base-forming properties, but in general these six successive elements in the periodic system exhibit similar chemical properties and undoubtedly perform similar physiological functions in plant nutrition. With respect to cobalt and nickel, these functions have not yet been studied experimentally sufficiently to determine whether they are really important in plant nutrition, and they are therefore tentatively listed both in Group IV and Group VII of this proposed scheme of classification.

With respect to manganese and iron, and copper and zinc, however, I believe that there is sufficient experimental evidence to justify the hypothesis that these are two pairs of mutually coordinating catalysts for oxidation-reduction reactions, the former for biological reactions in which the addition or removal of oxygen is the basis of the energy exchange and the latter for those in which loss or gain of hydrogen is involved. I recognize that the matter has not yet been investigated sufficiently to establish fully the soundness of this hypothesis of the balancing effect of these two pairs of catalysts, but believe that there is adequate proof that their major function is that of catalysts of oxidation-reduction reactions in biological processes. While it is impracticable to cite in detail in this preliminary paper the experimental evidence bearing upon this particular phase of the matter, it may be briefly summarized as follows:

Iron and manganese have been shown to be mutually antagonistic in their production of chloroses of plants due to improper conditions for the production of chlorophyll. This has frequently been explained as a calcium-manganese antagonism, but recent experiments have shown that the true explanation lies in the rendering insoluble and unavailable of iron by excessive proportions of calcium in the nutrient medium or cell protoplasmic contents, thus disturbing the iron-manganese balance, instead of the calcium-manganese balance.

The basis for the belief that copper and zinc are mutually counter-balancing catalysts for hydrogen exchange lies in their recently demonstrated striking and opposite effects upon the reversible oxidation-reduction reactions of both glutathione and ascorbic acid. The theory that these two compounds, which are now known to be almost, if not quite, universally present together in rapidly metabolizing tissues of both plants and animals, constitute linked factors in the system of oxidation-reductions in protoplasm, has been suggested recently by several different investigators.

In short, it is my hypothesis that the iron-manganese pair constitute the controlling catalytic factors in oxidation-reduction reactions of the oxygen exchange, or chlorophyll and hemoglobin type, and that copper and zinc act similarly in the hydrogen exchange, or glutathione-ascorbic acid type of similar reactions.

In my laboratory, we are now engaged in an experimental study of the soundness of the second phase of this hypothesis, as a part of our study of the general problem of the function of rarer elements in plant nutrition.

GROUPS V–VIII

The functions in plant nutrition of the elements tentatively placed in these groups have not been investigated sufficiently to provide a basis for their grouping according to known uses for this purpose. They are therefore grouped according to their chemical properties as connected with their place in the periodic table, with the thought that this may afford a basis for further study of their possible plant nutrition functions.

It is possible, of course, that further investigational work will show that some of the elements which are found in the higher orbits of the periodic table have definite functions in plant nutrition. In fact, it has been suggested that rubidium, iodine and even lead, for example, may have some such functions; but the observations which lead to such suggestions appear to refer to some abnormal growth or environmental conditions and not to the general normal processes of plant nutrition. The list here presented includes all those elements which have been reported to be frequently found in the ash of plants grown under generally-occurring conditions of soil nutrient supplies the world over, and is therefore believed to include all those elements which need to be considered in a systematic study of this problem.

ENVIRONMENTAL TRANSFORMATION OF BACTERIA[1]

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RECENT morphological and physiological studies of specific infectious agents have led to tentative conclusions, which bacteriologists and immunologists are frankly incompetent to harmonize with the currently accepted theories of genetics and organic evolution.

These studies suggest a Lamarckian rather than a

[1] Presented as part of the symposium on "Environmental Effects on Plants," Western Society of Naturalists, Asilomar, Calif., December 27, 1933.

Darwinian world, or at least a world in which both Lamarckian and Darwinian evolutionary mechanics are operative.

I

During the first fifty years after the initial discoveries by Koch, Lister and Pasteur, the basic generalizations of post-Darwinian biology, as taught and understood by the average college student, formed the unquestioned axioms from which bacteriologists and immunologists deduced many of their most important technical methods. Methods for the specific diagnosis of human and veterinary infections, for their specific prophylaxis and specific therapy were all either direct or indirect applications of Mendelian logic. No method was suggested, for example, that was contrary to the accepted law that each and every disease germ must arise from a pre-existing microbe of at least approximately the same type or species. Spontaneous generation was unthinkable. Environmental synthesis of infectious units was never conceived. Transmutation of pre-existing microbes into wholly new species or genera was beyond belief. Even the possibility of the environmental induction or ongrafting of a single new hereditary character in bacterial cells never entered into practical clinical logic.

The earlier bacteriologists, of course, did not assume that laws governing the evolution and racial stability of higher animals and plants are equally applicable to disease germs, without some attempt at their statistical or experimental verification. They observed, for example, that environmental bacteria do not appear in heat-sterilized broth, under conditions which exclude the possibility of their entrance from the outside world. This was accepted as proof that spontaneous generation of microorganisms is impossible.

It was further noted that the amount of pigment produced by certain bacteria varies at different temperatures or on transplantation to a different type of culture medium. The changes thus produced, however, were apparently always quantitative and never qualitative in nature, thus conforming with Mendelian expectations. Moreover, the changes were apparently never permanent, the microorganisms reverting to their original parent type on return to their original environmental conditions. Acquired characters, therefore, are apparently not hereditarily transmissible in the bacterial world.

If a pure-line bacterial culture is "plated out," so that each bacterium multiplies to form an individual colony, "natural variations" were noted in pigment production. These, also, were always quantitative in character. By selective propagation, intensely pigmented and quasi-albino strains were eventually obtained. Both strains, however, tended to revert to the original parent type, on prolonged non-selective sub-culture. This selective improvement and subsequent non-selective reversion were both in accord with conventional preconception.

Gradual losses of virulence were noted on prolonged non-selective cultivation of pathogenic microorganisms on routine or overly favorable culture mediums. On injecting the attenuated cultures into laboratory animals, however, the microorganisms recovered at autopsy were usually of restored virulence, an apparent "survival of the fittest" in the resistant animal body. By repeated animal passage, strains of "exalted virulence" were eventually obtained, apparently demonstrating the applicability of post-Darwinian evolutionary mechanics.

Swollen, distorted, atypically staining, granular or vacuolated cells were often noted in old cultures, as well as shrunken cells, "shadow cells" and granular "debris." On attempting routine sub-culture from such "atypical" bacteria, growth often did not occur. If multiplication did take place, however, the resulting daughter cultures were apparently always of "typical" morphology and staining reactions. Any departure from the assumed "typical" microscopical appearance, therefore, becomes synonymous with "degeneration," "involution" or "necrobiosis."

II

It seemed apparent from these earlier studies that the laws governing hereditary, natural variation, racial stability and selective evolution in higher animals and plants could be applied without change to bacterial species. Serious doubt as to the infallibility of this logic, however, began to be entertained about five years ago, when careful restudies of bacterial stability were begun in a dozen research laboratories.[2] Pedigreed strains, known descendants from a single ancestral cell, were now seen to "dissociate" into from two to a dozen morphological, tinctorial, physiological or antigenic types. This dissociation was apparently far beyond the analogous mutation of higher animals and plants. Many of the "dissociates" were so different from the parent culture as to simulate wholly unrelated families, sub-families or genera. It is as though in higher biological science rats should mutate into mice, gophers and guinea pigs, or primroses, daisies and nasturtiums should appear among hybrid sweet peas.

Both "reversible" and "non-reversible" new races, species, genera or families were afterwards grown from scores of clinically important bacteria, the terms denoting the relative stability of the new variants. Certain of the new races reverted in the first subculture to their original parent type. Others have already been cultivated for three years on routine

[2] Hadley, Delves and Klimik, 1931.

culture mediums without showing a demonstrable tendency to revert.[3] This means approximately seventy-five thousand cyto-generations, the equivalent of seventy-five centuries with shelled amoeba, or seventy-five millenniums with many higher animals or plants. During this time, of course, transplants have been made by non-selective methods and the environmental conditions have been kept relatively constant.

Dissociation may and often does occur under apparently optimum cultural conditions.[4] With certain bacterial species, however, racial plasticity is more marked under slightly adverse growth conditions, such as an unfavorable surface tension, reduced barometric pressure or the presence of dilute chemical or biological antiseptics, such as semi-immune animal serum. The mere addition of sterile milk[5] to a routine culture medium allegedly causes the acid-fast tubercle bacillus (Order II, *Actinomycetales;* Family II, *Mycobacteriaceae;* Genus I, *Mycobacterium*) to transmute into a non-acid-fast coccus (Order I, *Eubacteriales;* Family II, *Coccaceae;* Genus I, *Diplococcus*). With proper preliminary selective care, this quasi-coccus can be grown indefinitely as an apparently stable new species.[6] Non-flagellated typhoid bacilli, non-capsulated pneumococci, Gram-negative diphtheria bacilli, as well as a host of other unconventional species or genera can be produced at will. Many of them show a non-selective racial stability equal to or even greater than that of the "undissociated" parent culture. Even viable colloidal granules beyond the range of microscopic visibility are alleged,[7] the hypothetical "gonidial granules," "primordial phases" or "virus phases" of certain specific infections.

Of the eleven presumably unit characters thus far studied with certain bacterial species, there is apparently not a single character that is static under test-tube conditions, nor is there the least suggestion of a genetic "linkage" between any two of these eleven characters. The currently assumed unit characters include such diagnostic properties as: size, morphology, staining reactions, colony type, power to ferment certain carbohydrates, pigment production, toxin production, racial specificity of fractional antigens and type of disease produced in laboratory animals. Even the specific virulences for two closely related animal species or for two different organs or tissues of the same species[8] may vary independently of each other.

III

There is conclusive evidence that all test-tube variants can not be explained as the result of merely quantitative variations in preexisting hereditary unit characters. The environmental induction of one or more new unit characters is fully confirmed,[9] the "emergent evolution" of an apparently new protein specificity, for example, no trace of which is demonstrable in the parent culture. It is as though blue roses should appear among the predicted red, orange, yellow and albino hybrids, a genetic impossibility to modern horticulturists.

"Convergent evolution" is an interesting by-product of this emergence, two practically identical hypermutants arising from two presumably unrelated bacterial species. It is as though, under appropriate environmental conditions, crows and robins should each mutate into bluejays, or pines and cedars metamorphose into redwoods.

During the last two years it has been increasingly evident that emergent and convergent bacterial evolutions are not mere test-tube curiosities. Similar hyper-mutations are known to occur during the course of natural clinical infections or as a result of experimental inoculation into lower animals. The primary and tertiary phases of *Tr. pallidum,* for example, are of such qualitatively different antigenic properties as to simulate two relatively distinct microbic species. *B. tuberculosis* is apparently diphasic[10] and perhaps even triphasic in effective antigenicity during the course of the natural clinical disease. Poliomyelitis virus is allegedly transmuted into a qualitatively different antirhesus variant, on repeated injection into monkeys.[11] This transmutation is associated with a remarkable increase in its antirhesus virulence, strongly suggesting that this transformation must be considered as evolutionary rather than degenerative in character.

Recent fractional analyses of hemolytic streptococci isolated from clinical cases strongly suggest their convergent transformation in the human body, the appearance of a stable, human-diagnostic, polysaccharide fraction,[12] no trace of which is demonstrable in homologous streptococci of veterinary origin. An anti-human fibrinolytic function recently demonstrated in such streptococci[13] is also suggestive of the same convergence.

IV

There is apparently convincing evidence that hormones, chemical "organizers" or integrating enzymes play an important rôle in stabilizing bacterial populations, and in initiating or inhibiting dissociations or transformation phenomena. The integrating

[3] Mackenzie and Fitzgerald, 1933.
[4] Knaysi, 1933.
[5] Maher, 1933.
[6] Miller, 1931; Mellon, 1931.
[7] Hadley, 1931.
[8] Webster and Clow, 1933.
[9] Mackenzie and Fitzgerald, 1933.
[10] Thomas, 1932; Rice, Orr and Reed, 1933.
[11] Paul and Trask, 1933.
[12] Lancefield, 1933.
[13] Tillett and Garner, 1933.

factor thus far studied in greatest detail is the type-specific "activator" of the pneumococcus.

Under routine cultural conditions the pneumococcus breeds true as a capsulated diplococcus, whose type-specific diagnostic character is due to the presence of a highly specialized capsular polysaccharide. Some of these capsular sugars are of known chemical composition. Under certain slightly adverse cultural conditions, however, the capsulated pneumococcus dissociates into a non-capsulated variant, in which the most delicate immunochemical test fails to reveal a trace of its original type-specificity polysaccharide. This naked variant may breed true for innumerable test-tube generations.

If, however, under certain serological conditions, a pure culture of this naked variant is enriched by the addition of an aqueous extract of alien-type pneumococci, the naked variant is "activated" to a regeneration of its outer defensive capsule.[14] The regenerated capsule, however, is not of the original type-specificity. The pneumococcus now manufactures a new capsular polysaccharide, apparently identical with that of the alien extract with which it was "fertilized." Thus hormonally hybridized to an alien type, the recapsulated pneumococcus breeds true for innumerable test-tube generations. It is as though albino crows stained with bluejay extract should transmute into a new and stable race of blue crows.

Similar hormonal transformations are alleged for the tubercle bacillus, an unknown hormone, for example, capable of transmuting the conventional waxy tubercle bacillus into a stable, non-waxy variant.[15] Of even greater clinical interest, however, is the rapidly increasing evidence that hormonal transformations even take place between widely different bacterial species or genera. It is well confirmed, for example, that partial functional and chemical convergence takes place between certain pathogenic and non-pathogenic bacteria of the gastro-intestinal type if grown together in the same fluid medium.[16] Even such widely different microorganisms as the streptococcus and the diphtheria bacillus demonstrably hybridize.[17] Unfortunately, however, from the point of view of medical research, the resulting symbiotic or synergistic hybrids are rarely stable, reversion to type almost invariably taking place on separating the two associated species.

V

While the mechanisms of most bacterial dissociations or transformations are unknown, there is one type of metamorphosis that has been studied in considerable detail. These are the transformations which take place under the influence of the Twort colloid (1915).

Freshly isolated typhoid bacilli, for example, grown directly from clinical cases, are usually aggressive in character.[18] This is shown by their resistance to certain biological antiseptics and by their power of digesting or "hemolyzing" certain relatively refractory red blood corpuscles. By the second or third sub-culture, however, these aggressive bacilli usually dissociate into ordinary, non-aggressive laboratory types, setting free an aggressive "cytoplasmic gene"[19] into the surrounding fluid medium. This dissociated gene (Twort colloid) is of approximately the same size as the average protein molecule.[20]

Dissociated bacterial genes of this type are apparently not fully autonomous vital units. So far as known, they are incapable of self-proliferation. Added to a routine non-aggressive laboratory strain of typhoid bacilli, however, the free genes are adsorbed, absorbed or conjugated with the bacteria[21] and now multiply or are multiplied in symbiosis with these cells. The reassembled "bacterium-gene complex" has the same aggressive properties as those of the freshly isolated typhoid bacillus. By proper technical methods the reassembled complex can be bred true for innumerable test-tube generations. Under routine cultural conditions, however, redissociation usually takes place.

This transmissible gene apparently has a dual or reversible function, depending upon other environmental factors. Under certain non-colloidal conditions, for example, the ingrafted gene apparently functions as a hereditary lethal factor. Multiplication of the complex continues for a predictable number of generations, after which proliferation ceases and the complex disintegrates, presumably as a result of autolysis. On disintegration, many thousand times the original number of ingrafted genes is set free in the surrounding fluid medium.

VI

Transmissible bacterial genes are apparently widely distributed in nature, being found, for example, in almost any contaminated surface water. These native genes are usually highly specific for one bacterial type, being capable of fruitful symbiosis only with this one type. Occasionally, however, a relatively non-specific or "polyvalent" gene is found, which is capable of successful hybridization with two or more bacterial species. A recently described "trivalent" gene,[22] for example, functions as a successful ingrafted lethal factor with three relatively distinct bacterial strains.

[14] Alloway, 1932, 1933.
[15] Miller, 1932.
[16] Lommel, 1926.
[17] Roux and Yerain, 1890; Wygodtachikoff and Manuilowa, 1930.
[18] Sonnenschein, 1928; Bianchi and Callerio, 1931.
[19] Alexander, 1934.
[20] Krueger, 1932.
[21] Northrop and Krueger, 1932.
[22] Bronfenbrenner, 1933.

Bacterial genes are not immutable in function or in chemical composition. By careful sub-pasteurization, the above-mentioned trivalent gene can be fractionally denatured. Thus partially inactivated, it functions as a strictly monovalent gene, capable of symbiotic proliferation with only one narrow bacterial strain. Grown in repeated symbiosis with this strain, however, its two heterologous, heat-inactivated valences are regenerated.

In the relatively stable bacterium-gene-complex, the bacterial cell apparently does not function as an inert "carrier" of the ingrafted colloid. An aqueous extract of the stable complex yields an apparently new colloidal factor,[23] a gene-inhibiting component, capable of preventing its potentially lethal action.

SUMMARY AND CONCLUSIONS

About the only conventional law of genetics and organic evolution that is not definitely challenged by current bacteriologists is the nineteenth century denial of the possibility of spontaneous generation of bacterial cells. Even this is questioned by certain recent theorists in their hypothetical transformation of certain normal enzymes into "pathogenic genes" or "filterable viruses,"[24] and in their apparently successful synthesis of "Twort genes" by the chemical oxidation of certain heat-sterilized organic products.[25]

Whether or not future refinements in immunochemical technique can or will bridge the gap between the apparent Lamarckian world of bacteriology and the presumptive Darwinian world of higher biological science is beyond current prophecy.

OBITUARY

GEORGE OWEN SQUIER

MAJOR GENERAL GEORGE O. SQUIER (retired), distinguished army officer, engineer, inventor and applied scientist, died at Washington, D. C., on March 24, 1934.

George O. Squier was born at Dryden, Michigan, on March 21, 1865. He early showed marked ambition for advancement. At 18 years of age he entered the West Point Military Academy, where he graduated in 1887. In later years, he used to tell fascinating stories of West Point cadet life that would have made interesting reading had they been published.

After leaving West Point, he was appointed a second lieutenant in the 3rd Artillery. Desiring originally to pursue scientific studies in the field of ballistics, he entered the Johns Hopkins University as a graduate student, and received a degree of doctor of philosophy in 1893. He then returned to the Artillery Corps and became instructor at the U. S. Artillery School at Fortress Monroe, Virginia, from 1895 to 1898.

At the outbreak of the war with Spain in 1898, Dr. Squier sought service in the Signal Officer Volunteers and entered with the grade of captain. In this service, he was sent to the Philippine Archipelago, in 1900, where he commanded the cable ship, *Burnside,* and laid a system of submarine cables between strategic points in the islands. After the war, he was appointed first a captain, later a major in the U. S. Signal Corps and became chief signal officer in the California district.

It was during this period that he took up the study of army cable and radio communication, and published several papers in this field. Major Squier discovered that a growing tree could serve as a receiving radio antenna if a nail was driven into it fairly high up and a wire brought down from the nail to the receiving instrument. As a corollary to this proposition that trees and their branches have sufficient conductance to serve as antennas, he showed that forests, shrubs and vegetation generally, act as partially absorbent media for radio waves passing over land areas. He also made a study of aviation, then in its early stages of development. In 1908, Major Squier had been the first passenger of the world aviation pioneer, Orville Wright, in the latter's early form of airplane at Fort Myer. Twenty years later, the two men met in Washington to compare their aviation experiences.

From 1912 to 1916, Lieutenant Colonel Squier was a military attaché to the U. S. Embassy at London, where he made a special study of European military aviation and where the British army authorities gave him special facilities for investigation. He was a close observer of the British technical radio and aviation preparations during the first two years of the world war. The U. S. Ambassador to Great Britain at that time, Walter H. Page, wrote a glowing account in his Memoirs of Colonel Squier's services in London. Recalled to America in May 1916, Brigadier General Squier was put in charge of the U. S. signal service as chief signal officer. He organized and administered the electrical communication service between the American Expeditionary Force in Europe and its bases in America, using for that purpose radio, cables and wires. This service continued until two years after the war. He was raised to the rank of major general in 1917, and later received the distinguished service medal (D. S. M.) for his services.

[23] Levine and Frisch, 1933.

[24] Vinson, 1931.

[25] LeMar and Myers, 1933.