peptization with KI solutions essentially the identical behavior of the original gliadin

[7] Sinclair, W. B., and Gortner, R. A., *Cereal Chem.*, 10: 171–188 (1933).

preparation from which these fractions were derived. Accordingly in these experiments the properties of the original preparation were essentially regained, not by a recombination of polypeptide fractions as Sørensen's theory requires, but simply by reworking either the "soluble" fraction or the "insoluble" fraction by as nearly as possible the same standard technic which was used to prepare the original sample of gliadin. In this manner the dry, purified protein was brought back to approximately the same physical state, and the subsequent peptization behavior of the colloid aggregates in the dry gel reflected this similarity in physical state. It appears, therefore, that the difference in "solubility" may be accounted for as being due to a physical heterogeneity of the dry gel rather than to a chemical heterogeneity of mixed protein molecules.

Lustig[8], using non-standard technic, has fractionated the proteins of blood serum and has isolated three albumins which appear to differ sharply from each other in physical properties. Perhaps Lustig's fractions represent chemical entities, but there is no evidence that these preparations cannot be further fractionated by other technics so as to produce fractions possessing different physical properties. The whole question of protein individuality, protein classification, and protein isolation technics needs to be critically examined, particularly from the standpoint of colloid behavior and colloid peptizability. Abderhalden and Komm[9] have even gone so far as to suggest that those proteins which we consider as distinct entities may in reality be only fractions of a larger biochemical structure. If one accepts their viewpoint, then it may well be that chemical reagents bring about the peptization of a large micelle into certain of its component parts, the properties and composition of which are determined by the reagent and technic which were used. That this is likely to be the case with wheat proteins is emphasized by the study of McCalla and Rose[10] who studied the fractionation of gluten dispersed in sodium salicylate solution. By fractional precipitation of the dispersed protein, they obtained essentially a continuous series of fractions differing progressively and systematically from each other in both physical and chemical properties. They conclude their paper with the following significant statement:

The results of the extraction studies in this and previous investiga-

[8] Lustig, B., *Biochem. Z.*, 225: 247–263 (1930).
[9] Abderhalden, E., and Komm, E., *Z. physiol. Chem.*, 139: 181–204 (1924).
[10] McCalla, A. G., and Rose, R. C., *Canad. J. Res.*, 12: 346–356 (1935).

tions show that definitions of flour proteins based on solubility in a solution such as 70% alcohol are purely arbitrary, as the changes in physical and chemical properties are not sudden but gradual and regular. The precipitation experiment in the present study appears to give a much better picture of the nature and composition of gluten, and it therefore seems preferable that the terms "glutenin" and "gliadin" should be discarded. These suggest that gluten is composed of two distinct proteins, whereas the results of the present study, and those of earlier investigations, substantiate the view that it is a single protein complex which can be divided into a great many progressively different fractions.

It likewise seems essentially probable that protein coagulation and its reversal are largely if not entirely a coagulation-peptization phenomenon. Mona Spiegel-Adolph[11] and Anson and Mirsky[12] have reversed the heat coagulation of protein by treatment of the coagulum with very dilute alkalies or acids. It is the belief of these authors that heat coagulation involves terminal ring closure but does not result in the formation of peptide linkages, and that the new linkages are broken relatively easily by the dilute alkali or acid. It would seem that colloidal flocculation and subsequent peptization might for the present at least be as good an explanation. Unquestionably there are many stray valence forces arising from the various groups of a series of long polypeptide chains. The peptizability or non-peptizability of such a unit would be influenced by various adhesion forces, such as the adhesion of protein micelle for protein micelle, protein micelle for water molecules, water molecules for water molecules, salt ions for water molecules, and protein micelle for salt ions. The balance of all of these adhesion effects would determine whether or not the protein micelles were separated from each other or whether they adhered in an "insoluble" mass. At least the colloid viewpoint and the colloid chemical approach to the problems of protein coagulation and its reversal seem to offer a valuable tool for investigating the fundamental phenomena which are involved.

[11] Spiegel-Adolph, Mona, *Biochem.* Z., 170: 126–172 (1926); *Kolloid* Z., 38: 127–129 (1926); *Naturwissenschaften*, 15: 799–803 (1927).

[12] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 14: 597–604 (1931); 14: 605–609 (1931); 14: 725–732 (1931); *J. Phys. Chem.*, 35: 185–193 (1931).

Chapter IV

SOME FUNDAMENTAL PROPERTIES OF COLLOID SYSTEMS

It is desirable at this point to consider briefly some of the fundamental concepts which underlie colloid behavior.

Brownian Movement. Robert Brown, a botanist, in 1827, while viewing pollen grains suspended in a liquid under the microscope, observed that they were in continuous motion in the liquid. It was at first thought that this motion was due to cilia and was a manifestation of living forces. However, old pollen grains, plant spores, etc., obtained from the herbarium were found to possess similar motion, and it was later found that any particle small enough to remain in more or less permanent suspension would exhibit this characteristic motion which has come to be called Brownian movement.

For nearly a hundred years the cause of Brownian movement remained more or less obscure until the epoch-making investigations of Perrin[1] who experimentally demonstrated the correctness of the theory promulgated by Gouy[2] that the Brownian movement was caused by the bombardment of the particle by the molecules of the liquid in which the particle is suspended. Thus the kinetic energy of the molecules of the liquid causes Brownian motion.

Perrin's great contribution lay in his proof that the colloid particles which were suspended in the liquid distributed themselves according to the same law that affects the distribution of gas molecules under the influence of gravity, and he accordingly enunciated the law that colloidal systems obey the gas laws providing that each individual particle be regarded as a single molecule irrespective of the mass of the particle. By studying the distribution of particles at different depths in a gamboge sol Perrin calculated an Avogadro constant of 70.5×10^{22} for the expression

$$N = 3/2 \frac{RT}{W} \qquad (3)$$

[1] Perrin, J., *Compt. rend.*, 147: 530–546 (1908); 147:594–596 (1908); *Ann. chim. phys.* [8]18: 5–114 (1909); *Z. Elektrochem.*, 15: 269–277 (1909)

[2] Gouy, M., *J. de Phys.*, 7: 561–564 (1888); *Compt. rend.*, 109: 102–105 (1889)

where N = the number of particles in one gram molecule,
and W = the mean kinetic energy of a particle (a gas molecule).

Burton[3] has given an excellent presentation of the historical phases and theoretical significance of studies dealing with Brownian movement. Burton and Currie[4] have shown that the distribution is limited to a very thin surface layer and that below this layer the distribution of particles in the sol becomes uniform. Recently Levine[5] in an extensive theoretical paper has studied the problem of the sedimentation equilibrium in colloidal suspensions in the light of the Debye-Huckel theory for particles possessing different charge magnitudes and concludes that uniform distribution and close packing will be reached under gravitational forces at a limit of 2.1×10^{14} to 5.5×10^{15} particles per cubic centimeter of the sol.

Both von Smoluchowski and Einstein have developed other formulas based on the motion of the particle in a medium and find values for the Avogadro constant of 70.5×10^{22} and 65.0×10^{22} respectively. These are in fair agreement with 60.6×10^{22} that is generally accepted today as the value of Avogadro's constant in the gas equations.

From the above studies and other observations the conclusions have been drawn that colloid systems obey the gas laws providing that each particle is regarded as a single molecule. However, the mass of colloidal particles is usually very large. Thus for the gamboge sol which Perrin studied we would have a weight of 30,000,000,000 grams or 33,000 tons as the weight of one gram molecule. Truly we are dealing here with the long looked for ideal of the physical chemist! The infinitely dilute solution!

Diffusion and Osmotic Pressure.—Graham's contributions to the study of diffusion in colloid systems have already been emphasized, although it should be stated again that there is no sharp discontinuity between crystalloids and colloids. It is only a question of degree, the rate of diffusion being proportional to the radius of the diffusing particle. When diffusion takes place through a membrane or through a gel (and a membrane can be regarded as a gel), the phenomenon is called dialysis. Dialysis is often used to purify colloidal sols, the membrane restraining the movement of the colloidal particles while permitting crystalloidal molecules and ions to pass through into an external solvent phase. Graham demonstrated that the membrane

[3] Burton, E. F., "The Physical Properties of Colloidal Solutions," Chapter IV, Longmans, Green and Company, London, 1921.
[4] Burton, E. F., and Currie, J. E., *Phil. Mag.*, 47: 721–724 (1924)
[5] Levine, S., *Proc. Roy. Soc.*, 146A: 597–623 (1934)

had no influence on the final result and but little influence upon the rate of diffusion and showed that the coefficient of diffusion was an important physical characteristic of the various systems which he studied. Table I shows some of the characteristic coefficients of diffusion for various systems.

TABLE I

Coefficients of Diffusion of Certain Crystalloidal and Colloidal Systems

Substance	Coefficient of Diffusion
Nitric acid	2.10
Urea	0.81
Sucrose	0.31
Svedberg's nuclear gold	0.27
Egg albumin	0.059
Diphtheria toxin	0.014
Anti-tetanolysin	0.0021

It is thus seen that Svedberg's nuclear gold sol which has a particle size just within the lower limits of the colloid realm has a diffusion coefficient which is only slightly less than that of sucrose which is ordinarily regarded as a true crystalloid, and that there is no sharp break in the diffusion coefficient when one passes from the crystalloidal to the colloidal state.

From diffusion studies we can arrive at an estimate of particle size, inasmuch as

$$\Delta r = K \tag{4}$$

where Δ = the diffusion coefficient,
and r = the radius.

If therefore we know the diffusion coefficient and the radius for one substance and the diffusion coefficient for another substance we have the ratios

$$\frac{\Delta_1}{\Delta_2} = \frac{r_2}{r_1} \tag{5}$$

and we can find the unknown radius.

We can also calculate particle weight from Exner's equation

$$\Delta \times \sqrt{M} = K \tag{6}$$

where M = particle weight.

Svedberg uses the equation

$$\Delta = \frac{RT}{N} \cdot \frac{1}{6\pi\eta r} \tag{7}$$

With a nuclear gold sol having a radius of 1.33 mμ. (determined by Brownian movement) he found an Avogadro constant of 58×10^{22}, and using the above equation and the diffusion coefficient he found the radius to be 1.29 mμ.

If each particle behaves as a molecule and the gas laws hold, then

$$PV = NRT \tag{8}$$

and colloidal solutions should possess an osmotic pressure. Such an osmotic pressure is difficult to observe in most cases, since the sols are in general extremely dilute. As a rule the depression of the freezing point or the elevation of the boiling point is negligible, and direct measurements of osmotic pressure in most instances indicate only a few millimeters of water pressure and that only for those sols which can be obtained in relatively concentrated condition. Thus, for example, for a red gold sol with the particles 25 millimicrons in diameter, it would be necessary to have 300 pounds of gold in a liter of water to provide a normal solution. Concentrations of one gram per liter are about as concentrated as can be obtained. Such a sol would theoretically show an osmotic pressure of only approximately 0.12 mm. Hg or 1.6 mm. H_2O pressure. Thus the osmotic pressure of lyophobic systems is essentially zero, and that agrees with experimental findings.

Similarly the electrical conductivity of sols free from electrolytes is essentially zero, for theoretically each particle would behave as an individual ion, and from such a consideration we are dealing with infinitely dilute systems. Bikerman[6] in a theoretical paper concludes that any conductivity of a sol due to colloid micelles must almost always be negligible.

However, certain lyophilic sols (and presumably gels) do show osmotic pressure effects sufficient to be of physiological significance. Thus Sørensen's[7] studies on the osmotic pressure of egg albumin by direct measurement show egg albumin to have a molecular weight of approximately 34,000, measured with a water manometer. In these studies he was able to get a 10 to 15 centimeter water pressure rise.

Physiologists often refer to colloid osmotic pressure as a factor in keeping liquid within the blood stream. It will suffice at this point to say that this is in part at least an osmotic pressure effect. There are

[6] Bikerman, J. J., *J. chim. Phys.*, 32: 460–465 (1935)
[7] Sørensen, S. P. L., *Compt. rend. trav. lab. Carlsberg*, Vol. 12. (1917)

colloids in the tissues, colloids in the blood, and the arterial and venous walls act as membranes separating these systems. If the colloids in the blood fall below a certain level, water flows from the blood to the tissues, blood pressure falls, and shock ensues. Sir William Bayliss[8] originated the technic of injecting gum acacia into the blood stream in order to maintain blood volume following severe hemorrhage, and this technic has been credited with saving approximately 20,000 lives on the western front during the World War.

The problems of edema in nephritis or following excessive hemorrhage are in a considerable measure associated with reduced colloid osmotic pressure within the blood stream. In man colloid osmotic pressure of the blood varies from 27.5 to 42 centimeters of water pressure, although in extreme cases it may reach 55 centimeters of water pressure. It apparently increases with age, old persons having on the average 3 to 5 centimeters water pressure higher colloid osmotic pressure than younger persons, although the scatter is very great.

Filtration through the kidney and water absorption from kidney secretions as well as absorption from the alimentary tract all involve colloid osmotic pressure effects, so that the osmotic pressure of the biocolloids cannot be ignored in physiological studies, although it is difficult to separate the true osmotic pressure effects from imbibition effects (*vide infra*) which are of an entirely different order of magnitude. It should be added at this point that if colloids do possess an osmotic pressure, they must have a slow diffusion pressure. Accordingly they must diffuse, and again it is a question of rate and time.

Meyer[9] has recently summarized the entire field of colloid osmotic pressure insofar as human and animal physiology is concerned, and Peters[10] has summarized the literature of fluid exchange in man with particular reference to colloid osmotic pressure. It would take us too far afield to go into these problems in detail; consequently the reader is referred to the studies of Meyer and Peters for further elaboration.

Dialysis.—It has already been indicated that Graham made extensive use of membranes in studying diffusion of crystalloids and colloids. The membranes which Graham used were either gold beater's skin or animal bladders. Today collodion or cellophane membranes have largely replaced the animal membranes, although in

[8] Bayliss, W., *J. Physiol.*, 50: 23 (1916): *J. Pharmacol.*, 15: 29 (1919)
[9] Meyer, Paul, *Ergeb. d. Physiol.*, 34: 15–111 (1932)
[10] Peters, John P., "Body Water—The Exchange of Fluids in Man," Charles C. Thomas, Baltimore, Maryland (1935)

certain instances animal membranes, such as sheepskin, are advantageous. The animal membranes have the advantage of changing the sign of their charge in solutions with varying acidity or alkalinity. Thus one is able to dialyze through either positively or negatively charged membranes, whereas the vegetable membranes are in general negatively charged. The viscose sausage casings can be secured in various sizes and have, in general, a decided advantage over collodion tubes in that they do not shrink so greatly on drying. It should be emphasized again that the separation of colloids from crystalloids by dialysis is a relative phenomenon and depends upon the difference in the diffusion coefficients of the various components in the system. In a short period of time relatively small amounts of the more finely divided colloids will pass through the membrane, but with extended periods of time certain colloid systems will show appreciable diffusion. This is particularly true of the protein class known as albumins, and in order to dialyze lactalbumin free from crystalloids it is sometimes necessary to use a specially dense membrane so as to inhibit the diffusion of the small lactalbumin micelles.

Perstillation and pervaporation.—Kober[11] has described an important technic which appears to have been rather generally overlooked. He notes that if one encloses liquid in a collodion (probably cellophane would be preferable) bag and suspends such a bag over a free flame or an electric heater, one has in reality a ball of water suspended in air with evaporation possible on all surfaces. He notes that under these conditions it is practically impossible to raise the liquid inside of the bag to the boiling point and that evaporation is extremely rapid. This phenomenon he calls pervaporation. If the liquid contains both crystalloids and colloids, the crystalloids will diffuse through the membrane with the water and remain after the evaporation of the water on the outside of the membrane in crystal form completely free from colloidal contaminants which will remain inside of the membrane.

Ultrafiltration.—Ultrafiltration is defined as filtration through a gel or membrane using either vacuum or preferably pressure. In this way one can concentrate colloidal sols or one can secure the colloid-free dispersions medium without dilution such as takes place in dialysis. The older studies of ultrafiltration are largely due to Bechhold.[12] Ultrafilters are usually cloth, filter paper, porous porcelain,

[11] Kober, P. A., *J. Am. Chem. Soc.*, 39: 944–948 (1917)

[12] Bechhold, H., "Colloids in Biology and Medicine," translated by Jesse G. M. Bullowa, D. Van Nostrand Company, New York (1919)

alundum, sintered glass, etc., membranes impregnated with some colloid gel, such as collodion, or gelatin which has been hardened by formaldehyde or bichromate, etc. Cellophane membranes have recently come into rather general use, although they do not provide a graded series of pore size. Various workers have shown how a graded series of membranes can be prepared. Thus Eggerth[13] utilizes membranes which are prepared from collodion sols containing various percentages of alcohol and ether in order to prepare ultrafilters with a rather wide range in pore size. Krueger and Ritter[14] use various concentrations of collodion to prepare graded ultrafilters, and Schoep[15] mixes castor oil in various proportions with collodion to produce the same effect. Bauer and Hughes[16] have described a very usable apparatus for carrying out ultrafiltration studies.

The standardization of ultrafilters has been determined by various technics. Thus Krueger and Ritter (*loc. cit.*) standardized their ultrafilters by (1) the rate of water flow through the membrane under constant pressure or (2) testing colloidal sols containing particles of more or less known size. The pores of the filters which they prepared ranged from 6.60 mμ. to 475 mμ. Lundsgaard and Holbøll[17] tested the porosity of their ultrafilters by measuring the rate of diffusion of glucose through a membrane under standard conditions. They found that the coefficient of diffusion for glucose for a given membrane is given by the formula

$$\Delta = \frac{1}{t} \log \frac{C_1}{C_1 - 2C_2} \tag{9}$$

where Δ = the diffusion coefficient;
C_1 = the original concentration of glucose in the inner liquid;
C_2 = the increase in glucose concentration in the outer liquid in time (t).

The above methods of testing for size in ultrafilters sound very simple, and it would accordingly appear to be easy to determine pore size. However, a warning may be inserted at this point, that there are often other factors involved than merely pore size. Electrical effects on the walls of the pores, surface tension effects and probably a

[13] Eggerth, A. H., *J. Biol. Chem.*, 48: 203-221 (1921)
[14] Krueger, A. P., and Ritter, R. C., *J. Gen. Physiol.*, 13: 409 (1930)
[15] Schoep, A., *Kolloid* Z., 8: 80–87 (1911)
[16] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 18: 143–162 (1934)
[17] Lundsgaard, C., and Holbøll, S. A., *J. Biol. Chem.*, 68: 439 (1926)

number of other phenomena affect the measurements. Thus Brinkman and Szent-Györgyi[18] in studying surface tension effects found that a membrane which retained hemoglobin completely under a pressure of 3 atmospheres allowed the hemoglobin to pass freely, if a dilute solution of sodium oleate had been previously passed through the membrane. The hemoglobin sol which had passed the oleate-treated membrane would not later pass a similar membrane not so treated, so that the hemoglobin particle size had not been altered by passage through the membrane. Other surface tension depressants and certain physiologically active drugs altered the apparent permeability of the membranes, and no good theory has been proposed to account for their behavior. Similarly Mulvania[19] found that the pH of a solution containing the virus of tobacco mosaic determined whether or not the virus would pass a given membrane, and Varney and Bronfenbrenner[20] found that certain bacteria would not pass specific filters. If, however, certain sterile solutions were first passed through the filters, then the bacteria passed through the pores. Just what factors were involved in the above instances we do not know, but they illustrate the importance of surface forces.

Kramer[21] has likewise shown the importance of the electrical charge on the membrane. Kramer points out that the ordinary bacteriological filters are composed largely of silicates and are negatively charged. He prepared similar filters of plaster of Paris containing an excess of lime and showed such filters to be positively charged. Utilizing two sols of colloidal dyestuffs, Congo red which is negatively charged and Victoria blue which is positively charged, he demonstrated that the Congo red passed through the negatively charged filters but was retained by the positively charged filters, whereas the reverse behavior was shown by the positively charged Victoria blue. He furthermore showed that the viruses of smallpox and rabies, as well as bacteriophage, were retained by the positively charged filters, and he definitely raises the question as to the importance of electrical charge at the interface as compared to what has been generally regarded as simply pore size.

Ultrafilters have been largely used to determine particle size in

[18] Brinkman, R., and Szent-Györgyi, A. v., *Biochem. Z.*, 139: 261–269 (1923); 139: 270–273 (1923)

[19] Mulvania, M., *Phytopath.*, 16: 853–871 (1926)

[20] Varney, P. L., and Bronfenbrenner, J., *Proc. Soc. Exp. Biol. Med.*, 29: 804–806 (1932)

[21] Kramer, S. P., *J. Gen. Physiol.*, 9: 811–812 (1926)

colloid systems and of biochemical and biological preparations. Krueger, Howitt, and Zeilor[22] found the virus of equine encephalomyletis to be approximately 500 mμ. in diameter which is about the same size that Olitsky and Boëz[23] found for the virus of human encephalomyletis. These sizes are much larger than those which have been generally obtained for the virus of tobacco mosaic which is usually accepted as having a particle size about 30 mμ. in diameter. Ferguson[24] has brought together in a general discussion much of the literature dealing with the particle size of biological units. He lists eighty-three references in his paper.

Vividiffusion.—Abel and coworkers[25] have applied the technic of dialysis and ultrafiltration to the problem of determining diffusible constituents in the blood stream of the living animal. A canula is inserted in the carotid artery and another canula in the femoral vein. The blood is led out of the artery, passes through a series of collodion dialyzing tubes and back into the animal's body through the canula in the vein. An anti-coagulant (hirudin from the leech) was previously injected so as to prevent clotting of the blood in the collodion tubes. In this way Abel made the first positive demonstration that amino acids circulate freely in the blood stream following the ingestion of a protein diet. The free amino acids dialyzed from the blood stream through the collodion tubes into the external liquid from which they were recovered in quantity and identified. Unfortunately this pioneer work of Abel was interrupted during the World War due to his inability to secure a supply of hirudin, and was not resumed at a later date. The technic offers promise of interesting and valuable contributions to the physiology of digestion and metabolism. It has sometimes been referred to as an artificial and non-specific kidney.

Electrodialysis.—Electrodialysis may be defined as dialysis under the influence of electrical pressure. It is better adapted to the removal of electrolytes. In many instances ions of electrolytes are held on surfaces with great tenacity and cannot be removed by simple dialysis but will migrate under the influence of an electrical potential. Presumably such adsorption complexes have a decomposition voltage similar to that possessed by ordinary electrolytes. Electrodialysis has been used extensively in the purification of proteins, carbohydrates, soil colloids, etc.

[22] Krueger, A. P., Howitt, B., and Zeilor, V., *Science*, 77: 288 (1933)
[23] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 45: 673 (1927)
[24] Ferguson, John H., *J. Phys. Chem.*, 36: 2849–2861 (1932)
[25] Abel, J. J., Rowntree, L. G., and Turner, B. B., *J. Pharm. Exper. Therap.*, 5: 275–316 (1914)

Electrodialysis and Electro-ultrafiltration.—Bechhold and Rosenberg[26] have described an apparatus whereby one can use the electrical current for both dialysis and filtration. For sols containing negatively charged particles a perforated cathode is placed under the ultrafilter and the anode is placed in a collodion or cellophane bag in the liquid above the filter. When the current is turned on the ions of the electrolytes migrate out of the sol toward the anode or cathode respectively, and at the same time there is a streaming of the dispersions medium through the ultrafilter toward the cathode (electroendosmosis), thus bringing about the electro-ultrafiltration. In this manner the sol can be purified and concentrated simultaneously.

Viscosity and Plasticity.—Viscosity and plasticity studies are extremely valuable technics in the study of lyophilic systems. Poiseuille in 1847 enunciated the fundamental principles governing viscous flow. The Poiseuille formula is

$$\eta = \frac{\pi r^4 Pt}{8lV} \tag{10}$$

where η = the coefficient of viscosity;
r = the radius of the capillary through which flow is taking place;
P = the hydrostatic pressure;
t = the time in seconds;
l = the length of the capillary in centimeters;
V = volume of the liquid flowing through the capillary in time (t), all units expressed in centimeter gram seconds.

The unit of viscosity is the poise which is defined in cgs units as the force (1 dyne) which, when exerted on a unit area between two parallel planes one square centimeter in area and one centimeter apart, produces a difference in streaming between the two planes of one centimeter velocity per second. It will be noted that this is an absolute definition in that there is no specific reference standard. Water at 20° C. has a viscosity of 1.005 centipoise.

It will be further noted from formula (10) that there is a direct proportionality between the pressure which is applied and the rate of flow. Thus a truly viscous flow diagram is a straight line passing through the point of origin when plotted with rate of flow and pressure as the two axes. Such a plot is a test for truly viscous flow. True solutions and pure liquids as a rule exhibit true viscosity. Similarly

[26] Bechhold, H., and Rosenberg, A., *Biochem. Z.*, 157: 85–97 (1925)

when relative viscosity is plotted against concentration, one obtains for reasonably dilute true solutions an approximately straight line which passes through the point of origin.

When, however, we turn to colloidal systems we frequently encounter abnormal behavior. Most lyophobic sols in the concentrations which are usually obtainable show more or less the viscosity of the dispersions medium as indicated by the line in Fig. 6. Lyophilic sols on the other hand show a curvilinear relationship for viscosity plotted against concentration as indicated diagrammatically by the curve in Fig. 6. Many lyophilic systems, even in low concentrations, do not show true viscosity as tested by the simple viscosity diagram where the flow curve intersects the pressure curve at the point of origin. Instead they require that a definite force shall be applied before flow begins. In Fig. 7 the line AB shows what is to be anticipated if true viscous flow occurs, whereas the line CD indicates diagrammatically what occurs with many lyophilic systems. Such behavior has been designated as plasticity. It will be noted that the line AB can be defined by two constants, whereas plasticity involves three constants, one of which (AC in Fig. 7) is known as the yield value, *i.e.*, the force which must be applied before any flow begins. It is sometimes spoken of as the force of deformation, and it indicates that the system possesses some sort of structure. In reality it is highly probable that those lyophilic systems which possess a yield value are actually gels rather than sols, and probably the best definition of a gel is that of a colloid system which when tested by viscosity technic is found to possess a yield value.

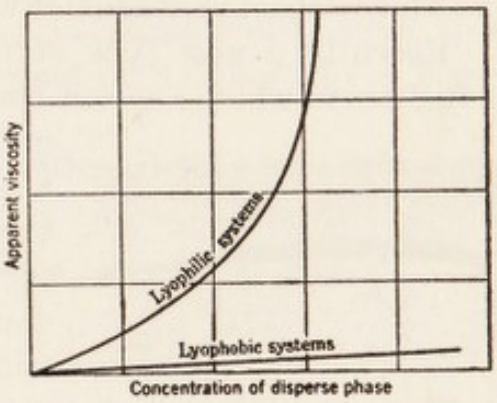


FIG. 6. A diagrammatic representation of the viscometric behavior of lyophilic and lyophobic colloid systems

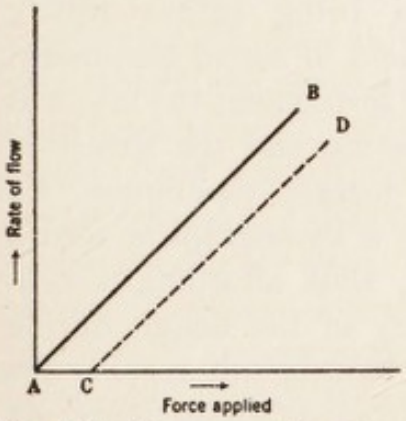


FIG. 7. A diagrammatic representation of the differentiation of viscous and plastic flow

Various types of instruments have been devised for studying solutions and colloid systems by viscosity technics. Among the most commonly used are the ordinary Ostwald glass capillary viscosimeter and tortion viscosimeters of various types. Hatschek[27] seems to have described one of the first forms of tortion viscosimeter. An instrument commonly used in industry, which employs the tortion principle, is the MacMichael viscosimeter which has been described by Herschel[28].

Recently a new type of technic has been adopted by certain industries in which a power-consumption meter has been attached to moving blades, thus recording the power requirement necessary to move the blades through a plastic mass. Thus Bailey[29] attached a micro-watt hour meter to a small dough-mixing machine driven by an electric motor and measured the power consumption necessary to turn the blades of the mixing machine for 100 revolutions in bread doughs. Dieterich[30] reports a somewhat similar set-up to measure plasticity changes which occur in rubber during the course of milling, and the Brabender farinograph, employing a somewhat similar principle, is being rather widely adopted in the field of cereal chemistry. In this particular instrument the motor is mounted free-floating and the thrust of the blades in the dough-mixing chamber rotates the motor. An arm attached to the motor frame draws on a kymograph a record of the thrust which the blades encounter, thus affording a continuous record of changes in the consistency of the dough during the process of mixing. Fig. 8 shows such kymograph records for several representative bread doughs made from different flours. At the top is the curve drawn by flour A which "develops" its gluten after a few minutes of mixing, and with continued mixing maintains its plasticity curve at a high level for a long period of time, only gradually breaking down with long continued mixing. In the middle curve a somewhat greater consistency is initially developed than in

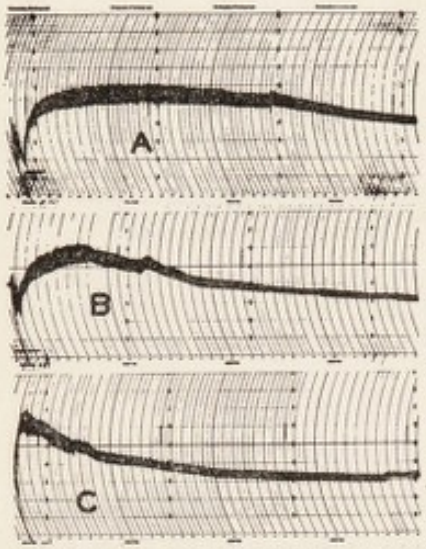


FIG. 8. Brabender farinograph (plasticity) curves of three wheat flours of widely differing characteristics

[27] Hatschek, E., *Kolloid Z.*, 12: 238 (1913)
[28] Herschel, W. H., *J. Ind. Eng. Chem.*, 12: 282–286 (1920).
[29] Bailey, C. H., *J. Rheology*, 1: 429–432 (1930)
[30] Dieterich, E. O., *Ind. Eng. Chem.*, 21: 768 (1929)

the case of flour A. Here, however, the consistency rapidly falls off with continued mixing; in other words, the "structure" of the gel is much more readily destroyed. In the bottom curve there is a sudden development of high consistency followed by an equally sudden and drastic breakdown. Flour A is typical of the high grade northern spring wheats and would be a good bread flour. Flour C is so sensitive that the baker would have to use extreme precautions in order to produce an acceptable loaf. It is a typical pastry flour. Flour B is intermediate, and its characteristics could be produced by a blend of flour A and flour C.

Probably at least a part of the deviation of the viscosity curves of the lyophilic colloids from a straight-line relationship is due to the solvation or hydration of the micelles. Thus the apparent concentration is much lower than is the actual concentration, in that a part of the dispersions medium has been removed from the solvent and becomes a part of the solute. This intimate relationship between the solvent and solute has led Wo. Ostwald[31] to state that there are at least eight additional factors (aside from concentration and temperature) that must be considered and controlled when viscosity studies are made on lyophilic systems. For truly viscous solutions straightline relationships are obtained by the rate of flow and pressure, if concentration and temperature are held constant. The eight additional factors which Ostwald notes are (1) degree of dispersion, (2) solvation, (3) electrical charge, (4) previous thermal treatment, (5) previous mechanical treatment, (6) the presence or absence of other lyophilic colloids, (7) the presence or absence of electrolytes including changes in hydrogen-ion concentration, and (8) the age of the sol. To these we should probably add also (9) the rate of shear. Probably most of these affect either the degree of dispersion or the solvation.

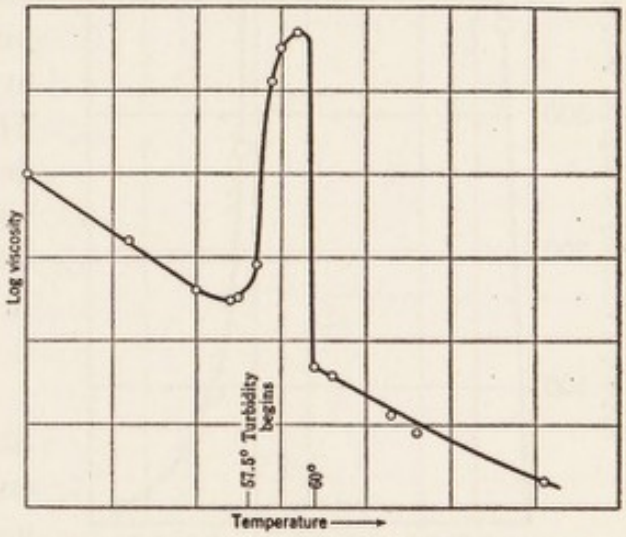


FIG. 9. The viscosity-temperature curve of an egg albumin solution (From Wo. Ostwald)

[31] Ostwald, Wo., *Trans. Faraday Soc.*, 9: 34–46 (1913)

Occasionally rather unexpected effects are noted in the viscosity studies of lyophilic systems, so that the prediction as to the viscosity behavior of any specific lyophilic system is extremely difficult. For example, Fig. 9, taken from the paper by Wo. Ostwald (*loc. cit.*) shows the viscosity curve of egg albumin plotted against temperature. It will be noted that there is a considerable portion of this curve which is characterized by a straight-line relationship but that this straight-line relationship is interrupted at 57.5° C. and resumed above 60° C. Accordingly if no measurements had been made in the relatively narrow range between these two temperatures, one might very well have been justified in drawing a straight-line over the entire temperature range. This straight line is in reality the decrease in viscosity of the water which is the dispersions medium. The break in the straight line is probably due to the denaturization of the egg albumin.

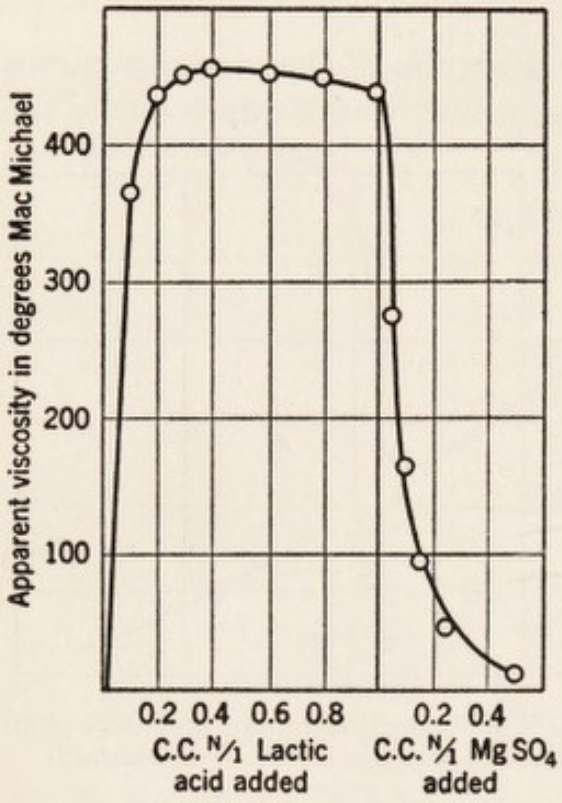


FIG. 10. The effect of hydrogen ion concentration and salt concentration on the apparent viscosity of a flour-water suspension (Data of Sharp and Gortner)

Fig. 10, taken from the work of Sharp and Gortner[32] shows the enormous viscosity changes which may be obtained by a change in the hydrogen-ion concentration or the electrolyte content of aqueous suspensions of wheat flour. Here we are apparently dealing with hydration ⇆ dehydration.

Some years ago, while studying the viscosity of aqueous suspensions of wheat flour, Sharp and Gortner[33] found that straight lines resulted when the logarithm of the apparent viscosity was plotted against the logarithm of the flour concentration. Such a straight line would be defined by the equation

$$\log \text{viscosity} = \log a + b \log C \qquad (11)$$

where b is a constant denoting the slope of the line and is in reality

[32] Sharp, P. F., and Gortner, R. A., *J. Phys. Chem.*, 27: 674–684 (1923)
[33] Sharp, P. F., and Gortner, R. A., *J. Phys. Chem.*, 27: 771–788 (1923)

the tangent of the angle which the line makes with a line drawn parallel to the axis of abscissa and a is a constant denoting the value on the ordinate axis where the straight line cuts the axis of ordinates at unit concentration.

In later work the author found that when the same experiment was reproduced on successive days it was relatively easy to secure reproducible values of the constant b, whereas it was very difficult to make the experimental lines coincide from day to day because of variations in the constant a. Typical experimental data of an experiment replicated on three successive days are shown in Fig. 11. It will be noted that the experimental data on the successive days graph in parallel lines all having the same slope but cutting the ordinate axis at different points. No reason for this anomalous behavior could be found at that time. Later Johnson[34] found that the amount of CO_2 which had dissolved from the air into the distilled water which was used for preparing the suspensions determined in some way the value of constant a. The problem was, however, much more complicated than is indicated by this statement. All viscosity measurements were being run at 30° C. The suspensions of flour were washed with several liters of distilled water in order to remove soluble electrolytes, brought to 30° C., made to volume with water which had been brought to 30° C., acidified with lactic acid to pH 3.0, and viscosities run at that temperature and hydrogen ion concentration. Such procedure yielded the irregular data shown in Fig. 11. If, however, the distilled water was drawn from the distilled water tank and placed in a container immersed in the constant temperature bath at 30° C. and the CO_2 which it contained was removed by vacuum

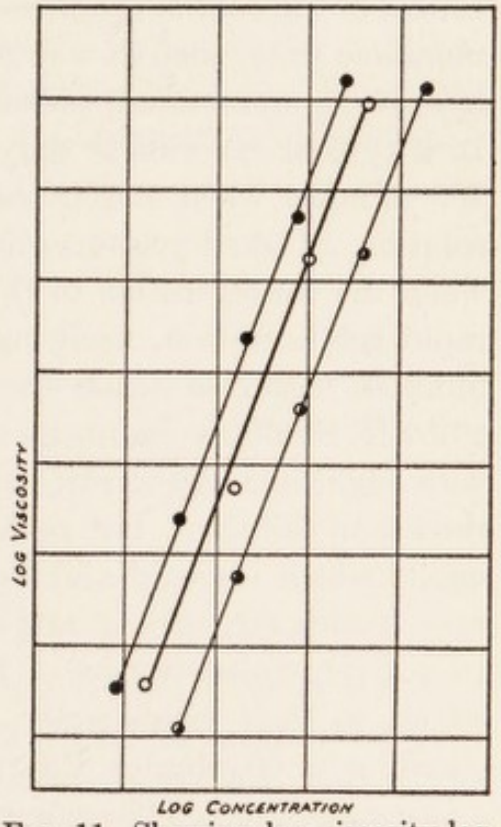


FIG. 11. Showing log-viscosity log-concentration curves for a flour-water suspension as affected by minor experimental conditions

[34] Johnson, A. H., and Herrington, B. L., *Cereal Chem.*, 5: 105–116 (1928)

and aeration and the water was then used as a dilutant for the flour suspensions, superimposible curves could be obtained on successive days. The illustration is inserted here simply to point out what detailed precautions must be taken and how sensitive lyophilic systems may be when studied by viscometric technic.

Equally remarkable were some of the effects of added lyophilic colloids in the studies on the cold gelatinization of starch by Wo. Ostwald and Frankel[35]. These authors point out that starch gelatinizes in the cold in the presence of critical concentrations of certain inorganic salts, such as sodium salicylate, hydrochloric acid, sodium hydroxide, ammonium, potassium, and sodium rhodinate, and urea. In a typical experiment only very slight gelatinization occurred in 260 minutes when starch was added to 1.000 N sodium salicylate solution. Marked gelatinization occurred under the same conditions when the concentration of the solution was 1.025 N, and extremely rapid gelatinization, resulting in the formation of a gel within 115 minutes, occurred when the sodium salicylate concentration was 1.050 N. Similarly enormous changes in the velocity of gelatinization with slight changes in salt concentration were found for the various rhodinate solutions, but perhaps the most striking series of experiments which Ostwald and Frankel record are those which are concerned with mixtures of raw starch from different botanical sources. In a given concentration of KCNS raw potato starch yielded a gel which, in their viscosimeter, had a viscosity of 1000 seconds after standing for 60 minutes. Raw maize starch, on the other hand, showed a viscosity of only 142 seconds after standing for 260 minutes. If now a mixture of 90 per cent of potato starch and 10 per cent of maize starch was studied under the same identical conditions, they found that this mixture in 60 minutes showed a viscosity of only 50 seconds, and in 260 minutes a viscosity of 254 seconds. Thus, mixing 10 per cent of maize starch with 90 per cent of potato starch yielded a preparation which behaved from the viscosity standpoint almost exactly like the pure maize starch. No theory at present explains such an unexpected effect, although in all probability it is in some way associated with the water relationships.

Various formulas have been proposed for applying viscosity data

[35] Ostwald, Wo., and Frankel, G., *Kolloid Z.*, 43: 296 (1927)

to lyophilic colloid systems. Einstein[36] proposed the formula

$$\eta = \eta_0 (1 + K\phi) \tag{12}$$

where η = the viscosity of the lyophilic sol;
η_0 = the viscosity of the dispersions medium;
ϕ = the volume of the system occupied by the disperse phase;
K = a constant.

In the original paper Einstein suggested that the value of K approximated unity. Later he[37] suggested that the value of the constant approximated more nearly 2.5. Hatschek[38] suggested a value for the constant of the Einstein equation of 4.5 and later[39] suggested a somewhat different equation of

$$\frac{\eta}{\eta_0} = \frac{1}{1 - \sqrt[3]{\phi}} \tag{13}$$

This formula he applied to rather concentrated suspensions of blood corpuscles and found that it yielded fairly satisfactory results. Recently Kunitz[40] proposed a somewhat different formula of

$$\frac{\eta}{\eta_0} = \frac{1 + 0.5\phi}{(1 - \phi)^4} \tag{14}$$

and applied this formula to sugar solutions, Oden's sulfur sols, and sols of glycogen, casein, and rubber dispersed in organic solvents. For glycogen sols varying in concentration from 20 to 40 per cent he found the specific volume (ϕ/C) to vary from 1.16 to 1.25, whereas, using the Hatschek formula, the variation was from 1.86 to 2.21. The specific volume of sucrose in concentrations ranging from 1 to 21.7 per cent varied only from 0.60 to 0.63 when determined by the Kunitz formula, whereas they varied from 1.04 to 1.69 when determinded by the Einstein formula. Casein sols in concentrations ranging from 4.35 to 9.39 per cent showed a constant specific volume of 5.5 when determined by the Kunitz formula and a range of specific volume from 8.04 to 9.72 when determined by the Hatschek formula.

[36] Einstein, Albert, *Ann. Phys.*, 19: 289 (1906)
[37] Einstein, Albert, *Ann. Phys.*, 34: 591 (1911)
[38] Hatschek, E., *Kolloid Z.*, 7: 301 (1910)
[39] Hatschek, E., *Kolloid Z.*, 27: 163 (1920)
[40] Kunitz, M., *J. Gen. Physiol.*, 9: 715–725 (1926)

In a later paper Kunitz, Anson, and Northrop[41] measured the hydration, molecular weight, and molecular volume of proteins in solution. The osmotic pressure was found by direct measurements, thus securing particle weight. The size of the particle was obtained by measuring the rate of diffusion, and from this the density of the particle in solution was determined. The difference between this value and the dry density enabled the authors to calculate the degree of hydration. Then the degree of hydration was determined independently by means of viscosity measurements, using the Kunitz formula. In the case of hemoglobin the particle weight was determined as approximately 67,000, the radius of the particle as 2.73 mμ, and the hydration from these data was found to range from 0.00 to 0.14 grams water per gram protein. The hydration from viscosity technic was 0.13 grams water per gram protein. Similarly isoelectric gelatin showed a particle weight of 61,500, a particle radius of 5.4 mμ, and a hydration from these data of 5.8 grams water per gram protein. The hydration from viscosity data was 5.9 grams water per gram protein. Crystalline trypsin showed a particle weight of 35,000, a radius of the particle of 2.60 mμ, and from these data a hydration of 0.54 grams water per gram protein. Hydration from viscosity data was 0.49 grams water per gram protein. The above representative data indicate a high degree of reliability for the Kunitz formula.

Gortner[42] applied the Kunitz formula to a study of the hydration capacity of starch. Since the formula is a fourth-degree equation, it

TABLE II

Value of Relative Viscosity (η/η_0) *and Volume of Sol.* (ϕ) *Occupied by the Disperse Phase for Plotting the Curve of the Equation* $\eta_r = \frac{1 + 0.5\,\phi}{(1 - \phi)^4}$

ϕ	η_r	ϕ	η_r
0	1.000	60	50.781
10	1.600	62	62.830
20	2.686	64	78.589
30	4.790	66	99.526
40	9.274	68	127.807
42	10.692	70	166.677
44	12.405	72	221.30
48	16.959	74	299.80
50	20.000	76	415.94
52	23.736	78	593.37
54	28.364	80	875.00
56	34.151		

[41] Kunitz, M., Anson, M. L., and Northrop, J. H., *J. Gen. Physiol.*, 17:365–373 (1934)

[42] Gortner, R. A., *Cereal Chem.* 10: 298–312 (1933)

is necessary to plot the theoretical curve on cross-section paper and use this theoretical graph for the determination of the per cent of the sol which is occupied by the disperse phase at the values of viscosity which are experimentally determined. Table II lists the essential data which can be used for plotting the theoretical curve. Table III shows the hydration capacity of starches from various botanical

TABLE III

The Hydration at 25° C. of Starches from Various Botanical Sources[43]
(All samples gelatinized in 2% concentration for one hour at 120° C. viscosities on 1% sols. at 25° C.)

	η_r	ϕ	Volume occupied by one gram
		P.ct.	*Cc.*
Potato (fat extracted, Kahlbaum)	6.63	35.00	35.00
Potato (ordinary)	5.05	30.75	30.75
Meadow-saffron (*Colchicum autumnale*)	3.34	28.33	28.33
Arrowroot (*Maranta arundinacea*)	3.13	22.75	22.75
Cassava (*Manihot utilissima*)	2.72	20.20	20.20
Zedoary (*Curcuma Zedoaria*)	2.59	19.40	19.40
Horse-chestnut (*Aesculus hippocastanum*)	2.47	18.50	18.50
Wheat (*Triticum vulgare*)	2.10	15.50	15.50
Glutinous rice (*Oryza glutinosa*)	1.96	14.50	14.50
Maize (*Zea mays*)	1.60	10.25	10.25
Rice	1.48	8.5	8.5

sources as calculated by the Kunitz formula from viscosity data at 25° C. Table IV shows the hydration capacity of starches from certain wheat sorts determined at 90° C. The interesting thing which is brought out by this table is that the hydration capacity of wheat starch at 90° C. is essentially the same as the hydration capacity at 25° C. (cf. Table III), *i.e.*, in the neighborhood of 15.5 cubic centimeters volume occupied by one gram of the heat-gelatinized starch.

Reference has already been made to the studies of Ostwald and Frankel on the cold gelatinization of starch. Their data did not prove suitable for recalculation by means of the Kunitz formula. However, Mangels and Bailey[44] utilized the cold gelatinization technic for studying the behavior of various wheat starches. Recalculation of certain of their data by the Kunitz formula yields the results which are shown in Tables V-VIII inclusive. It will be observed from these

[43] Viscosity data from Samec, M., Kolloidchemie der Stärke. Theo. Steinkopff. Dresden and Leipsig (1927)

[44] Mangels, C. E., and Bailey, C. H., *J. Am. Chem. Soc.*, 55: 1981–1988 (1933)

TABLE IV

The Hydration at 90° C. of Heat-gelatinized Wheat Starch[45]

Sample number and type	Concentration of starch	Viscosity	Relative viscosity	ϕ	Volume occupied by one gram of starch
	P.ct.	*Centipoise*		*P.ct.*	*Cc.*
9167	2.89	4.0	12.66	44.3	15.33
Hard red winter	3.86	13.0	41.14	57.93	15.01
	4.83	45.0	142.4	68.75	14.23
	5.79	158.0	500.0	77.06	13.31
9168	2.89	4.9	15.51	46.9	16.23
Soft red winter	3.85	16.6	52.53	60.37	15.68
	4.33	30.5	96.52	65.72	15.18
	4.81	56.0	177.2	70.5	14.65
	5.29	104.2	329.7	74.58	14.10
9255	2.87	4.8	15.12	47.1	16.41
Hard red spring	3.83	15.0	47.47	59.34	15.49
	4.31	26.0	82.28	64.42	14.95
	4.79	46.5	147.2	69.13	14.43
	5.74	146.5	464.0	76.63	13.35
9296	2.88	4.5	14.24	45.8	15.90
Soft white spring	3.84	14.0	44.30	58.63	15.27
	4.32	25.0	79.11	64.03	14.82
	4.80	44.5	140.8	68.72	14.32
	5.57	109.0	345.0	74.85	13.44
	5.76	135.5	429.0	76.17	13.22
9297	2.88	5.5	17.41	48.32	16.78
White club winter	3.84	20.0	63.29	62.10	16.17
	4.80	70.5	223.1	72.07	15.01
	5.28	132.0	417.7	76.03	14.40
	5.57	188.0	595.0	78.10	14.02
9327	2.88	4.2	13.29	44.95	15.61
Hard red spring	3.84	14.5	45.89	59.00	15.36
	4.79	50.0	158.2	69.65	14.54
	5.75	172.0	544.3	77.56	13.49
9328	2.85	6.2	19.62	49.8	17.47
Hard red winter	3.80	18.8	59.49	61.50	16.18
	4.27	33.0	104.4	66.60	15.60
	4.75	57.8	182.9	70.66	14.87
9329	2.87	5.0	15.82	47.14	16.43
Soft red winter	3.83	20.5	64.87	62.28	16.26
	4.31	39.0	123.4	67.75	15.72
	4.79	77.5	245.3	72.70	15.18
	5.27	157.0	496.8	77.05	14.62
9528	2.93	4.8	15.19	46.63	15.91
Marquis	3.91	13.0	41.14	57.93	14.82
	4.40	20.0	63.29	62.05	14.10
	4.89	32.0	101.3	66.34	13.57
	5.86	84.5	267.4	73.30	12.51
9530	2.88	4.9	15.51	46.90	16.28
Durum	3.84	16.2	51.27	60.0	15.63
	4.81	55.0	174.0	70.30	14.62
	5.28	103.0	325.9	74.50	14.11
9716	2.91	4.8	15.19	46.63	16.02
Dicklow Club	3.87	14.0	44.30	58.68	15.16
	4.37	25.0	79.1	64.04	14.65
	4.85	44.5	140.8	68.71	14.17
	5.82	131.0	414.6	75.98	13.05

[45] Viscosity data from Rask, O. S., and Alsberg, C. L., *Cereal Chem.*, 1: 7–26 (1924)

TABLE V

The Hydration (at 30° C.) of Wheat Starch Gelatinized by 0.5M NaOH for 1 Hour at 30° C.[46]

Conc. of starch	Hard red spring			Durum		
	η_r	ϕ	Volume occupied by one gram of starch	η_r	ϕ	Volume occupied by one gram of starch
P.ct.		*P.ct.*	*Cc.*		*P.ct.*	*Cc.*
2.0	58.4	61.3	30.65	39.8	57.6	28.8
1.75	34.2	56.0	32.0	27.5	53.67	30.7
1.50	22.3	51.25	34.10	18.0	48.75	32.5
1.25	12.6	44.25	33.2	11.1	42.50	32.0
1.0	7.1	36.0	36.0	6.7	35.12	35.1
0.75	3.7	25.56	34.0	3.9	26.50	35.3
0.50	2.1	15.50	31.0	2.1	15.50	31.0

TABLE VI

The Hydration of Wheat Starch Gelatinized in a 2% Suspension for 3 Hours at 30° C. by Various Concentrations of NaCNS Solutions[47]

Concentration of NaCNS	η_r	ϕ	Volume occupied by one gram of starch
Molar		*P.ct.*	*Cc.*
1.0	1.1	1.75	0.875
1.2	1.4	7.5	3.75
1.4	1.6	10.25	5.12
1.6	2.0	14.50	7.25
1.8	2.7	20.12	10.06
2.0	4.2	27.75	13.87
2.2	12.2	43.8	21.9
2.4	14.8	46.3	23.15
2.6	16.0	47.3	23.65
2.8	17.9	48.65	24.32
3.0	19.2	49.54	24.77
3.4	26.4	53.2	26.6
3.8	32.0	55.35	27.67
4.2	35.9	56.5	28.25
4.6	28.7	54.15	27.07

tables that cold gelatinization of wheat starch is a decidedly different phenomenon from heat gelatinization, inasmuch as the hydration capacity of the cold gelatinized starches is approximately twice as great as it is for the heat gelatinized starches. Incidentally it is of interest to observe that approximately the same maximum hydration capacity is reached for the various starches irrespective of whether they are gelatinized by solutions of sodium hydroxide, sodium or

[46] Viscosity data from Mangels, C. E., Ph.D. Thesis, University of Minnesota (1932)

[47] Viscosity data from Mangels, C. E., *loc. cit.*

TABLE VII

The Hydration of Wheat Starch Gelatinized in a 2% Suspension for 3 Hours (and for 24 Hours) at 30° C. by Various Concentrations of Sodium Salicylate Solutions[48]

Concentration of sodium salicylate	η_r (3 hrs.)	ϕ	Volume occupied by one gram of starch	η_r (24 hrs.)	ϕ	Volume occupied by one gram of starch
Molar		*P.ct.*	*Cc.*		*P.ct.*	*Cc.*
0.2	1.1	1.75	0.88	1.1	1.75	0.88
0.4	1.1	1.75	0.88	1.1	1.75	0.88
0.6	1.4	7.50	3.75	1.7	11.60	5.80
0.8	2.5	18.75	9.38	3.1	22.50	11.25
1.0	9.4	40.14	20.07	10.2	41.30	20.65
1.4	11.7	43.20	21.60	13.4	45.04	22.52
1.8	17.0	48.00	24.00	21.7	50.95	25.48
2.2	27.5	53.66	26.83	26.8	53.38	26.69
2.4	32.7	55.56	27.78	29.2	54.34	27.17
2.6	35.8	56.50	28.25	—	—	—
2.8	40.9	57.86	28.93	35.0	56.25	28.12
3.0	42.3	58.24	29.12	35.5	56.40	28.20

TABLE VIII

The Hydration of Wheat Starch Gelatinized in a 2% Suspension for 3 Hours at 30° C. by Various Concentrations of Urea Solutions[49]

Concentration of urea	η_r	ϕ	Volume occupied by one gram of starch
Molar		*P.ct.*	*Cc.*
2.5	1.1	1.5	0.75
3.0	1.2	3.5	1.75
4.0	1.4	7.25	3.62
6.0	3.7	25.62	12.81
7.0	8.2	38.12	19.06
8.0	15.8	47.1	23.55
9.0	25.2	52.7	26.35
10.0	29.9	54.6	27.30

potassium rhodinate, sodium salicylate, or urea, although the concentration of the particular reagent causing gelatinization is vastly different in the case of the different chemicals.

If the cold gelatinization data are plotted on the basis of log molarity of the reagent causing gelatinization vs. the log of the volume occupied by one gram of starch, a graph is obtained similar to that shown in Fig. 12. Microscopic observations showed that over the initial viscosity curve one was dealing with the swelling of the intact starch granules. This curve breaks sharply into a second straight line when the granules burst and a clear starch sol is formed. The

[48] Viscosity data from Mangels, C. E., *loc. cit.*
[49] Viscosity data from Mangels, C. E., *loc. cit.*

third straight line which appears at the higher reagent concentration seems to be a dehydration pehnomenon in which the osmotic pressure of the more concentrated reagent solution is competing with the starch granules for the water which is present.

Just why the hydration capacities of starches from various botanical sources differ as widely as is indicated in Table III is a problem which awaits further studies.

The above are only a few representative instances in which viscosity technics have yielded data of value and are given here in

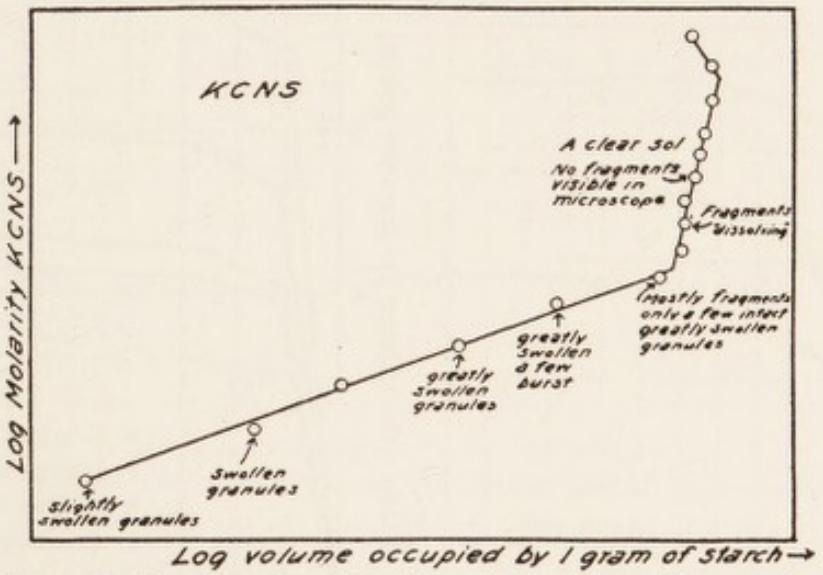


FIG. 12. Showing the behavior of wheat starch granules peptized with various concentrations of KCNS

detail only to illustrate the value of this particular technic. Similar data could have been cited in the fields of hemicelluloses, nitrocellulose, cellulose acetate, rubber, etc., and it should be emphasized again that viscometric technic is of importance second to none in the study of lyophilic colloid systems.

Hysteresis.—It has already been emphasized that time is an important fourth dimension which must be considered in the study of colloid systems. The term, hysteresis, as used by the worker in the field of colloids refers to the effect of past mechanical, thermal, etc., treatment on the present behavior of the colloid system and naturally involves predominantly the effect of time. It has been allegorically spoken of as the "memory of a colloid system," that is, the reflection of the past treatment on present behavior.

While unquestionably lyophobic sols show hysteresis to a certain degree, the effect is most marked in the case of lyophilic gels. Probably

the effect reflects structural changes, changes in particle size, or changes in the degree of solvation. Gortner and Hoffman[50] investigated the rehydration curves of gelatin which had been dried down to approximately 3 per cent moisture content from gels of different initial concentrations. Figs. 13 and 14 show the results which were obtained. The curves in Fig. 14 are particularly significant, inasmuch as in this instance the dried gelatin had been ground and sieved so as to secure uniform sized granules which would pass a 2 mm. sieve

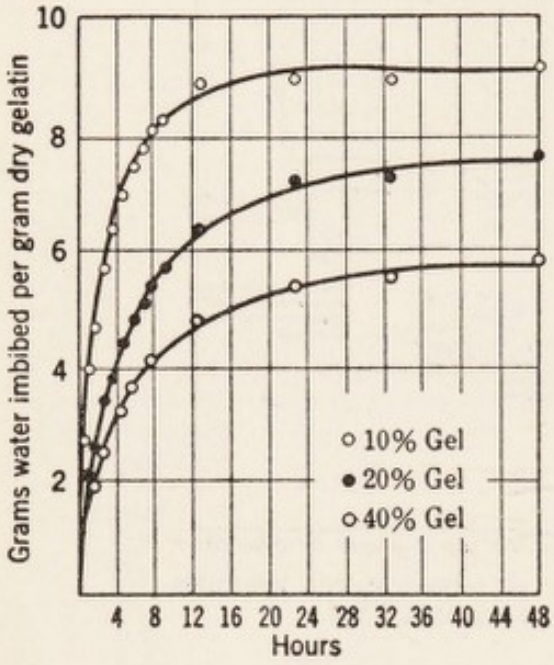


FIG. 13. Showing the effect of initial gel concentration on the subsequent imbibition behavior of gelatin sheets (Data of Gortner and Hoffman)

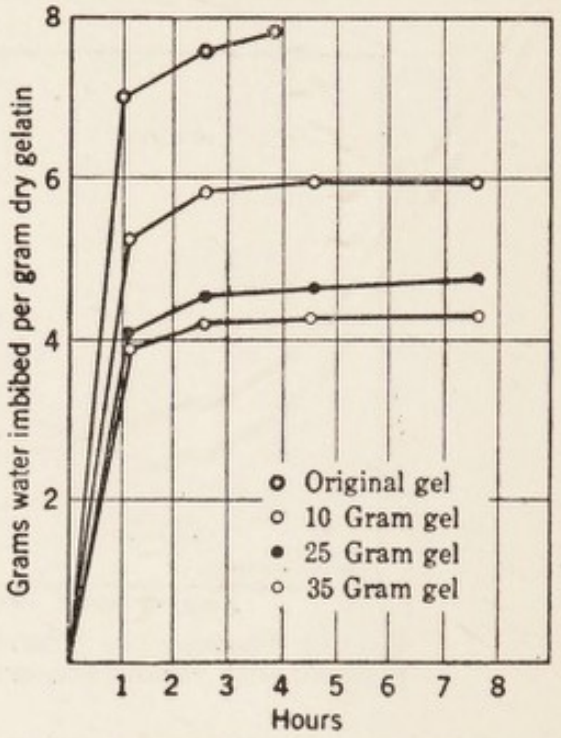


FIG. 14. Showing the effect of initial gel concentration on the subsequent imbibition behavior of uniform sized gelatin granules (Data of Gortner and Hoffman)

and be retained by a 1 mm. sieve. It will be noted that those granules which were derived from the more dilute gel had the higher hydration capacity, whereas those which were derived from the more concentrated gels had progressively lower hydration capacities.

The effect of time and temperature on the behavior of gelatin gels is excellently illustrated by experiments reported by Olsen[51] as shown in Table IX. In this table the striking difference is shown in the second and third series of experiments where the effect of holding

[50] Gortner, R. A., and Hoffman, W. F., *Proc. Soc. Exp. Biol. Med.*, 19: 257–264 (1922); *J. Phys. Chem.*, 31: 464–466 (1927)

[51] Olsen, Aksel G., *J. Phys. Chem.*, 36: 529–533 (1932)

the gels at 0°C. for one hour produces a marked alteration in the properties of the resulting system. One would anticipate the formation of a firmer gel structure at the lower temperature. Obviously, however, that is not the case.

TABLE IX

Showing the Effect of Various Temperature Conditions on the Physical Properties of Gelatin Gels

(9.6 grams gelatin, 2.1 grams tartaric acid, 472 cc. H_2O). The gelatin was dissolved in hot H_2O, brought to 50°C., and treated as indicated. (Data of A. G. Olsen)

Series No.	Treatment	Setting Time	Melting time at 22.5°C. after time indicated			
			6 hrs.	24 hrs.	48 hrs.	72 hrs.
1	Set at 0°C., kept at 0–1°C.	25 min.	5 min.	11 min.	13 min.	——
2	Set at 0°C., kept at 0°C. for 1 hr., then kept at 14.5–15°C.	25 min.	17 min.	74 min.	165 min.	220 min.
3	Set at 14.5–15°C., kept at 14.5–15°C.	270 min.	12 min.	96 min.	231 min.	303 min.

Hysteresis is a characteristic phenomenon of gels. Therefore gel behavior is very difficult to predict or to control, for in addition to knowing the exact composition of the present system, we should also know what the past treatment of the colloid was and this may go back to the treatment that some particular constituent has had in the factory. Again it should be emphasized that the time factor is of great importance. Unquestionably a great many of the divergent results which different workers have reported in the literature on what appear to be comparable series of experiments with colloid gels would be explainable, if one knew the exact history of the systems and the constituents of the systems under investigation.

Syneresis.—Under the term syneresis is included the extrusion of liquid from a gel. A typical example is the serum which extrudes from a blood clot on standing. Wo. Ostwald has stated that syneresis is probably the most characteristic property of a gel and is exhibited to a greater or less degree by all gels in the process of aging. The liquid which is extruded is not simply the pure dispersions medium but rather is a dilute solution of all the constituents present in the gel so that it may be looked upon as a dilute colloidal sol derived from a concentrated colloid system. The "bleeding" of agar slants noted

so often in the bacteriological laboratory and the breaking of custards noted by the housewife are typical examples of the phenomenon.

The fact that syneresis occurs on aging indicates very definitely that gels do not remain in an equilibrium state and accordingly are not amenable to physico-chemical treatment which presupposes the existence of a state of equilibrium. This is a fact which has often been overlooked, *i.e.*, that gels are dynamic and not static colloid systems and that attempts to deal with them as though all constituents were in complete equilibrium are likely to lead to erroneous conclusions. Thus in vapor pressure measurements on gels or concentrated lyophilic sols which from plasticity measurements are found to be actually gels, *i.e.*, they have a yield value, one is very likely to find that the vapor pressure determination reflects the vapor pressure of the liquid of syneresis rather than the vapor pressure of the dispersions medium intimately associated with the underlying gel structure.

Chapter V

ELECTROKINETICS

Freundlich[1,2] has designated certain electrical properties of colloid systems by the term, electrokinetic phenomena, in order to distinguish it from a similar but not identical electrical phenomenon known as the thermodynamic potential which exists at interfaces. Freundlich points out that the electrokinetic phenomena are very closely related to many of the physical properties of colloid systems, such as colloid stability, mutual precipitation, flocculation, adsorption, adhesion between particle and particle and between particle and solute, and the behavior of the colloid system under the influence of an applied E.M.F.

Abramson[3] has recently discussed in detail the historical background, the derivation of the various mathematical formulas, and the application of electrokinetic technic to a great variety of problems. In view of the ready availability of this excellent monograph it would be superfluous to repeat much of the historical background and enter into the involved mathematical derivations. The reader is accordingly referred to this monograph for such essential details. Suffice it to say that the first observation was made by Reuss[4], in 1808. He prepared a voltaic pile consisting of 92 silver rubles and 92 zinc plates separated by cloths moistened with salt solution. This battery was attached by wires to a U-tube 0.25 inches in diameter containing powdered quartz in the bottom of the U, the quartz being covered with water. He noted that on applying the current the water level rose approximately 9 inches above the quartz layer on the side in which the negative electrode was inserted and fell correspondingly on the side attached to the positive pole. This observation of Reuss was only eight years after the experiment of

[1] Freundlich, H., "Colloid and Capillary Chemistry," translated by H. S. Hatfield, E. P. Dutton and Company, New York, 1926

[2] Freundlich, H., "New Conceptions in Colloid Chemistry," E. P. Dutton and Company, New York, 1927

[3] Abramson, H. A., "Electrokinetic Phenomena," American Chemical Society Monograph, No. 66, Chemical Catalog Company, New York, 1934

[4] Reuss F. F., *Mem. Soc. Imp. Nat. Moskou*, 2: 327 (1809)

Nicholson and Carlyle (1800) who demonstrated the decomposition of water by the galvanic current and grew out of a repetition of that experiment. Reuss also demonstrated that a block of moist clay could act as a diaphragm.

In 1816, Porret further studied the phenomenon using both sand membranes and animal (bladder) membranes. He found that water migrated through bladder membrane toward the negative pole. He also coated filter paper with egg white, coagulated the egg white by heat and then showed a migration with this membrane similar to that which had occurred with the bladder membrane, and in the same paper suggested that minute electric currents may have a great influence in regulating the flow of water through minute pores in living tissues, adding, "Is not this electrofiltration jointly with electrochemical action in constant operation in the minute vessels and pores of the animal system?"

Becquerel, about 1830, observed transport of clay particles in an electric field, and in 1852 Wiedemann[5] showed that the amount of liquid flowing through a porous diaphragm was proportional to the E.M.F. which had been applied. The next major contribution was that of Quincke[6] who extended the observation of Wiedemann and measured the rate of streaming and the direction of streaming for membranes of various materials. He found that the direction of streaming was determined by the material of the membrane and might be toward either the positive or the negative pole. He also observed that the rate of streaming was greatly influenced by the nature of the material. He furthermore showed that since an E.M.F. would produce streaming, then conversely streaming would produce an E.M.F. He then turned to the study of suspended particles and showed that most suspended particles were negatively charged in water and positively charged in turpentine, and lastly, to account for the electrical effects, he developed a theory of a charge of one sign on the wall of the capillary and of an opposite charge in the liquid bathing the wall of the capillary. This is apparently the first suggestion of an electric double layer.

Here the theory remained until the epoch-making contributions of Helmholtz[7] in 1879. Helmholtz developed the theory of the double layer from the theory of a condenser. If Q is the charge and V_1 and

[5] Wiedemann, G., *Pogg. Ann.*, 87: 321 (1852)
[6] Quincke, G., *Pogg. Ann.*, 107: 1 (1859)
[7] Helmholtz, H., *Wied. Ann.*, 7: 337–381 (1879)

V_2 are the potentials on two plates, and K is a constant, then $V_1 - V_2$ is the difference in potential and

$$Q = K(V_1 - V_2) \tag{15}$$

the Capacity (C) at constant Q is

$$C = \frac{SK_1}{4\pi d} \tag{16}$$

where S = surface area;
d = distance between plates;
K_1 = a constant.

It will be noted that the capacity is directly proportional to the area and inversely proportional to the distance between the plates. Accordingly the capacity can be increased by increasing the area or by decreasing the distance which separates the opposite electrical charges. A small thickness and a great difference in potential gives a large ratio of $\frac{V_1 - V_2}{d}$ which is a measure of the mean electrical intensity of the field. Similarly the energy of the field (W) can be defined as

$$W = \frac{KS(V_1 - V_2)^2}{8\pi d} \tag{17}$$

Again the energy is directly proportional to the charge and inversely proportional to the distance.

The above elementary treatment of condensers would not have been introduced at this point except for the fact that Helmholtz in his derivations made certain assumptions, and these assumptions have until recently been overlooked or have been considered as fixed quantities. Thus he states that for simplifying the problem of a varying charge or potential, d (the distance between the plates) may be considered as fixed and equal to the diameter of one molecule. This statement was later interpreted as Helmholtz stating that d was a fixed quantity and equal to one molecular diameter. It remained for Gouy[8], in 1910, to point out that d varies and in fact may vary enormously. Gouy calculates that at the surface of 0.10 N NaCl solution d approximates 0.96 mμ, for a 0.001 N NaCl solution d approximates 9.6 mμ, and for pure water d approximates 1010 mμ,

[8] Gouy, M., *J. de Phys.*, [4] 9: 457 (1910)

assuming that the dielectric constant of 80 for water exists unchanged in the three instances.

The Distinction Between the Electrokinetic and the Thermodynamic Potentials. The thermodynamic potential is known by various designations, some of which are the boundary potential, the membrane potential, the concentration potential, the Nernst potential and the epsilon potential. Mathematically it is stated as

$$\epsilon = -\frac{RT}{nF} \log_e \frac{C_1}{C_2} \tag{18}$$

where C_1 and C_2 are two different concentrations and F is the Faraday. It will be noted that the potential can be calculated from the concentration gradient and must hold if thermodynamic reasoning holds. It is the difference in potential between two points well within the body of the two phases. For example, in the case of a block of gelatin immersed in a salt solution, it is the potential which exists between a point well within the interior of the block of gelatin and another point well within the body of the salt solution.

The electrokinetic potential or the zeta potential on the other hand is the difference in potential across the Helmholtz double layer which exists at the boundary between two phases, or rather it is the difference in potential which exists between the immovable liquid layer attached to the surface of the solid phase and the movable liquid layer immediately adjacent in the liquid phase. Thus the electrokinetic potential lies within the region governed by the thermodynamic potential and may actually be a part of the system within the thermodynamic potential, but at the same time the electrokinetic potential can vary both in sign and in magnitude from the thermodynamic potential. The behavior of the electrokinetic potential insofar as sign and magnitude are concerned depends upon what is happening in an extremely thin layer, probably rarely exceeding a few millimicrons in cross-section at an interface boundary. That the electrokinetic potential and the thermodynamic potential are entirely distinct phenomena is beautifully shown by the experiments of Freundlich and Ettisch[9] who measured both electrokinetic and thermodynamic potentials of identical samples of glass in contact with identical salt solutions. Fig. 15 shows the data which they obtained. It will be noted that in the case of the thermodynamic poten-

[9] Freundlich, H., and Ettisch, G., *Z. physikal. Chem.*, 116: 401–419 (1925)

tials we are dealing with essentially straight-line relationships as one would anticipate with a phenomenon directly related to changes in concentration, whereas in the case of the electrokinetic potential the form of the curves is unpredictable from the known physical properties of the systems.

Perhaps it is pertinent at this point to indicate that Loeb[10] in his studies of protein systems was dealing almost entirely with technics which measured the thermodynamic potential. It is accordingly not surprising that he found that protein systems in contact with acid,

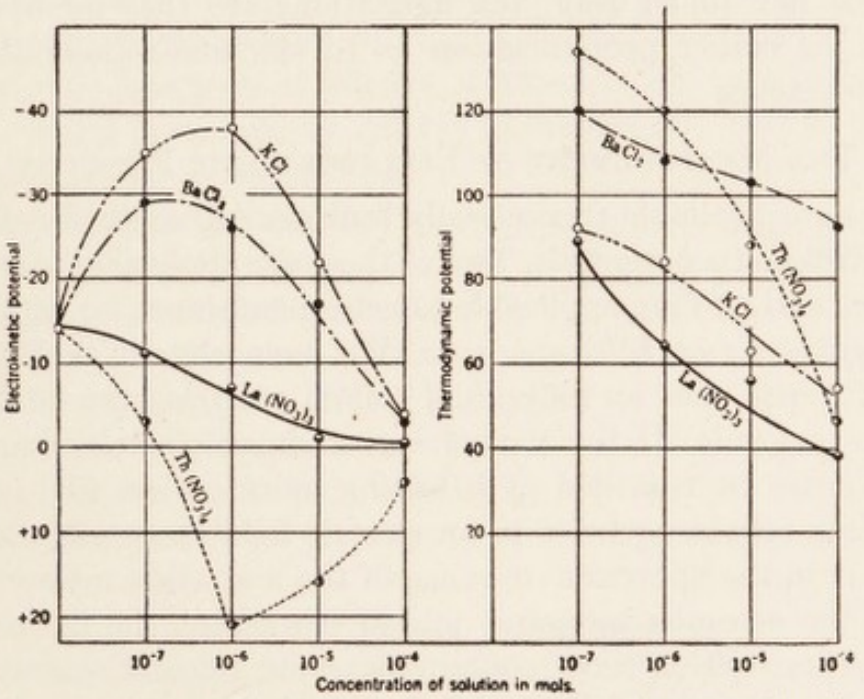


FIG. 15. A comparison of the effect of salts on the electrokinetic and the thermodynamic potentials at a glass-solution interface (Data of Freundlich and Ettisch)

basic, and salt solutions showed the expected electrical behavior. Such agreement with theory only indicated that the systems were being studied at equilibrium and did not indicate by what mechanism that equilibrium had been brought about. Hill[11] has rightly pointed out that the thermodynamic potential must vary with hydrogen-ion concentration in the manner that Loeb observed and that such findings only demonstrate that equilibrium has been reached in the system under investigation and that such readings tell us nothing as to how the equilibrium has been attained.

[10] Loeb, J., "Proteins and the Theory of Colloid Behavior," McGraw Hill Book Company, New York, 1922; also numerous papers in the Journal of General Physiology.

[11] Hill, A. V., *Proc. Roy. Soc.*, A, 102: 705 (1923)

In a few of the papers which Loeb published immediately before his death he began to use electrokinetic technics. In these papers he begins to speak of "aggregates of molecules" in the protein systems as causing deviations from the theory of a molecularly dispersed electrolyte. It is difficult to see any significant difference between "aggregates of molecules" and colloidal micelles, and it is to be greatly regretted that Loeb did not live long enough to pursue to their logical conclusions the experiments which were in progress at the time of his death. Had he been permitted to complete the experiments which he had under way, the indications are that he would have revised his earlier pronunciation as to the non-colloidality of the protein systems.

The Measurement of Electrokinetic Potentials

We have available theoretically four general methods of measuring electrokinetic potentials. Two of these are applicable to suspended particles, and two are applicable to gels, membranes, or capillaries.

Cataphoresis or Electrophoresis. We have already seen from the work of Perrin that an individual colloid particle may be treated as a single molecule. If the particles possess an electric charge, they may likewise be regarded as behaving more or less like individual ions. Thus colloid particles in an electric field show migration rates which are in the approximate range of the migration rates characteristic of the common inorganic ions if we except the hydrogen and hydroxyl ions which show abnormally rapid rates of migration. Unexpectedly, size has but little effect on the migration rate of colloid particles. Presumably this is because a greater size means a larger surface area and a correspondingly greater density of charge. Therefore the effect of size *per se* is largely nullified.

When electric migration studies are carried out with colloid sols, one finds, as might be anticipated, that the particles migrate to the pole possessing the opposite sign from the sign of the charge on the particle. The velocity of migration can be expressed as

$$V = \frac{\zeta E \epsilon}{4\pi\eta} \tag{19}$$

where ζ = the electrokinetic potential;
E = the drop in applied E.M.F. per unit length between the electrodes;
ϵ = the dielectric constant of the medium;
η = the viscosity of the medium.

From this we see that

$$\zeta = \frac{4\pi V\eta}{E\epsilon} \tag{20}$$

The actual experimental conditions for accurately conducting cataphoretic experiments require rather carefully controlled technics in contrast to the qualitative experiments of the earlier workers where simple U-tubes were used. It is rather essential to use non-polarizable electrodes, and precautions must be taken so that electrolytes diffusing from the vicinity of the electrodes do not reach the colloid particles with a consequent alteration in the charge carried by the particles. For biochemical studies micro-cells are largely employed. The cell which is commonly used is known as the Kunitz-Northrop cell[12] although the cell which has been recently described by Bull[13] has some desirable features.

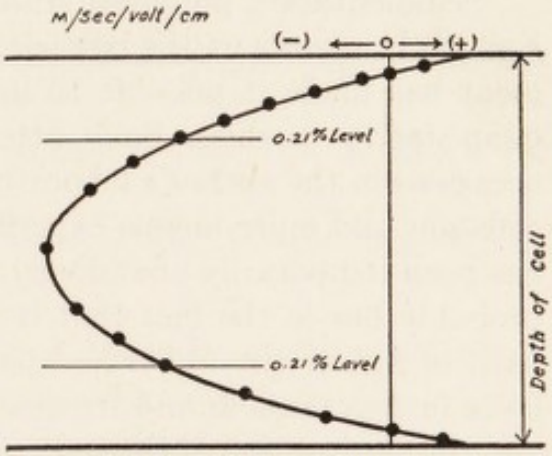


FIG. 16. Electrical mobility in μ/sec./volts/cm. for a flat microcataphoresis cell. Note that the true value is obtained only at the 0.21 per cent level. Also note that close to the walls even the sign may be reversed

A warning should be inserted at this point that there is a varying velocity of migration of the colloid particle in all portions of the cell. This is due to the fact that there is in addition to the cataphoretic migration of the particle toward the pole of the opposite electric sign an electroendosmotic streaming (*vide infra*) along the walls of the capillary in the opposite direction, and this streaming of liquid along the walls of the capillary returns through the center of the capillary, thus producing a retardation or in some cases an actual reversal in sign of the direction of migration of the particle close to the wall and intensifies on the other hand the rate of apparent migration in the center of the capillary. There are only two levels at which true cataphoretic velocity can be determined. In a flat cell these levels lie at 21 per cent of the distance from the top or from the bottom of the cell. For a micro cylindrical cell the levels lie at 14 per cent of the diameter of the capillary. Fig. 16 shows a cataphoretic velocity

[12] Northrop, J. H., and Kunitz, M., *J. Gen. Physiology*, 7: 729 (1924–25)
[13] Bull, H. B., *J. Phys. Chem.*, 39: 577 (1935)

curve for particles in a flat cell. The volume by Abramson is referred to for a discussion of the theory of this behavior.

Sedimentation Potential or the Dorn Effect. This may be regarded as the reverse of cataphoresis and was first observed by Dorn in 1878.[14] If an imposed E.M.F. causes the migration of particles, the particles moving in a liquid should generate an E.M.F. This is the basis of the sedimentation potential.

For non-conducting liquids (or gases) the potentials may be of rather large magnitude. Thus Stock[15] found in excess of 80 volts for powders falling through organic liquids. Probably electrical effects in dust storms where radio aerials often become highly charged and emit sparks, and lightning bolts falling from a clear sky are due to such origins.[16,17]

Sedimentation potential studies are of value in determining the sign of the charge on the particle,[18] but so far no mathematical treatment has made it possible to use the sedimentation potential as a quantitative method. Such attempts have been made on several occasions in the author's laboratories, but it was impossible to secure constant and reproducible experimental data, so that the experiment has been temporarily abandoned. Failure to secure reproducible data probably lies in the fact that it is impossible to control exactly the path of fall of the individual particles so that the particles take a more or less random and irregular path in falling through the liquid and the side motion of the particle together with convection currents in the liquid produce disturbing effects which interfere with a strict reproducibility of conditions.

Electroendosmosis. Electroendosmosis may be defined as the migration of the dispersions medium through a gel (or capillary) under the influence of an imposed E.M.F. One can think of the phenomenon as being due to a fixed charged surface which, if it were free to move, would tend to move toward the pole of the opposite charge. Since, however, the surface is fixed, the dispersions medium which carries the opposite electric charge is dragged past the fixed surface by the electrical attraction. Thus in electroendosmosis the dispersions medium flows through the gel or membrane toward the pole having the

[14] Dorn, E., *Ann. d. Phys.*, 5: 20 (1878); 9: 513 (1880)

[15] Stock, J., *Anzeiger. d. Akad. d. Wiss. Krakau* A (1913) p. 131, and (1914) p. 95

[16] Gortner, R. A., *Science*, 70: 118–119 (1929)

[17] Shaw, P. E., *Proc. Roy. Soc.*, [A] 122: 49–58 (1929)

[18] Bull, H. B., *J. Phys. Chem.*, 33: 656 (1929)

same sign as the charge on the surface of the capillaries of the gel. Formulas which apply to electroendosmosis are for a single capillary

$$V = \frac{r^2 \zeta H \epsilon}{4 \eta l} \tag{21}$$

or

$$P = \frac{2 \zeta H \epsilon}{\pi r^2} \tag{22}$$

or

$$\zeta = \frac{P \pi r^2}{2 H \epsilon} \tag{23}$$

where V = volume of flow;

r = the radius of the capillary;
l = length of the capillary;
P = hydrostatic pressure which is developed due to endosmotic flow;
H = the E.M.F. which is applied across the capillary (or membrane);
and η and ϵ have the same connotation as in the cataphoretic formula.

In these formulas H, the applied E.M.F., has a different connotation from the E of the cataphoretic formula. H in reality is equal to El and is the fall in potential per centimeter multiplied by the length through which the fall takes place.

For a bundle of capillaries of cross-section Q, we have

$$V = \frac{Q \zeta H \epsilon}{4 \pi \eta l} \tag{24}$$

and

$$P = \frac{2 \zeta El \epsilon}{\pi r^2} = \frac{2 \zeta El \epsilon}{Q} \tag{25}$$

and

$$\zeta = \frac{\pi r^2 P}{2\ El \epsilon} = \frac{QP}{2\ El \epsilon} \tag{26}$$

The dimensions of the capillary (r and l or Q and l) can be eliminated from the endosmotic equations providing that the current (i) is kept constant. Equation (24) would then appear as

$$V = \frac{\zeta i \epsilon}{4 \pi \eta \kappa} \tag{27}$$

and Equation (26) as

$$\zeta = \frac{4\pi\eta\kappa V}{i\epsilon} \tag{28}$$

Various workers have dealt with endosmotic flow. Pertinent references are those of Stamm[19] where flow through wood membranes was studied, Strickler and Mathews[20] for the flow of various liquids through filter paper membranes, Fairbrother and Balkin,[21] Fairbrother and Varley,[22] and Fairbrother and Mastin.[23]

Crowther and Haines[24] use the electroendosmotic principle in an attempt to reduce drawbar pull on the plowshare in heavy soils. The plowshare was made the cathode with the anode planted in the adjacent soil. When the current was applied a film of water formed on the surface of the plowshare, thus lubricating the plowshare so that the soil would not come in contact with the metal. These authors note that the reduction in drawbar pull approximately compensated for the increased cost of the electric current and observe that if the cost of gasoline for plowing purposes increased in the future with electrical energy costs remaining constant or decreasing, the technic might prove economically profitable.

In the same paper they reported an experiment in which the energy to move an iron slider across a level soil surface was studied. They note that when the slider was not electrified a very appreciable amount of energy was required to cause a uniform movement. When the slider was made the cathode, the amount of energy required to move it was very markedly decreased, in this case the slider being lubricated by a film of water. If, on the other hand, the slider was made the anode, the energy required to move it was sharply increased, for now the slider was in contact with a much drier soil than originally. They furthermore noted that below a moisture content of 14 per cent in the particular soil with which they were working there was no further endosmotic flow, indicating that the moisture content of this soil below the 14 per cent level was held by different forces than the balance of the water above this level.

Briggs[25] has considered various industrial applications of electroendosmosis.

[19] Stamm, A. J., *Coll. Symp. Monograph*, 4: 246 (1926); 5: 361 (1928)
[20] Strickler, A., and Mathews, J. H., *J. Am. Chem. Soc.*, 44: 1647 (1922)
[21] Fairbrother, F., and Balkin, M., *J. Chem. Soc.*, (1931) 389–403
[22] Fairbrother, F., and Varley, H., *J. Chem. Soc.*, (1927) 1584
[23] Fairbrother, F., and Mastin, H., *J. Chem. Soc.*, 125: 2319 (1924)
[24] Crowther, E. M., and Haines, W. B., *J. Agr. Sci.*, 14: 221–231 (1924)
[25] Briggs, T. R., Second Report on Colloid Chemistry, *Brit. Assoc. Advancement Sci.*, pp. 26–52 (1918)

In the author's laboratories attempts were made to use endosmotic flow for studying the electric behavior of the forces at the surface of cellulose fibers. It was found impossible, however, to duplicate the packing of the cellulose pads accurately enough so as to produce consistent experimental data.

It seems probable that a part of the difficulty workers have had in making quantitative endosmotic studies on biological materials may lie in a changing current [Equations (27) and (28)] due in part to a changing surface caused by a simultaneous electrodialysis whereby ions are removed from the interface (from the double layer) under the action of the applied direct current.

Streaming Potentials. The converse of electroendosmosis is the streaming potential. If an imposed E.M.F. sets up a liquid flow, it is obvious that a liquid flow will produce an E.M.F. Pioneer studies in this field are due to the work of Kruyt[26] and Freundlich and Rona[27]. These authors used single glass capillaries and showed that the E.M.F. which was developed was proportional to the hydrostatic pressure under which the liquid was streamed through the capillary. Briggs[28] and Martin and Gortner[29] have devised apparatus which makes it possible to utilize the streaming potential technic for the determination of the electrical forces at the interfaces of biochemical systems.

In the original streaming potential formulas the radius, the length, or the cross-section of the capillary appeared. Obviously it was impossible to utilize such formulas when one was dealing with a gel. Freundlich and Kruyt used the formula

$$\zeta = \frac{4\pi\eta\kappa H}{P\epsilon} \tag{29}$$

where H = the potential which is developed on the two sides of the diaphragm;

κ = the specific electrical conductivity of the liquid which is being streamed through the diaphragm;

and the other quantities have the same connotations as in earlier formulas.

Briggs (*loc. cit.*), in the author's laboratories, found that it was impossible to secure reproducible results when this formula was ap-

[26] Kruyt, H. R., *Kolloid Z.*, 22: 81 (1918)
[27] Freundlich, H., and Rona, P., *Sitz. preuss. Akad. Wis.*, 20: 397 (1920)
[28] Briggs, D. R., *J. Phys. Chem.*, 32: 641 (1928)
[29] Martin, W. McK., and Gortner, R. A., *J. Phys. Chem.*, 34: 1509 (1930)

plied to the experimental data resulting from streaming liquids through diaphragms made from cellulose fibers. The difficulty was found to lie in the fact that the specific electrical conductivity of the liquid as it existed in the pores of the diaphragm was higher than the specific electrical conductivity of the liquid in bulk. In other words, there was a surface conductance along the surface of the cellulose fiber greater in magnitude than the conductance through the body of the streaming liquid. This problem of surface conductance in gels and membranes has been considered at length by Briggs in a second paper.[30]

Briggs accordingly modified the streaming potential formula and proposed the formula

$$\zeta = \frac{4\pi\eta\kappa_s H}{P\epsilon} \tag{30}$$

where κ_s = the specific electrical conductivity of the liquid as it exists in the pores of the diaphragm, all values being expressed in electrostatic units.

Thus P must be expressed in dynes, H must be reduced to cgs electrostatic volts, *i.e.*, volts divided by 299.86, and κ_s in reciprocal ohms x 9 x 10^{11}. Accordingly for water at 20°C. ($\eta = 0.01$ and $\epsilon = 80$) Formula 30 becomes

$$\zeta = K\left(\frac{H\kappa_s}{P}\right) = 1.0596 \times 10^{22}\left(\frac{H\kappa_s}{P}\right) \tag{31}$$

where H is the observed E.M.F. expressed in millivolts, κ_s is the specific electrical conductivity of the liquid in the pores of the diaphragm expressed in reciprocal ohms, and P is the hydrostatic pressure under which the liquid is being streamed through the diaphragm expressed in centimeters of mercury pressure.

All of the above formulas contain ϵ, the dielectric constant. It is necessary to take for this value the dielectric constant of the liquid in bulk, although this in all probability introduces an erroneous assumption. The dielectric constant of water in bulk is 80. It has been suggested that in the Helmholtz double layer the dielectric constant may fall to as low as one, due to the intense electrical field existing within the double layer. It accordingly seems desirable to eliminate the dielectric constant from the streaming potential equation. Furthermore the streaming potential equations assume a constant

[30] Briggs, D. R., *Colloid Symposium Monograph*, 6: 41 (1928)

thickness of the double layer, which assumption is inherent in the original derivation of Helmholtz. Bull and Gortner[31] accordingly propose a formula which eliminates the dielectric constant and which introduces a thickness of the double layer factor. In the derivation of Formula 30, we have the relationship

$$\zeta = \frac{4\pi q\, d}{\epsilon} \tag{32}$$

where q = the charge density per sq. cm. of the double layer;
d = the thickness of the double layer.

Combining Equations 30 and 32 we have

$$q\, d = \frac{\eta \kappa_s H}{P} \tag{33}$$

All of the terms on the right-hand side of Equation 33 are measurable experimentally. The quantity qd may be regarded as the electric moment per sq. cm. of the double layer, that is, it is an expression for the determination of the symmetry of the double layer in much the same way that the electric moment of a molecule is an expression of the symmetry of a molecule and furthermore

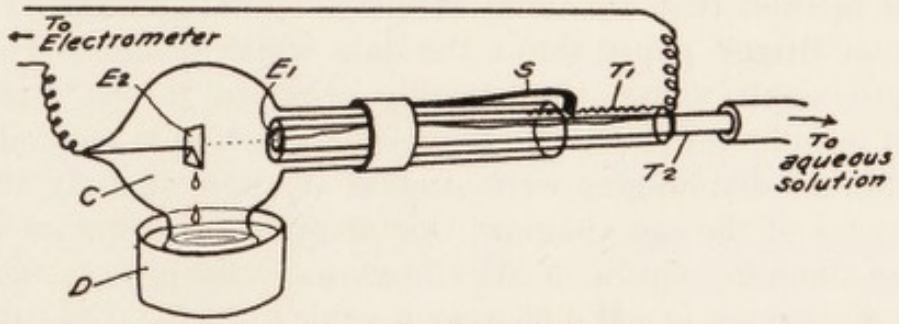


FIG. 17. A streaming potential cell for oil-water systems

Equation 33 does not involve any assumptions which cannot be measured experimentally. It seems probable that Equation 33 will prove more useful in electrokinetic studies than other electrokinetic equations which have been proposed.

One other type of technic deserves mention at this point, *i.e.*, the technic suitable for the study of electrical forces at liquid-liquid interfaces. In many instances such studies have been carried out by cataphoretic technics. Bull and Gortner[32] utilized a streaming potential cell originally devised by Martin. This cell is shown in Fig. 17.

[31] Bull, H. B., and Gortner, R. A., *Physics*, 2: 21 (1932)
[32] Bull, H. B., and Gortner, R. A., *Proc. Nat. Acad. Sci.*, 17: 288 (1931)

Studies were carried out as to the effect of various electrolytes on a paraffin oil—water interface, and the forms of the curves which resulted were similar in shape to the curves resulting from similar studies of the effect of electrolytes streamed through cellulose or quartz membranes using Briggs or Martin-Gortner type of apparatus. This liquid-liquid streaming potential apparatus should be of value in studying emulsification and in studying the physical and chemical behaviors of different samples of oil where slight impurities or changes in structure make great differences in surface behavior.

The Comparison of Various Electrokinetic Technics. It is perhaps pertinent at this point to ask the question as to how electrokinetic measurements conducted by the various methods compare with each other. Briggs[33] in a study of the electrokinetic potential has utilized the streaming potential methods on egg albumin adsorbed on a quartz—water interface. Briggs compared his data with data which had been reported by Abramson[34,35] for the electrokinetic potential of egg albumin adsorbed on quartz determined by cataphoresis. The comparison was carried out through a pH range of 3.38–7.50. The two series of experiments lay essentially on the same curve, although less scattering was shown by the data obtained by the streaming potential method than by those obtained by cataphoresis. Fig. 18 taken from Briggs' paper shows the data which resulted. The fact that reproducible values were readily obtained in the streaming potential technic was proven by the results which were secured when three different diaphragms were studied at approximately the isoelectric point of the egg albumin. One diaphragm in contact with a dilute egg albumin solution of pH 4.78 gave a value of ζ of -0.55 mv. Another diaphragm at pH 4.66 gave a value of ζ of $+0.54$ mv. Still another diaphragm at pH 4.70 gave a value of ζ of 0.00 mv. This last diaphragm gave a value of ζ of $+4.56$ mv. at pH 4.57 and -2.63 mv. at pH 4.85. Apparently therefore the streaming potential and the cataphoretic formulas were measuring identical electrical quantities.

Bull[36] compared the streaming potential technic, cataphoretic technic, and electroendosmotic technic on a pyrex capillary and pyrex particles coated with electrodialyzed gelatin or egg albumin. He found average ratios of 1.01 for ζ determined electroendosmoti-

[33] Briggs, D. R., *J. Am. Chem. Soc.*, 50: 2358 (1928)
[34] Abramson, H. A., *J. Am. Chem. Soc.*, 50: 390 (1928)
[35] Freundlich, H., and Abramson, H. A., *Z. physikal. Chem.*, 128: 25 (1927)
[36] Bull, H. B., *J. Phys. Chem.*, 39: 577 (1935)

cally and cataphoretically; 0.97 for values of ζ determined by the streaming potential technic and cataphoretically, and 0.99 for ζ determined by the streaming potential technic and electroendosmotically. These ratios indicate that at least for protein-covered surfaces and over the range of pH which was studied (pH 3.62–4.49) the three electrokinetic methods yield values which are within experimental error of each other.

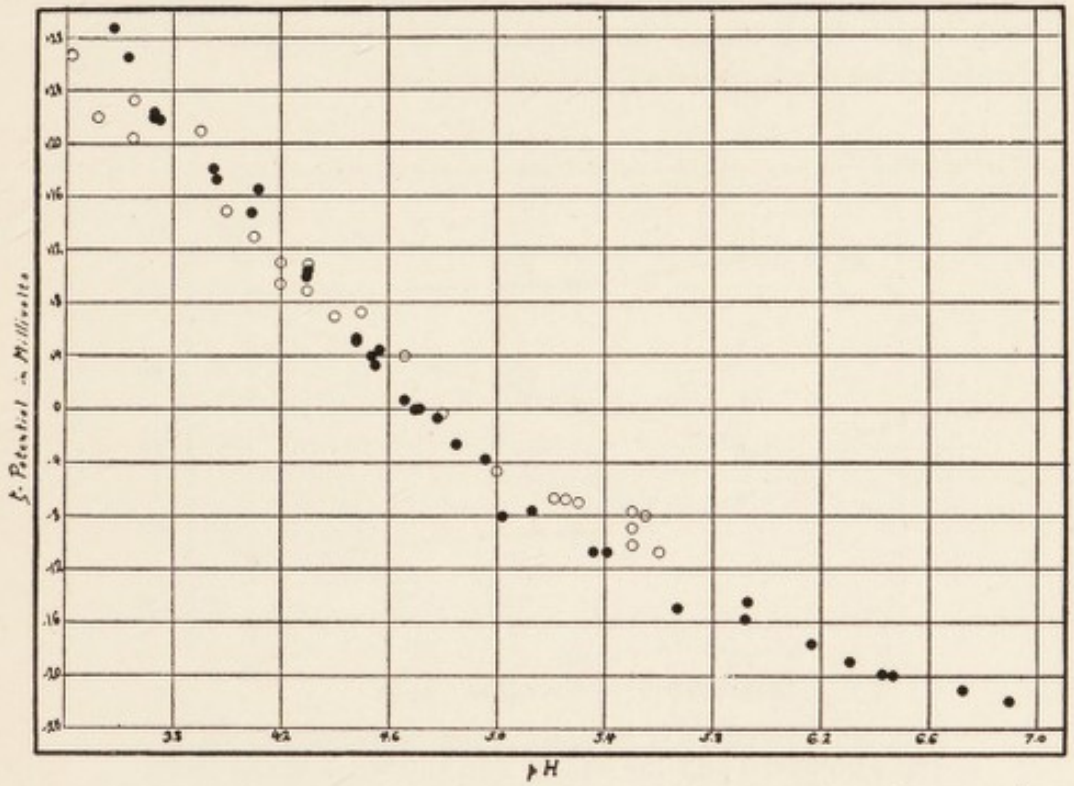


FIG. 18. Cataphoretic (open circles: data of Abramson) and streaming potential (solid: data of Briggs) measurements on egg albumin sols over a pH range

Bull did not find a ratio of one to hold for bare quartz surfaces or for cellulose fibers and suggests that studies on surfaces which derive their charge by adsorption rather than by ionization are needed. A somewhat similar disagreement is noted by White, Monaghan, and Urban[37], although they likewise report that when pyrex particles are coated with gelatin the three methods yield comparable data. These authors report that the same values are given by cataphoretic and electroendosmotic technic with somewhat different values by the streaming potential technics where bare glass surfaces are studied and suggest that in the cataphoretic and electroendosmotic technics

[37] White, H. L., Monaghan, B., and Urban, F., *J. Phys. Chem.*, 39: 611 (1935); 39: 585 (1935)

we are dealing with an electrical pull, whereas in streaming potentials we have a pressure force which they believe does not deform the fixed double layer, that is, they suggest that only a diffuse portion of the double layer moves under hydrostatic forces, whereas both the diffuse and outer portions of the fixed double layer move in an electrical field. Additional studies which would serve to clear up the behavior of bare surfaces are greatly needed.

Applications of Electrokinetic Technic. Abramson's book on electrokinetics lists many applications of electrokinetic technic, so that only a few examples will be noted here.

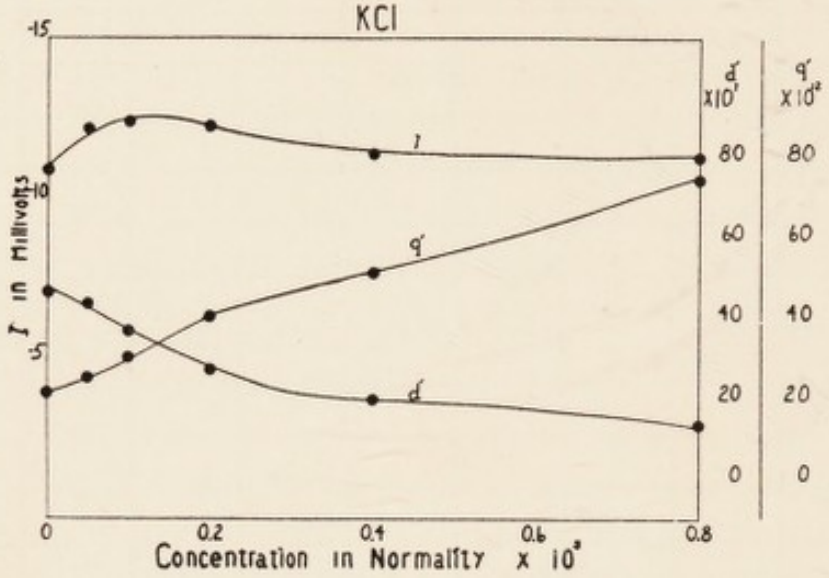


FIG. 19. The effect of increasing concentrations of KCl on the ζ-potential and the charge and thickness of the Helmholtz double layer at a water-cellulose interface (Data of Bull and Gortner)

It has already been suggested that the ζ-potential involves not only the magnitude of the charge per unit area of the double layer but likewise the distance which separates the positive and negative charges in the double layer. That this is true has only recently been made clear, and accordingly nearly all colloid texts carry the statement that "electrolytes may reduce the charge to zero or may reverse it." What this statement really means is that electrolytes may reduce the ζ-potential to zero or may reverse it, but this may be brought about by an alteration in either charge or distance. As a matter of fact the distance which separates the positive and negative charges in the double layer is usually the factor which is affected. This is shown by the studies of Bull and Gortner[38]. Figs. 19, 20, and 21,

[38] Bull, H. B., and Gortner, R. A., *J. Phys. Chem.*, 35: 309–330 (1931)

taken from this paper, show typical curves for potassium chloride, calcium chloride, and thorium chloride at a cellulose-salt solution interface. It is very evident from these curves that the distance

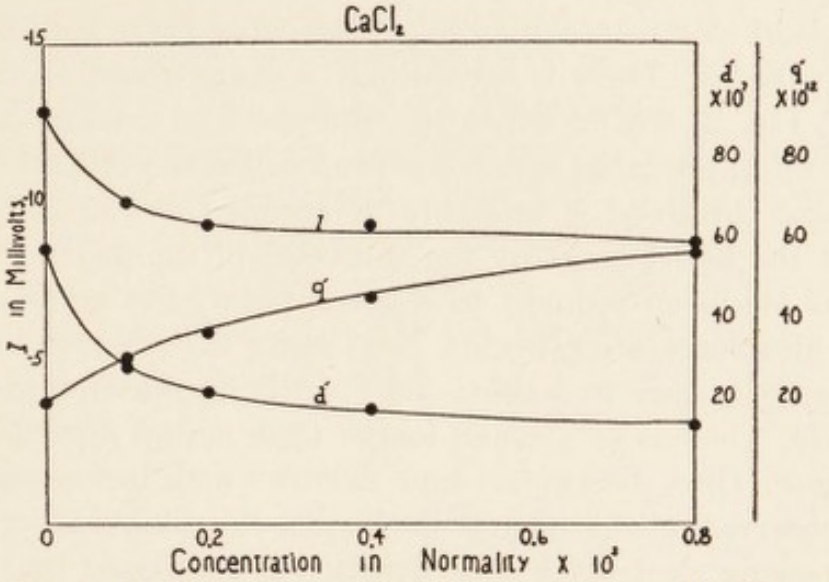


FIG. 20. The effect of increasing concentrations of $CaCl_2$ on the ζ-potential and the charge and thickness of the Helmholtz double layer at a water-cellulose interface (Data of Bull and Gortner)

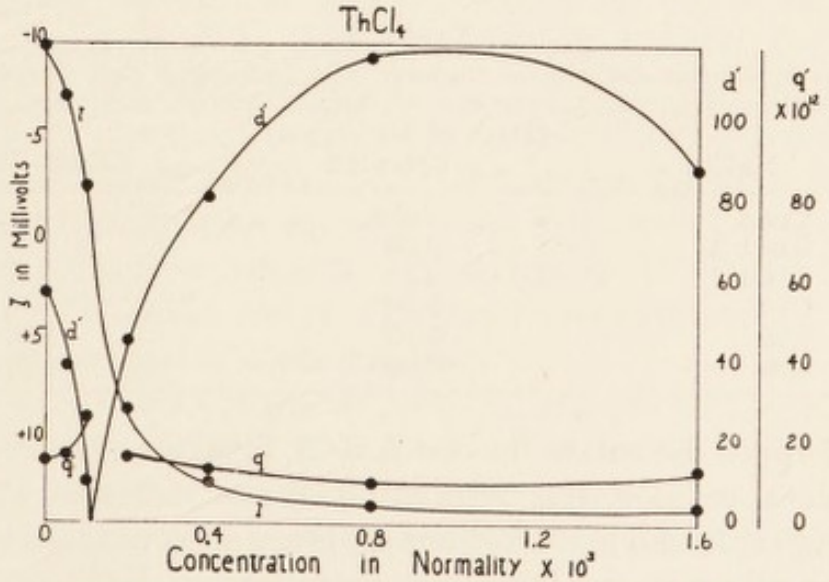


FIG. 21. The effect of increasing concentrations of $ThCl_4$ on the ζ-potential and the charge and thickness of the Helmholtz double layer at a water-cellulose interface (Data of Bull and Gortner)

which separates the charges in the double layer is the factor which causes the decrease in the ζ-potential and that q (charge density per sq. cm. of the double layer) is increasing with increasing salt concen-

tration while the double layer is collapsing. Apparently at a large value of q and a negligible value of d, the layer collapses and reverses. This is shown particularly in the curves for thorium chloride.

It has been recognized for a long time that the flocculation of lyophobic sols by electrolytes may take place when there is still an appreciable ζ-potential. There is accordingly a range about the isoelectric point (*cf.* Fig. 2) within which lies the so-called critical zone and a critical level of potential which has been called the critical threshold. The critical threshold is probably determined not so much by the charge at the interface as by the thickness of the double layer, and when this has been reduced to a given point, the system becomes unstable and flocculation begins. Here again the factor of *time* should be emphasized, since in a short time period flocculation may not be perceptible, whereas in a much longer time period flocculation may be complete. Thus the critical zone narrows with increasing time.

The observation that charge-density in the double layer increases with increasing electrolyte concentration is confirmed by the observation by Abramson[39] where he has calculated charge-density on typhoid bacteria as a function of increasing salt concentration. Certain of his data are given in Table X.

TABLE X

Changes in Charge-Density on Bacteria with Increasing Salt Concentration

NaCl	(Data of Abramson) ζ–Potential	Charge-Density
Conc.	Volts	e.s.u.
0.001 M	0.30	224
0.004	0.25	417
0.01	0.20	530
0.02	0.10	700
0.04	0.006	924

The form of the curves for NaCl, KCl, LiCl, etc., in electrokinetic studies at a cellulose-salt solution interface indicates that in all probability both anions and cations are being adsorbed into the double layer. For example, the lower concentrations of NaCl cause an increase in the ζ-potential from approximately -10 mv. to -14.10 mv. at a salt concentration of 1×10^{-4}. The ζ-potential then decreases to -10.90 mv. at a salt concentration of 1.6×10^{-3}. The increased negativity in all probability is due to an increased differential adsorption of anions into the double layer, whereas the decrease in the

[39] Abramson, H. A., *J. Bact.*, 27: 89 (1934)

negativity which occurs later is in all probability due to more of the cations entering the double layer.

The nature of the surface has a profound effect upon the electrokinetic behavior of many systems. Here we see striking examples of past treatment on the electrical properties at an interface. Moyer has studied extensively the electrophoretic mobility and isoelectric points of cholesterol sols which were prepared by various methods. He found no concordance in the literature as to the electrokinetic behavior of such sols, although they had been investigated by Keeser[40], Porges and Neubauer[41], Stern[42], Rona and Deutsch[43], Kermack and MacCallum[44], and Eagle[45]. Keeser reported an isoelectric point <pH 1.3, Eagle an isoelectric point of pH 2.1–3.6, and Remesow[46] an isoelectric point of pH 3.2. Remesow's description of the preparation of his sols was not sufficiently explicit to permit duplication of his technic. However, Moyer[47] attempted to repeat the experiments reported by other workers and secured the data shown in Table XI. Moyer then devised a new technic and prepared cholesterol sols by grinding crystals of cholesterol with ice in an agate mortar in a room maintained at $-10°$ C. All sols so prepared showed an isoelectric point of pH 3.0–3.3 with a velocity at pH 5.8 of 1.47–1.55μ/sec. When such sols were boiled for two hours, the isoelectric point dropped to approximately pH 1.7 and the velocity increased to approximately 2.44μ/sec.

The above experiments strikingly illustrate the divergent results which can be obtained when different methods are used to prepare colloidal sols. In all of the methods noted in Table XI where solvents were employed there appears to be a portion of the solvent remaining adherent to the surface of the cholesterol particles thus affecting the electrokinetic behavior of the material.

Abramson devotes pages 256–309 of his book to the question of biological application and in this section takes up the relationship of electrokinetic behavior to immunity, agglutination, the fertilization of eggs, etc. One striking series of observations has appeared

[40] Keeser, E., *Biochem. Z.*, 154: 321 (1924)
[41] Porges, O., and Neubauer, E., *Biochem. Z.*, 7: 152 (1908)
[42] Stern, R., *Arch. Exper. Path. Pharm.*, 112: 129 (1926)
[43] Rona, P., and Deutsch, W., *Biochem. Z.*, 171: 89 (1926)
[44] Kermack, W. O., and MacCallum, P., *Proc. Roy. Soc., Edinburgh*, 45: 71 (1925)
[45] Eagle, H., *J. Exp. Med.*, 52: 747 (1930)
[46] Remesow, I., *Biochem. Z.*, 218: 86 (1930); 218: 134 (1930)
[47] Moyer, L. S., *Biochem. Z.*, 273: 122 (1934); *J. Gen. Physiol.*, 18: 749 (1934–35); 19: 87 (1935)

TABLE XI

Properties of Cholesterol Sols Prepared by Different Methods from Same Cholesterol

Method	Solvent	Heated Hrs.	Heated °C.	Isoelectric Point pH	Velocity at pH 5.8 μ/sec.	Remarks
Keeser	Alcohol	0.75	100	1.3	2.35	
"	Alcohol	0.25	100	1.9	2.83	
"	Alcohol	2.00	100	1.3	2.56	
"	Acetone	2.00	100	2.4	1.84	
Porges-Neubauer	Acetone	7.0	50–60	1.7	2.37	
" "	Acetone	24.0	40	—	2.33	
Stern	Alcohol	24.0	85	2.4	3.27	
"	Alcohol	5.5	85	—	2.85	
"	Alcohol	1.0	85	—	3.61	Air bubbled through
"	Benzine	1.0	85	—	2.89	" " "
Rona-Deutsch	Acetone	2.0	60–70	1.2–1.3	3.12	" " "
" "	Acetone	1.0	60–70	—	4.00	" " "
" "	Acetone	0.75	60–70	—	4.50	" " "
Kermack-MacCallum	Alcohol	0.05	65	2.8	2.35	0.2% alcohol
" "	Alcohol	0.05	65	2.1	1.97	5.0% "
Eagle	Alcohol	0.00	—	3.00	2.30	
"	Dioxan	0.00	—	2.70	2.50	

since publication of Abramson's book. Moyer[48] has studied the species relationships in *Euphorbia*, as shown by the electrophoresis of latex particles derived from the plant sap, and shows that the various taxonomic groups in twenty-one species of *Euphorbia* can be differentiated by the form of the electrophoretic curves of the latex particles. In some species the isoelectric point of the latex particles lay close to pH 3.0. In others it was near pH 4.7. Apparently in the former the latex possessed a sterol surface and in the latter a protein surface. In one taxonomic group (the poinsettas) he found a marked difference in the form of the curves and in the isoelectric point for the latex particles of *E. heterophylla*. Fig. 22, taken from Moyer's data, shows this divergent curve. On investigation of the nature of the nucleus, *E. heterophylla* was found to be a tetraploid form possessing 56 chromosomes, whereas all of the others in this botanical group possessed the diploid number of 28 chromosomes. Later Moyer[49] studied the constancy of the latex isoelectric points and compared data on latex from plants grown from seed in Minnesota in 1933–34 with similar data from plants grown from seed in Penn-

[48] Moyer, L. S., *Am. J. Bot.*, 21: 293–313 (1934); *Bot. Gaz.*, 95: 678–685 (1934)
[49] Moyer, L. S., *Protoplasma*, 21: 588 (1934)

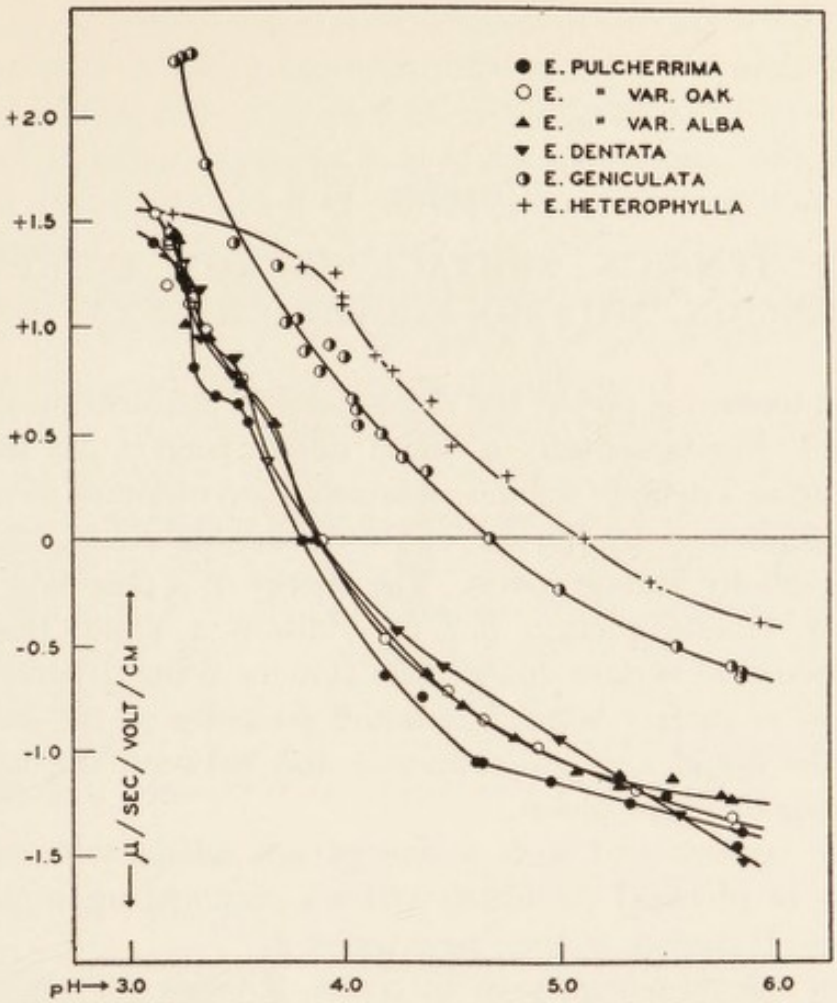


FIG. 22. Electrokinetic curves vs. pH for the latex particles of various species of Euphorbia (Data of Moyer)

sylvania during 1932–33, and in all instances found that the maximum deviation of the isoelectric point did not exceed 0.1 pH. Moyer accordingly suggests that electrokinetic studies may provide a useful tool for the taxonomist.

Chapter VI

SURFACE TENSION, SURFACE ENERGY, INTERFACIAL TENSION, AND MOLECULAR ORIENTATION

Surface tension is one of the characteristic physical properties of a liquid. Fundamentally a liquid differs from a gas in that a liquid occupies a definite volume, whereas a gas occupies an indefinite volume defined only by the size of the containing vessel. The energy of a gas is wholly kinetic energy. The energy of a pure liquid is predominantly kinetic energy, but in addition a liquid possesses a certain amount of surface energy, the amount being dependent upon the amount of surface which the liquid possesses at the boundaries between the liquid and the container and between the liquid and either the air or vapor phase.

Surface tension and surface energy are adequately treated in most texts of physical chemistry and are summed up concisely by Willows and Hatschek in their monograph.[1]

Surface energy is expressed in terms of "ergs per square centimeter" which is an expression of the work which must be done upon the system to increase the liquid-vapor area by one square centimeter. Surface energy contributes only an infinitesimal amount of energy to the total energy content of the pure liquid. When, however, we deal with colloid systems we find that the area of surface in the form of interfaces may reach very large values. Thus, for example, if we take one cubic centimeter of water in the form of a hypothetical cube, one centimeter on an edge, we would have a total surface area of six square centimeters. If this hypothetical cube of water were subdivided into colloid particles with a diameter of 0.1μ, we would have 1×10^{15} particles with a surface area of sixty square meters and a surface energy of 43,800,000 ergs. The original surface energy was only 438 ergs. If subdivision were continued further so that the particles had a diameter of 1.0 mμ, we would have 1×10^{21} particles with a surface of 6,000 square meters and a surface energy of 4,380,000,000 ergs, or 105 calories.

[1] Willows, R. S., and Hatschek, E., "Surface Tension and Surface Energy," Third Edition, J. and A. Churchill, London (1923)

Stefan[2], in 1886, suggested that a molecule expended one-half of its latent heat of vaporization in moving from the body of a liquid into the surface film, the remaining one-half of the latent heat of vaporization being expended in moving a molecule from the surface film into the vapor phase. The three steps in vaporization are (1) the moving of a molecule from the body of the liquid to the surface film, (2) pushing the molecule into the surface film so that it becomes a part of the surface, (3) the necessary kinetic energy to cause it to jump out of the surface into the vapor phase.

The latent heat of vaporization of water is 540 calories. Of this, forty calories represent the work which is done against the vapor phase, *i.e.*, atmospheric pressure, vapor density, etc., and accordingly 500 calories represent the work which is done against adjacent liquid water molecules. If Stefan were correct in his statement, one-half of this 500 calories would represent the force necessary to bring a gram of water into the surface film. Since 500 calories is equivalent to 21×10^9 dynes and since 10^6 dynes equals one atmosphere pressure, the cohesional pressure of one gram of water would be

$$\frac{1/2(21 \times 10^9)}{1 \times 10^6} = 10{,}500 \text{ atmospheres.}$$

Harkins and Roberts[3] have shown that Stefan's law is only a rough approximation. For approximately symmetrical molecules of non-associated liquids (*e.g.* liquid nitrogen) it holds fairly well. In the case of liquid nitrogen 44.29 per cent of the energy of vaporization is expended in pulling the molecules into the surface film and 55.71 per cent is expended in the molecule passing from the surface to the vapor phase. Water, however, is a highly abnormal and associated liquid. At 10° C. only 15.09 per cent of the latent heat of vaporization is expended in moving the molecule into the surface film and 84.91 per cent is expended in moving the molecule from the surface into the vapor phase.

Taking the 15.09 per cent of the latent heat of vaporization of water which is expended in moving a molecule into the surface, one can calculate the maximum surface area which can be occupied by the molecules in one gram of water, and this calculation indicates that the area would be approximately 55,292,000 square centimeters. Such a film would be about 0.18 mμ in thickness. Other studies have

[2] Stefan, J., *Wied. Ann.*, 29: 655 (1886)
[3] Harkins, W. D., and Roberts, L. E., *J. Am. Chem. Soc.*, 44: 653 (1922)

indicated that the cross-section of a water molecule is of approximately this order of magnitude. We are therefore dealing in the above instance with a monomolecular layer of water, at a water-water vapor interface.

Various technics are available in the physico-chemical literature for measuring surface tension. By appropriate modifications of these technics it has been possible to measure interfacial tension at liquid-liquid boundaries. In the case of solid-liquid interfaces, however, we can approach the problem only from the liquid side, so that direct measurements may not yield true surface tension values, and it is perhaps better to designate such energy relations by the term, "adhesion tension."

Bartell and his students[4,5,6,7,8,9,10] have published a number of important papers dealing with the technic of determination of adhesion tension and various applications of the observed results. Bartell and Osterhof point out that adhesion tension (A) is equal to the difference between the surface tension of the solid and its interfacial tension against a liquid.

$$A = \gamma_s - \gamma_{sl} \tag{34}$$

The greater the attraction of the solid, the smaller will be γ_{sl}, and the greater will be (A). (A) equals the work done when we substitute a liquid-solid interface for a vapor-solid interface and is therefore a direct measure of wettability.

Bartell and Osterhof further show how we can calculate the adhesion tension in ordinary dynes per centimeter units, providing that we know the radius of the capillaries in a diaphragm composed of the packed solid material. Table XII, taken from their paper, shows adhesion tension at silica-liquid interfaces and carbon black-liquid interfaces. In the case of the silica, the organic liquid was displaced by water. In the case of the carbon, the water was displaced by the organic liquid.

Certain applications of adhesion tension studies are perhaps self-evident. It is obvious that a suspension will be most stable in that

[4] Bartell, F. E., and Osterhof, H. J., *Colloid Symposium Monograph*, 5: 113 (1928)
[5] Bartell, F. E., and Osterhof, H. J., *Ind. Eng. Chem.*, 19: 1277 (1927)
[6] Bartell, F. E., and Sloan, C. K., *J. Am. Chem. Soc.*, 51: 1637 and 1643 (1929)
[7] Osterhof, H. J., and Bartell, F. E., *J. Phys. Chem.*, 34: 1399 (1930)
[8] Davis, N. S., and Curtis, H. A., *Ind. Eng. Chem.*, 24: 1137 (1932)
[9] Bartell, F. E., and Jennings, H. Y., *J. Phys. Chem.*, 38: 495 (1934)
[10] Bartell, F. E., and Walton, C. W., Jr., *J. Phys. Chem.*, 38: 503 (1934)

TABLE XII

Showing Adhesion Tensions of Silica and Carbon Black for Various Liquids
(Data of Bartell and Osterhof)

Liquid	Silica-Liquid Interface		Carbon Black-Liquid Interface	
	Displacement Pressure	Adhesion Tension	Displacement Pressure	Adhesion Tension
	dynes/cm.	dynes/cm.	dynes/cm.	dynes/cm.
Water	—	82.82	—	54.74
Aniline	8	82.00	1266	60.51
Carbon tetrachloride	409	40.69	6935	89.45
Hexane	395	42.13	3330	69.93
Benzene	295	52.43	5775	81.08
α-Brom naphthalene	397	41.92	—	—

liquid which shows the highest adhesion tension for the solid. Thus, from Table XII it is evident that SiO_2 will form the most stable dispersion in water and carbon black will be most stable in carbon tetrachloride. Furthermore in the case of two immiscible liquids the solid will pass to that liquid for which it has the highest adhesion tension. Such studies are of great importance in industrial processes and have a profound bearing on pigment stability in paints.

The above is perhaps another way of stating Reinders'[11] theorem which relates to the behavior of a solid and two immiscible liquids (*e.g.* water-oil). If $\gamma_{so} > \gamma_{wo} + \gamma_{sw}$, the solid will remain suspended in the water. If $\gamma_{sw} > \gamma_{wo} + \gamma_{so}$, the solid will leave the water and pass to the oil phase. If $\gamma_{wo} > \gamma_{sw} + \gamma_{so}$, or if no one of the interfacial tensions is greater than the sum of the other two, the solid will collect at the boundary between the two liquids. This latter phenomenon is of common occurrence when one attempts to shake out with ether many ether-soluble constituents from an aqueous solution in a separatory funnel, and demonstrates that colloidal particles were present in the original aqueous phase.

Since immersion in a particular liquid involves wettability, solid-liquid adhesion tensions become of great importance in the adherence of insecticides, dusts, oil sprays, paints, varnishes, etc. In the last paper cited, Bartell and Walton (*loc. cit.*) essentially propose adhesion tensions as a quantitative measure of the hydrophilic or hydrophobic properties of surfaces, *i.e.* wettability by water or wettability by oils with Al_2O_3 and carbon as solid extremes and water and *n*-heptane as extreme liquids. They were able to prepare by heat treatment samples of stibnite (Sb_2S_3) varying from extreme wettability by

[11] Reinders, W. von, *Kolloid Z.*, 13: 235 (1913)

water to extreme wettability by oil. Table XIII shows certain of their adhesion tension data.

Mudd and Mudd[12] suggested a technic whereby one could study the relative wettability of particles by oil or by water. The particles suspended in water were placed on a microscope slide and a drop of

TABLE XIII

Adhesion Tensions of Heat-Treated Stibnite Against Water and Benzene
(Data of Bartell and Walton)

Stibnite	Treatment	A_{sl} (H_2O)	A_{sl} (C_6H_6)
		dynes/cm.	dynes/cm.
A	Ground only	56.5	78.4
B	Heat 1 hr. at 170°	57.2	76.6
C	Heat 2 hrs. at 170°	58.1	76.0
D	Heat 3 hrs. at 170°	60.3	72.6
E	Heat 4 hrs. at 170°	64.2	—
F	Heat 5 hrs. at 170°	66.5	—
G	Heat 6 hrs. at 170°	70.5	55.0
H	Heat 8 hrs. at 170°	76.0	47.0
I	Heat 8 hrs. at 400–420° (in nitrogen)	77.1	45.4

oil was added adjacent to the water drop. When a cover slip was placed over this preparation, there was formed an oil-water boundary, and when the slide was observed under a microscope (preferably with dark field illumination), it was observed that the oil boundary advanced across the field. When the oil boundary reached a particle which was easily wetted by the oil, the particle progressed through the oil-water interface with little or no distortion of the interface. If, however, the particle was not wetted by oil or was very difficultly wetted, the interface was compressed and distorted, although eventually the particle might break through and be engulfed by the oil. In extreme cases such engulfment leaves the particle with a surrounding water film so that in reality there was no wetting of the particle by the oil.

Nugent[13] has diagrammed the appearance of the oil-water of the Mudd and Mudd phenomenon in eight degrees of wettability from an extreme case where a particle "dissolves" in the oil to one where a particle passes the interface only when surrounded by a water film or where the moving boundary simply pushes the particle ahead of it. Moyer[14] used this technic to determine the physical state of the

[12] Mudd, S., and Mudd, E. B. H., *J. Exper. Med.*, 40: 633 and 647 (1924)
[13] Nugent, R. L., *J. Phys. Chem.*, 36: 449 (1932)
[14] Moyer, L. S., *Am. J. Bot.*, 22: 609 (1935)

surface of latex particles from various species of *Euphorbia* and found that those species with a low isoelectric point (*vide supra*) of pH ± 3.0 showed preferential wettability by oil, whereas those with a higher isoelectric point of pH ± 4.5–5.0 possessed surfaces which were resistant to oil wetting and were preferentially wetted by water. These observations confirmed the electrokinetic studies where the assumption had been made that the former probably had a sterol-like surface, whereas the latter possessed a protein protective surface.

We have indicated earlier that large interfacial areas are characteristic of colloid systems. Surface tension or interfacial tension may be regarded as the intensity factor, whereas area may be regarded as the capacity factor. Surface energy would therefore be the product of the surface tension and surface area. For colloid systems surface energy may become the predominating form of energy, although it should be noted that it does not include the electrical forces which likewise may contribute greatly to the free energy of the system.

It is a theorem that every system tries to decrease its free energy, and since surface energy is a very active form of free energy, it is obvious that it can be decreased by a decrease in area, by a decrease in interfacial tension, and, of course, by a decrease in both. The phenomena of coagulation, crystal growth, etc., all point to a decrease in surface energy due largely to a decrease in area.

Long ago Willard Gibbs stated that those substances which decrease surface tension will concentrate at a surface. Gibbs' theorem may be stated as

$$C_2 = -\frac{C}{RT} \cdot \frac{d\gamma}{dc} \tag{35}$$

where C_2 = excess concentration in the interface;
C = equilibrium concentration in liquid phase;
dc = increment change in concentration of solution having increment change ($d\gamma$) in interfacial tension.

C_2 is positive when $d\gamma/dc$ is negative, i.e., when interfacial tension is decreased, and C_2 is negative when $d\gamma/dc$ is positive. In the one case we have positive adsorption, in the other case negative adsorption.

The validity of Gibbs' theorem is easily proven quantitatively for gas-liquid or liquid-liquid interfaces. It is more difficult to prove for solid-liquid interfaces, because of the difficulty of determining the interfacial tension of a solid. The surface tension of a solid, however,

has been obtained in a few instances indirectly. Ostwald[15] and Hulett[16] pointed out that the surface tension of a solid could be determined by the excess solubility of small particles, the surface energy replacing the mechanical energy of powdering or of solution, *i.e.*, the disruption of particle from particle (adhesion). The formula which is applicable to such calculations is

$$\gamma_{sl} = \frac{RTDr}{2M} \log_e \frac{C_2}{C_1} \tag{36}$$

where D = density of solid phase;
r = radius of particle;
M = molecular weight of substance whose solubility is being determined;
C_2 = concentration of solution in equilibrium with particles of radius r;
C_1 = concentration of solution in equilibrium with massive particles (r is large);
R = gas constant in ergs;
γ_{sl} = interfacial tension.

On the basis of such measurements Dundon[17], and Dundon and Mack[18] have presented the data shown in Table XIV. These values indicate that solid-liquid interfaces are characterized by relatively

TABLE XIV
Interfacial Tensions at Solid-Water Interface
(Data of Dundon, and Dundon and Mack)

Substance	Diameter of Particle	Increase in Solubility	γ_{sl}
	μ	per cent	dynes/cm.
PbI_2	0.4	2.0	130
$CaSO_4.2H_2O$	0.5	4.8	356
$CaSO_4.2H_2O$	0.2–0.3	12.3	385
Ag_2CrO_4	0.3	10.0	575
PbF_2	0.3	9.0	900
$SrSO_4$	0.25	26.0	1400
$BaSO_4$	0.1	80.0	1250

high interfacial tensions as compared with liquid-liquid or gas-liquid interfaces. Consequently solid-gas or solid-liquid interfaces should show high surface energy, particularly if the surface area is great.

[15] Ostwald, W., *Z. physikal. Chem.*, 34: 503 (1900)
[16] Hulett, G. A., *Z. physikal. Chem.*, 47: 357 (1904)
[17] Dundon, M. L., *J. Am. Chem. Soc.*, 45: 2658 (1923)
[18] Dundon, M. L., and Mack, E., Jr., *J. Am. Chem. Soc.*, 45: 2479 (1923)

Molecular Orientation.—The basic idea that molecules assume a definite space orientation at interfaces appears to have been first suggested by Sir W. B. Hardy[19] from his studies on lubrication. Hardy found that not all oils acted as lubricants and proposed the idea that there was contained in lubricating oils a certain fraction composed of molecules which had an attraction for the metallic surfaces of the journal and the bearing and that such molecules became oriented, one end of the molecule being attached to the metallic surface, and the other end extending toward the body of the oil. Accordingly when the journal moved within the bearing, the metallic parts never came in contact, due to this oriented layer of molecules which separated the journal and the bearing, and the frictional wear took place entirely between these two oriented films of molecules so that the eventual failure of the lubricant was due to the wearing out of those compounds which possessed the property of being oriented at the interfaces.

Somewhat later Harkins and co-workers[20,21,22,23], and Langmuir[24] independently came to views similar to those of Hardy, although Harkins' conception was derived from studies of surface tension and Langmuir's was derived from studies of the adsorption of gases upon metallic films in electric light bulbs.

The theory of molecular orientation and experimental evidence in its support have been extended by many workers, notable among whom are Adam[25], and Rideal[26] who sums up the literature in his book, "An Introduction to Surface Chemistry."

In general terms the theory of molecular orientation rests upon the conception that different groupings within a molecule possess different properties including different "solubilities" and that symmetrical molecules differ markedly in their surface behavior from non-symmetrical molecules. Examples of such behaviors would be *n*-pentane and amyl alcohol. *n*-Pentane is a pure hydrocarbon mole-

[19] Hardy, W. B., *Proc. Roy. Soc.*, A, 86: 610 (1912); A, 88: 303 and 313 (1913)
[20] Harkins, W. D., Brown, F. E., and Davies, E. C. H., *J. Am. Chem. Soc.*, 39: 354 (1917)
[21] Harkins, W. D., Davies, E. C. H., and Clark, G. L., *J. Am. Chem. Soc.*, 39: 541 (1917)
[22] Harkins, W. D., Clark, G. L., and Roberts, L. E., *J. Am. Chem. Soc.*, 42: 700 (1920)
[23] Harkins, W. D., and Cheng, Y. C., *J. Am. Chem. Soc.*, 43: 35 (1921)
[24] Langmuir, I., *J. Am. Chem. Soc.*, 39: 1848 (1917)
[25] Adam, N. K., *J. Phys. Chem.*, 29: 87 (1925)
[26] Rideal, E. K., "An Introduction to Surface Chemistry," Cambridge University Press, Second Edition, 1930

cule, symmetrical throughout and essentially insoluble in water. Amyl alcohol, which differs from *n*-pentane only by having an —OH group substituted for a hydrogen atom, possesses appreciable solubility in water. It likewise has an appreciable dipole moment, whereas *n*-pentane is electrically neutral, indicating that amyl alcohol is non-symmetrical both in its atomic configuration and in the distribution of electrical charges within the molecule. Reasoning from the analogy that "like attracts like," those compounds which show a similarity to the chemical composition of water should be more or less soluble in water, whereas those compounds which show a chemical composition similar to hydrocarbons should be more or less soluble in hydrocarbons. *n*-Pentane is miscible in all proportions in hydrocarbons. Amyl alcohol, which differs only from pentane by the substitution of an —OH group for hydrogen is rather poorly soluble in hydrocarbons. Harkins has coined the terms "polar" and "non-polar" to refer to specific groups within the molecules, or to particular molecular configurations of atoms, to indicate that they are attracted to or dissolved in water or hydrocarbons respectively. Thus amyl alcohol would be a polar compound, differing from the non-polar *n*-pentane by the introduction of the highly polar —OH group.

The above views can be easily illustrated by taking the non-polar hydrocarbon, methane, as an example. Methane is relatively insoluble in water. However, the introduction of polar groups, such as $-NH_2$ to form methylamine, —OH to form methyl alcohol, —COOH to form acetic acid, —CHO to form acetaldehyde, —CN to form acetonitrile, —CO— to form acetone, $-CONH_2$ to form acetamide, all cause the formation of highly water-soluble substitution compounds and convert a non-polar molecule into a polar molecule. In general, the introduction of groupings containing oxygen, sulfur, nitrogen, halogens, double and triple bonds, increases the polarity of hydrocarbon molecules, increases the dipole moment, causes the molecule to become highly unsymmetrical, and such molecules will assume specific orientations at interfaces.

The evidence that such molecules are oriented at interfaces has been beautifully set forth in Harkins' studies of surface tension. Harkins noted that when he studied an homologous series of the aliphatic acids, the surface tension depressing action at a water-air interface increased with length of hydrocarbon chain up to a critical value, following which increasing the length of the hydrocarbon chain produced little or no effect. He explained this behavior by suggesting

that at the critical value (at approximately lauric acid) there was a completely oriented layer of fatty acid molecules at the water-air interface, the molecules being so oriented that their carboxyl groups extended into the water and the hydrocarbon portion of the molecule extended toward the air. Thus, the surface tension measurement was actually being made at a hydrocarbon-air interface, and increasing the length of the hydrocarbon chain by a $-CH_2-$ group did not alter the nature of the surface which in both instances was essentially a surface of exposed $-CH_3$ groups.

Langmuir devised the so-called "surface tension balance." By the use of this apparatus he was able to measure the areas which were occupied by weighed amounts of a number of surface tension depressants, *i.e.*, compounds which would spread on water at an air-water interface. In these studies he was able to measure the thickness of the film so formed and to show that in general such films were monomolecular. Langmuir's studies have given us definite measurements of the cross-sectional area of many molecules. Table XV shows the cross-sectional dimensions of the area occupied by single molecules containing specific groupings in such interfacial films. Attention is called to the fact that the cross-sectional area of glycoldipalmitate is approximately twice the cross-sectional area of the hydrocarbon chain, and that triglycerides have approximately three times the cross-sectional area of the hydrocarbon chain. This is definite evidence that in the case of glycoldipalmitate both of the hydrocarbon residues of palmitic acid extend into the surface and in the case of triglycerides all three of the hydrocarbon residues of the fatty acids penetrate into the surface.

Most of the physico-chemical studies which have been made of molecular orientation have dealt with the formation of monomolecular films. Recently Blodgett[27], working in Langmuir's laboratory, has devised a technic for the production of polymolecular films and has built up such polymolecular layers to a depth of more than two hundred molecules. Blodgett uses a Langmuir surface tension balance upon which a surface tension depressant, (*e.g.* the calcium or barium salts of palmitic, stearic or other long-chain fatty acids) has been allowed to spread. This film is then compressed on the surface of the water by the spreading of another surface tension depressant (*e.g.* castor oil), the two films being kept separate by a waxed silk thread. In this way definite pressure can be brought to bear on the calcium

[27] Blodgett, Katharine B., *J. Am. Chem. Soc.*, 57: 1007 (1935)

TABLE XV

Showing the Cross Sectional Dimensions of the Area Occupied by a Single Molecule in an Interfacial Film

(Data of Adam)

Group	Cross Section
	sq. Å
Hydrocarbon chain	20.7
$-CH_2CH_2COOH$	25.1
$-CH=CH\ COOH$	28.7
$-CH_2CH_2COO\ C_2H_5$*	22
$-CH=CH\ COO\ C_3H_5$	28.7
$-CH_2OH$	21.7
$-CONH_2$	21
$-CN$	27.5
$-CH_2NH_2CO\ NH_2$	25.5
$-C_6H_4OH$	23.8
$-C_6H_4NH\ CO\ CH_3$	28.2 or 25.8**
Triglycerides	63
Glycoldipalmitate	42
Cholesterol	39
Hydrolecithin	53

*Ethyl, methyl, and allyl esters pack into the same area.

**According to temperature.

palmitate film by the spreading of the so-called "piston oil" (*e.g.* the castor oil noted above). The pressure so produced (± 16.5 dynes/cm) is not sufficient to crumple the film but is sufficient to cause it to move upon another suitable surface upon which it can spread. If now a clean glass or metal slide is *raised* through the film of calcium palmitate, the area occupied by the calcium palmitate on the water surface decreases by exactly the surface area of the slide, and a monomolecular layer of calcium palmitate is deposited over the surface of the slide with the carboxyl groups of the palmitic acid oriented toward the slide and the hydrocarbon "tails" extending outward from the slide. If now the slide is lowered through the calcium palmitate film, a second layer of calcium palmitate is deposited on the slide, in this instance the hydrocarbon portion of the molecule being attracted to the hydrocarbon surface already on the slide and the $-COOH$ portion of the molecule extending in the surfaces. Thus films may be built up, one film at a time, by successive raising and lowering of the slide through the surface film, the 1–3–5–7 . . . films being hydrophobic and being wetted by oil and not wetted by water, and the 2–4–6–8 . . . films being hydrophilic and being wetted by water and not wetted by oil. Such polymolecular films when sufficiently thick show a beautiful series of interference colors. They may be dried even by baking in an oven without destruction of the film structure.

Blodgett notes that Langmuir has suggested that "films could be built for use as diffraction gratings for soft X-rays by depositing $(2n + 1)$ layers of barium stearate, then $2n$ layers of stearic acid, then $2n$ layers of barium stearate, and so on in alternating succession. The stearic acid would be more transparent to radiations of short wave length than the barium stearate and would therefore serve to space the series of layers of barium stearate at known intervals apart."

These studies of Blodgett, demonstrating beyond peradventure of a doubt the possibility of polymolecular films of oriented molecules, are regarded by the writer as of great importance in extending the theories of membrane formation, etc., in biological systems, and we shall have occasion to again refer to Blodgett's studies.

We have already indicated that Gibbs predicted that those substances which decrease surface tension would concentrate at an interface. Such concentration has been designated as positive adsorption. On the other hand, those substances which increase surface tension will concentrate in the body of the liquid, in which case we have negative adsorption. Inorganic salts increase the surface tension of water, and at the surface of such salt solutions there is definite evidence[28,29] that we have a monomolecular layer of pure water molecules separating the bulk of the solution from the air or vapor phase. This layer of water ranges in thickness from 4.0 Å on a 0.1 M NaCl solution to 2.3 Å on a 5.0 M NaCl solution. In each case the layer is apparently monomolecular but is much more highly compressed in the case of the stronger salt solution. From these studies Harkins concludes that the water molecule probably occupies a cube 3.09 x 10^{-8} cm. on an edge.

It should be noted in passing that these monomolecular films are dynamic and not static films. The molecules oriented in the films still possess large amounts of kinetic energy. Harkins says that at 20°C. in a vacuum 7,000,000 molecules jump in and out each second from the area occupied by a single molecule in the film but that the time that is necessary for a molecule to orient itself is so small that at any given instant of time the percentage of oriented molecules is likely to be high. This viewpoint will be referred to later.

[28] Harkins, W. D., and McLaughlin, H. M., *J. Am. Chem. Soc.*, 47: 2083 (1925)

[29] Harkins, W. D., and Gilbert, E. C., *J. Am. Chem. Soc.*, 48: 604 (1926)

du Noüy[30] made use of surface tension studies on expanded and condensed films to secure size data of biocolloids. He reasons that if surface tension-concentration curves show only a single minimum, in all probability all three dimensions of the molecule are identical. If such studies show two minima, he concludes that two dimensions of the molecule are alike and one unlike, and if three minima are observed, he concludes that all three dimensions of the molecule are dissimilar. The two minima were observed in the case of water-egg albumin studies, and from his measurements he concludes that the egg albumin molecule has the dimensions, 30.8 x 30.8 x 41.7 Å. In the case of sodium oleate he finds three minima and concludes that the dimensions of this molecule are 6.64 x 5.76 x 12.30 Å.

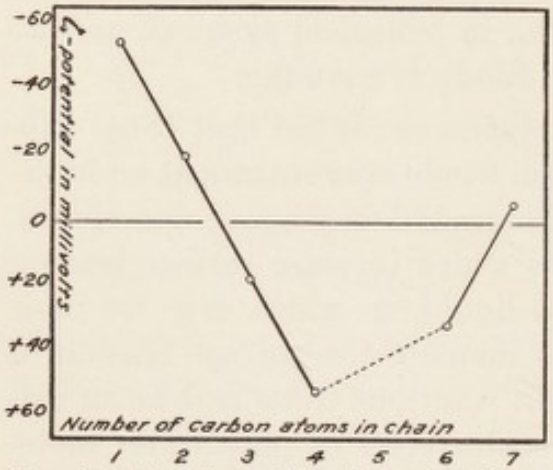


FIG. 23. The electrokinetic potential at an *n*-alcohol-cellulose interface as a function of the atomic structure of the alcohols (Data of Martin and Gortner)

Emulsion stabilizers apparently act by forming oriented films at the interfaces between the oil and water. Those stabilizers which are relatively hydrophobic, *i.e.* preferentially wetted by oil, form, in general, water-in-oil emulsions. Gum dammar would be an example of such a stabilizer. Those stabilizers which are preferentially wetted by water and accordingly are predominantly hydrophilic form, in general, oil-in-water emulsions. Gum acacia is the common example.

Another method of attacking the problem of molecular orientation is by studying the electrical behavior at interfaces. Compounds containing polar groupings are all electrical dipoles, and an oriented layer of dipoles should give rise to an electric double layer. Thus, in such systems we can study two problems at once, the electrical behavior of the double layer and the degree of specific orientation in the oriented molecular layer.

According to Gibbs' theorem surface tension forces need not draw the molecule into the interface with any particular degree of orientation. However, electrical studies made on such surfaces may form a means of determining the extent to which the molecules in the surface layer have assumed any specific orientation.

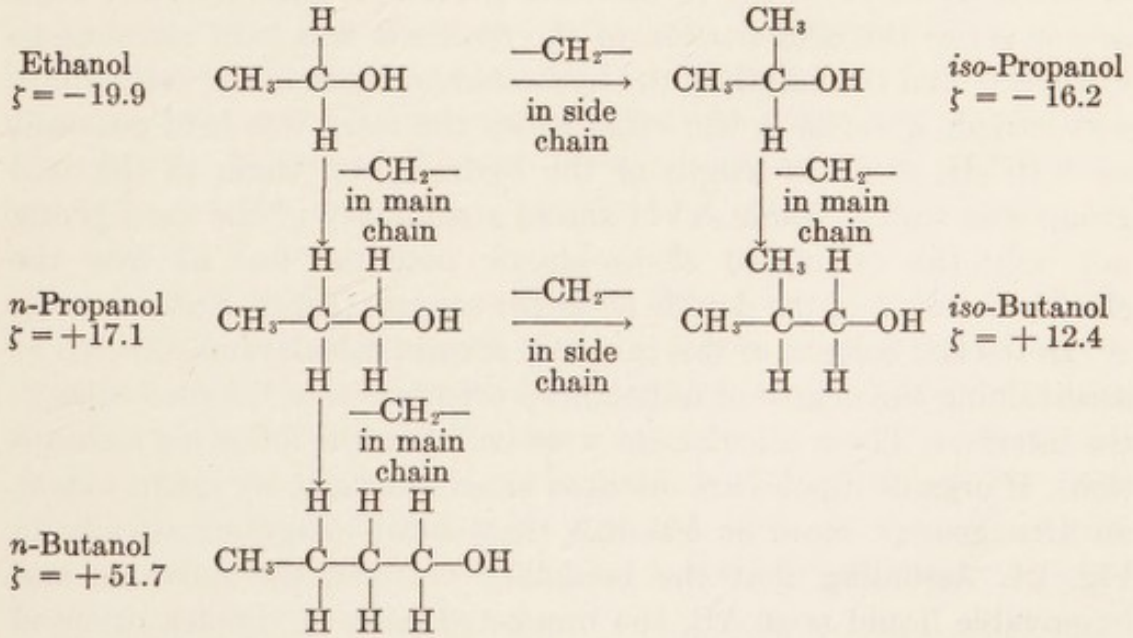
[30] du Noüy, P. L., "Surface Equilibria of Biological and Organic Colloids," Am. Chem. Soc. Monograph, No. 27, Chemical Catalog Company, New York, 1926

Martin and Gortner[31] in their study of interfacial energy as related to the structure of organic compounds measured the electrokinetic potential at the interface of cellulose and a number of pure organic liquids. They found that the structure of the molecule apparently determined not only the sign but the magnitude of the electrokinetic

TABLE XVI

Showing the Summary of the ζ-Potentials for Cellulose-Organic Liquid Interfaces
(Data of Martin and Gortner)

	ζ-Potentials	Difference due to one $-CH_2$-group in *n*-alcohols
	Millivolts	Millivolts
Water	− 5.4	
Methanol	− 55.3	
Ethanol	− 19.9	35.4
n-Propanol	+ 17.1	37.0
n-Butanol	+ 51.7	34.6
n-Hexanol	+ 31.0	36.8
n-Heptanol	− 5.8	
iso-Propanol	− 16.2	
iso-Butanol	+ 12.4	
Ethylene glycol	− 11.0	
Ethylene chloride	− 15.7	
Ethylene bromide	− 10.9	
Glycerol	−111.5	
Benzene	± 0.0	
Methyl benzene	− 0.2	
Chlorobenzene	− 1.0	
Bromobenzene	− 6.7	
Aminobenzene	− 49.7	
Nitrobenzene	−142.0	



[31] Martin, W. McK., and Gortner, R. A., *J. Phys. Chem.*, 34: 1509 (1930)

potential which was produced at the interface. Table XVI shows some of the data which they obtained. It will be noted that in the case of the normal aliphatic alcohols there is a definite relationship existing between the length of the hydrocarbon chain and the electrokinetic potential, and that the introduction of a $-CH_2-$ group into the main chain changes the electrokinetic potential by approximately 35 mv. (cf. Table XVI and Fig. 23), whereas the introduction of a $-CH_2-$ group into a side chain (*e.g.* replacement of $-H$ by $-CH_3$) changes the electrokinetic potential by only approximately 4 mv.

The data on the aromatic compounds present many points of interest. Thus benzene which possesses a completely symmetrical non-polar configuration showed no electrokinetic potential at the cellulose-liquid interface. Toluene which is slightly unsymmetrical showed a low but definite electrokinetic potential. Chlorobenzene, bromobenzene, aniline, and nitrobenzene showed increasing electrokinetic potentials, and it was suggested that studies such as this might be used to quantitatively evaluate the polarity of groupings substituted in a molecule.

These electrokinetic studies were extended by Jensen and Gortner[32] who investigated a series of *n*-aliphatic acids and the esters of these acids. The problem under investigation was at least in part the influence which a specific hydrocarbon residue would have on the electrokinetic potential when it was attached (a) to the acid portion of the molecule, and (b) to the ester portion of the molecule. Thus in one series the acid portion of the molecule was held constant as CH_3CO- and the length of the hydrocarbon chain in the ester group was varied, whereas in the other series the ester was held constant as $-OC_2H_5$ and the length of the hydrocarbon chain in the acid group was varied. Table XVII shows a summary of the data giving not only the calculated electrokinetic potential but likewise the electric moment of the double layer per sq. cm. (*qd*, *cf.* Equation 33).

In the last column of this table are certain calculations directed at ascertaining the degree of unbalanced orientation of the molecules in the interface. These calculations were based on the following assumptions. If organic dipoles are oriented at an interface, we might expect an arrangement more or less like that shown diagrammatically in Fig. 24. Assuming that the boundary between the movable and immovable liquid is at AB, the bracketed pairs of dipoles oriented

[32] Jensen, O. G., and Gortner, R. A., *J. Phys. Chem.*, 36: 3138 (1932)

TABLE XVII

Summary of Electrokinetic Data for Various Organic Liquid—Al_2O_3 Interfaces
(Data of Jensen and Gortner)

Liquid	ζ-Potential	qd × 10^5	Unbalanced orientation of molecules in interface
	millivolts	c.g.s. electrostatic units per cm^2	per cent
Acetic acid	+ 39.4	6.52	11.4
n-Propionic acid	+ 18.66	1.67	2.34
n-Butyric acid	+ 17.37	1.30	3.52
n-Valeric acid	+ 12.1	0.84	—
n-Caproic acid	+ 29.0	2.48	—
Methyl acetate	− 21.7	3.88	4.88
Ethyl acetate	− 24.8	4.14	5.05
n-Propyl acetate	− 19.2	3.17	3.75
n-Butyl acetate	− 20.6	2.75	3.27
n-Amyl acetate	− 9.53	1.26	1.45
Ethyl formate	+113	21.2	24.1
Ethyl acetate	− 24.8	4.14	5.05
Ethyl *n*-propionate	− 10.8	2.90	3.56
Ethyl *n*-butyrate	− 32.1	4.36	—
Benzene	0	0	0
Carbon tetrachloride	0	0	0

in opposite directions might be expected to neutralize each other, whereas the "unbalanced orientation" of the remaining molecules should give rise to a net negative charge on the immovable layer side of the interface with a corresponding positive charge in the streaming liquid. On this hypothesis, it should be possible to calculate the percentage of "unbalanced orientation" of the organic molecules in the immovable layer, assuming (1) a monomolecular, close-packed oriented layer, and (2) that the electric moment per unit area of the double layer is the product of the dipole moment of the organic molecule and the number of "unbalanced" molecules oriented per unit area.

In these calculations, the values for the cross-sectional area of the molecules (A) are those given by Rideal (*loc. cit.*) for the limiting areas per molecule in the liquid condensed form. For esters A = 22.0 $Å^2$ and for acids A = 24.4 $Å^2$.

The per cent of the total surface occupied by oriented but "unbalanced" molecules is given by the expression, $qd\ A/\mu 10^{14}$, where μ is the dipole moment, and qd and A have the meanings denoted above.

It will be noted that a relatively small percentage of unbalanced

orientation is sufficient to account for the electrical behavior which is measured in the interface. Furthermore it is of interest to note that ethyl formate behaves abnormally both in regard to sign of the electrokinetic potential and the percentage of unbalanced orienta-

FIG. 24. A diagrammatic representation of the oriented adsorption of organic dipoles at a solid-liquid interface, postulated as a source of the electric double layer.

tion. In all probability this means that ethyl formate has a reverse orientation at the interface from all of the other aliphatic esters which were studied. This is presumably due to the reactivity of the pseudo "aldehyde" group of the formate.

If the electrokinetic forces at a solid-organic liquid interface are due to the electrical dissymmetry of oriented organic molecules, the molecular orientation theory of Hardy, Harkins, Langmuir, Adam, *et al*, can well be extended to include surface electrical forces as well as surface tension and interfacial tension.

Chapter VII

ADSORPTION

We have seen from Equation 35 that those substances which reduce surface tension or interfacial tension tend to concentrate in surfaces or interfaces, thus giving rise to positive adsorption, whereas when the substance increases surface tension or interfacial tension, the solvent concentrates in the interface, giving rise to negative adsorption.

We have also seen that electrical forces cause ions and electrical dipoles to concentrate at interfaces.

Probably both of the above factors are operative in those phenomena which are designated by the colloid chemist with the term, adsorption, which in reality designates the phenomena of the concentration of substances at interfaces irrespective of the forces which bring such concentration about.

Freundlich has devised an empirical Equation (37) from a fit of the experimental data.

$$\frac{x}{m} = aC^b \tag{37}$$

where x = the amount of material adsorbed;
m = the mass of adsorbent;
C = the concentration of the solution at equilibrium;
a and b = constants.

Equation 37 is the equation for a parabola and can accordingly be expressed as

$$\log \frac{x}{m} = \log a + b \log C \tag{38}$$

Such an equation is the equation for a straight line where b is the slope of the line, *i.e.* the tangent of the angle which the line makes with a line drawn parallel to the axis of abscissa, and a is the value on the ordinate axis where the straight line cuts the axis of ordinates at unit concentration.

Equations 37 and 38 are equilibrium equations and accordingly

represent reversible reactions so that, if a large amount of material is removed from solution by adsorption from concentrated solutions and the adsorbent is washed with more dilute solutions, a certain amount of material will again pass into solution. These factors should be remembered when adsorption phenomena are utilized to purify solutions, *e.g.* the use of charcoal for decolorizing purposes, etc.

Equations 37 and 38 state that there is no equilibrium concentration beyond which an increase in concentration will not increase the amount of material which will be adsorbed. Langmuir has proposed a different equation which may be stated as

$$x = \frac{a\beta C}{1 + aC} \tag{39}$$

where x = the amount of material adsorbed;
C = the equilibrium concentration;
a and β = constants.

Equation 39 is the equation for a hyperbola and indicates that the adsorbent will become saturated at some definite value of C. This value of C is determined by constant β. Constant β becomes equal to x when C is a maximum, and this point is reached when the equilibrium concentration is the concentration of a saturated solution (cf. Linner and Gortner[1]).

In the initial portions of the curves both the Freundlich and the Langmuir adsorption isotherms yield straight lines when plotted on logarithmic paper, so that over those portions of the curves there is little choice between the two mathematical expressions. Since, however, the Langmuir expression does indicate a definite adsorption saturation, it appears to be the preferable expression for adsorption studies.

The Mechanism of Adsorption. There are in reality two schools of thought with respect to the phenomenon which we are discussing under the heading, adsorption. To one of these schools the ideas which will be propounded in this chapter, and even the term, adsorption, are anathema. This school has considered primarily the behavior of electrolytes toward surfaces and is interested in such phenomena as acid-base exchange in soils and minerals, in acid and alkali binding of proteins, and in the relationships which exist between the biocolloids and the electrolytes in biological systems and

[1] Linner, E. R., and Gortner, R. A., *J. Phys. Chem.*, 39: 35–67 (1935)

organisms. This school insists that stoichiometrical relationships account for the phenomena which we are calling adsorption and that the union between the electrolyte and the substrate is a true "salt" rather than an "adsorption complex."

Probably they are correct to a degree. It is well known that in order to replace one equivalent of calcium or magnesium in a zeolite, it is necessary to add approximately two equivalents of an alkali metal. However, the various alkali metals differ among themselves in their replacement ability. Thus, Jenny[2] points out that there is a 240 per cent difference between the replacement ability of the lithium ion and the caesium ion on ammonium permutit and a 700 percent difference in the replacement ability between the lithium ion and the potassium ion on hydrogen permutit, and that the monovalent ions arrange themselves in the lyotropic series Li < Na < K < Rb < Cs < H with respect to permutit systems.

Even if there were a stoichiometrical replacement in the case of zeolites, it is well known that it is very easy to prepare a series of zeolites having essentially the same chemical composition but which individually differ widely in their ability to bind the ions of the alkaline earths. Apparently the binding takes place on *surfaces*, and the *extent of surface area* determines not necessarily the type of reaction that the system will undergo but rather the magnitude of the reaction which will take place. Unquestionably stoichiometrical chemical combination and surface adsorption in many instances involve the same chemical forces. This can be illustrated diagrammatically, if we take a hypothetical mass of carbon and project a plane through it. Assuming that the carbon atoms are arranged with a definite space relationship to each other, we might postulate an arrangement similar to that shown in Fig. 25. Those carbon atoms which are imbedded in the body of the carbon mass and are not exposed at any surface will obviously not take part in an adsorption or a stoichiometrical chemical reaction. Those carbon atoms which are exposed in the surface of the plane have one free valence bond and accordingly may enter into stoichiometrical reactions to a limited extent. Those carbon atoms which are exposed on edges are indicated as having two free valence bonds and accordingly may be expected to be more reactive than those atoms which are exposed only in surfaces. Those carbon atoms which are exposed at corners are indicated as having three free valence bonds, and accordingly such atoms

[2] Jenny, Hans, *J. Phys. Chem.*, 36: 2217–58 (1932)

should be more reactive than those which are exposed in edges and still more reactive than those exposed in surfaces.

Now if we were to replace the carbon atoms in Fig. 25 with hydrous aluminum silicate radicals, we might have essentially those conditions which exist in zeolites. There might be a stoichiometrical binding of the alkali earths on the corners, the edges, and the faces of the exposed surfaces, but in the interior no active points of union would be exposed and the interior of the mass would be non-reactive. Thus, in order to prove the stoichiometrical nature of the binding, it would be necessary to know not only the surface area of the solid phase but also to know the ratios which exist between the "atoms" which are involved in the binding and which are exposed at the "corners," the "edges," and the "faces" of the exposed planes, since those portions which constitute the "corners" will be theoretically more reactive than those which form the "edges," and these in turn will have a higher binding capacity than those in the "faces."

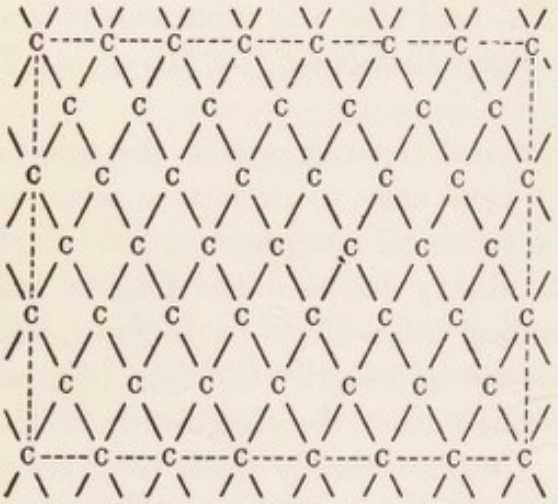


FIG. 25. A diagrammatic representation of the "free valences" in a hypothetical carbon surface

Taylor[3] has suggested that catalytic surface activity is due to surface atoms which have a high degree of valence freedom, thus approximating more nearly the gaseous state than the solid state.

In addition, in surfaces we have those energies which have been designated as the van der Waal's forces. These forces differ only in degree from primary valence forces, since both are probably electrical in origin.[4]

Inasmuch as the rare gases are positively adsorbed by charcoal and show fairly high heats of adsorption (argon, 3,450 calories per mole at 0°C. as compared with 3,654 calories per mole for nitrogen and 1870 calories per mole for hydrogen at the same temperature), it is evident that surface fixation and heats of adsorption do not

[3] Taylor, H. S., *J. Phys. Chem.*, 30: 145–171 (1926)

[4] Herzfeld, K. F., and Smallwood, H. M., Chapter IV in "Treatise on Physical Chemistry," edited by H. S. Taylor, Second Edition, D. Van Nostrand Company, New York (1931)

necessarily reflect compound formation. The demonstrated adsorbability of the rare gases is respectfully referred to that group of workers who insist that all apparent adsorption reactions can be accounted for on the basis of stoichiometry.

Probably the truth actually lies somewhere in the intermediate ground between strictly stoichiometrical combination and purely physical surface adsorption. In the case of the rare gases we are dealing wholly with surface behavior. In the case of relatively inert organic molecules we are probably dealing largely with surface energies and in the case of electrolytes we have probably in part stoichiometrical unions with a residuum of surface energy attraction. Recently the proteins have been singled out as a great class of the biocolloids in which the assumption has been made that only stoichiometrical reactions occur. Thus, Loeb[5] and his school interpret the reactions of proteins with acids and bases wholly on the basis of stoichiometrical salt formation. Hoffman and Gortner[6] concluded that adsorption also accounted for at least a part of the apparent acid and base binding in protein systems, and later Robinson, Gortner, and Palmer[7] showed that in contrast to succinic acid, casein did not possess a "maximum base-binding capacity," but that together with the stoichiometrical binding of the base by the acid casein there was a second reaction which apparently yielded an adsorption isotherm.

Recently in order to account for the osmotic behavior of the red blood cells, Peters[8] has suggested that the potassium "salt" of hemoglobin in the red blood corpuscles probably exists largely in an unionized form. It is difficult to picture a true potassium salt which fails to ionize. It is easy to picture an alternative potassium ion-hemoglobin adsorption complex which osmotically would behave as a unit, and that picture is suggested as an alternative to explain the osmotic behavior of the red cells.

Linner and Gortner (*loc. cit.*) studied the adsorption of thirty organic aliphatic acids, differing from each other in various structural relationships, upon a single comparable substrate, *i.e.* the decolor-

[5] Loeb, J., "Proteins and the Theory of Colloid Behavior," McGraw-Hill Book Company, New York, 1922

[6] Hoffman, W. F., and Gortner, R. A., *Coll. Symp. Monograph*, 2: 209–368 (1925)

[7] Robinson, A. D., Gortner, R. A., and Palmer, L. S., *J. Phys. Chem.*, 36: 1857–1881 (1932)

[8] Peters, J. B., "Body Water—The Exchange of Fluids in Man," Charles C. Thomas, Springfield, Illinois, and Baltimore, Maryland, 1935

izing carbon, Norit. These aliphatic acids could be grouped as (1) the saturated *normal* acids, (2) the saturated *iso* acids, (3) the mono- and dihydroxy acids, (4) the dicarboxylic acids, (5) the keto acids, (6) the unsaturated mono- and dicarboxylic acids including *cis* and *trans* isomers, and (7) certain halogen substituted acids. Attempts were made to study series differing only in a single structural unit

TABLE XVIII

Showing values of "1/n" and "α" of the Freundlich equation, $a = \alpha C^{1/n}$ for the various systems of Norit and organic acid at equilibrium, as well as the average per cent error and the number of points used for the calculations

(Data of Linner and Gortner)

Acid	α	$1/n$	Average Per Cent Error Between Obsd. and Calcd. a	Number of Points
Formic	2.47	0.435	2.6	15
Acetic	2.46	0.351	2.6	18
Propionic	2.46	0.236	1.8	15
n-Butyric	2.46	0.177	2.3	15
n-Valeric	2.84	0.182	2.8	12
Caproic	3.03	0.175	1.8	11
Isobutyric	2.36	0.273	12.3	10
Isovaleric	2.51	0.227	2.6	9
Glycolic	1.54	0.390	4.1	13
Lactic	1.66	0.335	3.6	18
Glyceric	1.29	0.267	6.7	13
Glyoxylic	3.89	0.455	2.3	12
Pyruvic	2.44	0.273	3.5	15
Levulinic	1.83	0.183	2.9	8
Oxalic	3.62	0.551	4.9	29
Malonic	3.88	0.410	3.3	11
Succinic	2.83	0.303	4.3	16
Glutaric	1.96	0.201	5.1	13
Adipic	1.79	0.163	4.5	22
Malic	1.28	0.252	2.2	15
Tartaric	0.94	0.275	3.3	15
Maleic	1.90	0.203	2.9	14
Fumaric	2.81	0.248	4.6	9
Mesaconic	1.80	0.133	2.4	14
Citraconic	1.69	0.167	1.8	15
Itaconic	1.54	0.148	1.9	15
Methylsuccinic	1.30	0.172	1.6	14
Citric	0.73	0.203	3.3	8
Monobromosuccinic	1.82	0.195	2.0	9
Dibromosuccinic	2.58	0.320	2.2	8

so that the effect of that unit difference could be ascertained. Calculations were made of the various constants in both the Langmuir and Freundlich isotherms of each acid. Tables XVIII, XIX, and XX show certain of the data which were obtained.

TABLE XIX

Showing values of α *and* β *of the Langmuir equation,* $a = \frac{\alpha\beta C}{1 + \alpha C}$ *for various systems of Norit and organic acid at equilibrium*

(Data of Linner and Gortner)

Columns 5 and 6 give the average per cent error over the range for which the Langmuir equation holds, together with the number of points considered.

Acid	$\alpha\beta$	α	β	Average Per Cent Error Between Obsd. and Calcd. a	Number of Points
Formic	0.273	0.159	1.710	1.0	13
Acetic	0.462	0.266	1.736	7.0	16
Propionic	0.925	0.491	1.885	2.0	13
Butyric	1.689	0.863	1.957	2.0	13
Valeric	1.878	0.872	2.154	5.8	11
Caproic	8.772	4.636	1.892	5.4	7
Isobutyric	0.883	0.497	1.776	5.5	7
Isovaleric	1.630	0.902	1.807	5.1	7
Glycolic	0.239	0.249	0.958	5.1	12
Lactic	0.437	0.415	1.054	4.0	18
Glyceric	0.668	0.812	0.823	8.5	13
Glyoxylic	0.508	0.223	2.275	3.0	11
Pyruvic	0.979	0.585	1.674	2.8	14
Levulinic	2.990	2.289	1.307	3.4	6
Oxalic	0.440	0.332	1.325	3.7	29
Malonic	1.897	1.540	1.232	5.6	11
Succinic	0.865	0.467	1.854	6.0	16
Glutaric	3.697	3.104	1.192	4.8	13
Adipic	2.347	1.886	1.245	4.5	19
Malic	0.531	0.574	0.927	2.3	14
Tartaric	0.322	0.468	0.687	2.5	14
Fumaric	7.097	5.798	1.224	4.1	9
Maleic	1.233	0.884	1.395	3.6	14
Mesaconic	2.706	1.886	1.435	2.9	14
Citraconic	1.356	1.014	1.337	4.7	14
Itaconic	1.167	0.904	1.291	4.0	14
Methylsuccinic	0.664	0.608	1.092	4.2	14
Citric	1.444	2.757	0.524	11.7	8
Monobromosuccinic	0.934	0.643	1.451	3.0	8
Dibromosuccinic	1.397	1.119	1.248	3.6	8

TABLE XX

Showing agreement between the experimentally determined values of β and the calculated values of a (at maximum adsorption) when C_s (equilibrium concentration) is that of a saturated solution

(Data of Linner and Gortner)

Acid	C_s in Millimoles per 75 cc.	β	a (Maximum) in Millimoles per Gram	Per Cent Error
Valeric	27.19	2.154	2.068	4.1
Caproic	6.60	1.892	1.862	1.6
Isobutyric	170.33	1.776	1.755	1.2
Isovaleric	31.13	1.807	1.745	3.5
Oxalic	97.92	1.325	1.286	3.2
Malonic	542.18	1.231	1.232	−0.1
Succinic	52.22	1.854	1.781	4.0
Glutaric	391.87	1.192	1.191	0.1
Adipic	8.11	1.245	1.169	6.0
Tartaric	696.23	0.687	0.685	0.7
Fumaric	4.52	1.224	1.179	3.7
Maleic	509.33	1.395	1.392	0.2
Mesaconic	20.55	1.435	1.399	2.5
Itaconic	53.11	1.291	1.265	2.0

It was found that the most reliable point of comparison between the various acids was that at maximum adsorption, *i.e.*, that point on the Langmuir adsorption isotherm where β is equal to x. The *iso* acids had essentially the same adsorption maximum as the *normal* acids, the branch chain apparently having but little effect upon the number of molecules which were adsorbed per unit area. On the other hand the introduction of a hydroxyl or a keto group markedly decreased the adsorption, and the introduction of a second hydroxyl group again further decreased the adsorption over the first one introduced. This is as would be anticipated considering the polar nature of hydroxyl and keto groups, *i.e.* their increased hydrophilic properties. Similarly the introduction of carboxyl groups was reflected in decreased adsorption, and the introduction of a double bond had a somewhat similar effect.

Making the assumptions (1) that maximum adsorption resulted in the formation of a mono-molecular layer of the acid molecules adsorbed at the solution-carbon interface, and (2) that the adsorbed oriented molecules are close packed at maximum adsorption, and (3) that the Langmuir value of 21 $Å^2$ represents the cross-sectional area of an adsorbed normal fatty acid molecule, it was possible to calculate

the cross-sectional area of the various series of aliphatic acids in the mono-molecular films under the conditions of maximum adsorption.

From the normal fatty acid data the surface area of the Norit used as adsorbent was calculated to be 240 square meters per gram. This is well within agreement of the area found by other investigators for decolorizing carbons.

Using this value as a basis, we have the cross-sectional area occupied by the various molecules as shown in Table XXI. A summary of the data of Table XXI is shown in Table XXII. Apparently

TABLE XXI

Approximate cross-sectional areas of solute molecules on the surface of the carbon interface

(Data of Linner and Gortner)

These are calculated on the assumption that the cross-sectional area of the fatty acid molecule is 21 sq. A.U.

Acid	Cross-Sectional Area of Molecule	Acid	Cross-Sectional Area of Molecule
	sq. A. U.		*sq. A. U.*
Fatty series	21.0	Acetic	21.0
Isobutyric	22.3	Glycolic	41.4
Isovaleric	22.0	Propionic	21.0
		Lactic	37.6
Oxalic	29.9	Glyceric	48.2
Malonic	32.2	Succinic	21.4
Succinic	21.4	Malic	42.8
Glutaric	33.3	Tartaric	57.7
Adipic	31.9		
Methylsuccinic	36.2	Acetic	21.0
Maleic	28.4	Glyoxylic	17.4
Fumaric	32.4	Propionic	21.0
Mesaconic	27.6	Pyruvic	23.7
Citraconic	29.7	Levulinic	30.4
Itaconic	30.7	Monobromosuccinic	27.3
Citric	75.7	Dibromosuccinic	31.7

the *iso* acids occupy approximately the same surface area as the *normal* acids. The areas occupied by the *normal* fatty acids and their dicarboxylic homologues stand to each other in the ratio of approximately 1 : 1.5. The introduction of a hydroxyl group approximately doubles the area which the molecule occupies in the interface, and the introduction of two hydroxyl groups further increases the area which the molecule occupies, so that the ratios of *normal* acid to mono-hydroxy acid to dihydroxy acid are approximately 1 : 2 : 2.5.

TABLE XXII

Summary of Approximations in Table XXI

(Data of Linner and Gortner)

Adsorbate	Mean Value	Per Cent Error Between Mean and	
		Low Value	High Value
	sq. A. U.		
Fatty acids	21*		
Iso acids	22	0.9	0.9
Dicarboxylic acids**	32	2.1	4.1
Monohydroxy acids	41	8.3	4.4
Dihydroxy acids	53	9.1	8.9
Geometric isomers (unsaturated)	30	5.5	7.7

*Assumed value

**Succinic excepted

The ratios are as follows:

(1) *Normal* fatty acids: *iso* acids = 1:1.

(2) *Normal* fatty acids: dicarboxylic acids = 1:1.5.

(3) Acid: monohydroxy acid: dihydroxy acid = 1:2:2.5.

The introduction of halogens or the double bond likewise increases the interfacial area which the molecule occupies.

Furthermore when the data for the maximum adsorption of the mono- and dicarboxylic acids were studied, it was observed that there was an alternation in the odd-even carbon atom series. Such an alternation is well known as affecting the melting point and other physical properties of the solid aliphatic compounds. The fact that an alternation is shown in these adsorption studies is further evidence that these aliphatic acids occur in the solid state when adsorbed at the interface. Jensen and Gortner (*loc. cit*) had earlier reported an odd-even carbon atom alternation for the electrokinetic effects of homologous series of aliphatic esters at an Al_2O_3 interface, and this observation, taken in conjunction with the alternation observed in these adsorption studies is further confirmation of the intense orienting and binding forces which are characteristic of surfaces. Apparently we are here dealing with solid films of oriented molecules, probably mono-molecular in cross-section but films wherein the molecules have their kinetic energy so greatly reduced that they no longer exhibit the properties characteristic of the liquid state. They are in reality pseudo-crystals, distinct from the ordinary crystals characteristic of these materials in that they possess length and breadth but only mono-molecular depth and presumably a different molecular orientation from that which they possess in their usual crystal forms.

But it may be asked, "Of what significance are such experiments

as the above to biochemical and biological problems?" We may answer this question somewhat as follows:

(1) We must know what occurs in simple systems which possess only a single variable before we can predict what will occur in a biological system containing many variables.

(2) Unquestionably biological systems contain surfaces and interfaces upon which adsorption and the orientation of molecules will take place.

(3) These surfaces and interfaces in biological systems are, in all probability, mosaics where very diverse kinds of chemical elements are exposed, and accordingly the adsorption which will take place on such surfaces may be anticipated to be more or less specific, one compound being attracted preferentially to one area of the mosaic interface and another compound to another area of the same mosaic interface. On one portion of the mosaic there may be one orientation arrangement, on another a different orientation, and because of these differences, specific reactions may occur (as we know they do occur) locally.

(4) Only by studying many simple systems and mixtures of simple systems, whose individual behavior is already known, can we hope to elucidate many of those complex reactions which are characteristic of the cells and tissues of a living organism.

An example of what in all probability can be regarded as directed adsorption and orientation followed by chemical reaction can be found in the summary paper by Quastel[9]. Quastel emphasizes adsorption as an essential feature of enzyme action, and he accounts for the specificity of enzyme action on the basis of specific adsorption. He studied the behavior of bacteria on 103 different organic compounds with particular reference to oxidation-reduction reactions. Of these 103 compounds he found that 56 were "activated" so that they acted as hydrogen donators in the presence of a suitable hydrogen "acceptor" such as methylene blue. From a strict application of the laws of enzyme specificity it would be necessary to postulate a minimum of 56 different dehydrogenases, an obviously ridiculous assumption, since many of the compounds were known not to be of biological origin. Quastel found, however, that all of the compounds could be grouped into classes, and he demonstrated a formic acid

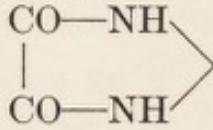
[9] Quastel, J. H., *Trans. Faraday Soc.*, 26: 853–864 (1930) *cf.* also Quastel, J. H., and Wooldridge, W. R., *Biochem. J.*, 22: 689–702 (1928)

class, a lactic acid class, and a succinic acid class. The significance of this finding lies in the fact that the various compounds within a class all interfere with each other insofar as oxidation-reduction reactions in the presence of the bacterial bodies are concerned, whereas there is no interference when compounds in two different classes are present, *i.e.*, in that case the rates of reaction are additive.

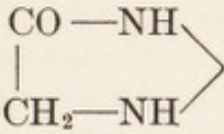
To account for these observations Quastel postulates different "centers" or areas on the surface of the bacterial mosaic which show specific adsorption for organic compounds possessing definite chemical configurations. Those compounds which are adsorbed on a single center interfere with each other in subsequent oxidation-reduction reactions, whereas those compounds which are adsorbed on different centers obviously could not interfere with each other and the reactions proceed independently of each other. Quastel further notes that in those instances where compounds are adsorbed and activated on a single center, that compound which is first added to the system is the most strongly activated, apparently because it was first present in the system and accordingly was able to occupy the major portion of the area upon which it could be specifically adsorbed.

The formic acid center appears to be specific for formic acid. Even acetic acid does not affect the reactions occurring at this center. We have seen (Table XVII) that Jensen and Gortner found that the formyl group throws ethyl formate out of line with the other compounds in the aliphatic acid ester series. Not only was it more powerfully adsorbed and more completely oriented but the direction of orientation was the reverse of that of the other esters. Probably somewhat similar effects differentiated formic acid from the other organic acids.

The lactic acid center adsorbs and activates lactic acid ($CH_3-CHOH-COOH$), oxalic acid ($HOOC-COOH$), glyoxylic acid ($CHO-COOH$), hydroxy malonic acid ($HOOC-CHOH-COOH$), glyceric acid ($CH_2OH-CHOH-COOH$), α-hydroxy butyric acid ($CH_3-CHOH-COOH$), mandelic acid ($C_6H_5-CHOH-COOH$), pyruvic acid ($CH_3-CO-COOH$). Apparently this center is specific for the groupings $-CO-COH^*-$ or $-CHOH-COH^*-$ where the H^* is activatable. Acetic acid, malonic acid, glycol, glycerol, glycine, formic acid, citric acid, and hydroxy *iso* butyric acid are not adsorbed or activated at this center. Parabanic acid,



was

powerfully adsorbed, whereas hydantoin,  , was not adsorbed; hydrogenating one carbonyl group destroyed the specificity.

The succinic acid center adsorbs and activates malonic acid, ($HOOC-CH_2-COOH$), succinic acid, ($HOOC-(CH_2)_2-COOH$), glutaric acid, ($HOOC-(CH_2)_3-COOH$), β-phenyl propionic acid, ($C_6H_5-CH_2-CH_2-COOH$), tri-carballylic acid, ($HOOC-CH_2-CH(COOH)-CH_2-COOH$), prototartaric acid ($HOOC-CH(CH_3)CH_2-COOH$). Acetic acid, glycine, glycerol, and sugars are not adsorbed or activated.

This center is apparently specific for the groupings $-C-CH_2-COOH$ or $-C-CH-COOH$.

An excellent demonstration of the specificity of the adsorption at the different centers is afforded by the observation that malonic acid is adsorbed at the succinic acid center, hydroxy malonic acid is adsorbed at the lactic acid center, and ethyl malonic acid is not adsorbed at any of the centers.

Quastel further notes that, whereas 56 of the 103 compounds tested were both adsorbed and "activated," there were a number of the remaining 47 compounds which were adsorbed but which were not "activated." If any of these compounds were first added to the system and subsequently another compound, which would normally be adsorbed on the same center and be "activated," were added, little or no activation of the second compound resulted. The initial adsorbed but non-activated compound covered the available area at the activating center and prevented the enzyme reaction from taking place. Thus Quastel states,

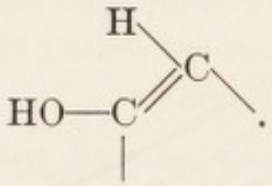
> Substances appear to act as "poisons" simply by competing with the substrate for the space available for adsorption at the centers. The "poison" and the substrate apparently compete with each other for adsorption on fairly equal terms. . . . A relatively large number of substances can be adsorbed in this specific manner, but out of this large number only a few can be *activated* to function as donators of hydrogen. . . . Specificity of enzyme action is seen to depend upon three factors:
>
> (1) Specificity of adsorption at the active centre.
>
> (2) The nature and strength of the polarizing field at the active centre.
>
> (3) The constitution of the adsorbed molecule.
>
> . . . . The reason therefore why an enzyme is so specific in its action is, in the first place, because only a limited number of substances—contain-

ing a certain type of structure—is accessible to or adsorbed by the enzyme, and in the second place, because out of this limited number of substances specifically adsorbed only a few are capable of being turned into the "active" molecules capable of the reactions under investigation. Thus each enzyme has a limited and definite range of specificity of action.

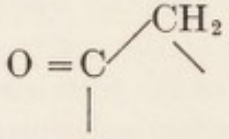
The bearing of the above experiments on various physiological and medical problems is obvious. We have as yet no specific information as to the types of surfaces which are exposed at the various centers of the bacterial mosaic surface to account for the areas characteristic of specific adsorption.

It was in the hope of securing such specific information that the molecular orientation studies of Martin and Gortner, Jensen and Gortner, and Linner and Gortner, already referred to, were undertaken. When the studies of Linner and Gortner are repeated using various other adsorbing surfaces, and when they are paralleled with electrokinetic data, we may find physico-chemical phenomena which will elucidate some of the factors which are involved. It is my belief that the most sure solution of these problems will come by the study of relatively simple systems in which we can control conditions so that we are dealing with only a single variable.

One other example from the field of medicine may suffice to illustrate the importance of specific adsorption and molecular orientation for biological studies. Morphine is well known to possess the desirable property of deadening pain but to have associated with this property the undesirable properties of habit formation, producing nausea, and favoring constipation. The morphine molecule contains the grouping



Alvarez[10] notes that when the hydroxyl group is converted to a carbonyl group and the double bond is hydrogenated so as to yield a structure containing the grouping



the pain-deadening properties of the drug are increased about five-fold,

[10] Alvarez, W. C., *Proc. Staff Mayo Clinic*, 7: 480 (1932)

the constipation and psychic effects are markedly decreased, and the habit-forming properties essentially disappear. In this case we are presumably dealing with adsorption and specific orientation of the original morphine on at least two brain centers, one which has to do with habit formation and the others with pain. Apparently a slight change in chemical configuration of the molecule intensifies adsorption and orientation on the pain center and destroys adsorption affinities (or alters the specific orientation) of the drug on the habit-forming center. Admittedly the above explanation is a hypothesis, but in the light of Quastel's observations it appears to be an extremely logical explanation. We need specific adsorption and molecular orientation studies on all of the physiologically active drugs and those compounds which are structurally closely related to them. When such data are available, it is believed that the pharmacological and physiological behavior of drugs can be much more rationally interpreted.

Chapter VIII

THE WATER RELATIONSHIPS OF THE BIOCOLLOIDS[1]

We have seen that surface energies are the characteristic energies of colloid systems. We have seen that surface energies may reach relatively enormous values, such for example as interfacial tensions of the order of 1400 ergs per square centimeter at a strontium sulfate-water interface, and we have also seen that non-symmetrical molecules are attracted to and oriented upon surfaces and that they are held upon such surfaces with relatively enormous forces. Furthermore we have seen that these adsorbed and oriented molecular films apparently often show the behavior of solids (*cf.* Jensen and Gortner, and Linner and Gortner, *loc. cit.*)

All of these relationships are associated with the phenomena of adsorption and become of especial importance when we consider the water relations of the biocolloids. We have seen that molecular dipoles are strongly attracted to and oriented upon interfaces, and from the work of Blodgett (*loc. cit.*) we learn that such oriented films may under certain conditions be many molecular diameters in thickness. Water has a rather high dipole moment (1.85×10^{-18}). Smyth[2] notes that, "In the water molecule, the positive ends of two large doublets lie near the surface causing a very strong field of force around the molecule, so that the molecules affect one another greatly, strong association occurs, and the liquid is highly abnormal."

[1] Papers which may be consulted as general references are:

Gortner, R. A., The State of Water in Colloidal and Living Systems, *Trans. Faraday Soc.*, 26: 678–686 (1930) (*cf.* also discussion, pp. 686–704)

Jones, I. D., and Gortner, R. A., Free and Bound Water in Elastic and Non-Elastic Gels, *J. Phys. Chem.*, 36: 387–436 (1932)

Gortner, R. A., The Rôle of Water in the Structure and Properties of Protoplasm, *Ann. Rev. Biochem.*, 1: 21–54 (1932)

Gortner, R. A., Water in Its Biochemical Relationships, *Ann. Rev. Biochem.*, 3: 1–22 (1934)

Gortner, R. A., The Rôle of Water in Living Organisms, "Outlines of Biochemistry," pp. 227–249, John Wiley and Sons, New York, 1929

Barnes, T. C., and Jahn, T. L., Properties of Water of Biological Interest, *Quart. Rev. Biol.*, 9: 292–341 (1934)

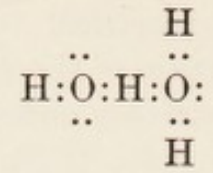
[2] Smyth, C. P., Dielectric Constant and Molecular Structure, A. C. S. Monograph, 55: 180, Chemical Catalog Company, New York, 1931

Unquestionably surface electrical forces (stray valences, etc.) would cause a similar attraction of the water dipole so that it would be "associated" with such surfaces under the same forces that cause the association of water in bulk. If the surface attraction is more intense than is the attraction of adjacent water molecules, then the intensity of the water-binding at surfaces will be correspondingly increased. In this connection it may be pertinent to call attention to the conception of Latimer and Rodebush[3] of a "hydrogen bond" as a factor in the phenomenon of association, and the comments which Hildebrand[4] has made on the rôle which the hydrogen bond may play in solubility relationships. Latimer and Rodebush make the following statement: "The phenomenon of association in liquids has long been recognized as related to dielectric constant and ionizing power as a solvent. According to one view, a so-called polar solvent contains dipoles of considerable moment, that is, positive and negative charges separated by a considerable distance. The high dielectric constant of such a liquid is considered to be due to the orientation of these dipoles in an electric field. Likewise association is supposed to take place because of the attraction of two dipoles for each other. This explanation is open to serious objections. In the first place it is hard to see why the compounds of very high dielectric constant should be chiefly hydrogen compounds. Also hydrogen chloride should contain dipoles of greater moment than water or hydrogen fluoride, yet it has a much lower dielectric constant both in the vapor and liquid. Nor does hydrogen chloride appear to be associated. It seems then that the explanation is to be sought along other lines.

"Let us compare again the compounds ammonia, water and hydrogen chloride. Ammonia adds a hydrogen readily but has little tendency to give one up. Hydrogen chloride, on the other hand, shows just the opposite tendencies. Water occupies an intermediate position and shows tendencies both to add and give up hydrogen, which are nearly balanced. Then, in terms of the Lewis theory, a free pair of electrons on one water molecule might be able to exert sufficient force on a hydrogen held by a pair of electrons on another water molecule to bind the two molecules together. Structurally this may be represented as

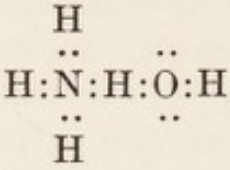
[3] Latimer, W. M., and Rodebush, W. H., *J. Am. Chem. Soc.*, 42: 1419–1433 (1920)

[4] Hildebrand, J. H., *Science*, 83: 21–24 (1936)



Such combinations need not be limited to the formation of double or triple molecules. Indeed the liquid may be made up of large aggregates of molecules, continually breaking up and reforming under the influence of thermal agitation.

"Such an explanation amounts to saying that the hydrogen nucleus held between 2 octets constitutes a weak 'bond.' Ammonium hydroxide



is an example in which the union is fairly strong. . . . There seems to be no reason for believing that gradations may not exist all the way from the case of ammonium chloride, where the hydrogen is definitely transferred from the chlorine to the ammonia, to the case in the association of water where the hydrogen is still held quite firmly to the original water molecule."

According to this view the known surface affinities of many substances for water may actually result in a more or less true compound formation where the vibrational energy of the hydrogen of the bond has been markedly reduced.

That certain surfaces strongly attract water is well-known. Aluminum oxide properly prepared is a better dehydrating agent for certain gases than is P_2O_5. Silica gel has a great affinity for water, although there is no evidence from the reaction curves for the formation of definite compounds of the type H_2SiO_3; rather the water appears to be held in adsorbed films of oriented water molecules on the surfaces and as water filling minute capillaries by capillary surface forces. Patrick and Grimm[5] observed that silica gel may retain as much as 4.8 per cent of water at 300°C. in a vacuum and that such water shows no appreciable vapor pressure at this temperature and pressure. Obviously such water is in a physical state vastly different from water in bulk.

Heat is usually evolved when adsorption takes place. This heat

[5] Patrick, W. A., and Grimm, F. V., *J. Am. Chem. Soc.*, 43: 2144–2150 (1921)

energy is derived from the reduction in surface energy in the interface and from the reduction of the kinetic energy of the molecules which are adsorbed. Such heat of adsorption may be very great. With a platinized grid and an organic vapor the heat may be sufficient to cause the grid to glow and ignite the vapor when oxygen is present. Nutting[6] has calculated from the heat of wetting of silica gel the intensity of the adsorption forces at a SiO_2—water interface and concludes that these are as great as those which would be exerted by a pressure of 17,410 atmospheres. He notes that if the solid pulls on the water with a pressure of more than 17,000 atmospheres, the water must pull on the solid with an equal pressure and furthermore that 17,000 atmospheres approximates the tensile strength of crystalline quartz. Surely this is an intense adsorption force for quartz-water at an interface. Nutting further calculates that at such an interface we are probably dealing with water films 100–120 molecules deep before the intensity of adsorption begins to fall off sharply. Keyes and Marshall[7] also argue in favor of polymolecular adsorption films at interfaces, pointing out that each new monomolecular layer constitutes a new surface obviously different from the first one but nevertheless a surface upon which, theoretically at least, adsorption and orientation of molecules can occur. Eventually in the building up of such polymolecular films we will reach a point where the energy inducing adsorption and orientation is no greater than the kinetic energy of the molecule which is being adsorbed and oriented and at this energy value the adsorption film will cease to increase in thickness. That there is a definite "adsorption pressure" is indicated by numerous experiments showing the increased density of liquids when present in the adsorbed state, *e.g.* Ewing and Spurway[8] who found a density of water adsorbed on SiO_2 equal to 1.0285 up to a water content of 4.36 per cent, such a density of water corresponding to a pressure of about 750 atmospheres.

Imbibition Pressure. Some of the earlier studies on the imbibition pressure of the biocolloids were carried out by Reinke[9] who measured the swelling of dried disks of the sea alga, *Laminaria*, against water. He constructed an apparatus in which the dry disk could be placed in a hollow metal cylinder fitted with a piston upon which weights

[6] Nutting, P. G., *J. Phys. Chem.*, 31: 531–534 (1927)
[7] Keyes, F. G., and Marshall, M. J., *J. Am. Chem. Soc.*, 49: 156–173 (1927)
[8] Ewing, D. T., and Spurway, C. H., *J. Am. Chem. Soc.*, 52: 4635–4641 (1930)
[9] Reinke, J., *Hanstein's bot. Abhandl.*, 4: 1–137 (1879)

could be placed, the bottom of the cylinder being perforated to allow water to come in contact with the material in the cylinder. When the cylinder was immersed in water, swelling took place, the piston was elevated against a known weight, and the swelling force measured in this manner. MacDougal's[10] auxograph is a more refined instrument which plots the swelling curve on a kymograph.

Shull[11] has discussed certain phases of imbibition as related to botanical problems, and he notes that dried seeds will absorb water from a saturated solution of lithium chloride until they attain a water content of about 8—9 per cent. Since a saturated solution of lithium chloride has an osmotic pressure approximating 1,000 atmospheres, it is evident that the imbibition pressure of the seed colloids may reach enormous values. Other experiments indicate that starch heated in the presence of water will swell against a pressure of 2500 atmospheres.

All of the above imbibition pressures are much greater than the maximum osmotic pressures characteristic of biological materials. The maximum osmotic pressure which has been observed for plant saps is apparently that measured by Harris, Gortner, Hoffman, and Valentine[12] who found a freezing point depression of 14.4°C. for the sap expressed from *Atriplex nuttallii*. Such a freezing point depression would correspond to an osmotic pressure of 169.3 atmospheres, assuming the conventional conversion formulas to hold. This particular osmotic pressure was largely due to the presence of soluble inorganic salts taken up by the plant, for the sample of sap investigated contained chlorides equivalent to 93.1 grams chlorine per liter. It seems probable that an osmotic pressure of 175 atmospheres is near the limit of true osmotic pressure values which may be attained by halophytes and that this group of plants probably represents extreme osmotic pressure values. Such an osmotic pressure, however, is far below the imbibition pressure which many biocolloids exhibit, and accordingly imbibition pressure must be regarded as being of an entirely different order of magnitude from true osmotic pressure effects.

The Bound-Water Concept.—The concept of bound water as a physiological factor apparently arose independently with three groups

[10] MacDougal, D. T., Hydration and Growth, Carnegie Institution of Washington, Publication No. 297, Washington, 1920

[11] Shull, Charles, *Bot. Gaz.*, 56: 169–199 (1913); *Ecology*, 5: 230–240 (1924)

[12] Harris, J. A., Gortner, R. A., Hoffman, W. F., and Valentine, A. T., *Proc. Soc. Exp. Biol. Med.*, 18: 106–109 (1921)

of investigators at approximately the same time. Balcar, Sansum, and Woodyatt[13], in 1919, made the suggestion that fever in the human organism might be due to a shift in the water relations of the body. They postulated that a part of the water in the normal individual might be bound to the collodial constituents and another part be a free liquid, and that pathological conditions might produce a shift in the bound $\rightleftarrows$ free water ratio. They note, however, that there were available at that time no technics by which their theory could be tested.

In the fall of 1920, Robert Newton began the investigation of the nature of winter hardiness in winter wheat, *Triticum vulgare*, in the laboratories of the Division of Agricultural Biochemistry, at the University of Minnesota. He planned on studying the physico-chemical properties of the sap expressed from hardy and non-hardy varieties following the technics which had been used so extensively by Harris, Gortner, *et al*[14]. The method consisted essentially of freezing the plant tissues so as to disrupt the protoplasmic structure, expressing the sap under high pressure, and determining the various physico-chemical constituents on such expressed sap. Newton made a collection of wheat leaves from the various plots on October 9, 1920, and secured approximately 60 cc. of sap from each 100 grams of leaves. No marked differences were observed between those varieties which were known to be winter-hardy and those which were known to be winter-tender. A similar collection was made on or about November 9, 1920, with similar results. In a third collection made early in December, Newton found that he was unable to express any appreciable quantity of liquid from "Minhardi," the most winter-hardy type, and this failure to obtain any appreciable amount of sap persisted even after the collected leaves had been exposed to a temperature of $-58°$ C. Here then was a clue to the nature of winter-hardiness in plants. Apparently by exposure to low temperature in the field plots the winter-hardy varieties had "hardened-off," and the water relations of the protoplasm had been so changed that the cells were not disrupted by freezing, nor could water be expressed from the tissues

[13] Balcar, J. O., Sansum, W. D., and Woodyatt, R. T., *Arch. Internal Med.*, 24: 116–128 (1919)

[14] For a general discussion of methods, objectives, and experimental data secured in a great variety of ecological habitats see, Harris, J. A., "The Physico-Chemical Properties of Plant Saps in Relation to Phytogeography: Data on Native Vegetation in its Natural Environment," University of Minnesota Press, Minneapolis, 1934, 339 pages.

under high pressures. Fig. 26 shows data which Newton[15] obtained in the winter of 1921-22 from similar experiments with a series of six wheat varieties. This series of experiments led Newton and Gortner[16] to propose a bound-water theory as a determining factor in the phenomena of winter hardiness. They suggested that in the hardening-off process a portion of the water became intimately associated with the hydrophilic colloids so that it was essentially removed from the liquid state and became to all intents and purposes a part of the solid phase, and they devised a technic whereby they could test this theory.

Independently of the work of Newton and Gortner, Rubner and his co-workers [17, 18, 19] arrived at essentially the same viewpoint with respect to the ability of protoplasmic colloids to bind water and remove it from the liquid state. Rubner likewise devised a technic for testing this viewpoint, which technic will be referred to later as the calorimetric method.

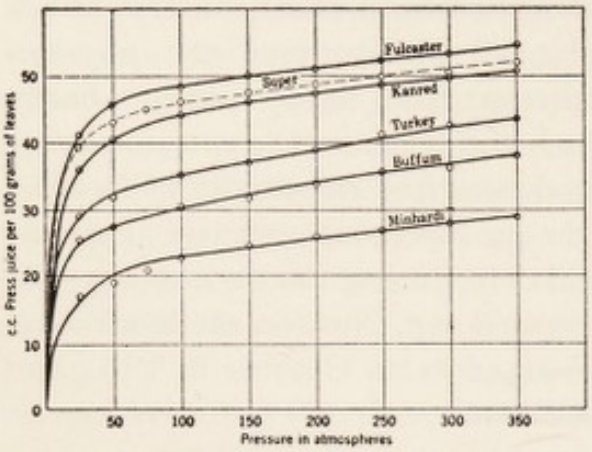


FIG. 26. Showing the amount of sap expressed from "hardened" wheat leaves of various wheat varieties as a function of the pressure applied (Data of Newton)

Methods for the Determination of Bound Water.—(1) The Cryoscopic Method.—The cryoscopic method was first suggested by Newton and Gortner (*loc. cit.*). The theory postulates that bound water is so intimately associated with the lyophilic colloids that it is no longer available to act as a solvent. The freezing point depression, Δ, of a sample of plant sap or of an animal fluid is accurately determined. Then to an aliquot of such fluid containing a definitely known amount of water there is added a definite amount of a solute, *e.g.* sucrose, and a second freezing point determination is made. If all of the water in the biological fluid is capable of acting as a solvent, the second depression of the freezing point should be the sum of the original depression plus the molar depression of the solute which was added. If, however, not all of the water was free to dissolve the solute, there would be observed an "excess depression" of

[15] Newton, Robert, *J. Agr. Sci.*, 12: 1–19 (1922); 14: 178–191 (1924)
[16] Newton, Robert, and Gortner, R. A., *Bot. Gaz.*, 74: 442–446 (1922)
[17] Rubner, M., *Abh. preuss. Akad. Wiss phys. math. Klasse* (1922) pp. 3–70
[18] Thoenes, F., *Biochem. Z.*, 157: 174–186 (1925)
[19] Thoenes, F., *Mschr. Kinderheilk.* 29: 378 (1925)

the freezing point, and from such data one could calculate the amount of water which was bound, *i.e.* not free to act as a solvent. Inasmuch as sucrose apparently forms a hexahydrate in solution[20], a gram molecular solution of sucrose would be a system containing a gram molecule of sucrose hexahydrate dissolved in 892 grams of water, and the molar depression (K_m) of a gram molecular solution of sucrose would be 2.085° C. instead of the theoretical 1.86° C. Newton and Gortner proposed the following equation for the determination of the amount of bound water by the cryoscopic method.

$$\frac{\Delta_a - (\Delta + K_m)}{\Delta_a - \Delta} \times 892 = \text{grams bound water per liter water in system} \qquad (40)$$

where Δ_a = the freezing point depression after the addition of a quantity of solute sufficient to form a molar solution in the amount of water which is present;

Δ = the depression of the freezing point on the liquid prior to the addition of the sucrose;

K_m = a molar constant for the depression of the freezing point.

Grollman[21] has criticized this method, pointing out that when salts or dissolved solutes are present, the difference between the two freezing point depressions will not be an accurate measure of the water which is not free to act as a solvent. Grollman accordingly proposed a modification of Equation (40).

$$\frac{\Delta_a - \left(\frac{1000}{892}\Delta + K_m\right)}{\Delta_a - \frac{1000}{892}\Delta} \times 892 = \text{grams bound water per liter water in system} \qquad (41)$$

where the quantities have the same notations as in Equation (40).

By recalculating certain of Newton and Gortner's data by the use of Equation (41), Grollman concludes that there is no evidence that bound water and winter hardiness are related. This criticism has been answered by Gortner and Gortner[22] who point out that Grollman's

[20] Scatchard, G., *J. Am. Chem. Soc.*, 43: 2406–2418 (1921)
[21] Grollman, A., *J. Gen. Physiol.*, 14: 661–683 (1931)
[22] Gortner, R. A., and Gortner, W. A., *J. Gen. Physiol.*, 17: 327–339 (1934)

criticism and Equation (41) apply only to plant saps and to solutions containing true solutes and that in a system containing only lyophilic colloids and water Equation (40) should hold. Likewise, they point out that Grollman's criticism of the lack of relationship between bound-water values and winter hardiness in wheats was arrived at from calculations on greenhouse-grown plants which had not been hardened-off and which accordingly were in a state in which no differences in bound water value between varieties would be anticipated. Table XXIII shows the calculations of bound water for greenhouse-

TABLE XXIII

The Determination of Bound Water in Certain Plant Saps and Lyophilic Sols by the Cryoscopic Method

(Taken from the paper of Gortner and Gortner)

Materials used		Calculations by Formula (40) of Newton and Gortner					Calculated by Formula(41) of Grollman
Leaves of		Δ	Δa	$\Delta a - \Delta$	$\Delta a - (\Delta + K_m)$	Bound water	Bound water
		degrees	*degrees*	*degrees*	*degrees*	*per cent*	*per cent*
Feb. 3–18, 1922. Collected from the open	Minhardi	1.741	4.226	2.485	0.400	14.4	7.49
	Buffum	1.719	4.158	2.439	0.354	13.0	5.49
	Turkey	1.273	3.612	2.339	0.254	9.7	4.10
	Kanred	1.461	3.753	2.292	0.207	8.1	3.65
	Super	1.085	3.279	2.194	0.109	4.4	−0.89
	Fulcaster	1.202	3.394	2.192	0.107	4.3	−1.60
From greenhouse. Feb. 10–16, 1922	Minhardi	1.147	3.284	2.137	0.052	2.2	−3.83
	Super	1.000	3.106	2.106	0.021	0.9	−4.37
	Cactus (stems)	0.505	2.803	2.298	0.213	8.3	5.80
	Gum acacia sols						
	1 per cent	0.005	2.147	2.142	0.057	2.37	2.32
	3 per cent	0.013	2.186	2.173	0.088	3.61	3.48
	5 per cent	0.025	2.221	2.196	0.111	4.50	4.37
	7 per cent	0.034	2.254	2.220	0.135	5.42	5.26
	10 per cent	0.048	2.294	2.246	0.161	6.39	6.15

and field-grown wheat varieties as well as for sols of gum acacia calculated by both Equation (40) and Equation (41).

Incidentally Gortner and Gortner introduced a somewhat different technic for calculating the freezing point depression of sucrose solu-

tions in the presence of lyophilic colloids and concluded that one mole of sucrose in water combines with 95.8 grams of water so that the 892 in Equations (40) and (41) should probably be 904.2.

Another objection of Grollman to the cryoscopic technic is that certain of the systems studied yield negative bound-water values, and Grollman believes that a negative bound-water value is an impossibility. Gortner and Gortner point out that a negative bound-water value may be expected under certain conditions. The assumptions which are made in the cryoscopic technic are (1) that none of the added sucrose dissolves in the bound-water and (2) that the addition of sucrose does not alter the bound-free water equilibrium. Probably neither one of these assumptions is strictly correct. However, the cryoscopic technic is valuable in that it should give minimal values for bound water. The amount of bound water which is indicated by the cryoscopic method will be a maximum if these two assumptions were strictly correct, *i.e.*, if the water is adsorbed by the lyophilic colloid to the strict exclusion of the solute. If both solvent and solute are equally adsorbed by the lyophilic colloid, there will be no bound water indicated. If, however, the solute is more strongly adsorbed by the lyophilic colloid, than is the solvent, then a negative bound-water value will be indicated. This is apparently what happens when electrolytes, such as potassium chloride, or certain non-electrolytes, such as urea, are used, as they were in Grollman's experiments.

The most extensive series of biochemical studies in which the cryoscopic method has been used are those carried out by Newton and Martin[23] on the nature of drought resistance in crop plants. Table XXIV shows bound-water values which these workers found for certain colloidal sols.

Newton and Martin find a remarkable parallelism between the drought resistance of different plant types and bound water as measured by the cryoscopic technic. They studied a transect of the vegetation in a habitat near Edmonton, Alberta, collecting native plants growing in a swamp land area with their roots perpetually moist as contrasted with other native plants growing on the sides and top of a hillside where moisture relationships varied from relatively moist conditions at the bottom of the hill slope to essentially arid conditions on the sandy hilltop. Table XXV, taken from their paper, shows the relationship of bound water, as determined by the cryoscopic method

[23] Newton, Robert, and Martin, W. McK., *Canadian J. Res.*, 3: 336–427 (1930)

TABLE XXIV

Water Bound by Different Colloidal Substances at Various Concentrations
(Cryoscopic Technic. Data of Newton and Martin)

Material	Actual Concentration		Bound Water	Bound Water per gm. colloid
	Per cent	°C.	Per cent	grams
Gelatin	0.93	0.002	1.9	2.05
	1.86	0.004	3.2	1.70
	2.77	0.006	3.7	1.31
	3.66	0.005	4.4	1.17
	4.55	0.006	5.0	1.04
	5.43	0.010	5.5	0.96
Agar	0.87	0.003	2.9	3.36
	1.77	0.013	3.4	1.87
	2.61	0.020	4.1	1.52
	3.48	0.029	4.2	1.17
	4.36	0.036	4.2	0.93
	5.24	0.047	4.5	0.82
	6.13	0.054	5.5	0.84
	7.02	0.060	6.0	0.79
	7.92	0.064	6.8	0.79
	8.81	0.067	7.2	0.74
Vegetable albumin	1.88	0.027	1.3	0.70
	3.78	0.041	2.3	0.60
	5.11	0.046	3.0	0.56
	5.70	0.055	3.4	0.56
	7.65	0.068	3.4	0.41
	9.62	0.080	3.8	0.35
Dextrin	1.83	0.050	2.2	1.19
	3.67	0.062	3.4	0.89
	5.12	0.102	3.8	0.71
	5.54	0.095	3.9	0.66
	7.43	0.120	4.3	0.54
	9.34	0.148	4.9	0.47
	11.27	0.182	7.0	0.55
Blood fibrin	1.91	0.014	3.1	1.61
	3.84	0.020	5.1	1.29
	5.79	0.032	5.4	0.88
	7.77	0.042	5.7	0.68
	9.78	0.052	6.1	0.56
	11.81	0.062	6.9	0.51
Gum arabic	1.79	0.015	0.7	0.38
	3.50	0.035	1.8	0.48
	5.16	0.046	3.1	0.57
	6.75	0.053	4.0	0.56
	8.28	0.062	5.4	0.60
	9.76	0.073	6.9	0.64
	11.19	0.084	7.8	0.62
	12.56	0.096	8.7	0.61
	13.90	0.114	9.3	0.58
	15.18	0.132	10.3	0.58
	16.43	0.148	11.4	0.58

on the plant saps, to drought resistance of these grasses, *Bouteloua gracilis* growing on the sandy hilltop and *Bechmannia erucaeformis* growing in the slough at the bottom. It will be noted that there is not

TABLE XXV

Grasses Arranged in Order of Drought Resistance as Indicated by Average Bound Water in Seasons of 1925 and 1926

(Data of Newton and Martin)

Species	1925		1926		Average	
	Osmotic Pressure	Bound Water	Osmotic Pressure	Bound Water	Osmotic Pressure	Bound Water
	atm.	per cent	atm.	per cent	atm.	per cent
1. *Bouteloua gracilis*, Blue grama	34.9	16.73	59.9	28.59	47.4	22.4
2. *Stipa comata*, Western spear	23.2	15.14	27.7	9.58	25.4	12.4
3. *Agropyron cristatum*, Crested wheat	20.3	11.70	—	—	—	—
4. *Agropyron tenerum*, Western rye	18.7	10.49	16.1	10.18	17.4	10.3
5. *Bromus inermis*, Awnless brome	19.7	10.60	18.7	9.99	19.2	10.3
6. *Agropyron smithii*, Western wheat	17.4	7.09	21.1	8.27	19.2	7.7
7. *Poa pratensis*, Kentucky blue	13.4	4.66	16.4	5.92	14.9	5.3
8. *Calamovilfa longifolia*, Sand grass	13.2	6.91	17.8	3.32	15.5	5.1
9. *Fluminea festucacea*, Prickle fescue	13.4	4.29	19.1	5.85	16.2	5.1
10. *Phleum pratense*, Timothy	15.7	3.43	15.7	5.62	15.7	4.5
11. *Calamagrostis canadensis*, Blue joint	11.9	3.71	13.4	3.22	12.6	3.5
12. *Panicularia grandis*, Tall manna	9.7	4.40	15.5	2.22	12.6	3.3
13. *Bechmannia erucaeformis*, Slough grass	11.3	3.09	12.2	1.48	11.7	2.3

Coefficients of correlation

	n	r
Osmotic pressure, 1925, and osmotic pressure, 1926	12	0.92 ± 0.03
Bound water, 1925, and bound water, 1926	12	0.82 ± 0.06

a single instance in which the bound-water values fall out of line with the known drought resistance of the vegetation.

Table XXV might be taken as indicating that osmotic pressure relationships were of equal importance with bound-water values in determining drought resistance of plants. However, Table XXVI, again taken from the data of Newton and Martin, indicates that this is not the case. Here we are dealing with various wheat sorts varying

widely in drought resistance and arranged in their known order of drought resistance as determined by years of agronomic testing. The osmotic pressure values of these various wheat sorts show no parallelism with their known drought resistance, whereas there is a strict parallelism of the bound-water values with the agronomic character-

TABLE XXVI

Wheat Species and Varieties Arranged in Order of Drought Resistance as Indicated by Average Bound Water in Seasons of 1925 and 1926

(Data of Newton and Martin)

Species	1925		1926		Average	
	Osmotic Pressure	Bound Water	Osmotic Pressure	Bound Water	Osmotic Pressure	Bound Water
	atm.	per cent	atm.	per cent	atm.	per cent
1. *Triticum dicoccum* var. Common emmer	14.3	7.10	11.6	7.39	12.9	7.2
2. *T. durum* var. Kubanka	13.9	6.73	13.8	7.23	13.8	7.0
3. *T. turgidum* var. Alaska	13.3	6.38	13.6	7.43	13.4	6.9
4. *T. vulgare* var. Caesium	13.9	6.67	14.5	6.43	14.2	6.5
5. *T. compactum* var. Hybrid 143	12.8	5.53	13.3	5.98	13.0	5.8
6. *T. spelta* var. White spelt	14.2	5.72	15.3	5.58	14.7	5.6
7. *T. monococcum*, Einkorn	12.7	5.42	12.6	5.84	12.6	5.6
8. *T. polonicum* var. White Polish	13.0	4.20	11.8	4.66	12.4	4.4
9. *T. vulgare* var. Marquis	14.6	3.87	12.5	4.30	13.5	4.1

Coefficients of correlation

	n	r
Osmotic pressure, 1925, and osmotic pressure, 1926	9	0.20 ± 0.22
Bound Water, 1925, and bound water, 1926	9	0.96 ± 0.02

istics. Whatever assumptions may lie behind the theory of the cryoscopic method and whatever objections may be brought against it from the theoretical standpoint, Tables XXV and XXVI demonstrate that it is measuring some property which is closely correlated with the response of plants to their environment.

Greathouse[24] studied the physico-chemical properties of plant saps in relation to environment, measuring osmotic pressure and bound-water by the cryoscopic technic on the saps of cabbage and milo subjected to drought and cold. He notes that a decrease in the soil water or a decrease in temperature both cause a shift of free water to bound water, and concludes that the bound-water measurements are a much more reliable index of winter-hardiness and of drought resistance than is osmotic pressure. He suggests that the osmotic pressure effects have

[24] Greathouse, G. A., *Plant Physiol.*, 7: 349–390 (1932)

TABLE XXVII

The Coefficients of Correlation between Certain Physico-Chemical Constants of Plant Saps

(Data of Gortner and Rude)

	Ligneous forms		Herbaceous forms		Both ligneous and herbaceous forms	
	No. of determinations	Coefficient of correlation	No. of determinations	Coefficient of correlation	No. of determinations	Coefficient of correlation
Osmotic pressure and bound water	23	+0.227±0.133	15	−0.345±0.153	38	+0.172±0.106
Total solids in sap, and bound water	23	+0.175±0.136	14	−0.289±0.165	37	+0.274±0.103
Total solids in sap, and osmotic pressure	25	+0.806±0.047	14	+0.546±0.127	39	+0.781±0.042
% of total water expressed and bound water	22	+0.373±0.124	10	−0.322±0.191	32	−0.013±0.119
% of total water expressed and total solids in sap	24	+0.044±0.137	10	−0.044±0.213	34	−0.207±0.111
% of total water expressed and osmotic pressure	25	−0.144±0.132	10	+0.319±0.191	35	−0.176±0.110
% of total water expressed and moisture content of leaves	31	+0.482±0.093	11	−0.658±0.115	42	+0.423±0.085
Water content of leaves and bound water	22	+0.318±0.129	15	+0.268±0.162	37	+0.011±0.111
Water content of leaves and osmotic pressure	25	−0.216±0.129	15	−0.703±0.088	40	−0.600±0.068
Water content of leaves and total solids in sap	24	−0.379±0.118	14	−0.732±0.084	38	−0.672±0.060

been over-emphasized and adds that drought resistance and cold-hardiness are not necessarily related.

Gortner and Rude[25] had earlier shown that bound water by the cryoscopic method was not correlated with any other of the usually determined physico-chemical properties of plant saps, and consequently that it is measuring a new, independent variable and as such should be of physiological and ecological interest. Table XXVII shows the correlation data upon which Gortner and Rude reached these conclusions.

Crist[26] studied the effect of fertilizer treatment on bound-water values in lettuce and tomatoes and found that the moisture content of the tissues and likewise the bound-water content of the tissues were markedly affected by the type and quantity of fertilizer applied. A fragment of his data is given in Table XXVIII.

TABLE XXVIII

Moisture Content and Bound-Water Content of Lettuce Tops as Affected by Fertilization with Calcium Nitrate

(Data of Crist)

Fertilizer Applied	Fertilizer Applied Per Pot	Water in tissue per gm. dry matter	Bound Water	Bound Water per gm. dry matter
	gm.	gm.	% of total water	gm.
None	0	7.90	9.1	0.93
Calcium nitrate	0.3	13.20	11.0	1.33
" "	0.8	13.70	17.9	2.76
" "	1.6	14.40	26.0	3.56

Kruyt and Winkler[27] applied the cryoscopic method and the viscometric method to the water relationships of starch sols. They conclude that the cryoscopic method measures the closely bound water in an oriented shell of dipoles, whereas the viscosity method measures not only this water but also the water which is held in a more diffuse layer. They find approximately 0.80 grams of bound water per gram of starch by the cryoscopic method, whereas there is a 2500 per cent increase in the volume of the micelle as measured by viscometric technics.

(2) The Calorimetric Method.—The development of the calorimetric method was due to Rubner (*loc. cit.*) and has been extended by

[25] Gortner, R. A., and Rude, Rachel, *Proc. Soc. Exp. Biol. Med.*, 25: 630–635 (1928)

[26] Crist, J. W., Tech. Bull., No. 74, Michigan Agricultural Experiment Station (1926)

[27] Kruyt, H. R., and Winkler, K. C., *Z. anorg. algem. Chem.*, 188: 200 (1930)

Thoenes (*loc. cit.*) and Robinson[28]. The theory of this method lies in the fact that ice has a latent heat of fusion of 80 calories per gram and that accordingly there is an absorption of 80 calories of heat energy whenever one gram of water in the form of ice changes to liquid water at 0° C. In determining bound water by the calorimetric technic the tissue under investigation is cooled to a low temperature, *e.g.*, −20° C. or −30° C., and held at that temperature for a sufficiently long period of time to presumably cause all of the free water to be frozen in the form of ice. The subsequent measurement of the heat required to bring that sample of material to some definite temperature above 0° C. permits of the calculation of the amount of water which had been converted into ice at the low temperature.

Robinson proposes the equation

$$X = \frac{FN\,(T_2 - T_3) - (WSR + W_1S_1R)}{80 - \frac{T_1}{2}} \qquad (42)$$

where F = correction for thermal capacity of calorimeter;
N = volume of water in cc. used in calorimeter;
T_2 = initial temperature of water in calorimeter;
T_3 = final temperature of water in calorimeter;
W = total weight of material;
S = specific heat of material;
R = range in temperature between T_1 and T_3;
W_1 = weight of tin foil container in which material (W) is placed;
S_1 = specific heat of tin foil which is 0.05;
T_1 = initial temperature of material in freezing cabinet. This being below zero appears to have a minus value but the formula is constructed so that the sign may be disregarded.

Thoenes has applied this method to the determination of bound water in certain animal and plant tissues as well as in gelatin and agar gels. Certain of his data are shown in Table XXIX. It will be noted that he found a decrease in the amount of bound water per gram dry material with dog muscle of increasing age. This is both interesting and suggestive. It is a general rule that lyophilic gels tend

[28] Robinson, W., *J. Biol. Chem.*, 92: 699–709 (1931)

TABLE XXIX

Percentages of Bound and Free Water in Certain Animal and Plant Tissues as well as of Gelatin and Agar Gels

(Data of Thoenes)

Material	Age	pH	Total Water	Free Water	Bound Water	Bound Water for each Gm. Dry Matter
			per cent	per cent	per cent	gm.
Dog muscle	24 hours		85.7	59.0	26.7	1.86
Dog muscle	3 weeks		83.8	60.4	23.4	1.44
Dog muscle	4 weeks		83.3	59.7	23.6	1.40
Dog muscle	Several months		79.3	55.1	24.2	1.16
Dog muscle	Several months		82.0	62.9	19.1	1.06
Dog muscle	Several months		79.7	58.3	21.4	1.05
Guinea pig muscle	Young (160 gm.)		81.6	61.5	20.1	1.09
Guinea pig muscle	Old (600 gm.)		79.6	60.5	19.2	0.94
Laminaria		5.5	57.2	21.25	37.8	0.92
Laminaria		6.2	69.8	32.4	37.4	1.19
Laminaria		8.0	62.5	28.3	34.2	0.91
Gelatin		5.3	87.0	62.8	24.2	1.86
Gelatin		4.3	86.4	60.25	26.2	1.92
Gelatin		3.0	87.1	59.5	27.6	2.14
Agar		5.5	94.1	69.55	24.55	4.15

to decrease their water-holding capacity with time, and perhaps the phenomena of old age may in part be due to a lower water-binding capacity.

Robinson's[29,30,31,32] studies have dealt largely with winter hardiness in insects. He points out that cold is one of the major factors in the natural control of insect population and that those insects which have the ability to live over under extreme cold conditions can be differentiated by bound-water technics from those which are winter tender. Robinson found that there was a "hardening-off" process in insects apparently analogous to the "hardening-off" process in plants, *i.e.*, when a winter-hardy insect was suddenly exposed to a low temperature, freezing occurred and the insect died. If, however, a cold-resistant insect was slowly acclimated to lower and lower temperatures, the bound-water content of the organism progressively increased to a point where complete hardiness had been produced. Fig. 27 shows certain of Robinson's data for the free- and bound-water changes of the pupae of *Telea polyphemus* as temperature was reduced over a period of days.

[29] Robinson, W., *Colloid Symp. Monograph*, 5: 199–218 (1928)
[30] Robinson, W., *Ann. Entomol. Soc. Amer.*, 21: 407–417 (1928)
[31] Robinson, W., *J. Econ. Entomol.*, 20: 80–88 (1927); 21: 897–902 (1928)
[32] Robinson, W., *J. Agr. Res.*, 37: 743–755 (1928)

Studies somewhat similar to those of Robinson have been conducted by Losina-Losinskii[33] who studied the diapause stages of *Euxoa segetum* Schiff which hibernates 15 to 20 centimeters below ground, *Loxostega sticticalis* L. which hibernates 3 to 4 centimeters below ground, and *Pyrausta nubilalis* Hübn. which hibernates in the stems of grasses and weeds above ground. Cold resistance increases in the order named. The first form is killed by under-cooling to approximately −6° C., the second by cooling to −7° C., whereas the form which hibernates above ground is resistant to temperatures as low as −21° C.

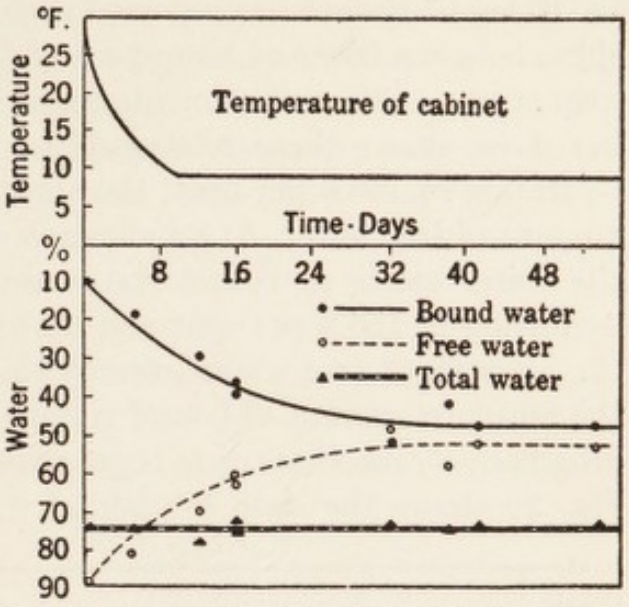


FIG. 27. Curves showing the total, "free," and "bound" water content in pupae of *Callosamia promethea* in relation to temperature (Data of Robinson)

Kehar and McCollum[34], using the calorimetric technic, studied bound water in cardiac muscle in relation to ventricular fibrillation and report 18.02 per cent of bound water in normal heart muscle which is reduced to 14.3 per cent in muscle after five minutes of fibrillation by electrical stimulation. In their studies the free water was frozen out by holding the tissue at −30° C. for two hours.

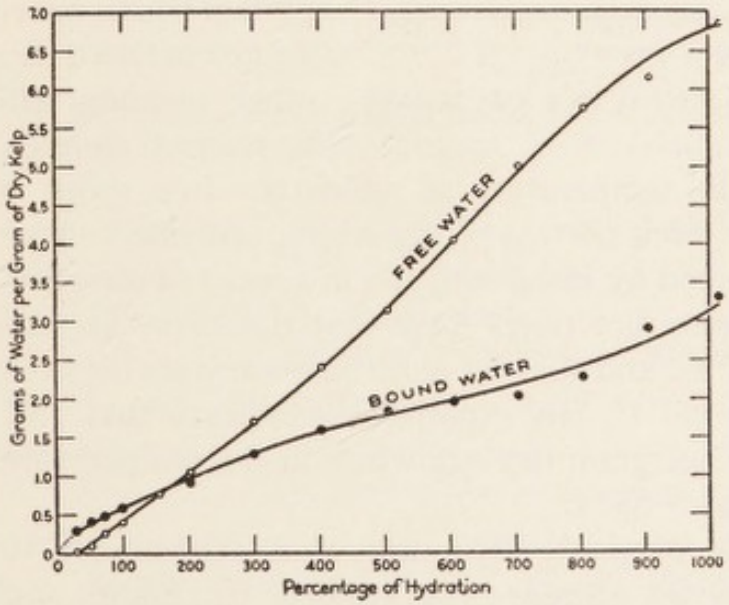


FIG. 28. Showing the "bound" and "free" water in kelp stipe at different degrees of hydration (Data of Chrysler)

[33] Losina-Lonsinskii, L., *Plant Protection* (U.S.S.R., No. 1, pages 15–22, 1935)
[34] Kehar, N. D., and McCollum, E. V., *Am. J. Physiol.*, 110: 485–487 (1934)

Helen Chrysler[35] has reported an interesting study of the relationships between free and bound water in a series of determinations on kelp stipe at different percentages of hydration. Fig. 28, taken from her data, shows these relationships. It will be noted that up to a hydration of 156.6 per cent, the amount of bound water exceeds the amount of free water. At a hydration of 156.6 per cent exactly half of the water exists as bound water and half as free water. Above a hydration of 156.6 per cent and up to a hydration of 1015 per cent, the amount of free water exceeds the amount of bound water, but the absolute amount of bound water in grams per gram dry material progressively increases up to the maximum degree of hydration studied. Fig. 29 shows the data for bound water plotted against degree of hydration on a logarithmic scale. Here a straight line is obtained as one would expect if one were dealing with an adsorption reaction. That similar straight lines are obtained from similar plots of bound water determined by other technics will be referred to later.

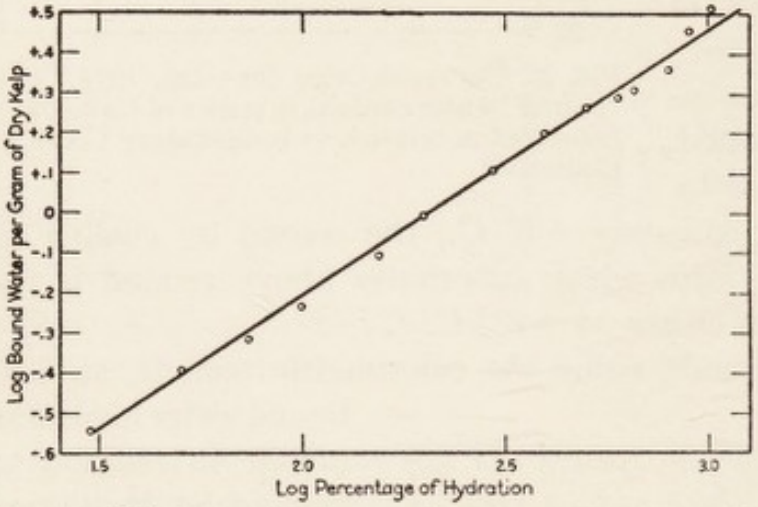


FIG. 29. The data of Fig. 28 plotted on a log-log scale (Data of Chrysler)

St. John[36] studied the temperature at which the free water is completely frozen in the thick portion of egg white, utilizing technic essentially like that proposed by Robinson, and in a series of measurements made over a temperature range finds that the curve flattens at approximately −12.5° C. and that no more water freezes between that temperature and −35° C. His experiments indicate that 1.97 grams of water is bound per gram dry egg white in the temperature range between −12.5 and −35° C.

(3) The Dilatometric Method.—The use of a dilatometer to measure water relations in colloids was studied by Foote and Saxton[37,38,39] in 1916 and 1917. They were, however, primarily in-

[35] Chrysler, Helen, *Plant Physiol.*, 9: 143–155 (1934)
[36] St. John, J. L., *J. Am. Chem. Soc.*, 53: 4014 (1931)
[37] Foote, H. W., and Saxton, B., *J. Am. Chem. Soc.*, 38: 588–609 (1916)
[38] Foote, H. W., and Saxton, B., *J. Am. Chem. Soc.*, 39: 627–630 (1917)
[39] Foote, H. W., and Saxton, B., *J. Am. Chem. Soc.*, 39: 1103–1125 (1917)

terested in the effect of freezing on the behavior of the colloid, but they conclude that in every system which they studied some of the water remained unfrozen, and they called this "combined water." Their studies were conducted on inorganic systems, such as SiO_2—H_2O, Al_2O_3—H_2O, $Fe(OH)_3$—H_2O, and lamp black—H_2O.

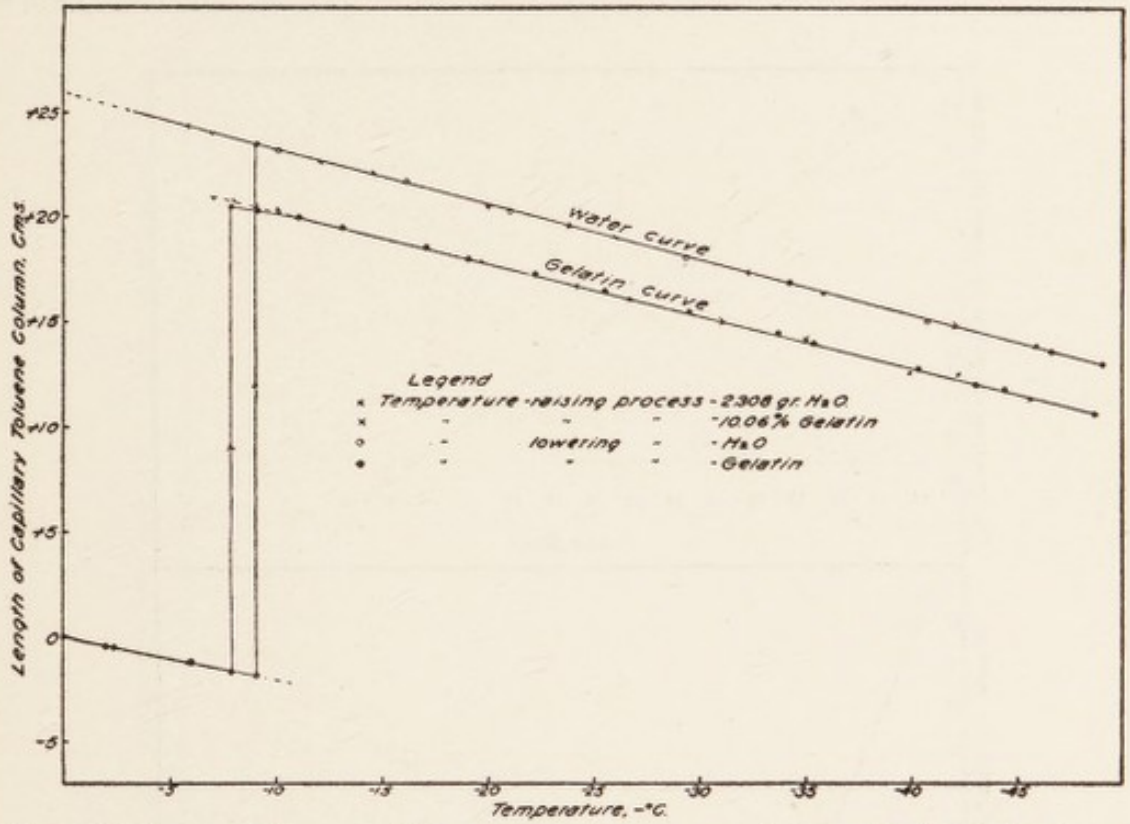


Fig. 30. Dilatometric curves for water, and water containing added gelatin, showing that part of the water is "bound" by the gelatin (Data of Jones and Gortner)

Bouyoucos[40,41] has applied the dilatometric technic to the determination of the "unfree" water in soils, *i.e.*, the water which is not available for plant growth, and McCool and Millar[42] have extended the work of Bouyoucos to studies of soil-plant relationships. Similarly, Lott[43], and Rosa[44] have applied the dilatometric technic to the problems of water relationships in winter hardiness.

Jones and Gortner[45] made a detailed study of the dilatometric

[40] Bouyoucos, G. J., *J. Agr. Res.*, 8: 195–217 (1917)
[41] Bouyoucos, G. J., *Tech. Bull.*, No. 36, Michigan Agricultural Experiment Station (1917)
[42] McCool, M. M., and Millar, C. E., *Bot. Gaz.*, 70: 317–319 (1920)
[43] Lott, R. V., *Research Bull.* No. 95, Missouri Agricultural Experiment Station (1926)
[44] Rosa, T. J., Jr., *Research Bull.* No. 48, Missouri Agricultural Experiment Station (1921)
[45] Jones, I. D., and Gortner, R. A., *J. Phys. Chem.*, 36: 387–436 (1932)

technic in its relationships to the bound-free water equilibrium, studying not only elastic gels, such as gelatin and egg white, but non-elastic gels, such as silica gel, ferric hydroxide gel, etc. Fig. 30 shows one of the curves taken from the paper by Jones and Gortner. In the case of the water curve it will be noted that contraction of the system occurred to approximately −10° C. Freezing then caused an

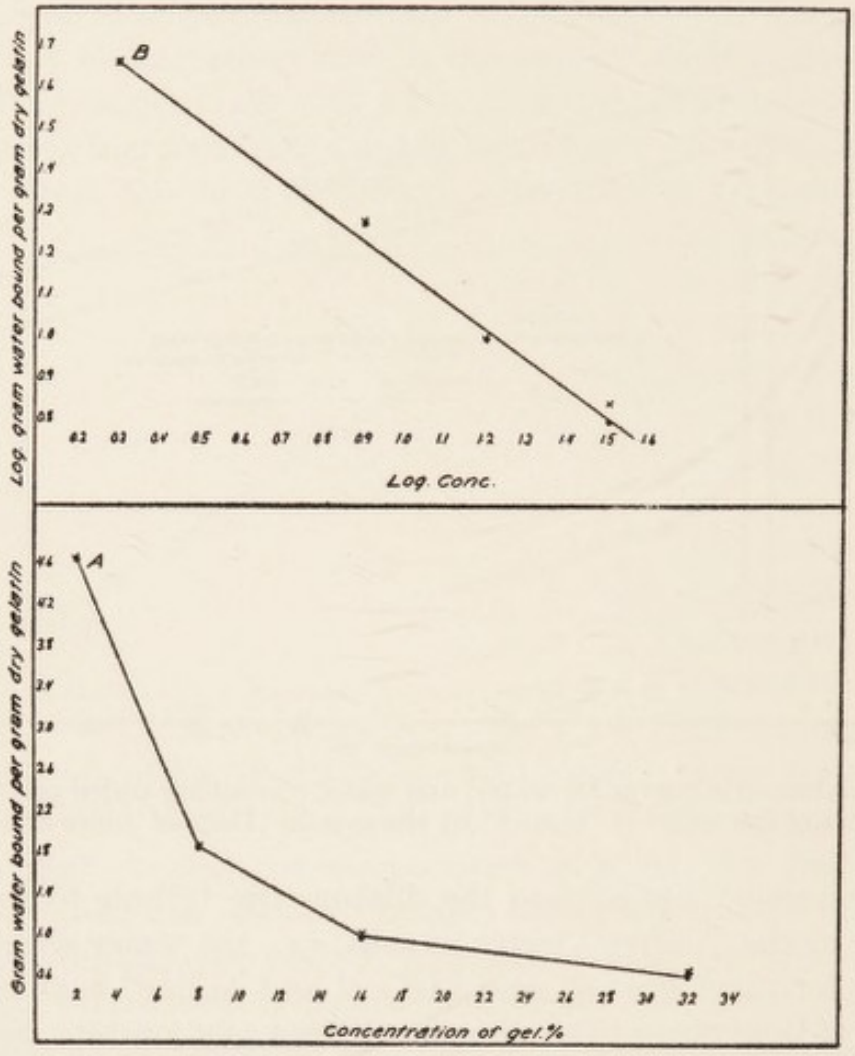


FIG. 31. Dilatometric curves of "bound" water in gelatin gels as a function of the gel concentration (Data of Jones and Gortner)

expansion of the system, and a continued lowering of the temperature caused a smooth straight-line contraction down to the lowest temperture studied, −48.6° C. When sufficient gelatin had been added to make a 10.6 per cent gelatin gel, the initial freezing began at a slightly higher temperature than in the case of the pure water, but the expansion of the system did not reach the expansion of the system

which is reached in the case of the water alone, and the gelatin curve paralleled the water curve down to the lowest temperature studied. Both the gelatin curve and the water curve were strictly reversible

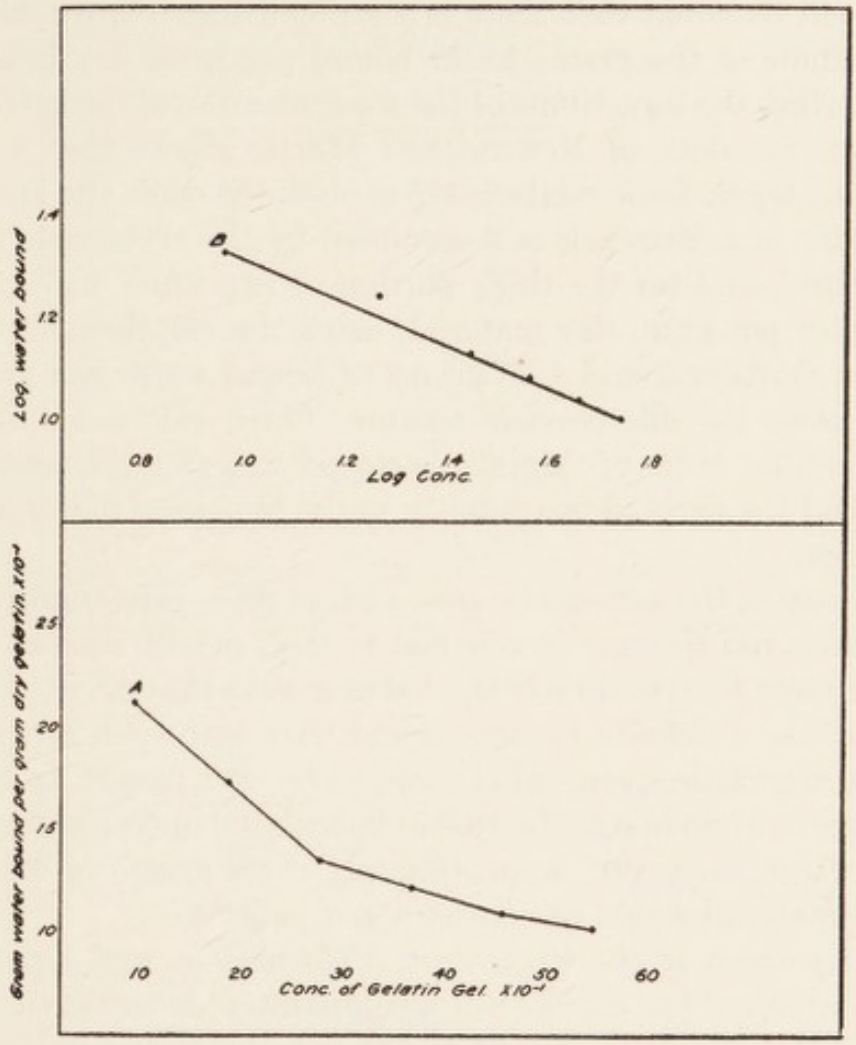


FIG. 32. Cryoscopic curves of the "bound" water in gelatin gels as a function of the gel concentration (Data of Newton and Martin)

TABLE XXX

The Bound Water in Gelatin Gels as a Function of Gel Concentrations

(Data of Jones and Gortner)

Gel Concentration	Bound Water Expressed as per cent of Total Water in System		Water Bound per Gram Dry Gelatin	
	−10°C.	−30°C.	−10°C.	−30°C.
per cent	per cent	per cent	gm.	gm.
2	9.35	9.35	4.675	4.675
8	15.10	15.19	1.888	1.899
16	16.16	16.82	1.010	1.051
32	20.56	22.43	0.643	0.701

upon raising or lowering the temperature. The difference between these two curves was taken as a measure of the bound water. Table XXX shows the percentages of bound water in gelatin gels determined at $-10°$ C. and $-30°$ C. These data are shown graphically in Fig. 31. Again it will be noted that there is a straight-line relationship when the logarithms of the grams water bound per gram dry gelatin are plotted against the logarithms of the concentration of the gel. Fig. 32, taken from the data of Newton and Martin shows that a similar straight-line logarithmic relationship is obtained when the amount of bound water in gelatin gels is determined by the cryoscopic technic.

St. John found for the thick portion of egg white 1.97 grams of bound water per gram dry material, using the calorimetric technic. Jones and Gortner found 1.55 grams of bound water per gram dry material using the dilatometric technic. These values are of a surprisingly similar order of magnitude considering the difference in the technics and the probable variability in the biological material under investigation.

In the case of the non-elastic gels, such as SiO_2—H_2O and $Fe(OH)_3$—H_2O Jones and Gortner in contrast to their gelatin studies did not find a complete reversibility of the freezing-thawing curves but found that there was a definite hysteresis and that with each series of decreasing temperatures somewhat more water was frozen. In the case of the ferric hydroxide gels the colloid flocculated upon freezing. Even in such cases, however, approximately 0.80 grams of water per gram dry material would not freeze above $-50°$ C.

A Comparison of the Cryoscopic, Calorimetric, and Dilatometric Technics.—Sayre[46] has carried out a comparative investigation of the cryoscopic, calorimetric, and dilatometric technics on a uniform substrate, *i.e.*, an 18.6 per cent gum arabic sol. Table XXXI shows the comparative data which he obtained. It will be noted that there is a remarkable uniformity in the average results obtained by the three methods. Consequently this is interpreted as definite evidence that these three independent methods are measuring the same physical property characteristic of this sol, and apparently at least on this particular system the methods could be used interchangeably.

(4) The Specific Heat Method.—Hampton and Mennie[47] have measured bound water by following the change in the specific heat of

[46] Sayre, J. D., *J. Agr. Res.*, 44: 669–688 (1932)

[47] Hampton, F. W., and Mennie, J. H., *Canad. J. Res.*, 7: 187–197 (1932); 10: 452–462 (1934)

TABLE XXXI

Comparison of the Three Methods of Measuring Bound Water in an 18.6 Per Cent Gum Arabic Solution

(Data of Sayre)

Cryoscopic Method	Calorimetric Method	Dilatometric Method
Bound Water	Bound Water	Bound Water
Per Cent	Per Cent	Per Cent
10.84	10.59	13.11
15.59	11.97	9.17
12.23	14.12	11.80
13.00	11.07	11.32
11.10	13.87	13.68
10.92	15.80	13.59
Mean = 12.28	Mean = 12.90	Mean = 12.11
σ = 1.83	σ = 2.02	σ = 1.74
$PE_m = \pm 0.50$	$PE_m = \pm 0.56$	$PE_m = \pm 0.48$

gel systems of various concentrations. They find that a portion of the water has a specific heat which is less than unity and that this portion of the water is regarded as "bound." They calculate the amount of bound water by the equation

$$H_{obs.} = aH_g + xH_x + (1-a-x)H_i - \lambda \qquad (43)$$

where a = the weight of dry gelatin;
x = grams bound water per gram of gel;
$H_{obs.}$ = the measured heat capacity of the gel;
H_g, H_x, and H_i = the heat capacity per gram of dry gelatin, bound water, and ice, respectively;
λ = a factor to correct for any evolution of heat which occurs when the frozen portion of the water is reabsorbed into the gel after melting.

Accordingly the amount of bound water (x) would be

$$x = \frac{aH_g + (1-a)H_i - H_{obs.}}{H_i - H_x} \qquad (44)$$

Hampton and Mennie find that the specific heat of the water in gelatin gels changes with the concentration of the gel and with temperature. Table XXXII, taken from their data, shows how the specific heat changes with gel concentration, and Table XXXIII shows their finding for the grams of bound water per gram dry gelatin in a 24 per cent gel at temperatures ranging from $-3°$ to $-78.5°$ C.

TABLE XXXII

Average Specific Heat of Water in Gels Between 0° and 25°C.

(Data of Hampton and Mennie)

% Gel	9.0	20.9	24.0	45.3	58.1	61.4	67.7	87.5
Av. sp. ht.	1.03	1.07	1.08	1.24	1.19	1.11	1.38	0.80

TABLE XXXIII

Bound Water per Gram of Dry Gelatin in 24 Per Cent Gel

(Data of Hampton and Mennie)

Temp., °C.	−3.0	−5.0	−10.0	−20.0	−40.0	−60.0	−78.5
Bound Water Equation (44)	0.69	0.57	0.46	0.37	0.33	0.26	0.24

It should be noted that they find approximately 25 per cent of the water in a 24 per cent gel to be bound at temperatures between −60° and −78.5° C. They conclude that in an 87.5 per cent gel all of the water is bound.

Hampton and Mennie furthermore point out that if the specific heat of water in a gelatin gel changes both with the gel concentration and with the temperature, then the assumptions which were made in Robinson's Equation (42) and in the equations of others who have used the calorimetric method will have to be modified and their equations will have to be corrected for variable specific heat factors.

(5) Direct Pressure Method.—The experiments of Reinke on imbibition pressure and the pressure curves of Newton (Fig. 26) have already been referred to. Perhaps the most important as well as the most recent contribution in this field is that of Lloyd and Moran[48]. These authors constructed a hydraulic press capable of relatively enormous pressures and so designed that the pressure could be held constant for long periods of time. Gelatin gels of various concentrations were wrapped in canvas impregnated with collodion and subjected to pressure in this hydraulic press. Lloyd and Moran found that, irrespective of the original concentration of the gel, the residual gel possessed essentially the same water content after equilibrium at a definite pressure had been established. They observed that there was a relatively sharp break in the pressure—gel concentration curve at approximately a 66 per cent gel and that this point is reached by a pressure of approximately 8000 pounds per square inch. They furthermore noted that in the pressure range between 8000 and 38,000

[48] Lloyd, D. J., and Moran, T., *Proc. Roy. Soc.*, London, 147A: 382–395 (1934) (*cf.* also Moran, T., *Proc. Roy. Soc.*, (B) 118: (No. 811) 548–559 (1935)

pounds per square inch only a comparatively small fraction of additional water is removed but that when the pressure is raised above 38,000 pounds per square inch, some additional water could be squeezed out. Fig. 33, taken from their data, shows the equilibrium gel concentration plotted against pressure in pounds per square inch.

Table XXXIV shows the calculations of Lloyd and Moran of the activity of the water under the observed pressures and the grams water per gram dry gelatin in equilibrium with the observed pressure. They then calculated the temperature at which water having these activities would be expected to freeze, and it will be noted that the freezing temperatures would range from −73° to −158° C. Accordingly it is not surprising that measurements of bound water by both the dilatometric and the calorimetric technics have indicated that an appreciable quantity of the water in gels is unfrozen in the temperature range of −20 to −30° C.

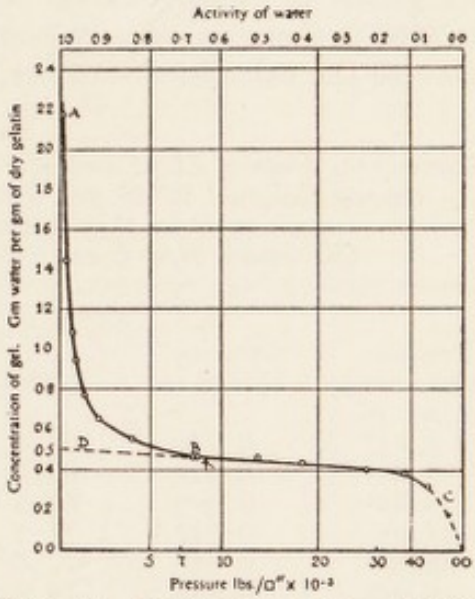


FIG. 33. Water content and "activity" of water in gelatin gels as a function of pressure (Data of Lloyd and Moran)

Lloyd and Moran accordingly reached the conclusion that there are two types of water in gelatin gels, loosely bound water which can be rather readily removed by pressure and closely bound water which is very resistant to removal. They regard this latter as being present in "chemical combination with the protein" and suggest that

> The hydration of proteins can occur by the formation of a co-ordinate link between a water molecule and certain groups in the protein structure for instance, the positively charged basic groups of the proteins readily accept a pair of electrons from the oxygen of a water molecule on to one (or more) of the hydrogen atoms.
>
> Conversely the negatively charged acidic groups of the proteins readily donate a pair of electrons from the singly bound oxygen atom to the hydrogen of a water molecule.
>
> Neutral groups, such as OH, NH_2, NH, COOH, can similarly form co-ordinate links with water molecules either by the donation or the acceptance of a pair of electrons. The formation of a co-ordinate link would be expected to show the usual characteristics of chemical change,

namely, heat changes (commonly evolution of heat) and considerable resistance to changes in physical conditions such as temperature, etc. It should be noticed however, that there is no way of constructing a co-ordinate link between a protein molecule and a water molecule except by donating a pair of electrons on to a chemically combined hydrogen atom. Since this already carries two electrons in its sheath and is in a stable condition, the link formed by making an outer sheath of four electrons will not be very resistant if compared with those links where the donation of two electrons leads to the completion of an electron sheath. Water co-ordinately bound to the protein molecule would be expected to show a considerable but not extreme resistance to, say, the forces of evaporation. . . . . .

The experimental data . . . . . show that 1 gram of dry gelatin carries 0.5 grams of closely bound water. One molecule of gelatin (mol wt. 34,500) is therefore closely bound to 960 water molecules, a figure very close to the calculated number of possible co-ordination centres.

TABLE XXXIV

Showing the Data of Lloyd and Moran for the Activity of Water, Gel Concentration, Grams Residual Water per Gram Dry Gelatin, and the Calculated Temperature at which that Amount of Water Would Freeze in Gelatin Gels which Had Been Held under Various Hydraulic Pressures

Pressure (observed) lb./sq. in.	Activity of Water	Conc. of Gel (observed)		Calc. Equivalent Freezing Temperature	Corrected Freezing Temperature
		% Gel	Gm. Water per Gm. Gelatin		
8,300	0.658	68.3	0.46	− 43° C.	− 73° C.
13,300	0.513	68.3	0.46	− 67° C.	− 94° C.
18,300	0.398	69.9	0.43	− 94° C.	−109° C.
28,300	0.242	71.5	0.40	−135° C.	−130° C.
38,300	0.146	72.6	0.38	−211° C.	−146° C.
48,300	0.088	75.8	0.32	−223° C.	−158° C.

Smyth (*loc. cit.* p. 190) states, "In polar liquids the greater molecular concentration caused by increased pressure should increase the interaction of the dipoles." If we regard a surface as polar and if we regard the water molecule as polar, the pressure either externally applied or induced by interfacial attractions should induce an increased immobilization of the water molecules, and that reasoning agrees with the experimental evidence.

(6) Drying Methods.—Nelson and Hulett[49], in 1920, studied the probable true water content of a number of biochemical substances with particular reference to the usual laboratory drying procedures. In their study they investigated the rate of moisture loss from these materials, *in vacuo*, at various temperatures. Certain of their data are shown in Figs. 34 and 35 and in Table XXXV.

[49] Nelson, O. A., and Hulett, G. A., *J. Ind. Eng. Chem.*, 12: 40–45 (1920)

TABLE XXXV

Showing the Difference between the Moisture Content of Various Biological Products as Obtained by the "Official" Method and the Probable True Moisture Content

(Data of Nelson and Hulett)

	Apparent Water Content at 100° *in Vacuo.*	Probable True Water Content.	Difference Due to Water Films Having No Appreciable Vapor Pressure at 100° *in Vacuo.*
	Per Cent	Per Cent	Per Cent
Wheat Flour	10.80	11.80	1.00
Cornmeal	11.34	12.25	0.91
Cornstarch	11.80	12.40	0.60
Cellulose (Swedish filter paper)	2.63	2.80	0.17
Cellulose (absorbent cotton)	5.49	5.90	0.41
Protein (edestin)	10.40	12.30	1.90

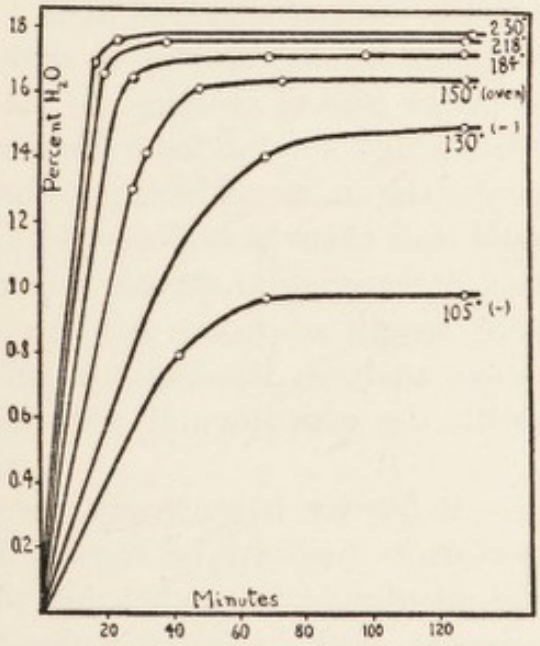


FIG. 34. Time versus moisture-loss curves for a sample of a biochemical product as influenced by the drying temperature (Data of Nelson and Hulett)

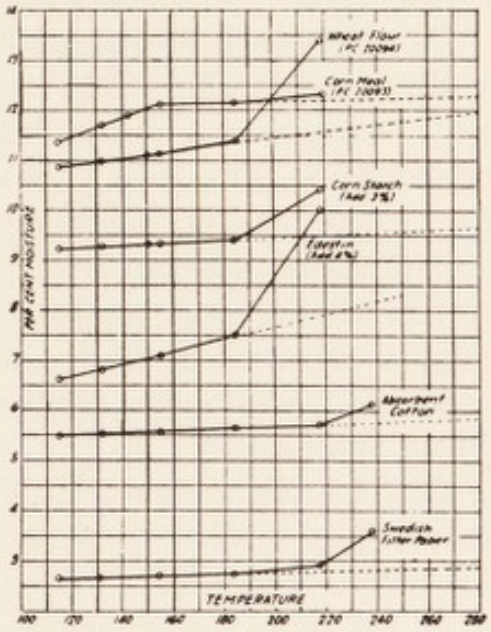


FIG. 35. Temperature versus equilibrium moisture-loss curves for a variety of biochemical products (Data of Nelson and Hulett)

It will be noted by reference to Fig. 34 that the drying procedure as ordinarily carried out in the laboratory is a purely empirical procedure and does not necessarily give the true moisture content. Nelson and Hulett assumed that the "probable true water content" could be determined from the graphs shown in Fig. 35. Over the initial portion of the curves there is a straight-line relationship between temperature

and per cent moisture. They conclude that over this portion of the curve only water from water films was being vaporized and that when the break in the experimental curve occurred, there was added an increment of water formed by the decomposition of the organic materials. Accordingly they extrapolated the initial straight-line portion of the curve to 365° C., the critical temperature of water, at which they state "water cannot exist as a liquid no matter how much pressure is applied," and assumed that the indicated moisture content at 365° C. represented the true moisture content of the material.

We have already seen that water does exist as adsorbed films in gels at temperatures above 365° C., and we have also seen that in all probability the firmly bound water in such adsorbed films is not liquid but is in reality a solid with a density appreciably greater than 1.0. It, therefore, appears probable that the values for the "probable true water content" in Column 3 of Table XXXV are too low and that even at 365° C. some water still remains in the form of adsorbed films.

This problem has been considered from a somewhat different angle by Rimington[50] who points out that in the case of proteins the elemental composition always indicated too high a value for hydrogen and oxygen to agree with what we know of the amino acid composition of a protein, and he therefore suggests that there is evidence, from this standpoint, of from 2 to 7 per cent of water still adhering to the protein when it had reached constant weight on being dried preliminary to carrying out the elementary analysis. Rimington's conclusion is in thorough agreement with the experimental work of Nelson and Hulett.

(7) The Refractometric Method.—Reference has already been made (Chapter I) to Graham's experiments whereby he showed a differential permeability of a bladder membrane to alcohol and to water. He explained the concentration of an alcohol solution confined within the bladder to the fact that water will wet the animal membrane more easily than will alcohol. In our present-day terminology Graham might have said that there was a preferential adsorption of water from the alcoholic solution by the animal membrane.

That such a preferential adsorption of water is not confined to biocolloids is indicated by the studies of Koets[51]. Working in the laboratories of Professor Kruyt, Koets investigated the differential adsorption of water and alcohol by silica gel, making use of a liquid

[50] Rimington, C., *Trans. Faraday Soc.*, 26: 699–702 (1930)
[51] Koets, P., *Proc. Konnij. Akad. Wetensch. Amsterdam*, 34: 420–426 (1931)

interferometer to measure change in concentration of the equilibrium solution. His data are shown in Table XXXVI. In these experiments 2 grams of silica gel were added to 20 grams of the water-alcohol mixture. Proper corrections were, of course, made for the original water contained in the silica gel.

TABLE XXXVI

Showing the Change in Concentration of Aqueous Alcohol Solutions Due to Selective Adsorption of Alcohol (−) or of Water (+) by Silica Gel

(2 Grams Silica Gel to 20 Grams Solution. Data of Koets)

Alcohol Conc.		Absolute Change	Relative Change
Blank c_1	After ads. c_2	$c_2 - c_1$	$\frac{c_2 - c_1}{c_2}$
Mole Per Cent	Mole Per Cent	Mole Per Cent	Per Cent
0.57	0.55	−0.02	−3.63
2.41	2.38	−0.03	−1.26
4.13	4.12	−0.01	−0.24
6.33	6.34	0.01	0.16
11.20	11.24	0.04	0.36
17.21	17.36	0.15	0.86
27.39	27.83	0.44	1.58
54.39	56.04	1.65	2.94
76.86	80.23	3.37	4.20
92.00	95.39	3.39	3.55
98.19	99.63	1.44	1.47

It will be noted that at low alcohol concentrations there is a slight positive adsorption of the alcohol. When the alcohol concentration equals about 6 mole per cent, the alcohol and water are adsorbed in the same ratio as in the original solution, and at higher alcohol concentrations there is a preferential adsorption of water with a maximum at about 90 mole per cent alcohol (*ca.* 95 weight per cent).

These experiments indicate that silica gel will "bind" water against the dehydrating forces of 95 per cent alcohol.

Dumanski[52] has also demonstrated by the use of a refractometer the preferential adsorption of water from sucrose solutions, using SiO_2, barium sulfate, talc, and aluminum oxide as the solid phase. The values for bound water which Dumanski obtained were low, but were greater than the experimental errors of the method.

(8) Polarimetric Method.—Koets (*loc. cit.*) has also used the polariscope to study the selective adsorption of water by silica gel from solutions of sucrose. Silica gel was added to solutions of sucrose of known concentration and after adsorptive equilibrium was at-

[52] Dumanski, A., *Kolloid Z.* 65: 178–184 (1933)

tained the concentration of the sugar solution in equilibrium with the solid phase was determined by reading the optical rotation. Table XXXVII shows the data obtained. It will be noted that equilibrium

TABLE XXXVII

Showing Selective Adsorption of Water (Bound Water) by Silica Gel in Contact with Sucrose Solutions (Polarimetric Method)

(Data of Koets)

	Gm. Gel		Rotation (α_D) (in 20 cm. tube)				
	Wet	Dry	Blank	Blank Corr.	After Ads.	Δ	Gm. H_2O withdrawn per gm. Gel
SiO_2 21% H_2O	5.00	3.95	3.92	3.84	3.93	0.09	0.30
	5.00	3.95	48.61	47.61	48.99	1.38	0.36
	10.00	7.90	48.82	46.86	49.12	2.26	0.30
	5.00	3.95	65.77	64.66	65.96	1.30	0.30
	5.00	3.95	65.06	63.95	65.35	1.40	0.33
	25.00	19.75[53]	3.92	3.24	3.92	0.84	0.31
SiO_2 25.4% H_2O	5.00	3.73	41.97	40.93	41.87	0.94	0.31
	10.00	7.46	41.97	39.94	41.75	1.81	0.30
SIO_2 5.7% H_2O	4.18	3.94	37.61	37.40	38.30	0.90	0.30
	8.29	7.82	37.61	37.19	39.00	1.81	0.30
	4.22	3.98	42.14	41.90	42.92	1.02	0.30
	8.19	7.72	42.14	41.66	43.66	2.00	0.30

in each case was reached at about 0.30 gram water per gram dry silica gel, irrespective of the initial water content of the gel. Koets interprets these data to mean that there is a layer of oriented water molecules at the surface of the silica gel which is so rigid that sugar molecules cannot penetrate into the layer, accordingly the water is positively adsorbed and the sugar is negatively adsorbed. The water adsorption by the silica gel appears to be independent of the concentration of the sugar solution, at least over the range of concentrations studied.

(9) Dielectric Constant Method.—Marinesco[54] has studied sols and gels of various concentrations with respect to changes in the dielectric constant, and from his studies he has concluded that appreciable quantities of the water in such systems possess a dielectric constant much lower than the value of 80 which characterizes water in bulk. He accordingly suggests that hydrophilic colloids are encased in a layer of oriented water molecules and that the surface pressure which is exerted on the water dipoles in this oriented layer is sufficiently great to immobilize the water molecules so that they

[53] In this case 25 gm. SiO_2 were added to 25 cc. solution instead of to 50 cc.

[54] Marinesco, N., *J. Chim. Physique*, 28: 51–91 (1931)

behave as a rigid system. For such immobilized water molecules he finds a dielectric constant which he suggests is analogous to that which would be characteristic of Bridgman's[55] ice VI which is stable above 0° in equilibrium with ice V and liquid water, when the pressure equals or exceeds 6,380 kilograms per square centimeter. It should be noted that Marinesco's conclusions are in agreement with the pressure data of Lloyd and Moran and with other data which have been previously referred to, indicating that water molecules which are adsorbed and oriented at interfaces are so immobilized that they assume the properties of solids and become to all intents and purposes a part of the solid phase.

(10) Vapor Pressure Method.—Hill[56], Hill and Kupalov[57], and Grollman (*loc. cit.*) have all studied the bound water problem, using relative vapor pressure methods. Hill studied the water relations in blood, centrifuged blood corpuscles, casein, egg white, and frogs' muscle. Hill and Kupalov made a detailed study of the vapor pressure of muscle, and Grollman studied the vapor pressure of a number of solutions containing either inorganic salts or organic solutes and the water relationships when gelatin or gum acacia was added to these systems. These workers agree in the conclusion that the vapor pressure method does not indicate any appreciable quantities of bound water in the systems which they studied, essentially all of the water being "free." Thus, Hill finds that only about three per cent of the water in blood can be regarded as "bound," and similar results were obtained for the casein and egg white systems. However, it should be noted that these systems contain from 70 to 85 per cent water, so that even 3 per cent of this would amount to from 7 to 17 per cent of bound water based on the dry matter content of the system studied.

Briggs[58, 59] has used the vapor pressure method in a physico-chemical study of water relations in colloids, using isoelectric casein and preparations of calcium and sodium caseinate as well as cellulose, fibrin, and agar, and points out that Hill applied the theory of dilute solutions to the systems under investigation, whereas in reality colloid systems appear to obey more nearly the laws of ideal concentrated solutions and, calculated from this standpoint, isoelectric casein appears to bind approximately 0.50 grams water per gram dry casein.

[55] Bridgman, P. W., *Proc. Am. Acad.*, 47: 439 (1912)
[56] Hill, A. V., *Proc. Roy. Soc.*, B, 106: 477–505 (1930)
[57] Hill, A. V., and Kupalov, P. S., *Proc. Roy. Soc.*, B, 106: 445–477 (1930)
[58] Briggs, D. R., *J. Phys. Chem.*, 35: 2914 (1931)
[59] Briggs, D. R., *J. Phys. Chem.*, 36: 367 (1932)

Briggs prefers to calculate the activity of the water in the colloid systems having various water contents and to measure "activity depressions" in the same way that freezing point depressions are used in the cryoscopic technic, and vapor pressure depressions are used in Hill's vapor pressure technic. Taking bound water to mean that water the activity of which is equivalent to a freezing point of $-20°$ C. as in the calorimetric method, Briggs finds that this point will be reached at an activity of water of 0.8221. Using this definition and the relative vapor pressure—water content curves, Briggs finds the following amounts of bound water per gram dry material: agar 0.37 grams, fibrin 0.33 grams, gelatin 0.33 grams, gum acacia 0.32 grams, and casein (isoelectric) 0.18 grams. These are appreciable quantities, somewhat comparable with quantities which have been obtained by other technics, and they indicate that even by the vapor pressure method appreciable quantities of bound water can be demonstrated to be present in lyophilic colloid systems.

There seems to be a logical explanation as to why vapor pressure technics indicate smaller bound-water values than do other methods. A necessary assumption in calculating data from vapor pressure measurements is that the system under investigation is in equilibrium. *A lyophilic colloid gel is never in equilibrium!* Every gel is a heterogeneous system consisting of a disperse phase and a dispersions medium. The bound water is assumed to be intimately associated with the disperse phase. Wo. Ostwald has stated that probably the most characteristic property of a gel is its tendency to undergo syneresis. A gel on aging tends to contract and to force out, from the interstices of the gel, liquid of syneresis. This liquid of syneresis is, as we have already seen, not pure water but a colloidal system rich in water, poor in solids, whereas the contracting gel tends to become more and more a colloid system richer in solids and poorer in water. The same argument will apply to a lyophilic sol, each micelle being thought of as an ultramicroscopic gel. Accordingly the hydrophilic colloid particles are not in strict equilibrium with the dispersions medium but undergo this phenomenon of syneresis, and the properties of the system change with time. That this is true is generally recognized by workers in the field of colloids who usually characterize the changing behavior of lyophilic systems under the term, hysteresis.

If the disperse phase and the dispersions medium are not in true equilibrium and if syneresis is continually taking place, it must be evident that the surface of a colloid gel is being continually bathed

with a thin film of the liquid of syneresis, a much more dilute colloid system than is characteristic of the body of the gel. Vapor pressure measurements on such systems would be measuring the vapor pressure of the more dilute portion of the system and accordingly should not be expected to yield maximum values for bound water nor indeed to yield average values but rather to yield minimal values for the bound water in the more dilute portion of the systems. The surprising thing, therefore, is that Hill found as much as 2 to 3 per cent of bound water in the liquid of syneresis of the muscle tissue which he was investigating. In similar tissue Thoenes, using the calorimetric technic, found a much larger portion of bound water. In the calorimetric technic the free water is immobilized in the form of ice, and theoretically the liquid of syneresis should be similarly immobilized as soon as formed. The same argument applies to the dilatometric technic. Accordingly these two technics, demonstrating approximately the same amount of bound water, would appear to be more reliable methods for the measurement of bound water than the vapor pressure technic. Persons who have worked with the cryoscopic technic have invariably recognized that repeated determinations on the same sample involving repeated thawings and refreezings tend to progressively lower bound water values. This result would again be anticipated if syneresis were continuing, and such syneresis probably accounts in a large measure for the change in the systems following repeated thawings and freezings.

General Conclusions.—We have discussed ten different methods which have been used by one or another series of investigators to study the state of water in hydrophilic colloid systems. Nine of these ten methods indicate clearly that a part of the water in hydrophilic sols and gels is immobilized or at least differs in its physical properties from water, as we know it, in bulk. The tenth method, the vapor pressure method, points in the same general direction but does not give the larger values indicated by some of the other methods. It has been pointed out that there is a theoretical basis for anticipating that the vapor pressure method should not yield as large values as are indicated by some of the other methods.

One other consideration points to the existence of water in colloid systems in a "bound" form, providing that by "bound" water we mean water molecules which have been so reduced in activity that they are not oriented into the crystal lattice pattern, characteristic of ice, when exposed to low temperature. This consideration lies in

the measurements which have been made of the "heats of wetting" and "heats of swelling" of dry materials which form colloidal systems. For example, Nutting (*loc. cit.*) presents the data taken from the papers of Bellatti and Finazzi,[60,61] and Parks[62] for the heat of wetting silica; Freundlich[63] gives the data for the heats of wetting of gelatin as taken from the papers of Rosenbohm[64], and Katz[65]. These data are shown in columns A and B of Table XXXVIII. In column B/A

TABLE XXXVIII

Calculations of the "Heat Loss per Gram of Water" by Adsorption of Water on SiO_2 and Gelatin

Silica[66]			Gelatin[67]		
(A) Water per Gram SiO_2	(B) Heat of Wetting per Gram SiO_2	(B/A) Heat Evolved per Gram H_2O	(A) Water per Gram Gelatin	(B) Heat of Wetting per Gram Gelatin	(B/A) Heat Evolved per Gram H_2O
Gm.	cal.	cal.	Gm.	cal.	cal.
0.0238	7.71	324	0.0053	2.1	396
0.0535	13.67	256	0.0168	5.3	316
0.0859	16.83	196	0.0315	7.0	222
0.1292	18.39	142	0.0406	8.9	219
0.1883	19.50	103	0.0514	10.0	194
0.2736	20.75	75.8	0.0738	13.1	177
0.3995	22.30	55.8	0.1030	18.4	177
0.4635	23.06	49.7	0.1070	19.1	178
0.5648	24.34	43.0	0.1328	22.2	168
0.6478	25.10	38.7	0.1932	30.6	158
0.7694	25.81	33.5	0.2420	33.2	137

is calculated *the heat of wetting in calories per gram of water involved* at the moisture contents shown in columns A. It will be noted in the case of silica that at 2.38 per cent water content the adsorption process has liberated 324 calories per gram of water adsorbed and that a similar evolution of heat takes place in the gelatin—water systems.

When liquid water is transformed into the ice crystal lattice, the heat of crystallization amounts to only 80 calories per gram. If we assume that the heat of wetting comes largely from the water molecules which lose a part of their kinetic energy when they become

[60] Bellatti, M., and Finazzi, L., *Atti. r. Ist. Veneto*, 61, II, 503–524 (1902)
[61] Bellatti, M., *Atti. r. Ist. Veneto*, 59, II: 931–947 (1900)
[62] Parks, I. J., *Phil. Mag.*, (VI) 4: 240–253 (1902)
[63] Freundlich, Herbert, "Colloid and Capillary Chemistry," p. 681, translated by H. Stafford Hatfield, E. P. Dutton and Company, 1926 (New York)
[64] Rosenbohm, E., *Kolloidchem. Beihefte*, 6: 177 (1914)
[65] Katz, J. R., *Kolloidchem. Beihefte*, 9: 1–182 (1917)
[66] Columns (A) and (B) from Nutting (*loc. cit.*)
[67] Columns (A) and (B) from Freundlich (*loc. cit.*)

oriented upon an interface, it is self-evident that those water molecules which have lost more than 80 calories per gram cannot rearrange themselves at a temperature below 0° C. into an ice crystal lattice which is characterized by a latent heat of fusion of 80 calories per gram. We may accordingly postulate that in those systems where the heat of wetting exceeds 80 calories per gram of water, such water will be "bound" in the sense that at temperatures below 0° C. it will not readily rearrange itself into ordinary ice crystals. In the case of silica gel noted in Table XXXVIII this limiting value of 80 calories per gram is reached in a gel containing 24 per cent of water. Jones and Gortner (*loc. cit.*) found in a 45 per cent SiO_2—55 per cent H_2O system that sufficient water remained unfrozen at −48° C. to give an SiO_2—H_2O gel containing 33.2 per cent of water. Admittedly this 33.2 per cent of water is appreciably higher than the 24 per cent of water indicated in Table XXXVIII, but the agreement is at least qualitative, and if we assume a small amount of condensed water in capillary spaces remaining undercooled at −48° C., the discrepancy could be easily explained. Similarly in the case of the gelatin figures of Table XXXVIII, we see that at the limit of Rosenbohm's experiments the adsorbed water at 0.24 gm. water per gram dry gelatin is still far above a limiting value of 80 calories per gram. The experiments of Lloyd and Moran (*loc. cit.*), as well as the cryoscopic, dilatometric, and calorimetric determinations indicate a probable value in the neighborhood of 0.5 grams bound water per gram dry gelatin, and the trend of the figures in column B/A for gelatin indicates that the 80 calories per gram limit will be reached somewhere in the neighborhood of this value.

From the above considerations it appears as if heat of wetting studies may afford an independent check on other bound water studies and contribute new and valuable information to the whole bound water problem.

In the discussions presented in this chapter the author has been interested not so much in the theoretical aspects of the problem as in the importance of water relations in determining the properties and reactions of living systems. The remarkable parallelism which has been demonstrated between the biological response and "bound water" as measured by a number of technics is so striking that it seems highly improbable that the relationships are due to chance. The author regards the biological observations as of even greater importance than the theoretical considerations of the nature of the

forces which are operating in these lyophilic colloid systems. There is no more important field in colloid chemistry than that field which deals with the water relations of the biocolloids, and there is abundant evidence that these relationships determine in a large measure the vital activities of organisms. More data are urgently needed, and the author recommends this as a fruitful field of research for the young physiologist interested in the border-line field between physiology and colloid chemistry.

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