logical questions, though it does not explain life itself any more than heredity is explained by the chemistry of nucleic acid.

SOME COLLOID-CHEMICAL ASPECTS OF DIGESTION, WITH ULTRAMICROSCOPIC OBSERVATIONS.

BY JEROME ALEXANDER.
Received February 16, 1910.

The changes which occur during digestion, and in fact in almost all physiological processes, are remarkable not only because of their very profound nature, but also because they are produced at comparatively low temperatures and in the presence of extremely dilute reagents. The living organism disintegrates proteins, oxidizes carbohydrates, and with the same apparent ease synthesizes substances of great complexity. Powerful reagents and high temperatures, which would be destructive to life, are necessary to bring about changes of this character under ordinary laboratory conditions.

The digestive process is preliminary to the actual absorption and use of food by the organism, and has for its object the modification or change of the ingested food into such forms or such substances as may be absorbed in the lower part of the digestive tube. To have a correct understanding of the absorption of the products of digestion, we must bear in mind the fact that the walls of the digestive tract act as semipermeable colloidal membranes, and that absorption consists in diffusion into or through these membranes or their constituent cells. Substances in crystalloidal solution, and colloidal sols whose particles are sufficiently small, represent then the two classes of digestion products which are diffusible and therefore absorbable.

Food as ingested consists mainly of substances that may be grouped into two classes:

1. Crystalloids—such as water, sugars, sodium chloride, etc.
2. Colloids—such as starch, proteins, etc.

The crystalloids are usually absorbed directly, although sucrose, for example, undergoes inversion. The colloids, as a rule, are not directly absorbable and for the most part digestion consists in the disintegration[1] of the colloidal complexes of the food, so that they can actually diffuse into the organism and there undergo further changes. Colloidal gels or even sols whose particles are of large size are, practically speaking, non-diffusible, and must therefore be reduced to a more finely dispersed state. So strong is the analogy between digestion and colloidal disintegration that Thomas Graham,[2] the father of colloid chemistry, coined

[1] It must of course be borne in mind that actual chemical changes may and very frequently do accompany changes of this character.

[2] "On the Properties of Silicic Acid and Other Analogous Colloidal Substances," *Proc. Roy. Soc.*, June 16, 1864.

the word *peptization* to express the liquefaction of a gel. He first speaks of the coagulation or pectization of colloids. "The pectization of liquid silicic acid," he states, "and many other liquid colloids is effected by contact with minute quantities of salts in a way which is not understood. On the other hand, the gelatinous acid may be again liquefied, and have its energy restored by contact with very moderate amounts of alkali. The latter change is gradual, 1 part of caustic soda, dissolved in 10,000 water, liquefying 200 parts of silicic acid (estimated dry), in 60 minutes at 100°. Gelatinous stannic acid also is easily liquefied by a small proportion of alkali, even at the ordinary temperature. The alkali, too, after liquefying the gelatinous colloid, may be separated again from it by diffusion into water upon a dialyzer. 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, with the difference that the solvent fluid here is not acid, but alkaline. 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 albumen, being peptized by minute quantities of hydrochloric acid."

Investigation has demonstrated that the high efficiency of the digestive juices is mainly due to small quantities of certain colloidal substances called enzymes (such as ptyalin, pepsin, and pancreatin) which act as catalyzers, enormously hastening reactions which would otherwise proceed so slowly that, practically speaking, they would not occur at all. The enzymes appear to act by forming with the substrate a combination of unstable character, which breaks down and liberates the enzyme again to continue the operation.[1] Recently Prof. W. M.

[1] In an unpublished thesis presented early in 1899, and read before this section on Dec. 8, 1899, I stated as follows:

"It appears then, that all fermentations are purely chemical changes brought about by substances usually highly complex and highly nitrogenous, as analysis shows. To confirm more fully this assumption, work should be done along the lines laid down by Büchner, and the active enzymes should be isolated from all fermentative microorganisms. In the meanwhile we may examine into the nature of the chemical action that takes place.

"There are numerous reactions which go by the name of 'continuous processes.' The decomposition of the diazo compounds by cuprous salts (Sandmeyer's reaction) is a case in point. It proceeds according to the equation $C_6H_5N : NR + Cu_2R_2 = C_6H_5R + N_2 + Cu_2R_2$, so that the process is theoretically continuous. The cuprous salt acts the part of a carrier or 'go-between,' just as do iodine, ferric chloride, aluminium chloride and many other substances under different circumstances. The catalytic decompositions of hydrogen dioxide and hypochlorous acid are apparently continuous processes, although the intermediate compounds have not been determined.

"Not alone can only a small quantity of one substance decompose a large quantity of another, but the same substance yields varied products depending on the nature

Bayliss, in his interesting monograph on "The Nature of Enzyme Action,"[1] has shown that in all probability "the 'compound' of enzyme and substrate, generally regarded as preliminary to action, is in the nature of a colloidal adsorption compound." Any one who has seen in the ultramicroscope the extremely active motion of the individual particles in colloidal solutions, can readily imagine the terrific bombardment a substance must undergo when a colloidal enzyme is concentrated on its surface by adsorption; and indeed it seems probable that enzymes actually produce their effects by virtue of their specific surface actions and the motion of their particles.

In order to find out if this idea could be verified by actual observation, I have watched, under the ultramicroscope, the action of diastase upon potato starch grains and the action of pepsin upon coagulated egg albumen.

In the first case actively moving ultramicrons in the diastase solution gradually accumulated about the starch grains, which after a time showed a ragged and gnawed margin. While the adsorption and motion of the larger ultramicrons was all that could be followed, the bright appearance of the field indicated that more numerous finer particles were present, and some apparently of intermediate size were seen.[2]

For observations on albumin, I used a dilute solution of white of egg which had been heated nearly to boiling. It was opalescent and in the ultra apparatus exhibited a field full of bright and rapidly moving ultramicrons. Upon allowing a droplet of essence of pepsin (Fairchilds, containing 15 per cent. of alcohol by weight) to diffuse in, an immediate coagulation occurred, the particles clumping into very large masses. A droplet of decinormal hydrochloric acid was then allowed to diffuse in, whereupon the large masses broke up in small groups and single ultramicrons, which once more resumed their original motion. Soon, however, the albumin particles began to grow smaller and disappear, the field all the while becoming brighter and brighter, indicating the concom-

of the 'go-between.' Ethyl acetoacetate, when hydrolyzed with dilute boiling acids, yields ketones; while if strong alcoholic potash be employed, acids are produced. The latter is not a continuous process because the potash is eliminated by combining with the acid produced; but it illustrates the point.

"Now I believe that the actions of diastase, and in fact of all other enzymes, are in the nature of continuous processes. I base this, as yet, almost entirely upon analogy, but can indicate the line of experimentation necessary to confirm the theory.

"*First*—Active enzymes must be isolated and their chemical constitution and structure investigated.

"*Second*—The constitution of the substances to be decomposed must be understood, and also the nature of the compounds they can form with the structurally determined enzymes."

[1] Longmans, Green & Co., 1908.

[2] This work will be repeated with purer enzyme and at greater dilution.

mitant appearance of smaller ultramicrons or amicrons. *In vitro* the addition of the pepsin to the opalescent albumin solution caused it to clear gradually, even at room temperature.[1]

Enzymes are inactivated to a greater or less extent by shaking, heating, electrolytes, etc., all of which, as is well known, cause the coagulation of colloidal solutions and a resulting decrease in the activity of the motion of their constituent particles. Another feature of interest is that the action of enzymes is reversible, a fact that does not usually come much into evidence because of the dilution and removal by diffusion of the products formed. In cells, tissues and organs, however, changes of concentration again occur and synthetic processes may result.

One principle of colloid chemistry is of the utmost importance in digestion, namely, the protective action of reversible colloids, which stabilize or protect from coagulation, irreversible or unstable colloids. Mucin and analogous colloidal substances undoubtedly have a function of this character, which may in some cases account for the variance between the action of natural and artificial digestive juices. The principle of colloidal protection[2] is in evidence in almost all physiological reactions and processes, and it is indeed extremely doubtful if there ever occurs *in vivo* any chemical reaction which is not greatly influenced by the colloids always present. How powerful this influence is, may be seen by adding hydrochloric acid to a solution of silver nitrate containing a little gelatin; instead of the usual precipitate, there results only an opalescent hydrosol which passes entirely through filter paper.[3] This directs our attention to a very important consequence of colloidal protection, namely that in the presence of protective colloids, colloidal sols pass through membranes otherwise impermeable to them.[4]

In the light of the principles outlined above, let us consider the digestion of milk, food which contains crystalloids (water, soluble salts, lactose) and colloids (casein, albumin), besides fat in suspension. The main interest centers in the proteins and in the fat, which will be considered in the order given.

The chief proteins of milk are casein and lactalbumin, both of which exist in colloidal solution. In the case of cow's milk the casein is readily coagulated by acids and by the ferment rennin, and therefore such milk curds soon after its ingestion. I will not attempt to mention the numerous and sometimes weird theories advanced to explain the phenomenon

[1] The experiment with pepsin must be repeated with purer enzyme and at greater dilution, in order to follow the course of the action more in detail.

[2] For particulars regarding "protective action," see "Colloids and the Ultramicroscope," by Prof. R. Zsigmondy. J. Wiley & Sons, 1909.

[3] For other reactions of this character, see *J. Soc. Chem. Ind.*, **28**, 280 (1909).

[4] See Zsigmondy, Chapter 14. Mucous membranes, when moistened with bile, allow fat to penetrate more readily than when not so treated (Jacobi).

of the curding of milk, most of which have been considered by Kastle and Roberts in their article on "The Chemistry of Milk."[1] Although many investigators regarded the rennin coagulation of milk as a colloidal or physical, rather than a chemical change, I was, so far as I am aware, the first to point out[2] that casein is an irreversible or unstable colloid which is protected from coagulation by the reversible colloid lactalbumin. A very simple experiment will suffice to show the significance of this fact: if we add to cow's milk some protective colloid such as gelatin or gum arabic, it becomes insensitive to quantities of acid and rennin which would otherwise produce coagulation.

Owing to the very great importance of milk as a food, especially for infants and children, the question of the digestibility of milk has for years received the closest study of the medical profession, and many facts have been established by clinical experiment. Thus, as far back as 1888, Dr. Abraham Jacobi[3] advocated the addition of gelatin and gum arabic to milk intended for infants, and in a recent paper he states:[4] "Ass's milk has always been recognized as a refuge in digestive disorders, when neither mother's or cow's milk or its mixtures were tolerated." These facts are readily understood if we look over the following table[5] showing the average constitution of various milks:

Kind of milk.	Casein.	Albumin.	Total proteins.	Fat	Sugar.
Cow	3.02	0.53	3.55	3.64	4.88
Human	1.03	1.26	2.29	3.78	6.21
Goat	3.20	1.09	4.29	4.78	4.46
Ewe	4.97	1.55	6.52	6.86	4.91
Mare	1.24	0.75	1.99	1.21	5.67
Ass	0.67	1.55	2.22	1.64	5.99

It will be seen that in mother's milk the casein is protected from coagulation by a much higher ratio of albumin than cow's milk, whereas ass's milk is even more highly protected than mother's milk. With such highly protected milks, the curd (if indeed coagulation does actually occur at all) is much more readily redissolved in the process of digestion.[6]

[1] See "Milk and Its Relation to the Public Health," *Bull.* 41, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Service, Washington.

[2] *Z. Chem. Ind. Kolloide*, 4, 86; *C. A.*, **3**, 1315; *Z. Chem. Ind. Kolloide*, **5**, 101; *C. A.*, 4, 350; *J. Soc. Chem. Ind.*, **28**, 280 (1909); *C. A.*, 3, 1672.

[3] "The Intestinal Diseases of Infants and Children," p. 62, *et seq.*

[4] "The Gospel of Top Milk," *J. Am. Med. Assoc.*, **51**, 1216–1219 (Oct. 10, 1908).

[5] Compiled by Leach from Koenig.

[6] Too low a percentage of albumin in mother's milk might make it indigestible for the infant. It is interesting to note here that Dibbelt (*Arb. Geb. path. anat. Bact.*, 6, No. 3; see C. A., 3, 2943) has shown that cases of richitis (rickets) are caused by deficient absorption of calcium, the infants not being able to utilize the larger percentage of calcium in cow's milk and artificial food.

The anomalous results of Kastle and Roberts (*Bull.* 41, 325), who found

Ultramicroscopic observation fully confirms the principle of colloidal protection above stated. Using a Leitz dark field condenser with $^1/_{12}$ oil immersion objective, and a 1200 candle power arc light for illumination, the individual particles of casein may be seen in very active motion. Upon allowing a little dilute acid to diffuse in under th cover glass, the agglutination or coagulation of the casein particles may be distinctly followed. They gather at first into groups of two or three, and gradually into larger and still larger masses. As the size of these groups increases, their motion decreases proportionately, until at last they float quietly and sink to the bottom of the fluid.

If, however, a little gelatin or gum arabic be added to the milk before the addition of the acid, the clumping is entirely prevented. The particles of casein continue the active motion, which in reality keeps them afloat and in solution.

These observations I first made on May 9, 1909, and since then they have been frequently repeated.[1] For ultramicroscopic examination a very dilute solution of skim milk (one drop to 100 or 200 cc. of water) should be used, for fat globules, or too great a degree of concentration produce a confused field. A very dilute solution of benzopurpurin (which dissolves as a colloid) shows exactly the same protection by reversible colloids as does milk, and in this case the macroscopic color changes as well as ultramicroscopic observation show that gelatin exercises a much greater protective action than gum arabic.[2]

Gelatin also protects casein against coagulation by rennin, as may be shown both in macroscopic and ultramicroscopic observations.[3]

Another very vital point to consider is that the colloidal protection of the casein has an important influence upon the digestion of the milk fat.

In the first place, the initial subdivision of the fat in milk is favored by the "lactalbumen," which acts as an "emulsifier." The use of colloids in forming and preserving emulsions has long been known in the arts and in pharmacy, and has also attracted the attention of physiologists. Thus Moore and Krombholz, in a paper entitled "On the Rela-

that frequently quite acid milks did not coagulate, whereas faintly acid milks did, are probably due to the fact that the former owed their stability to a larger relative percentage of albumin than the latter.

[1] Exhibited before the Cincinnati Section of the Am. Chem. Society on Dec. 8, 1909. Alexander and Bullowa, *Arch. Pediatrics*, Jan., 1910. *Ice Cream Trade Journal*, Dec., pp. 197–201, 1909. *Z. Chem. Ind. Kolloide*, 6, 197–201.

[2] Futher experiments with benzopurpurin, and their significance in dyeing are referred to in a paper submitted to the Seventh International Congress of Applied Chemistry. See *Trans. Am. Inst. Chem. Eng.*, 2 (in press).

[3] See Alexander and Bullowa, *loc. cit.*

tive Power of Various Forms of Proteid in Conserving Emulsions,"[1] state as follows:

"The action of acid and alkali albumins in so maintaining emulsions, must be of service in the digestion and absorption of fats. Protein food is invariably eaten along with fats, and as the fat becomes emulsified, it will be maintained in a finely subdivided form by the action of the acid and alkali albumin simultaneously formed. The fat does not become much subdivided in the stomach, and hence the acid albumin formed here does not come much into action, but afterward in the duodenum the alkali albumin present undoubtedly must aid in preserving the emulsion which is formed there."

The value of an emulsifying colloid is readily demonstrated by adding ferric chloride to a commercial emulsion of cod liver oil; this immediately coagulates the protective colloid and the oil at once separates out. It must also be remarked that protective colloids in the various digestive juices must be reckoned with, as well as those in the food, and those formed from the food.

In the case of milk the presence of a coagulative colloid, casein, introduces another factor, since the curd always carries down with it a very large percentage of the fat present. Now the smaller the degree of colloid protection, the greater is the probability of coagulation, and the less soluble the resulting curd. The total prevention of coagulation is highly desirable, especially in the presence of much milk fat, because fatty curds have a great tendency to adhere and form large masses which resist the action of the digestive juices.[2] In the so-called "fat indigestion" described by Czerny and Keller and others, the trouble has for the most part been ascribed to an excess of fat. But mother's milk, as a rule, contains even more fat than cow's milk, and I have heard of cases showing the typical hard, dry stools of "fat indigestion" where the child was fed cow's milk containing only 2 per cent. of fat. It would seem then that the protection of the casein is of the highest importance in favoring the absorption of the fat as well as the casein of milk.

Interesting confirmation of this is to be found by reading the results

[1] *Brit. J. Physiol.*, 22, 54 (1908).

[2] This process is well described by Dr. J. W. Schereschewsky, in his paper on "Infant Feeding," *Bull.* 41, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Service, p. 658, *et seq.*

In a recent paper entitled "Casein Curds in Infants' Stools," just published in the *Archives of Pediatrics,* 26, 924 (Dec., 1909), Dr. F. B. Talbot states in conclusion: "Furthermore there is sufficient clinical and scientific evidence to prove that tough curds are composed principally of casein, that they are due to the imperfect digestion of casein, and that an excess of casein in the food may result in a fat as well as a casein indigestion."

of Prof. C. A. Herter, as given in his book on "Infantilism."[1] He describes here a condition of arrested development, consequent upon the non-absorption of food and its subsequent putrefaction in the lower intestine. The patients excreted practically all the calcium ingested, this accounting for the failure of skeletal growth, and the feces contained neutral fat, fatty acids, and soaps in marked excess, indicating impaired fat absorption.[2]

Herter found that the addition of gelatin (which is a most efficient protective colloid) to the milk fed, caused an improved absorption and recommends its use.[3] He further observes that "in sparing protein small quantities of gelatin appear to have about as much effect as larger amounts," a fact quite in accord with protective action, for only a small percentage of gelatin is needed to accomplish protection.

In conclusion, I would point out that bald chemical analysis cannot express the digestibility and availability of a food any more than it can express or explain the action of the digestive juices, or in fact any other physiological process. In all these processes can be traced the influence of the colloidal substances everywhere present in the body, whose effects are quite out of proportion to their small mass. Striking examples are the enzymes which catalyze and direct chemical and physical changes, and the protective colloids which oppose crystallization, precipitation and coagulation, emulsify fats, and facilitate diffusion and absorption. As soon as we approach the chemistry of living organism, we are confronted with problems of colloid chemistry, and there is no doubt but that a correct understanding and application of its principles will throw much light upon many other obscure problems in biology, physiology and medicine.

LABORATORY OF NATIONAL GUM AND MICA CO., NEW YORK CITY.

[FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY OF THE HARVARD MEDICAL SCHOOL.]

ON THE ESTIMATION OF THE INTENSITY OF ACIDITY AND ALKALINITY WITH DINITROHYDROQUINONE.[4]

BY LAWRENCE J. HENDERSON AND ALEXANDER FORBES.

Received March 10, 1910.

Indicators serve two distinct purposes: The one is to mark sharply an end point in titration, the other to measure the concentration of ionized hydrogen or hydroxyl in solution. The properties which qualify a sub-

[1] "On Infantilism from Chronic Intestinal Infection," by C. A. Herter, M.D. The Macmillan Co., 1908.

[2] It is now well known that the cream layer or fat of milk contains from 10 to 500 times as many bacteria as the whole milk. See (U. S. Dept. Agr.) *Bull.* 56, 737; also Jacobi, *J. Am. Med. Association, loc. cit.*

[3] Herter, *loc. cit.*, pp. 101, 105.

[4] An investigation aided by a grant from the Elizabeth Thompson Science Fund