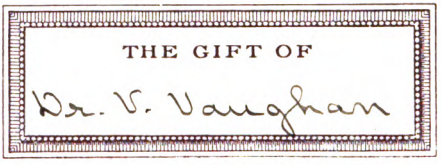

A CONTRIBUTION TO THE CHEMISTRY OF THE BACTERIAL CELL AND A STUDY OF THE EFFECTS OF SOME OF THE SPLIT PRODUCTS ON ANIMALS.

THE SHATTUCK LECTURE.

A CONTRIBUTION TO THE CHEMISTRY OF THE BACTERIAL CELL AND A STUDY OF THE EFFECTS OF SOME OF THE SPLIT PRODUCTS ON ANIMALS.

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PART I.—GENERAL STATEMENT.

Introduction.—For some years past the research work carried on in my laboratory has been along the line of the chemistry of the cell. The large tanks which I devised for growing bacteria in mass have enabled me to obtain cellular substance in large amount and free from extraneous matter. The results reached in the study of the material thus obtained have been given in contributions to current medical and scientific literature published from time to time by my students and myself. Inasmuch as this investigation has been quite different in conception and execution from any done by others, it has given me an unusual standpoint from which to view certain problems which are both chemical and biological, and it is my intention to present in this paper some of the conceptions which I reached concerning the structure and function of the living cell. In doing this I wish it plainly understood that, so far as the theory is concerned, I am speaking tentatively and with the light now before me, reserving the privilege of making any alterations in my conceptions which may be justified or demanded by future investigations. With this reservation and recognizing that my time and your patience are not inexhaustible, I will at once plunge *in medias res.*

The Composition of the Living Cell.—When matter becomes endowed with life, it does not cease to be matter; it does not lose its inherent properties; it is not released

from the laws that govern its structure, its attractions and its motions. In studying the organized cell of living things, whether vegetable or animal, it should always be borne in mind that it is material in composition and subject to the fundamental laws that govern matter and possessed of those properties essential to matter. In order that this point, so essential to a proper understanding of the subject, should be thoroughly appreciated, it may be well to review some of the properties of matter as taught by the most advanced science of the day.

Tait says: "Matter is that which can be perceived by the senses, or is that which can be acted upon by or can exert force." Since force is the result of motion, we may say that anything and everything that moves or can be moved, or whose position in space may be changed, is matter. There are many forms of matter that can neither be seen nor felt, and can be recognized only by their motions.

Matter is indestructible; it may be successively solid, liquid and gas, but in passing through these phases it neither loses nor gains. It has always been and it always will be. It is without beginning and will be without end. Matter consists of infinitely small particles, called atoms. According to the computation of Lord Kelvin, the diameter of an atom is not greater than 1-50,000,000 of an inch; however, all atoms are not of the same size and weight. When like atoms combine they form chemical elements, of which about seventy are known. When unlike atoms combine, chemical compounds are formed and the number of these is beyond computation. Until the discovery and study of radium it was supposed that one element is never converted into another, and consequently that the number of kinds of atoms is fixed and unchangeable. However, the evolution of helium from radium apparently demonstrates the formation of one element from another and it is within the range of sanity to suppose that all the elements

have been developed from a primordial ancestor, probably from the universal ether which pervades all space. Nothing has been created; everything has grown. Even silver, ron and other metals come into existence by being cast off from some ancestral element. It will be seen from this that even atoms do not represent the ultimate divisions of matter. Indeed, there are reasons for believing that the hydrogen atom consists of a nucleus about which some 700 particles or electrons revolve, and an atom of mercury is believed to consist of not less than 100,000 electrons. Atoms and electrons are in constant motion, and so small are they that the distances between them may be relatively as great as those between the planets of the solar system. The living cell is composed of molecules, made up of atoms, composed of electrons that are in constant systematic motion and may be compared to a group of stars with attendant suns, each of which is surrounded by its own planets.

Another property of matter is that it is gravitative. Every particle of matter attracts every other particle. When this attraction is manifest between masses it is called gravitation; between molecules it is called cohesion or adhesion, as the molecules held together are alike or unlike; between atoms it is known as chemical affinity or chemism.

Still another property of matter is inertia, by which term we indicate the inability of matter to change either its rate or direction of motion without being acted upon by other matter. It is of importance that this property of matter be held in mind in the study of cellular chemistry, and the proper mental picture of a cell molecule represents each of the atoms in the molecule, and each electron in each atom moving, each about its centre at a definite rate. Within the living cell molecule, change in number, kind and arrangement of atoms is constant; and changes in the direction and rate of the motion of the atoms are also susceptible to the influence of other matter and are of constant

occurrence. Whole groups of atoms are physiologically being dropped from the cellular molecule and being replaced by other groups split off from the pabulum upon which the cell feeds. In this way the cell renews itself and keeps itself supplied with energy.

Some eminent physicists are inclined to the belief that matter is made up of electric charges, but recognize that this is not a demonstrated fact as yet, and speak with caution. Lodge says: "There *may* possibly be two different kinds of inertia, which exactly simulate each other, one electrical and the other material; and those who hold this as a reasonable possibility are careful to speak of electrons as 'corpuscles,' meaning charged particles of matter of extremely small size, much smaller than an atom, consisting of a definite electric charge and an unknown material nucleus; which nucleus as they recognize, but have not yet finally proved, may quite possibly be Zero."

Cell Metabolism.—The only essential and constant difference between living and non-living matter is that within the molecules of the former there is constant metabolism, while in the latter no such process occurs. We are to conceive of the living molecule as made up of numerous atoms and each atom surrounded by its electrons; atoms and electrons are in ceaseless motion, groups of atoms are being constantly cast out of the molecule and replaced by new groups split off from matter outside the molecule. As soon as a molecule becomes the seat of assimilation and excretion it is no longer dead; it lives. As a result of assimilation it acquires the property of building up its own tissue; then polymerization follows and reproduction in its simplest form begins. The one phenomenon always manifested by living matter, and never exhibited by non-living matter, is metabolism. Verworn says: "Vital motion, metabolism, is a complex motion very strongly characterizing the living organism; it consists in the continual self-decomposition

of living substance, the giving off to the outside of the decomposition products, and, in return, the taking in from the outside of certain substances which give to the organism the material with which to regenerate itself and grow by the formation of similar groups of atoms, *i. e.*, by polymerization. This is characteristic of all living substance."

I have thought to give you some *new* conceptions of the living cell, and yet it must be admitted that Aristotle apparently recognized that metabolism is the one characteristic of living matter. He said: "Life is the assemblage of the operations of nutrition, growth and destruction." This Greek philosopher did not know about cells, molecules, atoms and electrons what is now known, but it must be acknowledged that he had a fairly clear conception of the most essential characteristics of living matter. Herbert Spencer has given three definitions of life and either may be applied to the conception which I am attempting to present. The first is: "Life is the coördination of actions." Coördination between assimilation and excretion is certainly essential to life, and failure of this coördination leads to death. The second is probably the best definition of life ever given, and fits our conception perfectly. It is: "Life is the definite combination of heterogeneous changes, both simultaneous and successive, in correspondence with external coexistences and sequences." The third is practically the same as the second, expressed in simpler terms, but in my opinion not so satisfactorily. It reads: "Life is the continuous adjustment of internal relations to external relations." Matter is alive when it feeds and excretes. Crystals grow and in a sense they multiply, but their growth is not intramolecular; it is by accretion. The living molecule not only absorbs, it assimilates. It chemically alters what it absorbs. The atomic groups taken into living molecules enter into new combinations. The living molecule is not stable, but is highly labile. Its composition is never con-

stant and it is never in a condition of equilibrium. There is a constant reaction between the living molecule and other molecules. Apart from other matter it could not exist. There is a constant interchange of atoms between it and other molecules. This is best seen in seeds, spores and ova. Matter existing in this form may be awakened into activity by proper stimuli; active life begins with the interchange of atomic groups, or with metabolism.

Why is there this constant atomic group interchange between the living molecule and outside matter? It is for the purpose of supplying the living molecule with energy. Allen has so ably expressed this fact that I make the following quotations: "The most prominent and perhaps most fundamental phenomenon of life is what may be described as the *energy traffic* or the function of *trading in energy*. The chief physical function of living matter seems to consist in absorbing energy, storing it in a higher potential state, and afterwards partially expending it in the kinetic or active form. We find in living matter a peculiar proneness to change its composition under the stimulus of slight changes in the energy-equilibrium between itself and its surroundings, energy being readily absorbed and readily dispersed. The absorption of energy coincides with deoxidation and the building up of large molecules; conversely the dispersion of energy coincides with oxidation and the disruption of the large molecules. The building up of these large molecules is always accomplished by slow steps; but when formed, the said molecules are very unstable, irritable, or, in modern phrase, *labile*. They may break down by degrees in some instances; in others their structure may be so precarious as to collapse on the slightest disturbance."

"The lability of such a molecule may be compared to that of a house of cards, which can be taken to pieces card by card, or may collapse at once. But the word lability

is applied, not only to *de*-structive, but also to *con*-structive instability. The molecules of living substance are prone to constructive as well as destructive changes; but as in the house of cards, the constructive changes are the most gradual; and as the structure grows more complex, construction becomes more difficult, and collapse is more imminent. It should be distinctly understood, however, that it is not the mere size of the molecules that makes them labile, but rather the manner in which they are linked together, and the amount of potential energy which is included in the molecule."

It is probable that in the absorption of energy by the living molecule oxygen is relieved from its combination with carbon or hydrogen and is attached to nitrogen, while in the liberation of energy the reverse takes place. Nitrogen and phosphorus, sometimes with iron and manganese, seem to be, as it were, the master elements within the living molecule. It is by virtue of their chemism that groups are torn from extra-cellular matter, taken into the living molecule and assimilated by an atomic rearrangement; and furthermore, it is on account of the lability of the compound thus formed that potential energy is converted into kinetic and cell work is accomplished.

That life resides within the molecule and that metabolic processes are intramolecular are shown by numerous investigations, some of the most important of which may be briefly stated as follows:

1. So long ago as 1867 it was shown by Hermann in his studies on the metabolism of isolated muscle that the carbonic and lactic acids produced in muscular contraction result from the action of intramolecular or combined oxygen. This was demonstrated by the observation that when a muscle is freed from all its uncombined oxygen under an air pump and then caused to contract in an oxygen-free medium it gives off carbonic and lactic acids. Contraction,

a vital muscle phenomenon, is thus shown to result from intramolecular changes.

2. In 1875, Pflüger kept a frog at a few degrees above zero in an atmosphere free from oxygen for twenty-five hours, and found that during that time the animal continued to give off carbonic acid and this could be produced under these conditions only through intramolecular changes. From these studies Pflüger concluded that the living content of the organism consists of proteid, which he designates "living proteid," in contradistinction to dead proteid, and the carbonic acid gas results from the decomposition of a labile proteid molecule, the nitrogenous constituents of which are capable, with the help of the fats and carbohydrates of the food, to regenerate "the living proteid molecule."

3. Recent research in my own laboratory has shown that both the toxic and carbohydrate groups of the cell of the colon bacillus are held in chemical combination with other constitutents of the cell. This microorganism will grow in a medium which contains organic nitrogen as amino compound only and with this nitrogen and inorganic salt as its sole food, it builds up by synthetical process a complex glyco-nucleo-proteid, forming a large molecule which contains as atomic groups, pentose, nuclein bases, amino and diamino compounds. These constituents are held chemically in the cell; they are constituent groups of the large cell molecule. They cannot be washed out by physical solvents, and can be isolated only by chemically breaking up the cell molecule.

Besides the above mentioned experimental data showing that cell life manifests itself by intramolecular reaction the following general considerations indicate the same thing:

(*a*) In taking its food the cell, whether it be vegetable or animal, whether it be that of a unicellular or that of a multicellular organism, manifests a selective action which

can be best explained—indeed, I might say, can only be explained—on the ground that it is due to ~~mass~~ chemical affinity. Mass attraction is, so far as I know, never specific; molecular attraction is specific only in general terms and not in the sense of forming synthetical compounds; but atomic attraction, or chemical affinity as it is usually designated, is specific, or at least selective. This fact, as is well known, is the basis of the side chain theory of Ehrlich, who, upon this principle, explains the nutrition of cells, the action of many therapeutical agents and the production and action of antitoxins. It is well known that certain poisons have a selective action on certain tissues, and this means that the chemical affinity between the poison and the constituents of certain cells is greater than that between this poison and other cells. If pharmacology and toxicology ever become exact sciences it will be, most probably, through investigations directed along this line.

(*b*) The fact that the secretions of cells are specific is a strong argument for the theory that action on the pabulum upon which they feed is intramolecular. The liver cells produce bile pigments and acids, each of the digestive fluids elaborates its specific products, the specific secretions of the adrenals and the thyroid gland have been studied and are now largely and successfully employed therapeutically. And still all these organs are supplied with the same blood and lymph. Certainly, the only possible explanation for these well established facts is that of a chemical reaction, or an intramolecular interchange, between the cells and the constituents of the substances with which they are brought into contact.

While other arguments might be adduced to show that metabolic processes, the only phenomena with which we are acquainted that are characteristic of all living matter and which do not occur in dead matter, are due to intramolecular reactions, it seems to me that those already given are sufficient to establish my thesis *i. e.*, life is molecular.

If I have made good my contention so far, it follows that life begins with the capacity of growth and reproduction. The life of such a molecule depends upon its continued reaction with matter outside of itself, or, in other words, it must feed; and reproduction in its simplest form results from polymerization. In this way the wonderful experiments of Loeb upon the artificial fertilization of certain ova may possibly be explained. The ovum is not alive; it possesses only latent life and when acted upon by certain stimuli it begins active life. The stimulus may be a spermatozoon or some inorganic salt in a certain definite strength of solution.

If life be molecular, it is possible that its lowest manifestations are without form. They may be infinitely small, and it is not beyond the range of possibility that they may exist in either or all the three known conditions of matter, solid, liquid, or gas.

Cellular life is the only form of life that we know at present, but the statement "omne vivum e cellula" is dogmatic and probably is not true. It is too early yet to predict what influence more exact studies of viruses that pass through porcelain filters will have upon this dictum.

The Cell not the Unit of Life.—The following quotation from Nussbaum, as given by Loeb, shows that the biologist recognizes that the cell is not the unit of life: "The cell is not the ultimate physiologic unit, even though it must remain such for the morphologist. We are, however, not able to tell how far the divisibility of a cell goes, and how we can determine the limit theoretically. Yet, for the present it will be well not to apply to living matter the conceptions of atoms and molecules, which are well defined in physical chemistry. The notion, introduced by Nægele, might also lead to difficulties, as the properties of living matter are based upon both nuclein and protoplasm. * * * The cell, consequently, represents a multiple of individuals."

Pflüger has shown that the egg, which has been thought to be a biologic unit, can give rise to many individuals, and Loeb states that his own experiments, as well as those of Driesch, confirm this finding.

It is highly probable that the lowest forms of life cannot feed upon proteids. This is true of the yeast cell. These cells grow rapidly when placed in a solution of sugar and nitrates, but proteids must be broken up by putrefactive bacteria before the yeast organisms can feed upon them. Indeed, many of the cells in the body of man cannot feed upon complex proteids, which must be split up by the digestive enzymes into much smaller and simpler groups before the cell molecules can feed upon them. Even the carbohydrate, starch, must be hydrated and its elements rearranged as constituents of a more labile molecule before it can become a source of energy in muscle. Proteid solutions injected into the blood of man are poisonous, but the same substance, after being properly split up, is an essential cell food.

Some light has been thrown upon the chemistry of the growth of the cells of germinating seeds by the researches of E. Schulze and his students. As is known, the seed consists of the germinating cell and the stored up food material. The cell in a lupine seed is not actively alive; it possesses only latent life. When placed under conditions favorable to the development of active life, a ferment begins to break up the proteid food material stored in the seed and as a result of the chemical cleavage induced by the ferment relatively simple nitrogenous bodies, such as the amino acids, tyrosin and leucin, and the diamino acids, arginin and lysin, are formed. These bodies serve as food material for the germinating cell and latent life is quickened into the active form and growth begins.

If the characteristic phenomena of life are due to intramolecular reactions, we must conceive of the living cell,

whether it belong high or low in the scale of development, as consisting in its essential or vital part of a chemical compound made up of complex molecules, composed of atoms, each surrounded by its electrons, all in motion and with a constant, probably a rythmical, absorption of atomic groups from other molecules, and with a like constant discharge of atomic groups.

This living molecule feeds by splitting off such groups as it may need from the pabulum within its reach, or it may absorb entire molecules, at the same time rearranging the atoms and making them a part of itself. When in ordinary physiological function, a portion of this molecule, which we may designate its chemical nucleus, remains undisturbed and regenerates the whole, supplying its waste by the absorption of new material. Cellular assimilation consists in properly locating the recently acquired atomic groups within the molecule.

Certain cell molecules, under proper stimuli, rearrange their atomic grouping, polymerize and thus multiply. This multiplication may be physiological or pathological. Rapid proliferation may tend to inability to function or to react with the food supply, and consequently destroy the molecule or lead to the death of the cell.

In his very interesting monograph on the "Biogen Hypothesis," Verworn objects to saying that a molecule lives. He states that it is illogical. "A living thing is only that which demonstrates the phenomenon of life,—something that changes itself. A molecule of a given compound, so long as it remains unchanged, cannot be said to be living." Then, in order not to speak of living molecules, he introduces the term "biogen molecule." Surely, this is a distinction without a difference. I certainly agree with the distinguished German physiologist that a molecule of a cell, *so long as it remains unchanged*, cannot be said to be living, but the point is that living molecules do not remain

unchanged. When life is latent, as it is in seeds and spores, the molecules cannot be said to be alive; but when placed under suitable conditions, then the change between atomic groups in the molecule and the external food substance begins, and life first manifests itself. However, it matters but little, I suppose, whether we speak of living molecules or designate them as "biogen molecules."

Cell Secretions.—With the conception of a living cell that I have attempted to present, it must be evident that its secretions consist of the atomic groups cast out as a result of its metabolism, and as the cells of different organs are unlike in their chemical composition, it follows that the secretions are specific. Outside the body hemoglobin breaks up, or may be broken up chemically, into hematin and a globulin. In this case the colored split product contains the iron; but the liver cells produce from hemoglobin bilirubin and an iron-containing proteid. In these two reactions the line of cleavage is quite different. The secretions of some cells enter into a more or less energetic reaction with certain extra-cellular compounds with which they come in contact. This is true of the digestive enzymes. Other secretions apparently are made for the purpose of reacting with or at least affecting the reactions of the molecules of other cells. This seems to be true of some, at least, of the so-called internal secretions, such as those of the thyroid and adrenals.

Ferments.—A most important group of cellular secretions consists of the ferments or enzymes. Without going into the history of the theories that have been advanced concerning the nature of these bodies, it seems to me that we are no longer justified in speaking of "organized and unorganized" ferments. All the ferments are cellular secretions. The work of Buchner on the ferment of the yeast plant seems to be positively convincing on this point. Oppenheimer has defined a ferment in a manner quite in

accord with the latest and best experimental investigation. His definition is as follows: "A ferment is a catalytically acting substance which is produced by living cells, to which it is more or less firmly bound, whilst it is not associated with the vital processes of the cells (which produce it); ferments are capable of inaugurating chemical processes which take place spontaneously (without the presence of the ferments), but proceed more slowly. In this process the ferment itself remains unchanged. Ferment action is specific, *i. e.*, each ferment manifests its activity only on substances of certain structural and stereo-chemical arrangement."

I am conscious that my translation of this definition is not altogether satisfactory, and, in order to give a more exact interpretation of it, as I understand it, I offer the following explanatory statements:

1. Every ferment is a cellular product; it is a cellular secretion; a substance of definite chemical composition formed by the rearrangement of the atomic groups within the cellular molecule.

2. The action of the ferment, while it is determined by the cell which produces it, is not concerned in the "energy traffic" constantly going on between the molecules of the cell which produced it and other molecules external to this cell. With our present limited knowledge of the chemistry of the cell molecule it is impossible, in many cases at least, to distinguish between the chemical reactions resulting from cell metabolism and those due to ferments. I am inclined to the opinion that more exact knowledge will show that the autolytic changes that take place in many cells after death, and which have furnished the theme of so many papers recently, will be found not to be due to ferments at all, but to the cessation of metabolic reaction and consequent dissociation of the constituent groups of the cell molecules.

3. The function of a ferment is to hasten chemical reactions which take place, but much more slowly, without the presence of the ferment. It seems to me that a clear conception of this point gives one a key to the action of ferments in general. I have already in this paper called attention to the fact that inertia is a universal property of matter; that the direction and rate of movement in matter cannot be altered spontaneously. A ferment is a substance of cellular origin which by its presence changes the *tempo* of chemical reaction. I borrow this word "tempo" from the musician because it expresses my meaning better than any other word in my vocabulary. I am fully aware that this does not explain *why* the ferment acts by its presence, but it is worth much to have a conception of *how* it acts, provided, of course, that this conception be correct. Furthermore, it must be admitted that the *modus operandi* of ferments is still beyond our ken. Some think that certain atoms or atomic groups are detached from one of the substances, combine with the ferment, and then are passed on to the other substances. On this supposition the ferment does enter into the reaction, but is constantly regenerated. Others hold that the ferment combines with the fermentable substance, making its molecule so labile that it falls to pieces and that in the dissociation the ferment is again set free. There are weighty objections to either of these theories, but time will not permit me to state them in this paper, which on this point at least is intended to be suggestive rather than exhaustive.

Part II.—The Bacterial Cell.

Preparation of Bacterial Cell Substance.—Bacteria of various species, both pathogenic and non-pathogenic, have been grown on the tanks. When the growth has reached its maximum, the time required for this varying with the species and the temperature, the growth is de-

tached from the subjacent agar with sterilized bent glass rods and drawn into sterilized flasks with the aid of a water pump. The *cellular* substance is then washed with dilute, and later with stronger, alcohol. After thorough extraction with this agent it is placed in Soxhlets and thoroughly extracted with ether; next it is rubbed up first in porcelain, then in agate, mortars and passed through fine meshed sieves. This treatment yields fine, impalpable powders, the color of which varies with the germ, but is generally white because the bacterial pigments are soluble in alcohol.* These powders, when examined microscopically, show the cells, broken more or less, generally but little, by the rubbing to which they have been subjected. They still take the stains to which the original cultures respond with some variations with certain species. In this way we have obtained the cellular substance of the following microorganisms: b. prodigiosus, b. violaceus, s. lutea, s. aurantiaca, b. coli communis, b. typhosus, b. pyocyaneus, b. anthracis, b. tuberculosis, b. diphtheriæ and m. pneumoniæ.

The Toxcity of Bacterial Cell Substance.—The cell substance obtained from each of the above mentioned microorganisms has been found to be toxic and its toxcity has been studied and reported upon by my students, Detwiler, Wheeler, Leach, Gelston, Marshall, J. W. Vaughan, and V. C. Vaughan, Jr. It is interesting to note that when injected intraperitoneally in animals, the cell substance of the b. prodigiosus is more potent than that of any of the pathogenic bacteria, being more than fifty times as active as the cell substance of b. anthracis; and even the lemon sarcine, the least toxic of the non-pathogenic organisms examined, surpasses the anthrax bacillus in the potency of its intracellular poison. This shows, as I have elsewhere

* A preliminary report was made on certain bacterial pigments by my student, Detwiler, in 1902. (Transactions of the Association of American Physicians.)

stated, that the prodigiosus is non-pathogenic to the higher animals, not from its inability to elaborate a poison, but because it cannot grow and multiply in the animal body; while on the other hand, the anthrax bacillus is highly infectious to some of the higher animals, not from the intensity of the poison which it elaborates, but rather from the fact that in these animals this bacterium finds the conditions favorable for its growth and multiplication. In a more general way this statement may be made as follows: a non-pathogenic bacterium may be capable of elaborating a highly active poison in artificial cultures or from dead material, but its chemism does not enable it to split up and feed upon the constituent tissues of the living animal.

The more finely bacterial cell substance is divided the more potent is it in its action. This was shown conclusively by the studies of Marshall and Gelston and is, in my opinion, due to the more ready solubility of the finely divided powders in the fluids of the body; the greater the relative surface of the germ surface exposed to the action of both the formed and the soluble constituents of the animal tissue, the more speedily is the poison set free and the more certain is death.

It may be interesting to give somewhat more in detail a statement of the amounts of cellular substance obtained from different bacteria found necessary to produce fatal results when injected intraperitoneally in guinea pigs. These amounts vary with the degree to which foreign matter is removed from the cell substance, the fineness to which the powder has been reduced and the length of time and the conditions under which the material has been kept. When frequently exposed to air and light the cellular substance decreases in toxcity, at times quite appreciably and suddenly. This I believe to be due to a hydration of the toxic group of the cell molecule and its consequent conversion into an inert or at least a less poisonous body. It is highly

probable that this occurs to a greater or less extent in cultures in which individual organisms must die from time to time and whose cells probably undergo autolytic changes. The following figures gives the average fatal amounts of the freshly prepared, finely dried cellular substance of some of the bacteria with which we have worked:

Table 1.—The toxcity of the finely powdered cellular substance when injected intraperitoneally in guinea pigs.

Name of Organism.	*Proportion of cell substance to body weight.*
Bacillus anthracis	1:1700
Sarcina lutea	1:2050
Micrococcus pneumoniæ	1:10000
Sarcina aurantiaca	1:25500
Bacillus violaceus	1:26500
Bacillus diphtheriæ	1:33000
Bacillus typhosus	1:40000
Bacillus pyocyaneus	1:50000
Bacillus coli	1:75000
Bacillus prodigiosus	1:90000

When suspended in water bacterial cell substance may be boiled without destroying its activity. Marshall and Gelston found that heating the cell substance of b. coli communis to 134° for 15 minutes did not appreciably lessen its toxcity, and Cooley and I heated the same substance in a sealed tube to 164° without rendering it inert. In a paper before the Association of American Physicians in 1901 I made the following statements concerning the toxcity of the colon cell:

1. The toxin is contained within the cell, from which it does not, at least under ordinary conditions, diffuse into the culture medium.

2. The toxin is not extracted from the cell by alcohol or ether.

3. Very dilute alkalis do not extract the toxin from the unbroken cells.

4. The unbroken germs may be heated to a high temperature with water without destruction of their toxin.

5. Heating the cell substance for an hour at the temperature of the water bath with water containing from 1 to 5 per cent. hydrocholric acid breaks up the cell content and lessens, but does not destroy, the toxicity of the cell content. Prolonged heating may render the toxin inert.

The Chemistry of the Bacterial Cell.—With the method employed and previously described, everything soluble in water, in dilute salt solution, in alcohol and in ether is removed from the cell substance. A discussion of the extractives obtained by the above mentioned solvents must be postponed and will, I hope, be presented to the profession at some future time. These extractives contain inorganic salts, fats, wax, traces of carbohydrates and several proteid bodies. These substances belong in part to the culture medium, certain constituents of which become more or less intimately adherent to the cells without being assimilated by them; others consist of split products formed by the action of the living cells upon the constituents of the culture medium; others still are secretions of the living cell, representing atomic groups dropped from the living molecule in the process of metabolism and in the traffic in energy constantly carried on between the living, growing and multiplying cell and the outside matter which constitutes its pabulum; and finally there are substances that result from the autolysis of the dead cells and some of these are identical with the bodies obtained by splitting up the cell molecules by chemical means and which will be described later. With some species of bacteria the important bodies that result from chemically breaking up the cell molecule can be best obtained by permitting the process of dissociation, which occurs sooner or later in all cells after death, to proceed until the greater part of the cell substance passes into the soluble form. When this happens the separation of the toxic from the non-toxic groups becomes easy of execution. There is much valuable information to be obtained

by the study of these extractives, but I have not done enough with them to enable me to make many positive statements at present. Moreover, it is the purpose of this paper to give the results of the studies of my students and myself on the cell molecule or that portion of the cell that remains after everything that can be washed out with physical solvents has been removed, and when I speak of the cell as being in its essential part a chemical compound of definite composition and structure it must be held in mind that I refer to that part of the cell that remains after the extractives have been removed. (Except of course, when autolysis has taken place.) I am anxious that all should clearly understand and fully comprehend this point.

The essential part of the cell, that which remains after the removal of the extractives, can be split up by chemical agents and these split products will now be discussed.

The toxic group of the bacterial cell molecule.—When bacterial cell substance, freed from extractives and prepared after the method already given, is heated in a flask with a reflux condenser with from 15 to 25 times its weight of a 2 per cent. solution of sodium hydroxid in absolute alcohol, the cell molecule is split into toxic and non-toxic groups. The poisonous portion is now in solution in the alcohol and in case of b. coli and b. typhosus it constitutes about one-third by weight of the cell substance. For the complete solution of the poisonous group three extractions at 78° for one hour each have been found to be sufficient. It is essential that the alcohol used in the extraction should be absolute; if it contains water, hydration proceeds too far, much of the toxic body is destroyed and it will be found on removal of the alcohol that a sticky, gummy mass, which it is quite impossible to dry, remains. The alcoholic solution of the toxic part is neutralized with hydrochloric acid, avoiding an excess, and the precipitated sodium chlorid removed by filtration. The alcoholic filtrate is evaporated in vacuo

below 40°, the residue redissolved in absolute alcohol, and this may be repeated several times. When the sodium chlorid has been removed the toxic part of the germ substance is obtained in a brownish mass which may be ground into a fine powder and weighed.

By the above mentioned treatment the cellular substance is broken into two portions, one of which is soluble in absolute alcohol and is poisonous, while the other is non-soluble in alcohol and non-poisonous. The dead cellular substance I have designated as the "crude bacterial poison," while the part of this rendered soluble in alcohol by treatment with an alcoholic solution of alkali I have called the "crude soluble poison."

The toxic part of the bacterial cell is soluble in water, ethyl and methyl alcohol, and insoluble in ether, chloroform, benzine and petroleum ether. It is more readily and freely soluble in absolute alcohol than in water, which is unusual in a proteid body. When a solution of it in absolute alcohol is evaporated in vacuo it forms a brownish hydroscopic powder which dissolves in water forming an opalescent, acid solution. The opalescence may be largely or altogether removed by filtration through hard paper. When neutralized with sodium bicarbonate, a brownish, non-toxic substance is precipitated and may be removed by filtration.

The crude soluble toxin in aqueous solution gives all the proteid color reactions with the exception of that of Molisch. It gives the Millon test beautifully and in very dilute solution. It does not reduce Fehling's solution, either directly or after boiling with dilute mineral acid. It contains sulphur, but not that sulphur group that is split off from most proteids on boiling with caustic alkali. Aqueous solutions are not coagulated by heat, but a precipitate is produced on the addition of mineral acid. The heavy metals, as mercury and copper, produce voluminous precipitates. Saturation of an aqueous solution with ammonium sulphate

throws down a sticky resinous portion and when this is removed by filtration, both the precipitate and the filtrate give the proteid reactions. Platinum chlorid produces a precipitate in both alcoholic and aqueous solutions of the "crude, soluble toxin" and the filtrate, after removal of the platinum, has but little or no poisonous action; but the platinum precipitate, which contains the whole, or the greater part, of the poison refuses to dissolve in any of the agents which we have tried and, consequently, we have been able to reach no satisfactory method of purifying the poison. Separation into two portions by saturation with ammonium sulphate is not sharp and distinct; sometimes the filtrate is more, again less poisonous than the precipitate, although the weight of evidence is in favor of the precipitation of the poisonous portion by ammonium sulphate added to saturation. Undoubtedly there are conditions influencing this separation which up to the present time have escaped our knowledge.

As to how to classify this poisonous bacterial substance we must admit that we are still very much in the dark. The fact that the "crude soluble toxin" gives all the proteid color reactions with the exception of that of Molisch leads us to say that it is a proteid body, while against this view stands its ready solubility in absolute alcohol. The precipitation of a portion of the "crude soluble toxin" by saturation with ammonium sulphate while both precipitate and filtrate, after removal of the salt, give all the proteid color reactions, with the exception of that of Molisch, leads us to say, for the present and until continued work increases our knowledge, that the poisonous group in the bacterial cell molecule consists of a bacterial albumose and that this differs from the better known vegetable and animal albumoses in its ready solubility in absolute alcohol. It seems probable that the albumose is the poisonous body and that it is easily converted into a pepton-like body which is either inert or

has but little poisonous effect upon animals. It may be that the real poison is mixed with this proteid body, while chemically it is no part of it, but we have tried all known methods of extracting a poison from this proteid and having failed in all these attempts we must for the present assume that the proteid itself is the poison. Indeed, we started upon this research and adopted the method that we have followed on the theory that the poison in the bacterial cell is neurin and that it loses its poisonous properties by easy conversion into cholin. We were led to suspect this on account of the similarity between the poisonous action of neurin and that of the active body in the bacterial cell. If this could be proven it would afford a ready and rational explanation of the injurious effect of the bacterial cell on the animal body; but we have labored earnestly now for some years to find neurin in the bacterial cell with constantly negative results. We do not say that a neurin group does not exist in the bacterial cell; indeed we still believe that the poisonous agent in the bacterial cell is physiologically at least a neurin, but we are compelled to admit that we have not been able to prove the presence of either neurin or cholin chemically.

The "crude soluble toxin" is an acid body with sufficient acidity to slowly decompose the alkaline bicarbonates forming salts which act like the free acid, but more tardily.

The Action of the Colon Bacillus and its Poisonous Group on Animals.—It is interesting to study the effects of the living colon bacillus, the dead bacterial substance and the soluble poison split off from the bacterial cell on animals.

We have used a colon bacillus 1 c.c. of a 12 hour or older culture of which has invariably killed guinea-pigs, when injected intraperitoneally, within 24 hours. When only 1 c.c. is given the animal shows no effects for from 10 to 12 hours; when a larger quantity is given the symptoms

appear earlier. This period of incubation represents the time necessary for the bacillus to multiply and be destroyed or broken up sufficiently to liberate enough of the poison to induce observable effects on the animal. In reality this is the critical period of the infection and the result depends upon whether or not all the bacteria are destroyed before they have multiplied sufficiently to furnish a fatal dose of the poison. "It is during this period that individual resistance and acquired immunity are important factors acting by causing increased bacteriolysis and the destruction of all germs before a fatal dose of poison has been set free. During this time the temperature of the animal may rise to a greater or less extent or may remain stationary; the animal remains active, eats; its coat is not roughened and it appears in all respects as well as a normal animal. At the end of this period, however, the appearance changes. The animal becomes less active. It remains in one corner of its

CHART I.

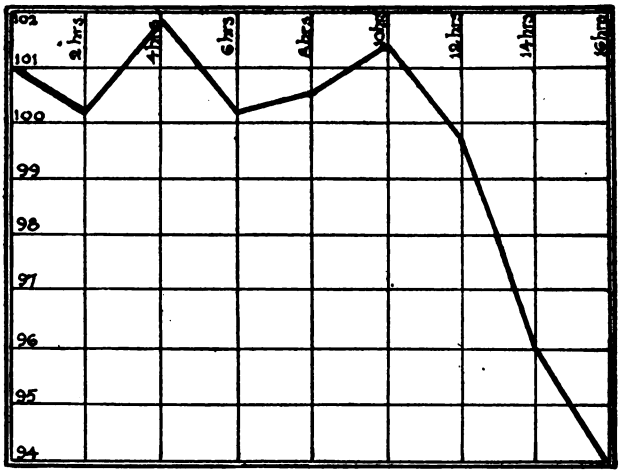


CHART I.—Temperature curve of guinea-pig after inoculation with 1 cc., 16 hr. bouillon culture of the colon bacillus. Death occurred 20 hours after inoculation.

cage; its coat becomes roughened; it hangs its head and apparently enters into a state of stupor. At the same time the rectal temperature begins to fall abruptly, as can be seen from a study of Chart I.

Indeed, this fall of body temperature is often the first marked symptom, and when occurring to a marked degree it is invariably a bad omen. The body temperature will often fall from 101° F. to 94° F. or even lower within from 2 to 4 hours, and this fall is progressive and continuous until the animal's death, immediately preceding which a temperature as low as 87° F. or 86° F. is not uncommon. At the same time the animal shows signs of the most marked peritoneal inflammation, as is evidenced by rigidity and spasm of the abdominal muscles on pressure. At autopsy, the only gross lesion present is a marked hemorrhagic peritonitis with a large amount of bloody fluid containing intact red corpuscles and leucocytes in the peritoneal cavity. The parietal and visceral peritoneum are studded with minute punctiform hemorrhages. Hemorrhage is an especially prominent feature in the great omentum, and is present to a less marked degree in the mesentery."

With the dead bacterial substance, or crude toxin as I have called it, the symptoms in the animal and the post mortem findings are exactly the same as when the living culture is used. The only difference is in the period of incubation. Of course the final issue when the dead germ is used depends upon the amount injected, since there is no possibility for increase in the poison after injection. The animal remains apparently well for about four hours and then its temperature begins to fall abruptly as is shown in Chart II, in which case the dose given was not sufficient to cause death.

When the "crude soluble toxin" is administered the period of incubation and the time necessary for the splitting up of the cellular substance are both done away with and

CHART II.

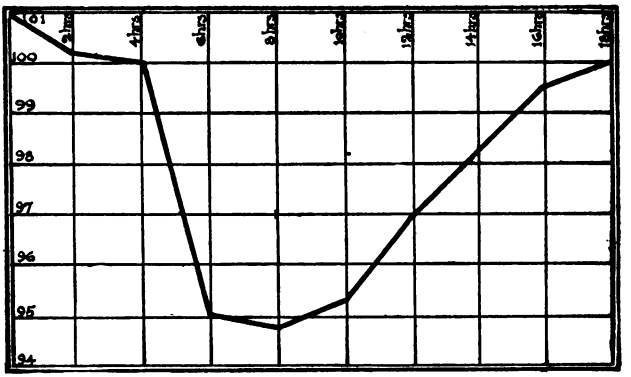


CHART II.—Temperature curve of guinea-pig after intraperitoneal injection of non-fatal dose of crude bacterial cell substance.

the fall of temperature begins within 15 or 20 minutes as is shown in Chart III. If the dose be a fatal one the fall continues until death; if it be less, the first indication of improvement is an upward move in the temperature.

CHART III.

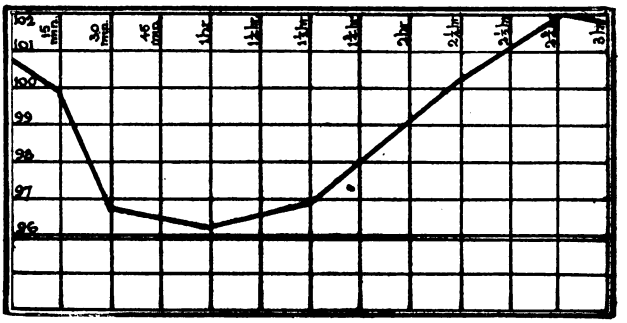


CHART III.—Temperature curve of guinea-pig treated with 45 mgs. of the soluble poison intraperitoneally.

The hemorrhagic peritonitis induced by both the living and the dead bacterial cells is wholly wanting when death results from the soluble poison. This seems to me quite

interesting and I offer the following explanation: This inflammatory condition manifesting itself in a severe and extensive hemorrhagic peritonitis is not due to any activity on the part of the living bacillus, because, as we have seen, it is just as marked when the unbroken dead germ is thrown into the peritoneal cavity as it is when a living culture is employed. For the same reason I must conclude that this inflammatory condition is not caused by, nor is it even connected with the growth and multiplication of the invading microorganism, but it is a result of the disintegration and destruction of the bacteria. It is in my opinion due to the chemotatic force acting between the constituents of the bacterial cells and constituents of the cells of the animal body, and this force is of sufficient intensity to break up both the animal and the bacterial cells. In other words, I believe that bacterial inflammation is essentially a chemical process or is due to the disruption of cell molecules through the chemical affinity between certain groups in the bacterial cell and certain groups in the cells of the animal. So long as the bacterial cells are alive the chemism that holds the living molecule together tends to resist this process of disintegration. I have attempted to investigate this point by placing the dead bacterial cell substance in collodion sacs in the abdominal cavity of rabbits, but in every instance, in which this experiment has been made, the animals have died and after death a localized area of inflammation about the sac has been observed. This point, however, deserves more extended experimentation.

The soluble poison diffuses through collodion sacs and animal membranes, but it does so slowly and when many times the fatal dose, determined by intraperitoneal injection, is introduced into the stomach of a fasting rabbit, the animal does not die. This I suppose to be due to the slow absorption of the poison rather than to its destruction. When the dead bacterial cell substance is subjected to arti-

ficial gastric digestion there is no diminution of its toxicity, but the poison is apparently slowly destroyed by pancreatic digestion.

The soluble poison of the bacterial cell apparently kills by its effect upon the respiratory center. The heart continues to beat normally for some minutes after respiration ceases. In this respect the bacterial poison bears a close resemblance to neurin. The quantity of the soluble poison necessary to kill a guinea-pig depends upon the extent to which purification of the poison has been carried. Usually it contains considerable sodium chlorid, and this can be removed only by repeated evaporation and re-solution in absolute alcohol. The most potent poison that we have obtained killed guinea-pigs in doses of 8 mg. given intraperitoneally, while the poison with which we have made most of our experiments required 60 mg. The fatal dose intravenously is about one-sixth, and that required when given subcutaneously is about twice the quantity needed intraperitoneally.

Our method of extracting the active poison from the bacterial cell is by no means satisfactory, and much of the poison is destroyed in the process. For instance, a bacterial cell substance 5 mg. of which will kill a guinea-pig yields less than one-third of its weight of this "crude soluble toxin," and when the best results are reached not less than 8 mg. of this kills guinea-pigs. I am of the opinion that the poison exists in the bacterial cell as a polymer, and much of this is hydrolized into an inert, or a relatively inactive, form by our process of extraction. If the bacterial cell could be broken up outside the body as it is within the body, we should have a larger yield of the active substance. We have tried various other methods of extraction, but up to the present time we have failed to improve upon that already described. We have detached from the cell substance of numerous toxicogenic bacteria bodies very

similar in their physical and chemical properties and in their effects upon animals. The general clinical symptoms of many of the infectious diseases are markedly similar, and we distinguish one from the other more by the organs involved than from differences in the effects of the poison upon the individual. A typhoid septicemia can hardly be distinguished from one induced by a paracolon or paratyphoid organism, and, indeed, a general tuberculous septicemia bears so close a resemblance to typhoid that it may lead astray, for a time at least, even a skilled diagnostician. A meningitis may be caused by any one of a number of micro-organisms, and no one can determine absolutely from the symptoms the exact name of the invading organism. Moreover, it has been shown by experiments in my laboratory that the molecules of egg albumen and of pepton contain poisonous groups very similar to, if not identical with, those of the cells of certain toxicogenic bacteria.

If I may be permitted to draw some inferences from these studies on bacterial endotoxins, I will state them as follows: The invading bacterium grows and multiplies. The tempo in which these processes proceed is variable, depending upon certain properties inherent in the infecting cell and at present quite unknown to us, and also depending upon the condition of the food material, in the animal, utilizable by the infecting cell and the resistance offered to its growth by the cells of the infected animal. All of these things, and possibly others as well, influence infection. From my own work using different strains of the same organism on individual animals of the same species, I have concluded that the virulence of the bacterium is the most important factor in determining the time and certainty of the death of the animal, and that the virulence of the bacterium is measured by the tempo with which it multiplies in the animal body. For the study of this question the colon bacillus is, in my experience, poorly suited, because

with this organism there are not the wide variations in virulence that we find in certain other pathogenic bacteria. In my investigation of this subject I have employed different strains of the pneumococcus. Some of these are only feebly pathogenic or virulent, while others are most highly so. One strain requires at least 1c.c. of a culture 12 hours old to kill a guinea-pig, while with another strain one-millionth of a c.c. kills. But when two guinea-pigs have died from the effects of these cultures, one from the feebly and the other from the highly virulent culture, so far as one can judge from a close inspection of the organs of the dead animal and the distribution and the number of bacteria present, the growth of the infecting cells has reached the same point in the two animals, and what is more scientifically exact when these two strains are grown artificially and the poison extracted from the cells, like weights of the purified cell substance yield like amounts of the poison. From this I conclude that differences in virulence between different strains of the same pathogenic organism depend upon the tempo or rate with which the organ multiplies in the animal body. This may not be true of all pathogenic bacteria, but it seems to be true of the pneumococcus. I have been surprised at the tenacity with which some strains of the pneumococcus hold their virulence through many generations of artificial cultures.

There is another point in favor of the idea that the virulence of the bacterial cell is dependent upon its rate of growth in the animal. The colon culture with which I have done much of my work varies but little, if at all, in virulence; 1c.c. of a culture 12 hours old invariably kills guinea-pigs when injected intraabdominally; I have a pneumococcus that kills, as I have already stated, in one-millionth of a c.c., and yet 5 mg. of the dead bacterial cell substance of the colon bacillus kills, while it requires about 60 mg. of the dead bacterial cell substance of the pneumo-

coccus to kill. It may be that the latter is not freed from foreign matter so thoroughly as the former, but this can hardly explain so wide a difference, and I must for the present at least hold that virulence in bacteria is largely due to rate of growth in the animals, and this is due to some form of energy in the bacterial cell, the nature of which is, at present, quite unknown.

I think it highly probable that the cells of the infecting organism feed upon the soluble, or at least on the lifeless and unorganized constituents of the animal body, and that the lesions of the infectious diseases are not due directly to the multiplication of the invading bacteria, but to the poisons elaborated by these invaders. Prudden and others have shown that tubercular lesions may be induced by the injection of dead tubercle bacilli, and we have seen that the hemorrhagic peritonitis caused by the injection of the crude colon toxin is undistinguishable from that found after death from the living bacillus.

The pathogenic bacterium assimilates the nutritious constituents of the fluids of the animal body, builds them into its own tissue, converts them into substances foreign to the host, and finally when the bacterial cell goes to pieces either from spontaneous dissolution or through the aggressive action of some animal cell these reconstructed chemical groups are set free and poison the animal, inducing lesions in various tissues, and, in many instances, so interrupting the vital functions as to cause death.

The Non-Toxic Residue of the Bacterial Cell.— When the cellular substance of the colon or typhoid bacillus has been thoroughly extracted with a 2 per cent. solution of caustic alkali in absolute alcohol about two-thirds by weight of the material is left insoluble in alcohol and this we have designated as the "non-toxic residue" or simply as the "residue." This residue responds to all the proteid color reactions with the exception of the Millon test.

This reaction, which is given in great perfection by the poisonous portion of the bacterial cell and which follows the poisonous part in all attemps to purify it, fails with the residue after purification by repeated solution in water and precipitation with alcohol. If a given residue responds to the Millon test it is proof that the poisonous part has not been wholly removed as can be demonstrated on animals. This is not, however, true of all bacteria; the non-poisonous residue of the tubercle bacillus does respond to the Millon test and I can positively assert its absence only in the colon and typhoid residues. This indicates a chemical relationship between these organisms and a chemical difference between them and the tubercle bacillus. Another interesting fact is that the Molisch reaction, which fails wholly with the poisonous portion of the colon and typhoid organisms, is given abundantly and promptly by their residues. Furthermore, the Millon test is positive not only with the poisonous portions of the colon and typhoid bacilli, but also with this portion of all the pathogenic bacteria, including the tubercle bacillus, which we have studied, as well as with the poisonous group of egg albumin and pepton. This indicates a chemical relationship between the poisonous portions of all these bodies. The fact that the toxic part of colon and typhoid bacilli gives the Millon reaction promptly and perfectly, while it fails absolutely to respond to Molish test, and the residue of these bacilli give the Molish test promptly and perfectly, while it fails in the Millon test, is strong chemical evidence that our method of splitting up these bacterial cells gives natural cleavage products. We can distinguish between these split products by chemical tests as well as by their effects upon animals. In neither case have we obtained bodies chemically pure, but it is a satisfaction to know that we have made a chemical separation.

From the fact that the toxic portion of the colon cell

fails to give the Molisch test while the residue responds to this reaction promptly I infer that the carbohydrate group of the cell molecule is confined to the residue. There are other chemical differences between the poisonous and the non-poisonous portions of the colon bacillus. The former contains about 12 per cent. of nitrogen and the latter not more than 5 per cent.; while the poisonous part yields less than one per cent. of phosphorus and the non-poisonous part contains more than 3 per cent. of this element. The chemistry of both of these split products is now being investigated, and I hope to be able at some future time to make more exact statements on these points.

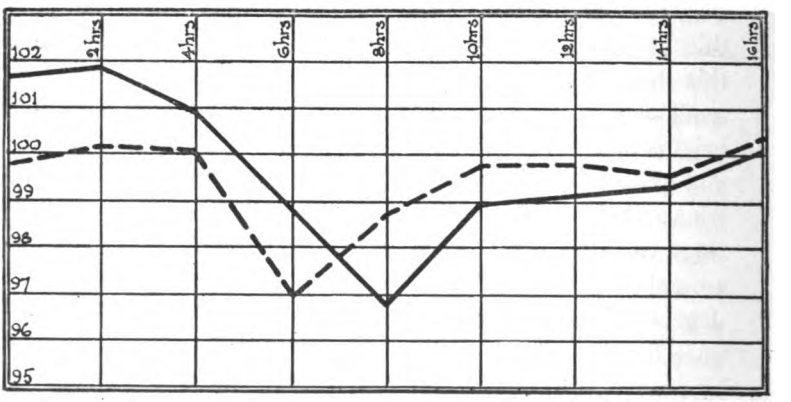
Part III.—The Production of Active Immunity with the Split Products of the Colon Bacillus.

Attempts to Establish Immunity with the Poisonous Portion.—The poisonous portion of the colon bacillus acts on animals so promptly and so powerfully that attempts to secure a tolerance to it frequently end in the death of the animal. If one rapidly increases the size of the successive doses this is certain to be the result. It may be found to be possible by combining the poison with an alkali and thus retarding its effect to secure better results, but this has not been systematically tried yet. Inasmuch as this subject has been discussed in some detail by one of my students in a recent paper* I will content myself with a brief condensation of his results. By proceeding carefully and gradually increasing the doses a point may be reached where the animal will bear treatment with from two to three times the amount that would surely kill an untreated animal. This indicates the development of either a slight degree of immunity or the establishment of a mild tolerance for the poison. Which of these actually results can be positively determined only after a satisfactory investiga-

* See bibliography at the close of this article.

tion concerning the production of an active antibody, and up to the present time our attempts along this line have not been followed by uniform results. Animals brought into this condition, whether it be one of increased tolererance or immunity, bear from two or four times the fatal quantity of living cultures of the colon bacillus. It is an interesting fact that animals that have had a single non-fatal dose of the poisonous portion are able to withstand twice the lethal dose of the living culture given the next day, but this increased resistance induced by a single dose is transitory and has usually disappeared or at least is markedly diminished by the second or third day. However, the increased resistance induced by many successive doses is more lasting, manifesting itself for thirty days. The temperature curve of an animal previously treated with the soluble poison when inoculated with twice the fatal dose of the living culture is practically identical with that of an untreated animal when inoculated with less than a fatal amount of the living culture. This is shown in Chart IV.

CHART IV.



——— Curve of normal animal inoculated with non-fatal dose of living germ.
- - - - - Curve of immune animal inoculated with living colon bacillus.

In both these animals the minimum temperature is reached within from six to eight hours, after which it gradually ascends to the normal. This similarity suggests that we have here a condition similar to that generally spoken of as natural immunity, and this suggestion is supported by the fact that this increased resistance to living cultures of the colon bacillus may be secured by previous treatments of the animal with the poisonous groups split off from egg albumin and pepton as well as with that from the colon bacillus itself. It is quite certain that the increased resistance to the living culture secured by treatment with the soluble poison is not specific. This point is an interesting one, especially in view of the well-known fact, attested by several independent investigators, that increased resistance to certain pathogenic bacteria may be secured by previous intraabdominal injections of various proteid solutions.

Immunization with the non-poisonous residue of the colon bacillus.—I have stated that the portion of the colon cell left insoluble in alcohol after the cellular substance has been heated with a 2 per cent. solution of sodium hydroxid in absolute alcohol is non-poisonous. As much as 500 mg. of it may be injected into the abdominal cavity of a guinea-pig without visible effect. The temperature does not fall as it does after treatment with the poisonous portion, nor is there any measurable or constant rise. Still the non-poisonous residue must react with certain cells of the animal body; otherwise it would be difficult to explain the formation of an antibody. It seems from our work that the toxcity of a substance for the animal body as a whole depends upon the function of the cells in the animal affected by the introduced substance. The poisonous group of the colon bacillus seems to have a special affinity for the cells in the respiratory center of the animal, and consequently in sufficient dose it kills the animal speedily; while the non-poisonous part of the same bacterial cell acts upon other cells

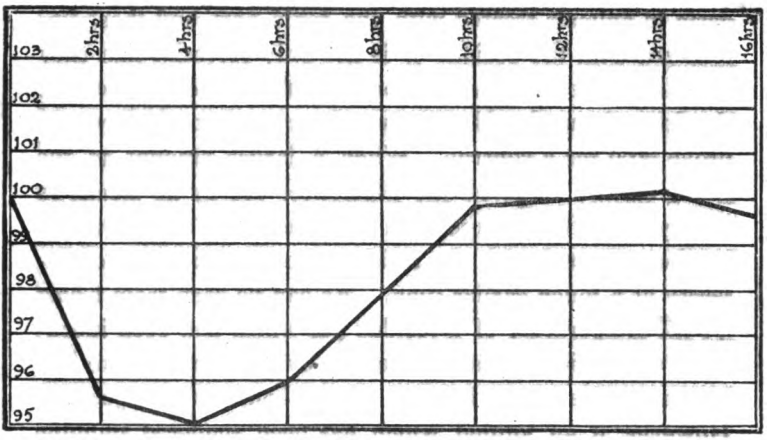
in the animal, and the function of these cells not being so immediately necessary for the continuance of the life of the animal no ill effects are directly observable. This is an important factor, and deserves farther study.

Guinea-pigs and rabbits treated with repeated doses of the non-poisonous part of the colon bacillus bear from six to eight times the minimum fatal amount of the living culture. The degree of immunity induced by these treatments depends not so much upon the total amount of the residue given as upon the number of injections and the length of time through which they are continued. There is, therefore, no object in using large doses of the residue in inducing immunity, because smaller doses given through a longer period induce a higher degree of immunity.

The clinical pictures obtained by immunizing animals to the poisonous and the non-poisonous portions of the colon bacillus differ in an interesting way. As has been stated, the symptoms induced in a pig by inoculation with twice the minimum fatal dose of the living culture after injections with the poisonous part are identical with those observed after inoculation of an untreated animal with less than a lethal dose of the culture. But animals immunized with the non-poisonous part and subsequently inoculated with twice the minimum fatal dose of the living culture are very ill within an hour, much sooner than the control, and after from six to eight hours, just when the control begins to manifest symptoms, the immunized animal begins to improve. All this is indicated in the temperature, as shown in the accompanying chart.

On this point one of my students, V. C. Vaughan, Jr., offers the following: "The difference between the behavior of animals treated with the toxic part and those which have been treated with the residue towards cultures of the living germ is easily explained, if we consider the fact that in the first case we are dealing with an animal which has acquired

CHART V.



a certain amount of tolerance for the intracellular poison of the colon bacillus as represented by the toxic part. In the case of animals treated with the residue, however, no tolerance for the poison contained within the colon bacillus has been developed. If now the process which takes place in both instances is a bacteriolytic one, it results that in the case of the animal immunized with the toxic group the effects of the poison contained within the bacterial cell and liberated upon its disintegration will not become manifest until a sufficient amount of poison has been set free to overcome the tolerance which the animal has attained during the process of immunization. In the case of the animal immunized with the residue there is no tolerance to be overcome other than that which is present in all animals, and the effects of the poison liberated through bacteriolysis become apparent sooner and to a more marked extent. Again, the fact that bacteriolysis may occur more rapidly in the case of residue pigs than in those immunized with the toxic group might explain in part the difference in behavior in the two cases. This is a point on which we are as yet unable to give any definite results.

"In order to study the differences in reaction to the living germ in animals treated with the toxic part and those immunized with the residue it is not only essential that they should receive the same amount of the same culture, but the dose given should not exceed twice that which would prove fatal for a control. When a larger amount of the living culture is given the differences are by no means so clearly defined, although even in this case the animal which has been treated with the residue shows symptoms of severity at a much earlier time. As can be seen from Chart V. the temperature of a residue pig which had been inoculated with twice the fatal dose of a living colon culture had begun to rise at an interval of six hours after injection. However, if an animal which has been rendered immune by treatment with the residue is inoculated with six to eight times the fatal dose of the living culture, we find that the temperature curve obtained is somewhat different in character. The temperature falls with the same initial rapidity, but instead of showing an early rise it continues for some time at a low point, and it is only at the end of from eight to ten hours that any appreciable rise is manifest. This, we think, is due to the fact that there has not been enough of the bacteriolytic substance directly available to destroy all the bacilli contained in the large amount of culture injected. The remainder of the germs are destroyed by the same factors which are operative in normal animals after the injection of a non-fatal dose of the living bacillus. As can be seen from Chart IV. it is only after an interval of six to eight hours that there is any appreciable fall in temperature in the case of a normal animal inoculated with a non-fatal dose of the living culture. This, we think, indicates that it is not until this time that any appreciable amount of poison is liberated by bacteriolysis, since, as we have seen in a previous paper, one of the first signs of the action of the intracellular poison is a fall in body temperature. In a pig

which has been immunized with the residue, and subseqently inoculated with a large amount of the living germ, we obtain evidence of hypothermia at a much earlier period, owing to the fact that bacteriolysis takes place very rapidly since the bacteriolytic substance is present in a form available for immediate use. If, however, the amount of this substance directly available is not sufficient to cause death and bacteriolysis of all germs present, those bacilli which remain are still capable of further reproduction. The same mechanism which causes destruction of the bacteria in normal animals, and which is probably connected with the phenomenon of phagocytosis, is, however, still operative in the immune animal. Thus we shall have two influences at work in the immune animal to cause bacteriolysis, one acting rapidly, and the other manifesting its action only after a considerable interval of time. We should, therefore, expect theoretically that we would find in the immunized animal a marked fall in temperature at an early time, due to the setting free of the poison from the bodies of the bacteria disintegrated by the directly available bacteriolytic substance followed by a secondary rise, and a succeeding fall due to the liberation of the poison by means of the factors present in the normal animal. However, this is not actually the case since the effect of the poison liberated at first has not worn off before the second period of bacteriolysis becomes well established. Consequently, the intermediate rise of temperature is absent."

Animals immunized against colon infection with the non-poisonous residue manifest no increased tolerance to the soluble poison, but are quite as susceptible to this as untreated animals are. This indicates that this form of immunity is not due to the production of an antitoxin, but is due to increased capability of the body cells to destroy the bacteria. Whether the bacteria introduced into animals immunized with the residue are destroyed by some bacter-

iolytic substance increased in amount by the successive treatments with the residue and present in the fluids of the body or by increased phagocytic action, I am not yet in a position to determine with certainty, but I am inclined to the opinion that the latter is the important factor. It seems probable that in some way the phagocytic warfare against the invading bacterial cells is rendered more effective. On this point I hope to have in the near future more positive and convincing evidence.

The immunity induced by the non-poisonous residue differs in yet another important respect from that secured with the soluble poison. We have seen that the latter is not specific, but may be secured with the similar, if not identical, poisons obtained from egg-albumin and pepton. On the other hand, the immunity obtained with the non-poisonous residue is specific, and is secured only with the colon residue, and not with residues prepared from albumin and pepton. I conclude from this that the residue contains that group in the colon cell which is peculiar to that organism.

Part IV.—Summary and Conclusions.

I believe that this work which has been done by my students and myself on the chemistry of the colon bacillus and the action of the split products on animals, and which I have briefly outlined in the preceding pages, gives us a glimpse, as it were, of the physiology and pathology of the living cell. You will, therefore, please pardon me if I make an attempt to offer an explanation of some of these things. This will be difficult, somewhat tedious, and more or less unsatisfactory to both you and me. It will be difficult, in the first place, because the subject is a new one, and I shall be compelled to use terms which have been used with other meanings, and the speaker and the listener will be likely to understand the statements differently. How-

ever, I will do the best I can. As I have already indicated, I believe that life is molecular; *i. e.*, that the essential phenomena of cell life are due to intramolecular changes. This idea is not wholly original, for, as I have stated, the morphologists, such as Nussbaum, and the physiologists, such as Verworn, have expressed similar conceptions, although both have objected to saying outright that life is molecular, because the word molecule gives one the idea of a relatively stabile compound, while the living molecule is never, for any measurable period of time at least, in a condition of equilibrium. As I have stated, Verworn has proposed the expression "biogen molecule," but this can be interpreted only as a molecule that produces or induces life. I wish to acknowledge my debt to the very interesting monograph by Verworn on the Biogen Hypothesis, for from this I have obtained many of my ideas, and I intend this statement as a general acknowledgment of this fact, but I will not hold him responsible for my interpretations, with some of which he certainly will not agree.

I believe that the living cell is in its essential and active part composed of a chemical compound of very complex, but of definite, structure. This chemical compound contains a chemical nucleus, which must not be confounded with the morphological nucleus of the cell. The cell is composed of a chemical compound, not of a single molecule. There are many molecules in a cell, just as there are in a speck of salt, and there are as many chemical nuclei as there are molecules. I wish that I knew of some better term to use to designate the part of the cell molecule to which I refer, but I do not. By chemical nucleus I mean the centre of the chemism of the cell molecule; a centre of special chemical activity. This, as in all known proteid compounds, contains a benzol ring, and to it numerous side chains are attached. The presence of this benzol ring in the colon cell is shown by its response to the Millon test

and by the fact that we obtain aromatic split products, such as tyrosin and indol, by breaking up the cell with strong acids and alkalis. The tyrosin we have obtained in crystalline form, and have positively identified by determining its melting point and by distinctive tests, while we have produced the characteristic odor of indol by heating the cellular substance with strong alkali. We have also shown the presence of a carbohydrate group in the colon cell.

Verworn makes the following statement concerning the functional dissociation of the biogen molecule: "The nitrogen oxid (which exists as a side chain to the benzol ring), as happens in the manufacture of sulphuric acid, splits the molecular oxygen, which is brought to the living substance in the medium by which it is surrounded, and is converted into nitrogen dioxid. The nitrogen dioxid, when brought by the intramolecular movement of the atoms near to the aldehyde (carbohydrate) group (another side chain attached to the benzol ring) gives up an atom of its oxygen, and in this way carbonic acid is formed along with water, lactic acid and other simple non-nitrogenous bodies. The nitrogen, now reduced to nitrogen oxid, remains attached to the benzol ring, and again becomes oxidized to nitrogen dioxid." In this way a nitrogen side chain serves as a receptor and transmitter of the oxygen, and thus the traffic in energy within the living cell molecule goes on rythmically. It is not to be supposed that the nitrogen side chain, which serves as the receptor and transmitter of oxygen, consists of so simple a body as nitrogen or nitrogen oxid, but it is probably a highly complex nitrogenous body in which the location of the nitrogen is central, as suggested by Allen. Nor is it to be supposed that only oxygen is broken off from the cell pabulum, but substances containing this element. This is the way in which the living cell molecule keeps up its constant, rythmic traffic in energy, absorbing heat by assimilation and giving it off by dissoci-

ation. Each living molecule has not only one, but many, of these nitrogenous side chains that act as receptors, and not only one, but many, carbohydrate side chains that furnish carbon and hydrogen for oxidation. Moreover, the metabolism within the molecule is not confined to the absorption of oxygen and the casting out of non-nitrogenous products of combustion. The whole molecule is labile, and there is probably in every living cell molecule a nitrogenous as well as a non-nitrogenous metabolism. The nitrogen absorbed with the oxygen is utilized in replacing the waste in this element, and the carbon brought into the molecule at the same time is in part detached by the free valences in the carbohydrate side chains and used to repair the loss in this part of the molecular structure. In the cell molecule it is probable that the nitrogenous metabolism takes place much more slowly than the carbon and hydrogen metabolism, and in both it goes on rythmically, and the tempo with which it proceeds depends upon the swing of the atomic groups that constitute the molecule, and this rate can be changed, hastened or retarded, by alterations, either physical or chemical, in the medium in which the cell lives. Under certain conditions all metabolism in certain cell molecules may be indefinitely arrested without disruption of its structure. The cell molecule is then not actively alive, but is possessed of latent life. This is true of spores, seeds and ova. In a seed, as I have already stated, the germ of life; *i. e.*, the molecular structure, is present, but there is no activity, probably because there is nothing in the seed to which the molecular side chains or receptors can attach themselves, but when the seed is placed under suitable conditions the ferment present in the pabulum surrounding the germ breaks up the complex bodies into simpler bodies, which react with the receptors, and active life is awakened. Even when the cell molecule is in active life its food is prepared for it by ferments, and there are many

who now believe that every kind of cell has its own special ferment. Be this as it may, it is certainly true that before a cell molecule can use outside matter in its reconstruction that outside matter, or pabulum, must be split up into relatively simple bodies. This is in part accomplished in animals by digestion in the alimentary canal; and in each organ this preparation of the food material is more specifically carried out by ferments supplied by the cells of each organ. It is most probable, indeed it is quite certain, that these ferments have their origin in the nitrogenous metabolism of the cell molecule. Certain side chains are detached from the living molecule, and in solution pass into the surrounding medium, splitting up complex bodies and fitting them for the receptors of the molecule.

I might enter into greater detail concerning the chemistry of the cell and my conception of the way in which it functions, but I am endeavoring to make this statement as brief as possible, and I think that anyone who has followed me so far can get my point of view, which, I take it, is not essentially different from the teachings of Ehrlich and Verworn.

I will now attempt to interpret the results of the investigations on the chemistry of the bacterial cell, and in order to avoid exhausting your patience I will condense as follows:

(1) The cellular substance of the colon bacillus, after being freed from all matter held by it and in it mechanically, consists of a complex chemical compound composed of chemical nuclei with numerous attached groups or side chains.

(2) The chemical nucleus, or centre, of chemism is contained in our "crude soluble poison." When the cellular molecule is split up by means of an alcoholic solution of sodium hydroxid, the molecular nucleus is found in that fraction which is soluble in alcohol.

(3) Since we have obtained similar "soluble poisons" from all the bacteria with which we have worked, also from egg-albumin and pepton, I conclude that the chemical nucleus in all these bodies is similar, but not identical. The presence of the benzol ring in the soluble poison is shown by its prompt response to the Millon test. However, the chemical nucleus consists of more than the benzol ring; it contains the benzol ring with the receptor nitrogenous side chains, and our soluble poison also contains the benzol ring with the nitrogenous receptors.

(4) The chemical nucleus has great affinity for other substances containing groups from which it can detach side chains, and for this reason is an active cell poison.

(5) The modus operandi of the chemical nucleus as a poison is explainable by its detaching from certain cell molecules of the animal body side chains, upon which the functioning of the animal cell depends.

(6) The intensity of the poisonous action of the chemical nucleus depends in the first place upon the intensity of its chemism, and in the second place upon the number and condition of its receptors. Our method of detaching the chemical nucleus and rendering it soluble is crude compared with the process as it occurs in the animal body. Five milligrams of colon germ substance thrown into the abdominal cavity of a guinea-pig kills, while the smallest fatal dose of soluble poison that we have obtained is eight mg., representing about twenty-five mg. of the germ substance. In our crude attempt to isolate the chemical nucleus we have probably destroyed, or at least inactivated, many of its receptors. Our soluble chemical nucleus acts much more promptly than the cellular substance, because we have broken up the cell molecule and set the nucleus free, which process in the animal body goes on slowly, but with less loss. The receptors of the chemical nucleus of the colon bacillus are acid in character and can be neutralized with

sodium bicarbonate, but when the sodium compound is introduced into an animal the alkali is replaced by atomic groups, for which the receptors have greater affinity, and these groups being taken from body cell molecules, the neutralized chemical nucleus is a poison with the same action as the free nucleus, but less prompt.

(7) The chemical nucleus of all the bacterial cells with which we have worked, as well as that of egg-albumin and pepton, appears to have a special affinity for certain side chains in the cell molecules of the respiratory centre of the animal. It tears off these side chains from the cell molecules of the respiratory centre, thus interrupting the functions of these cells and causing the death of the animal.

(8) It is probable that the chemical nucleus of every foreign cell, and of every foreign proteid as well, when brought into the circulation in a free state, is more or less of a poison to the respiratory centre.

(9) Whether a given proteid will prove poisonous to an animal or not, when introduced into the blood directly by intravenous injection or indirectly by subcutaneous or intra-abdominal injection, depends upon whether or not its chemical nucleus is set free intact and upon the rapidity with which it is set free.

(10) The chemical nucleus of a cell or a proteid does not absolutely destroy the cells of the animal body. It tears off their side chains, and impairs or interrupts their functions. If these side chains be detached from the animal cell molecule slowly and in only a few cells at a time, reconstruction lags behind destruction but little and the life of the animal is not endangered, but when these side chains are detached, as it were, in mass and from a large portion of the cell molecules of a given organ, then the function of that organ is perceptibly impaired, and it may be completely interrupted, and if this happens in an organ whose continued functioning is essential to the life of the animal, death results immediately.

(11) The chemical nucleus of the colon bacillus, at least, when administered by the alimentary canal, or when introduced into the abdominal cavity enclosed in a collodion sac, diffuses so slowly that it does not visibly affect the animal. It is also, in part at least, destroyed in the intestine, when taken by mouth. Furthermore, it is possible that the chemical nucleus even of the proteid foods largely passes through the alimentary canal, and is not absorbed at all.

(12) When the chemical nucleus is broken up its constituents are not poisonous, and inasmuch as it is a labile body its conversion into a harmless substance is easy under favorable conditions.

(13) The chemical compound of which the essential part of the colon bacillus is composed is not a poison, or at least not an active poison, until it is disintegrated by the aggressive action of the cell molecules of the animal body. The hemorrhagic peritonitis observed after death from intra-abdominal injection of the colon bacillus is not induced by, nor is it connected directly with, the growth and multiplication of the bacillus, for it is just as marked after the injection of the dead bacterial substance as it is after inoculation with a living culture. The hemorrhagic peritonitis results from the chemical reaction between the constituents of the bacterial cell and certain constituents of the body cells.

(14) That the soluble poison obtained by splitting up the colon cell is the poison that kills when an animal is inoculated with a living culture, is indicated by the following:

(*a*) The effect on the temperature. This is the same whether the animal be treated with a living culture, the dead germ substance or the soluble poison, except in the interval of time that elapses between the introduction of the foreign substance and the beginning of the fall, and when the soluble poison is combined with an alkali this period is lengthened.

(*b*) If with either of these the dose employed is less than a lethal one, the first trustworthy evidence of recovery is a rise in temperature.

(*c*) When a lethal dose is employed, the mode of death is the same; *i. e.*, it is due to failure of respiration.

(*d*) When the soluble poison is combined with alkali and time enough given for the saturation of the receptors with the alkali, the post mortem findings are the same.

(15) The production of increased tolerance, and the formation of an antibody, should further experimentation show that such a body can be produced, by successive treatments of animals with the soluble toxin, can be explained by Ehrlich's theory, or by a modification of this theory, which I have elsewhere discussed.

(16) Concerning the immunity secured against the living culture by successive treatments with the non-poisonous residue of the colon bacillus, I wish to say a few additional words. This I regard as the most interesting part of our work. It should be remembered that in these animals there is no increased tolerance to the soluble poison, and we must conclude that this form of immunity is due to bacteriolytic or phagocytic action. I have spoken quite at length concerning the chemistry of the bacterial cell, and I assume that the cells of the animal body are made up in a similar manner of living molecules, each of which contains a chemical nucleus with numerous side chains attached. These animal cell molecules split off from the bacterial cell molecules side chains upon which the functioning of the bacterial cell depends, and thus deprive them of life. Now by feeding the body cells with the detached side chains of the bacterial cell molecules the former are sensitized, as it were, to this kind of food, and acquire greater avidity for it with each successive administration. The chemism of the animal cell for these special side chains is intensified, and when the living culture is introduced the advantage is with

the body cell the lability and chemical structure of which have been adjusted to the absorption of the side chains of the bacterial cell molecule. This is probably due to some alteration in the chemical structure of the receptor side chains of the animal cell molecule. We recognized early in our work that if we could secure a perfect condition of this nature, enough poison should be set free in a short enough time, when a large amount of dead germ substance was introduced into the abdominal cavity, to kill the animal in the same time that it takes the free soluble poison to kill, and we made many attempts to reach this result. On this point V. C. Vaughan, Jr., in a paper published in 1905, wrote as follows: "Although we have as yet been unable to actually cause death in an immune guinea-pig at an early period, the animals are in every instance very ill within thirty minutes after the injection of the dead culture. In fact, several of them have shown signs of the commencement of the convulsive stage, as evidenced by slight convulsive movements of the head separated by considerable intervals of time. We have been unable to secure a fatal result in these animals up to the present time, simply because we have worked with pigs which did not possess a sufficient amount of bacteriolytic substance directly available to cause disintegration of enough bacilli to liberate a fatal amount of poison at one time. It is worthy of note that this behavior of animals immunized with the residue towards the dead bacterial substance furnishes additional proof of the fact that the poison of the colon bacillus is an intracellular one. If the poison existed free in the culture medium, we should expect that the control would show evidence of its action at as early a period as does the treated animal. However, as has been stated, this is not the case. The fact that the treated animal shows symptoms of poisoning to a much greater degree and at an earlier time than does the control, can be explained only on the ground that

the poison with which we are dealing is an intracellular one and is set free only after the disintegration of the bacillus by bacteriolysis."

It seems quite evident that the animal cell molecule does not absorb or destroy the chemical nucleus of the bacterial cell molecule, but when the other side chains are torn from the bacterial cell the chemical nucleus goes into solution. It should be understood that for the body cell molecule to break up the bacterial cell molecule it is not necessary for the two to come in contact in cellular form. Indeed, bacterial cells may be dissolved in body fluids in which there are no animal cells, but I imagine that this is due to ferments that have been formed in the body cells. As we have seen, the more complex bodies in the pabulum are broken into simpler bodies by soluble ferments, and I do not suppose that there is any intermolecular reaction between cells except of substances in solution.

Part V.—Bibliography.

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