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Mabel M. Esten

Albert G. Dannin

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Edited by

Ray C. Friesner

The *Butler University Botanical Studies* journal was published by the Botany Department of Butler University, Indianapolis, Indiana, from 1929 to 1964. The scientific journal featured original papers primarily on plant ecology, taxonomy, and microbiology. The papers contain valuable historical studies, especially floristic surveys that document Indiana's vegetation in past decades. Authors were Butler faculty, current and former master's degree students and undergraduates, and other Indiana botanists. The journal was started by Stanley Cain, noted conservation biologist, and edited through most of its years of production by Ray C. Friesner, Butler's first botanist and founder of the department in 1919. The journal was distributed to learned societies and libraries through exchange.

During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner, included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight. W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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CHLOROPHYLL THERAPY AND ITS RELATION TO PATHOGENIC BACTERIA

By MABEL M. ESTEN AND ALBERT G. DANNIN

Although the green parts of plants have been used in the treatment of various diseases for many centuries, it was not until the scientific investigations of Willstaetter and Stoll in 1913 (10) that actual proof of the worth of such remedies began to be found. The close similarity of the chemical character of the chlorophylls found in the chloroplasts of green plants to that of the hemoglobin in human blood led to the theory that the chlorophylls might be the factors in green plants which give them therapeutic value, so a great deal of scientific investigation has been undertaken in laboratories, hospitals and in the private practices of many physicians to determine the efficacy of chlorophyll therapy. Many reports of excellent results in treatment of various diseases with chlorophyll were made in scientific publications and medical journals prior to 1940, but research and applications of the results have been greatly accelerated since that date. Professors and physicians in medical and dental colleges and in private practices have cooperated with manufacturers of chlorophyll preparations and derivatives, and the results of their work are shown in a large number of reports in medical and dental journals. Much of this research, accompanied by relief and cure for hundreds of patients, has been due to the efforts of the Rystan Company of Mount Vernon, New York, who have furnished chlorophyll preparations to physicians and research workers, under the trade name of Chloresium. Although it is not the purpose of this paper to discuss fully all the results gained during the years since 1913, a few statements will be made as to the general therapeutic uses of chlorophyll preparations.

Chlorophyll and its derivatives are used primarily in the form of ointments or liquids in a hydrophilic or an oil base, with variations to meet the therapeutic need. It should be emphasized that these preparations are not the direct extract from green plants but are derivatives of chlorophyll. In Chloresium products, for example, only the water soluble derivatives of chlorophyll A are used, chiefly in a hydrophilic base. In the natural state chlorophyll occurs in a colloidal form but it is amorphous when isolated, and is ordinarily secured in a crystalline form for medicinal use. The preparations are applied topically, orally or systemically. In both topical and oral treatments they are used as wet dressings, ointments, irrigations and sprays. The systemic treatments are chiefly by means of packings, sprays and direct internal injections.

In the medical fields the following are the main types of diseases treated with chlorophyll preparations: cysts, ulcers and tumors of various kinds, wounds, burns, anal fistulae, ulcerative colitis, diseases of the perineal cavity, bed sores, gynecological and orthopedic conditions, ear, nose and throat infections, dermatoses, anemia and carcinoma. In dentistry the following have been treated with chlorophyll: Vincent's stomatitis, gingivitis, osteomyelitis, pyorrhea, post-operative conditions, malodorous breath and conditions requiring peridontal sepsis. A few of the outstanding results of chlorophyll therapy are the rapid acceleration of healing, rapid and sustained proliferation of normal tissue cells, formation of healthy granulation tissue, reduction of bacterial action, relief from pain, itching and burning, and the deodorization and clearing of malodorous suppurative conditions.

Chlorophyll products are apparently non-toxic, which gives them great advantage over many other substances. However, Burgi (3) reported in 1932 that cyanosis might be caused by extremely large internal doses of chlorophyll and that a cumulative effect might also occur. Becker (1) gave a report of one patient who showed a sensitivity to an isotonic solution of chlorophyll used as nasal packs in the treatment of sinusitis, but the absence of control conditions make this inconclusive. An extensive search of literature on the subject of chlorophyll therapy does not reveal any other reports of toxicity reactions and the concensus of opinion of all other writers is that chlorophyll preparations are completely non-toxic. Hein and Shafer (7) state that there are no toxic complications and the preparations may be used safely in concentrations up to 2% in the oral cavity. Gruskin (6) reports cases in which as much as 250 cc of a chlorophyll preparation was given intravenously for a period of six days without any toxic reactions whatever. Smith (9) states that 240 cc of a 2% solution was given orally for three days to a normal healthy male volunteer and there was no toxic effect. He also reports a case of subacute bacterial endocarditis in which 400 cc of a 0.5% cholophyll solution was given daily for eight days, with no toxic symptoms appearing at any time.

The excellent results gained in the treatment of various conditions in which pathogenic bacteria are involved have raised the question as to the exact relation of chlorophyll to the causal organisms. Impetus to research has been greatly stimulated by the work of Gruskin (6) and his colleagues at Temple University. Chlorophyll solutions and ointments prepared by the Department of Experimental Pathology at Temple University were used in the treatment of more than 1200 cases of "widely diverse character, ranging from acute infections of the upper respiratory tract and accessory sinuses to chronic ulcerative lesions of various types associated with varices, sinuses, and fistulae." Some cases were in the fields of proctology, gynecology, surgical infections, ear, nose and throat infections, and others were simple and complex dermatoses. Ulcerative carcinoma with much putrefaction and foul odor was cleared very promptly of odor and the chlorophyll preparation also stimulated production of connective tissue. The prompt relief from the odors of such suppurative diseases led Gruskin to conclude that chlorophyll has an apparent bactericidal effect upon the various pathogenic bacteria, although not actually bactericidal. He suggests that chlorophyll increases the resistance of cells in some physio-chemical manner so that enzymatic digestion of the cell membrane by invading bacteria or their toxins is checked, and that bacteria may be inhibited from forming their toxic compounds by the chlorophyll action. Since chlorophyll has the ability in the living cell where it is produced to break down carbon dioxide and free oxygen, he believes it may have the same action in the medicinal preparation and thus inhibit the action of anaerobic bacteria which cause malodorous lesions. Gruskin also reports a case of streptococcic septicemia which had an uneventful recovery with the sole use of chlorophyll derivatives. In a case of subacute bacterial endocarditis, the blood cultures remained negative for the bacteria as long as the patient received chlorophyll but became positive again after the treatment had been discontinued for three days, evidently as a result of a continuous discharge of septic emboli into the circulation. ' As a result of his experiments, Gruskin concludes that chlorophyll preparations do not have a definite bactericidal effect but do have some bacteriostatic effect, apparently by the stimulation of cells and tissues and the creation of adverse environmental conditions for the bacteria. However, he believes there may be a more direct action.

Goldberg (5), in a study of 300 dental cases, reports that odors due to anaerobic bacteria invariably disappear with a few chlorophyll applications, and he attributes this result to the oxidizing properties of chlorophyll. In cases of Vincent's stomatitis he came to the conclusion that chlorophyll therapy is almost a specific against the fusiform bacillus and the spirillum responsible for this condition. His results, however, seem to indicate a bacteriostatic and not a bactericidal action.

Rapp (8) reported a study of the effect of a chlorophyll preparation on the *Lactobacillus acidophilus* count in human saliva. Twentysix per cent of the subjects had a negative count at the end of ten days, 42% at the end of the fourth week, and 90% at the close of the twenty-sixth week of the experiment. The results in the control group of subjects who had used other normal oral hygiene procedures showed a slight reduction in count, but not of any significant value. While there is some question of the relation of *Lactobacillus acidophilus* to tooth decay, there seems no reason to doubt that chlorophyll does have an inhibiting effect upon the organism. The report does not attempt to explain the action of chlorophyll upon this particular pathogen.

Carpenter (4) has recently investigated the use of chlorophyll preparations clinically in a carefully selected and controlled series of cases. One outstanding result of his study is that the wounds which were resistant or slow in healing or failed entirely to heal contained a form of bacteria which he calls "*Pseudomonas aerogenes*." The percentage of failures, however, was remarkably low. Carpenter concluded that chlorophyll is bactericidal in vitro in large concentrations but that its action in vivo is by means of its stimulating effect upon tissue cells rather than as a direct bactericidal agent.

Bowers (2) records his own experiences and those of more than thirty officers of the surgical service in an army hospital in the treatment of various types of cases with water-soluble derivatives of chlorophyll. He states that "they possess direct antibacterial activity, especially in relation to the secondary saprophytic, proteolytic organisms associated with wound infection, which give rise to the foul odor associated with this type of lesion." He concludes that the action is actually bacteriostatic and that its beneficial effect is by means of stimulation of host cells instead of direct action against the bacteria themselves.

Smith (9) conducted experiments both in vitro and in vivo in an effort to determine the action of chlorophyll preparations upon various diseases, using a saponified metal complex derivative, such as sodium copper chlorophyllin, which was provided in a saline solution in strengths ranging from 0.2% to 5% and also as ointments in lanolin, cholesterol and hydrophyllic bases. His preliminary studies indicated that chlorophyll alone in dilutions of from 1:100 to 1:500 would not support the growth or viability of staphylococci, streptococci or coliform bacteria over a period of twenty-four to forty-eight hours. There was a definite bacteriostatic effect upon the growth of these organisms when dilutions up to 1:2000 were used in a broth medium, but growth was not completely inhibited in this medium beyond a 1:5000 dilution. The deodorization and clearing of such infected, ulcerated lesions as carcinoma and varicose ulcers in a short period led Smith to conduct experiments in vitro to attempt a determination of the action of the chlorophyll derivatives upon the causal organisms. He used eight pathogens with various dilutions of chlorophyll, with veal infusion and one per cent amigen broth as media. The innoculations averaged about 9000 colonies, except in the case of the two anaerohes in which 1000 colonies were used. Inhibition of growth was noted in Escherichia coli and Pseudomonas pyocyaneus in 1:50 dilutions; in Streptococcus mastitidis, two strains of Streptococcus hemolyticus, Clostridium perfringens and Clostridium histolyticum in 1:1600 dilutions; and there was no inhibition of the growth of Staphylococcus aureus. Repeated experiments indicated that chlorophyll might have an action which interferes with the oxidationreduction mechanism of anaerobic bacterial respiration, which might explain its bacteriostatic and even bactericidal effect in clinical cases. As a result of his studies, Smith states that the ordinary pathogenic bacteria will not survive for twenty-four hours in a simple isotonic saline solution of chlorophyll up to dilutions of 1:5000, which seems to be due to an oxidation action. The experiments on the more common pathogenic bacteria indicate that there is no direct action but bacterial growth is inhibited as a result of the production of an unfavorable environment. Smith states that "chlorophyll is not strictly bactericidal but that it does exert a definite bacteriostatic and even a bactericidal effect under suitable environmental conditions."

In brief summarization, it may be stated that investigations and experiments in chlorophyll therapy and in laboratory research indicate that strong solutions of chlorophyll preparations have a bactericidal action on some pathogenic bacteria in vitro. However, in vivo, the effect seems bacteriostatic rather than bactericidal, chiefly through the action of the chlorophyll in stimulating tissue cells and creating an environment unsuitable for bacterial growth. The ability of chlorophyll to release oxygen is cited as a possible cause of the inhibition of anaerobic bacteria which are particularly active in suppurative diseases. Research to date indicates that chlorophyll has a definite place in the treatment of diseases caused by bacteria, but further research is necessary to determine the exact nature of the effect of chlorophyll upon the causal organisms.

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